

RICE GENETICS

INTERNATIONAL RICE
RESEARCH INSTITUTE



RICE GENETICS

Proceedings of the
International Rice Genetics Symposium

27–31 May 1985

1986
International Rice Research Institute
P.O. Box 933, Manila, Philippines

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IRRI receives support, through the CGIAR, from a number of donors including: the Asian Development Bank, the European Economic Community, the Ford Foundation, the International Development Research Centre, the International Fund for Agricultural Development, the OPEC Special Fund, the Rockefeller Foundation, the United Nations Development Programme, the World Bank, and the international aid agencies of the following governments: Australia, Belgium, Brazil, Canada, China, Denmark, Fed. Rep. Germany, India, Italy, Japan, Mexico, Netherlands, New Zealand, Philippines, Saudi Arabia, Spain, Sweden, Switzerland, United Kingdom, and United States.

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Editing, design, and production by:
Island Publishing House, Inc.
Sta. Mesa P.O. Box 406, Manila, Philippines

ISBN 971-104-148-0

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FOREWORD

M.S. SWAMINATHAN
Director General

IRRI first organized a symposium on rice genetics and cytogenetics in February 1963. The proceedings of that symposium, published in 1964, contain an excellent summary of the knowledge then available concerning different aspects of rice genetics and cytogenetics. Drs. T. T. Chang and Peter R. Jennings played an important role in organizing that gathering. Unfortunately, since then no major international symposium on rice genetics seems to have been organized, either at IRRI or elsewhere. In contrast, in other major cereals like wheat and barley, international symposia are held once in 5 years to review the state of knowledge of genetics and to develop collaborative research in fields such as gene mapping, identification and conservation of genetic stocks, and gene symbolization and nomenclature.

In spite of the great importance of rice to both human nutrition and the rural economy in many nations of the world, there has been no systematic effort to organize at periodic intervals symposia on rice genetics. A continuous updating of knowledge of the genetics of the rice plant and the precise identification of the nature of the genetic control of important economic characters have become particularly urgent in the context of recent advances in molecular biology and genetic engineering.

We at IRRI, therefore, welcomed a proposal from our Japanese colleagues for organizing the present meeting. Scientists of the Japanese Committee on Rice Gene Nomenclature and Linkage Groups and IRRI joined together and planned the details of the symposium. I am particularly grateful to Prof. M. Takahashi, who agreed to serve as Co-chairman, and Dr. H. I. Oka, who assumed the role of Secretary of the International Organizing Committee. Most of the preparatory work at IRRI was done by Dr. G. S. Khush and his colleagues in the Plant Breeding Department. Our gratitude also goes to the other members of the Organizing Committee, namely, Drs. J. N. Rutger, R. C. Chaudhary, S.M.H. Zaman, B.H. Siwi, Min Shao Kai, T. Kinoshita, M.H. Heu, M. Jacquot, A. Abifarín, T. T. Chang, Ray Wu, and M. Van Montagu.

After the idea of organizing the symposium was finalized, we were fortunate to have had the benefit of discussions with Dr. Alva App, Director for Agricultural Sciences of the Rockefeller Foundation, who generously came forward with the assurance of financial support. His address discusses the new initiative of the Rockefeller Foundation in the field of genetic engineering in rice. We are most grateful to Drs. App and Gary Toenniessen and the Rockefeller Foundation for their support.

WELCOME ADDRESS

MS. SWAMINATHAN
Director General

During the course of this symposium we will give serious consideration to organizing a Rice Genetics Cooperative for the following purposes:

- To organize once in 5 years an International Rice Genetics Symposium, on the model of those already being organized in wheat, barley, etc.
- To develop standard rules for gene symbolization and nomenclature.
- To ensure the proper maintenance of genetic and chromosome markers including trisomics and translocation stocks and to make them available to interested researchers.
- To prepare and continuously update linkage maps.
- To monitor and promote the application of genetic engineering and tissue culture techniques.
- To assume responsibility for publishing the *Rice Genetics Newsletter*.

Under the Cooperative, Standing Committees could be appointed, each with an appropriate rice geneticist as convenor, to follow up and implement programs in the period between two international symposia. IRRI will be happy to provide secretarial and logistic support to such an endeavor. In consultation with several of you, we shall prepare some concrete proposals for your consideration during the concluding session.

I am glad that, with the help of our Japanese colleagues, a *Rice Genetics Newsletter* has been started. Dr. Khush and I are grateful to Drs. H. I. Oka, Y. Futsuhara, and T. Kinoshita for their labor of love in organizing the publication of this newsletter. The first issue was published last year under the joint editorship of Drs. Oka and Khush. This publication will provide an excellent vehicle for communicating information on the work of the proposed Rice Genetics Cooperative and its Standing Committees.

I welcome all of you and thank you for travelling long distances to be here with us today. Our group contains a happy blend of Mendelian and molecular geneticists. I hope this will help to generate some hybrid vigor in the work of rice geneticists. May I extend a particular welcome to the doyens of rice genetics like Profs. Matsuo, Takahashi, and Oka. I am also

happy to see among us today a few who attended the 1963 symposium. We are equally fortunate to have with us Profs. C. M. Rick and Ralph Riley. Prof. Rick's experience in heading the Tomato Genetics Cooperative for over 30 years will be invaluable in organizing the Rice Genetics Cooperative.

We have a very heavy program ahead of us. I hope that with the cooperation of all the participants we can make this first International Rice Genetics Symposium a very memorable and valuable one. To us at IRRI, this gathering is of particular significance, since it is the first of three symposia we are hosting to commemorate IRRI's 25th Anniversary, as well as a meeting of the International Rice Commission. The major purposes of these meetings are to review the current state of knowledge in different areas of rice science and to provide guidelines for our work during the rest of the century.

I welcome you all once again.

OPENING REMARKS

T. MATSUO
Professor Emeritus
University of Tokyo

I am very happy to be with all of you, the rice geneticists of the world. Today is a momentous day in the history of rice genetics, so many world leaders in the field are meeting on a common platform.

Our knowledge of rice genetics lags far behind that of other important food crops such as maize, wheat, barley, and tomato. Linkage maps of rice are still poorly understood. One reason is that there has been very little international coordination among rice geneticists. It is therefore high time to hold this symposium and discuss the present status of the field. I hope such international symposia will be held periodically.

There is also a common desire among rice geneticists to evolve an acceptable gene nomenclature and chromosome numbering system. We also need to establish gene stock centers to maintain and preserve the seeds of mutant stocks. An informal organization such as the Rice Genetics Cooperative (RGC) is needed to promote the exchange of materials and information among scientists.

I hope the rules of gene nomenclature and a chromosome numbering system will be agreed upon and that gene stock centers will be set up and the RGC established before this symposium is over. The RGC should be charged with the responsibility for monitoring gene symbols, linkage mapping on a cooperative basis, maintenance of gene stocks, and publication of a Rice Genetics Newsletter (RGN) annually. Fortunately, a beginning has been made in the publication of the RGN. The decision for publication was taken up after consultations among Drs. M. S. Swaminathan, G. S. Khush, H. I. Oka, Y. Futsuhara, and T. Kinoshita. The first volume of the RGN was published in December 1984 under the editorship of Drs. Oka and Khush. I hope the RGN will play a major role in promoting the cause of rice genetics.

I wish you best of luck for the success of this symposium, and I am looking forward to interacting with you during the next four days.

KEYNOTE ADDRESS

A. A. APP
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Please allow me to begin by pointing out what you already know — first, that rice is the most important food staple in the developing world, and second, that it is very probable that the population in the less developed countries (LDCs) will go from 3.6 billion in 1984 to approximately 6.4 billion in the year 2020. This means that cereal production in the LDCs will have to increase from 90 to 100% over this period of time. How will the LDCs feed all these people?

I believe we stand on the brink of a major revolution in the plant sciences, and I think that this is going to have an enormous effect on crop yields. It is not a question of whether or not it will have an effect: It is only a question of *when* and the *magnitude*.

Transformation of dicots is a reality today — a standard procedure. Many knowledgeable experts say that the same will be true of cereals within the next three or four years. Furthermore, we can expect the pace of development in genetic engineering to increase as time goes on. This new technology will produce breakthroughs in yield, grain quality, tolerance for environmental stress, resistance to pests and pathogens, and so on. This in turn will have an enormous impact on the economics of production, the environment, and the type and quantity of requirements for off-farm inputs. In addition, it will raise serious questions of equity for society within a given country, and between nations.

There is a problem. The first Green Revolution in rice was the result in part of the successful application of classical plant breeding. The principles and practice of plant breeding were well established. It was a matter of applying them to rice in the developing world. However, who is going to develop the methodology for genetic engineering of rice and assist in applying this to modern plant breeding programs in the LDCs? It is unlikely that the private sector will do this — the profit motive is missing. It is also unlikely that the public sector in the more developed world will do this, since rice is not an important cereal in their countries. The

ultimate responsibility and execution of this task resides with the rice-growing countries of the world. There is great concern that if this task is not accomplished, rice, the most important food in the world, will not benefit from the revolution in the plant sciences. The Rockefeller Foundation sees this as an important challenge, and within the limits of its resources and its sincere wish to help the LDCs, if asked, to obtain the scientific capability to develop and exploit biotechnology in their rice improvement programs.

We see the use of biotechnology as a second generation effort in rice variety improvement. However, we need a much better knowledge of the rice plant, and we need to stimulate research in the basic disciplines such as rice genetics, physiology, biochemistry, entomology, and pathology. Development of the knowledge base in these various disciplines will take time and funds. It is imperative that this research be expanded immediately. Otherwise, we run the risk of seeing a powerful new set of tools for genetic manipulation lie idle because of the lack of understanding of the systems we wish to modify and improve.

Classical plant breeding has served us well and is continuing to make a substantial contribution to increased rice production. It has been so successful in recent years that the area of rice genetics was felt by many to be somewhat irrelevant for varietal improvement, and as a consequence has been underfunded; has not attracted an adequate number of young, bright, well-trained scientists; and in general has been neglected.

It is for the above reasons that the Board of Trustees of the Rockefeller Foundation is particularly pleased and honored to join with the Japanese Committee on Rice Gene Nomenclature and Linkage Groups and with IRRI in support of this symposium.

Thank you very much.

ABBREVIATIONS

AC	-	analysis of correspondence
AC	-	anther culture
AICRIP	-	All India Coordinated Rice Improvement Project (new name) Directorate of Rice Research
BAP	-	benzylaminopurine
BC	-	backcross
BPH	-	brown planthopper
BRII	-	Bangladesh Rice Research Institute
BVG	-	basic vegetative growth
BVP	-	basic vegetative phase
CAAS	-	Chinese Academy of Agricultural Sciences
CARI	-	Central Agricultural Research Institute, Sri Lanka
CCRRFI	-	California Cooperative Rice Research Foundation, Inc.
CIAT	-	International Center of Tropical Agriculture, Colombia
CIRAD	-	International Center of Agronomic Research for Development, France
CLSU	-	Central Luzon State University, Philippines
CMS	-	cytoplasmic male sterile
CNRRI	-	China National Rice Research Institute
CNRS	-	National Center for Scientific Research, France
CRIFC	-	Central Research Institute for Food Crops, Indonesia
CRRI	-	Central Rice Research Institute, India
DAF	-	days after flowering
DAH	-	days after harvest
DAT	-	days after treatment
DGWG	-	Deo-geo-woo-gen
DH	-	days to heading
DLA	-	diseased leaf area
DMS	-	dimethyl sulfate
DNA	-	deoxyribonucleic acid
E	-	embryogenic
EI	-	ethylene imine
ENU	-	N-ethyl-N-nitrosourea
FAO	-	Food and Agriculture Organization
GA ₃	-	gibberellic acid
GCA	-	general combining ability
GEU	-	genetic evaluation and utilization

GLH	– green leafhopper
GMA	– glycol methacrylate
GMS	– genetic male sterile
HPAU	– Himachal Pradesh Agricultural University, India
IBPGR	– International Board for Plant Genetic Resources
IAEA	– International Atomic Energy Agency
IITA	– International Institute of Tropical Agriculture
INIA	– National Institute of Agricultural Research, Mexico
IPBNET	– International Plant Biotechnology Network
IRAT/GERDAT	– Research Institute for Tropical Agriculture and Food Crops (new name) IRAT/CIRAD, France
IRGS	– International Rice Genetics Symposium
IRRI	– International Rice Research Institute
ITEC	– Indian Technical and Economic Cooperation
MARDI	– Malaysian Agricultural Research and Development Institute
MBNL	– Mutation Breeding Newsletter
MNG	– N-methyl-N'-nitro-N-nitrosoguanidine
MNU	– N-methyl-N-nitrosourea
NAA	– naphthaleneacetic acid
NE	– nonembryogenic
NIAR	– National Institute of Agrobiological Resources, Japan
NIG	– National Institute of Genetics, Japan
ns	– nonsignificant
ORSTOM	– French Institute for Research and Development in Cooperation
OUAT	– Orissa University of Agriculture and Technology, India
PAS	– periodic acid—Schiff's
PAU	– Punjab Agricultural University, India
PEG	– polyethylene glycol
PMC	– pollen mother cell
PS	– photoperiod sensitive
R	– resistant
rc	– rank correlation
RGC	– Rice Genetics Cooperative
RGN	– Rice Genetics Newsletter
RRI	– relative resistance index
S	– susceptible
SCA	– specific combining ability
SD	– standard deviation

Seg	-	segregating
SES	-	Standard Evaluation System for rice
SSD	-	single seed descent
TARI	-	Taiwan Agricultural Research Institute
TBO	-	toluiding blue O
TCCP	-	Tissue Culture for Crops Project
TDAIS	-	Taichung District Agricultural Improvement Station, Taiwan
TLP	-	trisomic-like plant
TR	-	tolerance ratio
UKM	-	National University of Malaysia
UPLB	-	University of the Philippines at Los Baños
USDA-ARS	-	United States Department of Agriculture/Agricultural Research Service
WARDA	-	West Africa Rice Development Association
WAT	-	weeks after transplanting
WBPH	-	whitebacked planthopper
WCV	-	wide compatibility variety
ZLH	-	zigzag leafhopper

SYSTEMATICS AND EVOLUTION

SESSION 1

WILD PROGENITORS OF CULTIVATED RICE AND THEIR POPULATION DYNAMICS

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The common wild rice of Asia shows a perennial-annual continuum and wide variations in life history traits suggesting r - vs. K -selection. It also shows a trend of association of $KC10_3$ resistance and low temperature resistance among populations as is found among indica and japonica rice cultivars. The populations contain various isozyme alleles, and the most frequent genotypes are those found in intermediate indica-japonica cultivars. The genetic structure of populations reflects differences in breeding systems. When experimental populations raised from juvenile plants collected in natural habitats were examined, intermediate perennial-annual populations were mostly polymorphic. An example is presented showing that an intermediate population is ready to respond to environmental heterogeneity both spatially and temporally. These observations support our previous hypothesis that the intermediate perennial-annual type of wild rice is most likely to be the immediate progenitor of rice cultivars.

The common wild rice or the *Oryza perennis* complex is distributed throughout the humid tropics and is comprised of four major geographical forms: Asian, American, African, and Oceanian (8). It is generally accepted that *O. sativa* L. has been domesticated from the Asian form of this species complex (= *O. rufipogon* Griff.) in Asia, while *O. glaberrima* Steud. in Africa stems from *O. breviligulata* Chev. et Rheor (10). With my colleagues, I have continued investigations on the wild and cultivated rices concerning character variations, isozymes, breeding systems, F_1 sterility relationships, isolating mechanisms, introgression, population structure, and adaptive strategies, as partly reviewed by Oka (18) and Morishima (9). The main direction of differentiation in the Asian common wild rices is represented by that between the perennial and annual types (13). The two types also differ in many traits and habitat preferences and in adaptive strategy (26).

Whether the perennial or the annual type of wild rice is the ancestor of cultivars has been an unsettled issue. Chatterjee (3), Ramiah and Ghose (23), and Chang (1, 2) considered the annual type to be the ancestor in view of character similarities. Sampath and Rao (25), Richharia (24), and Oka (17, 18) considered the perennial type to be the ancestor. *O. sativa* is essentially a perennial plant, and a continuous array of grada-

tions between the perennial types and cultivars has been found in the semi-wild material from Jeypore Tract, India (19). Recently, we put forward another hypothesis that an intermediate perennial-annual type was more likely to be the progenitor in view of its high evolutionary potentiality and fairly high reproductive effort (27).

As to the site of incipient domestication, Chang (1, 2) considered a broad area extending over the foothills of the Himalayas and its associated mountain ranges; this broad area is comparable to Harlan's (6) noncenter concept. As to the time of origin, the eldest remains of rice grains or hulls have been excavated in India (Naharaga, Uttar Pradesh), China (Ho-Mu-Do, Xhejiang), and Thailand (Non Nok Tha), all being 6,500 to 7,500 years old. This suggests diffused origins of rice domestication (7).

DIFFERENTIATION OF ASIAN WILD RICE POPULATIONS

Compared with the perennial type, the annual type is characterized by a low regenerating ability, high reproductive allocation of resources, early flowering time, awn development, high selfing rate, and tolerance to drought and submergence (26). The annual types grow in relatively shallow, temporary swamps that are parched in the dry season, while the perennial types grow in deeper swamps having a more stable water condition. The mode of association of life history traits with habitat conditions indicates that the annual types are *r* strategists and the perennial types *K* strategists (4).

Wild strains from various localities of Asia are scattered according to anther length, representing outcrossing rate and percent reproductive allocation (Fig. 1). The two values are intercorrelated (20) and show a wide range of variation among strains for each locality, although Chinese strains are largely of perennial types having a low reproductive allocation. Strains with medium values in these traits can be regarded as intermediate perennial-annual types. Some of them showing traces of characteristics of a cultigen could have resulted from introgression between wild and cultivated plants. The intermediate types are relatively infrequent and often found in strongly disturbed habitats, suggesting that they are disturbance tolerant. They also contain a large amount of genetic variation in their populations and can be differentiated into perennial and annual types in response to microenvironmental conditions (14, 27).

Reproductive allocation (%)

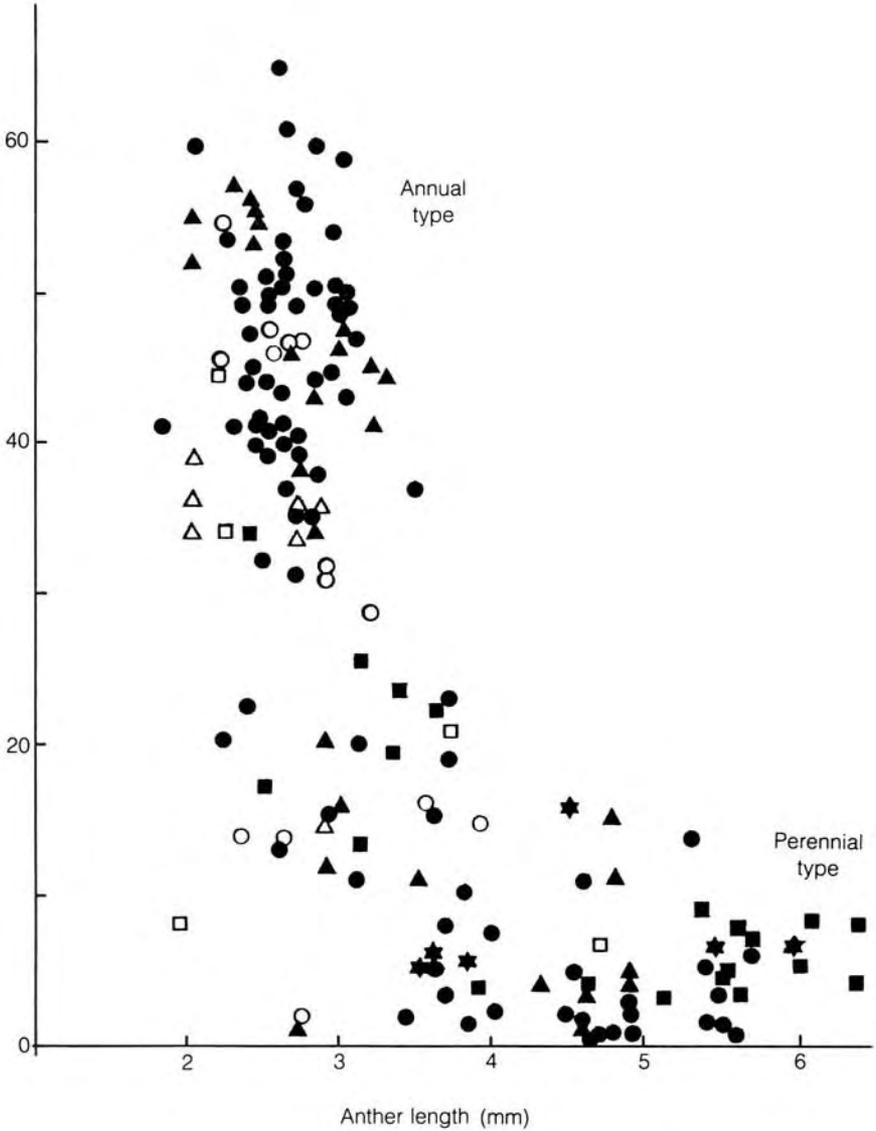


Fig. 1. Asian wild rice strains scattered by anther length and reproductive allocation. Solid symbols represent the strains showing high seed shedding and open symbols show low seed shedding. ▲ = India; ● = Thailand, Burma, Malaysia; ■ = China; ★ = Malayan archipelago.

In reproductive effort or seed productivity and selfing rate, wild annual types are more similar to cultigens than perennial types. In vegetative regenerating ability, seed dormancy, and awn development, wild perennial types are more similar to cultigens than the annual types. In view of the trend of evolution generally recognized in higher plants, it may be inferred that the perennial type is primitive and the annual type and cultigen have shared the evolutionary process to increase seed productivity. However, domestication has brought about other characteristics like uniform seed germination due to reduced dormancy, contemporary tillering and uniform seed maturity, and reduced seed shedding in addition to seed productivity, most probably in response to selection due to seeding and harvesting by man, which we have called cultivation pressure (21).

One may question which of the perennial and annual types is more similar to a cultigen in alleles for isozymes. Based on a multivariate analysis of data for 20 loci, Second (29) pointed out that the perennial and annual types could not be distinguished clearly. In our data, the perennial and annual types showed a difference in the frequency of alleles at *Sdh-1*, *Pox-1*, and some loci; at *Sdh-1*, allele 1 was predominant in the annual and allele 3 in the perennial types, while allele 2 was predominant among cultivars (J.C. Glaszmann, pers. comm.). At *Pox-1*, allele 2A was predominant in annual and cultivars, and both 2A and 4A in perennial types. Whether or not allozymic variations are associated with adaptive traits is under observation. In general, the wild populations are more polymorphic than cultivars and carry alleles that are not found among cultivars. Yet, it does not seem possible, at present, to detect the wild progenitors of cultivars on the basis of isozymic genotypes.

INDICA-JAPONICA DIFFERENTIATION IN WILD RICES

Rice cultivars are differentiated into the indica and japonica types, which are distinguishable by different patterns of character association or by correlations of diagnostic characters (12, 16). In wild rices, no significant correlations of diagnostic characters were found among strains from India and Thailand, so that the indica-japonica differentiation was not detectable (18, 22). Using a wider range of materials, we have recently found a tendency to such differentiation shown by a correlation between KClO_3 resistance and cold resistance (Fig. 2a). Most wild strains were sensitive to both KClO_3 and low temperature and were indica-like, but some of them, particularly those from China, were relatively resistant to both and were japonica-like in this sense.

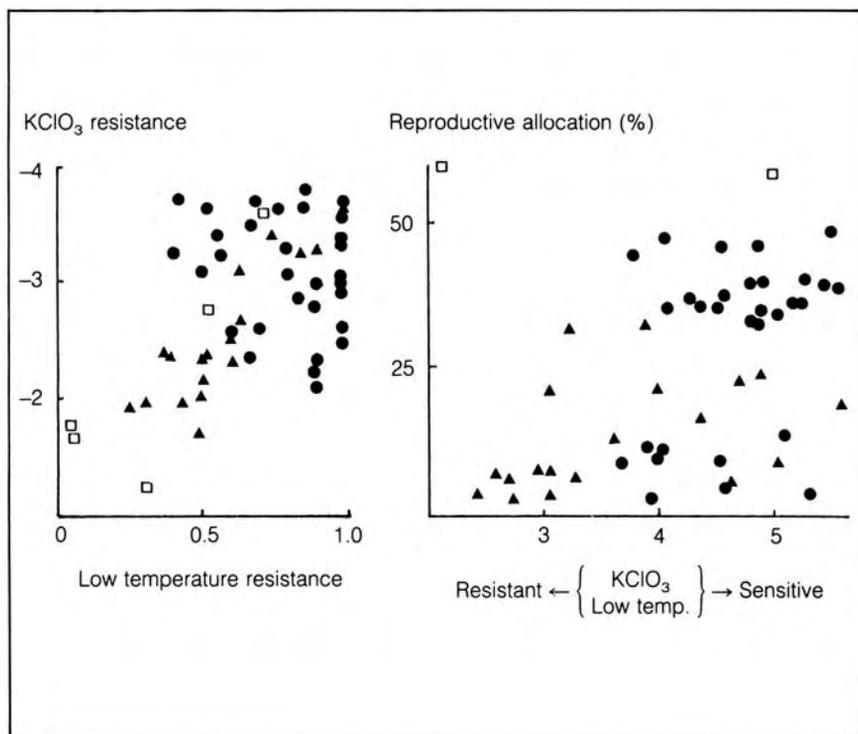


Fig. 2. Asian wild rice strains scattered (a) by resistance to low temperature and to KClO_3 and (b) by a score representing both resistance ($-K + 1.94 L$) and reproductive allocation. ● = India and Thailand, ▲ = China, □ = cultivated strains.

The wild strains were also scattered by an index obtained by combining the measurements of KClO_3 resistance and cold resistance and percent reproductive allocation (Fig. 2b). Strains with low reproductive allocation or perennial types were closer to japonica in the index value than those with high allocation or annual types, which tended to be scattered toward the indica type.

A survey of allozyme variations in wild and cultivated strains by Second (28) showed a certain trend of variation among rice cultivars that correspond to the indica-japonica differentiation. We are also engaged in this work; when examined with three diagnostic loci, *Cat-1*, *Pgi-1*, and *Acp-1*, 14 genotypes were recognized among cultivated strains. Two genotypes were most frequent and represented the indica and japonica types in cultivars (Fig. 3). The third most frequent genotype, which was a recombinant type, formed a group distributed mainly in the hilly areas of tropical Asia. When compared with the result of multivariate analysis of character values, varieties having those allozyme genotypes were inter-

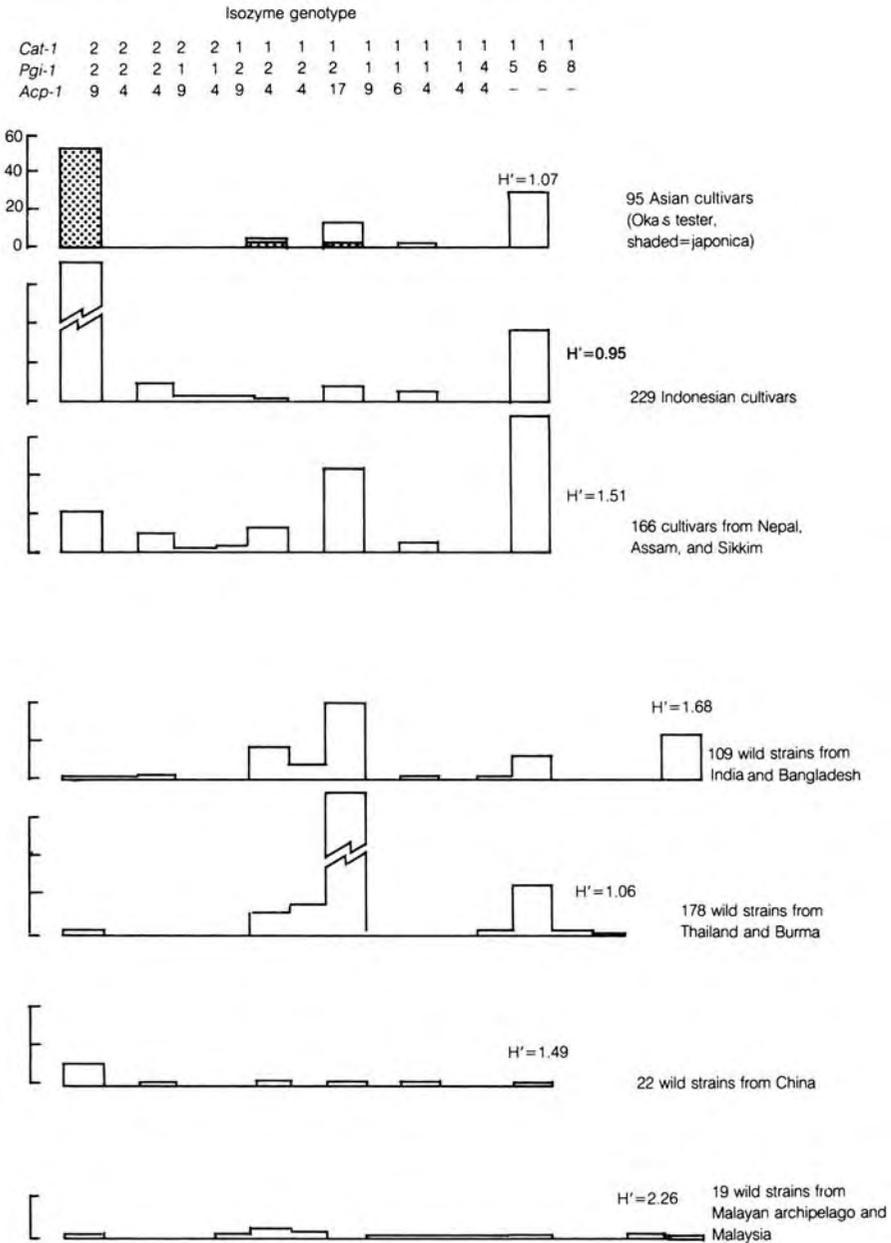


Fig. 3. Distributions of 17 isozyme genotypes for *Cat-1*, *Pgi-1*, and *Acp-1*, whose alleles differ in frequency between the indica and japonica types, among Asian rice cultivars and wild rice strains from different localities. $H' = -\sum x_i \ln x_i$, in which x_i represents frequency of the i -th genotype.

mediate indica-japonica types, although most of them were classified as indicas.

The wild strains showed a wider range of genotypic variation. Among them, the intermediate indica-japonica genotypes were most predominant. Among Chinese strains, the genotypes representing japonica were relatively frequent, as pointed out by Second (29). Allozyme similarity has often been used to assess phylogenetic relationships. In rice, however, the allozyme genotypes are various and their frequencies are subjected to sampling error and other conditions. It may be concluded that the genotypes representing intermediate indica-japonica cultivars are frequent among Asian wild rice strains.

GENETIC STRUCTURE OF WILD RICE POPULATIONS

Inter- and intrapopulational diversities were assessed on the basis of variations in isozymes and character values by using plants raised from seeds collected in their natural habitats. Generally, perennial populations were more polymorphic than annual populations in both isozymes and visual characters (Table 1). In contrast, interpopulational diversity was much higher among annual populations than among perennial ones. Intermediate perennial-annual populations were in an intermediate state. This pattern reflects the differences in breeding systems (11).

However, the data thus obtained represent the genetic diversity released by seed propagation, which is infrequent in perennial populations. To assess the realized or actual genetic diversity, we have raised experi-

Table 1. Genetic diversity and other parameter values estimated within and between wild rice populations.

Type	Average for <i>Pox-1</i> and <i>Acp-1</i>					Average for 6 characters		
	No. of populations	H_S^a	G_{ST}^b	Fixation index	Heterozygote %	No. of populations	C_S^c	C_{ST}^d
Perennial	24	.290	.532	.083	23.0	9	114	83
Intermediate	12	.117	.688	.427	9.1	7	77	63
Annual	14	.092	.670	.914	1.4	10	48	81

^a H_S = average gene diversity within population.

^b C_{ST} = relative magnitude of gene differentiation among population (15).

^c C_S = intrapopulation genetic variance shown by $\ln X \times 10^3$.

^d C_{ST} = relative magnitude of interpopulational generic variance to total genetic variance (%).

mental populations from juvenile plants collected in natural habitats in addition to those from seeds and have compared them (Table 2). In seed-derived populations, the average gene diversity for 10 isozyme loci was higher in the perennial and intermediate types than in annual types. In the juvenile-derived populations, however, there was no such difference between perennial and annual populations, and intermediate perennial-annual populations showed the highest diversity. The low genetic diversity in perennial populations indicates clonal propagation of a few competitive plants. The high diversity in intermediate populations may be attributed to their propagation by both seed and ratooning (27).

The difference in gene diversity between seed- and juvenile-derived populations differed according to the loci (Table 2). At *Sdh-1*, *Pgd-2*, and *Pox-1*, seed-derived populations seemed to be more polymorphic than corresponding juvenile-derived ones, but no marked differences were found at other loci. The fact that the perennial and annual types differed in allelic frequency at *Sdh-1* and *Pox-1* suggests that selectional mortality is involved in the demographic process of populations.

Table 2. Gene diversities at 10 isozyme loci observed in seed (S)- and juvenile (J)-derived populations, sampled at 6 sites in Thailand.^a

Site	Annual type						Intermediate type				Perennial type	
	NE-2		NE-3		NE-4		NE-1		CP-20		NE-88	
	S	J	S	J	S	J	S	J	S	J	S	J
Plants examined (no.)	33	7	18	78	26	118	64	14	71	9	10	24
Gene diversity ^b												
<i>Acp-1</i>	.000	.000	.000	.000	.076	.076	.295	.495	.661	.661	.500	.000
<i>Est-2</i>	.058	.240	.000	.000	.163	.170	.059	.500	.397	.466	.492	—
<i>Est-Ca</i>	.000	.000	.058	.000	.000	.000	.095	.000	.058	.000	.000	.000
<i>Pgd-1</i>	.226	.130	.448	.226	.496	.499	.113	.332	.379	.180	.320	.076
<i>Pgd-2</i>	.163	.000	.095	.058	.195	.000	.490	.000	.257	.168	.420	.000
<i>Pgi-1</i>	.196	.086	.466	.000	.000	.000	.435	.442	.255	.375	.000	.000
<i>Pgi-2</i>	.080	.086	.436	.108	.000	.080	.376	.526	.243	.643	.000	.076
<i>Pox-1</i>	.000	.000	.195	.000	.000	.000	.498	.000	.255	.500	.500	.499
<i>Pox-2</i>	.000	.000	.000	.036	.000	.262	.000	.000	.000	.095	.000	.000
<i>Sdh-1</i>	.467	.000	.265	.000	.166	.019	.498	.000	.382	.000	.180	.000
Average	.199	.054	.196	.043	.110	.111	.286	.230	.289	.313	.242	.112

^a Data from P. Barbier (unpublished data).

^b $h = 1 - \sum p_i^2$

OBSERVATIONS OF AN INTERMEDIATE PERENNIAL-ANNUAL POPULATION

In an attempt to look into the population biology and genetic changes of wild rice populations, we have continued monitoring several fixed sites in Thailand. An example is a population at site NE-1 near Saraburi that covers a roadside ditch and adjacent waste land. At our first visit in December 1977, seemingly intermediate perennial-annual plants scattered in a parched ditch were found to be an annual type by examining their seed-derived progeny. In December 1980 and May 1981, however, perennial-like plants dominated this site. A close observation in December 1983 revealed that different types were distributed, forming patches or subpopulations, some being persistent but others being more rapidly changing or short-lived (Table 3).

Examining seven subpopulations, we have learned the following:

- The plants in shallow water (December) were of annual type, propagating by seeds and having a low regenerating ability of excised stem segments and long awns, while those growing in deeper water were of perennial type, propagating by ratooning only and having a high regenerating ability and shorter awns.
- The regenerating ability of seed-derived plants was generally lower than that of juvenile-derived plants when plants sampled from the same patches were compared.

Table 3. Heterogeneity of subpopulations with different microenvironments in regenerating ability measurement, awn length, and allelic frequency from site NE-1 near Saraburi, Thailand.

Sub-population	Water depth Dec.83-Aug.84 (cm)	Plants/ m ^{2a}	Regenerating ability ^{b,c}	Awn length ^c (cm)	Frequency of ^c		Observation in 1985
					<i>Sdh-1</i> ¹	<i>Pox-1</i> ^{2A}	
A	0 – 25	—	0.2	11.0	100	100	Habitat destroyed
C	3 – 27	1760S	– (0.2)	10.4(8.1)	100(100)	100(100)	
F	27 – 55	0	0.6	7.1	86	100	Extinct
B	4 – 30	—	1.5	7.0	17	0	Persistent
D	16 – 50	412R	2.0 (2.4)	6.4 (6.1)	0 (0)	0 (0)	"
E	40 – 55	392R	0.0 (15)	7.4 (7.4)	55 (0)	33 (0)	"
G	25 – 64	304R	1.2 (1.9)	6.9 (6.8)	14 (0)	0 (0)	"

^aDensity of seedlings (S) and ratooned shoots (R) observed in June 1984.

^bRegenerating ability of stem cuts examined at Misima.

^cData obtained on plants raised from seed are shown. Those in parenthesis are due to plants raised from juveniles collected in the natural habitat and grown under the same condition.

- The perennial and annual types were fixed for different alleles at *Sdh-1* and *Pox-1*, but the perennial plants released some variants in their seed-derived progeny.
- All annual subpopulations observed in 1983 disappeared before January 1985 because of strong disturbance of the habitats by man, while perennial subpopulations persisted.

CONCLUSION

The intermediate population seemed to be a mixture of different perennial and annual types and readily responsive to selection imposed by environmental changes. The subpopulations can follow up environmental heterogeneity, both spatially and temporally, and can be differentiated into different perennial and annual types. They have high evolutionary potential. Even if this population is a product of hybridization between perennial and annual types, the evolutionary process is essentially due to differentiation-hybridization cycles (5).

Sano et al (27) considered that intermediate perennial-annual populations could be the immediate wild progenitor of *O. sativa*. This view is supported by the present observations, because it was found that:

- such intermediate populations carry a large amount of genetic variations, particularly when tested with juvenile-derived plants,
- they are highly evolutionarily flexible, and,
- some of the plants have a fairly high seed productivity and a perenniality as is found in *O. sativa* cultivars.

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EVOLUTION OF *ORYZA* *LONGISTAMINATA*

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Thirty-one populations of *Oryza longistaminata* A. Chev. & Roehr were classified on the basis of ecological data recorded during collecting missions and were studied through multivariate analysis for their breeding systems involving flowering components and self-compatibility. Only 14% of rhizomatous plants were self-compatible, but up to 75% showed a rate of self-fertilization below or equal to 1%. High levels of individual self-compatibility were observed only in populations found in cultivated areas. Some particular plants without rhizomes, called "obake" types, were released by scattered plants in cultivated fields; most of these plants were self-compatible and were thought to arise from natural hybridization between *O. longistaminata* and the cultivated rice species. The results suggest first that strict self-incompatibility acts fully only in the undisturbed isolated conditions of large populations and second that the crossing barrier that separates *O. longistaminata* from *O. breviligulata* could be strengthened, since no evidence of introgression was seen when the two species occurred sympatrically. On the contrary, acquisition of self-compatibility through introgression with cultivated rices would permit release of populations adapted to habitats perturbed by man and development of the weedy habit.

The perennial wild rice *Oryza longistaminata* A. Chev. & Roehr is also known as *O. barthii* A. Chev. or *O. perennis* Moench ssp. *barthii* A. Chev. *O. barthii* should correspond in fact to *O. breviligulata* (9); in order to avoid confusion we have used *O. longistaminata* to designate the perennial African wild rice and *O. breviligulata* for the annual African wild rice, both of which belong to the *O. sativa* species complex.

O. longistaminata has the widest distribution of all *Oryza* species in Africa. It shows a large range of ecological sites such as regularly flooded plains, where populations can cover huge areas, or temporary to almost permanent ponds in which *O. breviligulata* can be encountered sympatrically; *O. longistaminata* is a perennial with vigorous creeping and underground rhizomes and can overrun cultivated fields, forcing farmers to abandon them (2, 17). Both *O. longistaminata* and *O. breviligulata* are collected by local people in West Africa for traditional consumption.

Nayar (12) reported that *O. longistaminata* could be self-incompatible since the panicles, when bagged, produce only a few seeds. Chu et al (4) checked this partial self-incompatibility through cytological studies. *O.*

longistaminata is known to be isolated from the other rice species by a strong reproductive barrier (5,7). However, evidence for natural crossing between *O. longistaminata* and its related taxa was seen during field surveys (2, 13) and in experimental hybridizations (8). It was suggested that a balance between isolation and hybridization might play a role in the evolutionary dynamics of species such as *O. longistaminata* (8). This paper attempts, through extensive observations of breeding systems, to set forth challenging considerations on the evolution of *O. longistaminata*.

MATERIALS AND METHODS

Thirty-one populations composed of 750 plants were chosen to represent the geographical and ecological diversity of *O. longistaminata* in Africa (Fig. 1). These populations were original samples collected in 14 African countries by ORSTOM (Institut Francais de Recherche Scientifique en Coopération) and IRAT (Institut de Recherches Agronomiques Tropicales) within the framework of the preservation of rice genetic resources supported by IRRI and the International Board for Plant Genetic Resources (IBPGR). Two additional samples from Nigeria were kindly given to us by Dr. H.I. Oka of the National Institute of Genetics in Mishima, Japan, after his collecting mission in West Africa (15).

The characterization of populations was made at two different levels. First, the field data recorded during collecting missions were analyzed to set a coherent classification of *O. longistaminata* populations. A seed sample of each population was hulled to measure the frequency of abnormal or deteriorated kernels, since this may indicate the level of introgression pressure from the other rice species. Second, observations were made during 2 years on several characters pertaining to breeding system. The plants were grown in individual pots in concrete beds with water supply. They were rejuvenated periodically thanks to their perennial growth habit. Since the experimental site (Abidjan) is in a forest area at 4°N latitude, neither available light intensity nor critical day length was always suitable for abundant flowering. However, these marginal conditions provided valuable descriptors of flowering ability within *O. longistaminata* populations. The main flowering period ran from September to January. Self-compatibility was tested by bagging all the panicles at their emergence. Some rare flowerings occurred from February to June among rhizomatous plants that we considered insensitive to photoperiod. Perennial plants without rhizomes were also noted. Following Chu and Oka (8), we called these off-types “obake” since they were thought to be



Fig. 1. Location of *O. longistaminata* populations.

hybrids between *O. longistaminata* and the other rice species. Both field data on collecting sites and characters pertaining to breeding system are presented in Table 1. In order to perform multivariate analysis involving factorial correspondence analysis (1), data were computed as qualitative states of characters either directly at the individual level or as recorded frequencies at the population level.

RESULTS

Field data permitted *a priori* recognition of five population types that can be used in graphical representations of multivariate analysis. This classification agreed with our own field observations (16) as well as those made by the Japanese team (13,15). The first two types were found in isolated conditions in regularly flooded plains with little environmental variation or in temporary ponds of Sahelian countries, and the true wild form of *O. breviligulata* was expected when occurring sympatrically with *O. longistaminata*. The other three types were found in more or less cultivated areas and in rice fields; some of them were seen in various

Table 1. Rating of the characters used to describe the ecology and breeding system of *O. longistaminata* populations.

Character	System of rating
1. Population size	large, medium, small, scattered plants
2. Habitat	flooded areas, temporary ponds, cultivated areas
3. Proximity to rice fields	isolated, cultivated areas, weedy
4. Sympatry with <i>O. breviligulata</i>	absence, presence
5. Frequency of abnormal kernels	0-3%, 4-10%, 11-25%, 26-50%, >50%
6. Rhizome development	presence, absence
7. Flowering ability	presence, absence
8. Panicle number per plant	0, 1-4, 5-8, 9-12, >12
9. Photoperiod insensitivity	presence, absence
10. Self-compatibility	presence, absence
11. Self-fertilization rate	0, $0 \cdot 10^{-3}$, $10^{-3} \cdot 10^{-2}$, $>10^{-2}$

habitats recently perturbed by man but not in the weedy condition, which was restricted to small populations encountered in the vicinity of cultivated rice fields. An extreme situation consisted of a weedy type where the plants did not constitute populations but were scattered in cultivated fields, recent fallows, or alongside irrigation ditches. In these conditions, sympatry with *O. breviligulata* might have included weedy forms involving various origins as discussed subsequently.

Figure 2 shows the representation of the 31 populations according to our previous classification in the plane defined by axes 1 and 2 of a factorial correspondence analysis involving the 11 characters represented in Table 1. Axis 1 accounts for 28% of the total variability and axis 2 for 19%. The first axis is associated with flowering ability and flowering intensity. A cluster may be isolated that includes most of large isolated populations and that is characterized by low flowering components and only occasional self-compatible plants.

The second axis is related primarily to sympatry with *O. breviligulata* and secondarily to both absence of rhizomes and photoperiod insensitivity. Three groups may be observed. The populations growing sympatrically with *O. breviligulata* are quite similar to the large population except that their flowering ability is better. On the other side, scattered plants in cultivated fields yield predominantly "obake" types with continuous flowering habit. Most of them are self-compatible and can be considered as hybrid swarms.

Some populations are close to this group, since they contain many self-compatible rhizomatous plants with a high rate of self-fertilization and occasional "obake" types; these populations may represent posterior

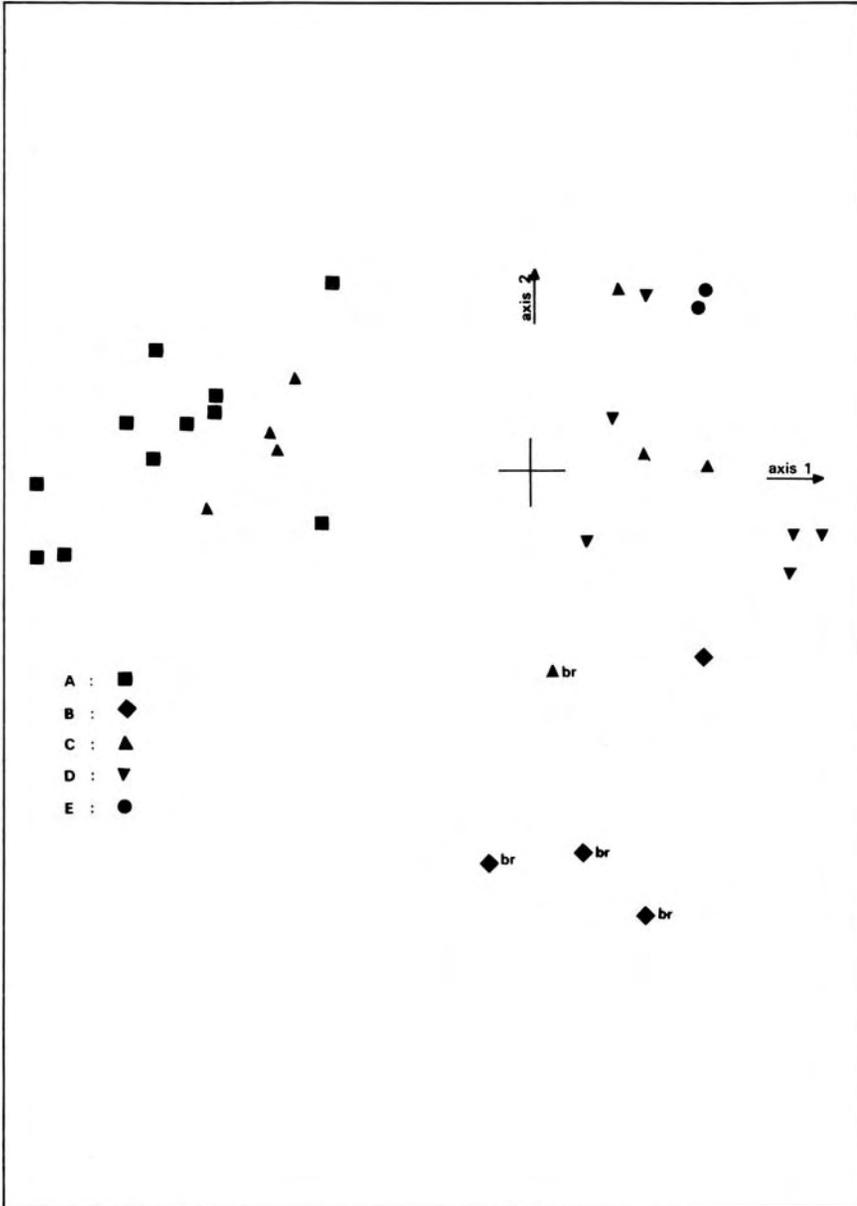


Fig. 2. Thirty-one populations of *O. longistaminata* plotted in the plane defined by axes 1 and 2 of an analysis of correspondences involving 11 characters relative to ecological factors and breeding system. A ■ = occurring in regularly flooded areas, B ◆ = in temporarily inundated ponds, C ▲ = in cultivated areas but not in the weedy condition, D ▼ := weedy, in the vicinity of cultivated fields, E ● := weedy, scattered plants in cultivated fields or recent fallows, *br* = sympatry with *O. breviligulata*.

evolutionary stages of hybrid swarms. Finally, most of the weedy populations constitute the last group, in which no “obake” types were recorded but frequent medium to highly self-compatible plants were observed. We can point out the intermediate position of a population sympatric with *O. breviligulata* that showed many self-compatible individuals. However, this population was found in cultivated areas, and the wild origin of *O. breviligulata* might be doubtful.

The third axis represents 15% of the whole variation and is linked only to high frequencies of abnormal or deteriorated kernels. This axis is characterized mainly by scattered plants as expected. However, Some large populations of East Africa have frequently shown small kernels filling the spikelets incompletely, although they could not be considered as deteriorated. Sympatry with *O. breviligulata* showed a high level of abnormal kernels, but no evidence of introgression was found such as “obake” types or highly self-compatible individuals. This suggests different relationships of *O. longistaminata* with its related species.

A second analysis was performed by extracting at random ten individuals among six populations representing each type previously described and where sympatry with *O. breviligulata* was involved in both wild and cultivated conditions. Factorial correspondence analysis was run by computing only the six characters pertaining to breeding system (Fig. 3). Axis 1 explains 53% of the whole variation and axis 2 explains 25%.

The first axis is related to flowering components and self-compatibility. The second axis permits separation among self-compatible plants of “obake” types from rhizomatous plants. Additional differences within each of the two groups can be seen along axis 2; the rhizomatous plants are distributed on the basis of their photoperiod insensitivity, and “obake” types are classified according to their rate of self-fertilization. Both large population and sympatry with wild *O. breviligulata* give a narrow range of variability based on flowering components. The self-fertilization rate of rarely occurring self-compatible plants is so low that it can be considered accidental or as having no evolutionary significance.

On the contrary, the populations found in cultivated areas show a large variability with high flowering ability even if the plants are self-incompatible. Both rate of self-compatibility and photoperiod insensitivity among rhizomatous plants are associated with the presence of “obake” types, and they increase as the cultivated fields become closer. Sympatry with *O. breviligulata* releases self-compatible plants only when it occurs in cultivated areas; this can be explained by the various origins of weedy *O. breviligulata*, since they may include some forms belonging taxonomically to *O. breviligulata* but that have arisen through introgres-

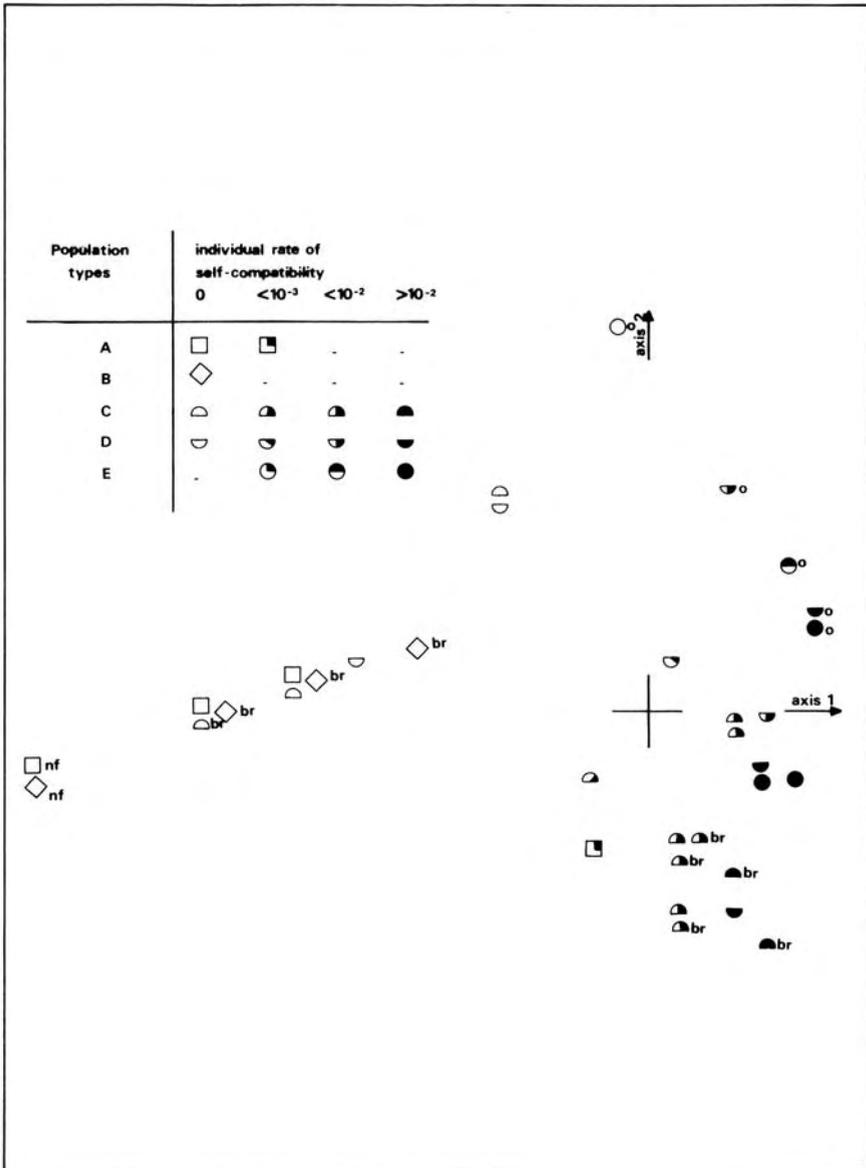


Fig. 3. Fifty-seven plants belonging to 6 population types and plotted in the plane defined by axes 1 and 2 of an analysis of correspondences involving 6 characters pertaining to rhizomatous, flowering, and self-compatibility habits. The ecological classification is the same as in Figure 2. Both population types and individual self-compatibility rates are represented by a conventional symbol. *br* = sympatry with *O. breviligulata*, O = "obake" type, *nf* = no-flowering ability.

sion between independently domesticated African and Asian rices (18).

When considering all the data available, 14% of rhizomatous plants are self-compatible; their rates of self-fertilization range from 0.1% to 27%, and up to 75% of these plants show a level of self-fertilization below or equal to 1%. High levels of self-compatibility occur only in populations from cultivated areas. On the other hand, 90% of "obake" types are found to be self-compatible, with a similar self-fertilization range (0.1% to 28%), and they can be thought as subsequent progeny of F_1 plants. Thus, our results suggest that self-compatibility and characters related to reproductive effort by seeds originate from natural introgression with cultivated rice.

DISCUSSION

O. longistaminata can be considered as the African form of *O. perennis*, which can be divided into four geographical groups based on phenotypic analysis and F_1 sterility relationships; the American, Asian, Oceanian, and African forms constitute different clusters that are assumed to have evolved independently of one another (11). Isozyme surveys on 24 loci showed a large genetic distance between *O. longistaminata* and a cluster made of Asian and American forms of *O. perennis*, *O. breviligulata*, and the cultivated rice species (19). Strong differences are observed between African and Asian wild rices belonging to the *O. sativa* species complex. In Asia, a continuous array of intermediate forms is seen from selfing to partial outcrossing and from annual to perennial habits (14). Since extensive hybridizations have occurred between *O. sativa* and wild rices, the classification of Asian *O. rufipogon* Griff. (= *O. perennis* Moench) into two species, namely *O. rufipogon* sensu stricto for the perennial form and *O. nivara* Sharma and Shastry for the annual one, can be thought only as conceptual because it may represent the situation in the paleoenvironment (3). On the contrary, no such continuum is found in Africa; annual autogamous and perennial allogamous forms give easily distinguishable, true species, namely *O. longistaminata* and *O. breviligulata*. According to genetic distances and climatic conditions prevailing in paleoenvironments, both species might have originated from Eurasia and invaded Africa, but the migration probably lasted longer for *O. breviligulata*, which is adapted to temporary ponds and to dissemination by cattle (19).

According to Stebbins (21), our results suggest that strict self-incompatibility acts fully only in large undisturbed populations that can

store up a large amount of genetic variation. Hardy-Weinberg expectations were tested on some loci and were in accordance with observed genotype frequencies (20). This can be an indication of predominant seed reproduction, but the low flowering ability and the poor seed production in open pollinated conditions suggest that a balanced breeding system between vegetative and sexual reproduction operates in these populations.

Sympatry with wild *O. breviligulata* gives no evidence for natural introgression, and the crossing barrier tends to be strongest when hybridization is attempted with that species (5,7). It is possible that *O. longistaminata* has strengthened its crossing barrier towards *O. breviligulata* in order to protect itself from natural hybridizations because the gene flow in their sympatric populations is directed only towards the cross-pollinated species.

Evidence for reciprocal genetic exchanges with cultivated rices was seen at the isozyme level (6, 10, 20). Hybrid swarms and self-compatible populations were found in weedy conditions, and some of these populations showed clear effects of inbreeding (unpublished data). The reproductive barrier may not be as strong with *O. sativa* as with *O. breviligulata* because of the recent introduction of Asian cultivated rice in Africa. However, the breaking of self-incompatibility through hybridization with *O. sativa* can also be promoted, since natural habitats of *O. longistaminata* are now reclaimed for rice cultivation. Consequently, large original populations are split into a lot of small weedy populations. In these conditions, the cost of reproduction by strict self-incompatibility increases, since the effective population size becomes smaller and natural interspecific hybridizations can be favored due to a lack of intercompatible lines. In addition, agricultural practices disturb habitats, which become unsuitable to rhizome propagation. Thus, the acquisition of self-compatibility permits elimination of lethal genes and genes causing inbreeding depression in order to release plants with improved flowering ability and high seed production. They can be adapted to habitats perturbed by man and can develop a vigorous weedy habit.

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ISOZYMES AND PHYLOGENETIC RELATIONSHIP IN *ORYZA*

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Isozymes have proven to be good markers for making systematic and phylogenetic inferences in many living organisms. A technique was developed to reveal isozymes at more than 40 loci in rice and was applied to the study of the genetic structure of the *Sativa* and *Latifolia* groups of species. This paper presents a summary of the results and their interpretation that were published elsewhere. The distinction of two cultivated species and of the *indica* and *japonica* subspecies of *O. sativa* was confirmed. No selection of particular alleles was observed in cultivated *O. glaberrima* compared with its direct wild ancestor. The interrelationships among wild species of both the *Sativa* and *Latifolia* groups can be interpreted as reflecting divergence due to genetic isolation. They suggest an evolutionary scenario coherent with tectonic and climatic modifications in the Old World since the mid-Tertiary era. Before domestication, the Himalaya range enhanced a differentiation of *O. rufipogon* in China and South/Southeast Asia, which is related to the *indica-japonica* genetic structure. Reciprocal introgression between the *indica* and *japonica* subspecies has probably been determinant in building the genetic diversity of *O. sativa*.

The term isozyme describes different molecular forms of enzymes with the same substrate specificity. They may represent products of different alleles at a given locus (allozymes) or products of different loci. Their study has gained popularity since the 1960s when the simple electrophoretic technique was developed and revealed an unexpectedly large amount of genetic polymorphism at the molecular level within populations and species of various organisms. Additional techniques coupled with electrophoresis, such as the study of isozyme thermostability, have unraveled still more variability, although it is now well established that these techniques are able to evidence only part of the total variability at the level of DNA. The polymorphism "hidden" by these techniques is large when individuals that are compared belong to more distantly related taxonomic units.

The isozyme approach has been widely applied to the study of plant genetics and breeding (20). Still, compared with animals, it has been applied very little to the study of phylogeny in plants. The reasons for this situation appear to be well exemplified in the case of *Oryza*.

Research on rice isozymes started early (4) but was limited to general enzymes that are stained by artificial substrate and show complex zymograms (peroxydases, esterases, acid phosphatases). Considering the large genetic diversity present in a single species group like the Sativa group in *Oryza*, it is not possible to infer loci and allelic relationship from such complex zymograms. The earlier studies failed to use the basic power of isozymes compared with morphological characters.

Another factor seems to have refrained researchers from using isozymes for phylogenetic studies on rice and other plants. Earlier studies showed that isozymes are often under apparently strong direct selection. Their polymorphism can be supposed to be subjected to genetic convergence according to a selection pressure like domestication (5). On the contrary, it has become more and more apparent that enzyme electrophoresis provides data that differ fundamentally from morphological, macroscopic characters. They represent primary products of structural genes that appear to be well conserved among organisms compared to other classes of DNA. They are thus genetically determined in a simple way and are generally neutral to direct selection or subjected to only slight selection coefficients (although they may be associated with selected genes on the chromosome segments). Besides, they can be compared on the same ground between the cultivars and the weedy or wild forms of a domesticated species complex.

We developed a technique on starch gel to strain more than 13 rice enzymes encoded at more than 40 loci. Provisional inferences on genetic determinism were based on the comparison of polymorphism observed in self-pollinated and closely related outcrossed species, and on general knowledge of the secondary structure of the enzymes considered. At present, more than 20 of these loci have been checked for Mendelian segregation of various alleles among the Sativa group of species (19). So far the results have always confirmed previous assumptions and have enhanced confidence in an assumed genetic determinism.

This technique was used to determine genetic structure and genetic distances among cultivated rice and the Sativa and Latifolia groups of species constituting the section *Eu-Oryza* of the genus *Oryza*. Because of the large genetic diversity studied, and because of some technical constraints, not all loci were always surveyed; but at least 17 or 24 were analyzed.

Detailed results have been published elsewhere (14, 15, 16, 17). This paper gives a general overview of the main results and their interpretations from the phylogenetic viewpoint.

THE GENETIC STRUCTURE OF CULTIVATED RICE

While none would dispute an Asian origin for *O. sativa*, Portères (12) suggested an independent domestication of *O. glaberrima* in Africa. This hypothesis was challenged, however, particularly by Nayar (9), who proposed that the origin of *O. glaberrima* was also India.

The relationship between *O. sativa*, *O. glaberrima*, and the latter's wild and weedy relative *O. breviligulata* was studied in detail at 40 loci. The following conclusions were reached:

- As shown in the multivariate treatment of the data in Figure 1, the cultivated, wild, and weedy African species form a group clearly distinct from *O. sativa*. The diversity of *O. breviligulata* is greater than that of *O. glaberrima*; domestication has reduced the gene diversity but has not selected new alleles.
- Two groups are clearly distinguished among *O. sativa* corresponding to the *indica* and *japonica* subspecies or to the traditional distinction (among others) of the Hsien and Keng types by the Chinese.
- Intermediate types between the *indica* and *japonica* subspecies exist that show particular alleles and/or particular characters such as inducing cytoplasmic male sterility or adaptability to upland or floating conditions. This point and the previous one are clearly corroborated by a study of a much larger collection of *O. sativa* (7).
- The distribution of F_1 pollen sterility relationships among *O. sativa* is related to some extent to its isozyme polymorphism as it leads to the extraction of two small groups of varieties with a complementary set of isozymes. All genotypes among *O. sativa* except rare alleles can be explained by hybridization between these varieties, which are assumed for that reason to represent the "ancestral" *indica* and *japonica* isozyme sets.
- An approximately equal genetic distance was found between *O. glaberrima* and the "ancestral" *indica* and *japonica* in the three combinations as shown in Figure 1.
- Some of the weedy strains (according to their occurrence in rice fields or recent fallows) of *O. breviligulata* show two alleles frequent in *O. sativa* but never found in wild strains of the same species, nor in *O. glaberrima*. In connection with field observations and artificial hybridization experiments, this strongly suggests that, according to Nayar's hypothesis, some of the weedy strains of *O. breviligulata* have their origin in the introgressive hybridization of *O. glaberrima* by *O. sativa*.

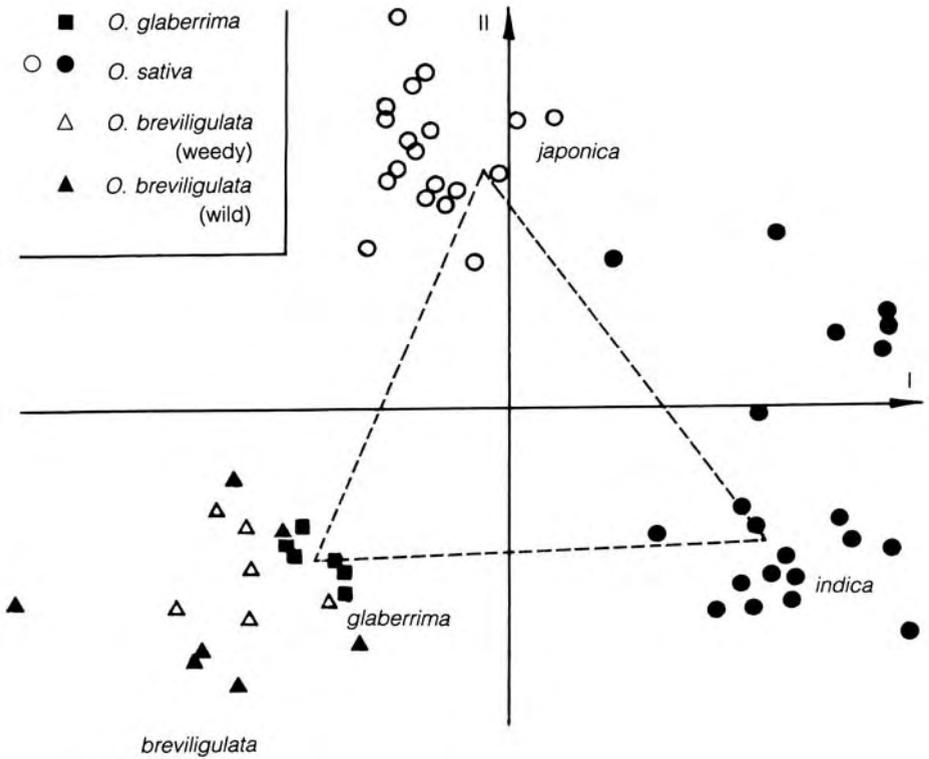


Fig. 1. Sixty strains of cultivated rice or wild and weedy *O. breviligulata* plotted in the first plane for a principal coordinate analysis of the genetic distances scored at 40 isozyme loci. Their classification is indicated by conventional symbols. Among *O. sativa*, an open symbol indicates a negative phenol reaction and allows the distinction of an *indica* and a *japonica* group. The dotted triangle shows that approximately equal genetic distances are found among the three groups of cultivated rice (about 15 allelic discordances over 40 loci scored).

It should be stressed here that, most unfortunately, the distinction of the *indica* and *japonica* subspecies made above does not correspond to the morphological distinction of the *indica*, *japonica*, and *javanica* morphological types widely in use, at IRRI in particular. However, it very closely corresponds to the “Continental” and “Insular” groups of Oka (10) and doubtless to a basic subspecific differentiation of *O. sativa* in which the *javanica* type is part of the *japonica* subspecies. Illustrative of this fact is the observation that both the *indica* and *japonica* subspecies present all morphological types of spikelets often used to distinguish the *indica*,

japonica, and javanica morphological types.

The above results are interpreted as reflecting a differentiation of the direct ancestors of cultivated rice into three species of subspecies prior to domestication. The rich ecotypic and varietal diversity of present *O. sativa* would have arisen (a) from introgressive hybridization between the *indica* and *japonica* incipient cultivars themselves and between the cultivars and the local wild rice in the various areas of their dissemination and (b) from selection by man superimposed on this basic genetic structure.

It is well known that hybridization may be an evolutionary stimulus, particularly in areas disturbed by man and for the origin of domesticates, both plants and animals (1). We followed that model for the origin of rice. However, two main questions arise:

- What kind of evidence do we have to warrant the assumption of a long time of isolation of the various ancestors of cultivated rice in different environments prior to their domestication?
- Is the introgressive hybridization of *O. sativa*, which has probably built most of its diversity at the isozyme level, a mere artifact of its success in bringing it to various ecological and geographical environments or, on the contrary, does this phenomenon explain its success to a significant degree?

Elements of the answers to these questions should lie in the study of the wild relatives of cultivated rice.

THE GENETIC STRUCTURE OF THE SATIVA GROUP

A fairly complete collection of the Sativa group with Australian, Chinese, South and Southeast Asian, African, and American origins can be constituted and studied at 24 isozyme loci. A striking result is apparent in Figure 2. Only *O. Longistaminata* and the Australian form of *O. rufipogon* can be unambiguously distinguished from the remaining taxa of the group. The maximum genetic distance was found between the Australian taxa and others with common alleles differing at 10 out of the 24 loci. On the other hand, the *glaberrima*, *indica* and *japonica* cultivated rice were all found to be relatively similar to each other and to *O. breviligulata* compared with the diversity within the Asian form of *O. rufipogon* itself.

When the Asian *O. rufipogon* was considered alone, some Chinese strains appeared divergent from any strain originating outside China, while being very close to the *japonica* isozyme set. They originated in Kwangsi Province and showed no trace of introgression from cultivars.

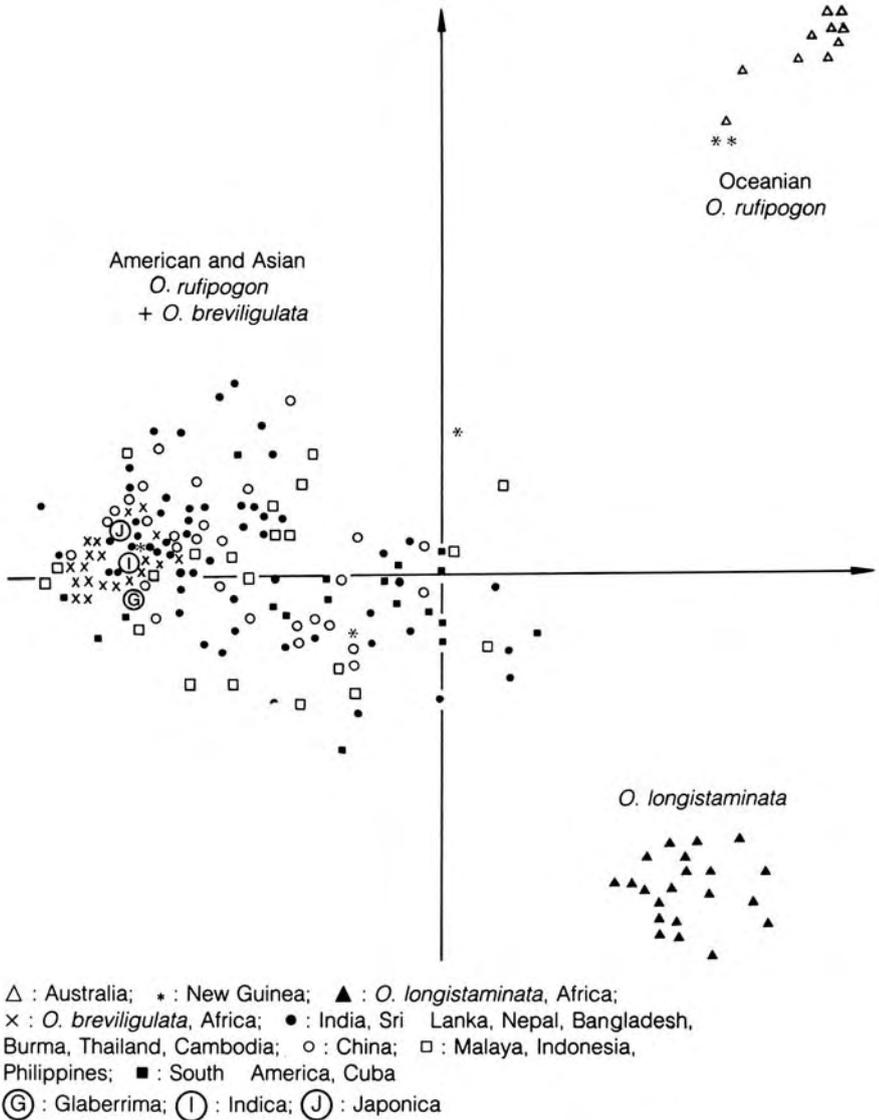


Fig. 2 Strains of spontaneous forms of the Sativa species group plotted in the first plane of a principal coordinate analysis of genetic distances scored at 24 isozyme loci. The geographical origins and taxonomic classification are indicated by conventional symbols. The position of cultivated rice is indicated by the three "ancestral" isozyme patterns of *indica*, *japonica*, and *glaberrima* types of cultivars introduced as supernumerary individuals.

On the other hand, the *indica* isozyme set was closer to many strains found in South or Southeast Asia.

In view of the large polymorphism in the Sativa group, another striking result was that the American form of *O. rufipogon*, although morphologically slightly divergent, shares all its common alleles with the Asian form of *O. rufipogon*. This fact definitely proves that the American strains studied did not evolve independently from their Asian relatives for a very long time. They have rather been recently introduced, probably by man, and naturalized in America.

In contradiction to the above statement, Barnes and Pental (2) found a divergence at the DNA level of some American strains compared to a few Asian ones. However, from our study (17), those Asian strains most closely related to American strains are found in Malaysia and Indonesia. These were not included in the DNA study cited above.

THE GENETIC STRUCTURE OF THE LATIFOLIA GROUP

As understood here, the Latifolia group includes *O. australiensis*, because its affinity at the isozyme level confirms macroscopic observations. The Latifolia group thus comprises three diploid genomes (defined on cytogenetic grounds) — BB with maximal distribution in Africa, CC in Asia, and EE in Australia — and two tetraploid genomes — BBCC found in Africa and Asia and CCDD found in America.

Strains representing most of the various forms and species described in this group were studied, but only one was probably from China (it corresponded to the CCDD genome). Figure 3 shows the interrelationships among them as analyzed at 17 loci.

Representatives of each diploid genome were found at extremes of the distribution, while allotetraploids, and also some diploids with the CC genome, were found intermediate between them. Intermediate diploids include *O. eichingeri* and a group of *O. officinalis* from Indonesia and the Philippines. Accordingly, two groups were formed within *O. officinalis*: one presumed ancestral (CC1) and one presumed introgressed (CC2) with genes from genome BB.

Compared to conventional taxonomy, the classification was made easy within such complexes as *O. eichingeri* and diploid and tetraploid *O. punctata* or between *O. officinalis* and tetraploid *O. minuta* or *O. malampuzhaensis*.

A remarkable observation was that the maximum distances found between the BB, CC1, and EE genomes (Nei's distance on the order of 1)

was not greater than those found between the Australian and other strains of the Sativa group with the single genome AA.

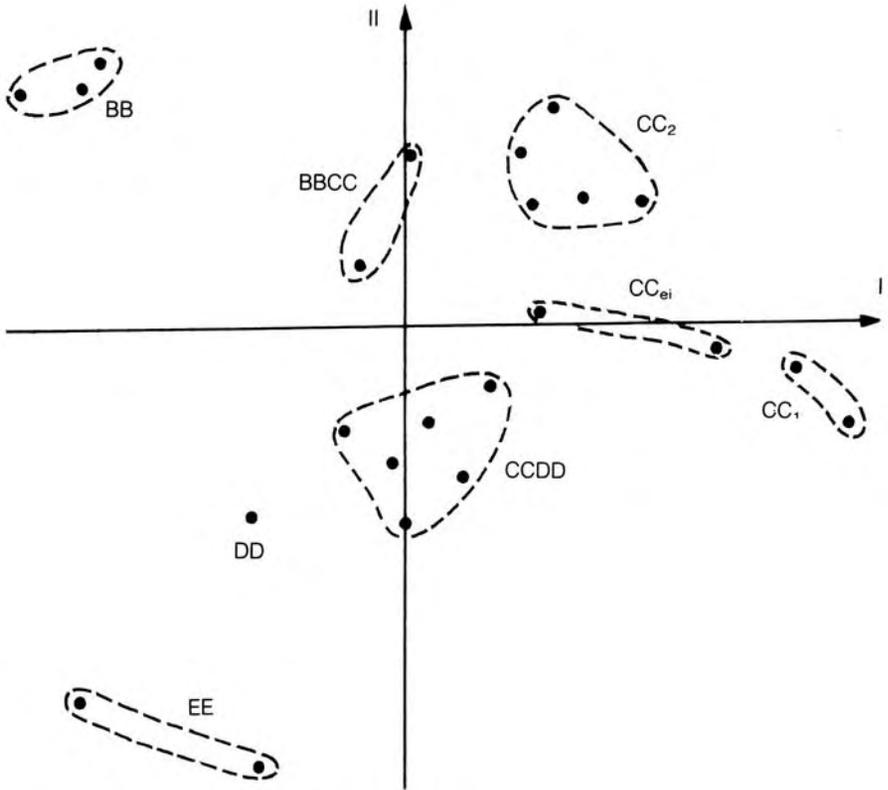


Fig. 3 Twenty-six strains representative of the various forms of the Latifolia species group plotted in the first plane of a principal coordinate analysis of the genetic distances scored at 17 isozyme loci. The groups are symbolized according to the genome or subgenome they represent and correspond to the following taxonomical classification. BB= *O. punctata* (diploid); CC1= *O. officinalis* "ancestral"; CC2= *O. officinalis* introgressed with the genes from genome BB; CCei= *O. eichingeri*; DD= a putative isozyme set of genome DD; EE= *O. australiensis*; BBCC= *O. punctata* (tetraploid), *O. malampuzhaensis*, and *O. minuta*; CCDD= *O. latifolia*, *O. alta*, and *O. grandiglumis*.

EVOLUTIONARY INTERPRETATION

A coherent evolutionary interpretation of the genetic structure found in both the Latifolia and the Sativa groups appears to be possible based on the two following postulates:

- In the case of genetic isolation, there exists a correlation between isozyme divergence and the time since divergence occurred (molecular clock).
- Migration of wild rice naturally occurs on land only and by short distances at a time, but man has promoted the migration of wild and cultivated rice (directly and indirectly) across oceans and high mountains.

A common ancestor of the genus *Oryza* in Eurasia seems to be a reasonable assumption, since only in Asia are found the forest-adapted species such as *O. meyeriana*, *O. ridleyi*, *O. officinalis*. (Based on their relationship with Asian species, *O. eichingeri* in Africa and the American species are assumed to be recently naturalized.) Both the Sativa and Latifolia groups may have migrated to Australia and Africa during the Tertiary era by land and remained subsequently isolated by geographical and/or climatic barriers. The recent uplift of the Himalaya range (8) can explain the divergence between Chinese and non-Chinese Asian populations of wild rice. As indicated in Table 1, a remarkably good agreement was found between electrophoretic dating and the sequence of events in the paleoenvironment that should have created barriers to the migration of wild rice within the Old World.

Because of the relative affinity of the wild annual *O. breviligulata* with all cultivated forms and also because accumulating favorable genes should be easier within self-pollinated types, it is assumed that incipient domestication of rice started from annual forms. It might have begun independently in various areas in Asia and Africa, as it does not involve catastrophic changes in the plant architecture nor physiology. As a matter of fact, incipient domestication of annual forms is apparently still going on in Western India (18). However, a differentiation of the wild progenitors in Africa, South/Southeast Asia, and China existed prior to domestication as well as in the respective cultures of the different ethnic groups in these areas. It may be assumed that a combination of these factors has resulted in the genetic differentiation of cultivated rice into three main subspecies or species.

Subsequently, disturbance of primitive habitats and seed transportation promoted introgressive hybridization between gene pools geographically, ecologically, or genetically isolated. In that sense, no doubt, the various forms of Asian *O. rufipogon* have all contributed to the origin of *O. sativa*. In turn, many strains of the wild and weedy *O. rufipogon* have introgressed genes from cultivars. The situation, which can be studied in Africa, with introgression going on between *O. glaberrima* and *O. sativa* on one hand and between *O. sativa* and the wild perennial *O. longistaminata*

Table 1. The correspondence between electrophoretic dating for the main genetic divergences within the Sativa and Latifolia groups and the tectonic or climatic events in the paleoenvironment that should have created a barrier to the migration of species of *Oryza* according to their environmental requirements.

Electrophoretic dating of genetic divergence	Tectonic or climatic events in the paleoenvironment
Australian vs non-Oceanic strains of the Sativa or Latifolia group: 15 My ^a	Collision of the Australasian plate with Southeast Asia
Asian vs African strains, Latifolia group (basically forest-adapted): 15 My	- Opening up of the Red Sea - Establishment of a climatic barrier between Asia and Africa according to the sequence:
<i>O. longistaminata</i> (adapted to humid savanna in Africa) vs Asian <i>O. rufipogon</i> : 7 MY	humid forest _____ humid savanna _____
<i>O. breviligulata</i> (adapted to dry savanna in Africa) vs Asian <i>O. rufipogon</i> : 2-3 My	dry savanna _____ desert (for rice)
Chinese vs South/Southeast Asian <i>O. rufipogon</i> : 2-3 My <i>Indica vs japonica</i> subspecies of <i>O. sativa</i> : 2-3 My	Emergence of the Himalaya as a barrier to land migration

^aMy=millions of years.

(6) on the other, could be illustrative of what has happened in Asia since prehistoric times.

Figure 4 diagrammatically depicts the proposed phylogenetic relationships of cultivated rice with its ancestors in the Sativa group. It is deduced from the isozymic genetic structure but appears to be coherent with all facts known to the author.

This model of evolution for rice seems to be in accordance with published knowledge as a whole, even if it is contradictory with many interpretations previously put forward (3, 11) as discussed in Second (16).

We may briefly answer now the two questions raised earlier:

- The role of the Himalayan mountain range as a geographic barrier becomes even more probable when one considers that (a) the Sativa group is found in open habitats (humid or only locally humid savannas) and (b) conditions suitable for *O. rufipogon* probably

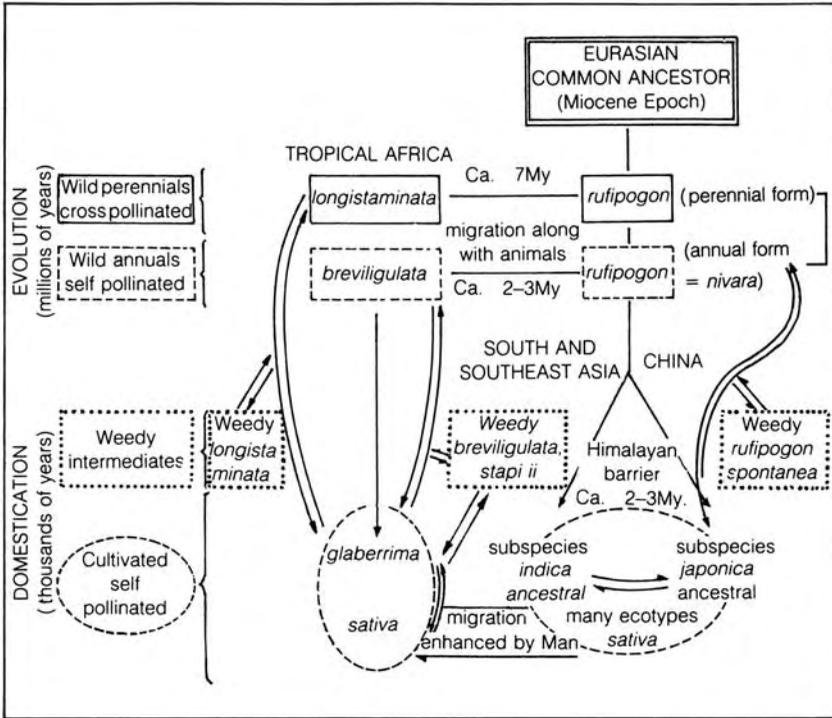


Fig. 4 Phylogenetic relationships of the two cultivated rice species in Asia and Africa. Taxa boxed by solid lines are wild perennials. Taxa boxed by broken lines are annuals. Taxa boxed by dotted lines are weedy types. Arrows in heavy lines indicate direct descent. Double arrows indicate introgressive hybridization, which seems to occur between all sympatric or parapatric forms. However, *O. longistaminata* and *O. breviligulata* are isolated in their natural habitat by reproductive barriers particularly developed. The subspecies *indica* and *japonica* are conceptual in the sense that they are meant to represent incipient cultivars in China and South/Southeast Asia. They correspond nevertheless to a fundamental dichotomy within *O. sativa*.

existed in Central Asia until its desertification during the Quaternary era (21) and in central China until historical times. Low levels of migration might have occurred among the areas of China and South Asia through the mountainous area of Indochina. However, there have never existed large, emerged plains between them, even when the sea was at its lowest and the islands of Southeast Asia were part of the continent.

- In the Latifolia group, allopolyploids seem to have developed by combination of genomes originating on two different continents — Africa and Asia for the BBCC forms in particular. They are adapted

to environments newly opened due to the disturbance of man. By analogy we should assume that in the Sativa group introgression between cultivars native to China and South/Southeast Asia and the Hsien type of Southern China are good candidates as representatives of such an event from the point of view of their genetic affinities, their growing environment; as well as historical considerations.

Also, besides introgressive hybridization *per se*, there is the possibility that the hybridization of two genomes previously isolated for a long time has a mutagenic effect as evidenced or presumed in other organisms like the mouse, frog, and *Drosophila* in particular (13). This could account for the rare electromorphs found in *O. sativa*, particularly in those varieties that do not really fall in the *indica* nor the *japonica* subspecies (7, A. Ghesquiere and A. de Kachko, personal communication). Some of these alleles are not observed in the wild species. This hypothesis represents an appealing model for the evolution under domestication of species polyphyletic in origin such as *O. sativa*.

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REPEATED DNA SEQUENCES AND RIBULOSE BISPHOSPHATE CARBOXYLASE/ OXYGENASE AS TOOLS FOR THE STUDY OF RICE EVOLUTION*

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The phylogenetic relationships of the cultivated rices, *O. sativa* and *O. glaberrima*, to the complex of species grouped under the heading of *O. perennis* is far from clear. We have analyzed a series of accessions from each of these species for the isoelectric focusing patterns of ribulose bisphosphate carboxylase/oxygenase (rubisco), and for the presence and organization of a number of coding and noncoding repeated sequence families. The results of these studies indicate a very close relationship between *O. sativa*, *O. glaberrima*, and *O. perennis* accessions from Africa, Asia, and South America. Two South American accessions of *O. perennis* differed from the others and from the cultivated rices, indicating that *O. sativa* and *O. glaberrima* must have diverged from the *O. perennis* complex more recently than some species that have been hitherto considered to be members of the complex. The results are certainly not consistent with the view that the cultivated rices have been isolated from one another and from their wild progenitors since the time of continental drift.

The phylogenetic relationships between the cultivated rices (*O. sativa* and *O. glaberrima*) and the *O. perennis* species complex are the subject of much discussion. *O. sativa* is the major cultivated rice on the Asian continent, while *O. glaberrima* is cultivated in West Africa. South America has no naturally occurring cultivated rice but shares with Southeast Asia, China, and Africa a complex of species, grouped under *O. perennis*. The complex includes *O. rufipogon* or *glumaepetula* in South America, *O. rufipogon* and *O. nivara* in Asia, and *O. longistaminata* and *O. barthii* or *O. breviligulata* in Africa (1,2,10,11,15). One school of thought (1) proposes that the cultivated rices *O. sativa* and *O. glaberrima* arose independently in Asia and Africa, respectively, from species of the *O. perennis* complex.

Nayar (11), on the other hand, suggests that *O. glaberrima* evolved from *O. sativa* within the last thousand years.

We have attempted to complement the existing understanding of morphological variation between the species by a study of wild and cultivated rices at the levels of repeated DNA sequences and rubisco polymorphism.

RESULTS

Fraction 1 protein analysis

Ribulose biphosphate carboxylase/oxygenase (rubisco) contains chloroplast-encoded (large) and nuclear-encoded (small) subunits. Rubisco was purified from a number of rice accessions by preparative polyacrylamide gel electrophoresis of total leaf protein. The proteins were then subjected to isoelectric focusing (Fig. 1). In all cases, the small subunit is seen as essentially the same pair of bands (visible most clearly for *O. nivara*). The accessions differ for their large subunits, however. *O. sativa*, *O. glaberrima*, *O. nivara*, Asian *O. perennis*, and African *O. perennis* all display similar isoelectric focusing patterns. Two of the South American *O. perennis* accessions—from Venezuela and Brazil (IRRI 100924)—also share this pattern; *O. perennis* from Surinam and Brazil (IRRI 100970), however, share a different pattern. *O. australiensis* has a unique isoelectric focusing pattern, confirming its identity as a distinct species.

Repeated DNA sequence analysis

Repeated DNA sequences constitute a considerable proportion of plant genomes. These sequences may be broadly categorized into *tandemly repeated* (or “satellite”) components, and *dispersed* repeated sequences (interspersed with either single copy or other repeated sequences). Most of these repeated elements are apparently noncoding and appear to evolve at fairly high rates (4). We have used two repeated sequences as hybridization probes to identify components shared by the genomes of *O. perennis* species, *O. sativa* and *O. glaberrima*.

Tandemly repeated DNA sequences. Figure 2 shows the results of probing various restriction enzyme digests of *O. sativa* DNA with a 350 bp fragment isolated as a prominent band in an EcoRI digest of *O. sativa*

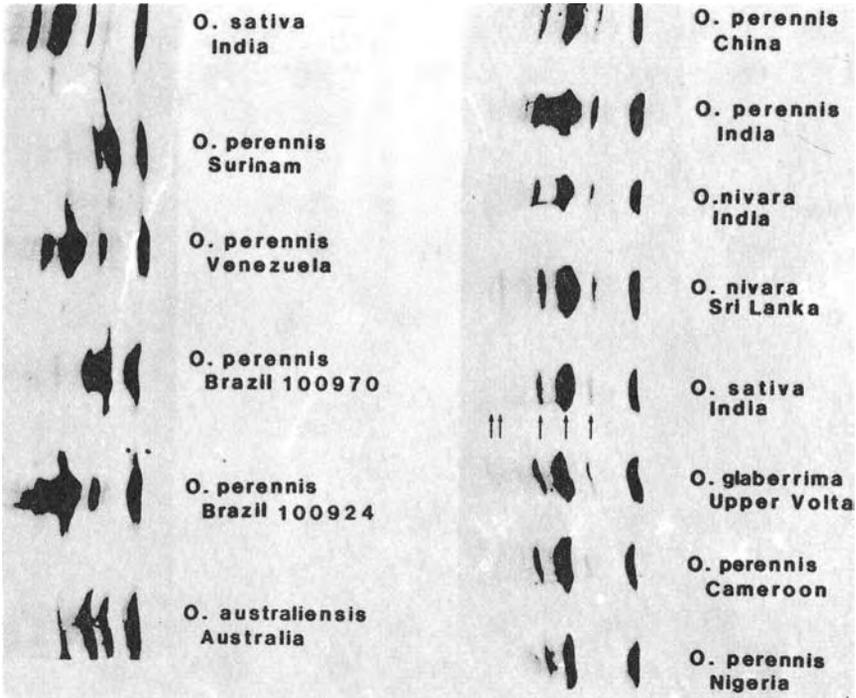


Fig. 1. Isoelectric focusing of ribulose biphosphate carboxylase/oxygenase (rubisco) from cultivated and wild rice accessions. All rubisco bands are arrowed in *O. sativa*. The purification of these proteins and the origins of all accessions used in this study are described in 14; accession numbers are those supplied by IRR1.

DNA and radiolabelled by nick translation using ^{32}P -dCTP. In several digests the probe hybridizes to a series of fragments that are multiples of 350 bp. The simplest conclusion is that members of the "RI 350" family are found in a tandem arrangement in the *O. sativa* genome. The fact that different restriction enzymes digest the arrays to differing extents (compare EcoRI, BstI, and BclI digests) suggests that some blocks of the satellite that have been recently reamplified (or homogenised) include these restriction sites. EcoRI sites are found in the vast majority of repeating units and are presumably part of the "ancestral" repeating unit.

The RI 350 sequence has been used to probe Southern blots of EcoRI-digested DNA from various *Oryza* accessions (Fig. 3). It can be seen that all DNAs possess sequences homologous to this probe except two *O. perennis* accessions from South America — from Brazil (No. 100970) and Surinam — and *O. australiensis*. All other DNAs have this sequence, with essentially the same repeating structure as *O. sativa*.

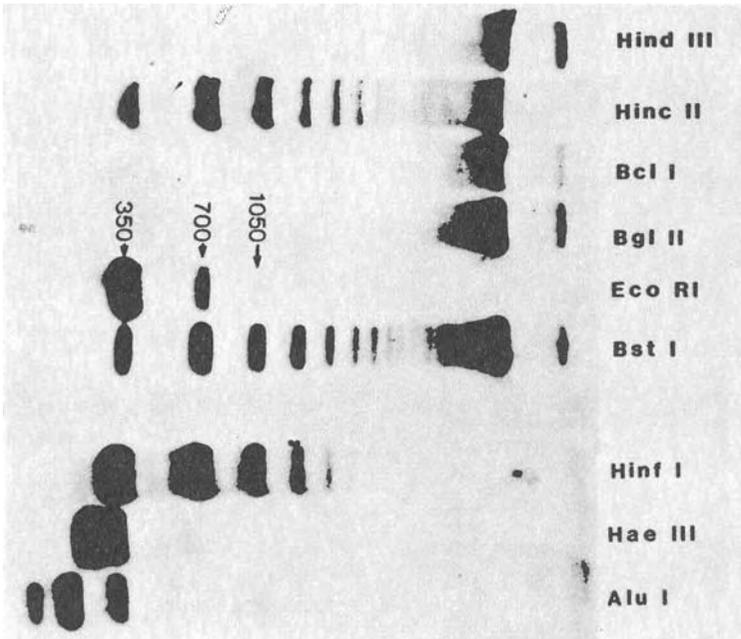


Fig. 2. Hybridization of *O. sativa* RI 350 probe to restriction digests of *O. sativa* DNA. Approximately 5 µg DNA was digested per track. Fragment lengths (base pairs) are indicated. DNA isolation, conditions for restriction, blotting, radiolabelling, and DNA hybridization were carried out as described by 6.

Interspersed repeated DNA sequences. Digestion of *O. sativa* DNA with EcoRI yields a prominent band 830 bp in length. This was radiolabelled and hybridized to EcoRI-digested DNAs (Fig. 4). The pattern of hybridization shows that the RI 830 family of sequences is found in all accessions, with some minor differences in organization. In all species, a band is seen at 830 bp, indicating conservation of this element since the divergence of these species. This band is somewhat reduced in *O. australiensis*, reflecting its distant relationship to the other species. Other minor bands are seen in all accessions. The fact that the bands are not related in intensity or molecular weight, along with the observation that the bulk of hybridization is to a broad smear of many different genomic fragments, suggests that the RI 830 sequences form an interspersed DNA family.

When DNAs from the various accessions are compared, it is clear that this family of sequences is relatively well conserved. The differences that can be seen in the minor bands of homology (corresponding to subfami-

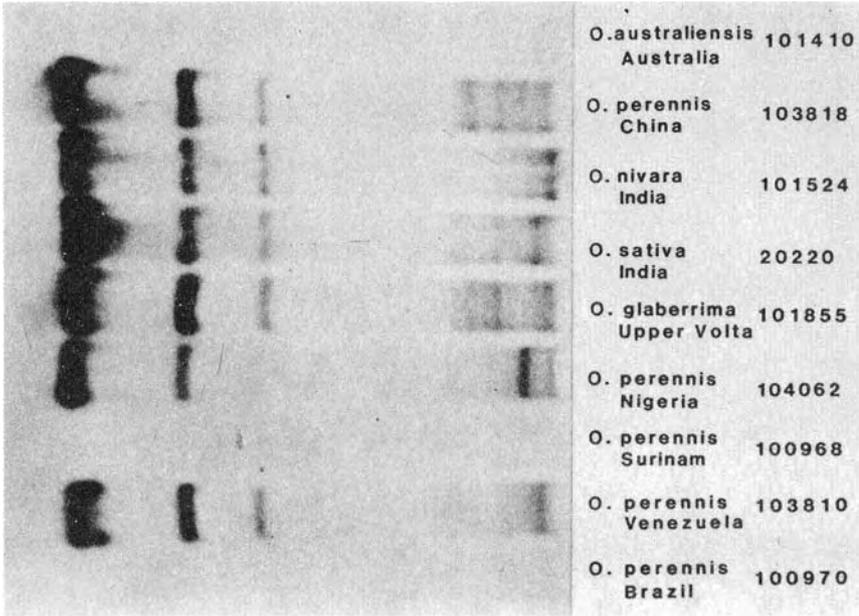


Fig. 3. Hybridization of the RI 350 probe to EcoRI-digested DNAs from nine *Oryza* accessions; equal amounts of DNA were loaded in each lane.

lies of the RI 830 sequences) generally reinforce the conclusion from the use of the RI 350 probe that all accessions have similar bands except *O. australiensis* and the *O. perennis* accessions from Surinam and Brazil (No. 100970).

Ribosomal RNA genes. Ribosomal RNA is encoded by two major types of gene: The 18S and 25S rRNAs are cotranscribed from the major rDNA repeats present in several hundreds of copies per genome; the 5S rRNA genes are found in separate tandem arrays.

In rice, the small genome size (0.6 pg/haploid genome) means that rDNA sequences may be visualized as a band when restricted DNA is electrophoresed on agarose gels (arrowed in Fig. 5). That these bands correspond to rRNA genes is shown by their hybridization to a wheat rDNA clone. Digestion with EcoRI results in single bands of around 9 kb in most accessions, suggesting that EcoRI cleaves the rDNA repeat once. The length of the EcoRI fragment is variable among accessions yet relatively homogeneous within genomes. This is in good agreement with observations in other eukaryotes, where rDNA repeats vary in length of

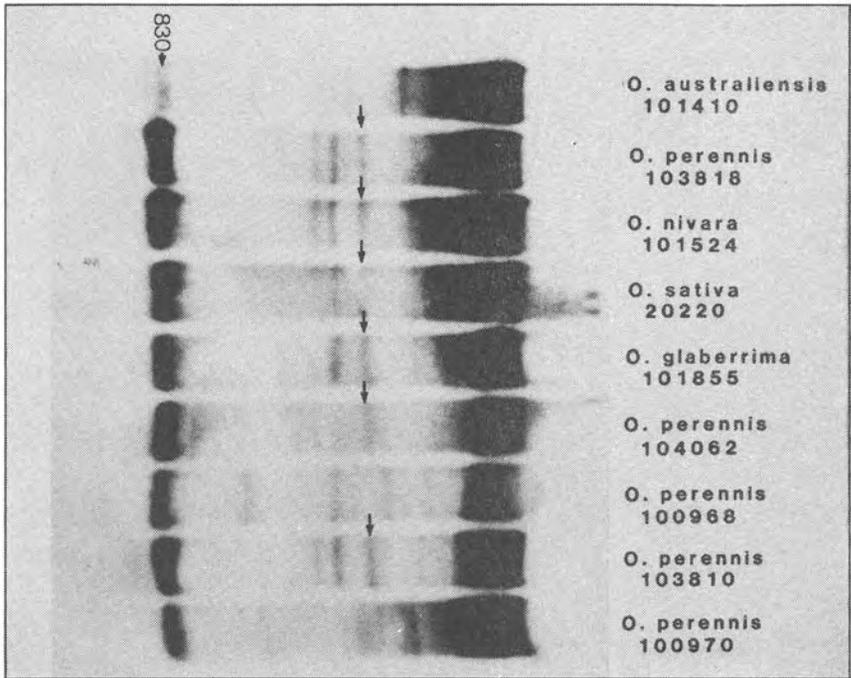


Fig. 4. Hybridization of the RI 830 probe to EcoRI-digested DNAs from nine *Oryza* accessions. A major band of hybridization at 830 bp is seen in all accessions. An additional conserved higher molecular weight band is indicated by arrows.

nontranscribed spacer sequences, although repeats within a single array are normally homogenous (9). In two of the species examined here there is evidence of heterogeneity: *O. perennis* (Brazil) and *O. perennis* (Venezuela) both have two major bands of rDNA in an EcoRI digest.

Figure 6 shows the patterns of rDNA digestion obtained with BamHI; bands are identified by hybridization with the wheat rDNA clone pTA71 (7). In all DNAs one 3.7kb band—presumably including the coding region—is conserved. The other band(s) vary in length between accessions; these fragments include both the variable (nontranscribed) spacers and a short region of the coding sequence. The heterogeneity seen in EcoRI digests of some DNAs is correlated with changes in the nonconserved band in BamHI digests. *O. perennis* (Brazil), for example, shows two clear spacer bands; *O. perennis* (China) shows three.

The origin of the heterogeneity is not clear from these data. It is apparent that most species have homogeneous sets of rDNA repeats. In those that have two or more classes of repeat, the intensity of hybridization suggests that the classes are of roughly equal abundance. Where

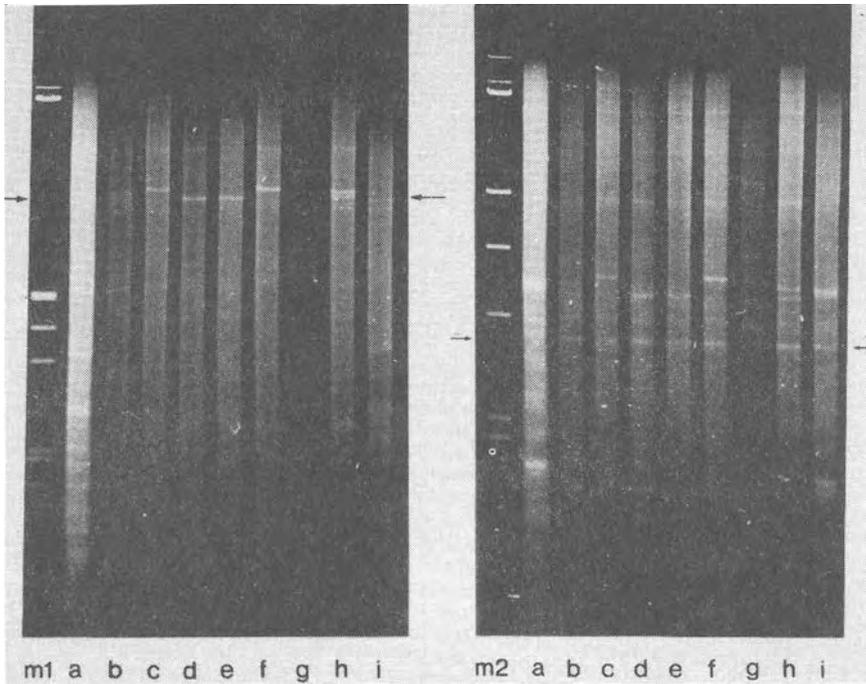


Fig. 5. Digestion of DNAs from nine *Oryza* accessions with EcoRI (left) and BamHI (right). DNAs were electrophoresed through 0.5% agarose gels and stained with ethidium bromide. Prominent bands (arrowed) correspond to the major rDNA repeat unit. a = *O. australiensis*; b = *O. perennis* (China); c = *O. nivara*; d = *O. sativa*; e = *O. glaberrima*; f = *O. perennis* (Nigeria); g = *O. perennis* (Surinam); h = *O. perennis* (Venezuela); i = *O. perennis* (Brazil); m1 = bacteriophage lambda DNA digested with HindIII and EcoRI; m2 = bacteriophage lambda DNA digested with HindIII.

there are only two classes, it may be that the two repeat lengths represent rDNA clusters on homologous chromosomes, each homogeneous for a different repeat length; alternatively, they may be present in different nucleoli, each homozygous for one type of repeat.

The restriction patterns of rDNA shown in Figure 6 do not permit any general conclusions with respect to rice phylogenetics. The rapid rates of change exhibited by rDNA clusters (5) could easily result in patterns that are effectively independent of their evolutionary ancestry.

Figure 7 shows the pattern of hybridization of a heterologous (flax) 5S rRNA gene probe (pBG13; 8) to *Rsa*I digests of nine rice DNAs. Several bands of hybridization are seen in most genomes.

All species (except *O. australiensis*) have a major band between 300 and 450 bp (open arrow), and in many species another band is visible with double this monomer length. The major repeat length is 310 bp (in *O.*

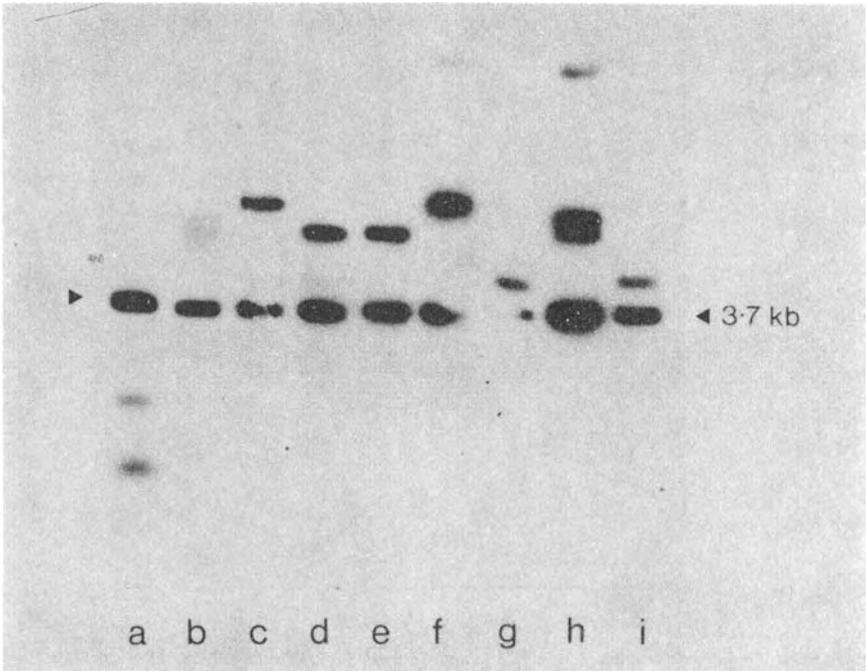


Fig. 8. Hybridization of wheat rDNA to BamHI-digested *Oryza* DNAs. The BamHI digests shown in Figure 5 were transferred to nitrocellulose and hybridized with 32 P-labelled pTA71. The repeating unit contains, in most cases, two sites for BamHI, both lying within the conserved coding region. Cleavage with this enzyme releases one fragment of constant length (arrowed), and a second fragment that displays considerable length variation both within and between accessions, presumably due to heterogeneity in the nontranscribed spacer.

glaberrima it is 295 bp), except *O. perennis* (Surinam), *O. perennis* (Brazil), and *O. australiensis*. The two exceptional *O. perennis* accessions share a repeat length of 425 bp. This heterogeneity in repeat length presumably reflects differences in spacer size, similar to that already described for the much larger repeat of the rDNA. All nine DNAs share a further conserved band (solid arrow) at around 1150 bp. We suspect that this does not represent the major 5S repeat in rice species, however, since this band is removed by washing the filter at higher stringency (for 90 min in 1xSSC (0.15 M NaCl, 0.015 M Na citrate) at 65 °C, compared with our normal conditions of 4xSSC at 65 °C 40 min). Hybridization to other bands is retained at higher stringency, however (unpublished data).

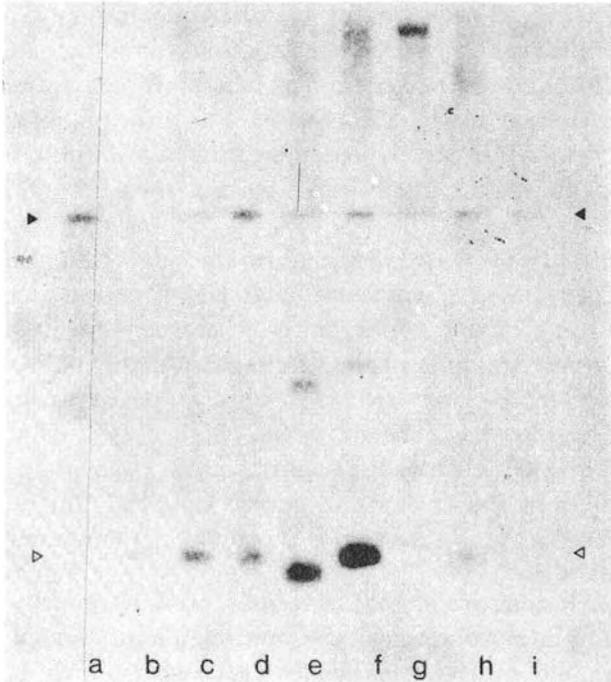


Fig. 7. Hybridization of flax 5S rDNA to *RsaI*-digested rice DNAs; digests were electrophoresed in 2% agarose gels. Accessions a - i are as listed for Figure 5 (for description, see text).

DISCUSSION

The results presented here shed considerable light upon the evolutionary relationships of the *Oryza* species studied. First, it is clear that *O. sativa*, *O. glaberrima*, and *O. perennis* are very closely related. The nuclear subunit of rubisco is homogeneous throughout all accessions studied, and all but two of the *O. perennis* accessions have identical chloroplast-encoded subunits. In these respects the accessions studied are considerably more homogeneous than the vast majority of wild rices that have been examined using these markers (D. Pental, unpublished data).

These relationships are supported by the results of repeated DNA analysis presented here. The RI 350 satellite DNA is present in all DNAs except *O. australiensis* and two *O. perennis* accessions from South America.

The RI 830 family of sequences is less discriminating in its patterns of hybridization; homologous sequences are found in all accessions examined here, although some of the minor bands support the relationships indicated by rubisco and RI 350 analysis. The principal repeat length of 5S rRNA genes is also in agreement, indicating overall similarity between all accessions except the same two *O. perennis* (those from Surinam and Brazil).

All of the results presented here confirm the close relationship between *O. sativa* and *O. glaberrima*, on the one hand, and *O. perennis* accessions from Asia, South America, and Africa, on the other. Some South American *O. perennis*, however, are quite distinct from these, implying that the cultivated rices are more closely related to some *O. perennis* species than the different *O. perennis* accessions are to one another, even within the same continent. Thus it is clear that *O. sativa* and *O. glaberrima* have evolved only recently from species of the *O. perennis* complex. Our results do not support Chang's (1) proposal that rice populations separated at the time of continental drift.

These conclusions are in good agreement with relationships that may be deduced from morphological and breeding data: *O. sativa* is virtually indistinguishable from *O. glaberrima* (13), with which it can cross (3, 11, 12). Similarly, *O. sativa* interbreeds with *O. nivara* in Southeast Asia.

Rubisco and, particularly, repeated DNA sequences serve as extremely useful markers for tracing the evolutionary history of rice genomes. The application of these techniques should indicate which wild rices are likely to be the most useful in wide-cross breeding programs and will in the future provide useful genetic markers for following genomes in such programs.

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NEW FINDINGS CONCERNING THE ORIGIN OF RICE

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Data on recently recorded wild species of rice in China are reviewed. The geographical distribution, taxonomical and morphological characteristics, ecological characteristics, genome structure, and potential of wild rice in breeding of improved cultivars are discussed. *Oryza sativa* f *spontanea*, *O. officinalis*, and *O. meyeriana* were found in Guangdong and Guangxi Provinces. *O. sativa* f *spontanea* was found in many places in large, pure populations of sometimes more than 28 ha. *O. sativa* f *spontanea* has a very wide area of distribution and has great polymorphism within the species concerning morphological, ecological, and physiological features that gave this species great potential for evolution into modern cultivated rice. *O. sativa* f *spontanea* has the genome AA and is closely related to cultivated rice. The common wild rice can be a very useful parent for breeding disease resistant varieties with high quality seeds and male sterile lines.

The problem of the origin and evolution of rice has been studied for a long time. The immediate wild relatives of the cultigen — *O. rufipogon* Griff, *O. nivara* Sharma et Shastry, and the *spontanea* forms of *O. sativa* — have been found in Guangdong, Guangxi, Yunnan, Taiwan, and Kiangsu and on Hainan Island (1). The Asian species of *Oryza* would have spread to adjacent areas following the drift and subsequent union of the South Asian plate with the Asian mainland. Most rice researchers now agree that the area of greatest diversity of *O. sativa* is located in a belt extending from the Assam-Meghelaya area in India to mountain ranges in mainland Southeast Asia and Southwest China. After a study of polymorphism in rice, Ting (9) divided cultivated rice into two subspecies — *O. sativa* ssp. Hsien Ting (usually called indica) and *O. sativa* ssp. Keng Ting (usually called japonica). Hsien is the dominant race in China, covering most of the production areas of the Yangtze River Basin. The oldest sample of Hsien rice found in the lower Yangtze Delta is from the Ho-mu-tu site in Yü-yao-hsien, Chekiang. The rice grains, hulls, and stems excavated from Ho-mu-tu are dated at 5008 BC (1). Even older examples have been found (10). The forms and the seed size are polymorphic and comprise a large population. So the large variability and complexity of ancient rices gave them great potential for evolution at a later

time, and they differentiated into the modern Hsien and Keng types. It is quite clear that the southern and the southwestern parts of China are very important places in the origin of rice. But the opinions of different scientists about the origins of rice are different. Ting (9) suggests that the southern part of China is the place of origin of rice, but others suggest the plateaus of Yunnan and Kweichow Provinces (6). So new research and field expeditions are very important in clarifying these problems.

From this point of view, the recent discovery of wild rice in Guangdong and Guangxi is very interesting. Intensive observation showed the geographic distribution, morphological and taxonomical variations, ecological characteristics, genome structure, and potential of wild rice in breeding.

GEOGRAPHIC DISTRIBUTION

In Guangdong Province, wild rice *O. sativa f.spontanea* is usually distributed in large populations 30–600 m above sea level. The area ranges from Taiwan in the east (121°15′ E) to Jinghungzhen, Jinghungshien, Yunnan (100°47′ E) in the west and from Hainan Island in the south (18°15′ N) north to Taiwan (25°00′ N). In this wide area *O. sativa f.spontanea* is found in almost all water-covered places. Sometimes it is found in almost pure populations in areas up to 100 ha as in Teluntag, Haiking County (4).

In Guangxi Province, wild rice is found in 41 counties out of 86, especially in the southern part of the province. It is concentrated in large populations in the counties of Xiangzhou, Laibin, Wuxuan, Kweiping, Kweishien, Binyang, Shanglin, Hengshien, Yulin, Hepu, and Bobai. Wild rice was discovered in 758 places and covered 317 ha. Pure populations larger than 2 ha were found in 13 places. The largest one — 28 ha in Malutang, Kweixin County — comprised 90% of the total area of Malutang. Along the River Dayung the wild rice population spread in a continuous population up to 17 ha (Table 1, 2).

MORPHOLOGICAL AND TAXONOMICAL VARIATION

The wide polymorphism of rice is another important feature. In the areas mentioned above usually three species of wild rice were found — common wild rice *O. sativa f.spontanea*, *O. officinalis*, and *O. meyeriana* — which showed variations as described on the following pages.

Table 1. Distribution of wild rice (2).

Provinces	Counties	North latitude	East longitude
<i>Oryza sativa f spontanea</i> Rosch.			
Guangdon, Guangxi,	111	18°09′	100°40′
Yunnan, Jiangxi,		(Aixian, G.D.) to 28°14′	(Jinghong, Y.N.) to 117°08′
Fujian, Hunan		(Dongxian, J.X.)	(Czhangpu, G.D.)
<i>Oryza officinalis</i> Wall			
Guangdon, Guangxi, Yunnan	36	18°18′ (Aixian, G.D.) to 24°17′ (Yingde, G.D.)	99°05′ (Gengma, Y.N.) to 113°07′ (Yingde, G.D.)
<i>Oryza meyeriana</i> Baill.			
Guangdon, Yunnan	27	18°15′ (Aixian, G.D.) to 24°55′ (Yingzhiang, Y.N.)	97°56′ (Yingzhiang, Y.N.) to 109°56′ (Lingshui, G.D.)

Table 2. Elevation and distribution characteristics of wild rice (2).

Provinces	Average elevation (m)	Highest and lowest elevation (m)	Characteristics of population
<i>Oryza sativa f spontanea</i> Rosch.			
Guangxi, Yunnan	130	215 (Hepu, G.X.) 553 (Jinghong, Y.N.)	large populations (6.7 ha in 23 cases; 33.3 ha in 3 cases)
<i>Oryza officinalis</i> Wall			
Yunnan	800	520 (Genma), 1,000 (Yongde)	small populations
Guangdon, Guangxi	200	25 (Tengxian), 450	small populations (sometimes up to 0.3 ha)
<i>Oryza meyeriana</i> Baill.			
Guangdon	50-400	50-800 (Wuzhi Mountain area)	small populations
Yunnan	600-800	425-1,000 (Yongde, Y.N.)	small populations

O. sativa f spontanea

The culm may be standing, semistanding, or creeping. The leaves may be wide or narrow, long or short, with small or large angle. The panicle varies greatly in the length, shape, and angle of the primary branches and in weight and density. The color of the lemma at anthesis may be green, pale, yellowish, gold, blackish brown, or purple. The awns may be fully awned, partly awned, or terminally awned.

O. officinalis

This species is relatively uniform. Plants are usually very high, up to 200-300 cm. The culm is strong. The leaves are long and wide, with smooth blades. The panicles are long, more than 80 cm. The seeds are wide, short, and 4-5 mm in length. The color of the lemma is pale brown or gray. Fertility is relatively low.

O. meyeriana

The height of this highly fertile plant is usually low, about 30-40 cm. The leaf blades are short and wide, like the leaves of bamboo. The axis of the panicle is short, and there are usually 10-15 seeds per panicle, all of which set closely at the axis. The lemma usually has a yellow-brown color. The seeds are of two types: elongated with 6-7 mm length and short ovaly with 4.5-5.5 mm length.

ECOLOGICAL CHARACTERISTICS

O. sativa f spontanea usually has a very complicated distribution in mountainous areas, hills, plains, and submerged places. It is usually found in low, waterlogged places. Wild rice loves light and high temperatures and is usually very sensitive to photoperiod in tropical and subtropical areas. The forms of common wild rice are different in photoperiod sensitivity (7), which has given them a large evolutionary advantage. Wild rices everywhere showed very high resistance to insects and diseases, especially to *Pyricularia oryzae* Cav., *Hypochnus sasakii* Shirai, and *Pseudomonas oryzae* Uyeda et Ishiyama. But the degree of resistartce varies from one form to another, so for breeding purposes it is necessary to test them each time separately. *O. officinalis* usually grows under shade and in humid

places and was never found in bright light or in submerged places. The soil was usually at pH 5.5–6.5. This wild rice grows along the banks of small streams.

GENOME STRUCTURE

The genome of *O. sativa* f *spontanea* is AA type and in crossing with cultivated rice gave fertile offspring, showing that it is quite closely related to cultivated rice. The genome of *O. officinalis* belongs to the CC genome, and it is very hard to get seed from crossing with cultivated rice. Even if seeds are obtained, the offspring are usually sterile. From genetic analysis we can suggest that *O. sativa* f *spontanea* has a closer relationship to cultivated rice and can be the direct ancestor of cultivated rice.

POTENTIAL OF WILD RICE IN BREEDING

Wild rice can be a very good resource for rice breeding programs for cold tolerance, for insect and disease resistance, and for high quality seeds. Many of these forms have high protein content in the seeds (3). As early as 1932 Ting (8) crossed cultivated rice with *O. sativa* f *spontanea* and bred an excellent variety, Zhongshan #1. IRRI breeders used *O. nivara* as a donor of disease resistance and crossed it with cultivated rice to get several new varieties like IR28, IR29, IR30, IR32, IR34, IR36, IR38, IR40, and IR42 (5).

Wild rice can be used as a source of male sterility for breeding male sterile lines of rice, which is widely done in China. For example, Yuan Longping's research group (personal communication), using male sterile forms of wild rices from Aixian on Hainan Island as the male parent crossed with cultivated rice, got a series of male sterile lines of rice that were later widely used for the production of hybrid rice. Chinese farmers have received great economic benefits from such hybrid rices.

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EVOLUTIONARY TRENDS IN GENUS *ORYZA*

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The primitive and advanced characters of genus *Oryza* were assigned numerical values and, on the basis of these characters, its species were assessed for their phylogenetic advancement. The phylogenetic position of various generic subdivisions (sections and series) were determined from pooled data of the species that delimit these groups. The phylogenetic sequence arrived at in this manner indicates certain evolutionary tendencies not emphasized so far in the phylogeny of genus *Oryza*. For example, it is inferred that the primitive species were characterized by shade-loving plants growing in the humid atmosphere of forests in well drained soils. The adaptation to open habitat marked a major breakthrough in the phylogeny of *Oryza*, and several other characters followed suit. Another significant trend has been the similar bipolar differentiation that has taken place in each section. This may be characterized by the differentiation in spikelet size of the two groups—one having small spikelets and the other large.

The genus *Oryza* has been classified into three sections, viz., Sect. *Padia*, Sect. *Angustifolia*, and Sect. *Oryza* (8). Sect. *Padia* represents the Southeast Asian elements that are perennial, shade-loving, and adapted to well drained soils. The plants are small and without awns or medium-sized with awns. This section is represented by *O. schlechteri*, *O. meyeriana*, *O. ridleyi*, and a few related species/subspecies. Sect. *Angustifolia* is an African group of small plants adapted to open habitat. They are perennials or annual plants growing in perennial or seasonal swamps. This section is represented by *O. perrieri*, *O. tisseranti*, *O. brachyantha*, and *O. angustifolia*. Sect. *Oryza* is distributed all over the tropics. The plants of this group are comparatively large, are adapted to hydrophytic conditions, and prefer an open habitat. *O. latifolia*, *O. australienris*, and *O. sativa* are a few of its representative species. These three sections have been further divided into series to represent intrasectional subgroups.

Sharma and Shastry (9) listed the variation available for the various characters in genus *Oryza*, especially at the level of supraspecific ranks (i.e., series and sections), and discussed their primitive and advanced nature. The aim of the present paper is to evaluate the phylogenetic position of each of these groups and indicate evolutionary trends in them.

MATERIALS AND METHODS

Collections of all the species of *Oryza* (except *O. schlechteri* and *O. angustifolia*) were examined, and data were collected on their specific characters. Published data on *O. schlechteri* and *O. angustifolia* were also taken into account. *O. perrieri*, *O. tisseranti*, and *O. angustifolia* are treated here as species of *Oryza*.

RESULTS

For the phylogenetic consideration of generic subdivisions, the characters were limited by three considerations: First, a character should be fairly consistent within a series but should vary at the level of series or section; second, data should be available for all the species for that character; and third, a phylogenetic assessment of the character should be possible. The cultivated species have been excluded from consideration, since human selection is not based on phylogenetic considerations.

The present approach and methodology are basically similar to those of Portkres (3). The author has, however, included a large number of characters, rated the characters on the basis of criteria established by him (9), and grouped the data according to his own system (8).

The characters utilized here for phylogenetic considerations are listed in Table 1. Associated characters are considered as a single group. Based on considerations discussed in detail by Sharma and Shastry (9), characters were rated as primitive-1, intermediate-2, or advanced-3.

The species/subspecies of genus *Oryza* are listed in Table 2. The primitive, intermediate, or advanced condition for each of the characters (listed in Table 1) is noted for every species/subspecies of *Oryza*. The mean phylogenetic value for each of the series and sections is assessed from Table 2 and presented in Table 3. A phylogenetic tree was erected on the basis of these data and is presented in Figure 1.

DISCUSSION

On the basis of the characters taken into consideration and the criteria employed, Sect. *Padia* seems to be the most primitive. Of the other two sections, Sect. *Oryza* occupies the more advanced position. The phylogenetic sequence arrived at in this paper bring out some novel facts.

Table 1. The primitive and advanced nature of characters in genus *Oryza* (after 9).

Character	Primitive—1	Intermediate—2	Advanced—3
I. Panicle			
Termination	in main rachis	—	in a branch
Ramification	profuse	—	poor
Pulvinus	absent	—	present
II. Fertile lemma and palea — sculpturing	smooth	not prominent	prominent
III. Fertile lemma and palea — hairs	ciliate	scabrid	spinulose
IV. Fertile lemma and palea			
Mucro	absent	—	present
Rachilla	straight	—	comma-shaped
Articulation	horizontal	—	oblique
Awn	slender	—	robust
V. Spikelet			
Lateral compression	not compressed	—	compressed
Size	large	—	small
VI. Sterile lemmas			
A. Shape	lanceolate	setaceous	absent
B. Size	large	small	absent
VII. Ploidy	diploid	—	tetraploid
VIII. Habit	perennial	weakly perennial	annual

The primitive groups of species are associated with a shade-loving character. For example, Sect. *Padia*, the most primitive section in genus *Oryza*, is characterized mostly by shade-loving plants. Within this section, Ser. *Meyerianae* comprises shade-loving species only. The plants of Ser. *Ridleyanae*, although shade-loving, tolerate more light and grow at the edges of the forest and by the side of forest roads. Similarly, within Sect. *Oryza*, Ser. *Latifoliae* is represented by many partially shade-loving species, but Ser. *Sativae* contains plants growing in sunny habitats.

A transition from shady to sunny habitat is also accompanied by change from mesophytic to hydrophytic habitat and from wide herbaceous leaves to linear rigidulous leaves. This can be explained if we consider the humid atmosphere to which the primitive shade-loving species would have been adapted. A hydrophytic adaptation would least disturb the internal physiology of plants when they come out of the shade. Every species of genus *Oryza* that does not have a shade-loving habitat is a hydrophyte.

Tale 2. Primitive and advanced nature of characters and their distribution in *Oryza* species.

Species/subspecies	Character ^a								
	I	II	III	IV	V	VIA	VIB	VII	VIII
a. <i>longistaminata</i>	3	3	3	3	1	1	2	1	1
b. <i>glumaepetula</i>	3	3	3	3	1	1	2	1	2
c. <i>rufipogon</i>	3	3	3	3	1	1	2	1	1
d. <i>nivara</i>	3	3	3	3	1	1	2	1	3
e. <i>breviligulata</i>	3	3	3	3	1	1	2	1	3
f. <i>australiensis</i>	3	3	3	1	3	1	2	1	2
g. <i>officinalis</i>	3	3	3	1	3	1	2	1	1
h. <i>punctata</i>	3	3	3	1	3	1	2	1	2
i. <i>eichingeri</i>	3	3	3	1	3	1	2	1	2
j. <i>minuta</i>	3	3	3	1	3	1	2	3	2
k. <i>malampuzhaensis</i>	3	3	3	1	3	1	2	3	2
l. <i>schweinfurthiana</i>	3	3	3	1	3	1	2	3	2
m. <i>latifolia</i>	3	3	3	1	3	1	2	3	2
n. <i>alta</i>	3	3	3	1	3	1	2	3	2
o. <i>grandiglumis</i>	3	3	3	1	3	1	1	3	2
p. <i>brachyantha</i>	1	1	3	3	1	2	2	1	3
q. <i>angustifolia</i>	1	1	3	3	1	3	3	? ^b	3
r. <i>perrieri</i>	1	1	1	1	3	3	3	1	2
s. <i>tisseranti</i>	1	1	1	1	3	3	3	1	2
t. <i>ridleyi</i>	1	1	2	1	1	2	1	3	2
u. <i>longiglumis</i>	1	1	2	1	1	2	1	3	2
v. <i>granulata</i>	1	2	2	1	1	2	2	1	1
w. <i>meyeriana</i>	1	2	2	1	1	2	2	1	1
x. <i>abromeitiana</i>	1	2	2	1	1	2	2	1	1
y. <i>schlechteri</i>	1	1	1	1	3	2	2	? ^b	1

^aRoman and Arabic numbers follow use in Table 1.^bNot known.**Table 3. Evolutionary status of the subdivisions of genus *Oryza*.**

Series/section	Species ^a	Score
Ser. Sativae	(a-e)	2.11
Ser. Latifoliae	(f-o)	2.22
Sect. <i>Oryza</i>	(a-o)	2.17
Ser. Brachyanthae	(p-q)	2.00
Ser. Perrierianae	(r-s)	1.78
Sect. <i>Angustifolia</i>	(p-s)	1.89
Ser. Ridleyanae	(t-u)	1.56
Ser. Meyereanae	(v-x)	1.44
Ser. Schlechterianae	(y)	1.50
Sect. <i>Padia</i>	(t-y)	1.50

^aLetters in parentheses refer to species/subspecies in Table 2.

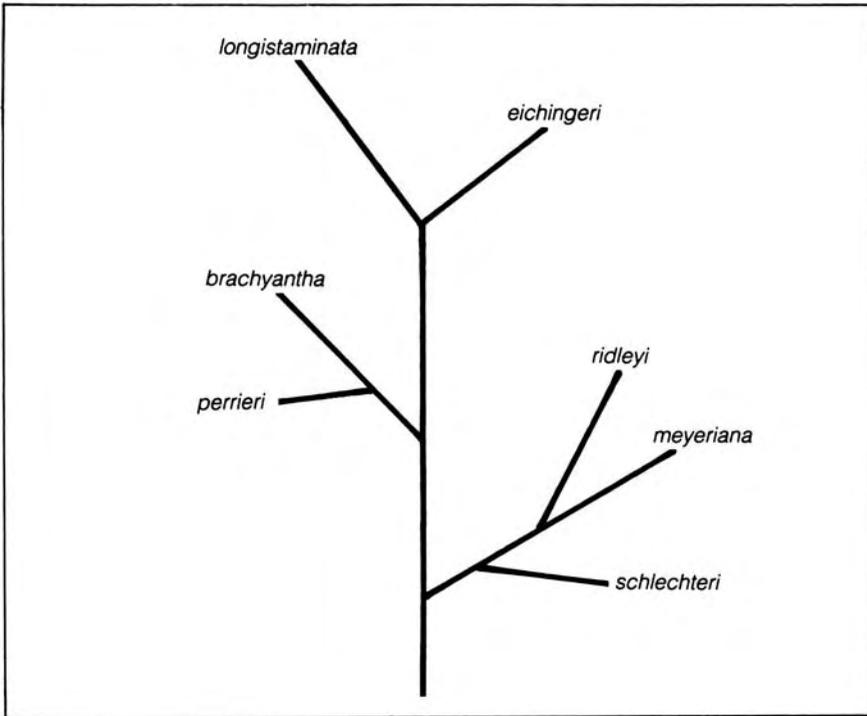


Fig. 1. Phylogenetic relationship of species in genus *Oryza*.

Plant size

The plants of primitive sections are small in size. Sect. *Padia* and Sect. *Angustifolia* are characterized by small plants (except *O. ridleyi* and *O. longiglumis*, which are medium-sized plants). The larger size of the plant does not manifest itself in the genus prior to Sect. *Oryza*. Even in Sect. *Oryza*, *O. collina* (the Asian form *O. eichingeri*) and *O. minuta* are small. Furthermore, species of genome AA (Ser. *Sativae*) are not as tall as those of genome CC or EE. This deserves special note because, in intersectional crosses, small size of plant (as in *O. brachyantha*) significantly dominates over large size (as in *O. alta* or *O. australiensis*). Even in intrasectional crosses, the small size of *O. collina* dominates over the large size of *O. officinalis* and *O. latifolia*. The dominance of smaller size of plant in *O. sativa/O. australiensis* also supports this generalization. If so, the tallness of CC, EE, and to a certain extent AA might be viewed as an advanced character.

Awn

Awn is another character that reveals the phylogenetic sequence of the three sections. In Sect. Padia, Ser. Meyerianae and Ser. Schlechterianae are represented by awnless spikelets. The third series, Ser. Ridleyanae, has nonmucronate awned spikelets. In the other two sections, all the (wild) species have awned spikelets with or without mucro. According to Hubbard (2) and Stebbins (11), development and elaboration of awn are advanced characters in Gramineae. Further, Sharma and Shastry (7,9) discussed the nonmucronate and mucronate conditions and argued that the former is an advanced character. If so, the absence of the mucronate condition in the whole of Sect. Padia and the absence of awns in some of its species reflect the primitive nature of this section.

Sect. *Angustifolia* has retained a more primitive vascular system in its awn than Sect. *Oryza*. According to Roy (4), "the single xylem patch of *sativa* and the double patch of *brachyantha* show that the awn of the former is more evolved than that of the latter. In the case of *sativa*, during the process of evolution, the xylem elements of the central bundle and those of papillar bundles have fused to form one group whereas, in the case of *brachyantha*, these two groups of xylem elements have not yet fused. Since reduction in size or volume is an advanced character, *sativa* awn with comparatively reduced xylem elements is more advanced than that of *brachyantha*."

Other Characters

Morphological features like elaborate sculpturing on the surface of fertile lemma and palea, presence of pulvini at the base of panicle branches, and robust plant habit manifest themselves in Sect. *Oryza* only. The evolutionary significance of these characters is not well understood. The tough lemma and palea (husk) may serve as better coats for the seeds, especially under hydrophytic conditions, and may protect the seeds during dormancy. The pulvinus at the base of panicle branches confers laxity in the panicle. This may be an adaptation for better seed dispersal. The robust plant habit may be another adaptation for hydrophytic conditions to ensure against unexpected rise in the depth of water.

The major selection pressure that has operated in the genus in the past thus seems to be for its adaptation from shade-loving habitat to open, sunny habitat. The other changes, namely from mesophytic to hydrophytic habit, from wide herbaceous leaves to narrow and rigidulous leaves, from thin lemma and palea to tough and sculptured ones, from a closed to

Tale 4. Bipolar differentiation in genus *Oryza*.

Section	Spikelet size	
	Large	Small
Sect. <i>Oryza</i>	Ser. <i>Sativae</i>	Ser. <i>Latifoliae</i>
Sect. <i>Angustifolia</i>	Ser. <i>Brachyanthae</i>	Ser. <i>Perrierianae</i>
Sect. <i>Padia</i>	Ser. <i>Meyerianae</i>	Ser. <i>Schlechterianae</i>

an open panicle, and from herbaceous to robust habit, may all be viewed as consequential to the above change in ecology.

In each section, morphological and ecological differentiation reveal two major directions — one characterized by large spikelets and the other by small ones (Table 4).

Spikelet size is often associated with several other characters: In Sect. *Oryza* and Sect. *Angustifolia*, larger spikelet size is associated with the presence of long and bold awns, presence of mucro, curved rachilla, and oblique articulation of the spikelet with the pedicel. On the other hand, small spikelet size is associated with characters like small and slender awns, absence of mucro, straight rachilla, and horizontal articulation of spikelet with pedicel. The species representing the smaller spikelets are often perennial and prefer a stable habitat. They grow in shady environments and have herbaceous and wider leaves; the species representing larger spikelets have developed an annual habit and prefer seasonal habitats. They are always hydrophytic, prefer sunshine, and have linear rigidulous leaves.

The above bipolar differentiation is not well marked in Sect. *Padia*. For example, Ser. *Meyerianae* has large spikelets but does not show the associated advanced characters. The absence of these associated (advanced) characters indicates again that Sect. *Padia* is probably a primitive section where all the associated advanced characters may not be expected.

PHYLOGENETIC TRENDS WITHIN SERIES OF SECT. *ORYZA*

The species of Ser. *Latifoliae* are well differentiated genomically into BB, CC, EE, BBCC, and CCDD species. The diploid species having the DD genome is still unidentified. Gopalakrishnan and Sampath (1) and Second (5) suspected some of the forms of *O. officinalis* to be DD. Sharma et al

(10) and Sharma (6) suggested that the genomic constitution of *O. officinalis* needs reinvestigation and might be DD.

Among the diploid species of Ser. Latifoliae, *O. eichingeri* (CC) is a comparatively small plant growing on moist but well drained soil in humid forests of equatorial Africa. On the other hand, *O. punctata* is adapted to sunshine and grows in the seasonal swamps of Sudan. In other words, the trend already manifest at the sectional level in genus *Oryza* is also visible among the species of Ser. Latifoliae. A parallel situation occurs in Asia, where *O. collina* corresponds with *O. eichingeri*, and *O. australiensis* with *O. punctata*. Ecologically, *O. officinalis* occupies an intermediate position between these two extremes.

Morphologically, the amphidiploid species (BBCC: *minuta*, *malumpuzhaensis*, *schweinfurthiana*; CCDD: *latifolia*, *alta*, *grandiglumis*) of Ser. Latifoliae hardly offer anything that is not available among the diploids. They have been cytogenetically successful because of sufficient genetic differentiation of their genomes at the diploid level, and ecologically successful because they fill a niche that diploids have not been able to occupy.

A steady trend of evolution from perennial to annual habit, from cross-pollination to self-pollination, and from adaptation to stable habitats to adaptation to unstable habitats is discernible in Ser. Sativae.

O. longistaminata and *O. rufipogon* are perennial species having long anthers, profuse pollen, and well exerted stigma—mechanisms that favor cross-pollination. They occupy permanent habitats indicated by their preference for sites where water remains deposited throughout the year. Among the perennial species, *O. longistaminata* has well branched suckers and a self-incompatibility system. *O. rufipogon* has a runner system in place of suckers and has lax panicles and long anthers with profuse pollen. *O. glumaepetula* has progressed more in the direction of an annual habit. This species has smaller and thinner anthers and less exerted stigmas, which are tendencies towards a self-pollinating mechanism. It has long and prominent awns, which indicate adaptation for seed dispersal expected in a species having a weak perennial system.

The trend towards an annual habit has received its fullest expression in the wild species *O. nivara* and *O. breviligulata*. These species are distributed in areas of less rainfall and occupy seasonal ponds and ditches. They channelize their potential for maximum seed production to guarantee survival beyond the dry hot summer.

In the annual species, the awns are prominently developed, robust, and scabrid. It is noteworthy that *O. breviligulata* has the most prominent awn in the genus. *O. nivara* is next only to *O. breviligulata*. The barbs on the awn are supposed to help seed dispersal and burial of the seed in the mud

until the advent of the next favorable season. The high degree of seed dormancy found in the annual wild species, especially in *O. nivara*, is useful for its survival under these conditions.

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DISCUSSION

SESSION 1: SYSTEMATICS AND EVOLUTION

Q – Wang, X.M.: Could you indicate the localities of origin of the Chinese wild forms that showed some similarities to japonica types?

A – Morishima: Most of the Chinese accessions examined were obtained from the Kwangsi and Kwangtung areas. Japonica-like characteristics were found in materials obtained from both areas.

C – Second: Concerning the possible change of isozymes according to the environment, a great amount of data has led to the so-called “neutral theory of evolution” concerning isozymes in particular. Assuming this theory, I was able to infer a reasonable evolutionary scenario while assuming an adaption value for isozymes, but I could not reach any conclusion. There might be exceptions to the rule of the neutrality of isozymes in adaptive values, but these must be rare.

C – Chang: I urge workers to re-examine their results in the light of plate tectonic data gathered in recent years. Many cultivars are already adapted to environments outside *O. sativa*'s original habitat. Data from these cultivars may be considerably biased by recent changes in environment.

Q – Rutger: How many entries of *Oryza australiensis*, *o. perennis* (Brazil), and *O. perennis* (Surinam) did you study? Is it now feasible to analyze more entries?

A – Barnes: A few samples of each species were studied. It would be feasible to analyze many more entries.

Q – Walbot: Did you show that large sub-unit heterogeneity is maternally inherited?

A – Barnes: No. We have not been able to do any genetic analysis of the characters described.

Q – Brar: Your data show a homogenous nature for the small sub-unit but a heterogenous one for large sub-unit. What could be the reason for this differential response of two genomes?

A – Barnes: It is possible that a higher rate of evolution might be possible for the large sub-unit, which is chloroplast encoded, because of the large number of chloroplast genomes per cell. If the mutation rate (per base pair) is the same in chloroplasts and nuclei, the large number of chloro-

plast genes would lead to a greater frequency of new mutations (per cell) in the chloroplast encoded sub-units.

C – Wu, R.: I agree with Dr. Barnes' interpretation that since there are many chloroplast DNA molecules there may be more chance of variation. At the DNA sequence level, we have found heterogeneity of the Rubisco large sub-unit gene.

VARIETAL DIVERSITY AND REPRODUCTIVE BARRIERS

SESSION 2

GEOGRAPHIC DISTRIBUTION OF GAMETOPHYTE GENES IN WIDE CROSSES OF RICE CULTIVARS

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When wide crosses were performed in rice cultivars, a large number of segregation distortions appeared in the selection of hybrid gametes due to the presence of gametophyte genes. The existence of these gametic genes reflects the genetic differences between two cultivars crossed. We observed loci of *ga-2*, *ga-3*, *ga-4*, and *ga-8* in the hybrid populations of the wide crosses including more than 2,700 combinations by the use of genetic tester lines. Loci of *ga-2* and *ga-3* are located on Chromosome 5 (linkage group XI), *ga-4* on Chromosome 6 (linkage group I), and *ga-8* on Chromosome 3 (linkage group III). The distribution of the gametophyte loci suggests that two major varietal groups of rices can be identified, corresponding to the japonica and indica groups. One of the groups shows simple variations such as those found in the Keng type rice of China and upland rices of the Philippines; the other group shows a marked diversity in the genotype for *ga* genes such as in Indian rices and the Hsien type of Chinese cultivar. These facts indicate that Indian and Chinese rices are genetically differentiated.

In wide crosses of rices, phylogenetic remoteness such as hybrid inviability, hybrid sterility, and hybrid breakdown are often suggested. Distorted segregations are present in many marker genes located on several kinds of chromosomes. The extent of the segregation distortion and hybrid semisterility indicates the presence of genetic differences between the varietal groups to which the parental cultivars belong.

The present study describes kinds of certation loci detected by the wide crosses and gives the geographic distribution of these reproductive genes in a wide range of genetic materials from Asia.

Japonica genetic tester lines were used to define the gametic loci of exotic indigenous cultivars. Marker characters used consisted of 40 loci on various kinds of chromosomes. Exotic cultivars used included 51 indigenous strains that originated in Asian countries. In the F₂ populations derived from 2,700 cross combinations, observations were made to determine whether the single major tester character segregates in a Mendelian ratio in reciprocal crosses. When the segregation distortion

was found, the genetic mechanism was analyzed in further generations or backcrossed populations. Then the distribution of reproductive isolation loci was determined. Here I use the system of chromosome numbers and linkage groups reported by Nishimura (7) and Nagao and Takahashi (2).

SEGREGATION DISTORTION AND LOCATION OF *ga-4* ON CHROMOSOME 6

Among the major marker genes on Chromosome 6 (linkage group I), *wx* (glutinous endosperm), *dp-1* (depressed palea), *alk* (breakdown in alkali solution), and *ws* (white striped leaves) represent the segregation distortion markers. The segregation ratios of the four markers in F_2 populations from wide crosses are skewed from the monogenic expectation because of linkage with the certation genes *ga-1* and *ga-4* (1,7). An example of distorted segregation for *wx* induced by the *ga-4* locus is shown in Table 1. Similar segregation ratios were obtained in the other three marker genes, depending on the extent of the linkage intensities between the marker loci and the *ga-4* locus. The locus of *ga-4* is between *dp-1* and *alk* as shown in Figure 1. Japanese tester strains and japonica cultivars from other areas harbor *ga-4*. Indian and Chinese cultivars usually harbor the *ga-4+* allele.

Table 1. An example of the distorted segregation of *wx* for glutinous endosperm in the F_2 of wide crosses.

Cultivar crossed with japonica tester	Segregation in F_2^a			Frequency of <i>wx</i> (%)	c^2 for 3:1	No. of crosses
	+	<i>wx</i>	Total			
Qing you	461	100	561	17.8	15.4	3
Kinandang puti	1690	306	1996	15.3	99.5	6
Kasalath	1145	210	1355	15.5	65.2	3
Dular	684	111	795	14.0	51.7	2
Chinsurah Boro 2	977	194	1171	16.6	44.4	7
Pusur	1536	299	1835	16.3	74.2	8

^aAll segregations showed significant distortions from the Mendelian ratio at the 1% level. The significance is also shown in the values in Tables 2-3.

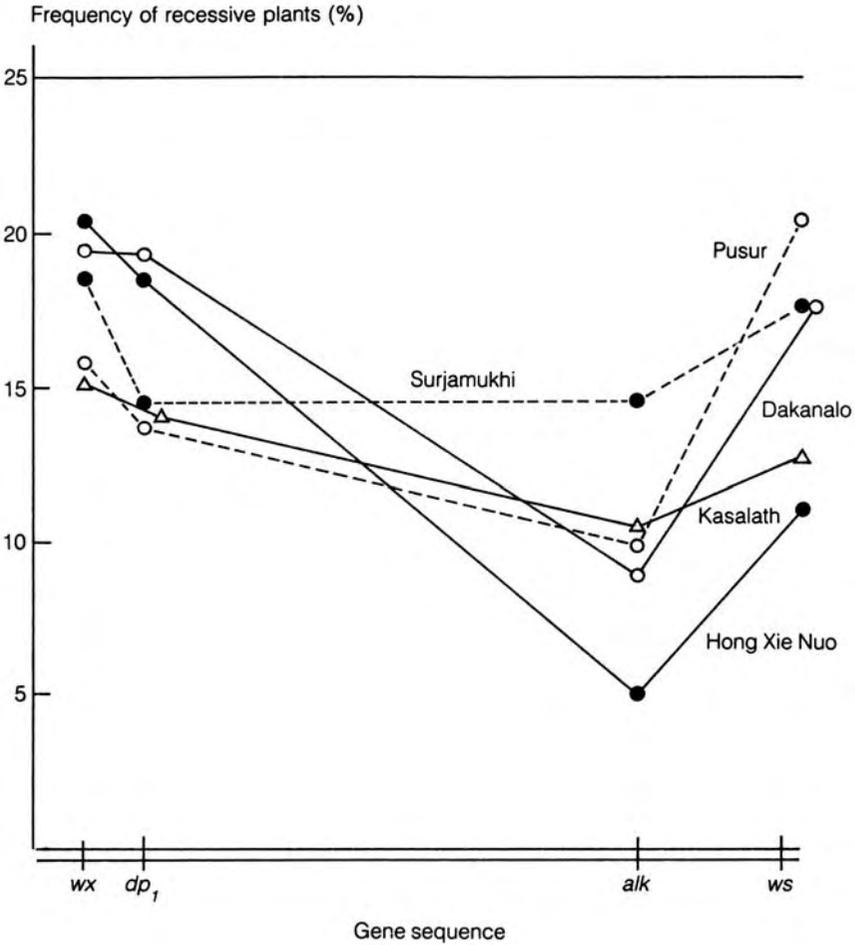


Fig. 1. Relationship between gene sequences and extent of distorted segregation of the four marker characters on Chromosome 6 in wide crosses of rice.

SKEWED SEGREGATION OF *ga-2* AND LOCI ON CHROMOSOME 5

When japonica tester strains are crossed with Chinese or Indian cultivars, significant segregation distortions are observed in many marker characters located on Chromosome 5 (linkage group XI). Some markers showing distortion are *chl-1* (chlorina-1), *v-1* (virescent-1), *bc* (brittle culm), *v-2* (virescent-2), *dl* (drooping leaf), and *spl-3* (spotted leaf-3). The frequencies of recessive plants with these characters are significantly

lower than the Mendelian expectation. Examples of segregation distortions are shown in the character of *dl* in various cross combinations (Table 2). These distortions are controlled by *ga-2*, a reproductive isolation locus (6). The extent of the skewness is expressed by the tightness of the linkage between the markers and *ga-2* (Fig. 2). Moreover, two types of distortions are found on the *ga-2* locus. One has a strong certation gap (A) between the alleles compared with the other (B). The former type is distributed in Chinese Hsien type cultivars, the latter in Indian and Nepali cultivars.

EXCESSIVE DISTORTION OF *ga-3* AND THE LOCUS ON CHROMOSOME 5

In some of the crosses between japonica tester lines and javanica or indica cultivars, the frequencies of recessive plants associated with sev-

Table 2. An example of the distorted segregation of *dl* for drooping leaves in the F₂ caused by the gametophyte gene *ga-2*.

Cultivar crossed with japonica tester	Segregation in F ₂			Frequency of <i>dl</i> (%)	No. of crosses
	+	<i>dl</i>	Total		
<i>A. Strong certation gap</i>					
Duang guang hua luo	3916	195	4111	4.7	11
Hu Nan Hsien	5190	526	5716	9.2	19
Dao Ren Qiao	2475	164	2639	6.2	10
Hong Xie Nou	7539	345	7884	4.4	34
Chi Hsien Dao	2758	139	2897	4.8	12
Deng Pao Zhai	2392	118	2510	4.7	13
<i>B. Weaker certation gap</i>					
Muha	3248	813	4061	20.0	13
Nepal No. 18	3106	569	3675	15.5	17
Kasalath	6831	1264	8095	15.6	27
Pusur	2511	337	2848	11.8	13

Frequency of recessive plants in F_2 (%)

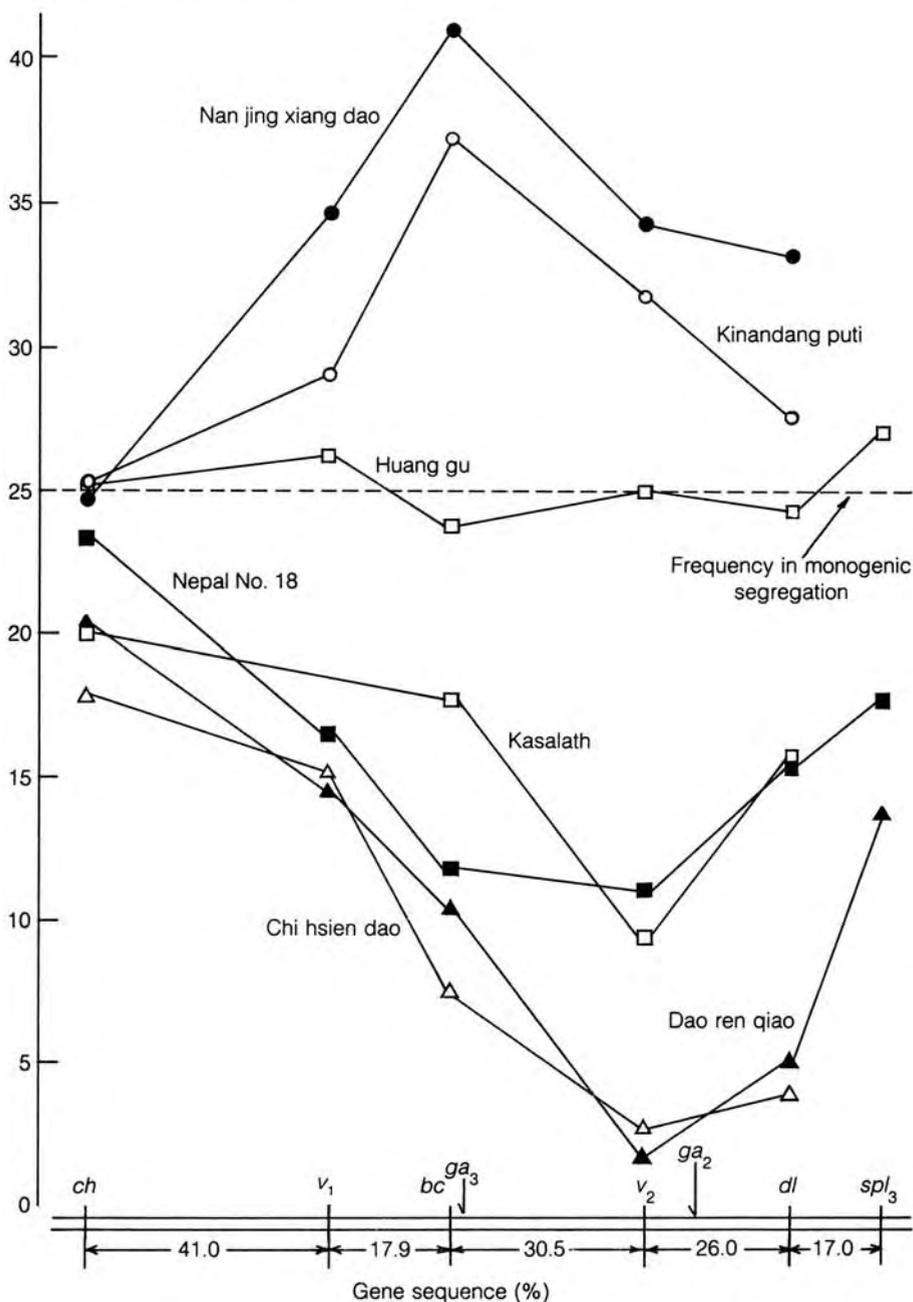


Fig. 2. Distorted segregation of marker characters and their gene sequences on Chromosome 5 in wide crosses of rice.

eral markers on Chromosome 5 significantly exceeded 1/4 ratio. These distortions occurred in the characters controlled by *v-1*, *bc*, *v-2*, and *dl* (Fig. 2). The causal mechanism is attributed to the linkage between the genetic markers and a certation gene, *ga-3* (3).

The *ga-3* character with a lower fertilization capacity is distributed in some of the Keng rices of southern China and in the upland type cultivars in the Philippines. In contrast, the dominant allele of *ga-3* is found in japonica tester lines, Indian rices, and Chinese Hsien type cultivars.

LOCUS OF *ga-8* ON CHROMOSOME 3 AND RELATED MARKER LOCI

The other reproductive barriers are observed in Chromosome 3 (linkage group III). Three markers — *d-10* (tillering type dwarf derived from Kikeibanshinriki), *lax* (lax panicle), and *eg* (extra glume) — are also found at segregation distortion loci (5). Segregations of F₂ populations for the three markers showed a significant excess in the ratio of the frequency of recessive plants. Examples of the distortion are shown in the *eg* character (Table 3). The relationships between the marker loci and the extent of the distortions are indicated in Figure 3. Finally, a strong gametophyte factor, *i.e.*, a certation gene (*ga-8*), is located near the *eg* locus.

Table 3. An example of the distorted segregation of *eg* for extra glume in the F₂ caused by the gametophyte gene *ga-8*.

Cultivar crossed with japonica tester	Segregation in F ₂			Frequency of <i>eg</i> (%)	No. of crosses
	+	<i>eg</i>	Total		
Liuzhou Baoya Zao	338	160	498	47.3	2
Kinandang puti	763	392	1155	51.4	4
Dakanalo	928	311	1239	33.5	4
Chinsurah Boro 2	726	394	1120	54.3	3
Jhona 2	624	356	980	57.1	3

STERILITY GENES OF CHROMOSOME 6

Sterility genes could be the causal factors for the reproductive isolation giving rise to hybrid breakdown in the progeny of wide crosses. Although it is not easy to detect sterility genes, several authors have reported that

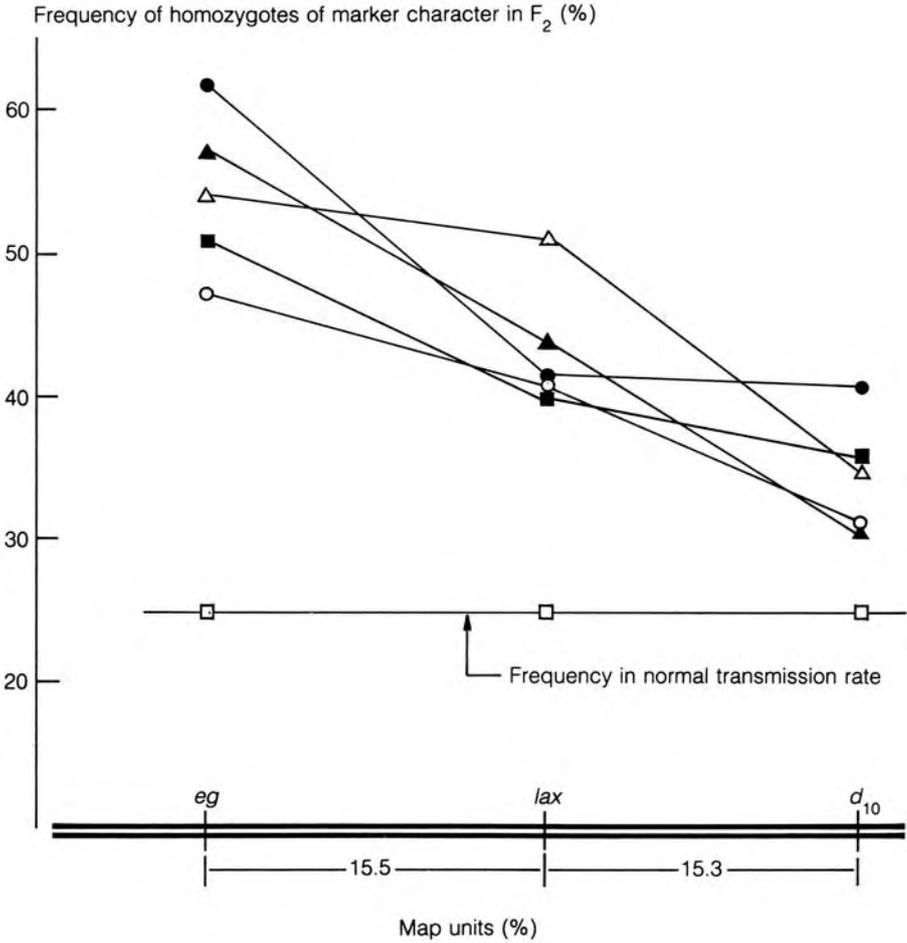


Fig. 3. Relationship between linkage intensity and distorted segregation in the three markers identified by various wide crosses. Parental cultivars: ○ = Liuzhou baoya zao, ● = Sensho-mai, ■ = Kinandang puti, △ = Chinsurah Boro 2, ▲ = Jhona 2.

sterility loci are found in Chromosomes 5 and 6 (4,9). Here, the sterility locus is given as an example of the distortion occurring in Chromosome 6 (Fig. 4).

GEOGRAPHIC DISTRIBUTION OF GENES FOR REPRODUCTIVE ISOLATION

The geographic distribution of loci associated with isolation mechanisms is summarized in Table 4. Japonica tester strains, exotic japonicas such as those from Northern China and the US, and upland rices belong to the group of the genotype with *ga-2 ga-3+ ga4 ga8+*. Some of the Keng cultivars from China belong to *ga-4 ga-2 ga-3 ga-8+*, Hsien type rice A to *ga-2+ (A) ga-3+ ga-4+ ga-8+*, Hsien type rice B to *ga-2+ (A) ga-3+ ga-4+ ga-8*, Indian rice A to *ga-2+ (B) ga-3+ ga-4+ ga-8+*, Indian rice B to *ga-2 ga-3+ ga-4+ ga-8*, and Indian rice C to *ga-2 ga-3 ga-4+ ga-8*.

Table 4. Distribution of gametophyte genes in Asian native cultivars.

Varietal group	Gametic genes			
	<i>ga-2, AorB^a</i>	<i>ga-3</i>	<i>ga-4</i>	<i>ga-8</i>
<i>Japonica type</i>				
Japonica testers	<i>ga</i>	+	<i>ga</i>	+
Japonica (Keng from China)	<i>ga</i>	+	<i>ga</i>	+
A part of javanica	<i>ga</i>	<i>ga</i>	<i>ga</i>	+
Upland rice from Philippines	<i>ga</i>	+	<i>ga</i>	+
<i>Indica type</i>				
Hsien rice A (China)	<i>A</i>	+	+	+
Hsien rice B (China)	<i>A</i>	+	+	<i>ga</i>
Indian rice A	<i>B</i>	+	+	+
Indian rice B	<i>ga</i>	+	+	<i>ga</i>
Indian rice C	<i>ga</i>	<i>ga</i>	+	<i>ga</i>

^aAllele A, designated *ga-2+(A)*, is stronger than allele B, designated *ga-2+(B)*, in fertilizing capacity compared with allele *ga-2*.

Frequency of heterozygotes of *wx* in F_2 (%)

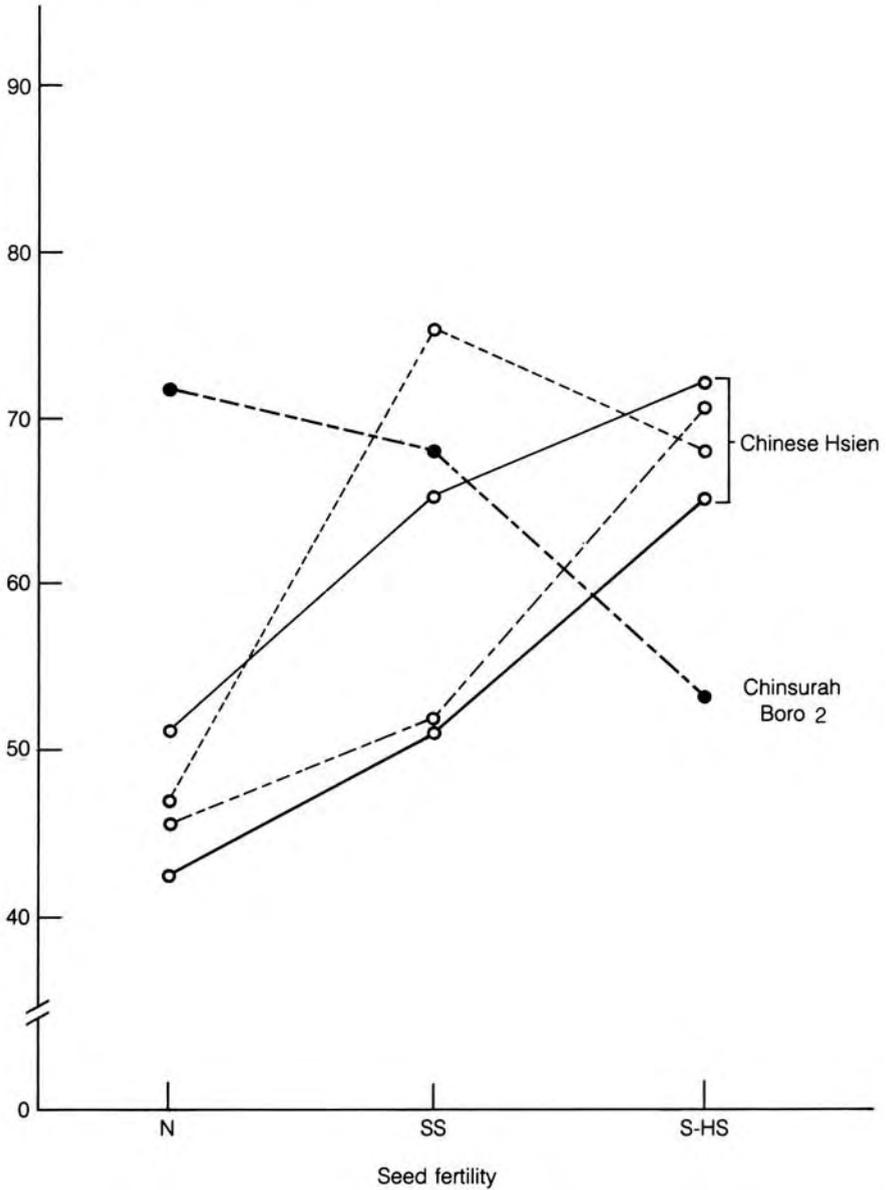


Fig. 4. Linkage *wx* locus with sterility genes on Chromosome 6. N = fertile, SS = partially sterile, S = semisterile, HS = highly semisterile.

It thus appears that japonica and javanica rices show a few genotypes in the reproductive barriers, but that Indian rices and Hsien type cultivars have many forms of genotypes. Accordingly, it is concluded that the indigenous cultivars of India and southern China can be genetically divided into several varietal groups as far as the gametophyte genes are concerned.

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A VARIETAL CLASSIFICATION OF ASIAN CULTIVATED RICE (*Oryza sativa* L.) BASED ON ISOZYME POLYMORPHISM

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Electrophoretically detected enzyme variation was surveyed among 1,688 varieties of Asian cultivated rice (*Oryza sativa* L.). The diversity was explained by gene polymorphism at 21 presumed loci. The analysis of the enzymatic variation in a representative sample of 120 varieties led to the identification of 6 varietal groups. Groups I, II, III, IV, and V consist of rices usually classified as indica. They are clearly differentiated from each other. Their correspondence with well known varietal types is presented. Group VI encompasses the classical japonica and javanica types, most upland rices from Southeast Asia, and most rices grown at high elevation in the Himalayas. The varietal diversity is very high on the Indian subcontinent, especially along the foothills of the Himalayas. Conversely, most varieties from Southeast Asia and East Asia belong to groups I and VI. The biological significance of this classification and its applicability for rice breeding are discussed.

A main part of the genetic improvement of a crop resides in the creation of new genic combinations from the available germplasm. In this perspective, the first concern of breeders is to know the genetic structure of the existing germplasm and its main factors. Isozyme studies can help reach this objective by elucidating the statistical distributions in the germplasm of various allelic combinations over several loci. A contingent linkage disequilibrium would indicate the existence of factors responsible for a restricted recombination between distinct components in the taxon. Determining whether recombining these components may create valuable novel variation requires further breeding experiments.

This paper presents results of a survey of electrophoretically detectable enzymatic variation in Asian domestic rice (*Oryza sativa* L.). Its specific objective is to describe the genetic structure of the species as it is perceived through a statistical analysis of the electrophoretic data. The procedure is an analysis of correspondences, which accounts for all the observed multilocus associations and provides a synthetic picture of the

organization of the species. Elements arise that permit a re-evaluation of traditional classifications.

The description of the electrophoretic technique, the enzymatic polymorphism, and its genetic interpretation will be presented elsewhere.

MATERIALS AND METHODS

A sample of 1,688 varieties provided by IRRI's International Rice Germplasm Center, Japan's National Institute of Genetics, and Thailand's Chiangmai University was chosen to cover the arrays of local varietal groups and crop environments for 20 Asian countries. It was analyzed for 15 presumed loci encoding 10 enzymes. The results permitted selecting a sample restricted to 120 varieties to represent the whole enzymatic variation as well as all the geographic origins. These 120 varieties were analyzed for 14 enzymes. The whole variation was interpreted as resulting from 21 polymorphic loci (Table 1).

For a given variety, two plants were individually analyzed. When heterogeneity was detected within a variety, three more plants were analyzed, and the most frequent genotype was used to represent the variety in the statistical analysis.

An analysis of correspondences (AC) (1) was performed on this set of

Table 1. Genetic interpretation of the electrophoretic variation observed for 14 enzymes in a survey of Asian cultivated rice.^a

Enzyme	No. of polymorphic markers	No. of loci alleles
Phosphoglucose isomerase	2	2, 4
Glutamate oxaloacetate transaminase	1	2
Shikimate dehydrogenase	1	4
Alcohol dehydrogenase	1	4
Isocitrate dehydrogenase	1	4
Phosphogluconate dehydrogenase	2	3, 2
Malic enzyme	1	2
Leucine aminopeptidase	2	7, 5
Alanine aminopeptidase	1	5
Arginine aminopeptidase	1	3
Esterase	4	2, 3, 2, 3
Acid phosphatase	2	3, 2
Catalase	1	3
Peroxidase	1	2

^aFrom 4, 6, 8, 9, and the author's unpublished data.

data (120 varieties, 21 loci, 59 alleles). This method treats qualitative data. Each variety is taken as a taxonomic unit, and each allele is considered as a character — of value 1 if the allele is present in the variety, of 0 if not. The AC identifies several axes (eigenvectors) that account for the largest part of the whole variation and are independent from each other. These axes are linear combinations of the characters. Each variety can be located on the planes formed by two of these axes, and varietal clusters may be identified from the distribution of all the varieties.

RESULTS

Analysis of correspondences

Axes 1, 2, and 3 of the AC account for more than 70% of the whole diversity, their respective parts being 50.8%, 12.3%, and 8.5%. The distributions of the varieties on planes (1,2) and (3,2) of the AC are shown in Figure 1. A synthesis of both representations results in the identification of six clusters, designated as group I to group VI. All groups appear on plane (1,2), although the distinction between group I and group II is not very clear. This distinction appears more clearly on plane (3,2).

The enzymatic groups

Group I corresponds to the typical indica group. It encompasses, among others, the three ecotypes traditionally considered as indica ecotypes, namely Aman (Bangladesh, Northeast India), Tjereh (or Cereh from Indonesia), and Hsien (or Sen from China).

Group II consists of varieties originating from the foothills of the Himalayas from Iran to Assam, India. The cycle of all these varieties is shorter than 120 days as measured in the wet season at Los Baños, Philippines. Their cultivation covers a wide range of water regimes, from irrigated conditions (e.g., in Pakistan) to dryland conditions (e.g., in Bangladesh). In Bangladesh and the surrounding Indian regions, this group clearly corresponds to the aus ecotype. Some boro varieties are also part of it.

Group III consists of two varieties from Bangladesh (Bhadoia, Aswina). They are deepwater varieties that present the peculiarity of being early maturing and nonphotosensitive.

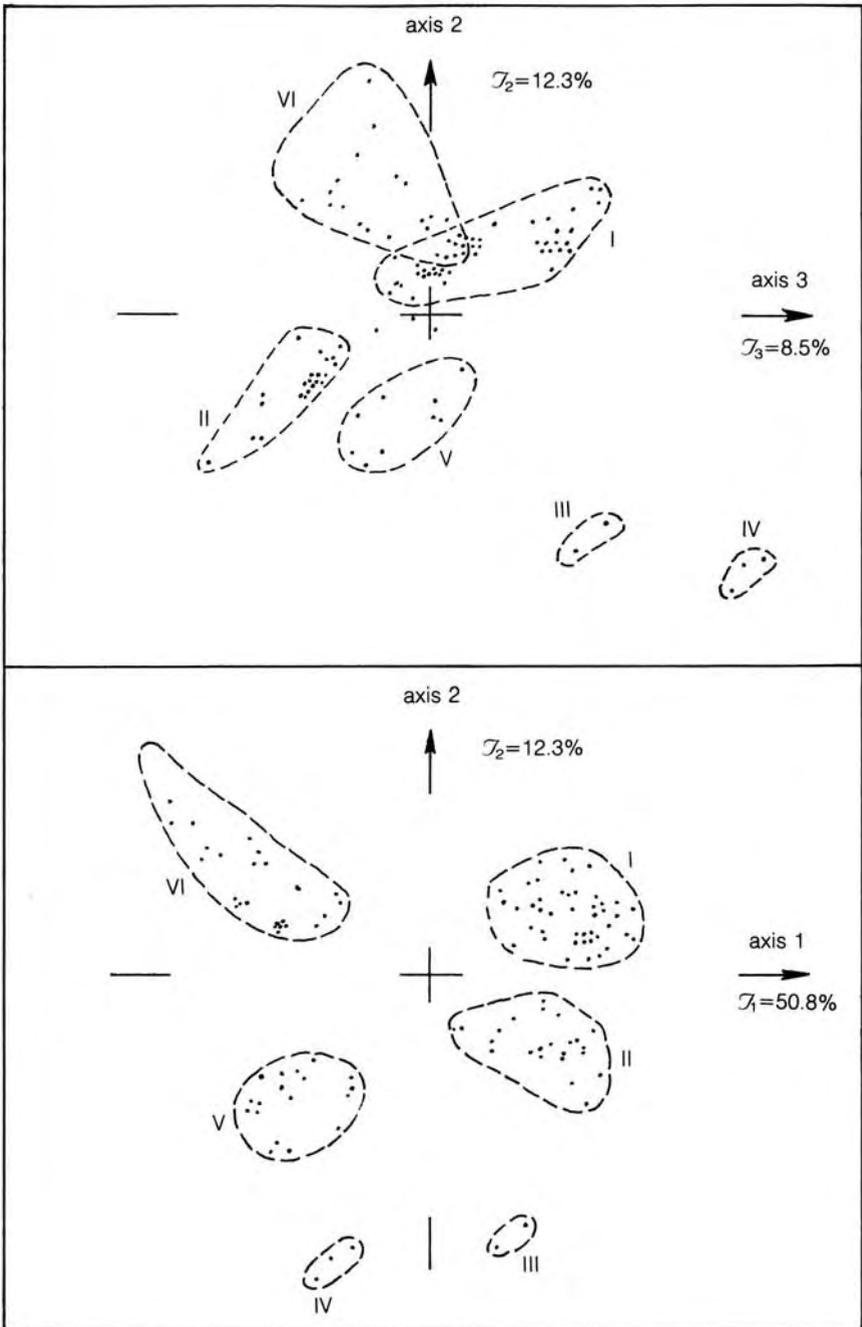


Fig. 1. Distribution of 120 varieties on planes (1,2) and (3,2) of an analysis of correspondences of their isozyme polymorphism at 21 loci, definition of 6 groups.

Group IV consists of the rayada varieties from the same area. They are floating rices, seeded with the boro rices, with cycles up to 12 months.

Group V spreads along the Himalayas from Iran to Burma. It consists of very diverse varieties. Many of them are known as high quality rices, such as the sadri rices from Iran; the basmati rices from Pakistan, India, and Nepal; and some special rices from Burma.

Group VI clearly corresponds to the japonica group in the scheme of Oka (7). Besides the Japanese and Korean varieties, and the keng varieties of China, it encompasses the bulu rices from Indonesia, most upland rices from Southeast Asia, and most rices grown at high altitudes in the Himalayas.

Table 2 draws the general correspondence between the enzymatic groups and the varietal types mentioned above.

DISCUSSION AND CONCLUSIONS

The ultimate aim of this study is to provide a clear picture of the organization of *O. sativa* in Asia. It may have a genetic significance applicable to plant breeding if it elucidates information on restrictions to recombination that occur (or have occurred) among the rice varieties; the tool that we used can be examined in this perspective:

- Hypotheses have been propounded on the genetic control of the bands of isozymes, but there are cases where they could not be verified by progeny tests. Alternative interpretations were possible, such as the involvement of several closely linked loci or the occurrence of regulatory events such as those reported by Shahi et al (10). Should this be the case, however, the value for classification of the

Table 2. General correspondence between well known varietal types and 6 groups based on analysis of isozyme polymorphism at 21 loci in Asian domestic rice.

Origin	Enzymatic group					
	I	II	III	IV	V	VI
Oka's testers (7) Iran, Pakistan, Northwest India	indica				sadri basmati	japonica
Bangladesh	Aman	aus	early deep water	rayada		
Southeast Asia	lowland					upland
Java, Bali	Tjereh					bulu
China	Hsien					keng

characters used in this study would not be much affected. They are oligogenic characters, expressed at a given developmental stage, in a given environment, and free from epistatic interrelationships. Nonrandom associations between such characters do result from linkage disequilibria among small genome fractions that themselves result from restriction to recombination. Nevertheless, it would be interesting to know more about the chromosomal location of the genes that govern the characters in order to determine which part of the genome is subjected to recombination restrictions.

- Electrophoretically detectable variation is only part of the whole variation. Thus, it is likely that what we called an allele can actually be a group of alleles. This restriction affects only slightly, if at all, the value of the methodology. However, it can have the consequence that only part of the multilocus associations has been perceived. In other words, the differentiation of the varieties in several groups remains valid, but these groups can still be heterogeneous.
- The loci we investigated are only a small part of the genome. This can be similarly taken into account by stating that the distinctions between the varietal groups we observed do exist but they certainly do not represent all the elements of the actual genetic structure of the species.
- The results of an AC are determined by the set of the individuals subjected to the analysis. One hundred twenty varieties constitute a very small sample, and one may question its representativeness. However, a computation of the partial data available for the 1,688 original varieties has provided a similar picture with the same basic 6 groups, supporting the cogency of the classification presented here (unpublished data).

Keeping these observations in mind, we can summarize the new information arising from this study as follows:

- The varieties usually classified in the indica type scatter in three major groups and two minor ones, clearly differentiated from each other. The largest group, group I, spreads all over Asia, while groups II, III, IV, and V are found only on the Indian subcontinent, especially in the foothills of the Himalayas.
- The temperate japonica varieties, the tropical japonica varieties (or javanica), most tropical upland varieties from Southeast and East Asia, and most high altitude rices of the Himalayas belong to a single group, group VI.

Regarding groups I to V, some elements tend to confirm the distinctions we made, although no particular attention has been given to them since they were usually all considered as indica varieties. Information is available for the aus rices (group II); they are known to produce partially fertile hybrids with both indica and japonica varieties, which differentiates them from typical indica varieties (Aman, Tjereh) (5). According to plant breeders, the exploitation of crosses between indica and aus varieties is usually made difficult by frequent partial sterility and little recombination.

The study of Engle (3) provides information on F_1 fertility in crosses between varieties of group I (Peta, Sigadis, Taichung Native 1) and varieties of group V (Basmati 370, Pankhari 203). Fertility was much lower than that within the two groups. As a rule, progenies of Basmati 370 with typical indica lines exhibit some sterility and poor recombination. The same is true for Burmese varieties also belonging to group V (G.S. Khush, personal communication).

Regarding group VI, there is available evidence to confirm the genetic similarity of its components. The bulu rices are known to exhibit close genetic affinity with the Japanese rices (5), and the tropical upland rices appear closely related to the Japanese and Ponlai (japonica from Taiwan) rices in the breeding programs (2).

Thus, although still diffuse, there is evidence that tends to prove biological significance for the groups proposed in the present paper. As factors responsible for this structure, spatial as well as seasonal isolation must have had important roles. To what extent these isolations are accompanied by sexual barriers and genetic imbalances in the hybrids still has to be investigated, at least for some of the intergroup combinations. Such knowledge can have practical applications in terms of breeding strategies and can provide a rational basis for the orientation of hybridization programs.

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GENETIC STRUCTURE OF AFRICAN TRADITIONAL RICE CULTIVARS

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Ten traditional African cultivars of *Oryza sativa* and 6 of *O. glaberrima* from Guinea Conakry (West Africa) were used to study the genetic structure of African traditional rice cultivars based on some morphological characters. The results obtained by principal components and variance analysis indicated that, while *O. sativa* could easily be separated into two distinct groups related to the indica and japonica subspecies, no such separation was obtained with *O. glaberrima*, although the results also showed that *O. glaberrima* seems to follow the same differentiation process as *O. sativa* but has remained at a very primary stage. In general, the results appeared to fit quite well with the results obtained through enzymatic analysis.

Traditional rice cultivars are generally assumed to be very heterogeneous for morphological traits. However, quantitative data to illustrate such heterogeneity are very scanty. This paper aims at giving the preliminary results of a study on the genetic structure of traditional African rice cultivars undertaken in the Ivory Coast. The main objectives of the paper are:

- to determine the sources of variability found in African traditional rice cultivars;
- to compare the variability found in the two rice species cultivated in West Africa — *Oryza sativa* and *O. glaberrima*; and
- to find out whether the variability based on morphological character can be associated with enzymatic variability and the phenol reaction.

MATERIALS AND METHODS

Materials

The study involved rice samples collected during various surveys in West and East Africa, but only results obtained from material from Guinea Conakry (West Africa) are discussed here. This is one of the very few countries in West Africa where *O. sativa* ssp. japonica, *O. sativa* ssp. indica, and *O. glaberrima* are all found in sufficient areas as pure stands and also in mixtures to enable a serious and in-depth study of the evolution of the two cultivated species. Ten cultivars of *O. sativa* and six of *O. glaberrima* were randomly used for this particular study. A detailed description of the sites where these cultivars were collected can be found elsewhere (1, 3).

Methods

In order to fit the type of study undertaken, a particular sampling technique was used besides that traditionally used for germplasm collection: a variety identified by a farmer by a known name is considered to be a cultivar. A farmer frequently identifies several cultivars in a given field in West Africa. It should be mentioned, however, that in a few cases there are fields made up of a homogenous mixture of several types of plants instead of patches of "homogenous" cultivars. Ten to 100 panicles are randomly harvested from each cultivar from the field. Each panicle is later planted in a row to give rise to individuals of the same family. At flowering, panicles are bagged in order to obtain a second generation of plants that will enable identification of the following sources of variability: cultivars, families/cultivars, individuals/families, and residual. The data presented here, however, were taken from the first generation of panicles collected from the field in such a way that the sources of variability were limited to cultivars, families/cultivars, and residual (including individuals/families) (5).

The following data were collected on individual plants:

- Days to heading (DH)
- Tiller number at heading (TH)
- Length of flag leaf (FL)
- Width of flag leaf (FW)
- Length of panicle (PL)
- Number of primary branches (B1)

- Number of secondary branches (B2)
- Number of spikelets per panicle (SP)
- Length of grain (GL)
- Width of grain (GW)
- 1000-grain weight (G1000)
- Width/length ratio of flag leaf (FS)
- Width/length ratio of grain (GS)
- Secondary branches/primary branches ratio (B2/1)

Analyses performed were:

- correlation analysis,
- analysis of principal components, and
- analysis of variance of the first three principal components.

RESULTS

Correlation studies

Correlation studies were undertaken at three levels:

- Global correlation analyses involving the two species (*O. sativa* and *O. glaberrima*) put together for a correlation analysis
- Correlation analysis involving only plants of the *O. sativa* species
- Correlation analysis for *O. glaberrima*

The results indicate that the global correlation coefficients reflect fairly well the relationship among the morphological characters measured for both *O. sativa* and *O. glaberrima* despite some slight differences between the two species. Indeed, while the tiller number seems to be in general negatively correlated with other traits in *O. sativa* this tendency appears to be significantly lessened in *O. glaberrima* (Table 1).

The highly positive correlation between SP and B2 is worth noting.

Analysis of principal components

An analysis of principal components was carried out using:

- all the individuals of the two species put together,
- *O. sativa* alone,
- *O. glaberrima* alone, and
- *O. sativa* on the basis of the principal axes determined by *O. glaberrima*.

Table 1. Correlation coefficients for global, *O. sativa*, and *O. glaberrima*^a among morphological traits.

	DH	TH	TN	FL	FW	PL	B1	B2	SP	G1000	GL	GW	FS	GS	B2/1
DH S		-	+	-	-	-	-	-	-	-	-	-	-	-	-
G		-	-	+	-	+	+	+	+	+	+	+	+	+	+
TH S	+18		-	+	+	+	+	+	+	+	+	+	+	+	+
G	-09		+	-	+	+	+	+	+	-	-	-	+	+	+
TN S	+01	-16		+	-	-	-	-	-	-	-	-	-	-	+
G	+44	-21		+	+	+	+	+	+	-	-	+	+	+	-
FL S	+26	+22	-26		+	+	+	+	+	+	+	+	-	+	+
G	-08	+18	-24		+	+	-	+	+	+	+	+	-	-	+
FW S	-47	+28	+24	+32		+	+	+	+	+	+	+	+	+	+
G	-47	+38	-57	+51		+	+	+	+	+	+	+	+	+	+
PL S	-34	+25	-28	+38	+56		+	+	+	+	+	+	+	-	+
G	-52	+33	-59	+41	+58		+	+	+	+	+	+	+	-	+
B1 S	+06	+50	-18	+18	+37	+37		+	+	+	+	+	+	+	-
G	-24	+40	-39	+27	+57	+51		+	+	+	+	+	+	+	-
B2 S	+32	+46	-23	+45	+16	+30	+45		+	+	-	+	+	+	+
G	-12	+26	-07	+38	+39	+45	+39		+	+	+	-	+	+	+
SP S	+27	+51	-23	+46	+29	+38	+66	+89		+	+	+	+	+	+
G	-18	+34	-15	+47	+56	+54	+60	+88		+	+	+	+	+	+
G1000 S	-10	+28	-68	+38	+45	+41	+36	+30	+37		+	+	+	+	-
G	-62	+21	-73	+25	+70	+59	+45	+07	+18		+	+	+	+	+
GL S	-06	+14	-56	+41	+34	+42	+16	+20	+21	+78		+	+	-	-
G	-52	+09	+65	+19	+54	+55	+24	-01	+03	+78		+	+	-	-
GW S	-18	+33	-52	+30	+48	+39	+39	+27	+35	+80	+50		+	+	-
G	-54	+32	-61	+28	+65	+56	+45	+16	+28	+82	+53		+	+	-
FS S	-60	+09	+08	-72	+35	+01	+06	-35	-27	-12	-23	+03		+	-
G	-36	+18	-32	-51	+42	+16	+17	+01	+08	+38	+28	+34		+	-
GS S	-15	+21	-02	-08	+18	+02	+24	+08	+15	+11	-41	+58	+27		+
G	-15	+29	-08	+14	+23	+14	+28	+19	+28	+21	-30	+64	+13		+
B2/1 S	+35	+29	-17	+41	+01	+18	+12	+92	+71	+18	+18	+11	-43	-06	
G	-05	+09	+08	+27	+19	+26	-01	+91	+68	-12	-09	-04	-08	+05	
	+13	+09	-03	+32	+17	+38	-05	+79	+25	+02	+21	-27	-29	-32	

^aS = *O. sativa*, G = *O. glaberrima*.

Global analysis. The first three principal components jointly account for over 67% of the total variance. The correlations between the measured traits and the three components are given in Table 2. Almost all the traits are associated positively with the first component except the tiller number (TN). This component reveals the overall plant vigor as it is associated with height, leaf size, panicle structure and size, grain size,

and weight. This vigor is, however, associated with tiller number in such a way that vigorous and tall plants appear to have fewer tillers at least at heading.

The third component is most highly correlated with the grain width/length ratio (GS), therefore opposing individuals with slender grains to those with shorter grains.

The distribution of the individuals in the plan determined by the first two principal axes is presented in Figure 1. It can be seen that *O. glaberrima* constitutes a distinct group from *O. sativa*. *O. sativa* appears to be more variable than *O. glaberrima*. For *O. sativa*, the superimposition of the phenol reaction on the distribution of *O. sativa* plants resulted in two distinct clusters: one with individuals showing a positive reaction and the other with individuals having a negative reaction to phenol. The two groups have been identified as "indica" for the positive phenol reaction and "japonica" for the negative reaction (7).

O. sativa. The first three principal components account for over 68% of the total variation. The correlation coefficients between the measured morphological characters and the three components are shown in Table 3.

The first component is negatively correlated with FW, PL, Bl, SP, G1000, GL, and GW and positively correlated with DH and TN. This component appears therefore related to duration and general aspect and opposes late maturing, high tillering, small plants with few and small grains to early maturing, vigorous plants with very few tillers.

The second component is highly positively correlated with B2 and SP. This component appears therefore to be related to grain number, opposing individuals with few grains per panicle to those with more grains.

The third principal component is highly correlated with GS.

The superimposition of the phenol reaction resulted in two distinct groups in the plan determined by the first two axes. These clusters have been identified as the japonica (negative reaction) and the indica groups (Fig. 2). It can also be observed that the japonica group appears to be more homogenous than the indica. YS29 appears to be distributed in both the japonica and indica groups. Indeed, they were found to be the only cultivars showing both positive and negative reactions. It is therefore a mixture of japonica type and indica type. This case is quite rare in the *O. sativa* material studied in West Africa.

O. glaberrima. As with *O. sativa*, the first three principal components account for over 68% of the total variation for *O. glaberrima*. Table 4

Table 2. Correlation coefficients between the first three principal components and the morphological characters: global analysis.

	1st component (34.57)	2nd component (19.51)	3rd component (13.21)
DH	+11	-.79	-.01
TH	+.57	-.05	-.39
TN	-.56	-.22	-.39
FL	+.65	-.32	+.29
FW	+.52	+.59	-.07
PL	+.60	+.34	+.07
B1	+.60	+.13	-.44
B2	+.76	-.45	-.30
SP	+.80	-.30	-.37
G1000	+.76	+.34	+.35
GL	+.61	+.17	+.68
GW	+.70	+.47	-.02
FS	-.31	+.74	-.37
GS	+.14	+.36	-.65
B2/1	+.59	-.58	-.14

indicates that the first component is highly positively correlated with DH, FL, PL, G1000, and GL and negatively correlated with FS and GS.

The second component is highly negatively correlated with TH, FW, B1, B2, and SP.

The third component positively correlated with G1000 and GW. The identification of the principal components appears to be more difficult than with *O. sativa*. However, it seems that the second principal component for *O. glaberrima* is identical to the first component in the *O. sativa* distribution (Tables 2, 3).

No distinct groups could be obtained on the basis of the phenol reaction as with *O. glaberrima*, although YG46, which was the only negative reaction glaberrima type found in our study, appears to be fairly well localized (Fig. 3). A larger number of *O. glaberrima* plants could have certainly given a better picture.

However, *O. sativa* individuals represented in the plan determined by the *O. glaberrima* first three principal axes showed a tendency to cluster in two groups that corresponded very well with the japonica and indica groups identified earlier. This is even more remarkable in the plans defined by the principal axes 1 and 2, and 2 and 3 (Figs. 4, 5).

Table 3. Correlation coefficients between the first three principal components and the morphological characters: *O. sativa*.

	1st component (39.06)	2nd component (19.34)	3rd component (10.34)
DH	+0.62	-0.30	-0.07
TH	+0.45	+0.17	+0.37
TN	+0.72	+0.38	+0.17
FL	-0.45	+0.41	-0.42
FW	-0.87	+0.00	-0.02
PL	-0.81	+0.08	-0.21
B1	-0.68	+0.13	+0.24
B2	-0.49	+0.80	-0.05
SP	-0.62	+0.71	+0.04
G1000	-0.82	-0.44	-0.08
GL	-0.63	-0.50	-0.49
GW	-0.82	-0.26	+0.26
FS	-0.37	-0.39	+0.45
GS	-0.35	+0.16	+0.74
B2/1	-0.24	+0.79	-0.18

Analysis of variance

A series of analyses of variance was carried out on the basis of the new scores defined by the first three components of *O. glaberrima*. The objective was essentially to identify the contribution of each source of variation to the total variance. The results are presented in terms of variance components expressed here in percentage to enable comparisons (Table 5).

Table 5 indicates that on the whole the differences among the cultivars accounted for most of the global diversity. The differences among the cultivars appear to be mostly due to the whole differences between the *glaberrima* and the *sativa* species (49.4%) and the diversity among the *sativa* cultivars (44.4%).

The diversity within the *sativa* species is due mostly to the variation among the cultivars (59.6%). The between families and within families variations contribute only 14.4% and 26.0%. The variation in the *O. sativa* populations appears to be essentially due to the differences between the japonica and indica groups, the former showing greater cultivar variability.

The variance components obtained from the analysis of variance of the

Table 4. Correlation coefficients between the first three principal components and the morphological characters: *O. glaberrima*.

	1st component (29.95)	2nd component (24.75)	3rd component (13.60)
DH	+0.63	+0.23	+0.43
TH	+0.08	-0.76	-0.10
TN	.00	-0.45	-0.06
FL	+0.89	+0.22	+0.09
FW	+0.32	-0.62	-0.03
PL	+0.67	-0.42	-0.02
B1	+0.15	-0.79	+0.34
B2	+0.46	-0.70	-0.31
SP	+0.38	-0.76	+0.14
G1000	+0.63	+0.26	+0.54
GL	+0.80	+0.38	-0.01
GW	-0.04	-0.17	+0.87
FS	-0.81	-0.40	-0.03
GS	-0.64	-0.38	+0.52
B2/1	+0.50	-0.20	-0.53

indica and japonica groups indicate that the within families variation contributed the most to the total variation of each group (52.4% for japonica and 41.7% for indica). The japonica cultivars appear to be very slightly more variable than the indicas. For both groups, the families accounted little for the total variation, although the indica families seemed to store more diversity than the japonica (26.9% and 10.3%), respectively.

The cultivars of *O. glaberrima* appear much less variable than *O. sativa*, accounting for only 15.7% of the total variation of the species. Most of its variation is due to the differences among families, which accounted for 58.2% of the total variation.

DISCUSSION

The results of the different analyses give a general picture of the structure of the African traditional rice cultivars. *O. sativa* and *O. glaberrima* are two different and distinct groups, each with its own specificity. The relationship among the morphological traits appeared to be mostly different for each species.

Table 5. Variance components, expressed in percentage. Variance components for global, *O. sativa*, *O. glaberrima*, *indica*, and *japonica*.

	Global	<i>Sativa</i>	<i>Glaberrima</i>	<i>Indica</i>	<i>Japonica</i>
Cultivars	60.3%	59.6%	15.7%	31.4%	37.3%
Families	20.6%	14.4%	58.2%	26.9%	10.3%
Within families	19.1%	26.0%	26.1%	41.7%	52.4%

Components of O. sativa cultivar variance (excluding YS29)

Indica vs japonica	46.1%
Indica	12.8%
Japonica	41.1%

Components of global cultivar variance

<i>O. glaberrima</i> vs <i>O. sativa</i>	49.4%
<i>O. glaberrima</i>	6.2%
<i>O. sativa</i>	44.4%

O. sativa appeared to be made up of two very distinct groups that are identified as the *indica* and *japonica* groups on the basis of the phenol reaction. The difference between the two groups is very important and contributed very significantly to the total variation of the species. Comparing the two groups, it was noted that while *japonica* populations appear more variable when the three principal axes are considered jointly, this tendency varies when the axes are taken individually. Thus *japonica* populations are quite uniform on the first axis compared to *indica* and 87.3% of the total variation in *japonica* on this axis is accounted for by the within families variation compared to 28.9% on the second axis and 18.5% on the third. On the basis of the morphological traits associated with the first principal axis, *indica* appears more variable in general appearance than *japonica*. The differences among the *indica* families are longer than in *japonicas*.

No such separated clusters could be obtained with *O. glaberrima*, even on the basis of the phenol reaction. On the whole, most of the variance in this species is accounted for by the between families variation. In fact it was reported that both positive and negative phenol reaction plants are frequently found in the same cultivar of *O. glaberrima* (4). However, plotting *O. sativa* individuals in the planes defined by the *O. glaberrima* principal axis resulted in two clusters: a *japonica* group and an *indica*

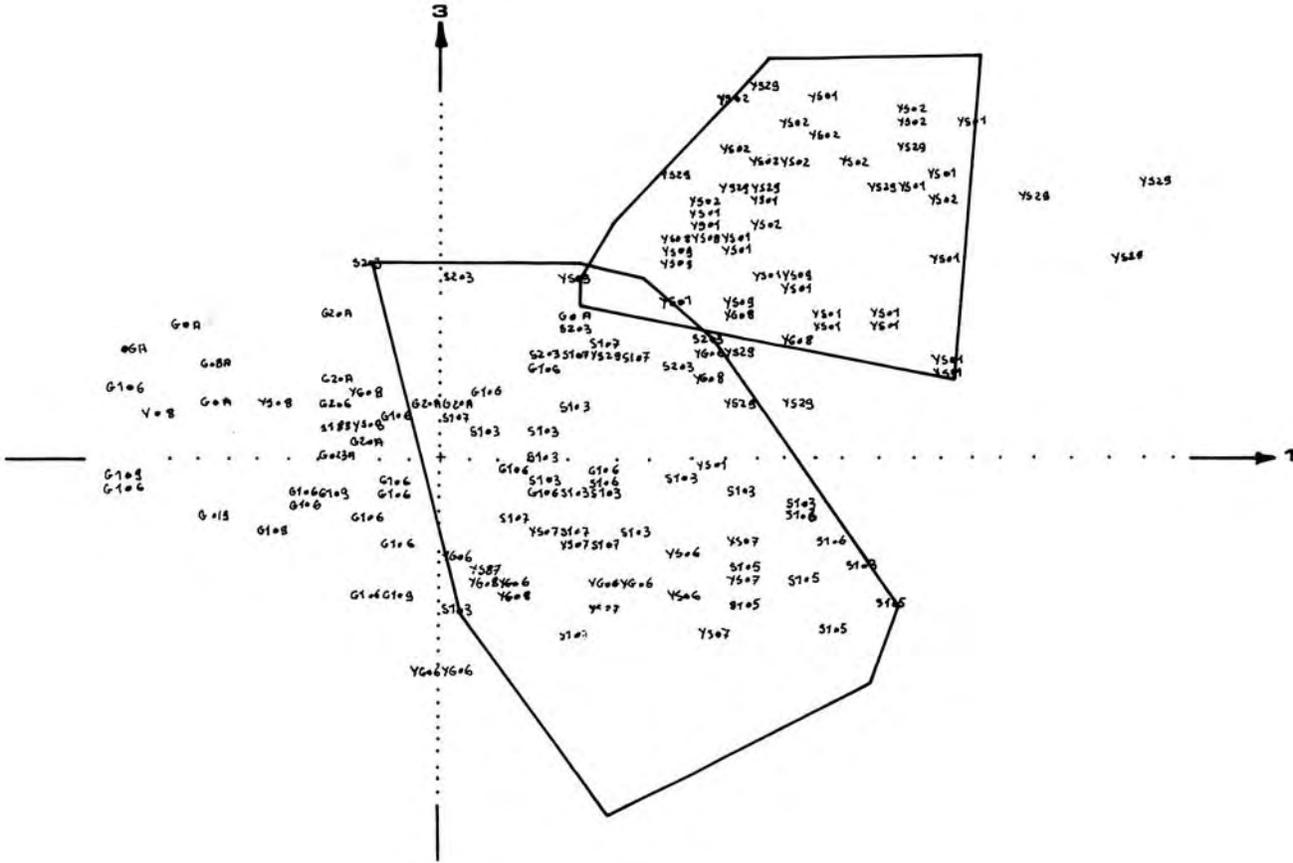


Fig. 4. Distribution of *O. sativa* (Axes 1 and 3 from *O. glaberrima*).

group. This indicated that *O. glaberrima* follows the same evolutionary process as *O. sativa* but has remained at a very primary stage in such a way that this process cannot be easily identified when *O. glaberrima* is considered alone. A more detailed analysis involving larger samples and a follow-up of the offspring of positive and negative phenol reaction plants for several generations, associated with enzymatic and morphological studies, would certainly enable a better understanding of this process. In the meantime, the use of such an advanced and well differentiated species as *O. sativa* gives a good idea of the final forms toward which *O. glaberrima* is evolving.

Extensive studies of isozyme polymorphism of African wild and cultivated rice species using electrophoretic techniques have been carried out in the Ivory Coast (2, 3, 4, 6, 7). It is found that in Africa the genetic diversity of traditional cultivars of *O. sativa* is very similar to that of the Asian land races. *O. sativa* tends to cluster in two groups, but intermediate strains are largely predominant. These two groups can be related to the japonica and indica subspecies, as seen by the phenol reaction and F_1 sterility relationships. It was found that the difference between the indica and japonica group accounted for most of the genetic diversity of *O. sativa*. It must be mentioned, however, that indica and japonica do not constitute two completely isolated groups. Instead, there is a continuous array of intermediate forms between two extreme forms that are identified as parental types, the intermediate forms being the most frequent, particularly on the japonica side. These intermediate forms can be identified only through electrophoretic analysis and F_1 sterility relationships. No attempt has been made yet to identify these forms through morphological traits. Very interesting information can be expected from such a study, particularly when the distinction between the indica and japonica subspecies seems to be closely related to geographical areas and the type of rice cultivation. Indeed, it has been reported that, while the japonica cultivars appear to be restricted to rainfed conditions in moist forest areas of Africa, the indicas are commonly grown in aquatic conditions, although they seem to have a wider distribution than japonicas (4).

The study of enzymatic polymorphism of *O. glaberrima* has indicated that, unlike *O. sativa*, *O. glaberrima* has a very low genetic diversity. Positive and negative phenol reaction plants were found, but it was not possible to relate them to a particular isozymic pattern.

Table 6 shows the components of genetic diversity for *O. sativa* and *O. glaberrima* obtained through enzymatic analysis on the basis of both 40 loci and 4 loci. The table indicates that the difference between japonica and indica explains most of the genetic diversity of *O. sativa*. As far as the

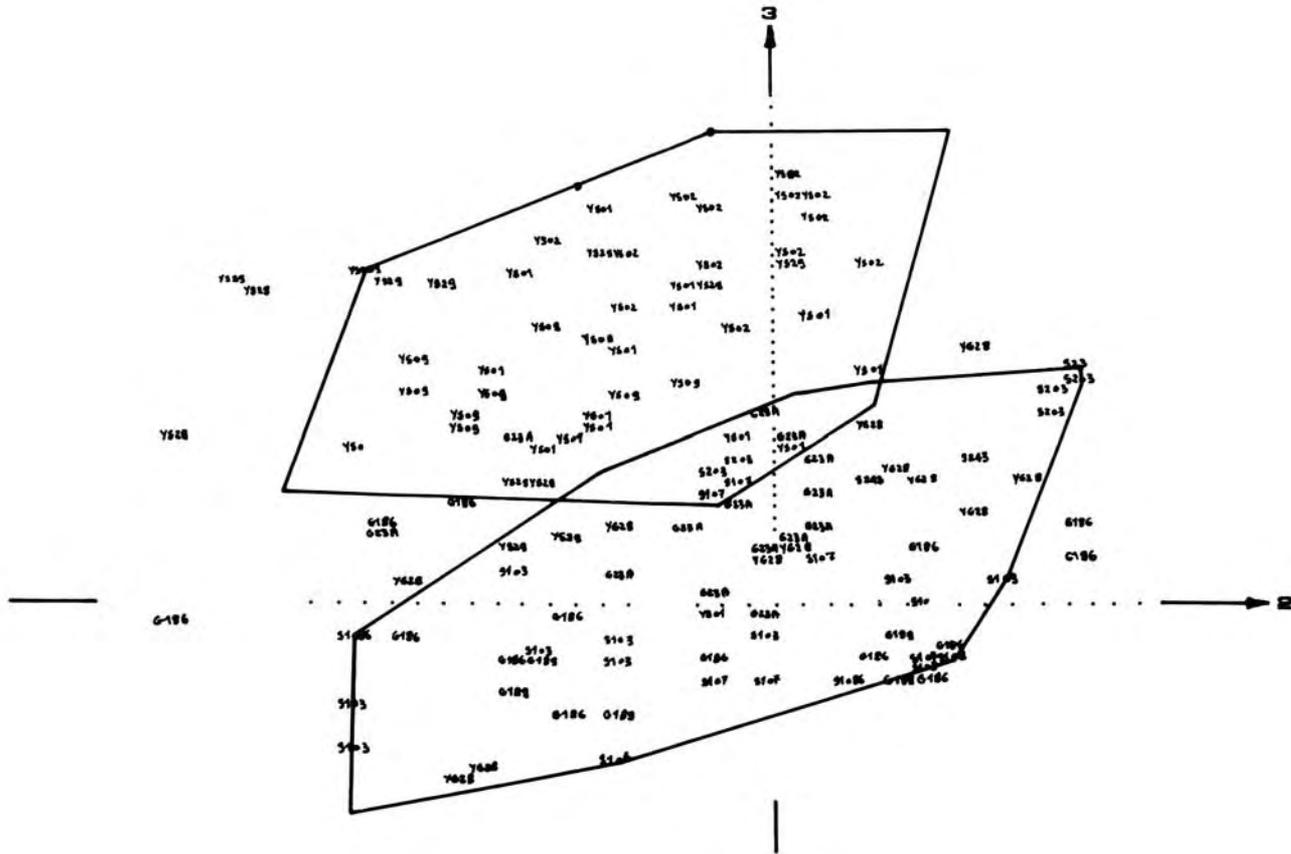


Fig. 5. Distribution of *O. sativa* (Axes 1 and 3 from *O. glaberrima*).

Table 6. Comparison between the components of enzymatic and morphological diversity.

	Japonca-indica	Populations	Families
<i>O. sativa</i>			
40 loci	54%	34%	12%
4 loci	49%	33%	18%
Morphological traits	57.7%	16.4%	8%
<i>O. sativa ssp. japonica</i>			
40 loci		84%	16%
4 loci		85%	15%
Morphological traits		37.3%	10.3%
<i>O. sativa ssp. japonica</i>			
40 loci		63%	37%
4 loci		44%	56%
Morphological traits		31.4%	26.9%
<i>O. glaberrima</i>			
40 loci		68%	32%
Morphological traits		15.7%	58.2%

two subspecies are concerned, japonicas present a higher population variability than indicas which in turn seem to have more interfamily variability than japonicas.

O. glaberrima is much less polymorphic than *O. sativa*. The total genetic diversity of this species determined on the basis of 40 loci appears to be due mostly to a difference among cultivars (populations) with, however, a significant contribution of the interfamily variation.

The picture fits quite well with the results obtained through the analysis of morphological traits shown in Table 6.

YS29, which is scattered all over the two groups, has been identified to be a mixture of types, both on the basis of the isozyme pattern and on the basis of the phenol reaction. This shows once more the convergency of the two methods in the study of African traditional rice cultivars.

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STERILITY BARRIERS BETWEEN *ORYZA* *SATIVA* AND *O.* *GLABERRIMA*

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The interspecific F_1 hybrid between the two cultivated rice species, *Oryza sativa* and *O. glaberrima*, is male sterile but female fertile. To look into the genetic mechanisms of sterility barriers, backcross experiments were carried out. Two gamete eliminators (S_1 and S_2) and a pollen killer (S_3), which induce sterility through allelic interaction, were detected. S_1 was tightly linked with *wx* and S_3 with *la*. Genetic backgrounds had an effect on the genic expression of S_1 , viz., the elimination of megaspores was incomplete in the japonica background of *O. sativa*. In addition, a one-locus sporophytic sterility gene (S_4) emerged from a near-isogenic line carrying S_1 . Further, male sterility was induced when the *O. sativa* cytoplasm was combined with the *O. glaberrima* nucleus. Indica cytoplasm of *O. sativa* appeared to be the same as japonica cytoplasm in terms of a fertility restoring system. A dominant gene from the *O. sativa* parent seemed to cause anther indehiscence. Thus, infertility arises from various nuclear gene interactions as well as the nucleus-cytoplasm interactions. Their genetic consequences were also examined in the experimentally introgressed population.

Isolation promotes genetic differentiation and permits different populations to coexist without losing their identity. If species are recognized as groups of potentially interbreeding populations, domesticated races generally belong to the same biological species as their wild progenitors, since domesticated ones are compatible with their progenitors when hybridized (3). The appropriate degree of isolation might result from a balance between advantages of gene flow and isolation. Usually, isolating barriers are much developed between distantly related taxa. It has been repeatedly suggested in maize, wheat, and sugarcane that not only their wild progenitors but also more distantly related taxa have contributed to crop evolution to some extent through gene flow across isolating barriers (2). A basic problem that needs to be solved is whether incorporation of genes from a secondary gene pool has played a role in the adaptation of crop species. To understand the genetic nature of gene transfer between species, we first need to know what sorts of genetic mechanisms are involved in reproductive barriers between species.

Oryza glaberrima is endemic in West Africa, while *O. sativa* is of tropical Asian origin. At present, the two cultivated rice species are often grown in mixture in farmers' fields. They are isolated by an F_1 sterility barrier, although their chromosomes normally pair in the hybrid (1). Hybrid sterility between distantly related taxa is caused by a complex of disharmonious gene interactions. This paper will describe various genetic mechanisms of F_1 sterility barriers found between the two species.

INTERACTIONS BETWEEN NUCLEAR GENES

Genetic basis of F_1 sterility

Assuming one or two loci, four possible models of genic systems controlling F_1 sterility have been proposed by Oka (5):

- duplicate gametic lethal (gametes with S_1S_2 deteriorate);
- one-locus sporo-gametophytic interaction (gametes with S^a deteriorate only in S/S^a);
- one-locus sporophytic sterility (heterozygotes for S and S^a have an adverse sporophytic effect on gametic development); and
- complementary sporophytic sterility (plants with two dominant genes, S_1 and S_2 , show sterility).

In each case, sterility factors may affect the development of male or female gametes, or both.

Various genic substitutions may be involved in processes of speciation. Successive backcrossings are relatively easy if the hybrid is male sterile but female fertile. To extract alien elements that disturb the normal development of gametes, the use of near-isogenic lines provided significant information for understanding the genic nature involved (5, 7, 8). The four models proposed can be distinguished from segregation patterns only in appropriate backcrosses and testcrosses (7).

Gamete eliminator and pollen killer

Although the F_1 hybrids between two cultivated rice species are highly pollen sterile, backcrosses are possible as some embryo sacs remain functional. Near-isogenic F_1 sterile lines having the genetic background of *O. sativa* (Acc108, indica type) and *O. glaberrima* (W025) parents were isolated from BC_8F_2 (7). They were self-fertile and showed semisterile in the F_1 's when crossed with the parents. However, all the selfed F_2 plants

were fully fertile. This F_1 sterility was explained by the one-locus sporo-gametophytic interaction model of sterility genes. Acc108 parents are assumed to have $S_1^a S_1^a S_2 S_2$ and W025 parents $S^I S^I S_2^a S_2^a$. If the S gene is present in the maternal tissue, gametes with S^a deteriorate. Interestingly, similar sterility genes S_1 and S_2 were detected in the two species. The S_1 gene appeared to be tightly linked with C in the first linkage group. The heterozygotes $S_1 S_1^a$ and $S_2 S_2^a$ showed 50% sterile and produced S_1 or S_2 gametes only, so, S_1 and S_2 act as gamete eliminators. If there were no other sterility elements between Acc108 and W025, the F_1 of $W025 S_2 / \text{Acc108} S_1$ would be fertile. Actually, the F_1 was highly sterile, suggesting that there are various other factors responsible for sterility barriers between them. A true breeding, partly sterile line with *O. sativa* background was obtained from $BC_8 F_2$. The plant seemed to be homozygous for duplicate recessive genes causing sporophytic sterility associated with F_2 breakdown.

A follow-up experiment was carried out using T65 (japonica type of *O. sativa*) and W025 (*O. glaberrima*) (7). Another type of sterility gene was extracted from $BC_8 F_2$. T65 was considered to be $S_3^a S_3^a$ and W025 to be $S_3 S_3$. The S_3 locus had no deleterious effect on the development of microspores in the homozygote but induced abortion of pollen not carrying it in the heterozygote $S_3 S_3^a$. There appears to be no adverse effect of S_3 on megaspores, which differs in genic action from S_1 and S_2 . Thus, S_3 acts as a pollen killer. S_3^a was tightly linked with gene *la* in linkage group VIII. In addition, plants homozygous for S_3 were photoperiod sensitive. The pleiotropic effect of S_3 or its tight linkage with photoperiod sensitivity is an example of coadapted gene complexes between species. The homozygotes for S_3 tend to be effectively isolated from plants carrying S_3^a by a difference in time of blooming. Therefore, gametophytic selection is able to influence the quality of the sporophytic generation. It seems likely that the phenomenon of gamete abortion through allelic interaction may be of wide occurrence between the two rice species (7).

Effects of genetic backgrounds

With regard to the one-locus sporo-gametophytic interaction model for F_1 sterility genes, further evidence was obtained from a sterility gene that was extracted from W025 into T65 (BC_8 – BC_{12}). The segregation patterns showed that the sterility gene was similar to a gamete eliminator such as S_1 . The waxy vs. nonwaxy character of pollen grains is testable by iodine reaction, and the heterozygote (+*wx*) produces two sorts of pollen

Table 1. Pollen analysis for detection of a linkage relation between *S* and *wx*.^a

Genotype of F ₁	Genotype of pollen	Fertility	Frequency	Observation ^b
+ S	<i>Noncrossover type</i>			
	+ S	fertile	(1-p)/2	+:wx = 7313:179 RV = 2.4%
wx S ^a	wx S ^a	sterile	(1-p)/2	
	<i>Crossover type</i>			
wx S ^a	+ S ^a	sterile	p/2	
	wx S	fertile	p/2	
wx S	<i>Noncrossover type</i>			
	+ S	fertile	(1-P)/2	+:wx = 114:3283 RV = 3.4%
+ S ^a	wx S ^a	sterile	(1-P)/2	
	<i>Crossover type</i>			
+ S ^a	wx S ^a	sterile	p/2	
	+ S	fertile	p/2	

^aIf a sterility gene (*S*) of the gametophytic type is located near *wx*, the linkage relation can be detected from pollen analysis of the heterozygotes, *S*₊/*S*^a *wx* and *S* *wx*/*S*^a +. In this case, microspores carrying *S*^a deteriorate through allelic interaction in the heterozygote (*SS*^a). A near-isogenic line of T65 (japonica type of *O. sativa*) carrying *S* from *O. glaberrima* was used.

^bRV = recombination value.

grains in 1:1 ratio. When the near-isogenic line with the sterility gene was crossed with T65*wx*, the F₁ showed semisterile, and reddish brown (waxy) pollen grains were found to be eliminated through gametic selection (Table 1). Out of 7,492 observed, only 179 were waxy. This implies that *S*^a and *wx* are closely linked and that *wx* was eliminated together with *S*^a. Moreover, the recombinants with *S* *wx* and *S*^a + were obtained from the hybrid derivatives. As expected, the plants showed semisterile and had only 114 blue (nonwaxy) pollen grains out of 3,397 observed. The recombination value between *S* and *wx* was calculated as 2.4 to 3.4% from pollen analysis.

The sterility gene (*S*) was located in the first linkage group, and then it was suspected to be identical to *S*₁, which was first examined in the genetic background of Acc108. However, the elimination of female gametes with *S*^a was incomplete in this case, viz., some 40% of female gametes with *S*^a remained functional in BC₉-BC₁₂F₁, suggesting that the sterility stems from neither the pollen killer nor the gamete eliminators and is instead intermediate between them. For the identification of the sterility gene, *S*₁ in the first linkage group was introduced from Acc108*S*₁ into T65*wx* by successive backcrosses (BC₇). Until the BC₅ generation, no *wx* and fertile segregant (*wxwxS*₁^a*S*₁^a) was found when semisterile plants

Table 2. Linkage relations between a sterility gene and wx .^a

Cross	Genotype of F_1	Fertility (%)		% waxy grains	
		Pollen	Seed	F_1 pollen	F_2 seed
T65 wx /T65S BC ₁₁	$S^a wx/S$ +	49.4	49.2	2.7	2.8
T65 wx /Acc108 S_1	$S_1^a wx/S$ +	40.3	16.2	2.4	0.0
T65 wx /Acc108	$S_1^a wx/S_1^a$ +	48.7	21.5	50.5	14.9
T65 wx /T65 S_1 BC ₇	$S_1^a wx/S_1$ +	48.3	50.7	2.6	1.4

^aT65 is Taichung 65 (japonica type) and Acc10B is Peiku (indica type) from Taiwan. T65S is a near-isogenic line of T65 with an unknown sterility gene from W025. T65 wx and Acc108 S_1 are near-isogenic lines carrying wx and S_1 , respectively. S_1 gene was extracted from Acc108 S_1 into the genetic background of T65 (T65 S_1) by successive backcrossings. The F_1 of T65 wx /Acc108 showed semisterile even though the F_1 is $S_1^a S_1^a$ indicating that there are other sterility genes as is often found in the hybrids of japonica and indica types. % waxy pollen grains represents recombination value between the sterility gene and wx . An unknown sterility gene S was proved to be identical to S_1 since the F_1 of T65 S_1 /T65S was fertile.

were backcrossed with the pollen of T65 wx , suggesting that elimination of megaspores with S^a was complete and S_1 acted as a gamete eliminator. The F_1 of T65 wx /Acc108 showed semisterile, but its pollen analysis revealed that there is no sterility gene with gametic selection that is tightly linked with wx . Therefore, the sterility gene extracted from Acc108 S_1 , which is located near wx , is S_1 from W025. In BC₆ – BC₇ F_1 , out of 27 plants 5 were fertile and homozygous for wx (Table 2), suggesting incomplete elimination of megaspores with S^a . The genetic background apparently has an effect on the genic expression of S_1 .

One-locus sporophytic sterility gene

A near-isogenic line of T65 with S_1 was established from BC₈ F_2 . No other gene responsible for infertility was detected in BC₇ F_2 and BC₈ F_2 . To purify the S_1 gene, backcrosses were continued up to BC₁₂. During the procedure, a sterility gene different from S_1 was obtained from BC₉ F_1 plants. A strange phenomenon was first detected in a semisterile plant with + wx . If the sterility gene is S_1 , pollen analysis should show a distorted segregation for waxy and nonwaxy pollen grains. However, its pollen analysis gave a normal ratio of 1:1. When the semisterile plants were backcrossed as the pollen parent, fertile and semisterile plants segregated into a ratio of 1:1. In the cases of the gamete eliminators and pollen killer, such crosses gave fertile plants only. Segregation patterns (Table 3) showed that the sterility is not gametophytic but of sporophytic origin. Selfing of semisterile plants always gave a ratio of 1: fertile to semisterile. The result is well explained by the one-locus sporophytic

model and not by others. This is the first case where this model was adopted for sterility in plants. The gene was designated S_4 . If this is true, selfing of semisterile plants ($S_4S_4^a$) gives 1:2:1 S_4S_4 (fertile) to $S_4S_4^a$ (semisterile) to $S_4^aS_4^a$ (fertile). Test crosses revealed that out of 30 fertile F_2 plants 14 had S_4S_4 and 16 $S_4^aS_4^a$, supporting the above assumption.

Of particular interest is the origin of S_4 . The sterility gene was detected after the establishment of a near-isogenic line of T65 S_1 . A possible assumption might be that S_1 mutated to S_4 during the course of experiments and a mutation altered the genic expression of S_1 . If so, S_4 should be tightly linked with wx . Since the sterility gene is of the sporophytic type, pollen analysis is not applied for its linkage relation. Linkage analysis from F_2 segregation showed that S_4 is independent of wx ($X^2 = 0.962$, $df = 2$). Possibly, a mutation took place at a locus independent of wx together with the elimination of S_1 . A similar alteration of linkage relation in fertility elements was reported in the *cms-S* strains of maize in addition to cytoplasmic reversion (4). It was proposed in maize that fertility elements can be located at different sites on different chromosomes and linkage alterations can be caused by an assumed transposable element. Further investigations are under way in order to examine if a similar mechanism is involved in the sterility genes of rice.

NUCLEUS-CYTOPLASM INTERACTION

Yabuno (12) reported that the combination of the cytoplasm of a japonica type (*O. sativa*) and the nucleus of *O. glaberrima* produces male sterility and that a dominant gene Rf_j is responsible for the full restoration of pollen fertility in the male-sterile lines. In order to verify differential nucleus-cytoplasm interactions between the two species, cytoplasmic substitution lines were made by using *O. glaberrima* (W025 S_2) and an indica type of *O. sativa* (Acc108 S_1). The *O. glaberrima* cytoplasm had no adverse effect on pollen development when combined with the nucleus of Acc108 S_1 . On the other hand, when the Acc108 cytoplasm was combined with the W025 S_2 nucleus the substitution line showed no seed set due to male sterility, although the pollen grains were normally stained with I₂-KI solution. Plants having dehiscent or indehiscent anthers were backcrossed to W025 S_2 . All the progeny tended to have dehiscent anthers, whereas when the plants with indehiscent anthers were backcrossed, both plants with dehiscent and indehiscent anthers segregated into a 1:1 ratio (Table 4). This indicates that a dominant gene from

Table 3. Segregation for fertility in the backcross generations between *Oryza sativa* (T65) and *O. glaberrima* (W025).^a

Generation	Segregation			Ratio	χ^2
	Fertile	Semisterile	Total		
BC ₁₀ -BC ₁₂ F ₁					
Semisterile/T65	31	34	65	1:1	0.138 ns
T65/semisterile	25	22	47	1:1	0.191 ns
BC ₁₁ -BC ₁₂ F ₂	120	137	257	1:1	1.125 ns
BC ₉ F ₃	134	114	248	1:1	1.613 ns

^aSemisterile plants showed infertility in both pollen and seed. F₂ and F₃ seeds were obtained from selfings of semisterile plants under bag pollination. ns = nonsignificance.

Table 4. Number of plants with dehiscent and indehiscent anthers in the substitution line with *O. sativa* cytoplasm (BC₅-BC₉F₁)

Cross	Segregation		Expected ratio	χ^2
	Dehiscent	Indehiscent		
Indehiscent type/W025S ₂	63	58	1:1	0.207 ns
Dehiscent type/W025S ₂	87	0	1:0	—

^aThe initial cross was Acc108S₁(♀)/W025S₂(♂). The two near-isogenic lines Acc108S₁ and W025S₁ were employed as the parents in this experiment since the effects of sterility genes S₁ and S₂ disappear in the hybrid. All plants of the substitution line showed no seed set under bag pollination due to male sterility. ns = nonsignificance.

Acc108S₁, which is responsible for anther indehiscence, is maintained in heterozygous conditions in plants having indehiscent anthers. No restorer gene was extracted from Acc108S₁ in this experiment. Then the male-sterile substitution line with Acc108 cytoplasm was crossed with an *O. glaberrima* strain carrying *Rf_j* from Akebono (japonica type) to examine the cytoplasmic difference between indica and japonica types of *O. sativa*. If *Rf_j* was not effective on pollen restoration in the substitution line with Acc108 cytoplasm, Acc108 cytoplasm would be considered to be different from the japonica cytoplasm. The F₁ was fertile, and fertile and sterile plants segregated into a 3:1 ratio in the F₂, suggesting that *Rf_j* is effective on pollen restoration in the Acc108 cytoplasm. The expression of the restorer was dominant and was the sporophytic type as found in the substitution lines with japonica cytoplasm (12). The detection of cytoplasmic differences depends highly upon whether the recurrent par-

ent employed possesses the fertility restoring gene(s). The Acc108 cytoplasm appears to be the same as those of japonica type in terms of a fertility restoring system.

The results suggest a wide occurrence of cytoplasmic differences between the two species. To ascertain this point, backcrossing experiments are now under way. Although their generation is BC₄ at present, all three *O. sativa* cytoplasm appeared to produce male sterility when combined with the nucleus of *O. glaberrima*, whereas six *O. glaberrima* and its wild progenitor (*O. breviligulata*) strains seemed to have normal cytoplasm. A wide distribution of the cytoplasmic difference and the restorer in *O. sativa* suggests that if *rf_j* carried by *O. glaberrima* were successfully introduced into *O. sativa* background, *O. sativa* itself would provide an additional and useful source of male-sterile cytoplasm.

GENETIC CONSEQUENCES AFTER HYBRIDIZATION

The two cultivated rice species were domesticated independently and became sympatric after speciation. They are often mixed in West African plantings at present and are forced into contact with each other (10). As a result, natural F₁ hybrids occasionally occur in farmers' fields (6). Successive backcrosses and introgression between them have been assumed but have never been conclusively demonstrated. Experimentally, gene transfer from either of the species is possible by backcrossing. The two species can be distinguished by a few distinct characteristics like short and tough ligules and few secondary panicle branches of *O. glaberrima*, which contrast with the long ligules and many secondary panicle branches of *O. sativa*. Parental characteristics in the hybrid progeny of the two species (Acc108 and W025) were observed in BC₁F₆ and BC₂F₆ lines that had different coefficients of relationship to the *O. glaberrima* parent ranging from 1/8 to 7/8 (9). A tendency for recombination restriction was detected in the hybrid derivatives, which might result from infertility in recombinants or so called M-V linkages. Among eight characters investigated, ligule length and secondary branch number per primary branch, which are useful as key characters (11), were practically nontransgressive and were subjected to M-V linkage more obviously than other characters. They are possibly able to coexist without losing their identity even after hybridization. Thus, the morphological recovery of the parental types occurred rapidly in the hybrid derivatives.

Infertility arises from nuclear gene interactions as well as nucleus-cytoplasm interactions between the two species, suggesting that differen-

Table 5. Fertilities of hybrid derivatives (BC_2F_7 and BC_1F_7) between *Oryza sativa* (Acc108) and *O. glaberrima* (W025) and their pollen fertility when crossed with the four strains Acc108, Acc108 S_1 , W025, and W025 S_2 .^a

Line	Cross	Cyto- plasm	Relationship to <i>O. glaberrima</i>	Fertility (%)		Pollen fertility (%) in crosses with			
				Pollen	Seed	Acc108	Acc108 S_1	W025	W025 S_2
1	GSSS	W025	1/8	85.1	73.3	22.1	73.1	0.0	0.0
3	GSGS	W025	3/8	94.0	81.1	24.1	55.2	0.0	0.0
4	SGSG	Acc108	5/8	91.8	32.7	34.6	44.8	0.0	0.0
7	SGSG	Acc108	5/8	94.2	79.4	0.2	4.0	28.9	44.8
11	GSSG	W025	5/8	80.1	76.9	0.0	0.0	39.1	76.4
12	GSG	W025	6/8	89.9	95.1	0.0	0.0	26.8	90.7
19	GSGG	W025	7/8	94.1	95.7	0.0	0.1	51.1	93.3

^aCrosses were made in different directions to obtain plants with different relationships to *O. glaberrima* (9). G = W025 (*O. glaberrima*) and S = Acc108 (indica type of *O. sativa*). W025 S_2 and Acc108 S_1 are near-isogenic lines carrying S_2 and S_1 .

tiation in both nucleus and cytoplasm has an important role for limitation or acceleration of gene transfer between them. If natural hybridization takes place between them, the sterility genes such as gamete eliminators may promote the process of interspecific gene exchange in spite of morphological recovery to the parental types observed in the hybrid derivatives, since pollen grains carrying such genes have a high selective advantage. Loci that are on the same chromosome should be incorporated in the introgressed population.

To look into sterility genes in the hybrid derivatives, each F_7 line was crossed with four strains — Acc108, Acc108 S_1 , W025, and W025 S_2 — and their F_1 pollen sterility was examined. Part of the data are shown in Table 5. All lines were clearly divisible into two parental types according to the occurrence of pollen sterility. In addition, the higher the relationship to *O. glaberrima*, the higher pollen fertility the line showed in crosses with *O. glaberrima*. Pollen fertility of the hybrids indicated that all the lines examined appear to have gamete eliminators S_1 and S_2 since near-isogenic lines ACC 108 S_1 and W025 S_2 always showed a higher fertility in the hybrids than did the parental strains Acc 108 and W025. This implies that S_1^a and S_2^a were eliminated in the introgressed population and S_1 and S_2 rapidly increased as expected. Each line has the cytoplasm of Acc108 or W025. Line 7 has Acc108 cytoplasm but showed a high fertility when crossed with W025 and W025 S_2 , suggesting that the nucleus of the line is similar to that of *O. glaberrima* in terms of hybrid sterility. Thus, the line possesses *O. sativa* cytoplasm and a nucleus

similar to *O. glaberrima*. This combination is expected to induce male sterility, but the line gave a good seed set under bag pollination. The line seems to carry a restorer(s) from Acc108 such as Rf_j . On the other hand, line 1 has W025 cytoplasm, but the nucleus is similar to *O. sativa*. The result predicts that if introgression occurs between the two species, gamete eliminators such as S_1 and S_2 tend to be incorporated into the other species and cytoplasmic gene flow might occur between the two species.

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GENETICS OF F₁ STERILITY IN REMOTE CROSSES OF RICE

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The F₁ hybrids of distant crosses show semisterility, while some wide compatibility varieties (WCVs) produce fertile F₁ plants when crossed to indica as well as to japonica varieties. To analyze the genes for sterility, some WCVs carrying marker genes were used in a pair of three-variety crosses: indica/WCV//japonica and japonica/WCV//indica. In both of the crosses, spikelet fertility was found to be closely linked with a marker gene *C* (chromogen for pigmentation). With the use of the marker, female gametes carrying an allele from the japonica parent were found to be aborted in the F₁ hybrids of indica/japonica crosses. Pollen sterility was independent of spikelet sterility and linked with none of the marker genes tested. A set of multiple alleles was located between the *C* and *wx* (waxy endosperm) loci: S_5^n for WCVs, S_5^i for indica varieties, and S_5^j for japonica varieties. The genotypes of S_5^n/S_5^i and S_5^n/S_5^j were fertile, but S_5^i/S_5^j was semi-sterile due to partial abortion of gametes carrying S_5^j . In addition, two sub-groups of javanica varieties were shown to possess different alleles in the S_5 locus. Spontaneous mutation at the locus was considered to develop the F₁ sterility barrier between distantly related varieties.

Rice improvement through distant crosses is not always easy because of various reproductive barriers. The partial F₁ sterility due to gamete abortion was once interpreted as the result of hypothetical structural differences between the chromosomes of indica and japonica types. In the past three decades, sterility in distant crosses has been attributed to genic differences, although there are two contrasting genetic explanations.

Oka (5, 6) proposed a model of duplicate gametophytic lethals, assuming that the genotypes of gametic lethals for two distantly related varieties, A and B, are Xy/Xy and xY/xY , respectively, and gametes carrying xy in the F₁ hybrid of Xy/xY become aborted due to some deficiency in gamete development. Varieties producing fertile hybrids, when crossed to A as well as to B, are assumed to be of XY/XY genotype. In this paper such a variety is called a wide compatibility variety (WCV). Oka's hypothesis was based on data obtained from a three-variety cross of WCV/A//B. A cross of WCV/A//B, the genotype of which can be written as $XY/Xy//xY$, segregated fertile (XY/xY) and semi-

sterile (Xy/xY) progeny in a ratio of 1:1. The result was interpreted as evidence of duplicate gametophytic lethals (Model 2 in Table 1).

Kitamura (3) reported contrasting results that female and male gametes were independently aborted by respective allelic interaction. It was assumed that indica and japonica varieties possess S^i/S^i and S^j/S^j alleles, respectively, at a locus, and the gametes carrying S^j were aborted in the maternal genotype of S^i/S^j . In this system, the genotype for WCVs can be given as S^n/S^n , assuming that S^n/S^i and S^n/S^j are fertile (Model 1 in Table 1). This system can be called one-locus sporo-gametophytic interaction (7). A similar case was described in tomato by Rick (8). Sano et al (11) reported a case of gamete abortion by allelic interaction in the crosses of isogenic lines from an indica variety and a strain of *Oryza glaberrima*.

In the present paper, the hypothesis of duplicate lethals is referred to as the two-locus model and that of one-locus sporo-gametophytic interaction as the one-locus model. The experimental result that Oka (5) initially cited as evidence for the two-locus model can also be interpreted by the one-locus model as shown in Table 1. Nevertheless, the two-locus model had been widely accepted before Oka's additional work in 1974 (7), which reported a sophisticated study on the two-locus model with isogenic lines. However, Kitamura's induction of the one-locus model was straightforwardly based on extensive tests of isogenic lines (3).

The two models predict contrasting outcomes from a distant cross. According to the two-locus model, a distant cross may eliminate the greater part of recessive lethal genes from its progeny and ultimately produce many lines that would behave like WCVs. The one-locus model envisages that only one of the parent alleles can be predominant in the progeny, and the WCV type would not be expected. Thus, the understanding of the nature of F_1 sterility is not only related to varietal differentiation of rice but also to basic strategies of rice breeding. Six varieties out of 74 were previously screened out as WCVs (1), most of which were from Indonesia or Bengal. As the second step, the genetic nature of the WCVs was investigated along with a critical test of the two models.

MATERIALS AND METHODS

Four groups of varieties were used as shown in Table 2: three of the six WCVs identified in our screening, indica testers IR36 and IR50, four japonica varieties, and three javanica varieties named after Morinaga (4). All of the WCVs tested possess C^+ (chromogen for pigmentation) and

Table 1. Design to test two theoretical models for hybrid sterility by means of marker genes.

Common premise for both models:
 Wide compatibility variety (WCV) produces fertile F_1 when crossed to variety A or to B. F_1 from A/B is semisterile.

Model 1: Sterility is caused by allelic interaction.
Assumed genotypes:
 S^n/S^n for WCV with S^n linked to marker genes;
 S^a/S^a for variety A and S^b/S^b for variety B.

Expression of F_1 genotypes:
 S^n/S^a and S^n/S^b are fertile; S^a/S^b is semisterile.

Linkage between marker and fertility in three-variety cross

Test cross	Genotypes produced	Linkage between marker and fertility
WCV/A//B	S^n/S^b (fert.) + S^a/S^b (semisterile)	detectable
WCV/B//A	S^n/S^a (fert.) + S^b/S^a (semisterile)	detectable

Model 2. Sterility is caused by duplicate recessive lethals.

Assumed genotypes:
 $X^W Y/X^W Y$ for WCV with X^W linked to marker genes;
 Xy/Xy for variety A and xY/xY for variety B.

Expression of F_1 genotypes:
 F_1 genotype of Xy/xY shows semisterility due to abortion of gamete carrying duplicate recessive lethal genes xy .

Linkage between marker and fertility in three-variety cross

Test cross	Genotypes produced	Linkage between marker and fertility
WCV/A//B	$X^W Y/xY$ (fertile) $X^W y/xY$ (semisterile) Xy/xY (fertile) Xy/xY (semisterile)	not detectable
WCV/B//A	$X^W Y/Xy$ (fertile) $X^W Y/xY$ (fertile) xY/Xy (semisterile) xY/Xy (semisterile)	detectable

Table 2. Marker genes of tested varieties.

Varietal group	Variety	Linkage group						
		I	II	III				
Wide compatibility variety	Ketan Nangka	<i>alk</i> , C^+ , <i>wx</i>	Ph^-	A^+				
	Calotoc	+, C^+ , +	Ph^-	A^+				
	CPSLO	+, C^+ , +	Ph^-	A^+				
Indica testers	IR36	}	+, C^+ , +	Ph^+	A^+			
	IR50							
Japonica variety	Akihikari	}	<i>alk</i> , C^- , +	Ph^-	A^-			
	Nihonmasari							
	Taichung 65					<i>alk</i> , C^+ , +	Ph^-	A^-
	Hata-koganemochi					<i>alk</i> , C^- , <i>wx</i>	Ph^-	A^-
Javanica variety	Banten	}	+, C^+ , +	Ph^-	A^-			
	Gamah							
	PenuhBaru					+, C^- , +	Ph^-	A^-

A^+ (anthocyanin activator). In the present experiment, C^+ and A^+ determined apiculus pigmentation, because P^+ (distribution gene) existed in all the varieties.

Three-variety crosses were designed to detect linkage between the marker genes and pollen or spikelet fertility. For testing the validity of the models, a paired cross scheme was conceived as shown in Table 1. According to the two-locus model, the assumed genotype for WCVs is XY/XY , and those for distantly related varieties A and B are Xy/Xy , and xY/xY , respectively. Then, the roles of X and Y from a WCV can be differentiated from each other in the paired crosses, WCV/A//B and WCV/B//A, as indicated in Model 2 of Table 1. If X^w of a WCV is effective for fertility in WCV/B//A, the effect of X^w cannot be detected in WCV/A//B, and vice versa. Therefore, if one of the dominant genes of a WCV is linked with some marker genes, the linkage between the marker and the fertility level can be detected only in one of the paired crosses, whereas, if fertility is controlled by a one-locus system, the effect of the WCV's S^n gene should be detected in both of the paired crosses (Model 1 in Table 1). Therefore, the linkage between fertility and the marker of the S^n can be detected in both of the paired three-variety crosses.

After preliminary tests, C^+ gene was found to be a key marker. The paired three-variety crosses were made primarily with a WCV, Ketan

Nangka, that possesses three markers, viz., *alk* (alkali digestion), C^+ , and *wx* (waxy endosperm) in the linkage group I. The indica testers and WCVs have A^+ , and only WCVs have C^+ , while the japonica varieties except Taichung 65 have none of these. Accordingly, any progeny possessing C^+ from the WCVs is able to be detected by apiculus pigment in the three-variety crosses.

As the first experiment suggested the validity of the one-locus model, it was expected that those gametes carrying S^i or S^j should be aborted in the F_1 genotype of S^i/S^j , thereby showing distorted segregation of the markers. To test such gamete abortion, F_1 hybrids of japonica variety/IR36 were backcrossed with IR36.

Javanica varieties were added to the experiments. Previously (1), 24 javanica varieties had been crossed with indica and japonica varieties to test their compatibility in terms of F_1 fertility. The results are summarized in Figure 1. The majority of javanica varieties were identified as Banten type, and four of them as Penuh Baru type, which showed semisterility when crossed with indica as well as with japonica testers. Both types produced fertile F_1 s when crossed with Ketan Nangka. Penuh Baru was tested in a three-variety cross of Ketan Nangka/Penuh Baru//IR50. Banten and Gamah from the "Banten group" of javanica varieties were added to the gamete abortion tests.

For each of the three-variety crosses, 50–60 F_1 plants were grown in an irrigated field in Okinawa. The pollen and spikelet fertilities were recorded on each plant by standard procedures (1).

EXPERIMENTAL RESULTS

As no linkage between the marker and pollen fertility was found, the results on spikelet fertility are described in this section.

Linkage between marker genes and spikelet fertility

The distribution of spikelet fertility in the paired three-variety crosses, viz., Akihikari/Ketan Nangka//IR36 and IR36/Ketan Nangka//Akihikari, are given in Table 3. It had earlier been confirmed that Ketan Nangka is a WCV, and the F_1 hybrid between IR36 and Akihikari showed semisterility. In the cross of Akihikari/Ketan Nangka//IR36, the genotypes of $wx/+$ and C^+/C^- clearly showed significantly higher fertility than the contrasted genotypes, $+/+$ and C^-/C^- . In the cross of IR36/Ketan Nangka//Akihikari, a relationship between fertility and the marker ge-

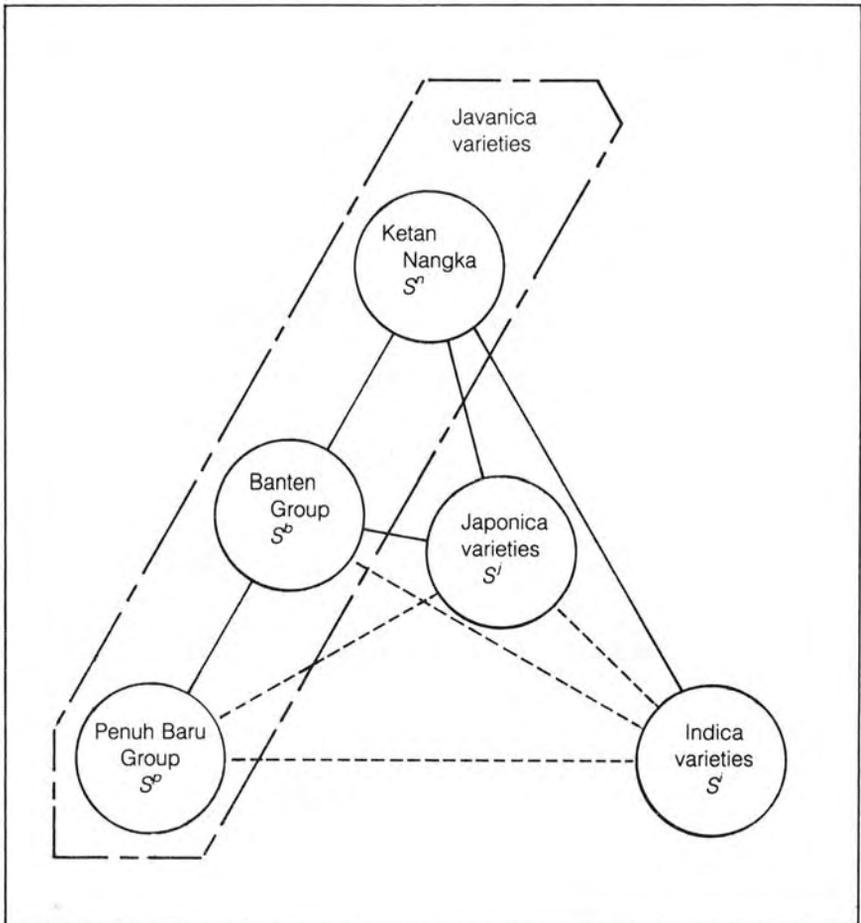


Fig. 1. F_1 fertility and pertinent alleles in the crosses between indica, javanica, and japonica varieties. ————— = varietal combination producing fertile F_1 . - - - - - = varietal combination producing semisterile F_1 . The Compatibility in terms of spikelet sterility indicated here agrees with the compatibility in terms of pollen fertility except that the F_1 of Banten group/IR varieties revealed normal pollen fertility.

notypes was also found, although it was not as clear as in the former cross.

Two reasons for the somewhat weak linkage between the marker and fertility in IR36/Ketan Nangka/Akihikari can be indicated. First, a genic interference was found to be associated with Ph^- . In that cross, the difference in fertility between $wx/+$ and $+/+$ and that between C^+/C^- and C^-/C^- was clear in the presence of Ph^+ gene (Table 3). The Ph^+ -related effect affected all the genotypes in Akihikari/Ketan

Nangka//IR36, since the donor of Ph^+ gene was IR36. Second, differential frequencies of genic recombination were observed between the paired crosses. The recombination value between C^+ and wx was higher in IR36/Ketan Nangka than in Akihikari/Ketan Nangka (Fig. 2). This fact implies that recombinations between the marker genes and the gene for fertility were more frequent in IR36/Ketan Nangka than in Akihikari/Ketan Nangka. The higher recombination frequency could lead to the weak linkage between the markers and fertility in IR36/Ketan Nangka//Akihikari.

Although there were such modifying factors, the linkage between the markers and fertility was clearly shown in the paired crosses. Therefore, fertility must be controlled by the S^m gene from Ketan Nangka, the locus of which was linked closely with C^+ .

The results of additional three-variety crosses are shown in Table 3. And the results with other WCVs are given in Table 4, where IR36/Calotoc//Akihikari and Nihonmasari/Calotoc//IR36 similarly showed close linkage between the C^+ gene and spikelet fertility. As the

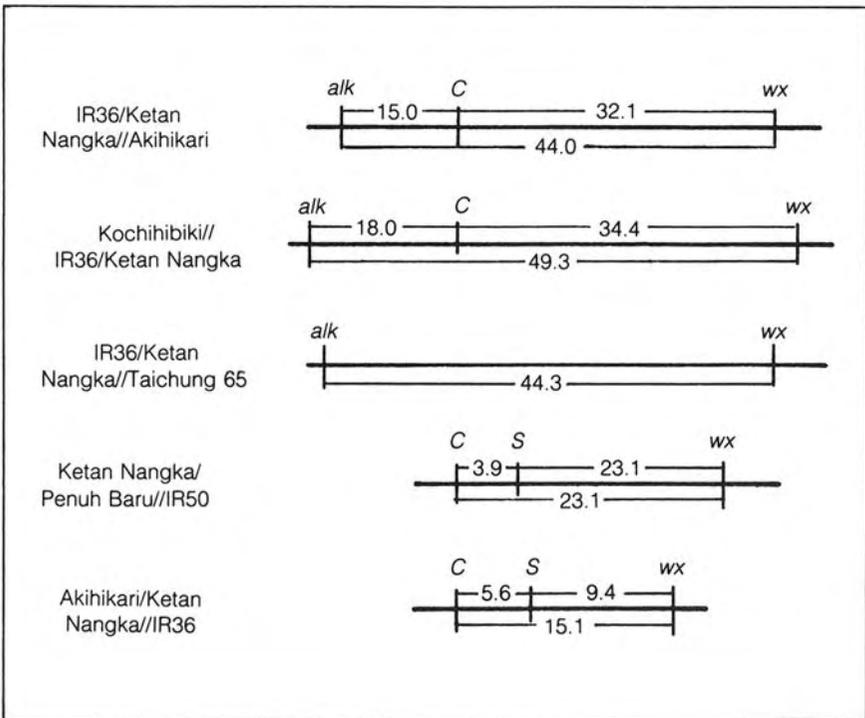


Fig. 2. Intensity of linkage among some markers in linkage group I and assumed locus of S^- alleles, allelic interaction of which causes spikelet semisterility.

two japonica varieties are known to behave identically in distant crosses, these crosses with Calotoc give additional evidence that segregation of fertility is basically controlled by one locus (Model 1 in Table 1).

Tests of gamete abortion by the frequency of marker genes

The results of the test crosses are shown in Table 5. In the cross of Hata-kogane-mochi/IR36//IR36 and Taichung 65/IR36//IR36, the frequency of the B_1F_1 plants carrying wx , alk , or C^+ was significantly lower, and many of the B_1F_1 carrying these markers showed lower fertility. These facts indicate that part of the gametes carrying the allele S^j from the japonica parent were aborted in the F_1 genotype of S^i/S^j .

Genetic analysis of the compatibility of javanica varieties

In the gamete abortion test, the cross of IR50/Banten or Gamah showed a decrease in gametes carrying C^+ as in the indica/japonica cross (Table 5). These two varieties seem to possess an allele similar to japonica varieties, although these two were differentiated from japonicas in their compatibility with Penuh Baru. Therefore, these two are assumed to have a different allele, S^b , at the S locus.

The three-variety cross Ketan Nangka/Penuh Baru//IR50 showed close linkage between spikelet fertility and wx or C^+ from Ketan Nangka (Table 3). Penuh Baru seemed to have a different allele, S^p , at the same locus.

Mapping of the S locus

Since the distribution of spikelet fertility was continuous and subject to environmental fluctuation, the recombinants between the S locus and the markers were not easily determined. However, in one cross with Penuh Baru (Table 3), the distribution of spikelet fertility was clearly separated into low and high groups. Hence, the recombination values were calculated as shown in Figure 2. In another cross of Akihikari/Ketan Nangka//IR36, clear linkage between fertility and two markers was shown, so that the bold numbers in Table 3 were tentatively assumed to be recombinants. The calculated recombination values show good fitness with the value between wx and C^+ (Fig. 2).

Table 3. Distribution of spikelet fertility in three-variety crosses with wide compatibility variety Ketan Nangka.

Marker genotype	No. of plants in each fertilitv class (%)									Total	Mean %	t-test ^b
	-20	-30	-40	-50	-60	-70	-80	-90	-100			
<i>Akikhikari/Ketan Nangka//IR36 (1983)</i>												
wx/+			1		1	10	12	9		33	73.1	**
+/+			1	4	7	5	1	2		20	57.9	
C ⁺ /C ⁻						8	12	9		29	75.8	**
C ⁻ /C ⁻			2	4	8	7	1	2		24	57.2	
<i>IR36/Ketan Nangka//Akikhikari (1983)</i>												
wx/+		1	5	4	2	6	5	2	1	26	59.2	
+/+	2	4	3	2	4	3	2	3		23	49.9	
C ⁺ /C ⁻		1	4	4	2	2	5	4	1	23	60.9	*
C ⁻ /C ⁻	2	4	4	2	4	7	2	1		26	49.4	
alk/alk		1	3	3	2	3	2	5	1	20	62.1	
alk/+	2	4	5	3	4	6	5			29	49.8	*
Ph ⁺ /Ph ⁻	2	3	5	3	3	6	6	3	1	32	55.6	
Ph ⁻ /Ph ⁻		2	3	3	3	3	1	2		17	53.4	
<i>IR36/Ketan Nangka//Taichung 65 (1984)</i>												
Ph ⁺ /Ph ⁻ , wx/+		1	2	2	1	4	4	2	1	17	62.8	
Ph ⁺ /Ph ⁻ , +/+	2	2	3	1	2	2	2	1		15	47.4	
Ph ⁻ /Ph ⁻ , wx/+			3	2	1	2	1			9	52.4	
Ph ⁻ /Ph ⁻ , +/+	2		1	2	1			2		8	54.6	
Ph ⁺ /Ph ⁻ , C ⁺ /C ⁻			2	2	1	1	4	3	1	14	66.4	
Ph ⁺ /Ph ⁻ , C ⁻ /C ⁻	2	3	3	1	2	5	2			18	47.2	**
Ph ⁻ /Ph ⁻ , C ⁺ /C ⁻		1	2	2	1	1	1	1		9	52.4	
Ph ⁻ /Ph ⁻ , C ⁻ /C ⁻		1	1	1	2	2		1		8	54.6	
Ph ⁺ /Ph ⁻ , alk/alk			2	1	1	2	1	3	1	11	61.6	
Ph ⁺ /Ph ⁻ , alk/+	2	3	3	2	2	4	5			21	50.1	
Ph ⁻ /Ph ⁻ , alk/alk		1	1	2	1	1	1	2		9	57.5	
Ph ⁻ /Ph ⁻ , alk/+		1	2	1	2	2				8	48.8	
<i>Ketan Nangka/Penuh Baru//IR50 (1984)</i>												
wx/+	1	1	2	4	3	4	7	10	1	33	66.0	
+/+		3	2	3		3	3	3	2	19	58.8	
alk/alk	1	1	1	1	1	1	5	9	1	21	70.6	
alk/+		3	3	6	2	6	5	4	2	31	58.5	*
Ph ⁺ /Ph ⁻		2		4	3	3	3	7		22	63.1	
Ph ⁻ /Ph ⁻	1	2	4	3		4	7	6	3	30	63.6	

^aBold figures indicate assumed recombinants.

^b*Significant at 5% level, **significant at 1% level.

Table 4. Distribution of spikelet fertility in three-variety crosses with Calotoc and CPSLO.

Marker genotype	No. of plants in each fertility class (%)									Total	Mean %	t-test ^a
	-20	-30	-40	-50	-60	-70	-80	-90	-100			
<i>IR36/Calotoc/Akihikari (1983)</i>												
C ⁺ /C ⁻		1		3	2	5	10	5	3	29	70.7	**
C ⁻ /C ⁻	1	1	5	11	7	3				28	45.6	
<i>Akihikari/IR36/Calotoc (1983)</i>												
C ⁺ /C ⁻			1		1	5	10	13	1	31	76.7	**
C ⁻ /C ⁻	2	3	12	5	8	2				32	51.1	
<i>Nihonmasari/Calotoc/IR36 (1981)</i>												
C ⁺ /C ⁻	1	2	2	2	8	3	3	2	1	24	56.4	**
C ⁻ /C ⁻	7	7	9	8	4	3		1		39	36.3	
<i>IR36/Nihonmasari/Calotoc (1982)</i>												
C ⁺ /C ⁻					2	3	8	10	4	27	79.5	**
C ⁻ /C ⁻	1	5	5	7	6	2	1			27	53.5	
<i>CPSLO/IR36/Nihonmasari (1983)</i>												
C ⁺ /C ⁻		2		1	5	4	6	1		19	70.7	**
C ⁻ /C ⁻		3	5	9	8	2	2			29	57.4	

**significant at 1% level.

DISCUSSION

From the experimental results, the one-locus model seemed to be valid, and a set of multiple alleles was identified: S^n for WCVs, S^i for indica varieties, and S^j for japonica varieties. The allelic interaction of S^i/S^j was shown to cause partial abortion of gametes carrying S^j . As the identification of the S locus with those reported earlier by Sano (9, 10, 11) in interspecific hybrids was not undertaken, the present locus is tentatively designated as S_5 .

From the viewpoint of the one-locus model, an antagonism between the heterozygous maternal tissue and the gametes carrying one of the alleles is the basis for F_1 sterility. The mutations at the S locus seem to produce new alleles antagonistic to each other and to develop reproduc-

Table 5. Female-gamete elimination and its effect on spikelet fertility detected in japonica/indica/indica or javanica, indica/indica crosses.

Marker genotype	No. of plants in each fertility class (%)										Total Mean %	t-test ^a	
	-40	-50	-60	-70	-75	-80	-85	-90	-95	-100			
<i>Hata-kogane-mochi//IR36//IR36 (1983)</i>													
<i>wx/+</i>		5	6	2	2	2	1				18	67.9	**
<i>+/+</i>			4	5	5	8	3	3			28	79.1	
<i>alk/+</i>		3	4	1							8	61.4	**
<i>+/+</i>		2	6	6	7	10	4	3			38	77.5	
<i>Taichung 65//IR36//IR36 (1984)</i>													
<i>C⁺/C⁻</i>	3		3	7		3	8	3			27	69.6	**
<i>C⁺/C⁻</i>				3	3	12	20	14	15		67	83.9	
<i>alk/+</i>	3		2	7		6	9	5	1		33	72.0	**
<i>+/+</i>			1	3	3	9	19	12	14		61	83.6	
<i>Banten//IR50//IR50 (1983)</i>													
<i>C⁺/C⁻</i>		1	2	5	1	2					11	63.8	
<i>C⁻/C⁻</i>		2	2	6	4	10	7	5	6		42	76.5	
<i>Gamah//IR50//IR50 (1983)</i>													
<i>C⁺/C⁻</i>		5	4	2				1			12	65.2	
<i>C⁻/C⁻</i>		7	8	5	4	6	2	2			34	72.2	

^a*Significant at 5% level, **significant at 1% level.

tive barriers. The diversity of the S locus in indica, japonica, and javanica varieties was demonstrated by the identification of some alleles. Of them, the role of S^i is of interest, because it eliminates the opposite allele with adjacent genes and forms an exclusive set of traits. In practice, the exclusion of the *alk-wx* segment of japonica or javanica varieties in the hybrids with indicas should be taken into account, as these genes determine the cooking quality of rice.

Citing the parentage of American WCVs like Century Patna 231 and CPSLO, Jennings (2) once discussed that progeny from indica/japonica crosses would produce wide compatibility. Theoretically, the two-locus model predicts such outcomes. However, the present results contradict this prediction. It is likely that the donor of S^n to CPSLO was some javanica WCV in the Philippines. The use of S^n may be desirable to facilitate distant crosses.

Through extensive tests of indica/japonica crosses in Japan, it has been realized that the fertility level in indica/japonica//japonica backcrosses is apparently lower than in indica/japonica//indica backcrosses. This can be explained by the elimination of S^j in the first cross and is not reasonably predicted by the two-locus model, which assumes a symmetrical genotype for each component of the indica/japonica crosses.

Throughout the present study, pollen fertility was recorded, but no link was found between fertility and the markers. It was also found that the javanica WCVs produce highly sterile F_1 s with aus varieties from Bengal. There may be some other loci for the F_1 sterility.

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DISCUSSION

SESSION 2: VARIETAL DIVERSITY AND REPRODUCTIVE BARRIERS

Q – Wu, H.K.: Which system of chromosome numbering did you use?

A – Nakagahra: The name of the chromosome used follows the system proposed by Nishimura (1961).

Q – Oka: What is the association between enzymic variation and other traits?

A – Glazmann: I have not personally investigated this aspect; however, it could be easily done. Since the enzymatic classification fits very well with the indica-japonica (temperate + tropical) classification based on character association, we should find a strong association between some isozymes and these characters.

Q – Oka: A senior Chinese scientist, K. S. Cheng, and his colleagues are most interested in your “intermediate groups” and have examined them with regard to six different traits. They need your detailed data for comparison.

A – Glazmann: Groups II, III, IV, and V look intermediate between indica and japonica when we consider the loci involved in the indica-japonica discrimination. However, these groups are characterized by specific alleles for some loci and thus cannot be considered as true intermediates. I will communicate my original data to K. S. Cheng and his colleagues.

A – Oka: I question the use of the name javanica because its definition is ambiguous.

A – Glazmann: I do agree that the term javanica is ambiguous. It is, however, widely used by many rice researchers to describe a morphological type that is often referred to as an intermediate type between indica and japonica. I used this term to point out that this type should rather be considered as a component of a “japonica in a wide sense” group.

Q – Kumar: In the light of your results, what breeding methodology do you suggest for improvement of high quality but poor plant type Basmati rice?

A – Glazmann: Prior to the elaboration of a breeding strategy on the basis of the isozyme classification, we need further genetic and breeding experiments to investigate the relationships between the groups for characters such as hybrid sterility, vegetative heterosis, and recombination in the progenies. As far as the genetic distances calculated from isozyme data

are concerned, the group that appears closer to group V is group VI or japonica in a wide sense.

Q – Oka: I assume that the genetic diversity in African rice fields plays a role in the stability of production in relation to pest and disease resistance. What do you think about it?

A – Miezan: I agree, but one has to be careful in drawing conclusions because of the ordinarily small size of rice fields. Cultural practices can also play a role.

Q – Abifarin: How many *Oryza glaberrima* populations were used in your analysis compared with those of *O. sativa*? If the sample populations are similar, one might expect similar variability.

A – Miezan: We used six *O. glaberrima* and ten *O. sativa* populations. Isozyme studies indicate that *O. glaberrima* is less variable than *O. sativa*. Sample sizes do not necessarily play a role here since even after splitting *O. sativa* into indica and japonica, indica appears to be more variable than *O. glaberrima*.

Q – Kinoshita: What is the molecular basis of cytoplasmic gene flow?

A – Sano: Cytoplasmic gene flow results from successive backcrosses. In addition, there might be a possibility that cytoplasmic instability is due to movable elements as found in maize.

Q – Second: Do you think that the mutational event you mentioned in your substitution line might have been prompted by the combination of two distantly related genomes?

A – Sano: I do not know at present. My recent work suggests that mutational events can occur in both the cytoplasm and nucleus of rice.

Q – Chaudhary: In your models, how do you explain high to complete sterility in indica/indica crosses?

A – Ikehashi: I do not try to explain all phases of F_1 sterility. We need more experimental work to explain various aspects of the sterility.

C – Oka: The F_1 sterility of sativa intervarietal crosses is complex. Different models can be applied to different cases.

KARYOTYPE, POLYPLOIDS, AND TRISOMICS

SESSION 3

RICE KARYOTYPE ANALYSIS

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In a survey of rice karyotype analysis, it was found that marked progress has been made in chromosome preparation in the past 25 years. Various techniques were evaluated, and some were judged more applicable and more reliable than others. Ways to eliminate discrepancies among workers and issues that need to be standardized during karyotyping are suggested.

Karyotype analysis is the basis of cytogenetics. Unfortunately, rice chromosomes are small and difficult to prepare. Karyotype analysis of rice was almost impossible before the advent of successful chromosome preparation techniques, which is why, although rice trisomics and translocation stocks have been available for more than 15 years, cytogenetic studies of them have been carried out only recently. This paper enumerates and discusses recent progress in rice karyotyping.

BRIEF REVIEW OF KARYOTYPING

Yao et al (30) attempted to examine cryptic structure differences in intervarietal hybrids at the pachytene stage. Shastry et al (26, 27) investigated the karyotype of Norin 6 and meiosis in an intersectional hybrid by pachytene analysis, while Hu (7) chose root tips of spontaneous haploid plants to measure the lengths of the 12 chromosomes and their arm ratios.

Since then, improved techniques of chromosome preparation have been emerging (11, 14, 15, 24, 25, 29). These techniques have been applied to karyotypic variation in *Oryza sativa* (19), to pairing at the pachytene stage in autotetraploids (22), and to analysis of interspecific hybrids (4, 9, 10, 21). Recently, the use of these techniques has been extended to identify reciprocal translocations (2, 23) and trisomics (12, 13, 18). The identified translocations and trisomics have been used to locate genes on rice chromosomes that were, in turn, corresponded to the

linkage groups (2, 12, 18, 23) established by genetic crosses. It should also be mentioned that Chen et al (3) applied root tip chromosome techniques to compare karyotypes of six species.

EVALUATION OF TECHNIQUES

When pachytene chromosome analysis was introduced to rice by Shastry et al (27), the smallness of rice chromosomes was no longer a serious problem. Smallness was later circumvented by the enzyme flame technique of Kurata (14) in preparing root tip chromosomes. From the beginning, to examine the centromere and to stain differentially in rice pachytene chromosomes were difficult tasks. These difficulties, however, have been partially resolved (29).

Technically speaking, a cell that is suitable for karyotype analysis should have its chromosomes well spread and differentially stained. As many workers have experienced, differential staining can be accomplished by overstaining and washing. That is why in the case of pachytene chromosome preparation a trace of FeCl_3 is added to 3:1 fixative (4, 11, 19, 21, 22, 23, 27, 29) and a trace of $\text{Fe}(\text{OH})_3$ is added to 1% aceto-carmin (29). Gently heating after covering the mixture of pollen mother cells (PMCs) and carmin with a coverslip also helps overstaining. Washing with drops of 45% acetic acid is necessary. If the overstained cells are squashed without washing, both cytoplasm and chromosomes will be deeply stained, and cells will be resistant to swell. Then, a good spread of pachytene chromosomes can usually be obtained by gently and evenly heating the slide that carries the overstained, washed cells to a point near boiling (29). Pachytene chromosomes can also be spread by KCl and differentiated by Giemsa stain and tap water washing, as Kurata has done (15). In preparing root tip chromosomes, cells are swelled simply by KCl, and staining differentiation is performed by rinsing the Giemsa overstained slides with tap water (14).

Although rice chromosome techniques have been substantially improved, their application has been limited to a small number of laboratories, especially for root tips. The reason for this is probably the lack of a medium such as the *Rice genetics newsletter*. Nevertheless, the following achievements in rice karyotype analysis have become evident:

- The length, relative length, and arm ratio of rice chromosomes can now be determined more accurately than before (2, 3, 13, 15).
- The short arm of chromosome 4 in *O. sativa*, as well as in many other species is totally heteromeric (2, 3, 12).

- In all species so far observed, there is only one major nucleolus in each cell, even though its shape varies (2, 3, 12, 13, 15).
- The chromosomes attached to the nucleolus are specific (3). In *O. sativa* and three other species only the tenth chromosome attaches to the nucleolus; in *O. australiensis* only the eighth. In *O. perennis* there are two chromosomes, the eighth and the tenth. The attachment is confined to the short arm of the nucleolar chromosomes via a micro-satellite toward the waist of the nucleolus (2, 3).
- The possibility of identifying translocation (2, 23) as well as trisomic stocks has been elucidated (12, 13, 18).
- Karyotypes among rice species have been proved to be different (3, 16).

The reliability and applicability of the techniques have thus been reflected. At the moment, it may be concluded that rice karyotyping has progressed through more or less sequential steps coincident with improvements in chromosome techniques.

ELIMINATING DISCREPANCIES

Parameters involved in karyotype analysis may include total chromosome length, relative length, arm ratio, and chromomere, knob, or band distribution patterns. However, the chromomere and knob patterns have not become popular in rice karyotype analysis.

A recent careful survey found that there are discrepancies among workers dealing with karyotypes of *O. sativa*. For example, the arm ratio of pachytene chromosome 9 of four cultivars had quite different values (3.2, 3.9, 2.0, and 2.2); the nucleolar chromosome of cultivars Taichung 65, Norin 6, and T1242 was assigned to five chromosomes (Table 1); and the correspondences of chromosome to linkage group have not yet been established (Table 2). These discrepancies could have come from the following sources:

- Cells with partially clumped and improperly differentiated chromosomes have been used.
- As the length of the chromosomes varies in the process of cell division, drawing of a random sample from various pachytene stages would give a variation in length. Pachytene chromosome length varies from 440 to 310 μm as the cell proceeds from early to late pachytene (2).
- In root tip cells, beyond the chromosome length, the number of heterochromatic regions varies. It decreases from prometaphase to

Table 1. Lengths of *O. sativa* chromosomes compiled from seven authors.

Phase/cultivar	Reference	Measurement ^a	Chromosome											
			1	2	3	4	5	6	7	8	9	10	11	12
Somatic metaphase/ Taichung 65	7	L+S	4.3	3.7	3.3	2.9	2.9	2.6*	2.0*	2.8	2.8	2.3	2.3	1.8
		L/S	2.2	1.5	1.3	1.1	1.7	2.1	2.1	3.0	2.5	1.6	1.3	2.1
		RL	13.0	11.1	9.8	8.7	8.6	7.8	7.8	6.0	8.3	6.7	6.7	5.4
Pachytene/Norin 6	27	L+S	79.0	47.5	47.0	38.5	30.5	27.5	26.5	23.0	21.0	21.0	20.5*	18.0
		L/S	1.8	2.1	1.2	2.1	2.1	4.0	1.0	1.7	3.2	6.0	1.6	3.0
		RL	19.8	11.9	11.08	9.6	7.6	6.9	6.6	5.8	5.3	5.3	5.1	4.5
Pachytene/T 1242	24	L+S	73.5	50.4	57.0*	48.4*	45.4	39.1	35.9	35.9	30.3	26.9	26.7	19.2
		L/S	1.4	1.4	1.1	1.4	1.9	2.1	1.0	3.1	3.9	1.3	2.2	1.6
		RL	15.0	10.3	11.7	9.9	9.3	8.0	7.3	7.3	6.2	5.5	5.5	3.9
Somatic/prometaphase/ Sekitori	14	L+S#												
		L/S	2.0	1.7	1.4	3.5	2.2	1.3	1.2	1.4	1.6	2.6*	1.3	2.0
		RL	15.2	12.2	11.2	9.6	7.6	7.6	7.0	6.5	6.2	6.2	5.5	5.1
Pachytene/Nipponbare	15	L+S#												
		L/S	1.8	1.7	1.2	4.7	1.8	1.2	1.9	1.2	2.0	3.5*	1.3	2.9
		RL	12.9	12.0	12.0	9.6	8.4	8.3	7.0	6.3	6.8	5.8	5.8	5.2
Pachytene/Chianung 242	2	L+S	54.2	48.3	41.8	34.0	31.9	30.1	28.2	26.4	24.4	24.4*	22.7	19.9
		L/S	1.4	1.2	1.9	2.1	1.2	1.2	1.4	3.8	1.2	1.8	1.8	2.6
		RL	14.0	12.5	11.0	9.0	8.3	7.9	7.4	6.9	6.3	6.1	5.7	5.2
Somatic prometaphase/ Taichung 65	3	L+S	6.9	5.9	5.2	4.4	4.2	3.8	3.4	3.2	3.0	2.7*	2.7	2.4
		L/S	1.9	1.5	1.9	4.6	1.5	1.7	1.6	1.4	2.2	3.7	1.2	2.6
		RL	14.3	12.3	10.9	9.3	8.7	7.8	7.2	6.8	6.3	5.8	5.6	5.1

^aL+S = length of long arm plus length of short arm, L/S = length of arm over length of short arm, RL = relative length, # = data not available, * = nucleolar chromosome.

Table 2. Relationships among chromosomes, trisomics, and linkage groups (8).

Chromosome K(14)	Trisomics Triplo (12)	Linkage group
10	9	VII, V
9	5	VI, IX, & XII
1	1	III
5	6	—
3	4	XI
6	3	I
12	10	—
2	2	X
8	11	VIII
11	7	IV
4	12	II
7	8	—

early metaphase, then to metaphase (13, 18). The distribution pattern of heterochromatin varies in a random cell sample involving different dividing stages.

- The size of the cell sample is often too small. Measurements of 10 cells, which appear in many reports, would give larger variations than those made of 30 or more cells.
- Length measuring is not a serious problem in root tip chromosomes but is in the case of pachytene chromosomes. Clever cytologists have performed the latter by coinciding fine, soft wires or threads with crooked pachytene chromosomes (2, 24), a tedious process with much chance for error.
- Different chromosome numbering systems have been adopted (8, 12, 14).

Knowing the sources of discrepancies, rice karyotype analysis can then be refined by adopting one of the chromosome techniques that are applicable and reliable, by choosing cells confined to a certain dividing stage, say midpachytene or prometaphase, by increasing the number of analyzable cells up to 30 or more, by using a digitizer attached to a computer instead of wires or threads to measure lengths, and by standardizing the chromosome numbering system.

STANDARDIZATION

In karyotyping, chromosomes should be numbered. Numbering was done in rice via an empirically assigned sequence of translocations before

the chromosomes could be morphologically distinguished. Currently, a system of descending chromosome magnitude has been adopted by many workers (Table 1). Although a minor discrepancy is obvious—the pachytene chromosome 9 of Kurata et al (13) is relatively longer than chromosome 8—this could be eliminated by rearranging. The prefix K was assigned to chromosomes by Kurata et al (13). This would be unnecessary if rice chromosomes, whether from PMCs or root tips, could be morphologically distinguished and the genome of a species, *O. sativa*, had its own characteristics. The use of arabic numerals should be encouraged.

The expression of arm ratio should also be standardized. Most workers except Sen (24) present arm ratio as length of long arm over that of short arm (Table 1).

Furthermore, whether the absolute lengths of chromosomes should be presented or not needs to be discussed. Shastry (25) emphasized the use of relative lengths. Although the absolute length of a chromosome does not reveal its DNA quantity, it directly indicates chromosome magnitude. For example, in *O. sativa* the longest pachytene chromosome is about 60 μm while that of prometaphase chromosomes is 7 μm .

PROSPECTS

Chromosome banding has been done in rye (5), wheat (20), and barley (17). In humans, the G-band has been helpful to distinguish chromosomes of similar magnitude (1). Developing techniques for G-, C-, and NOR-banding in rice will improve karyotype analysis and present a new challenge to rice cytogeneticists.

Karyotype analysis can be applied not only to cytogenetic studies of rice translocation, trisomics, and inversion, but also to the study of rice evolution. In the latter case, comparison of karyotypes among rice diploid species, among tetraploid species, and between diploid and tetraploid species would explain their evolutionary relationships as Yosida did in rat species (31).

Recently, the genes coding for zein, the major storage protein of *Zea mays* endosperm, have been located on the long arm of chromosomes 4 and 5, the short arm of chromosome 7, and the distal segment of the long arm of chromosome 10 by in situ hybridization (28). The improved technique of in situ hybridization can even localize the single copy insulin gene in the human genome at the distal end of the short arm of

chromosome 11 (6). In rice, several genes such as histone III have been cloned by Ray Wu (personal communication), a pioneer in rice genetic engineering. Based on rice karyotype analysis, it might be possible to localize the cloned genes in rice chromosomes by *in situ* hybridization.

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CHROMOSOME ANALYSIS OF MITOSIS AND MEIOSIS IN RICE

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Morphological characteristics of rice chromosomes in mitosis and meiosis are presented. Centromere position, relative length, and differentially staining patterns seen in mitotic metaphase are correlated with those seen in the pachytene stage in every chromosome, proving that our analytical system in rice chromosomes is reliable. In addition, morphological similarities among A, B, and C or F genomes were detected both in mitosis and meiosis. The analytical system was applied to the identification of extra chromosomes in mitotic metaphase in japonica rice. Chromosomes identified to be trisomic made it possible to correlate individual chromosomes with genetic linkage groups.

Clear morphological analysis of rice chromosomes, especially in mitotic metaphase, was lacking for a long time. We recently developed a new method different from the conventional squashing method. Our method uses enzyme treatment, flame drying, and Giemsa staining (3) to produce well characterized chromosomal features both in mitosis (6) and meiosis (7). Detailed morphological analysis has thus become possible.

CHROMOSOMES IN MITOSIS

Flame drying preparation of meristematic root tip cells and Giemsa staining brought on clearly characterized chromosome features not only in metaphase but in late prophase nuclei as shown in Figure 1. Relative chromosome length, centromere position, the nucleolar organizing region, and darkly stained chromosome segments were easily recognizable. Compared to pachytene chromosome features, as shown later, late prophase and prometaphase chromosomes in mitotic nuclei were endowed with equal characteristics. Twelve pairs of chromosomes of *Oryza sativa* (A genome), a japonica rice, appeared to be identifiable with these characteristics and so were designated K1 to K12 according to their length (Fig. 2A). Karyotype analysis showed that the 12 pairs are composed of 5 metacentric, 5 submetacentric, and 2 subtelocentric chromosomes and that K10 is the only nucleolar organizing chromosome.

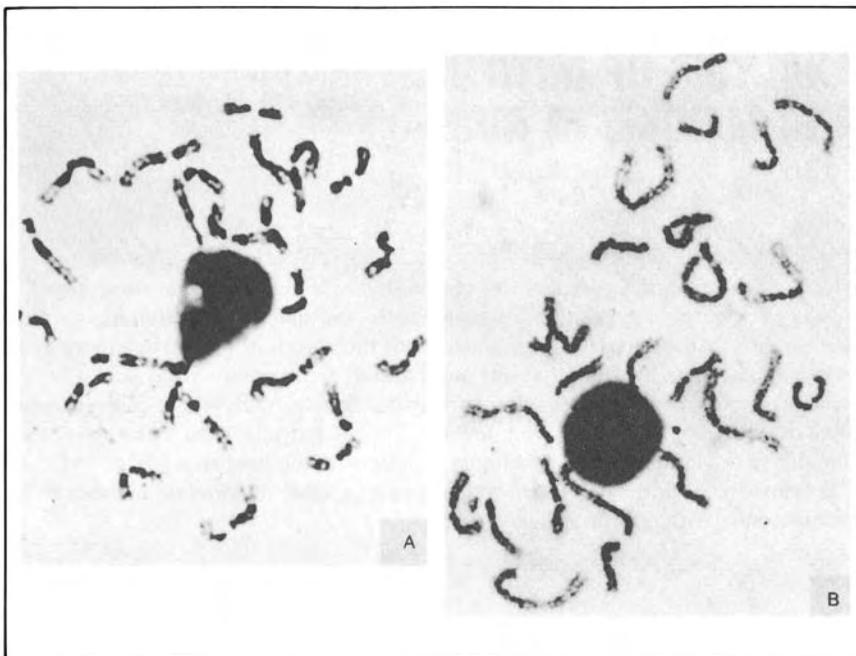


Fig. 1. Prometaphase (A) and late prophase (B) nuclei of rice root tip cells prepared by enzymatical maceration and flame drying method and stained with Giemsa. The longest chromosome in the late prophase nucleus is 16 μm .

Relative length and arm ratio of the 12 chromosomes are presented in Table 1.

Karyotype analysis was then carried out with other varieties, species, and genomes. Two *O. sativa japonica* strains, one indica rice, and one *O. perennis* were utilized as the A genome. *O. punctata* of the B genome and *O. officinalis* of the C genome were also analyzed. As shown in Figure 2 and Table 1, there was no difference in mitotic karyotypes even among genomes of A, B, and C (4).

Several trials to increase the precision of morphological characterization were done, and deoxyadenosine plus uridine pretreatment appeared to produce G-bands of differentially staining pattern. However, it was difficult to obtain complete G-banding patterns in one nucleus. Perhaps the air drying method using completely cell wall-digested protoplast is needed for the full detection of the G-bands. An example of G-banded chromosomes is shown in Figure 3. The reducing patterns of the bands with the advance of the cell cycle are shown in Figure 4.

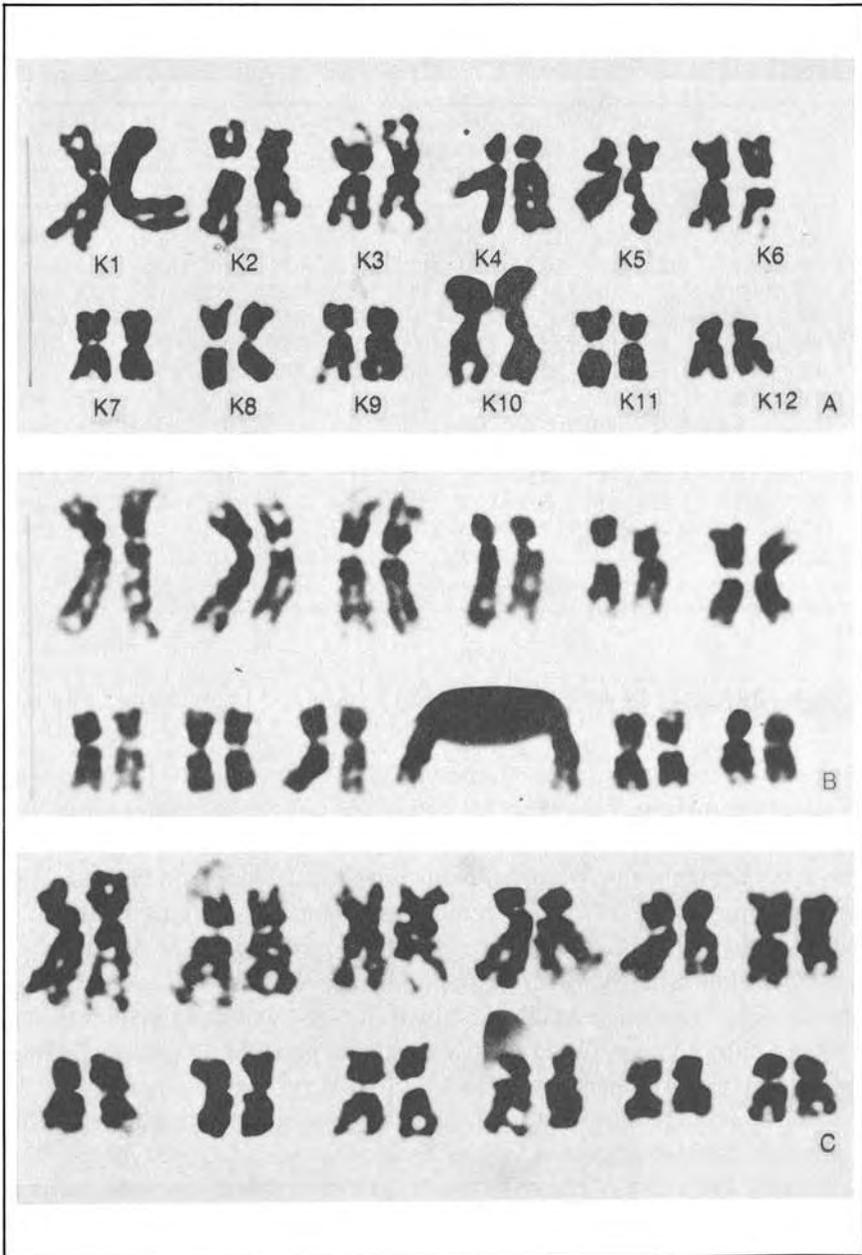


Fig. 2. Karyotypes of *Oryza sativa* (A genome) early metaphase (A), *O. punctata* (B genome) prometaphase (B), and *O. officinalis* early metaphase (C). Chromosomes are designated and arranged from K1 to K12.

Table 1. Means of relative length (RL) and arm ratio (AR) in *Oryza sativa*, *O. perennis*, *O. punctata*, and *O. officinalis* prometaphase chromosomes.^a

	<i>O. sativa</i> (japonica:20 cells)			<i>O. perennis</i> (W 1288:10 cells)			<i>O. punctata</i> (W 1514:10 cells)			<i>O. officinalis</i> (W 1274:5 cells)		
	RL	AR		RL	AR		RL	AR		RL	AR	
K1	14.0	1.82	(sm)	13.1	1.90	(sm)	13.4	1.92	(sm)	13.2	1.76	(sm)
K2	11.1	1.80	(sm)	10.7	1.90	(sm)	11.0	1.91	(sm)	10.5	1.85	(sm)
K3	11.7	1.32	(m)	11.5	1.28	(m)	12.3	1.35	(m)	11.7	1.23	(m)
K4	9.3	4.08	(st)	8.8	3.64	(st)	9.3	3.54	(st)	9.2	3.48	(st)
K5	8.0	2.18	(sm)	8.3	1.93	(sm)	8.0	2.26	(sm)	7.8	1.91	(sm)
K6	8.1	1.17	(m)	8.8	1.22	(m)	8.0	1.17	(m)	8.8	1.22	(m)
K7	6.9	1.30	(m)	7.0	1.48	(m)	6.7	1.57	(m)	7.7	1.22	(m)
K8	6.6	1.31	(m)	6.7	1.33	(m)	6.4	1.17	(m)	6.8	1.21	(m)
K9	6.9	1.83	(sm)	7.1	1.80	(sm)	7.0	1.95	(sm)	7.2	1.89	(sm)
K10 ^b	6.5	4.85	(st)	6.9	4.57	(st)	7.3	5.38	(st)	6.5	4.35	(st)
K11	5.5	1.35	(m)	5.6	1.36	(m)	5.4	1.39	(m)	5.3	1.24	(m)
K12	5.3	2.40	(sm)	5.6	1.93	(sm)	5.3	2.19	(sm)	5.4	1.75	(sm)

^a sm = submetacentrics, m = metacentrics, st = subtelocentrics.

^b Nucleolar chromosomes were calculated without the length of satellites and secondary constrictions.

CHROMOSOMES IN MEIOSIS

Several workers had presented analytical configurations of 12 pachytene bivalents in meiosis. However, the consensus pattern of bivalent features, especially in centromere positions, was difficult to obtain because the conclusive evidence on centromere position was lacking and because long chromosomes were difficult to resolve into constant marking features.

To pachytene analysis we also introduced enzymatic and/or uridine treatment following flame drying and Giemsa staining (7). Conspicuous centromeres were successfully obtained in late pachytene bivalents and distinct chromomere patterns in almost all pachytene nuclei. Centromeres and chromomere patterns are shown in Figure 5, with the 12 bivalents arranged from K1 to K12. Chromosome numbering of the pachytene bivalents is according to the analysis of mitotic karyotype. The arm ratio and relative chromosome length of 12 bivalents are shown in Table 2. Relative length, centromere position, and proportion of the darkly stained segment have good correspondence between mitotic chromosomes and meiotic bivalents. Well corresponded chromosome features throughout all stages, even between mitosis and meiosis, demonstrated that the chromosome characteristics detected by our procedures are fully reproducible and reliable.

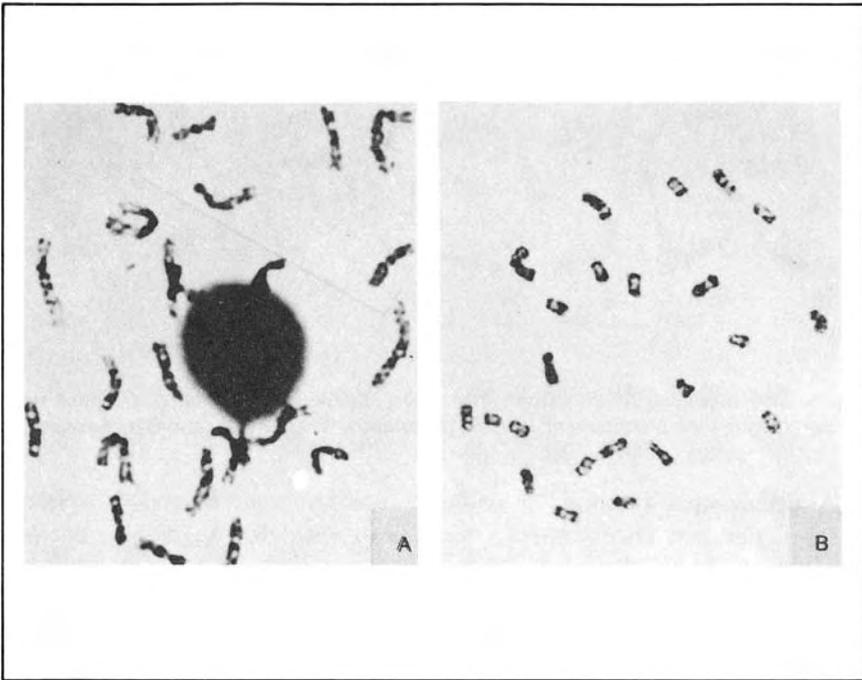


Fig. 3. Prometaphase (A) and mid-metaphase (B) nuclei with G-band structure of chromosomes. The cells were pretreated with deoxyadenosine and uridine. Most chromosomes are characterized by G-bands.

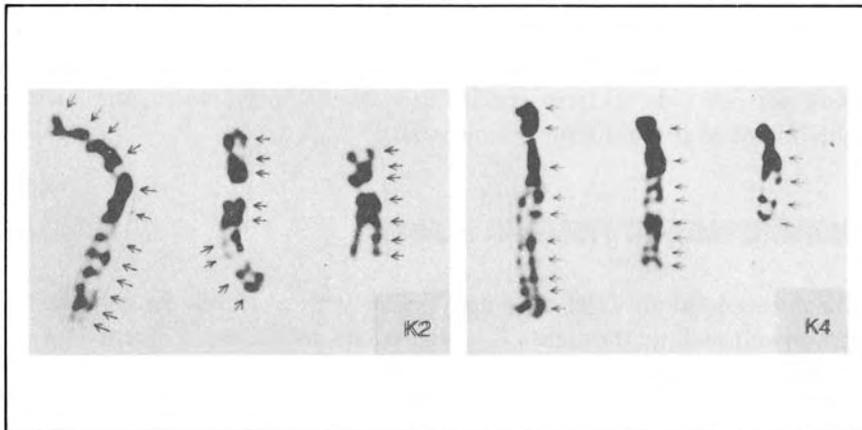


Fig. 4. Reduction patterns in the number of G-bands in accordance with cell cycle development. Late prophase, prometaphase, and early metaphase chromosomes of K2 and K4 are arranged from left to right. G-bands shown by arrows are different in number among the same chromosomes at different stages.

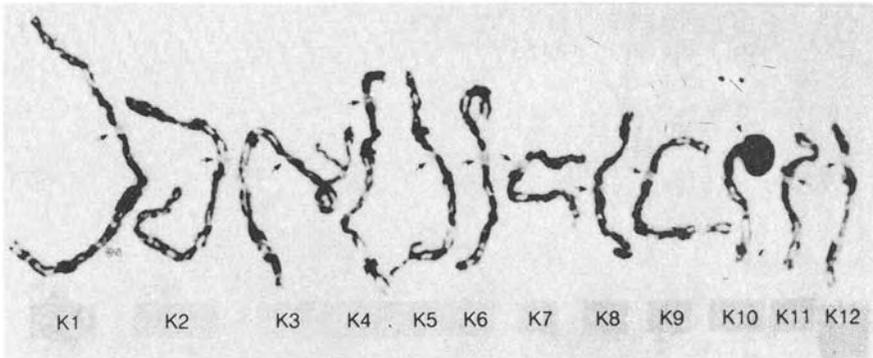


Fig. 5. Late prophase bivalents with their well characterized centromeres. These were prepared by uridine pretreatment, enzyme maceration, flame drying, and Giemsa staining.

Applying this method to pachytene analysis of B and F genome species, detailed chromomere comparison with the A genome became possible. Figure 6 shows the 12 pachytene chromosomes of *O. sativa* (A genome), *O. punctata* (B genome), and *O. brachyantha* (F genome). Chromosomes of the three species have remarkable morphological resemblance except that minor differences were detected in the K1 and K9 segments. Thus the morphological differentiation of rice chromosomes occurs by small degrees during genomic differentiation with regard to the A, B, F, and probably C genomes. In all species analyzed here, only one nucleolar organizing chromosome was detected in both meiosis and mitosis; K10 always attached to the nucleolus in all nuclei. On the other hand, the meiotic prophase nucleus carrying several micronucleoli besides a large nucleolus was seen in every species; the number of micronucleoli per cell differed from species to species (7). However, the sites of attachment of these micronucleoli varied.

CHROMOSOMES IN TRISOMIC PLANTS

Chromosome analysis of trisomics began with a search for mitotic trisomics and meiotic trivalents. Trivalents had complicated morphology in most nuclei and were difficult to identify without the simultaneous detection of all other 11 bivalent chromosomes. In mitosis, chromosomes in the trisomic condition were easily detected in the case of K4, K6, K10, and K12, but K5, K7, K8, K9, and K11 trisomics were somewhat difficult to identify in only one nucleus. Though the latter resembled one

Table 2. Relative length (RT), and arm ratio (AR) of pachytene chromosomes in *Oryza sativa* (A genome), *O. punctata* (B genome), and *O. brachyantha* (F genome).

Chromosome	<i>O. sativa</i> (A)		<i>O. punctata</i> (B)		<i>O. brachyantha</i> (F)	
	RL	AR	RL	AR	RL	AR
K1	13.2	1.74	13.9	1.47	13.4	1.47
K2	11.8	1.68	11.6	1.79	12.1	1.76
K3	11.8	1.20	11.0	1.15	11.4	1.14
K4	9.4	4.46	9.0	4.26	8.6	4.62
K5	8.7	1.80	7.8	1.86	7.9	1.90
K6	8.2	1.18	8.3	1.13	7.9	1.17
K7	6.5	1.48	6.9	1.67	7.1	1.47
K8	6.2	1.16	6.5	1.09	7.1	1.15
K9	7.3	2.10	7.3	2.06	7.2	2.32
K10	5.7	3.67	5.9	4.19	5.9	4.44
K11	5.8	1.29	6.0	1.30	6.1	1.43
K12	5.3	2.87	5.5	2.41	5.5	2.29

another, well characterized nuclei told us which was the extra chromosome. The subtelocentric and relatively longer feature of K4, small size and small short arm of K12, metacentric and medium size of K6, and the attachment to the nucleolus of K10 allowed positive identification of these trisomics. K5, K7, K8, K9, and K11 needed careful attention for the identification of extra chromosomes, which could best be seen when all 25 chromosomes were arranged. Examples of K4, K5, K8, and K10 trisomics are shown in Figure 7. Trisomic plants having K1, K2, and K3 as extra chromosomes were analyzed by Iwata et al (2), and the relationships between chromosomes and genetic linkage groups were investigated (1).

A RELIABLE ANALYTICAL METHOD

Recent progress in genetic engineering has presented us with a powerful technique for gene identification on a chromosome. It is possible to locate certain cloned DNA fragments on the authentic chromosomal position of the DNA by in situ hybridization methods. The cloned DNA fragment does not need to be a known gene or sequence but needs to be a single copy sequence in the genome. Twelve DNA fragments located on 12 chromosomes will work on DNA markers of individual chromosomes.

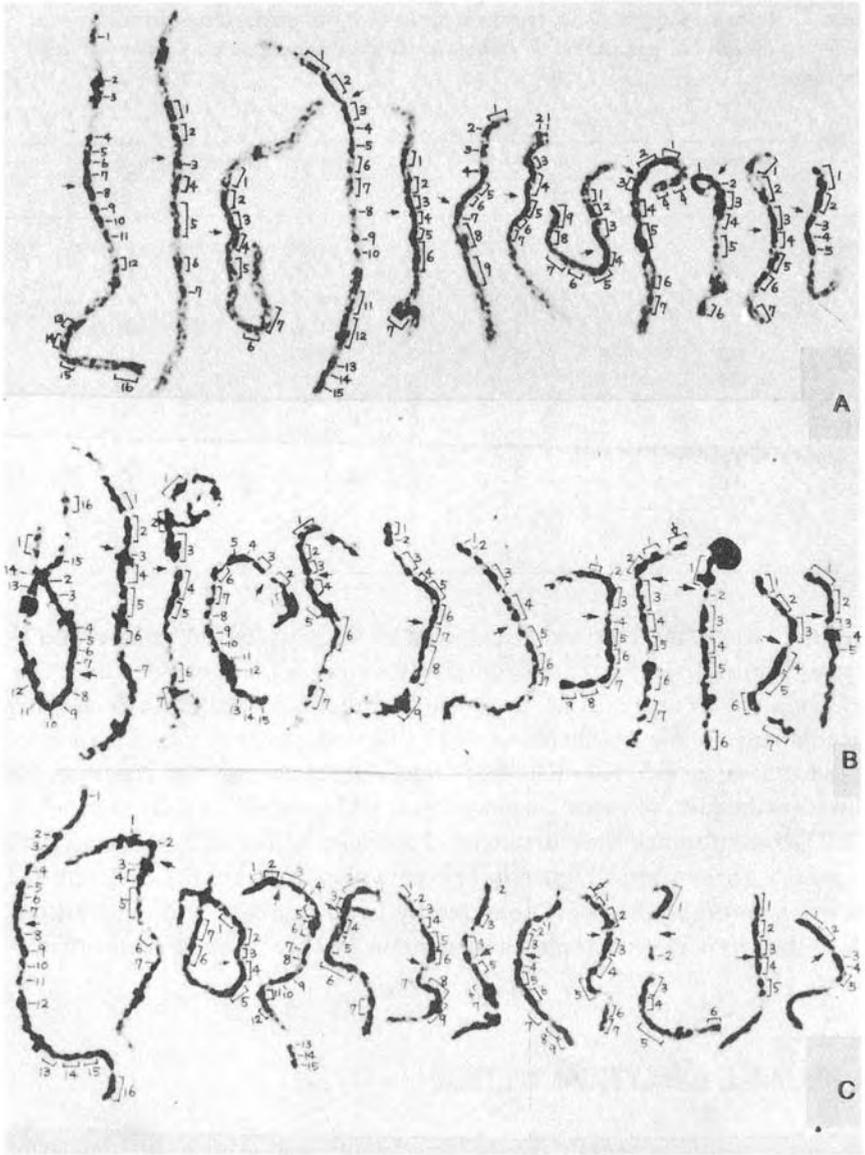


Fig. 6. Twelve late pachytene bivalents of *O. sativa* (A genome) (A), *O. punctata* (B genome) (B), and *O. brachyantha* (F genome) (C) arranged from K1 to K12. K1 showed about 40 μ m length in all three genomes. Centromeres are shown by arrows and chromomere blocks are numbered to correspond with each other among homologous chromosomes. The positions of centromeres and chromomere patterns are very similar in each homologous chromosome. Two minor differences in K1 and K9 are observed. Chromomeres numbered 4, 5, 6, and 7 in K1 seem different in length, and the number of chromomeres is different in the short arms of K9 as shown by white arrowheads.

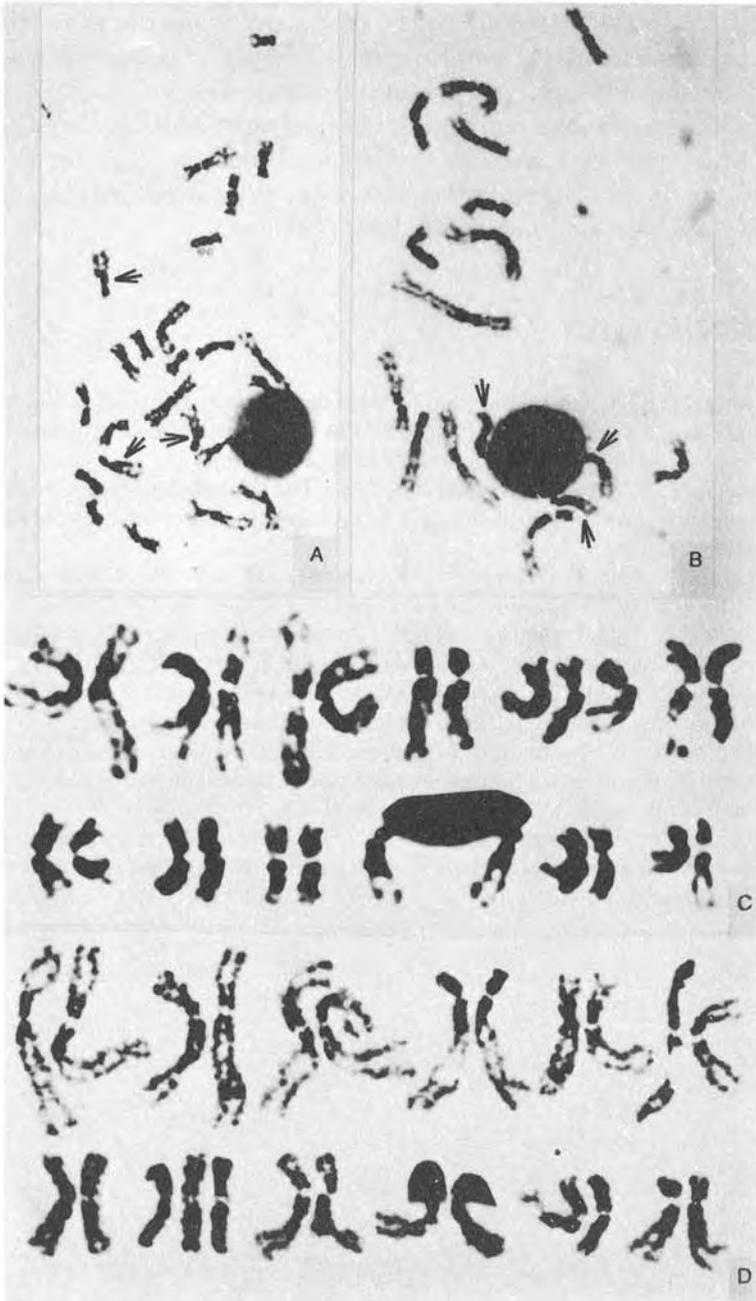


Fig. 7. Trisomic identification in K4, K5, K8, and K10. In a part of the nucleus of a K4 trisomic (A) and a K10 trisomic (B), the trisomics are shown by arrows. Karyotypes of a K5 trisomic (C) and a K8 trisomic (D) are shown.

These DNA fragments would also be good indexes of a rice gene library. The application of in situ hybridization methods to chromosome analysis would be a most reliable and powerful system to discriminate among the 12 chromosomes and to correlate cytological units with functional units. It is now time to start such a system in rice, because up to half the chromosomes of rice are still somewhat difficult to identify, though highly resolvable morphology has been obtained.

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CYTOGENETICS AND FERTILITY OF RICE AUTOTETRAPLOIDS

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Low and unstable fertility is the main problem encountered in the breeding of autotetraploid rice. The observed frequency distribution of pollen mother cells with different number of quadrivalents in autotetraploids fits excellently with the expansion of the binomial $(p + q)^{12}$, where p is the chance of forming bivalents and q the chance of forming a quadrivalent. The occurrence of quadrivalents has a negligible effect on the partial sterility of the rice autotetraploid. Fertility could be improved a great deal by conventional cross breeding. Elite plants with normal fertility and good agronomic characteristics have appeared frequently, but stable strains have not yet been isolated. Some improvement of fertility by monohybrid heterosis may occur, and in addition the quadruple state of each locus results in very low probability of occurrence of homozygotes in segregating generations. Diploidization by artificial induction of structural changes in chromosomes would be the best solution for the efficient breeding of tetraploid rice. Clone populations were raised successfully from elite plants by means of tissue culture, and the uniformity of the stands was better than the diploid cultivar. Cloning of elite plants may be a good short cut to the success of autotetraploid rice breeding.

The ultimate objective of the program of rice autotetraploid research initiated in 1951 is to develop promising varieties of tetraploid (4×) rice with comparable or better yield and quality than the cultivated diploid (2×) cultivars. In comparison with the original diploid rice cultivar, the newly converted autotetraploid form is inferior in tillering ability, number of spikelets per panicle, fertility, and grain yield per plant, although it has bigger kernels and higher protein content. About 200 cultivars, including both japonica and indica types with various agronomic characteristics, have been converted to the autotetraploid form by colchicine or Fumiren (3) treatment, thousands of cross combinations have been made among them, and their hybrid progeny have been examined for good agronomic characters and normal fertility. After 34 years of breeding work, we have obtained many elite plants with good tillering ability, large panicles, large grains, high protein content, normal fertility, and good yield per plant. Some of the clones derived from these elite plants by means of tissue culture have been tested by yield performance trials with replications, and the resulting yields are on same level as

the best newly released diploid rice varieties such as Zhong-Hwa 8 in the Beijing area (2). The normal fertility of the elite plant, however, cannot be maintained by seed propagation in later generations. The stability of fertility thus becomes the critical problem in developing tetraploid rice varieties for practical use. So it is time to have a working hypothesis about the cause of partial sterility in autotetraploid rice.

CYTOLOGY

The obvious cytogenetic property of autotetraploid rice is the quadruple state of both chromosomes and genes instead of the duplicate state in the diploid form. There are 12 homologous groups, and the maximum number of quadrivalents in a pollen mother cell (PMC) of an autotetraploid will be 12. However, the average number of quadrivalents per PMC at diakinesis is only 7.3 and the average number of quadrivalents per PMC at metaphase I is only 5.3, as shown in Table 1. There is thus a probability of 0.60 (7.3/12) for the four chromosomes of each homologous group to form a quadrivalent at diakinesis and a probability of 0.44 (5.3/12) at metaphase I. The frequency distribution of PMCs with different numbers of quadrivalents could be calculated by the expansion of the binomial $(p+q)^{12}$, where p is the chance of forming two bivalents and q is the chance of forming a quadrivalent, and $p+q = 1$. The agreement between the observed and expected values is excellent both at diakinesis and metaphase I as tested by goodness of fit. The twelve homologous groups have uniform behavior in quadrivalent formation. The difference of q 's at two meiotic stages is 0.16, meaning that 16% of quadrivalents at diakinesis are converted to bivalents at metaphase I due to terminalization of chiasmata. No significant differences have been found among the various $4\times$ material. Although their fertility varies greatly, it is obvious that the occurrence of quadrivalents in meiotic division has little or practically no effect on the partial sterility of autotetraploid rice. For this reason we paid little attention to cytological analysis of $4\times$ material after the 1950s.

GENETICS

In the 1950s we collected data on the fertility of various $4\times$ material and attempted to find some intrinsic mechanism of fertility fluctuation. Japonica and indica varieties are apparently different in response to the

Table 1. Frequency distribution of pollen mother cells (PMCs) with various numbers of quadrivalents (4).

4x varieties or crosses	PMCs with given quadrivalent number												Total	Mean IV/PMC	
	1	2	3	4	5	6	7	8	9	10	11	12			
<i>Diakinesis</i>															
Shui Yuan 52 (japonica)			1	1	4	2	4	6	3					21	6.8
Chuan Nong 422 (indica)					2	4	5	3	4	4	2			24	8.0
Shui Yuan/Chuan Nong (376-4, F ₆)			1	2	7	5	12	12	3	3	0	1		46	7.1
Shu Yuan/Chuan Nong (7143-12, F ₆)					3	4	5	5	3					20	7.1
B-III A/Chuan Nong (F ₁)							1	3	5	5	1			15	8.1
2x (Ning Fang/Nan Te Hao (F ₁ colchicine doubled)				3	2	9	6	6	4	3				33	7.0
Total	0	0	2	6	18	25	35	37	22	11	2	1	159	7.3	
Expected (p+q) ¹² , q = 0.60 (7.3/12)	0	0.4	2.0	6.7	16.0	28.0	36.1	33.8	22.0	10.2	2.8	0.4	159	x ² = 2.58 p = 0.99	
<i>Metaphase I</i>															
Shui Yuan 52		3	3	5	2	6	1	1						21	4.6
Chuan Nong 422			1	4	3	1	3	2	1					15	5.7
Shui Yuan/Chuan Nong (376-4, F ₆)				1					1	1				3	6.7
Shui Yuan/Chuan Nong (7143-12, F ₆)	1	1	2	2	2	5	3	1						17	5.1
B-III A/Chuan Nong (F ₁)				8	11	12	5	4	1					41	5.7
Total	1	4	7	19	18	24	12	9	3				97	5.3	
Expected (p+q) ¹² , q = 0.44(5.3/12)	0.9	3.8	9.9	17.4	21.9	20.1	13.5	6.6	2.3	0.5	0.1	97	x ² = 4.22 p = 0.95		

primary effect of chromosome doubling. As shown in Table 2, japonica varieties seem to have a very serious primary effect, resulting in very low fertility, usually below 20%, and after two or three generations they recover to some extent to around 50%. On the other hand, the newly formed autotetraploids of indica varieties usually have a fertility around 50% and remain at that level in later generations.

Table 2. Fertility (%) variation in primary autotetraploids of japonica and indica varieties (4).

	Variety	Cheng Tu		Beijing			Year of doubling
		1954	1956	1957	1958	1959	
Japonica	Ning Fang	13.1	42.1	45.4	46.2	51.7	1951
	Shui Yuan 52	19.4	33.5	35.0	26.6	40.9	1951
	B-III A	—	—	—	11.1	11.3	1957
Indica	Chuan Nong	57.8	49.0	36.1	73.0	69.6	1951
	Nan Te Hao	—	—	51.1	40.0	47.2	1956
	Zhe Chang 3	—	—	—	71.6	62.9	1957

In diploids, the F_1 hybrid of a cross between typical japonica and indica varieties is very low in fertility, usually only a few percent. Unexpectedly, in tetraploids the F_1 is much more fertile, but fertility varies widely among individual plants, from 18.0% to 63.5% as shown in Table 3. The large tillers detached from the mother plant could be propagated freely. The fertility of the tiller plant fluctuates from 23.3% to 74.6%, a range more wide than that of the mother plant. An apparent difference can also be found in hybrid plant 49-1, whose mother plant had a fertility of 29.4% while the tiller plant had a fertility of 74.6%. These fluctuations in fertility could not be attributed to genetic variation. Most probably the reason was environmental. So in the 1950s we got the strong impression that the newly formed rice autotetraploids, either primary varieties or their hybrids, are very sensitive to fluctuations in external conditions. If this were true, then the selection of plants with high fertility in hybrid progeny should be low in efficiency. Since then, this kind of reasoning has been used to explain why the progress of tetraploid breeding work has been so slow, and why the selected elite plants with normal fertility cannot be maintained in later generations.

CLONING

Since 1981, autotetraploid rice clones have been raised successfully by tissue culture from elite plants selected from hybrid progeny of various crosses. A clone population not only will retain the high fertility of the elite mother plant but also can express excellent uniformity, even better than the widely used diploid cultivar, as shown in Table 4. The corres-

Table 3. Fertility variation among the individual plants of 4× japonica/indica hybrid F₁, 1954 (1).

Cross	Plant code number	Mother plant		Tiller plant	
		Fertility (%)	Spikelets per panicle	Fertility (%)	Spikelets per panicle
Ning Fang/Chuan Nong 442	49-I	29.4	119.0	74.6	149.5
	49-II	26.5	147.0	23.3	133.0
	49-III	45.1	144.0	46.4	132.5
	50	33.2	183.5	46.6	206.0
	Average	32.4	148.4	47.7	155.3
Shui Yuan/Chuan Nong 422	52-I	63.5	100.5	48.3	159.5
	52-II	62.3	92.3	38.1	214.0
	52-III	18.0	97.5	—	—
	Average	47.9	96.8	43.2	186.8

Table 4. Comparison of fertility between clones of elite 4× plants and their sexual progeny (2).

Code number of elite plant	Clone			Sexual progeny		
	Number of plants	Fertility (%)	SD	Number of plants	Fertility (%)	SD
V 049 F ₃	150	85.2	10.53	154	83.3	11.15
V 072 F ₃	134	84.3	7.34	142	77.7	13.70
V 1032 F ₃	109	85.0	7.80	131	79.9	11.80
Jing Yue 1 (2× cultivar)	—	—	—	152	87.4	10.84

ponding sexual lines, without exception, cannot maintain the fertility level of their mother plants. The standard deviation (SD) of the mean fertility could be used as a measure of uniformity of the plant sample. Without exception, the sexual lines always have a higher SD than the clones. Autotetraploid rice clones are even more uniform in fertility within the population than the diploid pure line varieties. This fact reveals that autotetraploid rice cannot be more sensitive to environmental fluctuation than diploid rice. So the great variation of fertility in autotetraploid rice is mainly genetic rather than environmental.

HYPOTHESIS

When a diploid variety of rice with normal fertility is converted to an autotetraploid with low fertility, some quantitative balance among the genes might be disturbed. For example, we may suppose that the gene combination $a^+a^+b^+b^+$ is favorable for fertility in diploids, but its quadruple state $a^+a^+a^+a^+b^+b^+b^+b^+$ may have unfavorable effects on fertility due to the different dosage effect of different genes. When some of the genes with stronger dosage effects are substituted by their mutant allelic genes, as $a^+a^+a^+a^+b^+b^+bb$, the favorable effect on fertility may be recovered or partially recovered. Then both $b^+b^+b^+b^+$ and $bbbb$ are low in fertility, while b^+b^+bb is better. The partial recovery of fertility in this way in autotetraploids may be considered as monohybrid heterosis and thus cannot be isolated in the homozygous condition. This may be a part of the reason why stable strains are so difficult to develop from elite plants with normal fertility. They are just melted away in later generations, or they may be maintained at a certain level of fertility by careful selection but always fluctuate to a certain extent.

But another situation is also possible — the quadriplex mutant gene b may be favorable for fertility restoration. In this case, the improvement of fertility could be maintained by the homozygous state of the genes concerned. This partial restoration of fertility was manifested in some crosses having better average fertility level in the progeny. However, as the number of such genes increases, the population size required for the occurrence of the homozygous individual would increase tremendously. In diploids, the F_2 population of a monohybrid consists of 50% heterozygous and 50% homozygous individuals. In the case of n genes, the chance of homozygous individuals for all n genes in the F_2 population is $(1/2)^n$. The corresponding value in autotetraploids is $(1/18)^n$. It would be very difficult to isolate homozygous individuals in autotetraploid rice hybrids when the n value reaches 5 or more, as frequently happens in quantitative traits.

Theoretically, it is possible to diploidize the autotetraploid by artificial induction of structural changes in chromosomes, and the four homologous chromosomes would be thus separated into two pairs and form bivalents instead of a quadrivalent. Consequently, the mode of inheritance would change radically. For instance, b^+b^+bb could be bred true if the chromosome with the b locus has been changed structurally and would not pair with the original chromosome carrying the b^+ gene.

The segregation ratio would be different, too. For duplicate genes, the dihybrid F_2 would segregate in a ratio of 9:3:3:1, four of which or 25% are homozygous instead of 1/18 in the quadruple condition. So the selection would be more efficient when an autotetraploid is diploidized.

CONCLUSION

In conclusion, we now have three trends in the research work on autotetraploid rice, namely: conventional cross breeding, diploidization by structural changes in chromosomes, and cloning of elite plants from cross breeding by tissue culture.

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GENETIC STUDIES ON TRISOMIC-LIKE PLANTS OF *ORYZA SATIVA* L. DERIVED FROM THE INTERCHANGE HOMOZYGOTE RT 2 - 3 b · T 65

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To learn why many abnormal or trisomic-like plants segregate in F_2 plants involving the interchange homozygote, genetic and cytological studies were made using Taichung 65, its isogenic interchange homozygote RT2-3b·T65, and linkage testers. The appearance of trisomic-like plants was about one quarter of the F_2 . Further breeding behavior of the trisomic-like plants was the same as that of the trisomics. However, it was evident from cytological observations that the plants were not trisomics; associations of 4 chromosomes were formed at diakinesis, and cells at M-11 had 12 chromosomes. It was estimated that the unbalanced gamete 2^3-3 produced by the adjacent-I disjunction developed normally in both gametogeneses, and that trisomic-like plants resulted from the unbalanced female gamete fertilized with one of two balanced gametes — normal (2-3) and interchanged (2^3-3^2) — since most of trisomic-like plants had the nl_1^+ allele on the nl_1 (neck leaf) locus located on the second chromosome derived from RT2-3b·T65 and the *A* gene (anthocyanin activator) located on the third chromosome from the linkage tester T65·A *Pn*.

Interchange homozygotes are one of the important materials for looking into relations between genes and chromosomes. In linkage studies by the use of interchanges, abnormal or trisomic plants are rarely observed. Nishimura (4) suggested that trisomics in rice are produced by nondisjunction of chromosomes involving associations of four chromosomes. There was, however, an exceptional interchange homozygote RT2-3b·T65; many abnormal or trisomic-like plants were segregated in a selfed progeny of the interchange heterozygote. To learn why trisomic-like plants frequently segregated, the author studied these plants genetically and cytologically.

MATERIALS AND METHODS

Four linkage testers, Taichung 65 (a japonica cultivar from Taiwan), and the interchange homozygote RT2-3b·T65 were used in this study. The linkage testers had the same genetic background as that of Taichung 65 except for their respective marker genes, *nl*₁ (neck leaf), *gl*₁ (glabrous leaf), *A* (anthocyanin activator) and *Pn* (purple node), and *Hg* (hairy glume). They were obtained by recurrent backcrossing with Taichung 65 (Table 1). The interchange homozygote was cytologically examined by using a series of reciprocal translocation homozygotes that were established by Nishimura (4) and Iwata (1) in order to determine which chromosomes are involved (6, 9). Nomenclature of the chromosomes followed Nishimura's (4) formula. The interchange homozygote was selected from Taichung 65 after x-ray treatment and was backcrossed ten times with Taichung 65 (the first three backcrosses by H. I. Oka and Y. Sano at the National Institute of Genetics, Misima, and the subsequent seven by the present author). The linkage testers and the interchange homozygote could then be considered as isogenic lines of Taichung 65. Out of the isogenic linkage testers, T65·*nl*₁ was bred by Oka and Sano, while the others were bred by the present author.

Table 1. Linkage testers used and their marker genes.

Linkage tester	Marker gene ^a	Character expression	Backcross generation
T65· <i>nl</i> ₁	<i>nl</i> ₁	Neck leaf	BC ₇
T65· <i>gl</i> ₁	<i>gl</i> ₁	Glabrous leaf and hull	BC ₁₄
T65· <i>APn</i>	<i>A</i>	Anthocyanin activator	BC ₁₁
	<i>Pn</i>	Purple node	BG ₁₁
T65· <i>Hg</i>	<i>Hg</i>	Hairy glume	BC ₈

^a Obtained from linkage testers by Nagao and Takahashi (3).

Segregation of trisomic-like plants was studied in F₂ plants between the interchange homozygote and five normal lines, four linkage testers, and Taichung 65, and in BC₁F₁ plants (RT2-3b·T65/Taichung 65/Taichung 65). Segregations in selfed progeny of trisomic-like plants selected from the BC₁F₁ plants, and those in reciprocal crosses between trisomic-like plants and Taichung 65, were also examined. Five trisomic-like plants carrying the *nl*₁ gene in the heterozygous state were selected from the F₂ plants between RT2-3b·T65 and T65·*nl*₁, and their selfed progeny were

grown to learn the combined segregation of the gene and the plant type. Ten selfed progeny of trisomic-like plants having the *A* gene were also cultivated.

Segregating populations were grown in the experimental field of the College of Agriculture, University of the Ryukyus, from 1980 to 1984 at a spacing of 15×20 cm with a single plant/hill. Fertilizers (150 kg/ha each of N, P, and K) were supplied in a ratio of 5:3:2 basal to topdress 1 to topdress 2.

Several agronomic characters of normal chromosome homozygotes, interchange heterozygotes, and trisomic-like plants were examined using 40 plants each selected randomly from BC_1F_1 plants.

Young panicles were fixed for cytological observation in 1:3 acetic-alcohol fluid containing a trace of $FeCl_3$. The cytological preparations for meiosis were made by the usual acetic-carmines smear method.

RESULTS

Trisomic-like plants were distinguished morphologically from normals or interchange heterozygotes by the presence of a lopped flag leaf. When the plants were recessive homozygotes for the nl_1 gene, the second leaf from the top was lopped. Several other agronomic characters of trisomic-like plants were also significantly different from those of normals: trisomic-like plants showed poor tillering, short ear and culm lengths, and long and narrow grain, whereas their leaf blade and leaf sheath lengths were similar to those of normals (Table 2). Shortening the culm of trisomic-like plants seemed to be caused by shortening all of the internodes. Their pollen and spikelet fertilities were quite high. The interchange heterozygotes showed remarkably high fertilities compared with those of other interchanges of rice, which are generally lower than 50%. It was also observed that the uppermost internode length of interchange heterozygotes (IN 1) was significantly reduced.

Many trisomic-like plants were observed in the F_2 and in BC_1F_1 when the interchange heterozygote was used as the maternal plant (Table 3). The appearance of trisomic-like plants was about 25.2%, and segregation of plant types fit well in the ratio of 3:1 normal to trisomic-like plants. Two sorts of plants segregated nearly evenly in selfed progeny of trisomic-like plants selected from BC_1F_1 plants (Table 4). The same segregation data were obtained in a cross between trisomic-like plants and Taichung 65. However, none of the abnormal plants were observed in the reciprocal cross between Taichung 65 and trisomic-like plants (Table 5).

Table 2. Comparison of several agronomic characters between normals (Nor), interchange heterozygotes (IH), and trisomic-like plants (TLP) in BC₁F₁ of RT2-3b.T65/T65//T65.^a

Group	Character									
	No. of ears	Ear length (cm)	Culm length (cm)	Internode length (cm)				Leaf blade length (cm)		
				In 1	In 2	In 3	In 4	Lb 1	Lb 2	Lb 3
Nor	7.6	20.2	101.7	41.6	25.2	18.9	11.8	26.3	33.5	47.8
IH	7.6	19.8	99.9	39.0***	24.5*	19.1	12.8*	24.9*	33.1	46.7
TLP	3.5***	16.7***	83.4***	34.9***	21.2***	13.6***	10.5***	24.9*	33.2	45.4**

Group	Character						
	Leaf sheath length (cm)			Unhulled grain (mm)		Fertility (%)	
	Ls1	Ls 2	Ls 3	Length	Width	Spikelet	Pollen
Nor	31.1	24.7	24.5	6.68	3.49	87.1	98.0
IH	30.7	24.5	24.0	6.63**	3.49	61.7***	70.7***
TLP	28.8*	24.3	24.8	7.11***	3.22***	76.2***	86.1***

^a* = significant at the 5% level, ** = significant at the 1% level, *** = significant at the 0.1% level.

Table 3. Segregation of normals (Nor) and trisomic-like plants (TLP) in BC₁F₁ and F₂.

Cross combination	Segregation mode		Plants (no.)	TLPs (%)	c ² value (3:1)
	Nor	TLP			
BC ₁ F ₁ ; (RT2-3b-T65/Taichung 65 ²)	186	62	248	25.0	0.000
F ₂ ; (RT2-3b-T65/Taichung 65)	188	72	260	27.7	1.005
F ₂ ; (RT2-3b-T65/T65-Hg)	752	248	1000	24.8	0.021
F ₂ ; (RT2-3b-T65/T65-gl ₁)	771	244	1015	24.0	0.500
F ₂ ; (RT2-3b-T65/T65-nl ₁)	1240	427	1667	25.6	0.336
F ₂ ; (RT2-3b-T65/T65-A Pn)	431	148	579	25.6	0.097
Grand total	3568	1201	4769	25.2	0.086

Table 4. Segregation of normal fertiles (Nor F), normal semi-steriles (Nor S), and trisomic-like plants (TLP) in selfed progenies of TLPs selected from BC₁F₁ plants.

Generation	Segregation mode			Plants (no.)	c ² value (1:1)
	Nor F	Nor S	TLP		
F ₂	212	0	178	390	2.964
F ₃	158	0	139	297	1.216
Grand total	370	0	317	687	4.089* ^a

^a* = significant at the 5% level.

Table 5. Segregation of normal fertiles (Nor F), normal semi-steriles (Nor S), and trisomic-like plants (TLP) in reciprocal crosses between TLP and Taichung 65.

Cross combination	Segregation mode			Plants (no.)	c ² value (1:1)
	Nor F	Nor S	TLP		
TLP/Taichung 65	184	0	150	334	3.461
Taichung 65/TLP	310	0	0	310	

Trisomic-like plants were transmitted by the female gamete, as in true trisomics.

Cytological observations showed that trisomic-like plants form associations of four chromosomes at diakinesis and produce pairs of cells having 12 chromosomes (Table 6). This means that the plants are not trisomic, and that one or two interchange chromosomes are taking part in the expression of abnormality.

Linkage relations between the breakpoint 2-3b and marker genes were studied by the use of normal-type plants segregated in the F_2 between RT2-3b·T65 and four linkage testers (Table 7).

Table 6. Cytological observations of pollen mother cells of trisomic-like plants.^a

Chromosome association at diakinesis			Cells (no.)
12II	10II + (4) R	10II + (4) C	
25	2	88	115

No. of chromosomes in MII cell		Cells (no.)
12 + 12	Others	
28	0	28

^aC = chain, R = ring.

Sato et al (7) reported that the two genes nl_1 and gl_1 are located on the second chromosome, and that the nl_1 gene is closely linked with the breakpoint 2-3b, whereas the gl_1 gene is independent of it. These linkage relations were confirmed in this study. Two genes, A and Pn on the third chromosome (5), are closely linked with the breakpoint, having recombination values of 0.0 and 12.2%, respectively. The Hg gene is independent of the breakpoint (Table 7). The linkage intensity between the two genes A and Pn is 11.7% (Table 8).

Segregations of genes linked with the breakpoint 2-3b were all significantly different from a ratio of 3:1 in trisomic-like F_2 plants, while those of genes independent of the breakpoint fit the ratio well (Table 9). In the first trial of F_2 plants between RT2-3b·T65 and T65· nl_1 , only six plants showed the neck leaf character. In the second, no such plants were observed, and dominant homozygotes and heterozygotes for the gene nl_1

Table 7. Combined segregations of spikelet fertility and marker genes in normal F₂ plants between RT2-3b·T65 and linkage testers.

Marker gene	Segregation mode ^a				Plants (no.)	c ² value for indep. (df = 1) ^b	Goodness of fit	
	AF	AS	aF	aS			R.C.V. (%)	c ² value
<i>Hg</i>	308	285	90	69	752	0.496		
<i>gl</i> ₁	306	289	86	90	771	0.364		
<i>nl</i> ₁	1	151	253	142	8	554		
	2	155	343	184	4	686		
Total	306 (321.0)	596 (609.0)	326 (299.0)	12 (11.0)	1240	408.017***	1.8 ± 0.5	3.507 (df = 3)
<i>A</i>	123 (107.8)	203 (215.5)	105 (107.8)	0	431	120.669***	0.0	2.954 (df = 2)
<i>Pn</i> ^c	120 (107.0)	182 (194.1)	3 (24.9)	21	326	17.734***	12.2 ± 2.9	2.347 (df = 2)

^aAF = dominant fertile, AS = dominant semisterile, aF = recessive fertile, aS = recessive semisterile. Numbers in parentheses indicate expected numbers based on their recombination values.

^b*** = significant at the 0.1% level.

^cCharacter expression is caused by the *A* gene.

Table 8. Combined segregation of two genes *A* and *Pn* in normal F_2 plants between RT2-3b·T65 and T65·A *Pn*.

Phase	Segregation mode ^a Plants			(no.)	χ^2 value ^b (9:3:4)	Goodness of fit	
	<i>A</i> _ ₋ <i>Pn</i> _ ₋	<i>A</i> _ ₋ <i>Pn</i> + <i>Pn</i> + ₋	<i>A</i> + <i>A</i> +_ ₋ ₋			R.C.V. (%)	χ^2 value ^b
Couple	302 (299.5)	24 (23.8)	105 (107.8)	431	54.671***	11.7 ± 2.5	0.095

^aNumbers in parentheses show expected numbers based on the recombination value.
^bDegrees of freedom = 2, *** = significant at the 0.1% level.

were segregated evenly (Table 9). Normals segregated in selfed progeny of trisomic-like plants carrying the *nl₁* gene in the heterozygous condition were all recessive homozygotes, whereas trisomic-like plants were mostly heterozygotes (Table 10). Trisomic-like plants obtained from the cross RT2-3bT65/T65·A *Pn* all had the *A* gene, and normals appeared in their progeny showing the recessive character for the *A* gene, while trisomic-like plants in these progeny and all plants in other progeny carried the *A* gene (Tables 9, 11).

Table 9. Segregations of marker genes in trisomic-like F₂ plants between RT2-3b·T65 and linkage testers.

Marker gene	Segregation mode			Plants (no.)	χ ² value	
	AA	A+	+ +		3:1 ^a	1:1:0
<i>Hg</i>		189	59	248	0.194	
<i>gl₁</i>		190	54	244	1.071	
<i>nl₁</i>	1	190	6	196	50.313***	
	2	110	221	231	77.000***	0.524
<i>A</i>		148	0	148	49.333***	
<i>Pn</i>		139	9	148	28.252***	

^a*** = significant at the 0.1% level.

DISCUSSION

The fact that trisomic-like plants segregated constantly at a high frequency, about 25.2% in F₂ and BC₁F₁ (Table 3), shows that the plants did not appear spontaneously. Although further genetic behavior of the plants was the same as that of trisomics (Tables 4, 5), it is obvious that they were not trisomic, for they produced pairs of cells having 12 chromosomes at the M11 stage, which corresponds to the chromosome number of a haploid plant of rice (Table 6). Since the plants formed associations of four chromosomes at diakinesis, some structural aberrations involving one or two interchange chromosomes were estimated to be responsible for the development of trisomic-like plants. As the interchange heterozygote produced many trisomic-like plants, some unbalanced gametes by adjacent disjunctions are estimated to be functional. The present author (5) reported from the tetrad analysis of interchange

Table 10. Combined segregation of plant type and the nl_1 gene in selfed progenies of trisomic-like F_2 plants carrying the nl_1 gene in heterozygotes.

Family	Normal plant			Trisomic-like plant		
	+ +	+ nl_1	nl_1nl_1	+ +	+ nl_1	nl_1nl_1
1	0	0	35	0	30	0
2	0	0	32	1	21	0
3	0	0	28	0	22	0
4	0	0	25	0	26	0
5	0	0	38	0	26	0

heterozygotes involving the first or nucleolus chromosome that the adjacent-II disjunction occurred in 5.5% to 11.0% of cases, and that the adjacent-I and the alternative disjunctions appeared nearly as often. It was then considered that one of two unbalanced gametes, 2^3-3 and $2-3^2$, derived from the adjacent-I disjunction, was developed normally in both gametogeneses, and that the male gamete was not fertilized by certation with other balanced gametes, $2-3$ and 2^3-3^2 , for trisomic-like plants showed the same feature. Segregation of plant types in the F_2 and BC_1F_1 was, however, significantly biased from the ratio of 2:1 expected from the frequency of two disjunctions, the alternative and the adjacent-I, and it fit well the ratio of 3:1 (Table 3). These results suggest that the survival value of unbalanced female gametes is low, which is supported by the observation that segregation in selfed progeny of trisomic-like plants was also biased from the ratio of 1:1 (Table 4). The pollen fertility of the interchange heterozygote fell in the expected fertility range of 66.8% to 70.9% based on the appearance of three disjunctional types (5) as shown in Table 2. All trisomic-like F_2 plants carried the gene *A* on the third chromosome derived from T65·*A Pn*, and most of the plants had the nl_1^+ gene on the second chromosome, which originated in RT2-3b·T65; this shows that the unbalanced gamete 2^3-3 was functional (Table 9), which was confirmed in pedigree tests of trisomic-like plants carrying the nl_1 gene in the heterozygous state or the *A* gene (Tables 10, 11). Normals observed in offspring of heterozygously trisomic-like plants for the nl_1 locus are expected to be recessive homozygotes, while trisomic-like plants are heterozygous, as observed in all five families (Table 10), since mother plants might be developed from eggs with $2^3-3/2-3$ and the gene was closely linked with the breakpoint 2-3b (Table 7). The gene *A* was always carried by the normal third chromosome (Table 7). Conse-

quently, when trisomic-like plants were $2^3-3/2^3-3^2$, normals in their offspring might show the recessive character for the *A* gene, as observed in three families, and if the plants were $2^3-3/2-3$, all of their offspring carried the gene, as actually seen in seven (Table 11).

Table 11. Combined segregation of plant type and the *A* gene in selfed progenies of trisomic-like F_2 plants carrying the *A* gene.

Family	Normal plant		Trisomic-like plant	
	<i>A</i> ⁻	<i>A</i> ⁺ <i>A</i> ⁺	<i>A</i> ⁻	<i>A</i> ⁺ <i>A</i> ⁺
1	0	18	16	0
2	0	26	26	0
3	0	27	24	0
4	28	0	25	0
5	18	0	19	0
6	28	0	19	0
7	23	0	21	0
8	24	0	21	0
9	20	0	22	0
10	24	0	19	0

The trisomic-like plant is estimated to be partially monosomic for the second chromosome and trisomic for the third. Then the plant may be useful for linkage studies.

The phenomenon that an unbalanced gamete produced by an interchange heterozygote was functional was detected for the first time in a plant, having been previously observed in humans and *Drosophila*; Down's syndrome involving a "15/21" interchange is estimated to result from the same causes noted in this study, whereas the appearance of fertile unbalanced gametes and their breeding behavior in *Drosophila* is considerably different. Four sorts of unbalanced gametes from the adjacent-I and -II disjunctions were fertile; viable zygotes, however, come from the union of unbalanced gametes, providing the two gametes concerned carry complementary segregation products (8).

The linkage intensity of 1.8% obtained between the breakpoint 2-3b and the *nl*₁ gene (Table 7) was significantly different from that reported by Sato et al (7). The discrepancy seems to come from the fact that they examined all F_2 plants, without discarding trisomic-like plants; their segregation for the gene was significantly different from the ratio of 3:1. The arrangement of *A*-2-3b-*Pn* was estimated from a linkage study

among them (Tables 7, 8). The recombination value for $A-Pn$ of 11.7% obtained in this study was significantly lower than the value of 29.9% obtained in an experiment using normal chromosome lines (3). The reduction of the recombination value was estimated to result from the presence of the breakpoint 2-3b between two genes, as Sato (5) observed.

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CYTOGENETICS OF TRISOMICS IN INDICA RICE

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Twelve primary trisomics corresponding to the haploid chromosomes of rice were isolated from the progeny of a spontaneous autotriploid of the cultivar Sona. These trisomics are morphologically different from each other as well as from their disomic parent. Conspicuous morphological differences were observed with regard to growth habit, panicle length, leaf shape and size, ligule length, spikelet size, presence of awn, and size of anther and pistil. The trisomics showed pollen and spikelet sterility ranging from 0 to 100%. Except Triplo-12 and -11, all trisomics showed reduced vigor. The reduced vigor and fertility of trisomics were interpreted to be due to disturbed genic balance caused by the addition of an extra chromosome. The female transmission rate of the extra chromosome of each trisomic ranged from 16% in triplo-1 to 50% in triplo-12, the shortest chromosome. The extra chromosomes were numbered in descending order of length, and accordingly primary trisomics were designated as triplo-1 to triplo-12.

Although the utility of trisomics in assigning genes and respective linkage groups to specific chromosomes has long been realized following the classical discovery of different trisomics types in *Datura* (2,3,4), such an elegant technique could not profitably be employed in rice, as the earlier investigators could not isolate a complete set of 12 trisomics (8,9,16,17,21). Only recently, however, a beginning was made in this direction after the identification of a complete set of primary trisomics in Taiwanese and Japanese rices (6,7,26). Since the two subspecies of rice, japonica and indica, are known to have nonidentical linkage groups (14,15), it is desirable to develop a complete set of primary trisomics in indica rice to use them in genetic studies.

IRRI in the Philippines and the Central Rice Research Institute (CRRI) in India have independently established all the 12 primary trisomics in the background of two different indica varieties, IR36 and Sona (11). In this paper, the morphological and cytological identification and breeding behavior of the primary trisomics of Sona are reported.

MATERIALS AND METHODS

A spontaneous triploid was identified in the rice variety Sona. Of the 241 seeds obtained from the triploid, only 104 plants grew to maturity, 21 of which were found on chromosome analysis to be primary trisomics. These were grown in earthen pots each containing 5 kg of soil mixed with 500 g of well decomposed farmyard manure.

These 21 plants were divided into 12 different groups based on morphological features. Diploid Sona was grown under similar conditions for comparison. One representative trisomic from each group was pollinated with diploid Sona. The seed progeny of each trisomic was grown in a small plot and morphological observations were taken of those plants that resembled the parent trisomic maintained in pots. Chromosome counts were done on these plants for further confirmation.

Identification of extra chromosomes was done at the pachytene stage of meiosis following the chromosome numbering system described by Shastri et al (22). Each extra chromosome was identified on the basis of length, arm ratio, and chromomeric pattern. The trisomics were designated triplo-1 to triplo-12, triplo-1 being the trisomic for chromosome 1 and so on.

All 12 trisomics were grown at CRRRI during the 1982 wet season along with the 12 trisomics developed at IRRI for comparison.

RESULTS

Morphology of primary trisomics

The primary trisomics were identified on the basis of distinct morphological characters and were grouped into 12 classes. Characters like plant height, leaf type and disposition, panicle size, and presence of awns were most reliable for identification of trisomics. Based on the presence of an extra chromosome present in them, they were designated as triplo-1 to triplo-12. The identification of each trisomic in the background of the two varieties agreed with each other. Thus triplo-1 in Sona was identical to triplo-1 in IR36 and so on. A representative picture of all trisomics with a disomic is shown in Figure 1. Salient morphological features of these primary trisomics are given below.

Panicle length of the trisomics is one of the most distinguishing features. Except triplo-9, triplo-11, and triplo-12, trisomics have shorter panicles in comparison to the disomic. The longest ligule is the salient

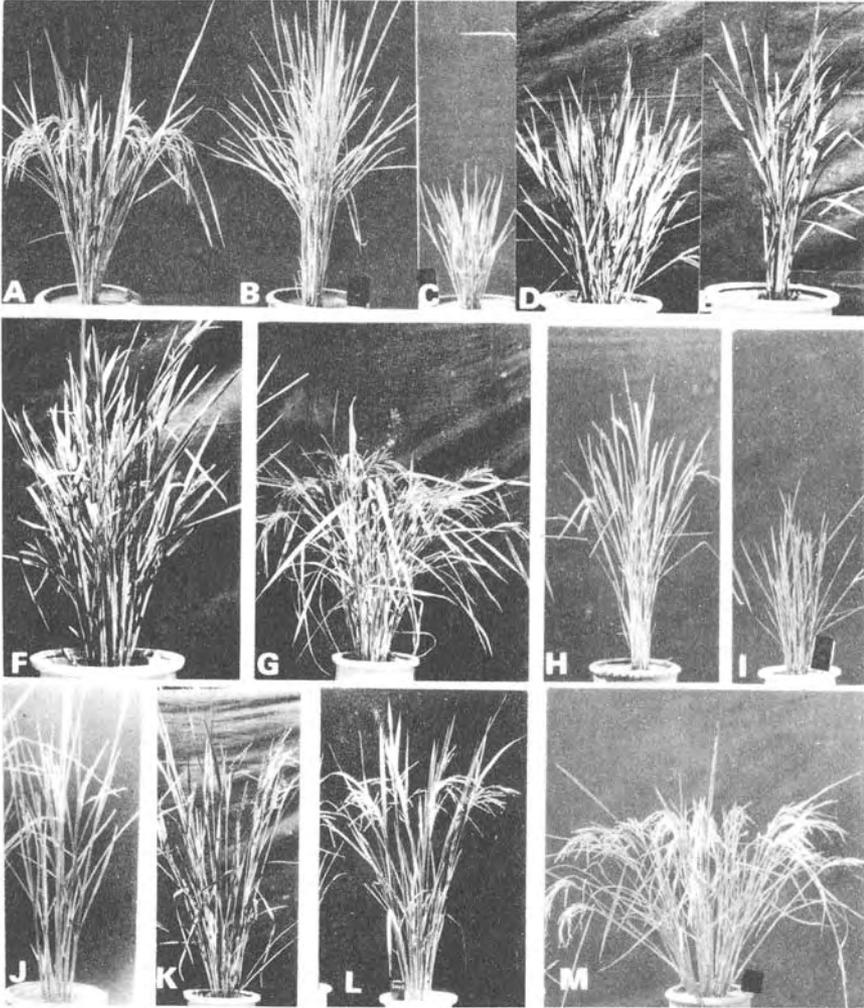


Fig. 1. Typical plant morphology of 12 primary trisomics with disomic Sona. A = disomic, B-M = triplo-1 to triplo-12, respectively.

feature of triplo-11. The comparative morphological features of trisomics and the disomic are given in Table 1. Morphological observations are given for the reproductive parts of trisomics and the disomic (Table 2). The shortest anther occurs in triplo-4 followed by triplo-5, whereas triplo-6 and triplo-12 have the longest anthers. Small ovary and short style are features of triplo-4 and triplo-8.

Table 1. Morphological features of primary trisomics of rice.

Trisomic	Plant height (cm)	Flag leaf		Ligule length (cm)	Culm diameter (cm)	No. of panicles	Panicle length (cm)	No. of spikelets/panicle	Spikelet	
		Length (cm)	Width (cm)						Length (cm)	Width (cm)
Triplo-1	74.0	34.0	0.8	1.5	3.9	19	22.9	157	0.59	0.20
Triplo-2	45.0	16.0	0.8	1.2	4.0	18	14.0	62	0.75	0.32
Triplo-3	65.0	23.0	1.2	2.0	4.4	19	17.0	49	0.80	0.25
Triplo-4	47.0	14.0	0.9	0.3	4.5	21	15.0	110	0.60	0.15
Triplo-5	51.0	27.0	1.0	1.0	3.2	17	17.0	98	0.80	0.20
Triplo-6	63.0	19.0	0.9	1.2	4.5	38	26.0	105	1.00	0.20
Triplo-7	67.0	24.0	0.8	0.8	4.6	15	28.0	102	0.90	0.20
Triplo-8	54.0	16.0	0.8	1.3	4.5	15	17.0	101	1.00	0.30
Triplo-9	65.0	36.0	1.0	1.3	5.1	14	28.0	90	1.20	0.20
Triplo-10	101.0	18.0	0.9	1.3	4.1	19	25.0	120	0.58	0.20
Triplo-11	65.0	25.0	1.0	2.9	4.2	13	26.0	126	1.00	0.25
Triplo-12	110.0	38.0	1.5	1.4	4.8	20	30.0	150	0.85	0.35
Disomic	76.0	35.0	1.0	2.6	4.5	14	27.0	125	1.10	0.30

Triplo-1 (grassy). Plants are slightly shorter in height than the control and show slender culm, profuse tillering, and pale, narrow, drooping leaves. The presence of thin and droopy leaves gives the characteristic grassy appearance. Spikelets are short and slender. It flowers very late—almost 20 days later than the disomic. Spikelet fertility is very low.

Triplo-2 (dwarf). This is the shortest among the trisomics and has a semispreading appearance due to nodal bending after flowering. Leaves are short and slightly twisted at the base. The panicle is small, with poor exertion; spikelets are short and broad with a slight tip awn. Sterility is high.

Triplo-3 (awned). Due to the presence of long awns in the spikelets, this can be easily distinguished from other trisomics as well as from the disomic. It has short height with short, erect, dark green leaves. Panicles are short and spikelet fertility is moderate.

Table 2. Reproductive features of primary trisomics of rice.

Trisomic	Pollen fertility (%)	Spikelet fertility (%)	Anther		Pistil		Style/ovary
			Length (μ)	Width (μ)	Ovary length (μ)	Style length (μ)	
Triplo-1	3.9	1.0	1191.6	308.0	620.0	340.0	0.54
Triplo-2	52.9	1.0	1470.0	390.0	1133.3	883.3	0.77
Triplo-3	81.3	17.0	1475.0	335.0	1125.0	495.0	0.44
Triplo-4	0.0	0.2	655.0	315.0	490.0	260.0	0.53
Triplo-5	62.0	15.0	815.0	270.0	1055.0	520.0	0.49
Triplo-6	86.5	50.5	1670.0	310.0	905.0	450.0	0.49
Triplo-7	48.0	14.0	1410.0	380.0	830.0	460.0	0.55
Triplo-8	4.5	1.0	1285.0	350.0	535.0	375.0	0.70
Triplo-9	86.3	58.0	1435.0	365.0	1100.0	500.0	0.45
Triplo-10	77.0	34.0	1370.0	520.0	1462.5	981.2	0.67
Triplo-11	86.7	72.0	1410.0	305.0	855.0	490.0	0.57
Triplo-12	68.2	13.0	1910.0	395.0	1035.0	385.0	0.37
Disomic	96.1	95.0	1652.5	316.2	880.0	570.0	0.64

Triplo-4 (sterile). This plant is sterile and low tillering and has short, thick, dark green leaves and short, compact panicles. The spikelets are shorter, hairy, and the palea is smaller than the lemma. The stamens are small, rudimentary, and vary from 5 to 6. The stigma is mostly tri- to quadrifid. The ovary is comparatively the shortest, the ratio of style to ovary being 0.53. It is completely pollen sterile, and only 0.2% of the seeds set on open pollination.

Triplo-5 (twisted leaf). This is characterized by the presence of pale green, narrow, twisted, hairy leaves. Plants are short with compact, short panicles with poor exsertion. Spikelets are partially fertile. The lemma and palea often remain open after fertilization.

Triplo-6 (bushy). Short height, light green leaves, long flag leaves, and a profuse tillering habit are characteristic features of this trisomic. Panicles are long with moderate spikelet fertility. Spikelets have short tip awns. Degenerated spikelets at the tips of the panicles are a characteristic feature of this trisomic.

Triplo-7 (narrow leaf). Plants are short and have low tillering with characteristic long, narrow leaves that sometimes appear slightly rolled. Spikelets are slender with low fertility.

Triplo-8 (rolled leaf). Short height; low tillering; rolled erect, accicular leaves with short bold spikelets; and poor panicle exertion are the distinguishing features of this trisomic. Pollen and spikelet fertility are low.

Triplo-9 (stout). This has a stout habit with thick, dark green leaves and large spikelets. The stout culm due to a larger culm diameter panicle is long with high pollen and spikelet fertility.

Triplo-10 (short grain). This is slightly taller than the disomic with an erect habit. Its long leaves have the margin curved somewhat inwards. The panicles are long and the rachis wavy with small, round spikelets. It has the longest ovary among the trisomics.

Triplo-11 (pseudonormal). Morphologically, this trisomic is not distinguishable from the disomic except by the presence of a lax type panicle and the longest ligule. It has high fertility, with a style to ovary ratio of 0.57.

Triplo-12 (tall). This is the tallest among the trisomics and the disomic. It has a robust habit in all morphological traits; long, broad leaves; long panicle with bold spikelets; long anthers; and a style to ovary ratio of 0.37. It has moderate fertility.

Identification of extra chromosomes

For the identification of extra chromosomes from the pachytene chromosome pairing of all the trisomics, the pachytene chromosome morphology of disomic Sona was studied. The salient landmarks of each chromosome helped in the identification of the extra chromosome of each trisomic. A disturbing feature of the pachytene chromosome configuration in the trisomics is the formation of a triradial configuration. The extra chromosome, when it remained as a univalent, was difficult to identify because of the lack of chromomeric expression. A full pachytene chromosome complement of disomic Sona as well as the karyotype of each chromosome is shown in Figure 2. A typical trivalent configuration of all trisomics is shown in Figure 2. All the three homologous chromosomes were found to be paired for a considerable part of their length. In the case of nonhomologous pairing of the chromosomes, the extra chromosomes are identified following the chromomeric pattern and centromeric position. In the case

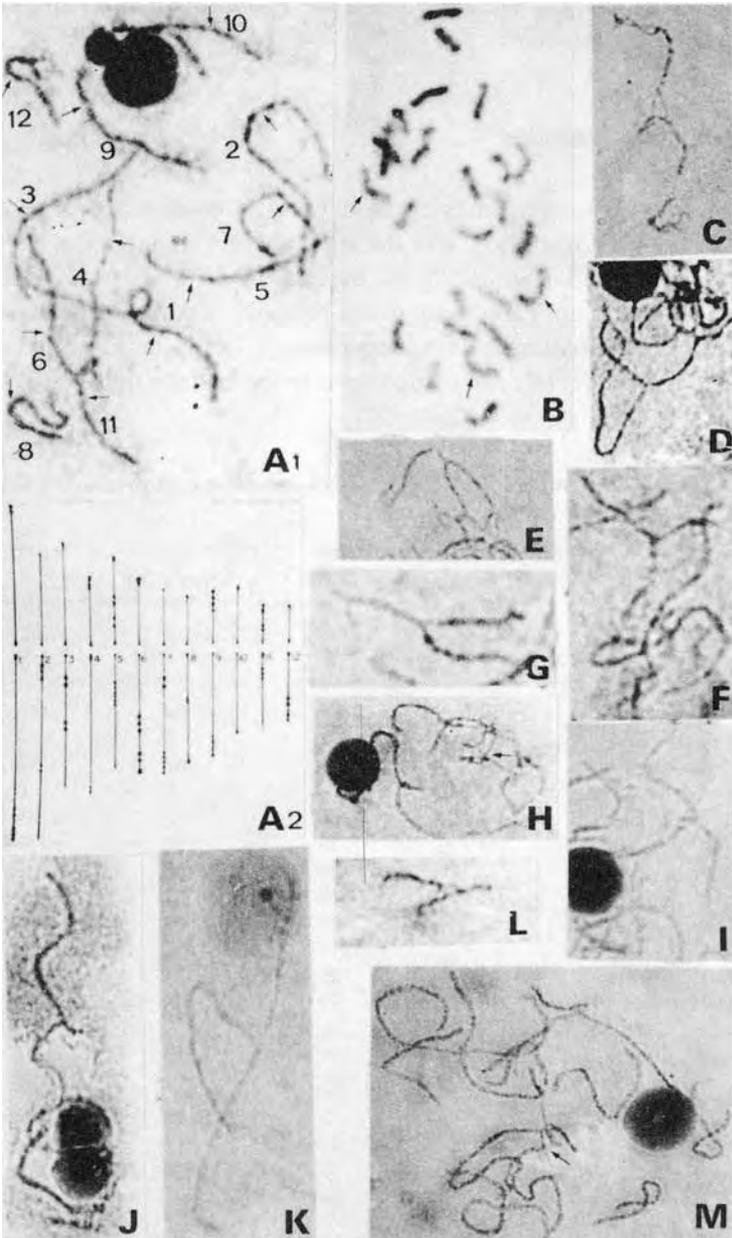


Fig. 2. Pachytene chromosomes of disomic and trivalent configurations of trisomics. A1, A2 = pachytene chromosomes of disomic with idiogram of the chromosome complements (X1250). B = mitotic chromosome complements with three chromosomes of triplo-1 indicated by arrows. C-M = trivalent pairing of triplo-2 to triplo-12, respectively.

of triplo-1, the longest extra chromosome is identified from the mitotic chromosome complement.

Transmission of trisomics

The female transmission rates of the extra chromosomes of all trisomics except triplo-4 were studied, and the transmission frequency of the extra chromosome varied from 4.5% in triplo-2 to 50% in triplo-12. Non-parental trisomics appeared only in the progeny of triplo-11 at the rate of 0.7%. Among the trisomics with longer extra chromosomes, transmission rates through the female are comparatively lower than those of trisomics with shorter chromosomes (Table 3).

Table 3. Female transmission rate of extra chromosome in primary trisomics of rice.

Trisomic	Total no. of plants	Progeny (no.)		
		2n	2n + 1	2n + 1 (%)
Triplo-1	150	126	24	16.0
Triplo-2	200	191	9	4.5
Triplo-3	173	106	67	38.7
Triplo-4	0	0	0	0.0
Triplo-5	201	158	43	21.4
Triplo-6	169	139	30	17.7
Triplo-7	200	122	78	39.0
Triplo-8	63	42	21	33.3
Triplo-9	200	158	42	21.0
Triplo-10	141	116	25	17.7
Triplo-11	146	105	41	28.3 ^a
Triplo-12	198	99	99	50.0

^aOnly one (0.7%) nonparental trisomic appeared.

DISCUSSION

The 12 primary trisomics obtained here exhibited conspicuous variation with regard to a number of morphological traits and fertility. Only triplo-11, designated "pseudo normal," largely resembled the disomic, but it could be morphologically distinguished from the latter by the presence of a long ligule (2.9 cm compared to 2.6 cm in the disomic). A

similar type of trisomic was reported in japonica rice by Iwata et al (7) and Watanabe et al (26). Since these trisomics are developed from one triploid of a single variety, the observed morphological variations are due largely to the addition of an extra chromosome in their complements. Heterogeneity of genetic background has apparently caused considerable differences in the morphological expression of trisomics (7). To test this, the primary trisomics obtained at IRRI (12) were compared with our trisomics, and the comparison revealed that the trisomics for the same chromosome in both genetic backgrounds are similar except in some quantitative characters. Hence, it is apparent that the morphological differences among the different trisomics are due mainly to the addition of different extra chromosomes.

In barley (23), *Datura* (1), tomato (19), and sorghum (20), it is easy to distinguish the trisomics morphologically from each other and from the disomic. At CRRRI we have developed the 12 primary trisomics in 3 different backgrounds and have observed that, irrespective of genetic background, the trisomics for a specific chromosome are largely similar (R.N. Misra, unpublished data).

All trisomics except triplo-9 and triplo-12 exhibited reduced pollen and spikelet fertility. Such morphological and physiological retardation has been attributed to the genetic imbalance caused by the addition of the genes from the extra chromosome (5). In tomato, the differences in trisomics are more likely to be due to imbalance of the genomes, especially with regard to gene-determined quantitative characters brought about by the addition of extra chromosomes (19). The morphological and physiological differences among rice trisomics are therefore more likely to be due to the effect of the extra chromosomes, each of which imparts a certain definite effect on the cellular and development rhythm of the individual trisomics.

It is very often fruitful to employ pachytene chromosome analysis for correct identification of extra chromosomes in trisomics as has been done in maize (18), tomato (19), sorghum (24), and solanum (25). In rice, we are able to identify the extra chromosomes of trisomics at the pachytene stage. The trivalent configuration is clearly analyzable by confining the analysis to three to five good pollen mother cells (PMCs), and for perfect identification of the extra chromosome emphasis was placed on PMCs where 11 bivalents and 1 trivalent are clearly discernible. This identification method is similar to that followed by Khush et al (12). In the present study close pairing was observed for a considerable part of their length, which complicates the identification (13). The pairing path of trivalent formation is traced for homologous and nonhomologous association, from

which some were clearly identifiable and some were not.

The triradial configuration at pachytene, which normally would be mistaken as due to the isochromosome of a secondary trisomic, has been observed in solanum (25). But by carefully tracing the constituent chromosomes, the peculiar pairing of the nonhomologous regions of two homologous chromosomes due to stretching and adjustment of chromosome arms was revealed. These configurations resemble the T-configuration recorded by McClintock (13) in maize.

The complete pollen sterility of triplo-4 and the small number of progeny did not permit the correct estimation of male transmission. The low rate of male transmission of the extra chromosome revealed that the extra chromosome in rice trisomics does not transmit through male. Transmission of the extra chromosome through the female is quite good except in triplo-4 because of high sterility. Khush (10) provided many other examples where transmission of the extra chromosome in trisomics occurred with far greater frequency through the female than through the male.

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USE OF PRIMARY TRISOMICS OF RICE IN GENETIC ANALYSIS

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Nine primary trisomics of the indica subspecies of *Oryza sativa* were used to study character-chromosome relationships. Based on deviated F_2 segregation ratios in crosses of different trisomics with appropriate donor parents, critical chromosomes for characters of Mendelian inheritance were identified. Trisomic F_2 families showing significantly different mean values from the disomic F_2 mean were taken as chromosomes of major influence for quantitative traits. With respect to simply inherited characters, more chromosomes than expected on the basis of the disomic F_2 ratio appeared critical with, however, two or three showing profound influence. The number of critical chromosomes influencing tri- to tetragenically inherited anthocyanin pigmentation on different plant parts varied from two to seven. Chromosomes 12 and/or 11 appeared to carry basic genes like *A* and *P*. Localizing genes and batteries of regulators for different plant parts seemed to be largely located on chromosome 5. Genes for resistance to bacterial blight were found to be located on triplo-3 and -9, whereas those for aroma were on triplo-5 and -9. Several genes of varied strengths distributed over different chromosomes were governing quantitatively behaving traits. Of six to eight chromosomes influencing qualitative traits, triplo-3, -5, -6, -7, and -9 appeared to show a pronounced effect.

Successful development and study of the trisomic series in the indica and japonica varietal groups of *Oryza sativa* have helped immensely in recent years to further our understanding of character-chromosome relationships, the independence of linkage groups, the genomic constitution of cultivated rice, and linkage similarities of the subspecies (6). By testing several marker genes against each of the trisomics, Khush et al (6) located as many as 20 on different chromosomes. They also provided an updated linkage map by relating cytologically identifiable chromosomes with established linkage groups (8). In light of the foregoing and of other reports by Japanese workers, our findings on character-chromosome

relationships made from trisomic analysis using nine primary trisomics of the indica varietal group are reported here.

MATERIALS AND METHODS

Only nine primary trisomics of the indica group, developed and made available to us by G. S. Khush of IRRI, could be used, as the rest either failed to survive or remained sterile. The parental background and method of their development have been detailed by Khush et al (6), whose triplo numbers were retained. The triplos and diplo were crossed with appropriate donors, viz., BJ 1 and Type 3, using the triplos as females. After cytological confirmation, F_1 's with extra chromosomes were harvested and, depending on the character, seed harvested from trisomic F_1 's was used either to raise the F_2 population or as F_2 seed for quality analysis. Some 250–500 F_2 plants or seeds were studied in each of the trisomic families.

Critical trisomics were identified for each of the simply inherited characters based on the extent of deviation of the observed frequencies in the F_2 populations of crosses of each of the trisomics with the donor parent from the observed frequencies in the F_2 's of diploid/donor crosses. Trisomic families showing significant deviation at the 5% and 1% levels in heterogeneity tests were marked as critical. The segregation pattern for each of the characters was determined by χ^2 analysis. When more than one Mendelian ratio showed goodness of fit, the ratio that was genetically explainable with the least χ^2 value was taken to reflect more nearly the inheritance of the character. Independence of segregation of any two traits and presence of common genes were determined by χ^2 tests based on expected joint segregation ratios calculated by taking into account the nature of common genes and the type of gene interactions.

In the case of quantitatively behaving traits, the critical chromosomes were identified on the basis of deviation of the mean of the trisomic F_2 population from that of the disomic F_2 . For testing the significance of deviation of the mean the Z test was employed (17).

Observations on morphological traits and yield components were made at appropriate stages of growth, using standard methods. Screening for resistance to bacterial leaf blight was done by the clipping method using a virulent isolate XO₄₁. Basmati aroma was determined by the alkali method (16). F_2 seeds were analyzed on a single grain basis for estimation of seven indices of consumer quality. Water absorption, volume expansion, and kernel elongation values were computed on the

basis of differences in the values of uncooked and cooked rice. For estimation of amylose content the modified method of Williams et al (18) was followed, whereas gelatinization temperature was measured using a 9-point scale (5). The procedure of Cagampang et al (1) was adopted for estimating gel consistency.

GENETIC SEGREGATION FOR QUALITATIVE TRAITS

Anthocyanin pigmentation on plant parts

Genetic study of anthocyanin pigmentation has shown it to be a complex trait governed by three basic complementary genes — *A*, *C*, and *P* — each one remaining in multiple alleles (2,9,14) in association with one or more of about 20 localizing genes. Besides, numerous inhibitory, anti-inhibitory, intensifying, and diluting genes have been reported (11). Keeping the foregoing in view as well as conflicting reports on the inheritance of pigmentation on different plant parts (2,3,4), the segregation pattern of pigmentation on nine plant parts was studied in the F_2 's of crosses of the disomic and nine trisomic lines with BJ 1. The data summarized in Table 1 reveal that the trait follows a multigenic mode of inheritance involving three to four variously interacting genes. Pigmentation of the leaf axil is determined by the interaction of basic, inhibitory, and anti-inhibitory genes, while internodal pigmentation follows tetragenic inheritance involving one inhibitory, one anti-inhibitory, and two complementary genes. In the case of junctura three interacting genes — one basic and two complementary-inhibitory—govern as against the combination of basic, inhibitory, and anti-inhibitory genes controlling septum color. A basic gene with two complementary genes determines the inheritance of nodal pigmentation, while the pigment on the stigma shows a trigenic ratio involving one complementary and two duplicate genes. Pigmentation on the sterile lemma was found to be controlled by three complementary genes and pigmentation on the apiculus by one basic gene plus three complementary-duplicate genes. As with the septum, pigmentation of the awn is governed by a basic gene interacting with inhibitory and anti-inhibitory genes.

Critical chromosomes influencing expression of pigment on different plant parts as inferred from the deviated ratios in the trisomic F_2 's varied from two to seven. The deviation was due to skewed segregation of either pigmented or nonpigmented phenotypic class. Five triplos (triplo-10, -7, -12, -6, -5) were found to be critical for the expression of pigment on leaf

Table 1. Segregation pattern of anthocyanin pigment on some plant parts in the F₂'s of crosses of donor parent (BJ 1) with diploid (2n) and primary trisomics (2n + 1).

Anthocyanin pigmentation on	F ₁	Disomic F ₂		Trisomic F ₂ , (2n + 1 and 2n progeny)										Direction of skew of critical triplos ^a
		Valid ratio ^a	χ^2 values for disomic ratio ^c											
			Triplo-2	Triplo-3	Triplo-5	Triplo-6	Triplo-7	Triplo-9	Triplo-10	Triplo-11	Triplo-12	Diplo		
Leaf axil	purple	39(P): 25(Gr)	0.66	0.30	6.55**	8.75**	23.45**	2.72	42.82**	5.26**	18.14**	3.41	5 → P; others → Gr	
Internode	purple	117(P):139(Gr)	2.05	0.19	5.37*	0.77	0.53	0.39	6.03*	1.88	0.72	0.00	5,10 → Gr	
Juntura	purple	21(P): 43(Cl)	9.18**	5.03*	0.90	6.33**	44.98**	39.93**	14.45**	3.42	17.76**	0.03	12 → P; others → Gr	
Septum	purple	39(P): 25(Cl)	0.24	2.83	10.38**	3.08	62.85**	16.26**	1.69	15.80**	10.37*	3.15	6,7,9, → P; 11,12 → Cl	
Node	colorless	21(P): 43(Cl)	0.02	9.63**	2.94	2.43	1.90	1.82	19.39**	4.15*	16.31**	3.67	all → Gr	
Stigma	purple	45(P): 19(Cl)	637.94**	35.59**	36.36**	0.77	6.57**	13.52**	0.12	56.33**	30.83**	0.54	2,3,5,11, → Cl, 7,9,12 → P	
Sterile lemma	colorless	27(P): 37(Cl)	2.54	11.29**	1.42	15.71**	3.07	33.81**	3.65	0.39	2.43	0.80	3,9 → P; 6 → Cl	
Apiculus	colorless	189(P): 67(Cl)	43.34**	8.21**	9.82**	11.29**	14.54**	3.36	1.01	62.20**	105.82**	3.61	7 → P; others → Cl	
Awn	purple	39(P): 25(Cl)	0.27	1.46	28.19**	10.79**	4.74**	0.04	5.60*	12.30*	21.34**	0.04	all → Cl	

^a P = purple; Gr = green; Cl = colorless → = towards

^b* = significant at the 5% level, ** = significant at the 1% level.

axil, two (triplo-10, -5) on internode, five (triplo-7, -9, -12, -10, -2) on junctura, five (triplo-7, -9, -11, -12, -5) on septum, three (triplo-10, -12, -3) on node, seven (all except triplo-6, -10) on stigma, three (triplo-9, -6, -3) on sterile lemma, seven (all except triplo-9, -10) on apiculus, and five (triplo-5, -12, -11, -6, -10) on awn. Among the nine trisomics studied triplo-12 was found to influence predominantly the expression of pigment on seven of the nine plant parts, followed by triplo-11 and -5.

The findings of genetic analysis were broadly in agreement with our understanding of the complex genetics of pigmentation. Results of trisomic analysis showed that the number of critical chromosomes in the majority of cases was more than expected on the basis of disomic ratio, which appears to be in direct conflict with genetic and linkage findings. Assigning a character to a specific chromosome, as is customarily done in the study of independence of linkage groups using marker genes, is understandable when it follows monogenic inheritance. When a character is controlled by more than one gene and still a single chromosome is found critical, one has to assume that all influential genes including structural and regulator-modifier components are located on the same chromosome. With more and more understanding at the molecular level of the gene and its complex mode of functioning, the observed character-chromosome relationship warrants satisfactory explanation.

Of several possibilities, the dosage effect of genes brought about in the aneuploid state is one. Phenotype being the result of a series of genetically guided biochemical reactions, genetic alterations at any step in the pathway amount to either nonexpression or altered phenotype of a character. Dosage increase of genes in the trisomic state might act either in complementary or additive fashion for making or altering the phenotype. Similarly, involvement of common genes might be yet another reason for the manifestation of skewed segregation of several triplos. Instances of the same gene controlling pigmentation on more than one plant part were found in the present study (Fig. 1). For instance, leaf axil has two genes, an inhibitory and an anti-inhibitory, in common with internode, and only an anti-inhibitory gene in common with septum. Stigma and apiculus have two duplicate genes in common, while junctura and internode, internode and sterile lemma, sterile lemma and apiculus, and sterile lemma and awn have only one basic gene each in common. The location of structural and regulator genes on different chromosomes as demonstrated in maize (12), wherein anthocyanin pigment genes controlling the same pathway have been reported to occur on different chromosomes, could be another reason. The existence of certain genes or sequences of genes on more than one chromosome —

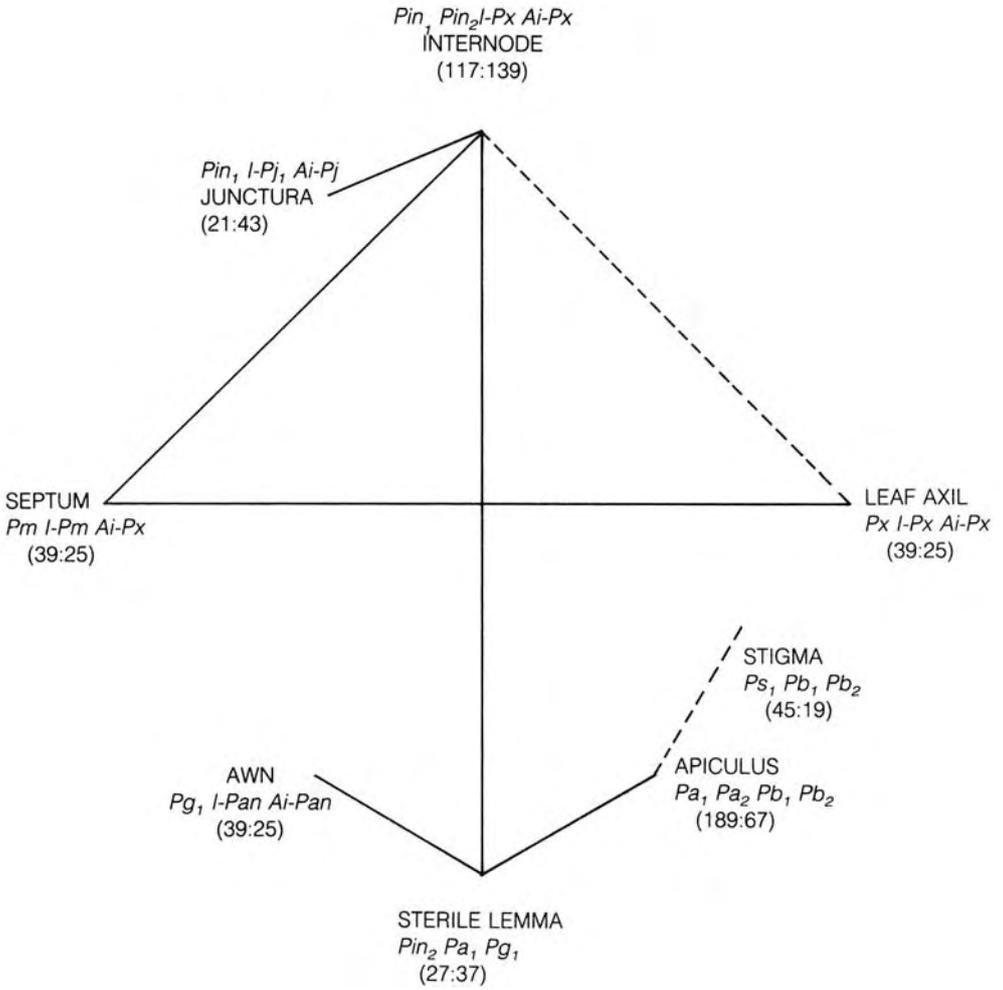


Fig. 1. Number of common genes for anthocyanin pigmentation in indica (I)/BJ I. - - - - = 2 genes common, _____ = 1 gene common.

possibly due to selective polyploidization of a few chromosomes of the genome and distribution of the same in small segments over chromosomes in the course of karyotypic evolution of rice — could be yet another factor, though there is no direct evidence in its support. While mapping the same character on different linkage groups or chromosome by different workers lends indirect evidence to such possibilities, studies of Sadananda (13) indicated that the linkage group *mp* is located on two different chromosomes and the group *Era-Lxai* on a few chromosomes.

Although the data made it difficult to assign a character to a specific chromosome, linkage groups could be assigned with reasonable certainty as more characters were taken into consideration at a time. For instance, the linkage group $Pa_2-Ps = Ai-Pm = Ai-Px = Ai-Pin = Pn$ (*Sp* group) could be assigned to triplo(I)-5, as this trisomic has been found critical for most of the characters mapped in this group. Similarly, linkage belonging to the *mp* group appeared to be on triplo(I)-12 and/or triplo(I)-11. It is quite probable that chromosomes 12 and/or 11 carry basic genes like *A* and *P*, while chromosome 5 carries the localizing genes, with batteries of regulators and modifiers remaining on other critical chromosomes.

Bacterial blight resistance and basmati aroma

Disomic segregation in the cross involving BJ I indicated that resistance to the isolate XO₄₁ was conferred by any two of three recessive genes (54:10 susceptible to resistant). Resistance being isolate-specific, this observation is at variance with that of Petpisit et al (10). Assuming that *Xa5* confers resistance to this isolate as well, the genotype of BJ I was constituted as $xa_5 xa_5' xa_5''$ and that of IR3265-193-3 as $Xa_5 Xa_5' xa_5''$. Trisomic analysis revealed triplo(I)-3 and triplo(I)-9 to be critical, thus agreeing to a great extent with the ratios obtained from genetic analysis (Table 2).

While agreeing with the recessive nature of aroma reported earlier (16), the present study is at variance with regard to the mode of inheritance in that it is controlled by three recessive genes, two of which are essential for the expression of the trait. Trisomic analysis revealed the aroma genes to be located on triplo(I)-5 and triplo(I)-9.

BEHAVIOR OF QUANTITATIVE TRAITS

The location of polygenes determining quantitative traits on almost all the chromosomes of a genome has been amply demonstrated by aneu-

Table 2. Segregation pattern of aroma and resistance to bacterial blight (XO₄₁) in the F₂'s of crosses of donor parents^a with diploid (2n) and primary trisomics (2n + 1).

Character	F ₁	Disomic F ₂ Valid ratio ^b	Trisomic F ₂ (2n + 1 and 2n progeny)										Direction of skew of critical triplos ^d
			c ² values for disomic ratio ^c										
			Triplo-2	Triplo-3	Triplo-5	Triplo-6	Triplo-7	Triplo-9	Triplo-10	Triplo-11	Triplo-12	Diplo	
Bacterial blight	susceptible	54(S):10(R)	0.21	11.53**	0.68	0.36	0.03	4.31*	0.08	1.01	0.83	1.42	both → R
Aroma	nonscented	54(NSc):10(Sc)	0.19	0.17	32.07**	1.54	0.66	7.50**	1.21	—	0.91	0.68	9 → Sc 5 → NSc

^a BJ 1 for blight resistance, Type 3 for aroma.

^b S = susceptible, R = resistant, NSc = nonscented, Sc = scented.

^c * = significant at the 5% level, ** = significant at the 1% level.

^d → = towards.

ploid analysis in crops like wheat (7). Using trisomics, chromosomes having a major influence on a set of agronomic and quality characters were identified in the present study (Tables 3, 4).

Agronomic traits

Mean values of practically all trisomic F_2 populations were significantly less than diploid populations for plant height and more for flowering duration. On the basis of maximum effect, however, triplo(I)-9 and triplo(I)-10 were found to influence maximally the height and triplo(I)-5 and triplo(I)-3 the duration. With respect to panicle length, at least five chromosomes showed pronounced effects; for tiller number, four; and for spikelets/panicle, two. Triplo(I)-5 had the shortest and triplo(I)-2 the longest panicles, whereas triplo(I)-7 had the lowest and triplo(I)-9 the highest tiller number; triplo(I)-10 had the highest number of spikelets/panicle (Table 3). These observations suggest that (a) many genes governing quantitative traits of this kind are distributed over different chromosomes, (b) the number of genes and the relative effects of each might vary from chromosome to chromosome as inferred from differential standard mean values, and (c) the critical chromosome for a given trait might carry either genes of positive effect or negative effect or both. Intensive study of these aspects might throw more light on the genetic architecture of quantitative traits.

Indices of consumer quality

Consumer quality is a complex trait determined by a syndrome of physicochemical indices. Keeping in view the conflicting reports on the mode of inheritance of these indices (15), nine major indices were studied through trisomic analysis. The results summarized in Table 4 suggest that six to eight chromosomes influence the expression of all the indices and that the few chromosomes that play the major role vary with the character. Triplo(I)-3 exhibited maximum positive and triplo(I)-7 maximum negative deviations with respect to water absorption and volume expansion. Besides, four more chromosomes were common, showing close relationships between them. Triplo(I)-3 and triplo(I)-9 appeared to exert maximum influence on kernel elongation. The trait, however, seemed to be independent of volume expansion, as they differed on certain critical chromosomes. In the case of gelatinization temperature, although the magnitude of deviation was significant in all, triplo(I)-2 appeared to show maximum effect. Triplo(I)-3 and triplo(I)-7 showing highly significant negative deviation and triplo(I)-2, triplo(I)-6, and

Table 3. Mean performance of various agronomic characteristics in trisomic and disomic F₂ populations.^a

Cross of BJ I with	Plant height (cm)	Days to flowering	Panicle length (cm)	Tiller number	Spikelets/ panicle
Triplo-2	87.54±0.69	110.33±0.48**	20.71±0.11**	14.31±0.31	84.48±0.97
Triplo-3	89.65±0.85	112.58±0.54	19.67±0.15	14.30±0.17**	86.94±1.16
Triplo-5	83.19±1.41**	114.92±0.41**	17.53±0.11**	15.50±0.15	92.76±1.05*
Triplo-6	89.18±0.79	109.34±0.53**	18.24±0.11**	14.30±1.00	95.36±2.12**
Triplo-7	86.48±0.42**	98.88±0.50	20.0±0.07*	16.67±0.16**	86.32±0.95
Triplo-9	79.44±0.68**	107.29±0.53**	19.48±0.08	13.34±0.21**	80.24±1.36
Triplo-10	81.77±0.68**	105.08±0.51**	19.55±0.10	15.00±0.19	99.32±1.25**
Triplo-11	86.49±0.77**	107.06±0.49**	19.08±0.14**	15.44±0.13	85.30±0.86
Triplo-12	93.55±0.78*	108.23±0.52**	20.07±0.10**	15.00±0.13	92.46±1.25*
Diplo	92.99±0.81	99.94±0.62	19.64±0.11	15.44±0.13	84.20±1.20

^a* = significant at the 5% level, ** = significant at the 1% level.

Table 4. Deviations of mean values of crosses of trisomic lines from the crosses of disomic line in the F₂ generation for various indices of quality.^a

Triplo/diplo	In crosses with Type 3								In cross with BJ I
	Water absorption (mg)	Volume expansion (ml)	Kernel elongation (mm)	Gelatinization temperature (Alkali score)	Amylose content (%)	Kernel length (mm)	Kernel breadth (mm)	Kernel L/B ratio	Gel consistency (mm)
Diplo	2.69±0.04	3.69±0.07	0.13±0.02	2.87±0.13	22.18±0.43	6.20±0.04	1.79±0.02	3.40±0.05	33.29±0.70
Triplo-2	3.17±0.05**	4.37±0.08*	0.19±0.03	5.34±0.06**	24.90±0.59**	6.45±0.05**	1.96±0.02**	3.29±0.06	33.03±0.70
Triplo-3	3.54±0.04**	4.89±0.08**	0.48±0.05**	3.06±0.11**	19.06±0.45**	6.77±0.04**	2.14±0.01**	3.10±0.04**	24.30±0.52**
Triplo-5	2.80±0.02**	4.44±0.05**	0.32±0.04*	3.33±0.10**	20.40±0.52**	5.47±0.05**	1.94±0.03**	2.82±0.03**	—
Triplo-6	3.14±0.04**	4.42±0.06**	0.34±0.04*	3.50±0.12**	24.55±0.47**	6.37±0.04**	2.11±0.01**	3.00±0.03**	40.53±1.06**
Triplo-7	2.57±0.05**	3.22±0.06**	0.28±0.05*	3.11±0.07**	19.13±0.51**	5.90±0.04**	1.90±0.02**	3.16±0.04**	46.43±0.84**
Triplo-9	2.71±0.03**	3.93±0.08**	0.45±0.03**	3.06±0.09**	23.97±0.31**	6.32±0.04	2.10±0.01**	2.97±0.03**	36.90±0.69**
Triplo-10	3.03±0.04**	3.74±0.07**	0.22±0.04	2.79±0.08**	24.90±0.41**	5.99±0.03**	1.64±0.02**	3.71±0.05**	34.88±1.13**
Triplo-11	—	—	—	—	—	—	—	—	30.81±0.73**
Triplo-12	2.76±0.03**	4.32±0.08**	0.46±0.03**	3.13±0.09**	23.60±0.60**	6.16±0.04	2.06±0.01**	2.89±0.03**	43.30±0.85*

^a = significant at the 5% level, ** = significant at the 1% level.

triplo(I)-10 showing positive deviation seemed important for amylose content. Genes affecting gel consistency appeared to be located on triplo(I)-3, triplo(I)-6, triplo(I)-7, and triplo(I)-12, with some showing positive and others negative deviations. In the case of physical grain characteristics, triplo(I)-3 showed maximum positive and triplo(I)-5 maximum negative effects on kernel length, and triplo(I)-3, triplo(I)-6, and triplo(I)-9 affected kernel breadth.

An overall analysis suggests that five chromosomes, namely triplo(I)-3, triplo(I)-5, triplo(I)-6, triplo(I)-7, and triplo(I)-9, probably carry the genes or gene blocks pertaining to the majority of the quality indices. Of these, triplo(I)-3, which has been found to affect as many as ten traits, seems to carry the gene(s) basic and common to most of the physico-chemical properties of starch. Also, each of the remaining four chromosomes has been found to affect three to four indices of quality. Thus, instances of many genes distributed over different chromosomes affecting one trait, as well as of one chromosome carrying genes that influence the expression of more than one trait, suggest a complex quantitative genetic constitution of most of the indices of consumer quality in rice.

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PRODUCTION OF MONOSOMIC ALIEN ADDITION LINES OF ORYZA SATIVA HAVING A SINGLE CHROMOSOME OF *O. OFFICINALIS**

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Eighteen accessions of *Oryza officinalis* from different countries were crossed as males with three improved plant type breeding lines of *O. sativa*. Crossability of the two species was 1.0% to 2.3%. Viable F_1 hybrids could be obtained only after embryo culture. The F_1 hybrids had 24 chromosomes, showed no chromosome pairing during meiosis, and were completely sterile. Upon backcrossing with the respective female parents they produced a few BC_1 progenies, most of which were allotriploid and also completely male sterile. The second backcross yielded 94 plants. Of the 70 BC_2 plants so far examined, 35 have $2n + 1$ chromosomes. These represent monosomic alien addition lines that have the complete chromosome complement of *O. sativa* and one alien chromosome of *O. officinalis*. These monosomic alien addition lines resemble the primary trisomics of *O. sativa*.

Diseases and insects are the major threat to the continued yield stability of improved rice varieties. Therefore, many rice improvement programs are emphasizing the development of improved varieties with multiple resistance to major diseases and insects. A large number of varieties of cultivated rice *O. sativa* L. have been screened for resistance, and donors for resistance have been identified (6). These donors have been utilized in hybridization programs, and varieties with multiple resistance have been developed (7, 8, 11). The wild germplasm of *Oryza* has also been screened, and many accessions of wild species have been found to be resistant to several insects (3).

The wild species of *Oryza* have rarely been used in rice improvement. Use of *O. nivara* for developing grassy stunt resistant varieties is an exception. To date, *O. nivara* is the only known source of resistance to grassy stunt. The dominant gene *Gs* for grassy stunt was successfully transferred from *O. nivara* to *O. sativa*, and several resistant varieties were developed (9). *O. nivara* has the same genome as *O. sativa*, and no serious barriers to gene transfer were encountered. However, gene transfer from some other species whose genomes are nonhomologous to the A genome

Table 1. Accessions of *O. officinalis* (CC) and *O. sativa* (AA) used in the study.

Species	Acc. no.	Origin	Reaction ^a to BPH
<i>O. officinalis</i>	100179	Japan	R
<i>O. officinalis</i>	100180	Malaysia	R
<i>O. officinalis</i>	101121	Philippines	R
<i>O. officinalis</i>	101113	Philippines	R
<i>O. officinalis</i>	101117	Philippines	R
<i>O. officinalis</i>	101077	Philippines	R
<i>O. officinalis</i>	101078	Malaysia	R
<i>O. officinalis</i>	101150	Malaysia	R
<i>O. officinalis</i>	101152	Malaysia	R
<i>O. officinalis</i>	101155	Malaysia	R
<i>O. officinalis</i>	102385	Indonesia	R
<i>O. officinalis</i>	102382	Indonesia	R
<i>O. officinalis</i>	101414	India	R
<i>O. officinalis</i>	101412	India	R
<i>O. officinalis</i>	100947	India	R
<i>O. officinalis</i>	100896	Thailand	R
<i>O. officinalis</i>	100878	Thailand	R
<i>O. officinalis</i>	102399	Vietnam	R
<i>O. sativa</i>			
	IR15 29-680-3-2	IRRI	S
	IR25 587-109-3-3-3-3	IRRI	S
	IR31 917-45-3-2	IRRI	S

^aR = resistant, S = susceptible.

of *O. sativa* is extremely difficult; these species are difficult to cross with cultivated rice, and their genomes do not pair and recombine with the A genome.

This study was undertaken to determine the possibility of establishing alien monosomic addition lines having the full chromosome complement of *O. sativa* and single chromosomes of *O. officinalis*. The latter species has several useful traits of economic importance such as resistance to brown planthopper (BPH). The alien addition lines would be useful for transferring the genes or chromosome segments bearing these genes from the alien chromosomes to the A genome of *O. sativa*.

MATERIALS AND METHODS

Eighteen accessions of *O. officinalis* (Table 1), which has the C genome, were obtained from the International Rice Germplasm Center of IRRI. These accessions are resistant to all the known biotypes of BPH. They

were crossed with three breeding lines of *O. sativa* (Table 1) having short stature and high yield potential but being susceptible to BPH.

A number of crosses between *O. sativa* lines as females and accessions of *O. officinalis* as males were attempted. The seeds were imperfectly developed and started to abort 14 days after pollination. To overcome this problem, 14-day-old embryos were excised under a stereo microscope in an aseptic condition and were cultured on 1/4 MS medium devoid of auxin. The cultured embryos were incubated in the dark until germination and were subsequently transferred to a light incubation room. The young seedlings at the three-leaf stage were transferred to liquid nutrient solution. After ten days the seedlings were transferred to soil.

The F_1 plants were pollinated with the pollen from the respective female parents to obtain BC_1 plants. The BC_1 plants were again crossed with the same male parents to obtain BC_2 progenies.

The F_1 , BC_1 , and BC_2 progenies were cytologically examined to determine their chromosome number. The pollen and seed fertility as well as the morphology of the different progenies were studied.

RESULTS

Crossability of species

Seed set in the crosses when *O. sativa* lines were used as females and *O. officinalis* accessions as males was very poor. Moreover, when pollen from different accessions of *O. officinalis* was used, the results were similar. Therefore, the data on seed set from different crosses of *O. officinalis* accessions have been pooled in Table 2. A total of 26,034 spikelets of the three lines of *O. sativa* were pollinated, and the seed set varied from 8.82% to 17.30%. Since the seeds started to abort about 14 days after pollination, 14-day-old embryos were excised and cultured on 1/4 MS medium (4). The germination of embryos varied from 56.57% to 70.05%. The crossability of the three breeding lines of *O. sativa* with *O. officinalis* as calculated according to the formula of Wu et al (22) was 1.0% to 2.3% (Table 2).

F_1 progenies

A total of 400 F_1 hybrid plants between the three breeding lines of *O. sativa* and *O. officinalis* were obtained. They were intermediate between the two parents in some respects but showed a preponderance of the

Table 2. Crossability of three breeding lines of *O. sativa* and *O. officinalis* accessions.

<i>O. sativa</i> line used as female	Spikelets pollinated (no.)	Seed set (%)	Embryos cultured (no.)	Embryo germination (%)	Crossability
IR1529-680-3-2	8,104	8.82	449	56.57	1.8
IR25587-109-3-3-3-3	10,145	10.02	266	63.50	1.0
IR31917-45-3-2	7,785	17.30	374	70.05	2.3

traits of the wild species. They were robust and extremely vigorous (Fig. 1). All of them were completely male sterile. Most of them had 2n chromosome number. There was almost complete absence of chromosome pairing in the F₁'s at diakinesis and metaphase I, and only an occasional bivalent was observed (Table 3).

BC₁ progenies

Seed set upon backcrossing of the F₁'s with the respective female parents was extremely poor. Of the 41,437 spikelets of the F₁'s that were pollinated, only 1.3% set the seed. Some of the seeds failed to germinate, but 367 BC₁ plants were obtained. Out of these, 357 plants turned out to be triploid and 10 were hypotriploids, thereby indicating that the unreduced gametes on the female side were mostly functional. These triploids were thus of AAC constitution. The hypotriploids were extremely weak and stunted, but all the triploid plants were vigorous. They had intermediate height, profuse tillering habit, long panicles, and large spikelets with long awns. All of them were completely male sterile. The modal chromosome pairing at diakinesis or metaphase I was 12 II + 12 I (Table 4, Fig. 2).

All of the 357 triploid plants were backcrossed with the respective recurrent parents. Out of 41,487 spikelets so far pollinated, 153 imperfectly developed seeds were obtained. Upon germination of these seeds in vitro, 94 BC₂ plants were obtained.

BC₂ progenies

To date, chromosome counts of 70 of the 94 BC₂ plants have been made. Eighteen of these turned out to be disomic (2n = 24), 35 had 25 chromosomes, 9 had 26, 6 had 27, 1 had 29, and 1 had 30.

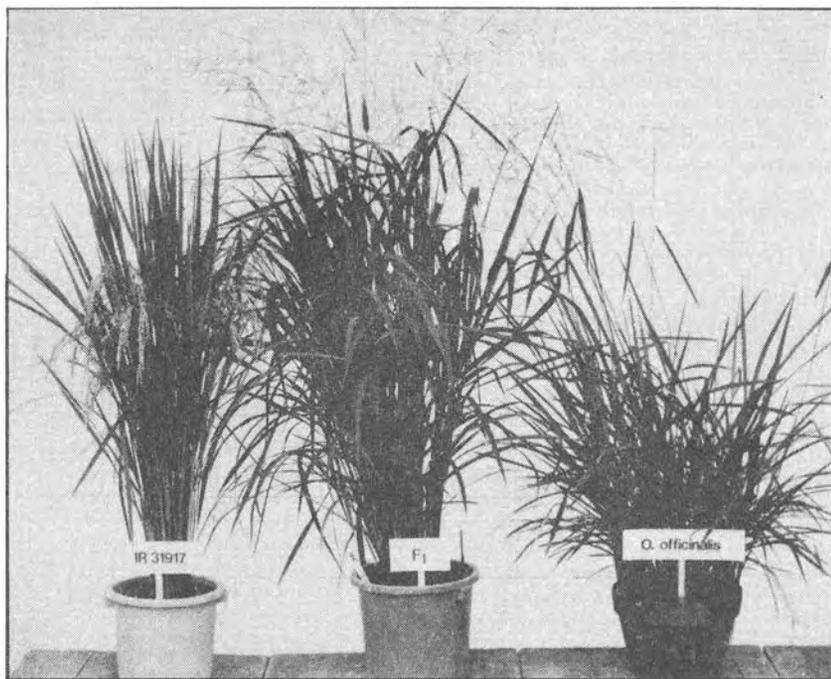


Fig. 1. Morphology of IR31917-45-3-2 (left), F_1 hybrid (center), and *O. officinalis* (right).

The disomic plants are fully fertile and mostly resemble the recurrent parents. However, they exhibit slight differences from each other and from the recurrent parents. Some of them have a modal chromosome association of $11 \text{ II} + 2 \text{ I}$ at diakinesis. The cytology, morphology, and breeding behavior of the plants will be further studied.

Plants with 26 and 27 chromosomes are highly sterile and greatly modified.

The most interesting group of plants are those with 25 chromosomes. Since the modal chromosome pairing in the allotriploids from which these plants were obtained was $12 \text{ II} + 12 \text{ I}$, it is reasonable to conclude that the BC_2 plants with 25 chromosomes have the complete chromosome complement of *O. sativa* and an alien chromosome of *O. officinalis*. They thus represent the monosomic alien addition lines.

Monosomic alien addition lines

When the morphology of the BC_2 plants with 25 chromosomes was compared with that of primary trisomics of *O. sativa* (10), some plants showed a striking resemblance to specific trisomics. Three plants re-

Table 3. Chromosome associations at diakinesis in the interspecific hybrids between three breeding lines of *O. sativa* and *O. officinalis*.

<i>O. sativa</i> line used as female	Pollen mother cells studied (no.)	Bivalents/cell (no.)		Univalents/cell (no.)	
		Mean	Range	Mean	Range
IRI529-680-3-2	236	1.73	0-4	22.27	20-24
IR25587-109-3-3-3-3	232	0.55	0-3	23.45	21-24
IR31917-45-3-2	50	0.48	0-3	23.52	21-24

Table 4. Chromosome pairing in the allotriploids of AAC constitution.

Meiotic stage	Pollen mother cells (PMCs) examined (no.)	% PMCs with 12 II + 12 I	% PMCs with 11 II + 14 I
Diakinesis	95	97.9	2.1
Metaphase I	179	97.8	2.2

sembled triplo-4; two each resembled triplo-2, triplo-6, and triplo-10; and one each resembled triplo-1, triplo-3, triplo-5, triplo-7, triplo-8, triplo-9, triplo-11, and triplo-12. Thus monosomic alien addition lines corresponding to the twelve chromosomes of *O. officinalis* have been identified. However, there are some $2n + 1$ plants that cannot be corresponded with any of the trisomics of *O. sativa*. The extra alien chromosome of these plants is probably a modified chromosome.

The modal chromosome pairing at diakinesis or metaphase in the monosomic alien addition lines is $12 II + 1 I$.

We are still in the process of counting the chromosome numbers of the remaining BC_2 plants, and investigations will be continued.

DISCUSSION

Monosomic alien addition lines have been thoroughly studied in polyploid species such as wheat (14, 15), oats (20), and tobacco (1). In the diploid species very few such studies have been undertaken. In genus

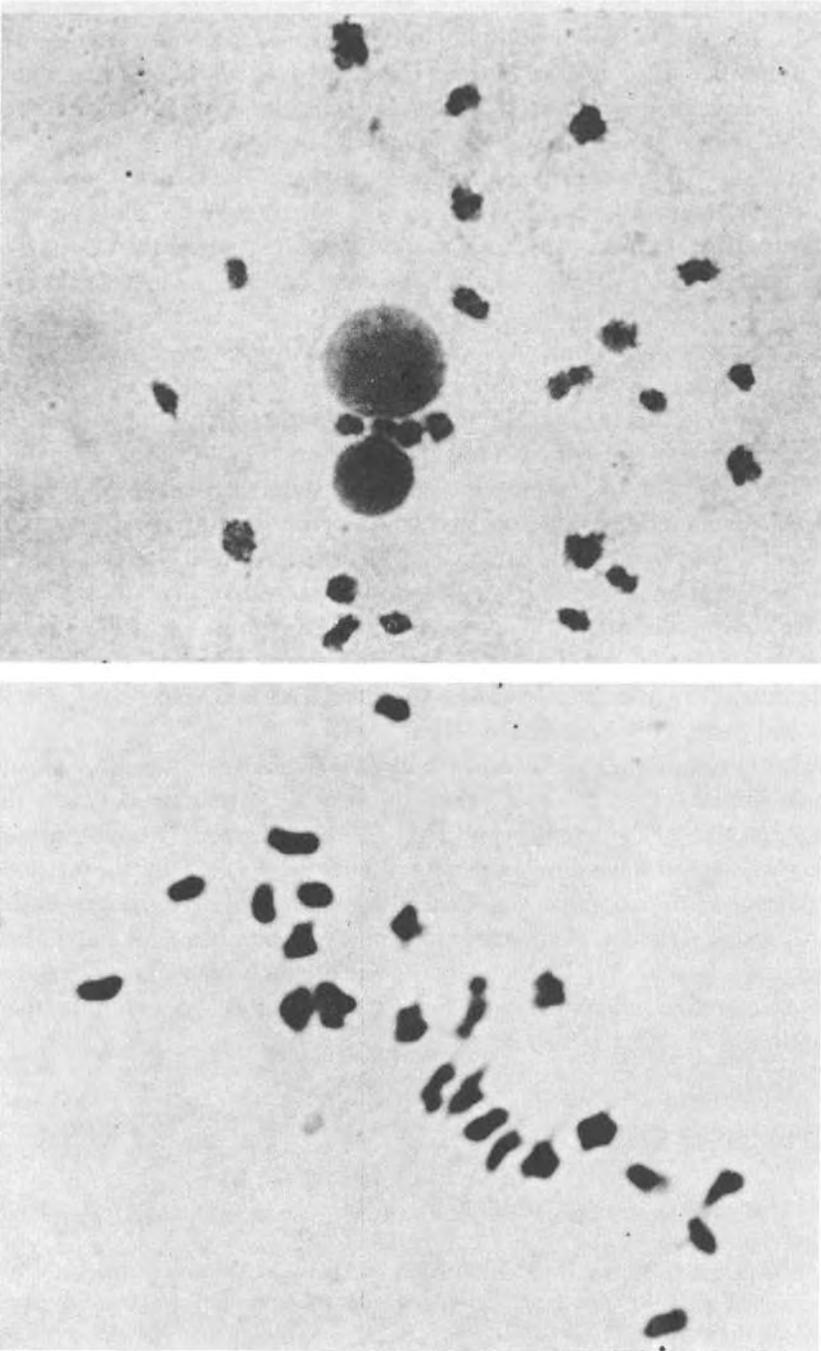


Fig. 2. Diakinesis and metaphase I of a BC₁ plant showing 12 II + 12 I (x 1000).

Oryza, monosomic alien addition lines of *O. sativa* having single alien chromosomes of *O. officinalis* were established by Shin and Katayama (19). Their main interest, however, was in the cytogenetics of alien addition lines and in the study of homology between the genomes of the two species. In our study the primary objective is the transfer of traits of economic importance from wild species to cultivated rice. Thus we plan to study the reaction of various monosomic alien additions to diseases and insects such as BPH. The alien chromosomes having loci of genes for resistance will be identified, and attempts will be made to transfer the alien chromosome segments to the *O. sativa* genome either through rare recombinational events or through radiation treatments. This will require careful examination and evaluation for pest resistance of the BC₂ plants and their subsequent progenies.

This approach for interspecific gene transfers has been employed for transferring useful genes from the wild species to cultivated wheat by several workers (5). Sears (16) transferred a segment of *Aegilops umbellulata* chromosome carrying rust resistance to wheat by irradiating a monosomic addition line. Similar transfers were later made by Knott (12) and Sharma and Knott (18) from *Agropyron elongatum* to wheat, by Wienhues (21) from *A. intermedium* to wheat, and by Driscoll and Jensen (2) and Sears (17) from rye to wheat.

The chromosomes of *O. sativa* and *O. officinalis* are morphologically quite similar (13). However, there is very little pairing between the chromosomes of the two species. That the homologous chromosomes of the two species have similar gene contents is revealed by the extreme similarity of the monosomic alien addition lines to the primary trisomics of *O. sativa*. Further characterization of the monosomic alien addition lines is underway. We plan to identify the alien chromosomes of each of the monosomic alien addition lines cytologically and examine their stability.

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DISCUSSION

SESSION 3: KARYOTYPE, POLYPLOIDS, AND TRISOMICS

Q – Oka: To what extent do you expect discrepancies in chromosome numbering based on length? Dr. Kurata showed a marked variation in arm ratio of a chromosome from cell to cell. To what extent can arm ratio or banding pattern be a characteristic for identifying individual chromosomes?

A – Wu, H.K.: Arm ratio is an important criterion in karyotyping. No discrepancy is expected from cell to cell. Discrepancy in the arm ratio of the same chromosome might result from difficulty in determining the location of the centromere accurately. Some discrepancy may also result from differential stretching of chromosome arms.

Q – Barnes: Have any of the chromosome workers tried the use of restriction enzymes for chromosome banding?

A – Kurata: In rice, there are no such studies as yet. But in animal chromosomes, for example in the Chinese hamster and the Indian muntjac, successful banding was obtained after restriction enzyme treatment.

Q – Second: There are some reports about higher 2C value and higher heterochromatin content of the species of the *latifolia* group vs. the *sativa* group. Would you like to comment on that?

A – Kurata: I have no information on the subject.

Q – Sharma S. D.: It appears that Giemsa banding is helpful in identifying the individual chromosomes within a karyotype but not in studying the karyotypic differentiation of species. What are your comments?

A – Kurata: Giemsa banding is helpful in studies of the karyotypic differentiation of species. However, careful attention must be paid to the selection of the cell stages that will be compared. It is better to use C-banding for such purposes.

Q – Dalmacio: Secondary constriction and satellites are parts of the chromosome. What made you decide not to include these regions while measuring chromosome length? Is this a standard procedure? If you had included these regions in measuring the length of the chromosome, then this satellite a-K10 chromosome should have been K8 or maybe K9.

A – Kurata: In 99.5% of the cases, satellites and secondary constriction regions are buried in the nucleolus and we cannot measure them accurately. Also, the length of secondary constriction varies greatly because of

its flexibility. The chromatin content of the satellite and the stretched thin fiber of secondary constricted region is very low, so I think it is not necessary to include them in chromosome length.

Q – Ikehashi: How do you prove your hypothesis for the restoration of fertility in the progeny of autotetraploids? It seems that unless there is a sound theoretical base, the use of autotetraploids may not be productive.

A – Bao: My working hypothesis is concerned with the maintenance of normal seed fertility reached by the elite plants. The high yield of several clone lines from elite plants has been proved by yield performance trials with replications for two years. The improvement of techniques for the efficient mass propagation of rice buds in test tubes may make the use of autotetraploid rice clones possible in practical cultivation in the near future.

Q – Second: A theoretical possibility to diploidize the tetraploid rice could be in using a wild A genome species distantly related to cultivated rice. Did you try this?

A – Bao: No, we did not.

Q – Riley: Have you allowed for the occurrence of univalents at meiosis in 4x rice? They could be a principal cause of infertility and could also give rise to aneuploids. Formulae that assume that all four homologous chromosomes are either in two bivalents or in a quadrivalent will not accurately represent the true situation.

A – Bao: We observed 256 PMCs at diakinesis and metaphase I. Not a single case of a univalent accompanying a trivalent was found. Therefore, it seems to us that aneuploids could not be a principal cause of partial sterility in 4x rice. Although, we did not make any chromosome counts of individual plants of progeny derived from an autotetraploid parent, aneuploids could be present, but only rarely.

Q – Riley: The majority of your trisomics arose from euploids, so there must have been nondisjunction at anaphase I or II of meiosis. Presumably this means that normal rice varieties used on farms will contain aneuploids. Have you examined the meiosis of normal euploid rice to predict the proportion of aneuploids in rice varieties?

A – Yisra: Normal euploids (diploids) have regular meiosis, and abnormal disjunction at anaphase I and anaphase II does not occur. However, the triploids show high trivalent frequency and the types of disjunctions that lead to such a high frequency of trisomics.

Q – Oka: I wonder why one examines the effect of triplo chromosomes on quantitative characters. For this purpose the translocations would be more useful.

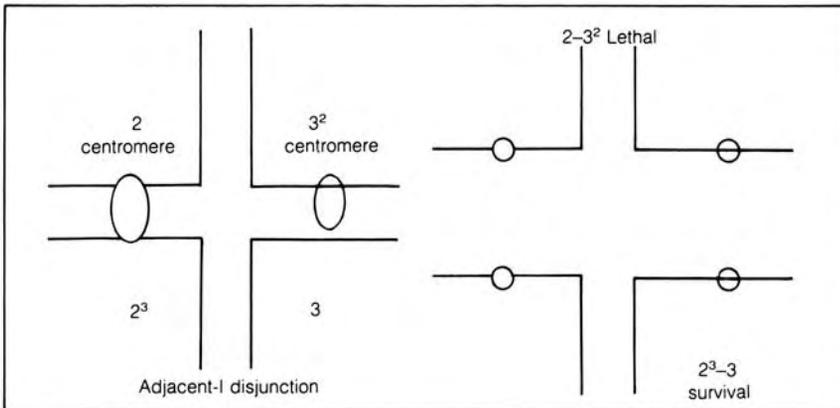
A – Siddiq: No doubt, in the study of quantitative traits, translocation stocks would be useful. At the same time, aneuploid series including

primary trisomics have been widely used more than translocation stocks in crop plants for the study of metric characteristics like yield and its components. It is logical to think that an additional chromosome (triplo state) would modify the expression of all traits irrespective of their mode of inheritance by the dosage effect (if relevant genes are located on the specific chromosome), as witnessed in polyploids.

Q – Oka: What are trisomic-like plants? Are they secondary or tertiary trisomics?

A – Sato:

Since the functional unbalanced gamete coming from the adjacent disjunction carries the normal third chromosome and the interchange chromosome 2^3 has the centromere of the second, the zygote or the trisomic-like plant derived from the fertilization of the unbalanced gamete with a normal or balanced translocated gamete is considered as a partially monosomic-trisomic plant.



Q – Alzona: What is the stability of these monosomic alien addition lines? Are not these transferred traits lost after several generations, thus defeating the purpose/objective of gene transfer?

A – Jena: Of course there is addition decay. But after identification of the particular chromosome bearing the desirable gene(s), efforts will be made to transfer this gene(s) to the genome of *O. sativa*, and thereafter it will be stable.

C – Riley: I would like to congratulate Drs. Jena and Khush on isolating what I believe to be the first set of lines in which alien chromosomes have been added to the complement of a diploid crop species.

LINKAGE MAPS

SESSION 4

STANDARDIZATION OF GENE SYMBOLS AND LINKAGE MAPS IN RICE

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A review is made of the general rules and gene symbols and some additions and amendments are proposed. New linkage maps portraying new information in both japonica and indica rice are presented. Differences in the genetic scheme for organ coloration and scarcity of identical genes involved in japonica and indica groups make it difficult to establish coordinated linkage groups through indica and japonica rice. Future problems related to linkage maps are mentioned, and the coordinated numbering of linkage groups, chromosomes, and trisomics is advocated.

Since the first monograph by Ikeno (2), much important information on the mode of inheritance and linkage relations of genetic markers and their related characters has been accumulated. In a recent review by the author (6), the nature of over 450 marker genes was listed together with their loci and references.

Because of new developments in rice genetics during the last two decades, the need for the reexamination of gene symbols and linkage groups has been progressively intensified. Realizing this, the Japanese Rice Genetics Information Committee initiated this examination of gene symbols. In this paper, our proposal for rice gene symbolization is presented.

STANDARDIZATION OF GENE SYMBOLS

In 1963, a committee of the International Rice Commission proposed general rules and standard symbols for known genes. The Japanese committee chaired by Y. Futsuhara reconfirmed that it would follow the recommended international rules and gene symbols. Besides that, the following principles were stated:

- The symbols commonly used by many workers can be retained even if they do not fit the rules completely, e.g., *lax*, *sd*, *Xa*.
- When a new gene is identified but its allelic relationships with previously reported mimic genes are not known, it is denoted by adding (t) to its symbol, meaning tentative, e.g., *d-50(t)*.

- The list of genes includes those for which seed stocks are maintained.

Newly designated genes and the revision of the symbols are shown in Table 1. Temporarily, the old symbols will be shown in parentheses. Numbering of suffixes attached to the basic symbols was coordinated with the genes preserved in Japan. Our committee desires that the adoption of uniform gene symbols will promote cooperation and exchange of information on rice genetics. During the course of discussion, some problems remained for further discussion, as follows:

- Classification of chlorophyll aberrations: albino, virescence, chlorina, zebra, stripe, fine stripe, and other characters
- Genes for heading date: *E*, *Ef*, *m-Ef*, *Se*, *se*
- Genes for leaf spot: *bl*, *spl*, *sl*, *ysl*, *zn*
- Genes for sterility: *s-a-1*, *s-a-2*, *S^a-1*, *S-A-1*, etc.
- Genes for lethality: *L-1-a*, *L-1-b*, *D-a*, *W-a*, etc.
- Suffix number of basic symbols: *al-na*, *chl-no*, *d-no*, *ga-no*, *ms-no*, etc.
- Needs for allelism test: *v-1(t)*, *nal*, *rl*, *bl*, *spl*, etc.
- Semidwarfness and dwarfness: *sd-1* = *d-47*
- Disease and insect resistance: *Pi*-series, *Ydv* (mycoplasma disease), *Grh*, and *Glh*
- Cytoplasmic male sterility: [*ms-bo*], [*ms-ld*], etc.
- Isoenzymes: *Acp-1* = *Acp-B*.

LINKAGE GROUPS

As early as 1948, Jodon (4) provided eight linkage groups based on accumulated data at that time. Following this, Nagao and Takahashi (10) first constructed the 12 groups corresponding to the haploid number of chromosomes in 1963. Three years later Misro et al (9) also presented complete linkage maps in indica. However, because of differences in the genetic scheme for anthocyanin coloration and scarcity of identical genes involved in the two series of linkage groups at that time, it has been difficult to establish 12 groups common to indica and japonica types. For reference, the two different genic propositions on the anthocyanin coloration in different organs are shown in Table 2. According to the scheme of Takahashi (16), there was multiple differentiation of alleles at the three loci *C*, *A*, and *P* to account for the intensity of pigmentation and color hues, depending on the various combinations of the three genes. The coloration of parts other than the apiculus can be partly explained by the

pleiotropic action of the basic genes. However, the majority of coloration patterns was the end product of the pleiotropic effect of distributing and localizing the pigment into respective parts in coexistence with the basic color-producing genes. In contrast with this, the genes were allotted to the coloration localized in only one or two organs and the linkage relationships were considered to be among genes in indica rice (8). In addition, at least six kinds of inhibitors inserted their actions on the coloration of various parts in Takahashi's scheme, while the interactions of inhibitor (*I*) and anti-inhibitor (*Ai*) were postulated to describe the various segregation ratios in the coloration by Dhulappanavar's system (1). Recently it was found that the *C-A-P* gene scheme for anthocyanin pigmentation in japonica is also applicable to indica, except for the nature and number of localized genes (13). Any two of the genes *Pa*, *Pb*, and *Pc* act together as basic genes instead of the alleles at the *P* focus. Thus, further experiments are needed for the identification of color genes by exchanging materials between Japanese and Indian workers. According to Khush et al (5), complete correspondence between the linkage groups of japonica and indica was demonstrated. On the other hand, distorted segregations exist in the intervarietal crosses in rice caused by several reasons. These problems must be pursued by using uniform linkage maps.

The linkage maps presented in Figure 1 include 119 marker genes and amendments to Takahashi and Kinoshita's (17) maps based on recent findings in japonica and indica rice. Linkage relations other than those included in the maps are shown in Table 3. The sixth and ninth groups have been combined, since the point of interchange RT2-3d connecting the two groups was detected by Sato et al (12). However, there is no definite map combining the markers of the fifth and seventh groups. The twelfth groups were retained except that *gl-1* and *An-2* were shifted to sixth plus ninth group. *Hg* and *d-20* belonging to the twelfth group have not been subjected to trisomic analysis.

Different systems of numbering the chromosomes, linkage groups, and trisomics have been employed by different authors, as presented in Table 4. The relative lengths of chromosomes are in agreement except for two chromosomes in the karyotypes determined by Shastry et al (14) and Kurata et al (7). The correspondence between the extra chromosomes of the trisomics and the chromosomes of Nishimura's designation (11) was also ascertained by the analysis of translocation. However, the identification of the extra chromosomes corresponding to the respective trisomics was significantly different in the systems of Kurata et al (7) and Khush et al (5), except for the first (largest) and second chromosomes. Accord-

Table 1. Supplement and revision of gene symbols.

a. Addition to the gene list by IRC

<i>Acp-1</i>	Acid phosphatase-1	<i>Pj</i>	Purple junctura
<i>alk</i>	alkali degeneration	<i>Pjb</i>	Purple junctura back
<i>bgl</i>	bright green leaf	<i>Plm</i>	Purple leaf margin
<i>Bp</i>	Bulrush-like panicle	<i>Pmr</i>	Purple midrib
<i>Bph</i>	Brown planthopper resistance-1	<i>Pnr</i>	Purple nodal ring
<i>Cat-1</i>	Catalase-1	<i>rcn</i>	reduced culm number
<i>drp-1</i>	dripping-wet leaf-1	<i>Rf-1</i>	Pollen fertility restoration-1
<i>du</i>	dull endosperm	<i>rfs</i>	rolled fine striped leaf
<i>eg</i>	extra glume	<i>Sb</i>	Stem borer resistance
<i>Est-1</i>	Esterase-1	<i>sd-1</i>	dee-geo-woo-gen dwarf
<i>eui</i>	elongated uppermost internode	<i>shr-1</i>	shrunken endosperm-1
<i>fes</i>	female sterile	<i>sl</i>	sekiguchi lesion
<i>ga-1</i>	gametophyte gene-1	<i>Sm</i>	Stem maggot resistance
<i>gf</i>	gold furrows of hull	<i>sp</i>	short panicle
<i>Glh-1</i>	Green leafhopper resistance-1	<i>spl-1</i>	spotted leaf-1
<i>gm</i>	gall midge resistance	<i>su</i>	sugary endosperm
<i>Grh</i>	Green rice leafhopper resistance	<i>ts</i>	twisted stem
<i>Hbv</i>	<i>Hoja blanca</i> virus resistance	<i>Tuv</i>	Tungro virus resistance
<i>HI</i>	Hairy leaf	<i>Un</i>	Uneven grain
<i>Lap-1</i>	Leucine amino peptidase-1	<i>Ydv</i>	Yellow dwarf resistance
<i>lgt</i>	long twisted grain	<i>ylb</i>	yellow banded leaf blade
<i>lhs</i>	leafy hull sterile-1	<i>ysl</i>	yellow leaf spot
<i>lp</i>	long palea	<i>zn</i>	zebra necrosis
<i>Mdh-1</i>	Malate dehydrogenase-1		
<i>Mi</i>	Minute grain		Cytoplasmic male sterility
<i>mls</i>	malformed lemma		

(Table 1. continued)

<i>ms-1</i>	male sterile-1	<i>[ms-bo]</i>	Cytoplasm from 'Chinsurah boro II'
<i>nbs</i>	nonbearing of spikelets	<i>[ms-ld]</i>	Cytoplasm from 'Lead rice'
<i>ops</i>	open hull sterile	<i>[ms-TA]</i>	Cytoplasm from 'TA 820'
<i>Pa</i>	Purple apiculus	<i>[ms-CW]</i>	Cytoplasm from Chinese wild rice
<i>Pc</i>	Purple coleoptile	<i>[ms-WA]</i>	Cytoplasm, WA-group
<i>Pd</i>	Pendant panicle	<i>[ms-HI]</i>	Cytoplasm, HL-group
<i>Pgi-1</i>	Phosphoglucose isomerase-1	<i>[ms-ip]</i>	Cytoplasm from japonica cultivar 'Akebono'
<i>Pgl</i>	pale green leaf		

b. Revision of gene symbols

Gene symbol		Character	Gene symbol		Character
New	Old		New	Old	
<i>Bsv</i>	<i>Bs</i>	Black streaked dwarf virus resistance	<i>Prp-a</i>	<i>Pp</i>	Purple pericarp
<i>chl</i>	<i>ch</i>	chlorina	<i>Prp-b</i>	<i>Pb</i>	Purple pericarp
<i>dl</i>	<i>lop</i>	drooping leaf	<i>Scl</i>	<i>En-CI</i>	Super clustered spikelets
<i>Ef-1</i>	<i>E</i>	Earliness-1	<i>sd-1</i>	<i>d-47</i>	dee-geo-woo-gen dwarf
<i>fgl</i>	<i>fl</i>	faded green leaf	<i>Sdr</i>	<i>Sd</i>	Seed dormancy
<i>G-2</i>	<i>Gm, Gl</i>	Long sterile lemmas-2	<i>Se-1</i>	<i>Lm, Lf</i>	Photosensitivity-1
<i>Gsv</i>	<i>Gs</i>	Grassy stunt virus resistance	<i>Shp</i>	<i>Ex</i>	Sheathed panicle
<i>Hg</i>	<i>Lh</i>	Hairy glume	<i>st-1</i>	<i>ws</i>	stripe-1
<i>lax</i>	<i>lx</i>	lax panicle	<i>st-2</i>	<i>gw</i>	stripe-2
<i>lhs-1</i>	<i>op</i>	leafy hull sterile-1	<i>st-3</i>	<i>stl</i>	stripe-3
<i>Pcs</i>	<i>ops-2</i>	parthenocarpy sterile	<i>st-4</i>	<i>ws-2</i>	stripe-4
<i>Pi-se-1</i>	<i>Rb-1</i>	<i>Pyricularia oryzae</i> resistance-se	<i>Stv</i>	<i>St</i>	Stripe virus resistance
<i>Pi-is-1</i>	<i>Rb-4</i>	<i>Pyricularia oryzae</i> resistance-is	<i>Wph</i>	<i>Wbph</i>	White backed planthopper resistance
<i>Pox</i>	<i>Px</i>	Peroxidase	<i>Xa</i>	<i>Xe</i>	<i>Xanthomonas oryzae</i> resistance

Table 2. Genic scheme of anthocyanin coloration.

a. Japonica rice

Fundamental genes	Distribution gene	Coloration of organs
Combination of C (1:44) and A (III:68)	Pleiotropic action of <i>C.A.P.</i>	coleoptile, leaf sheath (line), internode (line), midrib apiculus, sterile lemma, stigma
$C^{Bs} > C^B > C^{Bp} > C^{Bt} > C^{Br} > C^{Bd} \geq C^{Bk} \geq C^{Bc} \geq C^{Bm} \geq$	$P \geq P^k \geq P^c \geq P^+$ (II:2% from <i>Pl</i>)	
C^+	<i>Pr</i> (II:137)	lemma, palea
$A^S \geq A^E > A > A^d > A^m > A^+$	<i>Ps-1</i> (V:100), <i>Ps-2</i> (II:163), <i>Ps-3</i> (II:141) <i>Pl</i> (II:61)	stigma
	<i>Pl^w</i> (allelic with <i>Pl</i>)	leaf blade, sheath, collar, pulvinus, internode
	<i>Plⁱ</i> (allelic with <i>Pl</i>)	leaf blade, sheath, internode, part of collar and pulvinus, pericarp
	<i>Pn</i> (III:96) <i>Pin-1</i> (II:31% from <i>Pl</i>)	leaf blade, sheath, internode (dilute color) pulvinus, collar, leaf margin internode leaf sheath, part of collar and pulvinus
Inhibitors:	<i>I-Pl-1</i> inhibits the coloration of leaf blade in the interaction with <i>Pl</i> . <i>I-Pl-1,2,3</i> partly inhibit the coloration of leaf blade and sheath in the interaction with <i>Pl^w</i> . <i>I-Pl-45</i> inhibit the coloration of pericarp in the interaction with <i>Pl^w</i> . <i>I-Pl-6</i> completely inhibits the coloration of leaf blade and sheath in the interaction with <i>Plⁱ</i> .	

b. Indica rice

Character	Gene (linkage group) ^a	F ₂ segregation ratio (P:W or G)
Coleoptile	<i>Pc</i> ₁ (III), <i>Pc</i> ₂ , <i>I-Pc</i> , <i>Ai-Pc</i> (IV)	9:7,39:25,195:61
Apiculus	<i>Pa</i> (IV), <i>Pb</i> , <i>Pc</i> , <i>I-P</i> , <i>Ai-P</i> (IV), <i>Ap</i> (IV)	3:1,9:7,3:13,39:25,9P:3R:4W
Lemma	<i>Pr</i> ₁ (X), <i>Pr</i> ₂ , <i>Pr</i> ₃ , <i>A Pr</i> _a (IV)	9:7,9:55,405:619
Stigma	<i>A Ps</i> _{a1} , <i>Ps</i> _{a2} , <i>Ps</i> _{a3} , <i>Ps I-Ps</i> (III)	9:7,3:13,162:94
Glume, outer glume	<i>Pg</i> ₁ (X), <i>Pg</i> ₂ (III), <i>Pg</i> ₃ , <i>A1-Pg</i> (IV) <i>A Pg</i> (IV), <i>Gp I-Gp</i> (III)	9:7,3:13,9:55,27:37,117:139
Leaf blade	<i>CA LSP</i> ₁ , <i>Lsp</i> ₂ , <i>Il</i> _p	241G:15P,3:13, various
Leaf axil	<i>Px</i> (III), (four or five)	162:94,567:457
Leaf sheath	<i>CA Lsc</i> , <i>Psh</i> (III)	9:7,27:37,3:253

(Table 2. continued)

Character	Gene (linkage group) ^a	F ₂ segregation ratio (P:W or G)
Auricle	<i>Pau_a</i> (III), <i>Pau_b</i> , <i>l-Pau</i> , <i>Ai-Pau</i> (IV)	9:7, 27:37, 117:139, 387:637
Ligule	<i>Plg_a</i> (X), <i>Plg_b</i> (X), <i>Ai-Plg</i> (IV)	45:19, 27:229, 117:139
Juntura	<i>Pj_a</i> (IV), <i>Pj_b</i> , <i>Pj_c</i> , <i>Pj_d</i> , <i>Pj_e</i> <i>Pj_a</i> (III), <i>Pj_{b1}</i> , <i>Pj_{b2}</i> † <i>Pj</i>	3W:1P, 9:7, 45:19, 162:94, 243:781
Juntura back		247W:9P
Nodal ring	<i>Pnr₁</i> (X), <i>Pnr₂</i> , <i>Pnr₃</i>	9:55
Node	<i>Pn₁</i> (X), <i>Pn₂</i> , <i>Pn₃</i> , <i>Pn</i> (III)	9:55, 117:139, 9:247, 117:139
Pulvinus	<i>Pu_a</i> (III), <i>Pu_b</i> , <i>Pu_c</i> , <i>Pu_d</i>	81:175
Septum	<i>Pm_a</i> (III), <i>Pm_b</i> , <i>Pm_c</i> , <i>Pm_d</i>	9:7, 189P:45Y:22W
Internode	<i>Pin_a</i> (III), A <i>Pin_a</i> (IV), <i>Pin_{a1}</i> (IV), <i>Pin_{a2}</i> , <i>Pin_a</i> (X), <i>Pin_b</i>	9:7, 9P:6P1:1Y, 9P1:6G:1Y, 27:37

^aAfter 9.

ingly, the author proposes to revise the numbering of chromosomes in the karyotype, and the coordinated numbering of chromosomes and linkage groups will be adopted when a reliable correspondence between the karyotype and the series of trisomics is made and agreed upon by most rice geneticists.

FUTURE PROBLEMS

Besides the coordinated numbering of chromosomes and linkage groups, the following matters in relation to linkage groups will need attention.

- Allelism tests between marker genes with similar effects are most important. For this purpose, an exchange of gene stocks and information among rice workers is proposed.
- Different workers are building up multiple markers, induced mutants, and cytogenetic materials. Visible but easily identifiable mutants would be valuable for use in linkage studies. The accuracy of recombination values will be much improved by the use of male sterile genetic stocks. The author did a trial as shown in Table 5.
- A complete set of primary trisomics has already been established in both japonica and indica rice (3,5). Chromosome staining techniques have progressively improved. The use of in situ hybridization, chromomere number, and chromosome banding is important for the reliable identification of chromosomes. The extensive

Fig. 1. Linkage maps of rice.

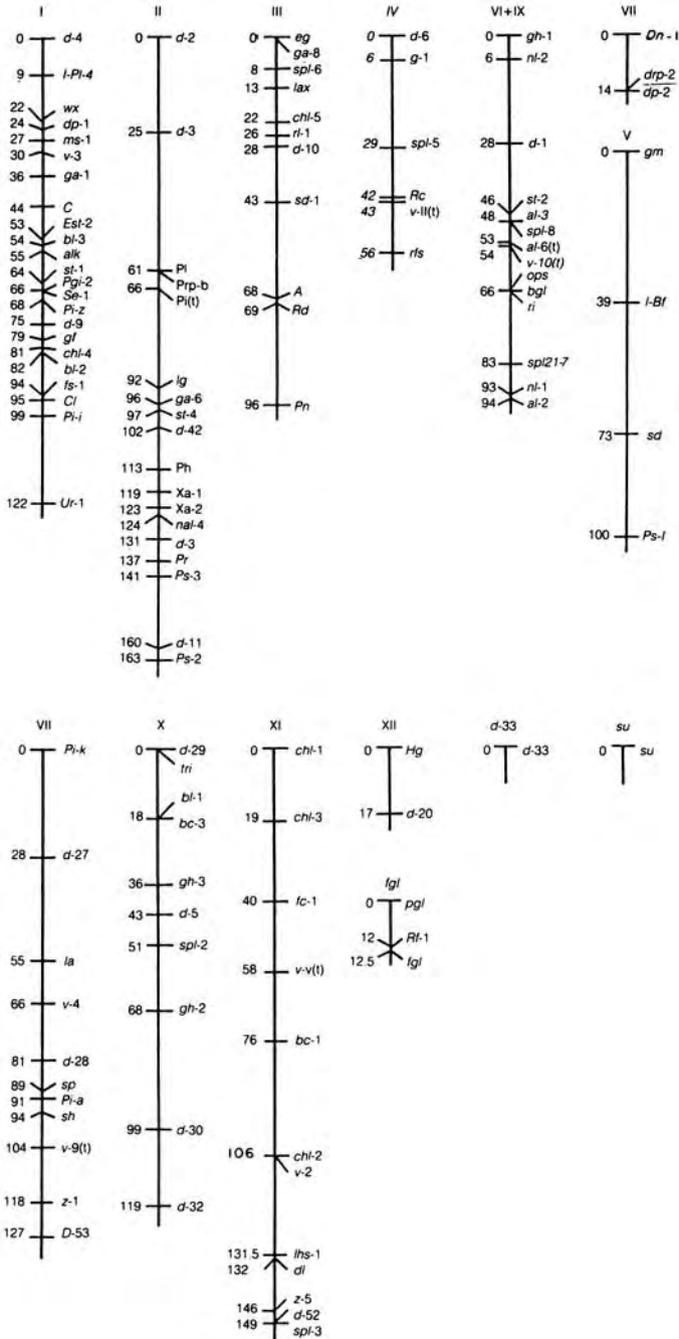


Table 3. Linkage relations not shown on the map.

Group I (<i>wx</i> group)		RCV ^a
<i>al-1</i>	<i>wx</i>	7.1%
<i>al-9</i>		trisomic B
<i>chl-7</i>	<i>Pl-z</i>	27
<i>d-21</i>	<i>wx</i>	8.3
<i>ga-4</i>	<i>wx</i>	34
<i>ga-5</i>	<i>wx</i>	27
<i>Hl-a</i>	<i>fs-1</i>	21
<i>I-Pl-2</i>	<i>I-Pl-4</i>	10
<i>rcn</i>	<i>C</i>	32
<i>s-a-1</i>	<i>wx</i>	21
<i>S-b-1</i>	<i>wx</i>	18
<i>s-c-1</i>	<i>C</i>	8.6
<i>s-d-1</i>	<i>wx</i>	33
<i>S-1</i>	<i>C</i>	close
<i>S-A-1</i>	<i>C</i>	9.5
<i>S-B-2</i>	<i>wx</i>	28
<i>spl-4</i>	<i>dp-1</i>	2.5
<i>Stv-a</i>	<i>wx</i>	38
<i>Un-a</i>	<i>Cl-a</i>	22
<i>v-1</i>	<i>C</i>	25
<i>zn</i>	<i>C</i>	20
Group II (<i>Pl</i> group)		
<i>al-5</i>	<i>lg</i>	34%
<i>al-7(t)</i>	<i>lg</i>	31
<i>An-1</i>	<i>d-11</i>	5.4
<i>Bph-1</i>		trisomic E
<i>bph-2</i>	<i>d-2</i>	39
<i>drp-1</i>	<i>d-2</i>	39
<i>drp-5(t)</i>	<i>lg</i>	17
<i>ga-10(t)</i>	<i>lg</i>	27
<i>nal-1</i>	<i>d-2</i>	25
<i>nal-5</i>	<i>lg</i>	9.5
<i>P</i>	<i>Pl</i>	2.7
<i>Pin-1</i>	<i>Pl</i>	31
<i>rk-1</i>	<i>lg</i>	35
<i>rl-2</i>	<i>d-2</i>	35
<i>s-c-2</i>	<i>Ph</i>	31
<i>s-e-2</i>	<i>lg</i>	15
<i>Sc-1</i>	<i>lg</i>	26
<i>ssk</i>	<i>Pl</i>	6.8
<i>Wh</i>	<i>lg</i>	8.0
<i>Xa-kg</i>	<i>Xa-1</i>	2.1
<i>ylm</i>	<i>lg</i>	10
<i>z-5</i>	<i>lg</i>	11

^a Recombination value.

Table 3. continued

Group III (A group)		
<i>al-4</i>	<i>lax</i>	13
<i>al-8</i>	<i>d-18</i>	11
<i>bph-4</i>	<i>Bph-3</i>	close
<i>chl-6</i>	<i>lax</i>	31 %
<i>d-18</i>	<i>RT3-8b</i>	0.6
<i>d-26(t)</i>	<i>A</i>	37
<i>d-54</i>	<i>rl-4</i>	30
<i>d-55</i>	<i>eg</i>	12
<i>fs-2</i>	<i>d-18</i>	13
<i>ga-7</i>	<i>A</i>	29
<i>ga-9</i>	<i>d-18</i>	0.6
<i>Glh-3</i>	<i>bph-4</i>	34
<i>I-PS-b</i>	<i>A</i>	link.
<i>lgt</i>	<i>d-26</i>	16
<i>Prp-a</i>	<i>A</i>	7.3
<i>rl-4</i>	<i>A</i>	20
<i>shr-1</i>	<i>rl-4</i>	24
<i>ts-a</i>	<i>A</i>	23
<i>v-6</i>	<i>lax</i>	27
Group IV (g-1 group)		
<i>d-7</i>	<i>d-6</i>	39
<i>ge</i>		trisomic F
<i>lp-1</i>	<i>Un-b</i>	12
<i>m-Ef-1</i>	<i>Rc</i>	23
<i>se-2</i>	<i>g-1</i>	23
<i>Un-b</i>	<i>g-1</i>	18
<i>Xa-4</i>		Triplo 7
Group VI+IX (d-1 group)		
<i>An-2</i>	<i>gl-1</i>	33
<i>bd</i>	<i>gl-1</i>	22
<i>er</i>	<i>gh-1</i>	38
<i>eui</i>	<i>nl-1</i>	27
<i>gl-1</i>	<i>RT2-3d</i>	12
<i>I-PI-1</i>	<i>gh-1</i>	31
<i>v-10(t)</i>	<i>ri</i>	12
<i>xa-5</i>		trisomic L
<i>ylb</i>	<i>nl-1</i>	32
Group VII (Dn-1 group)		
<i>Bp</i>		trisomic H
<i>d-57</i>	<i>dn-1</i>	21
<i>Pi-ta</i>	<i>RT1-4</i>	4.5
<i>sl</i>	<i>Pi-ta</i>	10

Table 3. continued

Group VIII (<i>la</i> group)		
<i>drp-7</i>		trisomic G
<i>Ef-1</i>	<i>la</i>	38
<i>nal-2</i>	<i>la</i>	36
<i>Pi-f</i>	<i>Pi-k</i>	15
<i>Pi-se-1</i>	<i>la</i>	9.5
<i>Pi-is-1</i>	<i>la</i>	23
<i>S-3</i>	<i>la</i>	1
<i>z-2</i>	<i>d-27</i>	5.9
Group X (group)		
<i>d-29</i>	<i>bl-1</i>	14
<i>Pi-b</i>	<i>RT-7-8</i>	5.8
Group XI (<i>bc-1</i> group)		
<i>An-3</i>	<i>bc-1</i>	38
<i>al-10</i>	<i>dl</i>	22
<i>bl-4</i>	<i>bc-1</i>	29
<i>d-14</i>	<i>dl</i>	32
<i>d-56</i>	<i>dl</i>	7.2
<i>drp-3</i>	<i>dl</i>	22
<i>drp-4</i>	<i>dl</i>	6.0
<i>ga-2</i>	<i>dl</i>	11
<i>ga-3</i>	<i>dl</i>	34
<i>Lk-f</i>	<i>bc-1</i>	19
<i>Mi</i>	<i>Lk-f</i>	24
<i>rl-5</i>	<i>chl-1</i>	13
<i>s-e-1</i>	<i>bc-1</i>	16
<i>st-3</i>	<i>bc-1</i>	1.1
<i>v-5</i>	<i>chl-1</i>	2.0
<i>v-7</i>	<i>bc-1</i>	1.7
Group XII (<i>Hg</i> group)		
<i>lhs-2</i>	<i>Hg</i>	8.2
<i>fgl</i> group		
<i>Bph-3</i>		trisomic C
<i>bph-4</i>	<i>rk-2</i>	30
<i>du</i>		trisomic C
<i>Ef-2</i>	<i>RT3-7</i>	7.8
<i>Glh-3</i>	<i>bph-4</i>	34
<i>rk-2</i>	<i>RT7-9</i>	2.5
<i>d-33</i> group		
<i>d-33</i>		trisomic A
<i>nal-3</i>	<i>RT3-4b</i>	19
<i>rl-3</i>	<i>RT4-12</i>	13
<i>spl-1</i>	<i>RT3-4a</i>	1.7
<i>su</i> -group		
<i>An-4</i>	<i>RT10-12b</i>	5.0
<i>d-51</i>		Trisomic D
<i>Stv-b</i>	<i>RT3-12</i>	link.
<i>su</i>		trisomic D
<i>ur-2</i>		trisomic D
<i>V-8</i>		trisomic D
<i>2-4</i>		trisomic D

Table 4. Relationships among various systems of numbering linkage groups, chromosomes, and trisomics.

Linkage groups		Chromosomes			Trisomics	
Kinoshita (6)	Misro ^a (8)	Nishimura (11)	Shasttry et al (14)	Kurata et al (17)	Khush et al (5)	Iwata and Omura (3)
I	I	6	3	K6	triplo-3	B
II	II	11	12	K4	triplo-12	E
III	III	3	1	K1	triplo-1	O
IV	IV	10	7	K11	triplo-7	F
VI+IX	VI,XII	2	5	K9	triplo-5	L
V	IX	1	—	—		H
VII		1	9	K10	triplo-9	H
VIII	VIII	9	11	K8	triplo-11	G
X		8	2	K2	triplo-2	N
XI	XI	5	4	K3	triplo-4	M
XII						
fgl		7	10	K12	triplo-10	C
d-33		4	6	K5	triplo-6	A
su		12	8	K7	triplo-8	D

^a Groups V, VII, and X were not correlated with Kinoshita's groups.

Table 5. Recombination values calculated from backcrossings and F₂ segregations.

Linkage group	Gene pair	Recombination value (%) ^a		
		Backcross		F ₂
I	C — <i>st-1</i>	27.6	c	25.5
	<i>fs-1</i> — <i>Cl</i>	0.3		
	<i>fs-1</i> — <i>Ur-1</i>	27.3	c	27.5
II	<i>d-2</i> — <i>lg</i>	55.6	c	47.2
	<i>d-2</i> — <i>Pr</i>	43.7	c	50.0
	<i>d-2</i> — <i>P</i>	52.5		
	<i>Pl</i> — <i>lg</i>	25.6	c	30.9
	<i>Pr</i> — <i>lg</i>	30.3	c	28.2
	<i>Pr</i> — <i>P</i>	32.2	c	
III	A — <i>Pn</i>	20.0	r	29.9
	A — <i>bl-7</i>	29.0		
	<i>Pn</i> — <i>bl-7</i>	33.8	c	36.3
IV VI+IX	<i>d-6</i> — <i>g-1</i>	7.7	r	14.0
	<i>gl-1</i> — <i>An-2</i>	42.3	c	39.4
	<i>gh-1</i> — <i>st-2</i>	32.0	r	40.9

^ac = coupling, r = repulsion.

use of cytological mutants such as telotrisomics and tertiary trisomics and induced deficiencies should be encouraged to locate the gene loci on the respective arms of chromosomes. The problem of inter-varietal hybrid sterility must be pursued by cytological analysis.

- Linkage information should be utilized in breeding programs. Linkage relations between marker genes and agronomic characters such as cold tolerance and germinability at low temperature have also been detected (15).
- Recently, plant tissue culture techniques have progressed, and single cell systems such as suspension culture, pollen culture, and protoplast culture are going to be established. With the development of the cell fusion technique, somatic cell genetics of rice will be used as an advanced method. On the other hand, recombinant DNA techniques will also be important for the development of rice genetics and breeding. The construction of elaborate chromosome maps will be important as a basis for new technologies in the advancement of rice genetics.

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THE RELATIONSHIP BETWEEN CYTOLOGICALLY IDENTIFIED CHROMOSOMES AND LINKAGE GROUPS IN RICE

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A complete set of primary trisomics, which were obtained from the progeny of $2x/3x$ in a japonica cultivar, and 52 lines of reciprocal translocations were used as cytological tools in a study of the association between the 12 cytologically identified chromosomes and linkage groups. Karyotype analysis by a new technique was also used for identification of the extra chromosome of trisomics. Eighty-eight marker genes were described by our laboratory, about half of them induced by treatment of fertilized egg cells with N-methyl-N-nitrosourea or irradiation. Development of new marker genes and use of trisomics to identify the chromosome on which they are located were effective for the establishment of linkage groups. Linkage groups consisting of only the new marker genes were found. As the numbers assigned to chromosomes and linkage groups have been independently used until now, a standardized numbering system for rice chromosomes based on karyotypes is proposed, and some problems are pointed out and discussed.

Nagao and Takahashi (16) first proposed 12 linkage groups in japonica rice cultivars. Cytological evidence supporting these groups, however, was not clear. Since then, trisomics and reciprocal translocations have been used as cytological tools for establishing the relationship between the linkage groups and the 12 chromosomes (2, 3, 4, 5, 7, 12, 18, 19, 20, 21, 23, 28). Karyotype analysis was also used for the identification of the extra chromosomes of trisomics (13). Consequently, the complete association between the 12 chromosomes and the linkage groups was elucidated in japonica rice (6, 8, 9) at almost the same time as in indica rice (11).

The present paper, which deals mainly with the results of the author and his colleagues at Kyushu University, discusses the relationships among chromosomes, trisomics, and linkage groups in japonica rice. The

results are summarized in Table 1. Based on the results, some comments follow.

RECIPROCAL TRANSLOCATIONS

Nishimura (17) first studied the reciprocal translocations of rice in Japan and designated the numerical order of chromosomes on the basis of identification of chromosomes concerned with reciprocal translocations. The numbers adopted for chromosomes (RT) in Table 1 are those used by Nishimura (17), and those used for linkage groups are those of Nagao and Takahashi (16). Using 37 lines with reciprocal translocations from Nishimura (17) and others, the linkage relation between the interchanged point of reciprocal translocations and the marker genes was examined and the 9 linkage groups were clarified for corresponding chromosomes. On the other hand, reciprocal translocations were available to identify the extra chromosome of trisomics. If the extra chromosome of a trisomic is the same as one of the translocated chromosomes, these chromosomes will form one pentavalent in the pollen mother cells (PMCs) of trisomic F_1 plants of the cross between the trisomic and the reciprocal translocations. This method made it possible to identify the extra chromosome of three types of trisomics—A, C, and D—as chromosomes 4, 7, and 12, none of which corresponded to any linkage group of Nagao and Takahashi (16).

Now we have 52 lines of reciprocal translocations available for linkage studies (2, 3, 8, 28), providing one of the most useful tools for the establishment of a chromosome map of rice in the future.

TRISOMICS

The trisomic method is the most positive way to test the independence of linkage groups, but with this method it is impossible to determine the position of genes on the linkage map. Iwata et al (7) and Watanabe and Koga (27) succeeded in establishing a complete set of 12 trisomics in japonica rice. Our trisomics were distinguished morphologically into 12 types, A–L, and their extra chromosomes were identified by crosses with marker stocks.

First, the relationship between six trisomic types—B, E, F, G, H, and L—and linkage groups was clarified. Seven marker genes of the three linkage groups—VI, IX, and XII—showed trisomic segregation in the

Table 1. Relationships among chromosomes, trisomics, and linkage groups in rice.

Chromosome		Trisomics		Linkage group	Marker genes ^a
RT	Karyotype	Japonica	Indica		
1	K10	H	triplo-9	VII V	BP* , Dn , dp-2* , <i>drp-2*</i> I-Bf
2	K9	L	triplo-5	VI IX XII	bgl* , d-1 , nl-2 : ops* , v-10(t)* nl-1 , rl , <i>spl-7*</i> , <i>spl-8*</i> gl-1
3	K1	O	triplo-1	III	<i>A</i> , <i>ch-5*</i> , <i>ch-6*</i> , <i>d-10*</i> , d-18 , <i>eg*</i> , <i>fs-2</i> , lax , <i>Pn</i> , <i>Rd</i> , <i>rl-2*</i> , <i>spl-6*</i> , v-6* , <i>shr-1*</i>
4	K5	A	triplo-6	—	d-B* , nal-2* , rl-1 , spl-1*
5	K3	M	triplo-4	XI	<i>bc-1</i> , <i>ch-1*</i> , ch-2* , <i>ch-3*</i> , <i>ch-7*</i> , <i>d-k-2*</i> , dl* , <i>drp-3*</i> , <i>drp-4*</i> , <i>fc*</i> , <i>gh-1</i> , <i>op*</i> , <i>rl-3*</i> , <i>spl-3*</i> , <i>stl*</i> , <i>v-1</i> , v-2* , <i>v-5*</i> , <i>v-7*</i> , <i>z-3*</i>
6	K6	B	triplo-3	I	<i>C</i> , ch-4* , Cl , dp-1* , <i>spl-4*</i> , <i>v-3*</i> , ws* , wx
7	K12	C	triplo-10	—	du* , fl* , pgl* , Rf-1 , rk-2*
8	K2	N	triplo-2	X	bl-1 , bc-3* , d-K-1* , <i>d-k-4*</i> , <i>d-W*</i> , gh-2* , <i>gh-3*</i> , <i>spl-2*</i> , <i>tri*</i>
9	K8	G	triplo-11	VIII	<i>d-C*</i> , DK-3* , <i>d-t*</i> , la , sp* , v-4* , z-1* , z-2* , v-9(t)*
10	K11	F	triplo-7	IV	d-6 , g , ge* , Rc , <i>rfs*</i> , <i>spl-5*</i> , v-11(t)*
11	K4	E	triplo-12	II	<i>d-2</i> , <i>d-11*</i> , lg , <i>nal-1*</i> , <i>Ph*</i> , <i>Pl</i> , rk-1* , <i>ylm*</i>
12	K7	D	triplo-8	—	d-51* , su* , ur-2(t)* , v8* , z-4*

^aGenes in bold showed trisomic segregation in the F₂ or BC₁F₁ in crosses with the trisomics concerned, * = found and designated by the author's laboratory.

F₂'s with trisomic type L having an extra chromosome 2 (Table 2). So these linkage groups were considered to reconstruct a new linkage group borne on chromosome 2. The linkage map of chromosome 2 consisting of the genes *d-1*, *ups*, *nl-2*, *bgl*, *nl-1*, and *ri* of linkage groups VI and IX is presented in Figure 1, but none of the linkage relations between these genes and *gl-1* of linkage group XII were found. Although *gh-1* was regarded as a marker gene of linkage group VI, it showed disomic segregation in the F₂ with trisomic type L. Moreover, *gh-1* was found to be located on chromosome 5 by analysis using reciprocal translocations and to link closely with *ch-1* of linkage group XI, with a recombination value of 3.6%.

Similarly, linkage groups VII and V were found to be associated with chromosome 1, which is the extra chromosome of trisomic type H,

Table 2. Trisomic segregations of the marker genes belonging to three linkage groups in the F₂'s of crosses with trisomic type L.

Linkage group	Gene	Portion of population	Observed number			χ^2 value ^a		
			Dominant	Recessive	Total	Disomic	Trisomic	
						3:1	8:1 for 2x	44:1 for 2x+1
VI	<i>d-1</i>	2x	259	48	307		6.362*	
		2x + 1	131	0	131			2.977
		Total	390	48	438	46.047***		
	<i>ops</i>	2x	271	36	307		0.118	
		2x + 1	131	0	131			2.977
		Total	402	36	438	65.781***		
	<i>nl-2</i>	2x	288	31	319		0.627	
		2x + 1	189	1	190			2.515
		Total	477	32	509	95.063***		
	<i>bgl</i>	Pooled	63	1	64	18.750***		
	<i>gh-1</i>	2x	49	15	64		9.846***	
		2x + 1	29	10	39			98.439***
		Total	78	25	103	0.029		
		Pooled	302	96	398	0.164		
	IX	<i>nl-1</i>	2x	168	24	192		0.016
2x + 1			113	0	113			2.568
Total			281	24	305	47.739***		
<i>ri</i>		2x	257	15	272		8.625**	
		2x + 1	167	1	168			2.047
		Total	424	16	440	107.103***		
XII	<i>gl-1</i>	2x	245	23	268		1.736	
		2x + 1	165	1	166			2.005
		Total	410	24	434	87.745***		

^a* Significant at 5% level, ** significant at 1% level, *** significant at 0.5% level.

because *Bp*, *Dn*, and *dp-2* of linkage group VII and *IBf* of linkage group V showed trisomic segregation in crosses with this trisomic type.

The construction of three new linkage groups has been proposed for the three trisomic types — A, C, and D — that did not correspond to any linkage groups of Nagao and Takahashi (16) by the fact that they associated with several new marker genes. Three types of trisomics —

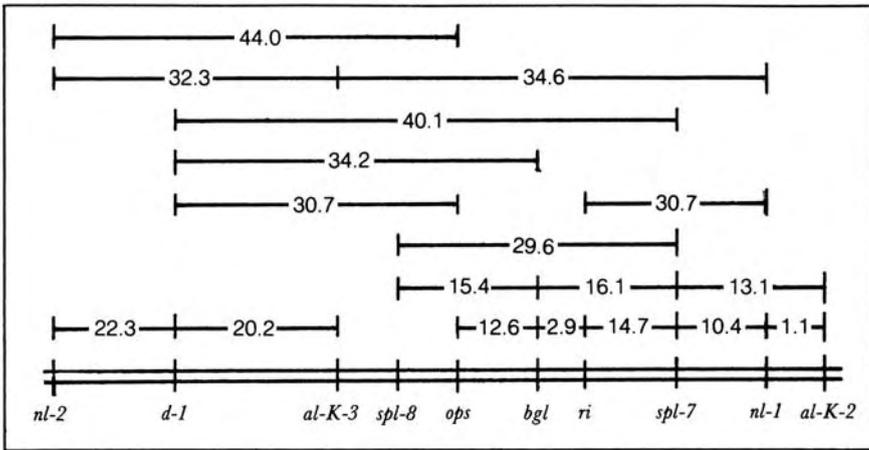


Fig. 1. A linkage map of 10 genes on chromosome 2.

having either chromosome 3, 5, or 8 as the extra chromosome—have never been found in our trisomics. Kurata et al (13) found by karyotype analysis of these trisomics that four types—G, I, J, and K—had the same extra chromosome, K8. Three types—having chromosome K1, K2, or K3 as the extra chromosome—have not been found in the trisomics (13). Numbers of chromosomes, K1 to K12, were designated according to the length of somatic chromosomes by karyotype analysis as described later. Recently, the remaining three trisomic types—M, N, and O having chromosomes, 5, 8, and 3, respectively, as the extra chromosome—were obtained from the progeny of a cross between triploid and diploid rice. Now we have two complete sets of trisomics derived from japonica cultivars Nipponbare and Kinmaze. The morphological features of the 12 trisomics are summarized in Table 3. Type M is perfectly self-sterile, but it set some seeds when pollinated by fertile disomics.

A set of 12 trisomics triplo-1 to triplo-12 derived from indica cultivars has been established by Khush et al (11) as shown in Table 1. According to the numbering system proposed by Shastry et al (26), those were designated in descending order of chromosome length of extra chromosomes by karyotype analysis at the pachytene stage of the PMCs. Correspondence of extra chromosomes between japonica and indica trisomics has been done by the use of our reciprocal translocations.

Marker genes written in bold letters in Table 1 showed trisomic segregation in the F₂ or BF₁ in crosses with the trisomics concerned.

KARYOTYPES

Kurata and Omura (14) developed a new method for karyotype analysis of chromosomes in rice and designated the chromosomes K1 to K12 according to the descending order of chromosome length at somatic prometaphase. In this method, enzymatically macerated protoplasmic cells of root meristems were prepared by flame drying and stained with Giemsa. Kurata et al (13) identified the extra chromosomes of japonica trisomics by this method. Kurata et al (15) also examined the pachytene chromosomes of microsporocytes by this method and could designate the chromosomes as K1 to K12, because the relative length and the arm ratio correspond to somatic chromosomes, though small disagreements exist between them.

MARKER GENES

The marker genes listed in Table 1 are those used mainly for linkage studies in our laboratory. Marker genes with an asterisk have been found and designated by our laboratory. Although the Japanese Rice Genetics Information Committee (10) has recently discussed the standardization of gene symbols and has recommended new symbols for some genes, the original gene symbols are used in this paper to avoid confusion, because the recommended symbols have still not been authorized internationally.

We tried to find or induce new marker genes useful for linkage studies and obtained numerous ones. These actually contributed to the establishment of the 12 linkage groups. For example, almost all the marker genes of chromosomes 4, 7, and 12 are described newly by us. The number of marker genes we used and their origin are shown in Table 4. Two-thirds of 115 genes have been described by members of our laboratory. Half of them are spontaneous mutants, and the other half were induced by irradiation or chemicals. The treatment of fertilized egg cells with N-methyl-N-nitrosourea (24, 25) is very effective in inducing mutations, and more than 3,000 mutant lines have already been obtained. Of these, mutants for dwarf, chlorophyll, physiological leaf spot, and embryo or endosperm properties are very useful for linkage analysis at an early stage.

There are some linkage groups consisting of only a few mapped genes or consisting of unmapped genes that are identified with a linkage group only by the trisomic method. Therefore, we are trying to find marker genes useful for making detailed linkage maps. In our linkage studies, the

Table 3. Morphological features of 12 primary trisomics derived from a japonica cultivar, Nipponbare.

Type	Short name	Morphological features
A	pale	pale green leaves at heading stage, fertile
B	awned	somewhat rough and lax panicles, awned spikelets
C	small grain	fine stature, bushy, small grain
D	erectoides	dark green leaves, erect panicles, short grain
E	spreading	open tiller, more or less narrow grain
F	rolled leaf	semi-rolled leaves, imperfectly emerged panicles
G	pseudo-normal	nearly the same morphological features as disomics
H	large grain	dark green leaves, large grain, excess of nucleolar chromosomes
L	short panicle	short in height, short panicles, small grain
M	sterile	dark green leaves, short in height, perfectly sterile
N	smooth glume	dark green leaves, small and smooth glume, highly sterile
O	grassy	pale green and droopy leaves, bushy, small and narrow grain, highly sterile

Table 4. Number of marker genes used by Kyushu University researchers and their origin.

Character of marker genes	Total	First described by		Origin		
		Kyushu Univ. researchers	Others	Spontaneous	Irradiation	Chemicals
Coloration	10	2	8	9	1	0
Dwarfness	18	14	4	10	3	5
Chlorophyll character	41	38	3	9	16	16
Morphological character	33	23	10	25	3	5
Physiological character	13	11	2	6	2	5
Total	115	88	27	59	25	31

following routine system is applied: First, the chromosome bearing a new gene tested is clarified by the trisomic method. Then the linkage relations of this gene with marker genes of the chromosome are examined by the conventional method. The effectiveness of this routine system is verified in the process of our linkage studies. Moreover, this system should be successfully applied to the genetic analysis of agronomical or quantitative characters such as resistance to disease or insects, grain quality, and so on by the use of the isogenic trisomics set.

The establishment of chromosome maps based on cytological evidence

might be the next approach for linkage studies in rice, as has been done in maize, barley, and tomato. For this purpose, Sato (22) and Chen (1) tried to make preliminary cytological maps by using reciprocal translocations. In addition to the use of reciprocal translocations and karyotype analysis of pachytene chromosomes, the positive use of deficiencies, telotrisomics, and tertiary trisomics has been planned to this aim, and the preparation of materials is being carried on now in our laboratory.

As seen in Table 1, the chromosome numbers by using reciprocal translocations (17), chromosome numbers based on the karyotype analysis of somatic cells (14), trisomic types based on morphological features (6,7), and linkage group numbers (16) were used independently. As the relationships between chromosomes and linkage groups are completely established, the numbering system of rice chromosomes and linkage groups suitable to both japonica and indica should be standardized. There are various ways to number the chromosomes, but it seems most reasonable to number them according to the length of somatic or pachytene chromosomes in descending orders as in maize and tomato. However, some problems still remain in this system, because there is no correspondence of the karyotypes among the results obtained by various workers. While Khush et al (11) established a set of trisomics and clarified their correspondence with 12 linkage groups, Chen et al (1) proposed a different order of chromosomes by karyotype analysis of a Taiwan cultivar. These results are largely different in chromosome number or descending order of chromosome length, though the other correspondences have been completely confirmed. In order to adopt a suitable numbering system of chromosomes, it is necessary to compare strictly and standardize the method of karyotype analysis.

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RELATIONSHIPS BETWEEN LINKAGE GROUPS AND CYTOLOGICALLY IDENTIFIABLE CHROMOSOMES OF RICE

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Twelve linkage groups of rice have been established. The 12 chromosomes have been identified and numbered according to decreasing order of length either at the pachytene stage of meiosis or in somatic cells. Primary trisomic series have been established by several workers. Extra chromosomes of the trisomic series of IRRI, Los Baños, and CRRI, Cuttack, have been identified according to the numbering system of pachytene chromosomes, and those of the University of Kyushu series according to the numbering system of somatic chromosomes. The IRRI trisomic series and the Kyushu trisomic series have been utilized for associating linkage groups with respective chromosomes and for testing the independence of different linkage groups. Linkage groups corresponding to each of the 12 chromosomes were identified both at IRRI and at Kyushu. There is a need to adopt one of the two chromosome numbering systems universally. Future investigations should aim at determining the arm locations of markers and the positions of centromeres on the linkage maps. Series of tertiary, secondary, and telo-trisomics are needed for this purpose. The exact location of the marker genes on the cytological map can be determined by radiation induced deficiencies.

The establishment of all linkage groups and their association with cytologically identifiable chromosomes is the primary goal of classical genetic studies of crop species. To date, this level of understanding of basic genetics has been achieved only for maize, barley, wheat, tomato, and rice. Rice is the latest entrant to this elite group. The studies leading to the establishment of complete chromosome maps of diploid crop species have proceeded in five steps.

- establishment of linkage groups corresponding to the haploid chromosome number of the species
- cytological identification of the individual chromosomes of the complement

- establishment of the complete primary trisomic series or translocation stocks involving different members of the chromosome complement
- identification of the extra chromosomes of each of the primary trisomics or the chromosomes involved in the translocation tester stocks
- association between the linkage groups and the chromosomes through the modified ratio technique characteristic of trisomic segregations or linkages between marker genes and the translocations

The first case of linkage in rice was reported by Parnell et al (29), and linkage summaries were prepared by Yamaguchi (34), Jodon (13,14), and Ramiah and Rao (30). Nagao and Takahashi (26,27) were the first to propose 12 linkage groups of rice, which were designated linkage groups I to XII.

Shastry et al (32) identified the 12 chromosomes of rice at the pachytene stage of meiosis and described the criteria for identifying individual members of the complement. These included total length, arm ratio, presence of darkly staining chromomeres, and several other morphological features such as nucleolus. The longest chromosome was called chromosome 1, the next longest chromosome 2, and so on. A complete pachytene chromosome complement is shown in Figure 1. Ishii and Mitsukuri (4) and Hu (2) measured the somatic chromosomes of rice, and the longest was numbered 1, the second longest 2, and so on. Kurata and Omura (21) numbered the somatic chromosomes K1 to K12, K1 being the longest and K12 the shortest.

Primary trisomic series of rice were established by Sen (31), Hu (3), Watanabe and Koga (33), Khush et al (19), Iwata et al (12), Iwata and Omura (11), and Misra et al (25). All the trisomics are readily identified from disomic sibs and from each other at different growth stages (Table 1) and are thus easily utilized in gene location work.

Nishimura (28) described reciprocal translocations and arbitrarily numbered the chromosomes involved in these translocations I to XII in the order in which they were discovered. Later on roman numerals were changed to arabic numerals. Nishimura's system of numbering chromosomes is not based on cytological identification. The associations between the linkage groups of Nagao and Takahashi (27) and the chromosomes of Nishimura's system were determined by Iwata and Omura (7,8) by translocation analysis.

To date, extra chromosomes of only three of the trisomic series have been identified cytologically. Khush et al (19) and Misra et al (25) identified the extra chromosomes at the pachytene stage of meiosis

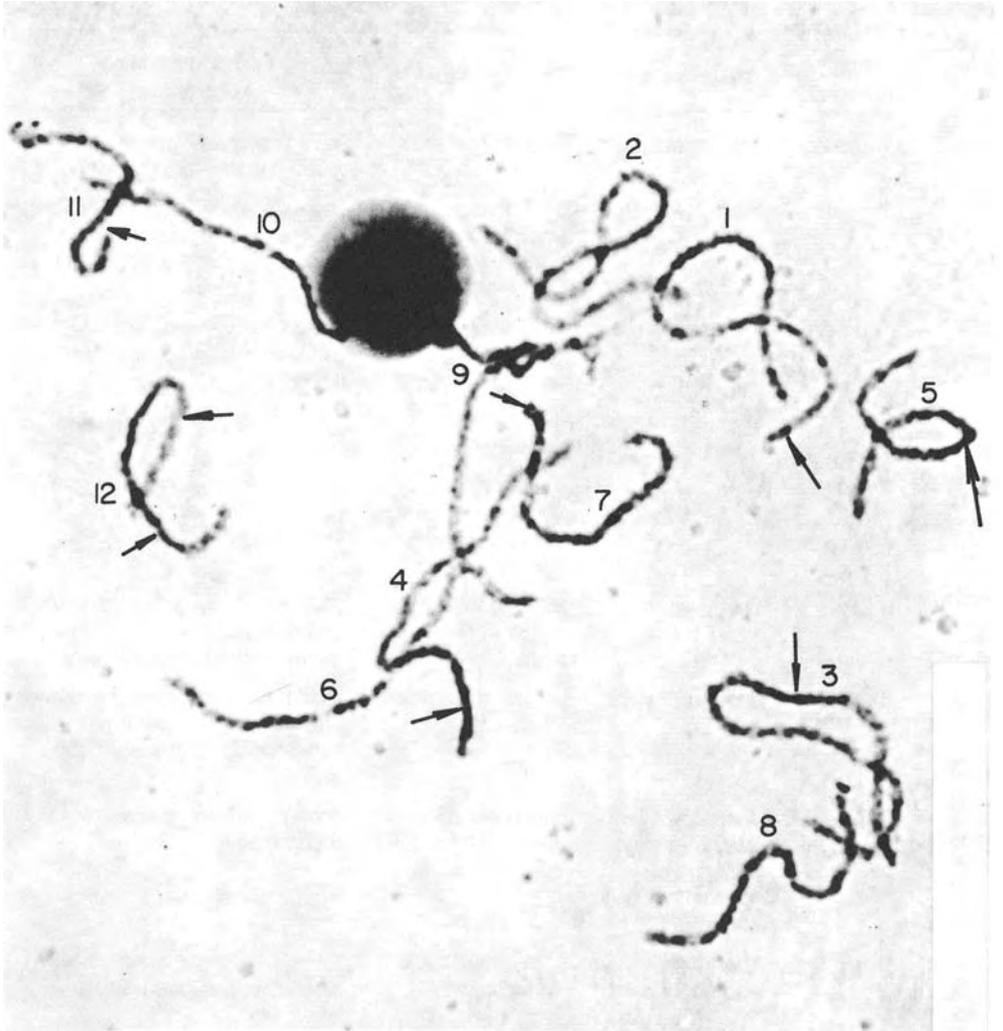


Fig. 1. Pachytene chromosome complement of rice.

according to the numbering system of Shastry et al (32). The trisomic series of Khush et al (19) established at IRRI and those of Misra et al

Table 1. Diagnostic morphological features of primary trisomics of rice.

Primary trisomics	Growth habit	Leaf characters	Other outstanding characters
Triplo-1	short, grassy	long, narrow, thin leaves	seed fertility low, grains narrow and triangular, late flowering
Triplo-2	short, few tillers	short, thick, dark green leaves	short ligule, small anthers, short panicle, round grain, long glume, high female fertility but highly self-sterile
Triplo-3	short, many tillers	short, thick, semi rolled leaves	awned, lax panicle, highly self-sterile, long ligule, early flowering
Triplo-4	short, slow growth	short, thick, dark green leaves	late flowering, highly sterile
Triplo-5	short	twisted, short, fine, white, shiny hairs on leaves at maximum tillering stage	short ligule, compact and short panicle, short anthers, high self seed fertility
Triplo-6	bushy, short, many tillers	light green (pale) leaves at booting stage	lax panicle, few grains, terminal grains deformed, long narrow grains, high seed self-fertility
Triplo-7	short, few tillers	narrow, dark green, rolled leaves	short ligule, incompletely exerted panicles, lax and partially self-fertile, long grains with tip awns
Triplo-8	short, few tillers, slowest growth, recovers at maximum tillering stage	narrowest, dark green, rolled leaves	short ligule and panicles, fertile, round grains
Triplo-9	short, many tillers, spreading habit	thick, short, dark green leaves	panicle lax, large grains, highly self-fertile, panicles partially exerted
Triplo-10	short, slender	normal	hairy ligule, small and narrow grains, completely self-fertile
Triplo-11	normal	normal	normal
Triplo-12	spreading growth habit, tall, many tillers	long, droopy, light green leaves	panicles lax, grains of upper half of panicle possess short awn while lower ones have no awn, completely self-fertile

(25) established at the Central Rice Research Institute (CRRI), Cuttack, India, were morphologically compared by growing the two series at IRRI as well as at Cuttack. Complete correspondence between the chromosomal identification of the two series was found (18). The extra chromosomes of the primary trisomic series studied by Iwata and his colleagues at Kyushu University, Japan, were identified by Kurata et al (20) and Iwata and Omura (11) according to the chromosome numbering system proposed by Kurata and Omura (21).

The correspondence between the chromosome numbering system of Shastry et al (32) and that of Nishimura (28) was determined by crossing each of the trisomics of the IRRI series with the translocation stocks of Nishimura. Trisomic F_1 populations were cytologically examined to determine whether an association of five chromosomes or an association of four chromosomes and a trivalent were formed during meiosis. The presence of a pentavalent indicated that one of the two chromosomes involved in the translocation was the extra chromosome in that trisomic. When the same trisomic formed an association of five in crosses with two translocations, we concluded that the extra chromosome of the trisomic corresponded with the chromosome involved in both of these translocations. The relationships between the extra chromosomes of each trisomic and the chromosomes of Nishimura's designation were ascertained by using this technique (6).

Only two of the trisomic series — those of IRRI and of Kyushu University — have been utilized for determining the association between linkage groups and cytologically identifiable chromosomes. The extra chromosomes of the IRRI trisomics were first identified and the respective linkage groups were associated with each of the 12 chromosomes through the modified ratio technique (19). Iwata and Omura (9, 10) determined the associations between nine of their trisomics and the linkage groups and then identified the extra chromosomes of these trisomics (20). Iwata and Omura (11) discovered three remaining trisomics, identified the linkage groups corresponding to them, and identified the extra chromosomes of these trisomics. Thus, associations between the linkage groups and rice chromosomes have been determined independently at IRRI and Kyushu University. However, the chromosome numbering systems differ. IRRI has followed the pachytene chromosome numbering system of Shastry et al (32), and Kyushu University has used the somatic chromosome numbering system of Kurata and Omura (21). Another chromosome numbering system in existence is that of Nishimura (28). Fortunately, the relationships among the three systems are clearly understood; they are shown in Table 2. We should now

Table 2. Relationships among various systems of numbering chromosomes, trisomics, linkage groups, and marker genes of rice.

Chromosomes			Trisomics		Linkage groups	Marker genes
Shastry et al (32)	Nishimura (28)	Kurata and Omura (21)	Khush et al (19)	Iwata and Omura (9)	Nagao and Takahashi (27)	
1	3	K1	1	O	III	<i>eg, lax</i>
2	8	K2	2	N	X	<i>tri</i>
3	6	K6	3	B	I,V	<i>wx, ws, Ps₁</i>
4	5	K3	4	M	XI,XII	<i>bc₁, ch₁, dl</i>
5	2	K9	5	L	VI, IX, XII	<i>Hg</i>
6	4	K5	6	A	—	<i>gh₁, nl₁, gl₁</i>
7	10	K11	7	F	IV	<i>spl₁, rl₁</i>
8	12	K7	8	D	—	<i>g</i>
9	1	K10	9	H	VII, V	<i>v₈, su</i>
10	7	K12	10	C	—	<i>dp₂, drp₂, l-Bf</i>
11	9	K8	11	G	VIII	<i>pgl, fl</i>
12	11	K4	12	E	II	<i>la, z₂</i>
						<i>lg, Pl</i>

adopt one of the chromosome numbering systems universally and remove the confusion created by the multiplicity of systems.

In our opinion there are several advantages in adopting the pachytene chromosome numbering system. For example:

- The pachytene chromosomes are easier to identify than somatic chromosomes because of their size and numerous other distinguishing features. Because of their paired nature the number of chromosomes is half of the somatic number, thus rendering the task of identification easier. In Figure 1, the distinguishing features of each of the pachytene chromosomes are clearly evident.
- The chromosomes involved in translocations can be identified at pachytene, whereas this task is almost impossible to accomplish with the somatic chromosomes. Similarly, the extra chromosome of the tertiary and secondary trisomics can be identified only at pachytene.
- Chromosomal deficiencies are best identified through the detection of a deficiency loop in the paired condition at pachytene. This cannot be done with somatic chromosomes.

Now, since we have associated all the linkage groups with specific chromosomes, our next task is to map the centromere positions on the linkage maps and determine the precise locations of important marker genes on the chromosome arms (15). This will have to be accomplished

through radiation-induced deficiencies and through the establishment and utilization of secondary, tertiary, and telo-trisomics (17). Adoption of the pachytene chromosome numbering system would thus facilitate and stimulate further cytogenetic investigations of the rice genome. There is no denying the fact that the linkage groups of maize and tomato are well understood because of the adoption of pachytene chromosome numbering systems proposed by McClintock (24) for maize and Barton (1) for tomato.

It is gratifying to note that Kurata et al (20) have also examined the pachytene chromosomes of rice and have stated that “comparing the bivalents [with those] of Shastry et al (32) the characteristics of the bivalent length and centromere position considerably correspond with each other in 12 bivalents except that the order was replaced between bivalents 6 and 7.” It is thus obvious that, because of their distinctive features, chromosome identification at the pachytene stage is easier and more reliable.

As pointed out by Khush et al (19), 12 linkage groups of Nagao and Takahashi were assigned to 9 chromosomes. Iwata and Omura (10) clearly showed that three genes — *d-1*, *nl-1*, and *gl-1*, which were assigned to linkage groups VI, IX, and XII, respectively — belonged to the same chromosome. These findings were confirmed by Khush et al (19), who associated the markers of these linkage groups with chromosome 5. However, our recent data show that at least some markers of linkage group XII belong to chromosome 4. As the data in Table 3 show, *Hg* and *lhs*, previously considered to belong to linkage group XII (16), show very close linkage with *dl*. We also found that *op* — renamed *lhs-1* by Kinoshita (15) — and *lhs* are in fact allelic. Thus, half of linkage group XII (*Hg* and *lhs*) belongs to chromosome 4 and the other half (*gl-1* and *An-2*) to chromosome 5. The weak linkage of 39 crossover units between *gl-1* and *Hg* reported by Nagao and Takahashi (27) must have been spurious. Similarly, linkage group V, proposed by Nagao and Takahashi (27) on

Table 3. Linkage relations among some marker genes of rice.

Gene pair	Segregation data				No. of plants	Recombination value (%)
	AB	Ab	aB	ab		
<i>Hg-lhs</i>	117	9 ^a	38	0	164	0.0
<i>dl-lhs</i>	259	112	121	0	492	0.0

^a Segregation for *lhs* in the *Hg-lhs* cross was highly distorted.

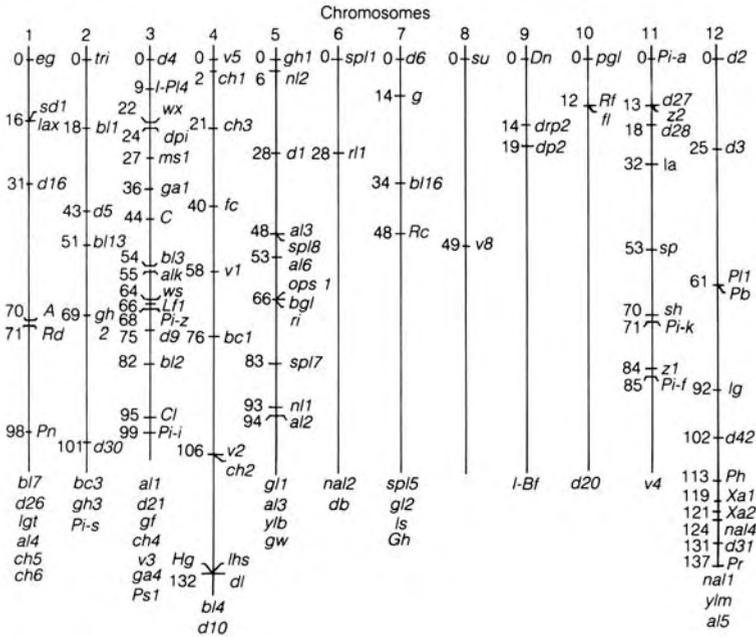


Fig. 2. Linkage map of rice.

the basis of the weak linkage of 42 crossover units between *I-Bf* and *Ps-1*, was assigned to two different chromosomes by Khush et al (19), with *I-Bf* going to chromosome 9 and *Ps-1* to chromosome 3 (Table 2). Three new linkage groups were discovered in the course of study of trisomic segregations by Iwata and Omura (9), Isono et al (5), and Khush et al (19).

To summarize then, the 12 linkage groups of Nagao and Takahashi (27) were assigned to 9 chromosomes. Two of their linkage groups had to be split up, with the markers going to different chromosomes. The latest chromosome map based on these findings is shown in Figure 2.

As is evident from Figure 2, some of the chromosomes such as 6, 7, 8, 9, and 10 are still poorly marked. We are now testing unlocated marker genes with our trisomic series to find more marker genes for these chromosomes. The paper in these proceedings by Librojo and Khush (23) reports the results of one such study.

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CHROMOSOMAL LOCATION OF SOME MUTANT GENES THROUGH THE USE OF PRIMARY TRISOMICS IN RICE

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The chromosomal location of eight mutant genes was determined through primary trisomic tests. Each of the mutant genes was crossed with 12 primary trisomics, and genetic segregation was studied in the F_2 or backcross populations. Of the 96 possible combinations 78 were examined. The results permitted the localization of gene *mp-1* on chromosome 1; *mp-2*, *ch*, and *bc-4* on chromosome 3; *Hg* on chromosome 4; *eui* on chromosome 5; *ygl* on chromosome 10; and *aul* on chromosome 12.

Twelve linkage groups of rice corresponding to the haploid chromosome number were proposed by Nagao and Takahashi (11). The independence of these linkage groups was tested through trisomic analysis by Iwata and Omura (5, 6) and Khush et al (9) and Sato et al (15). As a result of these studies, three linkage groups of Nagao and Takahashi (11) were associated with one chromosome and two linkage groups with another. Thus the 12 linkage groups were associated with 9 chromosomes. Three new linkage groups were discovered by Iwata and Omura (5), Isono et al (2), and Khush et al (9). Associations among the 12 linkage groups and the cytologically identifiable chromosomes were determined by Khush et al (9) for the first time.

Several of the linkage groups such as 6, 8, 9, and 10 are very poorly marked. However, a large number of well defined mutants of rice have been produced by different workers. In order to find additional markers for these chromosomes we are systematically determining the chromosomal location of the unlocated genes through primary trisomic tests. This study was undertaken to determine the chromosomal location of eight mutant genes.

MATERIALS AND METHODS

The eight mutant genes studied are listed in Table 1 and described below.

- Hg* (hairy glume). This gene conditions longer trichomes on the glumes, leaf margins, and auricles. The longer trichomes impart a shiny appearance to the grain. The mutant trait is best expressed after flowering. This mutant was provided by M. H. Heu under the designation *Lh* (long hair). However, it was found to be allelic to *Hg* (8) earlier described by Nagao et al (12).
- mp-1* (multiple pistil 1). The florets of this mutant have two or more functional pistils, and two kernels are enclosed within one lemma and palea (Fig. 1). Multiple pistil mutants were earlier described by Parthasarathy (13) and Misro (10), but seeds of these mutants were not available for allele tests.
- mp-2* (multiple pistil 2). This mutant is similar to but nonallelic to *mp-1*, as the F_1 between the two was normal.
- ch* (chlorina). This chlorophyll mutant has distinct yellowish leaves and is early maturing. The character is expressed from seedling to maturity. The mutant was induced in the breeding line CR113-32 by mutagenic treatment by R. N. Misra of CRRI, Cuttack, India. It is nonallelic to *ch-1* and *ch-3*. Allele tests with other described chlorina mutants have not yet been made.
- ygl* (yellow-green leaves). This chlorophyll mutant exhibits yellow-green leaves at all stages of growth. It is similar to but nonallelic to *pgl*. The original mutant was in the background of wx 126-17-26-B-99-3, a Korean breeding line.
- eui* (elongated uppermost internode). This character is manifested only at flowering. The uppermost node is elongated in this mutant (14).
- aul* (auricleless). This mutant lacks auricles, and the ligule is rudimentary (Fig. 2). However, it is nonallelic to *lg*.
- bc-4* (brittle culm). This mutant has a brittle culm and leaves that break very easily. The mutant trait is expressed at all growth stages. It is nonallelic to *bc-1*, *bc-2*, and *bc-3*. Hence it is designated *bc-4*.

The mutant plants and primary trisomics were grown in the screenhouse of the Plant Breeding Department at IRRI. The 8 mutants were crossed with 12 primary trisomics to obtain 96 F_1 progenies. The trisomic F_1 progenies were grown in the screenhouse, and the disomic plants were rouged as soon as the identification of $2n+1$ plants was possible. The trisomic F_1 plants of triplo-1, triplo-2, triplo-3, and triplo-4 were highly sterile and were backcrossed to respective marker stocks except for the

trisomic F_1 's of *Hg*, which were backcrossed to IR36. Trisomic F_1 plants of triplo-5 to triplo-12 were fertile and were allowed to self-pollinate to obtain F_2 seeds.

Table 1. Mutants studied.

RGS number	Gene symbol	Phenotype	Source
209	<i>Hg</i>	hairy glume	M. H. Heu, College of Agriculture Suweon, Korea
237	<i>mp-1</i>	multiple pistil 1	Bangladesh Rice Research Institute Joydebpur, Bangladesh
266	<i>mp-2</i>	multiple pistil 2	M. H. Heu, College of Agriculture Suweon, Korea
263	<i>ch</i>	chlorina	R. N. Misra, CRRI, Cuttack, India
264	<i>ygl</i>	yellow-green leaves	M. H. Heu, College of Agriculture Suweon, Korea
291	<i>eui</i>	elongated uppermost internode	J. N. Rutger, Univ. of California Davis, California, U.S.A.
292	<i>aul</i>	auricleless	R. P. Thakur, Rajendra Agricultural Univ. Sabour, Bihar, India
293	<i>bc-4</i>	brittle culm	R. Marie, INRA, Montpellier, France



Fig.1 Kernels of *mp-1* (top), IR36, and *mp-2* (bottom).

Backcross and F_2 populations were grown in the field and classified for mutant versus normal and trisomic versus disomic traits. Seventy-eight backcross or F_2 populations were available for study (Table 2). The remaining 18 combinations could not be studied due to insufficient seeds.

Table 2. Summary of primary trisomic segregation tests of eight markers in rice trisomics.

Gene	Primary trisomic and type of segregation obtained ^a											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Hg</i>	-	D	D	T	D	D	D	D	D	D	D	D
<i>mp-1</i>	T	D	D	D	D	D	D	D	D	D	-	D
<i>mp-2</i>	-	D	T	D	D	D	-	D	D	D	D	D
<i>ch</i>	-	D	T	-	D	D	D	D	D	D	D	D
<i>ygl</i>	-	D	D	-	D	D	D	D	D	T	D	D
<i>eui</i>	-	D	-	-	T	D	-	D	D	D	D	D
<i>aul</i>	-	D	D	-	D	D	D	D	D	D	-	T
<i>bc-4</i>	-	-	T	-	D	D	D	D	D	D	D	D

^aD = disomic, T = trisomic.

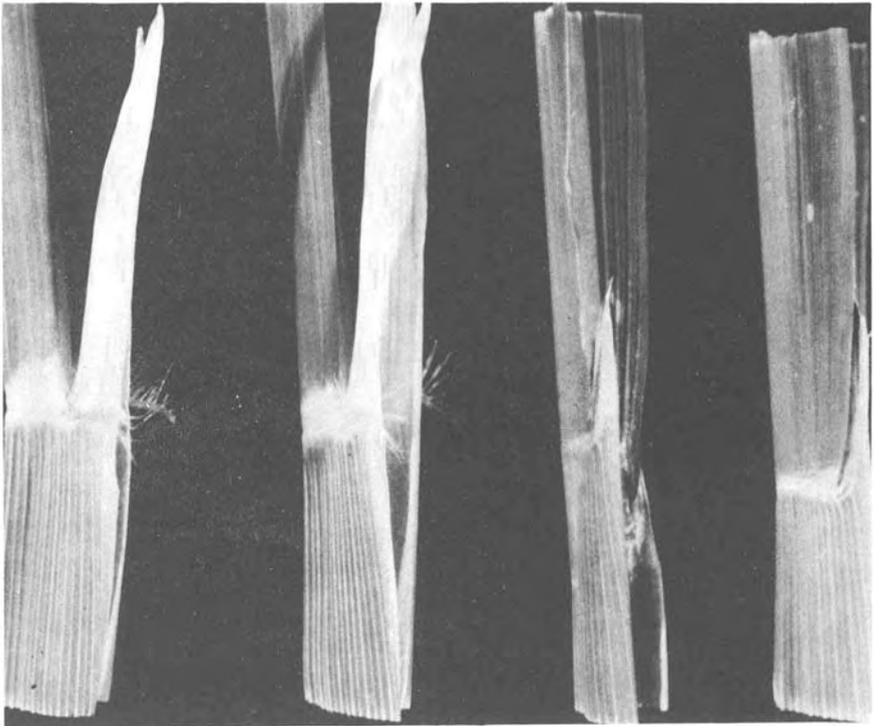


Fig. 2. Sections of leaf blades and leaf sheaths of normal (left) and auricleless (right).

RESULTS AND DISCUSSION

Of the 78 progenies studied, 8 segregated in the trisomic fashion (Table 3). As expected, each of the mutant genes showed trisomic inheritance with one trisomic only. This permitted the localization of all the eight genes on specific chromosomes.

The dominant gene *Hg* segregated in trisomic fashion in the BC population of triplo-4 and is thus located on chromosome 4. Gene *mp-1* showed trisomic segregation with triplo-1 and was delimited to chromosome 1. Genes *mp-2*, *ch*, and *bc-4* segregated in trisomic fashion with triplo-3 and were thus located on chromosome 3. Although the χ^2 values for trisomic ratios in the progenies of these markers and triplo-3 were somewhat higher, the trisomic inheritance of these progenies is beyond doubt. The results suggest that *mp-2* may be located on chromosome 3, but the number of plants in the backcross population of *mp-2* with triplo-3 was not adequate to draw definite conclusions. Heu and Suh (1) designated this gene *pc* (polycaryopsis) and indicated that it might be located on chromosome 7. Thus the location of *mp-2* on chromosome 3 is tentative. Trisomic inheritance was observed for *eui* with triplo-5 and for *ygl* with triplo-10. Thus *eui* was located on chromosome 5 and *ygl* on chromosome 10. The results for *aul* are somewhat unclear. The disomic portion of the progeny gave 3:1 segregation for the normal vs mutant, but all the trisomic plants were normal. The absence of mutant plants among the trisomic fraction establishes beyond doubt that *aul* is located on chromosome 12, but the disomic segregation among the 2n fraction indicates that the F₁ trisomic plant heterozygous for *aul* that gave rise to this F₂ may have been a secondary trisomic rather than a primary. As discussed by Khush (7), secondary trisomics occur in the progenies of primary trisomics as the result of misdivision of the univalent. This suspicion regarding secondary trisomism will be verified by further studies.

On the basis of these analyses, the eight useful marker genes have been located on their respective chromosomes. Three of the markers were assigned to linkage group 3, which is already well marked. However, *ygl* has been assigned to linkage group 10, which lacks good markers.

Table 3. Segregation ratios for eight genes in the F₂ or BC generation of primary trisomics of rice.

Gene	Trisomic	F ₂ or BC	Progeny							
			2n			2n + 1		Total		
			Normal	Mutant	c ² 8:1 (F ₂) or c ² 2:1 (BC) ^a	Normal	Mutant	Normal	Mutant	c ² 12.5:1 (F ₂) or c ² 3.5:1 (BC) ^a
<i>Hg</i>	triplo-4	BC	129	81	2.59	10	6	139	87	0.22 ^b
<i>mp-1</i>	triplo-1	F ₂	42	12	6.75*	40	0	82	12	3.94*
<i>mp-2</i>	triplo-3	BC	31	5	6.12*	13	1	44	6	3.02
<i>ch</i>	triplo-3	BC	79	18	9.53**	59	0	138	18	10.30**
<i>ygl</i>	triplo-10	F ₂	97	14	0.26	65	2	162	16	0.65
<i>eui</i>	triplo-5	F ₂	129	17	0.04	53	0	182	17	0.38
<i>aul</i>	triplo-12	F ₂	107	28	12.68**	68	0	175	28	12.06**
<i>bc-4</i>	triplo-3	BC	133	30	16.35**	103	4	236	34	10.58**

^a* = significant at the 5% level, ** = significant at the 1% level.

^bc² for 1.5:1 ratio.

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DISCUSSION

SESSION 4: LINKAGE MAPS

Q – Second: Rule number 6 for gene symbolization states that for any revision, contact with the original author is necessary. You have modified our isozyme gene symbols; for example LAP-E has become LAP-L. But you never contacted us. Therefore, I do not understand this revision.

A – Kinoshita: Your argument is valid, but we followed the common basic rules for designation of genes. As to the suffix letter or numerals, we would like to ask for your cooperation to discuss these matters once the gene list committee of the Rice Genetics Cooperative is established.

Q – Kumar: Are the linkage groups of indica and japonica varieties comparable or are there some differences between them?

A – Kinoshita: According to the trisomic analysis by Khush et al (1984), there was no indication of differences between the linkage groups. However, the linkage maps are considerably different, as shown in the figure in my paper. There is still a possibility that structural differences exist between the chromosomes of the two types as suggested by some cytogeneticists.

A – Khush: So far, the gene mapping work has been for morphological traits. Very few genes for other traits have been analyzed. Regarding stress tolerance traits, even their mode of inheritance is not known as yet. However, some enzyme markers have been mapped.

GENETICS OF MORPHOLOGICAL TRAITS

SESSION 5

INHERITANCE OF SEMIDWARF AND OTHER USEFUL MUTANT GENES IN RICE

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The inheritance is reported of mutations for semidwarfism and a recessive tall type, early maturity, endosperm characteristics, genetic male sterility, and hull color. Semidwarf mutants have been induced at three or more different loci, the most important of which is the *sd₁* locus also present in DGWG. The *sd₁* mutants have been used to develop several improved cultivars. A recessive gene for tall plant height (*eui*) that may be useful in hybrid seed production has been found to be independent of *sd₁*. Several mutants for early maturity have been found. In the only one characterized genetically, early maturity is controlled by a single, weakly dominant gene. Both waxy (*wx*) and opaque (*o*) endosperm mutants have been found. Opaque, which is useful as another recessive marker gene, is nonallelic to waxy. Nine genetic male sterile mutants, each controlled by a single recessive gene, were induced. Three hull color mutants—goldhull, yellow panicle, and light green panicle—were induced. Inheritance was studied of the latter two, which are new mutants. The yellow panicle mutant is controlled by a single recessive gene *yp*, which also has a pleiotropic effect for early maturity. The light green panicle mutant is controlled by a single recessive gene *lgp*. Both *yp* and *lgp* may be useful as marker genes for cultivar identification and/or genetic studies.

Numerous mutants have been induced or selected from adapted Japonica rice cultivars in the cooperative federal-state-industry rice improvement program in California (5, 12). Useful mutants, defined as those that either enhance the value of the plant or have application as breeding tools, fall into five general categories: semidwarfs and a recessive tall type; and those showing early maturity, endosperm characteristics, genetic male sterility, and hull color.

SEMIWARFS AND A RECESSIVE TALL TYPE

Induced semidwarf mutants have played an important role in rice improvement in California, both as direct releases as cultivars and as parents in cross-breeding programs. The first semidwarf rice cultivar in California, Calrose 76, originated as a single gene semidwarf mutant from the very well adapted tall cultivar Calrose (15). Calrose 76 possesses a single recessive gene for semidwarfism, designated sd_1 . The sd_1 gene reduces plant height about 25%; panicle length remains essentially unchanged (16). The sd_1 mutant gene is allelic to the major semidwarfing gene in Deo-geo-woo-gen (DGWG) (6). Thus, in F_2 generations of crosses between sd_1 and the DGWG semidwarf, no truly tall recombinants have been recovered, although considerable variation exists in the height of the F_2 semidwarfs. In crosses with tall cultivars, the sd_1 mutant gene shows rather discrete semidwarf vs tall segregation. On the other hand, the DGWG semi-dwarfing source shows more continuous variability, which is usually attributed to the presence of a single major gene plus minor gene modifiers (1).

Breeders of the California Cooperative Rice Research Foundation, Inc. (CCRRFI) have used both the Calrose 76 source and the DGWG (or IR8) source as semidwarf donors in the development of numerous semidwarf cultivars (5). Semidwarfs, which are 80–90 cm tall, yield 15–25% more than the tall cultivars they have replaced. CCRRFI breeders also induced and directly released the semidwarf cultivar M-401 from a tall cultivar of particular interest because of its premium quality (4). M-401 has a semidwarfing gene allelic to sd_1 (12), indicating that this is a recurring mutation.

At least three independent, recessively inherited semidwarf genes were induced in the original mutagenic treatments of the tall cultivar Calrose: the sd_1 locus present in Calrose 76, the sd_2 locus in CI 11033, and the sd_4 locus in CI 11034 (6, 8). Typically, F_1 's among nonallelic semidwarfs are tall, and 9:6:1 tall to semidwarf to doubledwarf ratios are observed in the F_2 . Neither the sd_2 nor the sd_4 source has been as agronomically useful as the sd_1 source. The sd_2 source reduces height only 15 cm and thus is still somewhat lodging susceptible (sd_1 reduces height about 30 cm, a more desirable reduction). The sd_4 source also reduces height only 15 cm and has an additional pleiotropic effect for a 20% reduction in seed size.

After the three independent semidwarfing alleles sd_1 , sd_2 , and sd_4 , were identified, subsequent genetic studies were concentrated only on determining if new mutants were allelic to sd_1 . These allelic relationships are depicted in Figure 1. Two semidwarf mutants (CI11045 and CI11046),

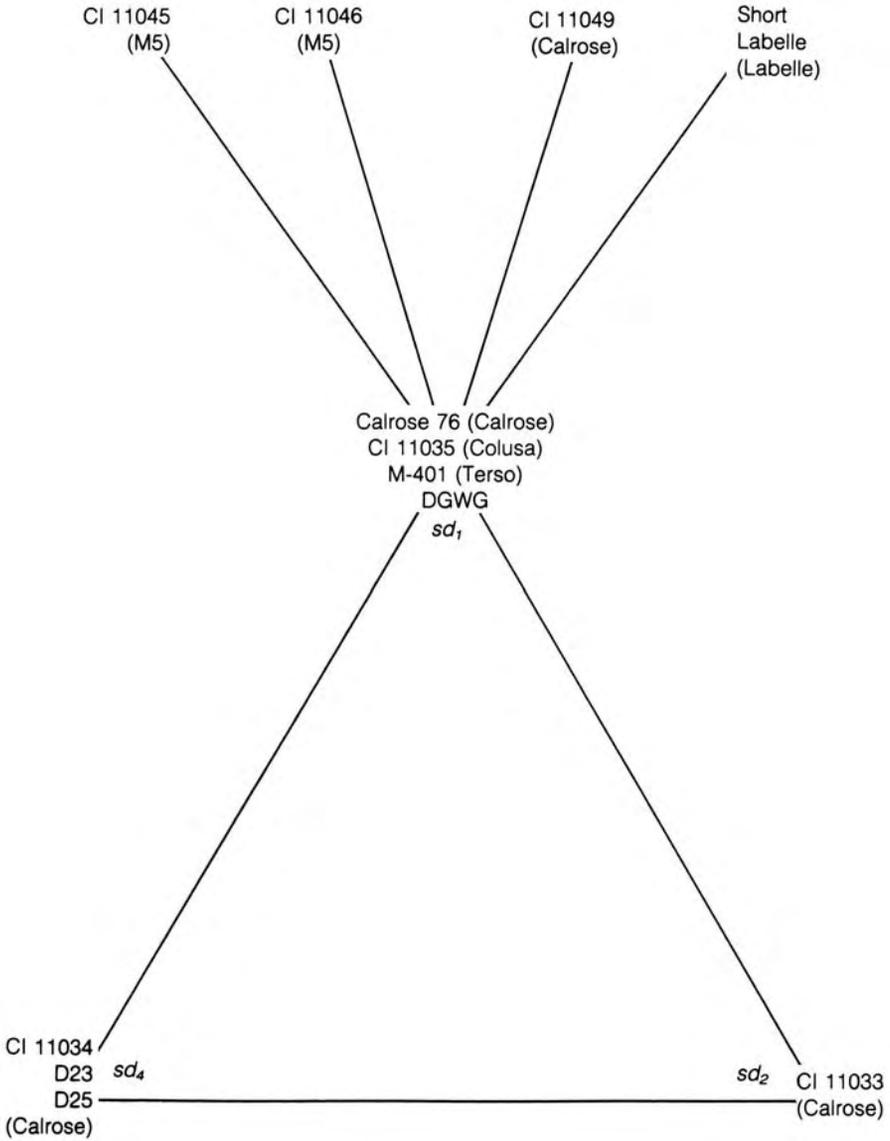


Fig. 1. Allelic relationships of induced semidwarf mutants and DGWG. Genotypes at the same corner of the triangle are allelic; those at different corners are nonallelic. Genotypes in the "fan" at the top are nonallelic to the sd_1 locus, but their relationships to other loci are unknown. Parent cultivars are shown in parentheses.

induced by Carnahan and co-workers from the tall cultivar M5, were thus found to be nonallelic to *sd*₁ (14). Both are about 30 cm shorter than their parent and, except for a tendency to show discolored hulls at harvest, both are phenotypically identical to the *sd*₁ source. Although neither of the M5 "raw" semidwarfs has been more productive than its tall parent, neither has been evaluated for yield potential after crossing with other genotypes.

A narrow leaf semidwarf mutant CI11049, selected from Calrose, has also been found to have a gene nonallelic to *sd*₁ (14). The semidwarfing gene in CI11049 has a pleiotropic effect for short, narrow leaves and reduced seed size.

Another semidwarf mutant, Short Labelle, selected from the tall cultivar Labelle, has a semidwarfing gene nonallelic to *sd*₁ (10). Yields of Short Labelle were generally lower than those of its tall parent. Curiously, Short Labelle, though fully fertile itself, shows considerable F₁ sterility in crosses with its tall parent.

In all of the semidwarfs noted above, tall plant height is dominant over short plant height. In contrast, a recessively inherited tall plant type was found in a breeding population that had been selected for semidwarfism (13). The tall plant height resulted from a recessive gene for elongated uppermost internode (*eui*), which produces a near doubling in length of the uppermost internode (Fig. 2). It was proposed that this gene would be a useful fourth element that would complement the other three genetic elements — cytoplasmic male sterility, maintainers, and restorers — generally used in hybrid seed production. The *eui* gene would be incorporated in pollen fertility restoring parents in hybrid seed production in situations where a semidwarf F₁ generation is desired. The tall paternal plant type would be desirable for windblown pollen dispersal onto semidwarf female plants, and the resulting hybrid plants would be semidwarf, unlike the usual case of tall hybrids from semidwarf/tall crosses. Additionally, the increased height of the paternal plant would permit a co-mingling of the hybrid parent seed stocks to maximize crossing and would facilitate mechanical removal of the paternal parent before mass harvest of the commercial hybrid seed.

The original study (13) did not differentiate whether the *eui* gene was nonallelic to the semidwarfing gene *sd*₁ or was a different allele at the *sd*₁ locus. Therefore, the *eui* plant type was hybridized with the standard tall cultivar M5, and F₂ segregation, determined from F₃ progeny tests, was examined. In the present study, segregation for *eui* again fit a single gene model, as did segregation for semidwarfism (Table 1). The *eui* gene was found to be independent of the *sd*₁ gene; thus extremely tall plants,

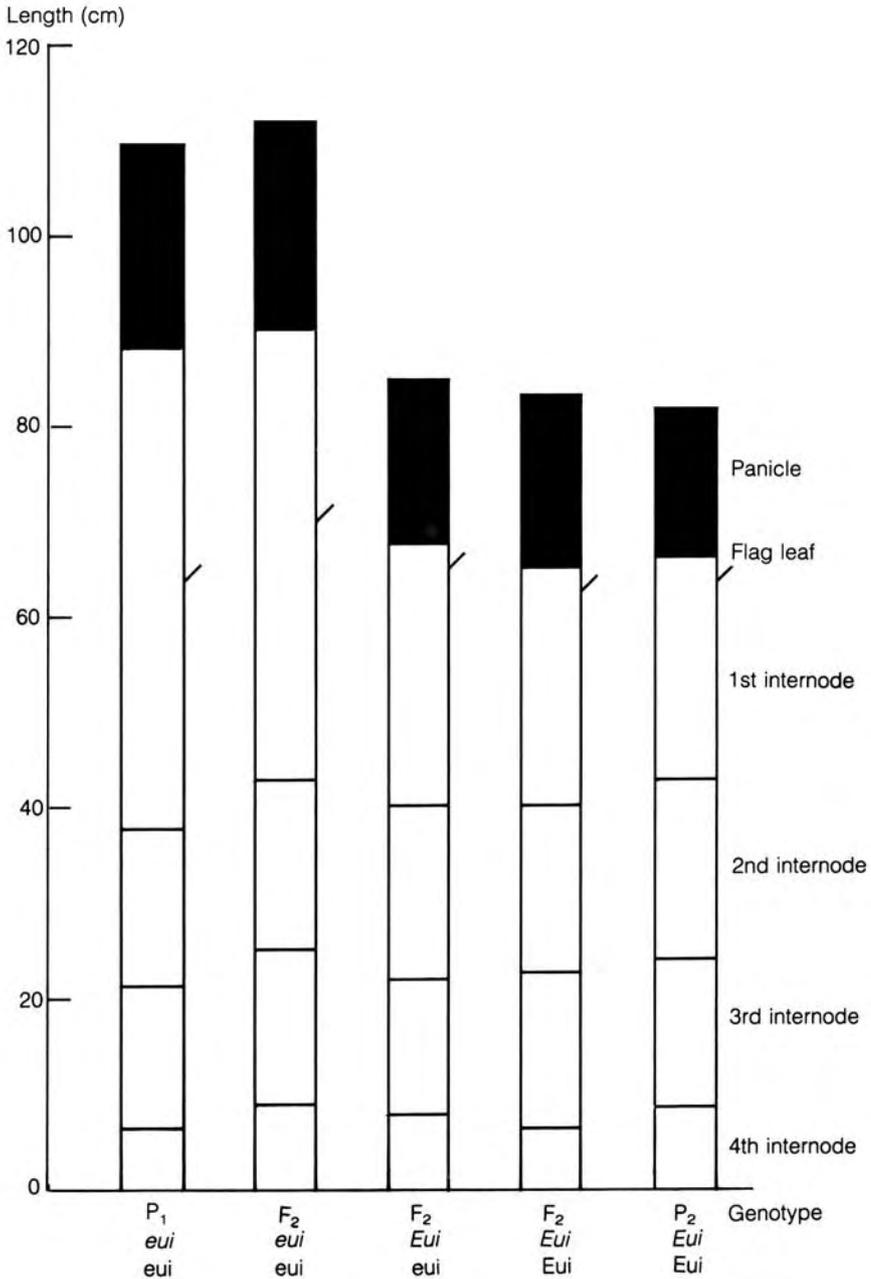


Fig. 2. Internode length, panicle length, and position of flag leaf of parents and F_2 genotypes from the cross between an elongated uppermost internode parent and a normal semidwarf parent (from 13).

homozygous for both tall plant height and elongated uppermost internode, were among the recombinants.

EARLY MATURITY

Both spontaneous and induced mutants for early maturity have been used in California breeding programs (5, 12). The inheritance of early maturity has been studied for only one mutant, CI11037, which carries a single, weakly dominant gene for early flowering (*Ef*). Its early maturity was independent of the *sd*₁ gene and the glabrous (*gl*) hull and leaf gene (9). Early maturity mutants are the easiest mutants to find, as one needs only to inspect a population a few days before normal flowering and to mark the occasional plants that are already flowering. However, relatively little work has been done on the inheritance of flowering, mainly because plants must be monitored daily during the flowering period in order to obtain segregation data. By contrast, many other mutant characters can be observed on a single day at harvest time.

ENDOSPERM CHARACTERISTICS

The waxy endosperm gene *wx* is readily induced by mutation. Carnahan et al (3) irradiated the adapted early maturing tall cultivar S6 and induced a *wx* mutant that was directly released as the cultivar Calmochi-201. Similarly, the present authors induced a *wx* mutant in the semidwarf cultivar M-101 (unpublished data). It is assumed that the induced *wx* alleles are at the same locus as the *wx* allele in existing rice cultivars.

In searching for *wx* mutants, the authors frequently found a phenotype, designated opaque (*o*), that was initially thought to be *wx*, but that has an amylose content approaching normal. The opaque mutant cannot be visually distinguished from waxy grains but has softer grains that are more easily broken. The opaque mutant shows similar I-KI staining to normal translucent endosperm types, i.e., blue-black. Satoh and Omura (17) described what appears to be a similar mutant, which they designated floury.

While the opaque mutant has no known grain quality value, it is subject to xenia effects like the waxy mutant and has thus been used as a marker gene for detecting selfs in outcrossing experiments (2). In the F₂ generation of a cross between the opaque endosperm mutant ESD7-3-*o*

and a normal endosperm line carrying a mutant gene for yellow panicle ESD7-3-*yp*, segregation of the opaque gene provided a satisfactory fit to a single gene ratio and was independent of the yellow panicle gene (Table 2). When the same opaque mutant source was crossed with the waxy endosperm cultivar Isao Mochi, a satisfactory fit to a 9:7 translucent to white (waxy plus opaque) ratio was observed in half of the families (Table 3). The heterogeneity χ^2 was highly significant. Although the opaque mutant is clearly nonallelic to waxy, further tests are needed to determine if linkage may be involved or if environmental effects obscured the segregation ratios.

GENETIC MALE STERILITY

Nine genetic male sterile mutants were induced by irradiation of the semidwarf cultivar M-101 (11). Each mutant is controlled by a recessive gene (*ms*); allelism tests have not been conducted. Anther and pollen characteristics of these nine plus two additional male sterile mutants are presented elsewhere in this volume (7). Two of these mutants, I-2 and I-17, are in use in population improvement programs. Mutant I-17 is unusual in that its florets remain open for several days, which would seem to be a desirable attribute. However, out-crossing on I-17 is only 8% compared with 18% on I-2, which has florets that remain open only the usual I-2 h.

HULL COLOR

Three hull color mutants — goldhull, yellow panicles, and light green panicle — have been found in irradiated populations. The goldhull mutant is visible at maturity; the other two mutants are visible until kernels approach maturity, at which time they become indistinguishable from normal straw color hulls. The goldhull mutant was found independently by USDA Agricultural Research Service researchers at Davis and by CRRFI researchers at Biggs in the semidwarf cultivar M-101 in 1981. The goldhull mutants appear to be the same as the goldhull gene *gh* previously used in several cultivars in the southern US, e.g., Bluebelle, but have not been genetically investigated.

The yellow panicle mutant, which also produces yellow-green leaves, was found in an irradiated population of the experimental semidwarf ESD7-3. When hybridized with a sister mutant for opaque endosperm

Table 1. Classification of F₂ plants on the basis of F₃ progeny tests in the cross M5/80:14337 (*Sd*₁ *Sd*₁ *Eui Eui/sd*₁ *sd*₁ *eui eui*), grown at Davis, 1983.^a

Plant height type	Upper most internode type			Total
	<i>Eui Eui</i>	<i>Eui eui</i>	<i>eui eui</i>	
<i>Sd</i> ₁ <i>Sd</i> ₁	12	35	10	57
<i>Sd</i> ₁ <i>sd</i> ₁	17	53	28	98
<i>sd</i> ₁ <i>sd</i> ₁	11	25	16	52
Total	40	113	54	207

^a χ^2 for 1:2:1 segregation of semidwarfism = 0.82, 0.50 < P < 0.75,

χ^2 for 1:2:1 segregation of elongated uppermost internode = 3.54, 0.10 < P < 0.25, χ^2 for independence (4 df) = 3.53, 0.25 < P < 0.25

(see previous section), the F₂ segregation for yellow panicle failed to fit a single recessive gene model, mainly because of a deficiency of yellow panicle segregates (Table 2). However, among progeny of 29 F₃ families that were segregating for yellow panicle, the segregation ratio fit a single recessive gene model (Table 2). Therefore it was concluded that yellow panicle was conditioned by a single recessive gene, designated *yp*. The *yp* gene has a pleiotropic effect for early maturity: The 16 homozygous yellow panicle F₃ lines flowered in ± 86 days, compared with ± 102 days for the 43 homozygous normal lines.

The light green panicle mutant, which also produces light green leaves, was found in an irradiated population of the cultivar M-101. When hybridized with the normal panicle color line ED7, F₂ segregation ratios, based on F₃ progeny tests, of 98:219:127 homozygous normal to heterozygous to homozygous light green panicle fit a single recessive gene model (0.50 < P < 0.75). The gene conditioning light green panicle is designated *lgp*.

Hull color mutants, if they have no detrimental effects on production, are potentially useful as marker genes for cultivar identification. The mutants for yellow panicle and light green panicle, as well as a waxy endosperm mutant, yielded as much as the check cultivar M-101, while the goldhull mutant suffered a significant reduction in yield (Table 4).

Table 2. Classification of F₂ plants on the basis of F₃ progeny tests in the cross ESD7-3-opaque endosperm/ESD7-3-yellow panicle (*o o Yp Yp/O O yp yp*), grown at Davis, 1980.^a

Endosperm type	Panicle color			Total
	<i>Yp Yp</i>	<i>Yp Yp</i>	<i>Yp Yp</i>	
OO	7	19	5	31
Oo	25	36	9	70
oo	11	15	2	28
Total	43	70 ^b	16	129

^a χ^2 for 1:2:1 segregation of opaque endosperm = 1.08, 0.50 < P2 < 0.75,

χ^2 for 1:2:1 segregation of yellow panicle = 12.24, P < 0.005. χ^2 for independence (4 df) = 2.85, 0.50 < P < 0.75.

^bAmong a random sample of 29 F₃ segregating for yellow panicle, the ratio was 844:302 normal to yellow panicle, χ^2 for 3:1 segregation = 1.12, 0.25 < P < 0.50.

Table 3. Classification of endosperm characteristics of F₂ seeds in the cross ESD7-3-opaque endosperm/Isao Mochi (*o o Wx Wx/O O wx wx*), grown at Davis, 1981.

Family (F ₁ plant)	Endosperm type		c^2 (9:7)	P
	Trans-lucent	White (waxy and opaque)		
81:9331-1	102	106	4.39	.025 - .05
-2	198	184	3.03	.05 - .10
-4	317	260	0.41	.50 - .75
-8	368	271	0.46	.25 - .50
-11	587	519	4.53	.025 - .05
-12	290	343	28.02	< .005
-13	298	300	10.01	< .005
-14	436	361	0.77	.25 - .50
Total			(8 df) 51.62	< .005
Composite	2596	2344	(1 df) 27.47	< .005
Heterogeneity			(7 df) 24.15	< .005

Table 4. Days to heading and yield of four marker gene mutants in M-101 and plants with closely related background, grown at Davis, 1983.

Genotype	Days to heading	Yield (g/0.60 m ² plot)
M-101 (check)	90	638
Yellow panicle ESD7-3 (<i>yp</i>)	88 ^a	629
Light green panicle M-101 (<i>lgp</i>)	91	628
Goldhull M-101	91	565
Waxy M-101 (<i>wx</i>)	90	638
LSD.05	1	35

^aDays to heading of the ESD7-3 parent = 102.

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DEVELOPMENTAL GENETIC STUDIES ON INDUCED DWARF MUTANTS IN RICE

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Induced dwarf mutants in rice are valuable materials not only for practical use but also for fundamental research. To clarify the mechanism of dwarfing, the present study investigated character expression and mode of inheritance of some induced dwarf mutants. The dwarf gene *d-18^h* of the Akibare dwarf mutant is considered to exert its effect immediately after seed germination. When treated at 1 or 2 day intervals from the seedling stage with gibberellic acid, this dwarf mutant grew as tall as the original cultivar, Akibare. By diallel analysis on culm length of some dwarf and long culm mutations derived from Fujiminori, the extreme dwarf mutants appeared to be controlled by a single recessive gene for dwarfness. On the contrary, the slightly shortened mutants are governed predominantly by incomplete or partial recessive genes. The dwarf mutant line Fukei 71, which differs from the original type by a single recessive gene *d-50*, was depressed more under high temperature than under moderate temperature due to abnormal cell differentiation from the intercalary meristem of the internode.

In rice plants, dwarfness is one of the most important breeding objectives because of its characteristics closely relating to plant type (light-intercepting form, harvest index), fertilizer response, and lodging resistance. In addition, the induced dwarf mutants, even if they are deleterious forms, are used as materials for developmental genetic research to trace the path leading to character expression at successive stages of genetic, physiological, and biochemical development.

Furthermore, since dwarf mutation is comparatively easy to induce and detect, many induced dwarf mutants have been reported, most characterized by shortened culm internodes, a very few having a decreased number of internodes, and one exceptional many-node dwarf (1, 3). However, there are very few, if any, detailed studies about character expression in induced dwarf mutants.

The present study was carried out to find a clue to the genetic and physiological mechanisms of dwarfing by analyzing the effects of dwarf genes on culm elongation, with special reference to the interaction of mutant genes and environmental factors.

CHARACTER EXPRESSION AND INHERITANCE OF AKIBARE DWARFISM

By either X-ray or ethylene imine treatment, two extreme dwarf mutants were easy to induce compared with the other dwarf types; one is conspicuously responsive to gibberellic acid (GA_3), the other nonsensitive (2). The F_2 population of a cross between the Akibare dwarf mutant sensitive to GA_3 and its original cultivar, Akibare, segregates in a monohybrid ratio, 3:1 tall to dwarf, indicating that the former carries a single recessive gene responsible for dwarfness. By an allelism test, this dwarf gene was shown to be allelic to the dwarf gene of Hosetsu dwarf, $d - 18^h$, which is located on linkage group III. According to Kinoshita and Shinbashi (8), multiple allelism was found in the locus $d - 18$, the order of dominance being $\pm > d - 18^k > d - 18^h$. By a further allelism test, all the same dwarf mutants that were induced from the other cultivars — Fujiminori, Reimei, and Fukei 71 — were proved to have the same dwarf gene as the Akibare dwarf mutant gene. Accordingly, the locus $d - 18$ is considered to be considerably mutable.

When treated at 1 or 2 day intervals from the seedling stage to maturity with 10–20 ppm GA_3 , the Akibare dwarf mutant grew as tall as the original cultivar Akibare (Figure 1). Almost all of the characters that showed simultaneous alterations were restored to the same magnitude as the original cultivar by GA_3 treatment. This suggests that the simultaneous alterations are due to pleiotropic effects of the dwarf gene, which are achieved through GA_3 metabolism.

It is important to know in which developmental stage or in which part gene action is expressed. It was found that plumule length and radicle length in dormant seeds of the Akibare dwarf mutant are not much different from those of the original cultivar Akibare, demonstrating that the effects of the dwarf gene are not yet expressed in the process of embryonic growth. Since this mutant becomes shorter at the time when the coleoptile grows, the dwarf gene is considered to exert its effect immediately after seed germination. This is in accord with the results of the dwarf genes daikoku, ebisu, and shodaikoku reported by Nakayama (13). The inhibition effect of this dwarf gene on root elongation is not so effective and is especially meager in the beginning stage of development compared with that of culm elongation (5).

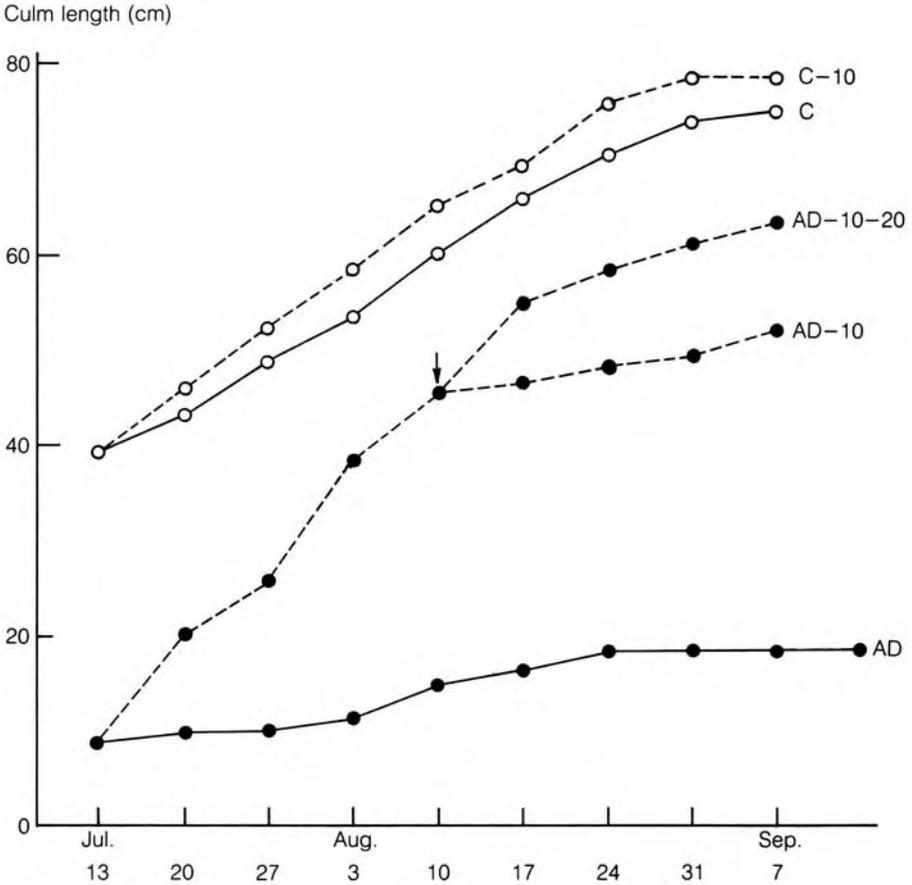


Fig. 1. Effects of gibberellic acid on plant height of Akibare dwarf mutant and its original cultivar Akibare. Arrow shows the time when GA_3 was enhanced to 20 ppm (2).

C = untreated Akibare, D = untreated Akibare dwarf mutant,

C-10 = Akibare treated at 1 or 2 day intervals with 10 ppm GA_3 .

AD-10 = Akibare dwarf mutant treated at 1 or 2 day intervals with 10 ppm GA_3 ,

AD-10-20 = Akibare dwarf mutant treated at 1 or 2 day intervals with 10 ppm GA_3 in the early vegetative stages and then with 20 ppm GA_3 in the later stages.

DIALLEL ANALYSIS OF DWARF AND LONG CULM MUTANTS

Some dwarf and long culm mutants induced by re-irradiation of Reimei and Fukei 71, which were derived from the Japanese cultivar Fujiminori by gamma irradiation, were chosen in order to clarify the genetic mechanisms of the dwarf and long culm mutations. The genealogical relationships among these materials are shown in Figure 2. To simplify the

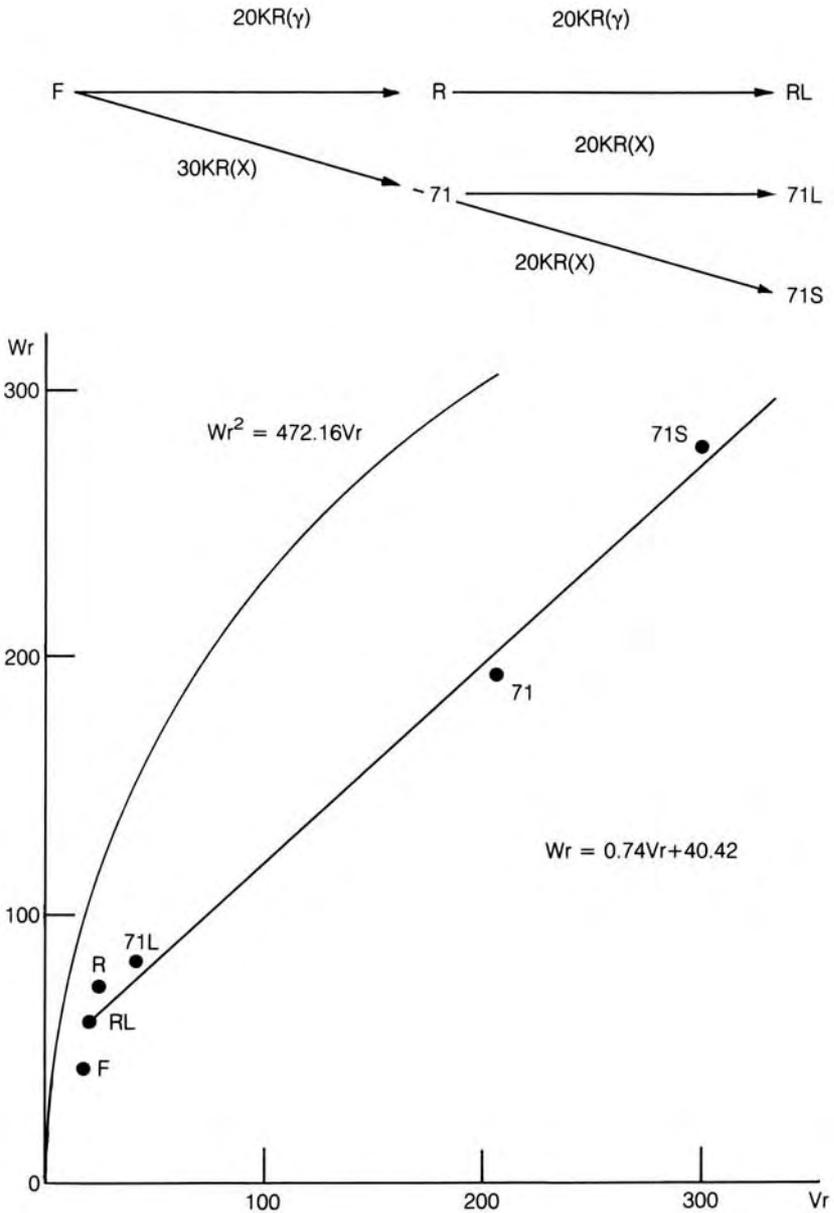


Fig. 2. W_r , V_r graph of culm length and genealogical relationship among the materials tested (4).

F = Fujiminori, R = Reimei, 71 = Fukeyi 71,
 RL = Reimei long culm mutant,
 71L = Fukeyi 71 long culm mutant,
 71S = Fukeyi 71 extreme dwarf mutant.

presentation, the abbreviations of line names used in Figure 2 are used below.

A diallel analysis (6 times 6) of culm length was performed on these mutants and their original cultivar (4). Culm length of each F_1 and parent was measured as the average of ten plants grown under a randomized block design including two replications. The variance (V_r) of each array and covariance (W_r) between the parents and their progeny in each array were estimated according to Hayman's method (6, 7). From the W_r , V_r graph of culm length (Figure 2), it was found that incomplete dominant genes are responsible for culm length. The dwarf mutant Fukei 71 (11) and its derivative extreme dwarf mutant (71S) appeared to be controlled by a single or relatively small number of recessive genes for dwarfness. On the contrary, the slightly shortened mutants were governed predominantly by incomplete or partial recessive genes for dwarfness. Chang and Li (1) also reported that the quantitative and rather complex nature of semidwarfism in indica cultivars is shown by the incomplete dominance of tallness. On the other hand, the ratio of dominance genes of each parent ($W_r + V_r$) showed a negative regression against the average values of culm length of each parent, suggesting that the dwarf mutant genes are generally recessive for the tall gene.

It was found that the long culm mutant derived from Reimei (RL) is somewhat longer than Fujiminori (F) and that the long culm mutant derived from Fukei 71 (71L) is as tall as Reimei (R), suggesting that these two long culm mutants are not due to true back mutation. The extreme dwarf mutant (71S) is shorter than 71, from which it was derived. The reason why the culm length of the F_1 of a cross between 71 and 71S is phenotypically as tall as that of Fujiminori is not clear. Further research is needed to explain fully the mechanism of this phenomenon.

EFFECTS OF TEMPERATURE ON CHARACTER EXPRESSION OF INDUCED DWARFS

In relation to the interaction between the character expression of the dwarf gene and environmental factors, Kitano and Futsuhara (9) reported that the dwarf mutant line Fukei 71, which differs from the original type by a single recessive gene *d-50*, shows a large variation in culm elongation (Figure 3). In order to get more detailed information on this peculiar character expression, Fukei 71 was compared with the other semi-dwarf mutant cultivar Reimei and their original cultivar Fujiminori in mode of culm elongation under various temperature conditions (9, 10, 11).

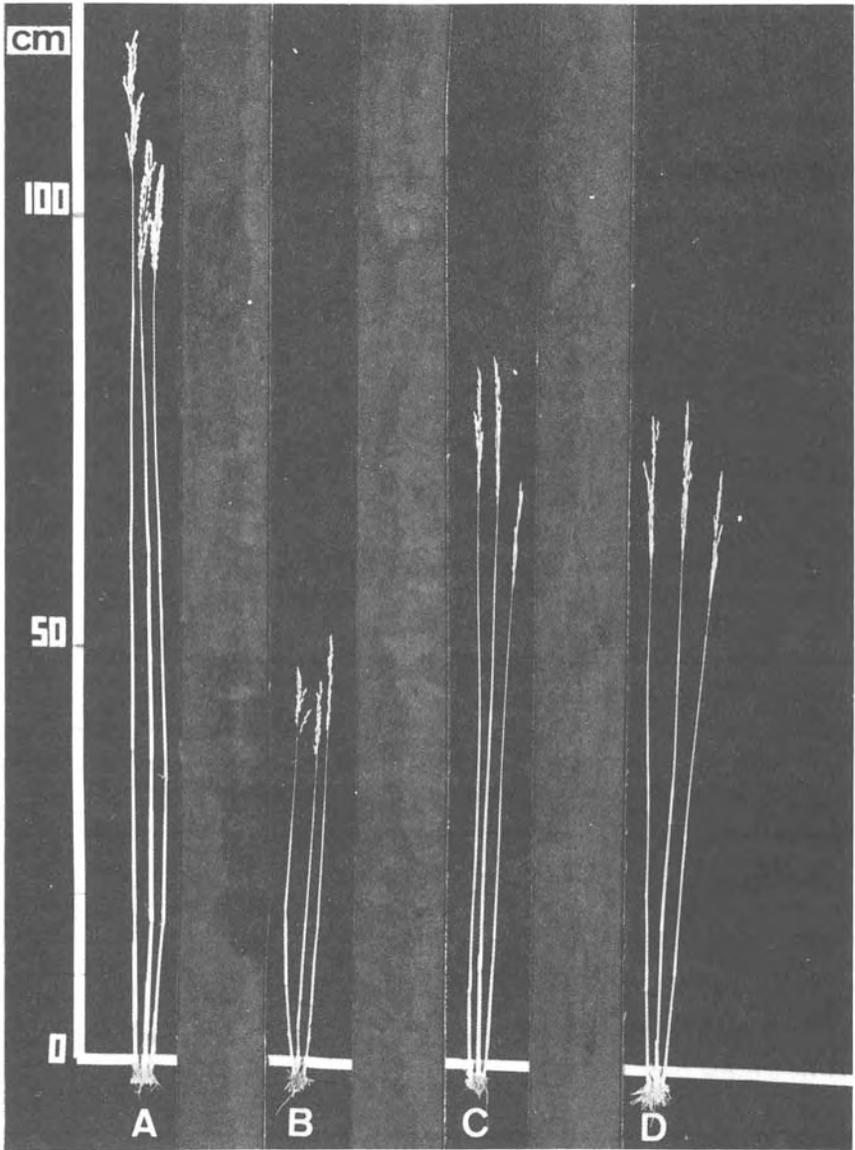


Fig. 3. Naked culms of the dwarf mutant line Fukei 71 and its original cultivar Fujiminori (10). A and C show Fujiminori grown under high and low temperature conditions, respectively; B and D show Fukei 71 grown under high and low temperature conditions, respectively.

In combination with three different fertilizer treatments, three different temperature treatments were performed from the seedling stage to maturity as follows: a) high temperature condition (day 30°C, night 25°C), b) intermediate temperature condition (23°C, 18°C), and c) low temperature condition (16°C, 12°C). Figure 4 shows the plant height of the two mutants and their parent measured weekly under different temperature conditions. Although no varietal difference was detected in plant height at the early vegetative stages, it became more remarkable with age. Of these three lines, Fukei 71 showed a specific response to temperature in plant height as shown in Figures 3 and 4. This line was considerably lower in plant height than the other two lines under high temperature conditions but grew as tall as Reimei under intermediate temperature conditions and became higher than Reimei and almost the same as Fujiminori under low temperature conditions. On the other hand, no particular response to fertilizer was found in these lines.

In further investigations under eight temperature conditions composed of the combination of two temperatures, high (30°C, 25°C) and low (23°C, 18°C), and three growth periods (vegetative, early reproductive, and late reproductive or culm elongation stages), it was shown that Fukei 71 has a greater response to the treatments with regard to culm elongation than Fujiminori, the culm length of the former being reduced significantly by high temperature treatment, especially at the culm elongation stage.

So far, investigation into dwarfing has concentrated mainly on external morphology. But growth activity takes place at the cellular level and is expressed at the organ level; the structure and function of plant organs are determined by the growth and function of their cells. So the present study tried to investigate character expression of dwarf genes histologically.

To elucidate the dwarfing mechanism of Fukei 71, some histological observations were carried out on the elongated internodes of Fukei 71 and Fujiminori grown under high and low temperature conditions. When Fukei 71 was exposed to high temperature, the number of parenchyma cells tended to decrease in the longitudinal direction and to increase in the transverse direction of each elongated internode (Figure 5). It was also observed that partially nonuniform parenchyma cells occurred only in Fukei 71 when treated with high temperature. These results demonstrate that the dwarfness expressed under high temperature conditions in Fukei 71 is induced by abnormal cell differentiation from intercalary meristems of the internodes.

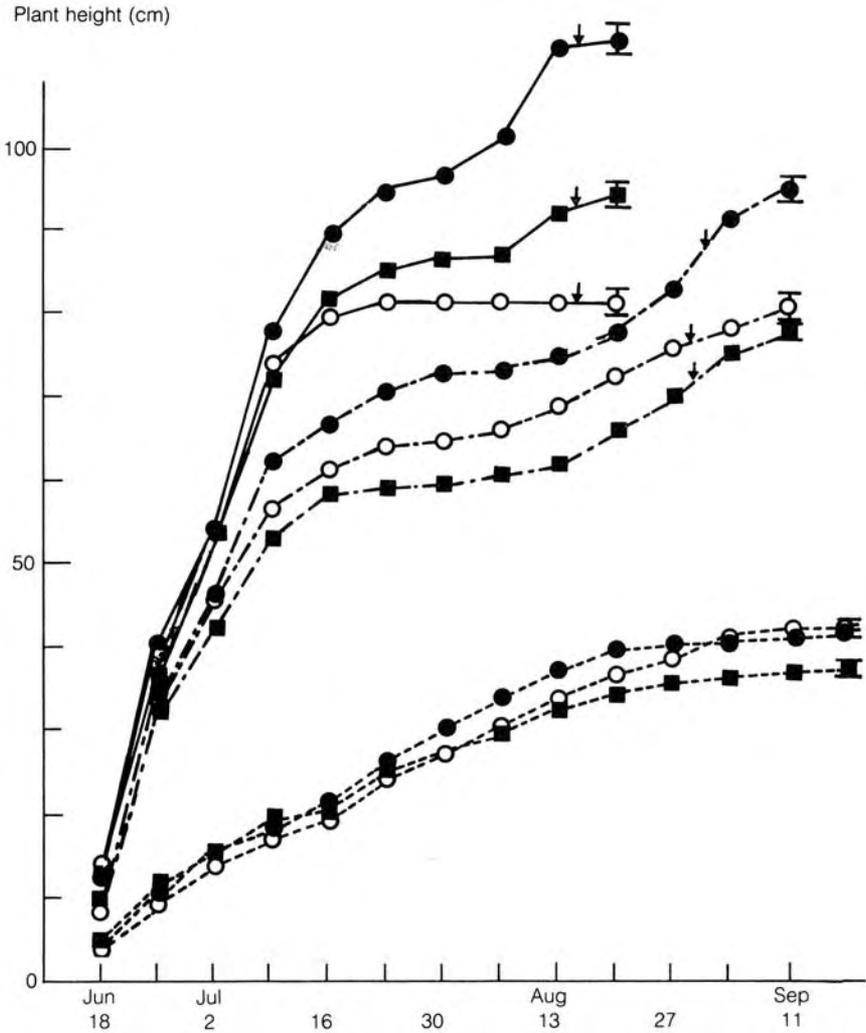


Fig. 4. Plant heights of dwarf and semi-dwarf mutants and their original cultivar Fulminori measured weekly under three different temperature conditions. Arrow shows heading date (9).

- = Fujiminori, ■ = Reimei, ○ = Fukei 71,
- = High temperature (30°C, 25°C),
- - - = intermediate temperature (23°C, 18°C),
- · · = Low temperature (16°C, 12°C).

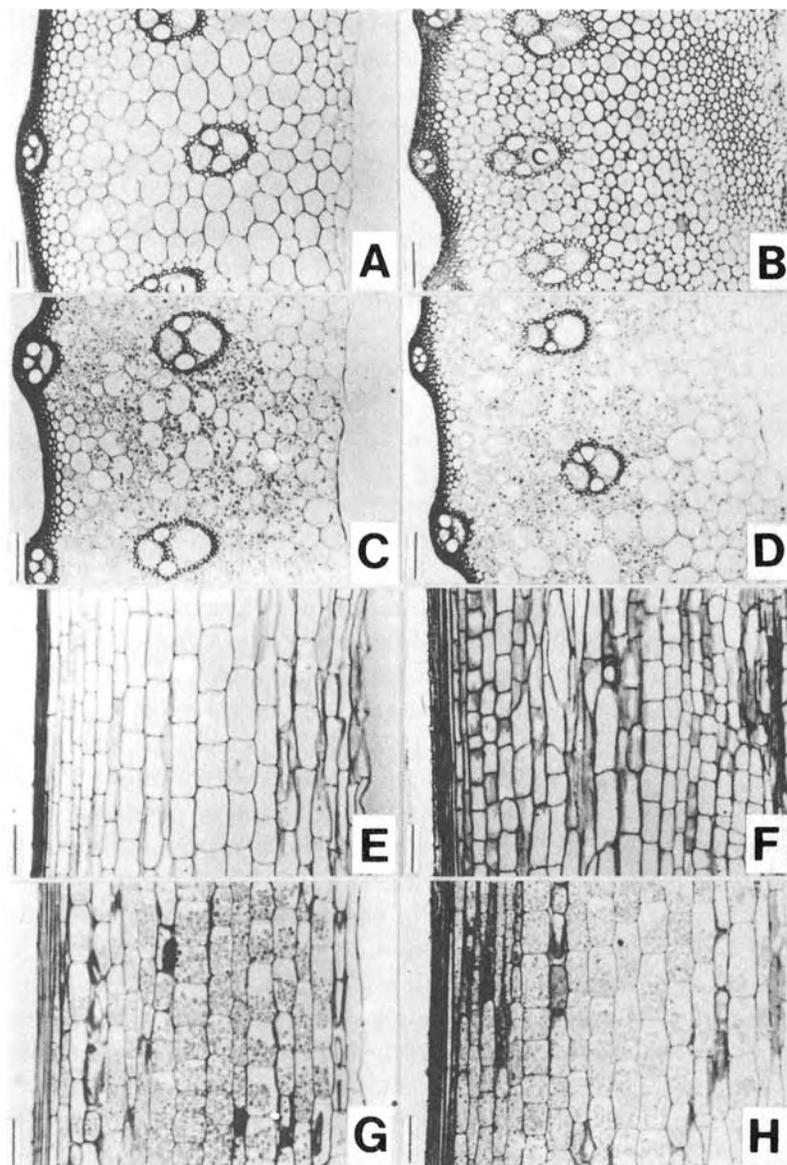


Fig. 5. Transverse (A-D) and longitudinal (E-H) sections of the third internode of Fujiminori and Fukei 71 grown under high and low temperature conditions (10).
A and E = Fujiminori grown under high temperature conditions,
B and F = Fukei 71 grown under high temperature conditions,
C and G = Fujiminori grown under low temperature conditions,
D and H = Fukei 71 grown under low temperature conditions.

In dwarf rice and maize, many genes have been proved to affect the various steps in the GA_3 biosynthetic pathway (14). Furthermore, in dwarf mutants of tomato and maize, a high peroxidase activity as a cause of dwarfing was noticed (12). These results suggest that most mutants classified as morphological mutants are dependent on biochemical mutations affecting enzyme activity.

In order to clarify the effect of GA_3 on culm elongation, Fukei 71 and its original cultivar Fujiminori were treated at 3 or 4 day intervals from the seedling stage with various concentrations of GA_3 . Under high temperature (30°C, 25°C) conditions, culm elongation of Fujiminori was strongly promoted, while Fukei 71 did not respond to GA_3 as strongly as Fujiminori. It is especially noteworthy that Fukei 71 showed similar responses at three levels of GA_3 concentration, although the culm length of Fujiminori was elongated approximately in proportion to the increase in GA_3 concentration. However, under intermediate temperature (23°C, 18°C) conditions the response of culm length to GA_3 in Fukei 71 was very nearly that of Fujiminori.

Endogenous gibberellin-like substances were ascertained by bioassay with the two dwarf lines Tangin-bozu and Waito-C in the acidic ethyl acetate fraction of 70% acetone extracts from the culm and shoot of Fukei 71 and Fujiminori at various growth stages. A similar level of GA_3 activity was detected in Fukei 71 with Tangin-bozu assay, compared with that of Fujiminori. No GA_3 activity was detected in both lines with Waito-C assay. It was concluded that the action of the dwarf gene on culm elongation of Fukei 71 is not altered by GA_3 treatment, and that this dwarf gene is not directly concerned with GA_3 biosynthesis.

The process of morphogenesis in higher plants such as rice is determined by the types of differentiation, development, and growth, all of which depend primarily on genetic control. Thus, in order to know the mechanism of morphological mutants such as dwarfness, it is necessary to understand at the molecular level how these specific genes are effective in the meristem of nodes, root tips, and the shoot apex, which are the sites of differentiation. Yet not much is known about dwarf genes. With further research in developmental genetics, a breeder's dream—the artificial control of the gene to work at the time and site desired—might be possible.

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SEMIDWARFING GENES OF HIGH YIELDING RICE VARIETIES IN JAPAN

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The inheritance of semidwarfism in three Japanese high yielding varieties was studied in relation to the *Dgwg* dwarfing gene. Two types of near-isogenic lines were developed by transferring the semidwarf trait from Taichung Native 1 having the *Dgwg* gene and another semidwarf trait from Shiranui with the gene of Jikkoku into the Japanese tall variety Norin 29 through four backcrossings. The genetic behavior of the crosses of the near-isogenic line with Norin 29 demonstrated clearly that the semidwarfism of DGWG variety and that of Jikkoku are controlled by a single recessive gene. Allelism tests among the isogenic lines showed that DGWG and Jikkoku have a common semidwarfing gene. An allelism test between Reimei, an induced mutant, and the isogenic line carrying the *Dgwg* gene indicated that Reimei also has a similar semidwarfing gene at the same locus as that of *Dgwg*, although it has modifier genes that increase the culm length. In the same way, Kochihibiki is assumed to be allelic to the *Dgwg* gene. The existence of a linkage relationship between the semidwarfing gene and a recessive shattering gene is suggested.

A breakthrough in rice yield was attained with the development of semidwarf varieties characterized by lodging resistance, N responsiveness, and erect leaves (2). The success of the Green Revolution is directly related to the intensive use of these tropical semidwarfs. The short-statured indica varieties, represented by Dee-geo-woo-gen (DGWG) and Taichung Native 1 (TN 1), were principal sources of short stature and have been extensively used in breeding programs. TN 1 is a progeny of Tsai-yuan-chung/DGWG and has derived the short-stature gene from DGWG (2).

In Japan, two distinct sources of semidwarfism have made an important contribution to rice production. In southern Japan, a series of new high yielding varieties — Hoyoku, Kokumasari, and Shiranui — were released from a cross between Zensho 26 and the native semidwarf variety Jikkoku (11). Many short-statured leading varieties with high yielding potential have been developed through subsequent hybridization with other varieties (Fig. 1). In northern Japan, the semidwarf Reimei, which was derived from Fujiminori by gamma irradiation, was released in 1966 (4,5) and is now grown over wide areas. It could also

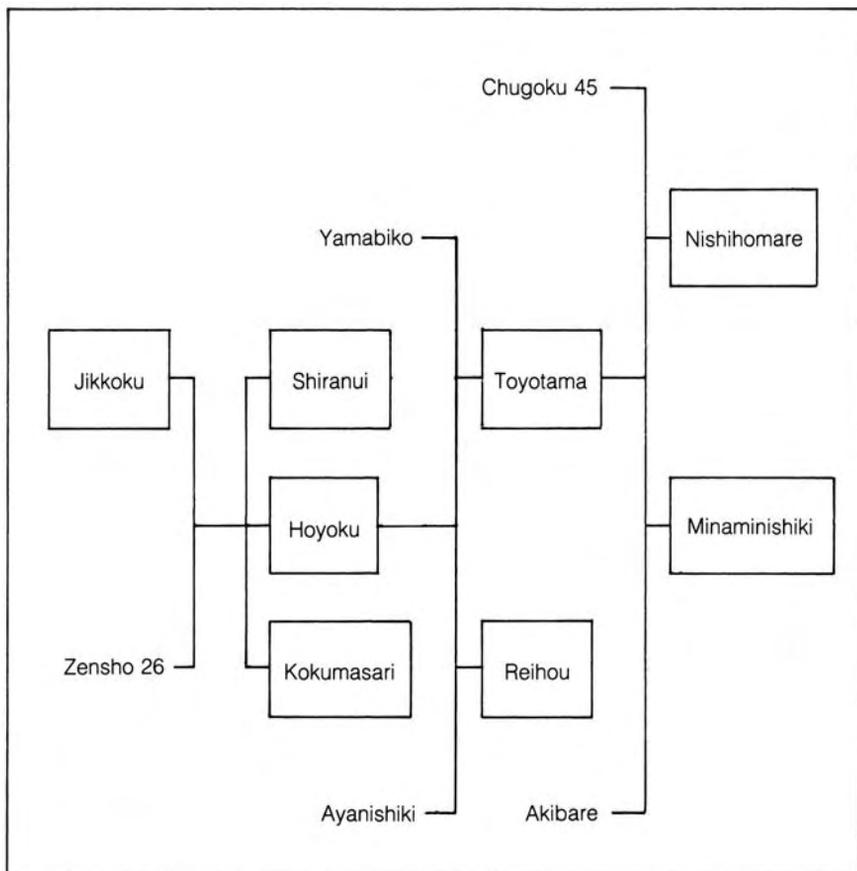


Fig. 1. Improved rice varieties released in Kyushu, southern Japan, that can be traced to Jikkoku, a semidwarf Japanese variety. These varieties are enclosed in boxes.

serve as a useful source of lodging resistance and high yielding ability. One of its descendants, Akihikari, which is a progeny of Toyonishiki/Reimei, was planted on 120,000 ha in 1979 and ranked fourth or fifth on the list of planted acreage in Japan (16). Another high yielding variety, Kochihibiki, was developed from the cross Kochikaze/Akibare//Kochikaze at the Central Agricultural Experiment Station in 1976. It has short stature, erect leaves, and good grain quality and also shows adaptability for late planting. Kochihibiki has been grown in central Japan. The source of short stature is assumed to be the local variety Shiro Senbon.

Extensive studies have been carried out to identify the genes for semidwarfism. As a result, the semidwarf traits of TN 1 and DGWG were

found to be controlled by a single recessive gene (1, 8). The semidwarf trait of the induced mutant Reimei is controlled by a single gene lacking dominance, designated as *d-49(t)* (5). So far, however, little is known about the allelic relationship between semidwarfing genes in the Japanese varieties and those in the indica varieties. The frequent use of the semidwarf indicas such as Chinese, IRRI, and Korean varieties as gene sources for higher grain yields has made it necessary to investigate the allelic relationships of these indica varieties with Japanese short-statured varieties. The present study was conducted to identify genes controlling the culm length of the Japanese high yielding semidwarf varieties Shiranui, Reimei, and Kochihibiki.

INHERITANCE OF SEMIDWARFISM

Characteristics of TN 1, Shiranui, Japanese tall variety Norin 29, and their F_1 's are presented in Table 1. TN 1 and Shiranui are about 20 cm shorter than Norin 29. The F_1 's between TN 1 and the Japanese varieties showed a high level of seed sterility and overdominance for lateness. Therefore, attempts to identify semidwarfing genes by performing a direct cross between TN 1 and Shiranui were not successful since these

Table 1. Characteristics of TN 1, Shiranui, Norin 29, and their F_1 's, 1971.

Variety or F_1	Heading date	Culm length (cm)	Panicle length (cm)	Grain length (mm)	Seed fertility (%)
Taichung native 1 (TN 1)	13 Aug	68.7	25.7	7.5	
Shiranui	7 Sep	67.1	21.2	6.9	
Norin 29	23 Aug	89.9	23.8	7.1	
Shiranui/TN 1	25 Sep	76.2	22.8	6.9	10.9
Norin29/TN 1	19 Sep	105.5	25.7	6.8	24.9
Norin 29/Shiranui	31 Aug	86.3	23.4	6.8	96.5

phenomena interfered with the full expression of genes controlling culm length.

These difficulties were successively overcome by the use of isogenic lines. By transferring the semidwarf trait from TN 1 carrying the *Dgwg* gene and another semidwarf trait from Shiranui carrying the *Jikkoku* gene into the Japanese tall variety Norin 29 through four backcrossings, two series of near-isogenic lines were developed. These were designated

Table 2. Characteristics of near-isogenic lines and the recurrent parent Norin 29, 1979.

Line	Source of semidwarfing genes	Heading date	Culm length (cm)	Panicle length (cm)	No. of panicles per plant	Grain length (mm)
SC 2	TN 1	16 Aug	60.5	18.3	21.8	6.5
SC 3	TN 1	20 Aug	61.7	19.6	20.1	6.4
SC 4	Shiranui	16 Aug	65.7	20.2	18.4	6.6
SC 5	Shiranui	15 Aug	64.2	20.2	17.3	6.6
Norin 29		17 Aug	91.8	21.0	17.3	6.8

as SC 2 and SC 3 (Norin 29/TN 1//4*Norin 29) and SC 4 and SC 5 (Norin 29/Shiranui//4*Norin 29). As shown in Table 2, the four near-isogenic lines were similar to the recurrent parent Norin 29, although they were somewhat different from one another. However, their culm lengths were 26-31 cm shorter than that of Norin 29 at maturity. The reduction in culm lengths of the semidwarf lines was due mainly to decrease in lengths of internodes, not reduction in the number of internodes (Fig. 2).

Each of the near-isogenic lines was crossed with the tall variety Norin 29 to investigate its genetic behavior. The F_1 and F_2 progenies, along with the parents, were planted at the National Institute of Agricultural Sciences (Tsukuba) in 1981. Culm length was measured from the soil surface to the panicle base. The mean F_1 values in the two crosses were higher than the mid-parent values and close to those of the taller parent Norin 29, indicating partial dominance of the genes controlling tallness. F_2 distributions for SC 2/Norin 29 and SC 4/Norin 29 clearly segregated into the 3:1 tall to short ratio for a one gene model ($P = .90-.95$ for SC 2/Norin 29 and $P = .25-.50$ for SC 4/Norin 29), indicating that the semidwarf trait of TN 1 and that of Shiranui are controlled in each case by a single recessive gene (Fig. 3). The results obtained with TN 1 are in agreement with the findings obtained by Aquino and Jennings (1) and Heu et al (8). SC 4 carrying the Jikkoku gene was further crossed with SC 2 carrying the *Dgwg* gene to determine the allelic relationship between genes for semidwarfism in the two isogenic lines. The F_1 hybrid was similar in culm length to both parents, and the F_2 population showed a narrow range of variation in culm length around that of the F_1 and parents (Fig. 3), indicating that Jikkoku has the same dwarfing gene as DGWG.

The semidwarf trait of Reimei is known to be controlled by a single gene with incomplete dominance (5). This variety was crossed to SC 2 to

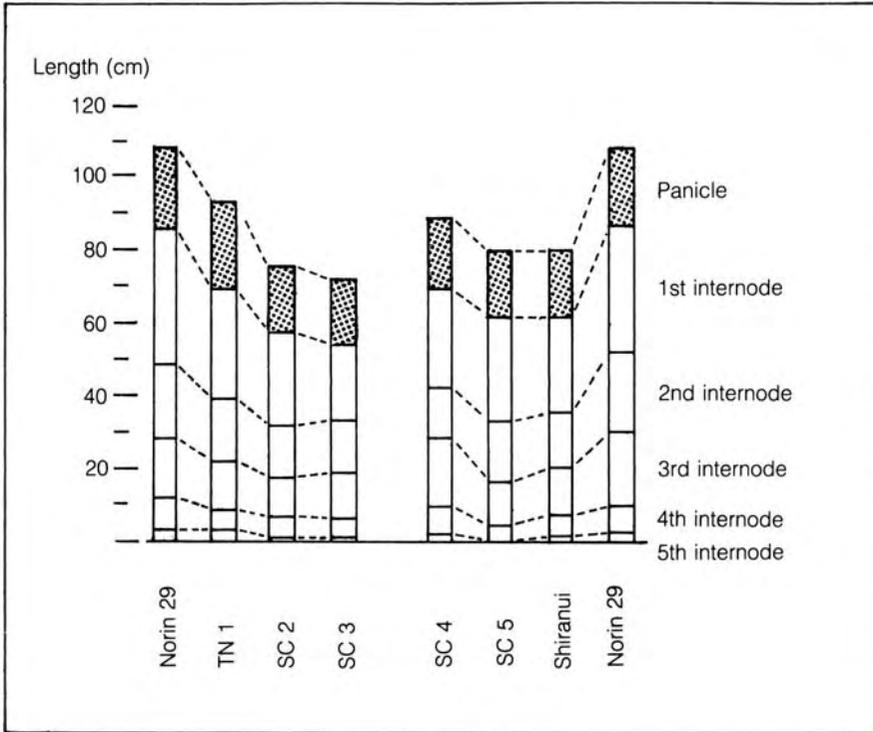


Fig. 2. Panicle and upper internode lengths of Norin 29, Taichung Native 1 (TN 1), Shiranui, SC 2, SC 3, SC 4, and SC 5.

determine the allelic relationship between the semidwarfing genes in both Reimei and DGWG. The F_1 plants had slightly shorter culms than those of Reimei. The variation in culm length in the F_2 population was continuous, with the distribution skewed toward Reimei (Fig. 4). The range of the segregation in F_2 was narrower than that between the two parents. There was no complete recovery of the type of the shorter parent SC 2 in the F_1 . These results suggest that Reimei also has a similar semidwarfing gene at the same locus as *Dgwg* although it may have different modifying genes for culm length, which could explain the failure to recover short segregates that are similar in culm length to SC 2.

Inheritance of culm length in Kochihibiki was studied in the cross of SC 2/Kochihibiki. The distribution of culm length in the two parents, F_1 plants, and F_2 plants is shown in Figure 5. The mean F_1 value was higher than the mid-parent value and was near to the taller parent Kochihibiki, indicating partial dominance of the genes controlling tallness. The F_2 distribution gave a satisfactory fit ($P = .25-.50$) to the ratio of 3:1

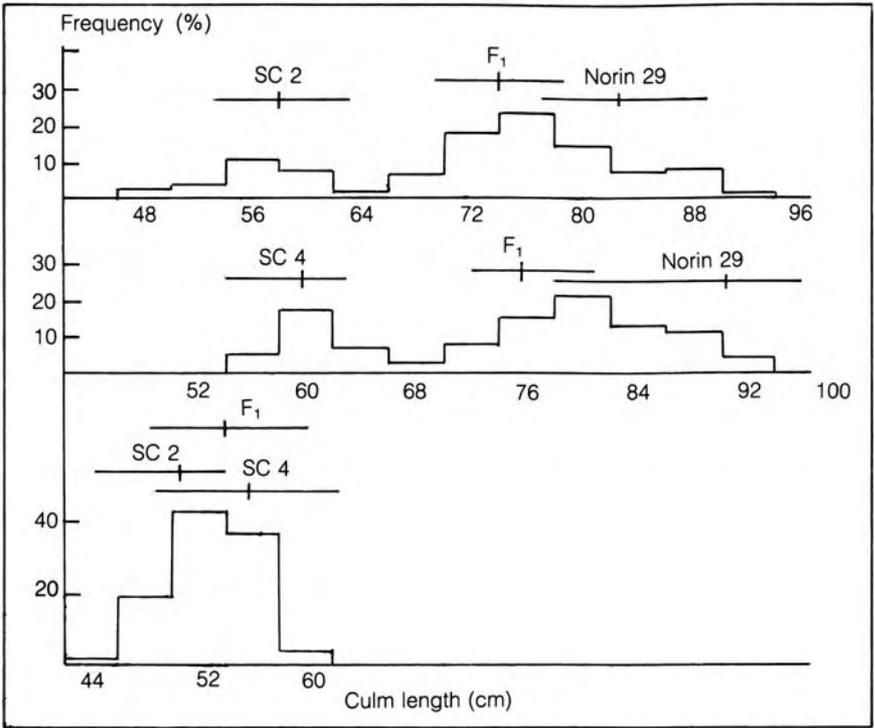


Fig. 3. Frequency distribution for culm length in F_2 in the crosses SC 2/Norin 29, SC 4/Norin 29, and SC 2/SC 4. Solid horizontal lines show the range of parents and F_1 's.

Kochihibiki stature to SC stature, indicating that the difference in culm length is controlled by one pair of alleles. Therefore, Kochihibiki was found to be dwarfed by another gene that is allelic to the *Dgwg* gene.

ASSOCIATION BETWEEN SEMIDWARFISM AND GRAIN SHATTERING

We observed that in the semidwarf near-isogenic lines SC 2, SC 3, and SC 4 the grains can be easily shattered from the panicle compared with those of the recurrent parent Norin 29. To investigate the genetic association between semidwarfism and shattering, these isogenic lines were crossed to Norin 29. The F_1 's and F_2 's were grown in the field. The degree of shattering was expressed by the number of grains shed when one panicle was gripped tightly by hand at the maturity stage. As shown in Figure 6, all F_1 plants were resistant to shattering, indicating that the

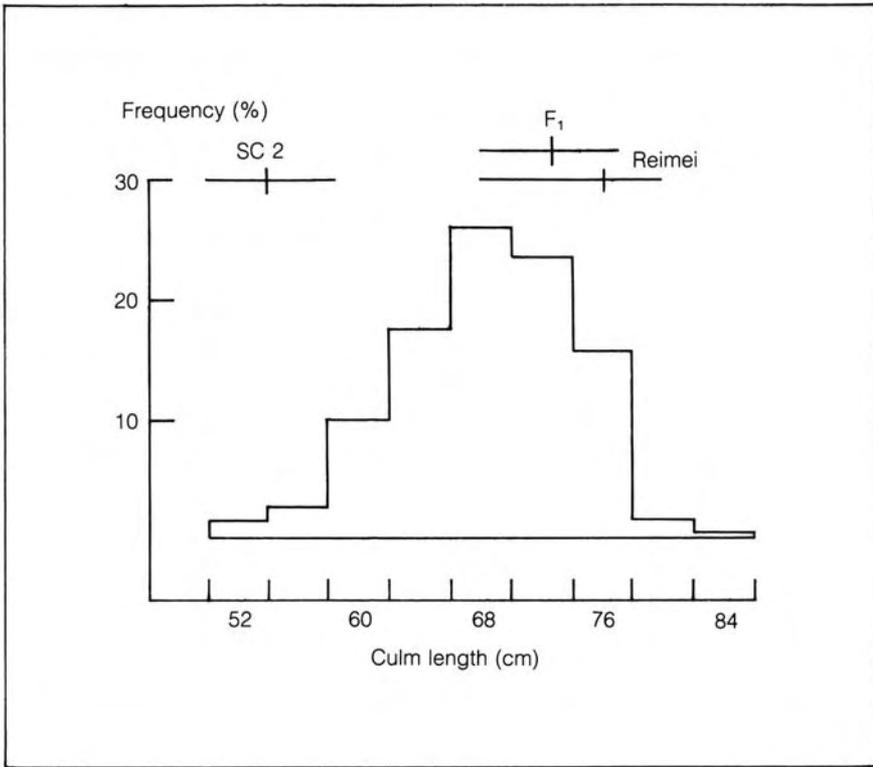


Fig. 4. Frequency distribution for culm length in F_2 in the cross SC 2/Reimei. Solid horizontal lines show the range of parents and F_1

trait for resistance to shattering is dominant over that for susceptibility to shattering. A close association between the two characteristics was observed in the F_2 population. Almost all of the short-statured plants had the shattering habit. However, a few recombinants were observed, indicating that the association is due mainly to the linkage between genes controlling the two characters. Therefore, it is advisable to select recombinants with the nonshattering habit in selecting semidwarf plants in the early generations after hybridization.

IMPLICATIONS OF SEMIDWARFISM FOR RICE BREEDING

The use of semidwarfism is one of the most significant achievements in rice breeding. However, it should not be overlooked that the semidwarf-

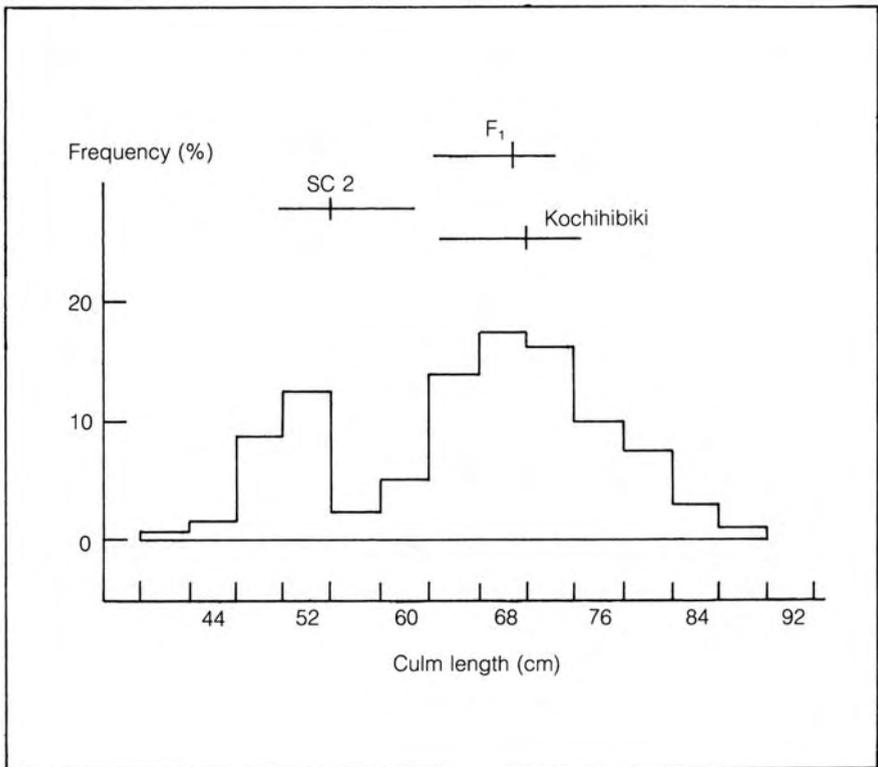


Fig. 5. Frequency distribution for culm length in F_2 of the cross SC 2/Kochihibiki. Solid horizontal lines show the range of parents and F_1 .

ing genes carried by the short-statured varieties of economic importance are the same and/or at the same locus as the *Dgwg* gene in spite of their different origin. This fact is widely known.

Calrose 76, an induced semidwarf mutant grown in California, carried a single recessive gene for semidwarfism, designated *sd₁*, which is allelic to the *Dgwg* gene (3, 10, 15). Short-statured mutant lines induced from tall native varieties, selected for high yielding potential, also had the dwarfing gene at the same locus (9). All named IR varieties except IR5 and virtually all other semidwarf varieties in major rice growing countries carry the semidwarfing gene from the Chinese semidwarf variety DGWG (6, 7).

The semidwarf trait of the Korean variety Tongil (IR8//Yukara/TN 1) was found to be controlled by a single recessive gene *d-t*, which is linked with marker genes of linkage group III such as *A* (anthocyanin activator) with recombination value of 24.8%, *Pp* (brown pericarp) with 35.1%, *Pn*

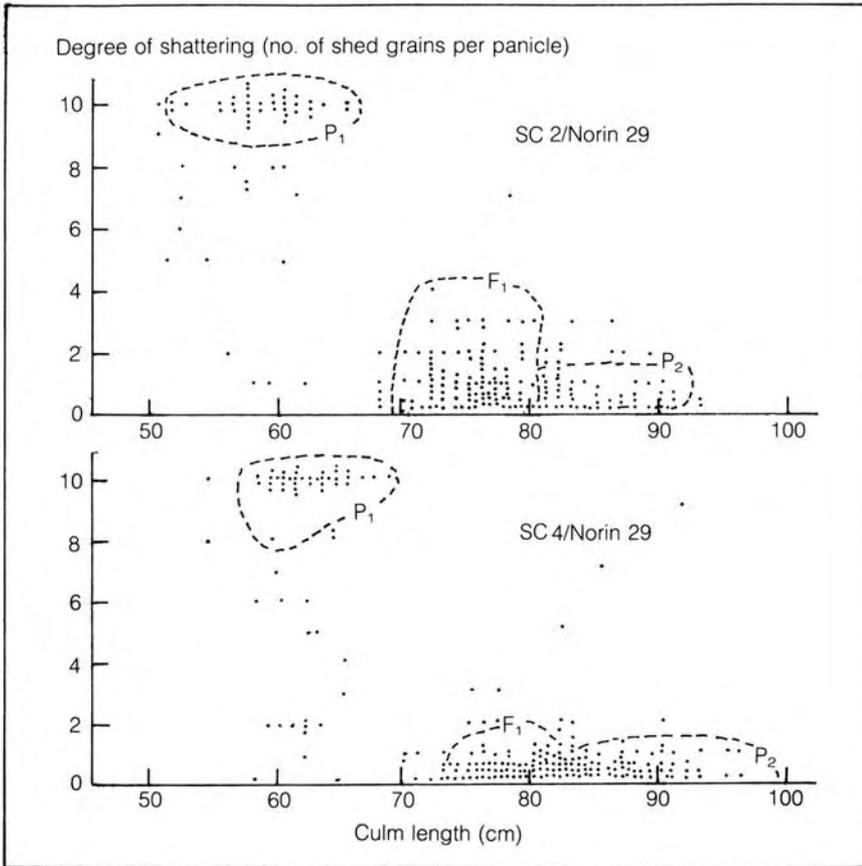


Fig. 6. Scatter diagrams of the relationship between culm length and shattering in the F_2 of the two crosses SC 2/Norin 29 and SC 4/Norin 29. The areas surrounded by dotted lines refer to the range of parents and F_1 's.

(purple node) with 40.9%, and pau (purple auricle) with 42.9% (17).

In indica/japonica hybridization programs, if the two parents carry the same gene for semidwarfism, the undesirable transgressive segregation for long culm, which is frequently observed in the segregating generations, will not occur. This is also the case for choice of parents in hybrid rice.

On the other hand, it is pointed out that the frequent use of the same semidwarfing gene may reduce genetic diversity and bring about genetic vulnerability. New sources of semidwarfism are necessary to broaden the genetic base of the high yielding varieties (2).

The five semidwarf mutants induced from the Indian variety Tellakattera by ethylmethane sulfonate treatment carried new genes that are

nonallelic to the *Dgwg* gene (13, 14). In California, two additional semidwarfing genes, *sd*₂ and *sd*₄, which are nonallelic to the *sd*₁ locus present in Calrose 76, were induced in the tall variety Calrose. Because of slightly taller height and/or associated pleiotropic effects, neither the *sd*₂ nor the *sd*₄ source has been as useful as *sd*₁ (15). Therefore, in the case of the identification of new semidwarfing genes from induced or spontaneous mutants, careful studies should be undertaken on their pleiotropic effects and linkage relationships between semidwarfism and other characters (12). All induced and hybrid-derived semidwarf varieties were found to be highly susceptible to bacterial leaf blight. The improved short-statured varieties such as TN 1 and IR8 are also susceptible to this disease (9). The semidwarf variety Jikkoku is very susceptible to bacterial leaf blight (11).

Genetic association of semidwarfism with other agronomically important characteristics will provide valuable information for the breeding of semidwarf varieties.

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GENETIC ANALYSIS OF NOTCHED GRAIN IN RICE

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The average frequency of notched grain type in a total of 1,366 rice varieties and lines investigated was 5.63%, and the correlation between notched type frequency and grain weight was positive, with $r = 0.6195$. In 1978, 1979, and 1981, 17 combinations were made among notched and normal grain varieties. F_1 , F_2 and BC_1F_1 populations were analyzed. It was found that notching is controlled by two pairs of complementary dominant genes. Environmental conditions affect the degree of notching only. Notching can be removed by successive selection.

In the 1960s semidwarf rice varieties became popular in China and significantly increased yields. In recent years, plant breeders have focused their attention on improving grain number/panicle and grain weight to increase yields. The authors worked to increase grain weight during 1972–1980 and developed a new indica variety Zhou yun nu, whose 1,000-grain weight reaches 48 g and whose small area yield reaches 9 t/ha, but the increase in grain weight was accompanied by a series of unfavorable traits such as declined tillering ability, notched grain, and sprouting on ears.

The notched grain of rice is characterized by a hollow of variable size in the belly of the mature kernel. This character severely affects milling quality, especially in some indica varieties with bold grains. This detrimental trait exists in the new variety Zhou yun nu (Fig. 1). Among notched grain rice varieties, the degree of notching is different. In some varieties the notch is called “kidney rice” by farmers. Such grains are apt to be broken during processing, with the yield of whole kernels consequently decreasing.

Ghose and Butany (2) considered notched grain to be controlled by a dominant gene and an inhibitor; the segregation ratio was 13:3 in F_2 progeny. Pavithran (4) thought the ratio should be 9:7. Ratios of 3:1 (3), 27:37 (5), and 27:229 (1) were proposed. Takeda (6, 7, 8) stated that the

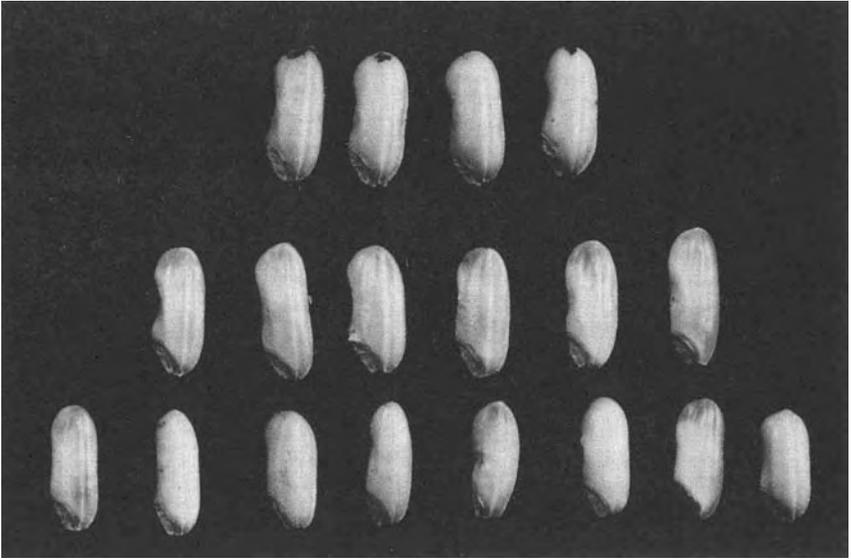


Fig. 1. The appearance of Zhou yun nu kernels.

heritability of notching in the F_3 - F_4 reaches 0.63-0.69 and relates to grain shape. This study was carried out to determine the characteristics and inheritance of notched grain in rice.

OCCURRENCE OF NOTCHED GRAIN

In 1979, the occurrence of notched grain was investigated in various rice germplasms of China, including 258 Yunnan varieties, 850 Southern Chinese semidwarf varieties, and 258 breeding lines. The location of the notched grains on the panicle was analyzed in 1983, as well as the influence of environmental factors.

Distribution of notched grain in rice germplasm. A total of 1,366 rice varieties were analyzed, and the average frequency of notched grain was 5.63% (Table 1). Notching can occur in either big or small grain varieties (Fig. 2), and the 1,000-grain weight of notched grain varieties ranged from 23 to 48 g. But there was a positive correlation between notched type frequency and grain weight, with $r = 0.6195$. In this paper, notched grain variety refers to those of which more than 90% of the grains are notched, though degrees of notching vary.

Table 1. The relation of notching and grain weight to germplasm.

	1,000-grain weight (g)							Total
	17.1- 20.0	20.1- 23.0	23.1- 26.0	26.1- 29.0	29.1- 32.0	32.1- 35.0	>35.0	
Observed (no.)	72	286	498	298	127	53	32	1,366
Notched (no.)	0	4	15	29	18	6	5	77
Notched (%)	0	1.39	3.01	9.73	14.17	11.32	15.56	5.63

Distribution of notched grains on panicle. As shown in Tables 2 and 3, the location of notched grains on the panicle differs. Table 2 shows the location on F_2 progeny of crosses of normal grain varieties, while Table 3 shows the location on notched grain varieties. Such location differences were less obvious for the genetically notched grain varieties (Table 3), while the occurrence of physiologically notched grain greatly differed for the upper and lower parts of the panicle (Table 2). However, the general tendencies for both genetic and physiological notching were similar, i.e., the frequencies in the upper and middle parts were less than in the lower part. For the notched grain varieties, all the grains were notched, but a few grains in the upper and middle parts of a panicle were full, most of which were located on primary branches (Table 3).

GENETIC ANALYSIS OF NOTCHED GRAIN

Four combinations (including reciprocal crosses) of a notched grain variety (Zhou yun nu) and normal grain varieties (Zhou ke No. 2, Hang Jian dao No. 2) were made in late spring of 1978 in Hangzhou. In late spring of 1979, 11 combinations were made among notched (Zhou yun nu Fan₃, Fan₄, and Ke qing) and normal (Hu nan te da li, Si mei No. 2, Fu qing, Hao jing kang, 2248, IRA215, and Zheng rong 13) varieties, including notched/normal and notched/notched crosses. Two combinations between notched (Zhou yun nu and Lu te ke No. 3) and normal (Yuan feng zao and Guang lu ai No. 4) varieties were made in 1981. Backcrosses were made for the combination of 1981. F_1 , F_2 , and BCF_1 of the above combinations were observed and analyzed. All F_1 plants were transplanted. In the F_2 , 500 plants were planted for each combination, about 100 plants were selected at random for observation, and 10 grains/plant were examined. Notches induced by incomplete maturity were not counted.

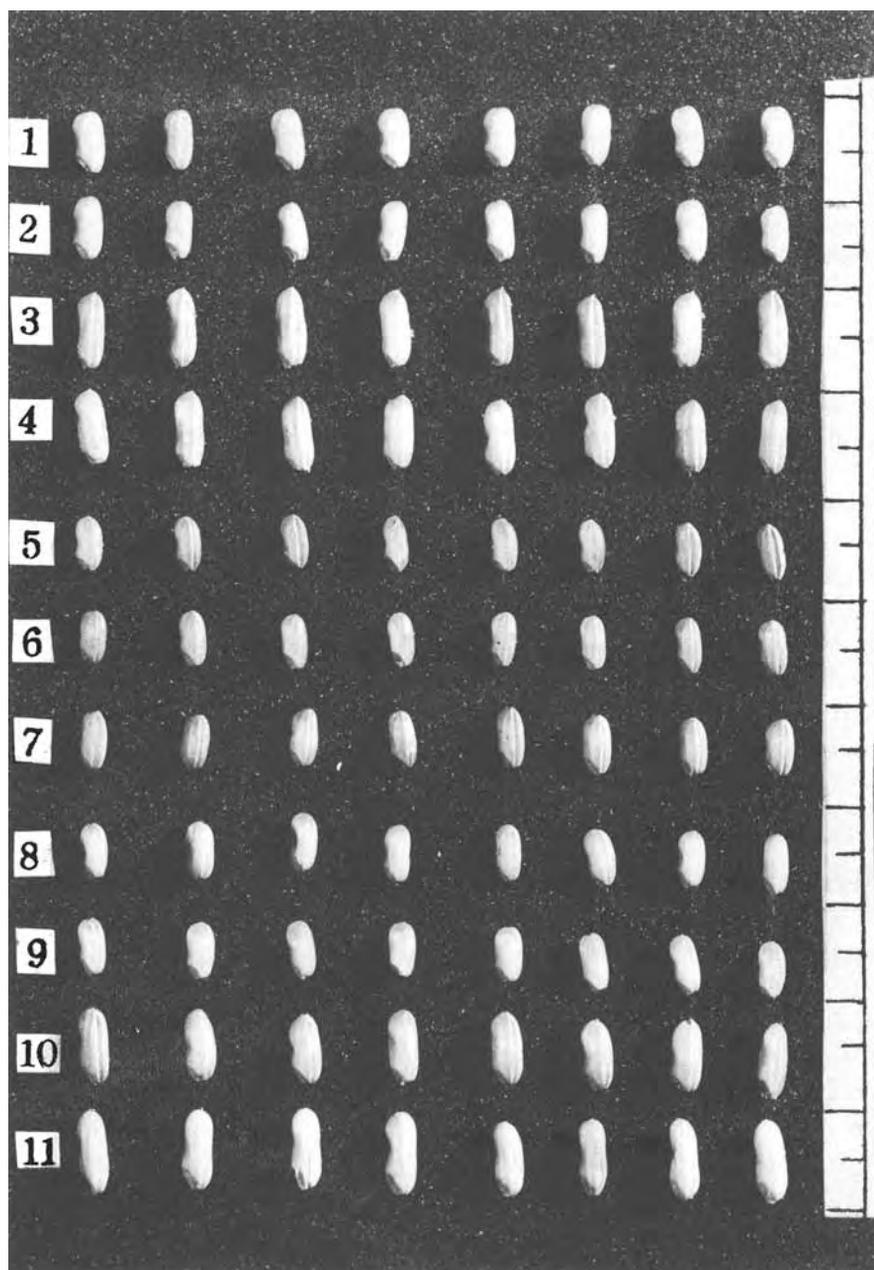


Fig. 2. The notched kernels of rice germplasm. 1. Lu ri zao, 2. Zao bai, 3. Lu te ke, 4. Zhou quang 50, 5. Yun nan-5099, 6. Yun nan-50155, 7. Yun nan-50118, 8. Si hong, 9. Ke qing, 10. Qing ke hui, 11. Si lu wen.

Table 2. The location of notched grains on the panicles in normal crosses.^a

Position	F ₂ of Dian Yui 405/Fu 8105		F ₂ of Zhou 154/Fu 8105	
	Grain weight (g)	Notched kernel (%)	Grain weight (g)	Notched kernel (%)
Upper	30.0	17.65	28.4	5.26
Medial	30.0	11.11	28.4	5.26
Lower	28.6	33.33	28.0	17.65

^a Average of 20 panicles/cross.

Table 3. The location of notched grains in notched varieties.

Position	Notches (%)	
	Zhou yun nu	79-selection 3
Upper primary branches	92.63	97.87
Upper secondary branches	100.00	100.00
Medial primary branches	100.00	97.56
Medial secondary branches	100.00	97.05
Lower primary branches	100.00	100.00
Lower secondary branches	100.00	100.00

Performance of notch in F₁. In the combinations of 1979, the grains of all F₁ plants from notched/normal, normal/notched, or notched/notched crosses were notched, which was consistent with the F₁'s of the combinations of 1978 (Fig. 3). The only difference among combinations was the degree of notching. So it is obvious that notching on the kernel is controlled by dominant gene(s) but influenced by grain weight or type.

Segregation of notching in F₂ populations. For the seven combinations of notched and normal grain varieties made in 1978, 1979, and 1981, segregation ratios of notching were all 9:7 (Table 4), indicating that notching is controlled by two pairs of complementary dominant genes. For the combinations between notched varieties (e.g., Ke qing/Zhou yun nu), grains of F₁ plants were notched, as were grains of all F₂ plants. The absence of obvious segregation of notching implies that the genes for notching in these varieties are allelic.

Backcross test. A backcross test was made using Guang lu ai No. 4/Zhou yun nu from the combinations of 1981. When F₁ plants were

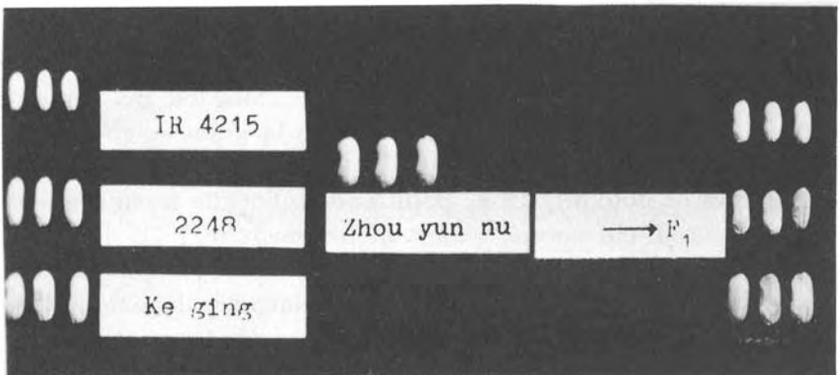
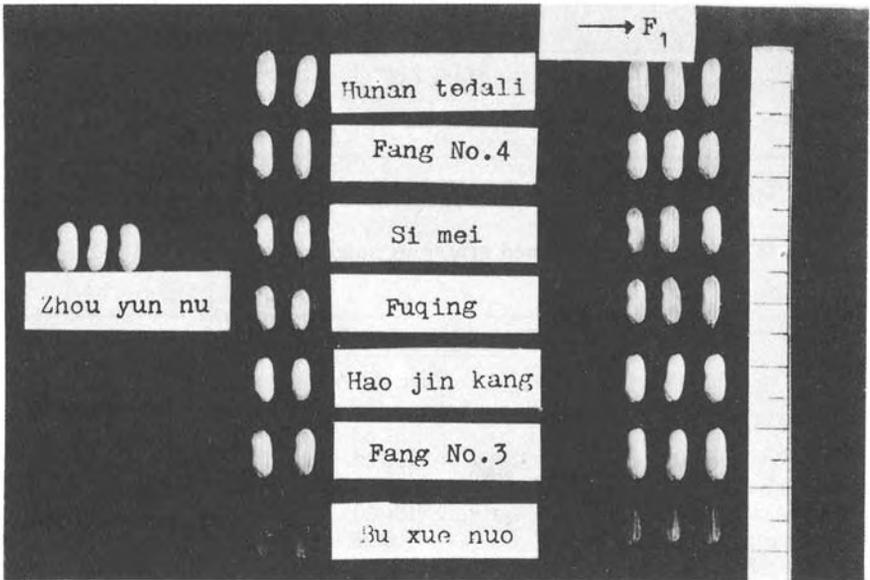


Fig. 3. The appearance of notches in some crosses of rice (upper = Zhou yun nu ♀, lower = Zhou yun nu ♂).

backcrossed to Zhou yun nu, 21 single plants were derived, all the grains of which were notched. When F_1 plants were backcrossed to Guang lu ai No. 4, 37 single plants were derived, among which notching was absent from 27 and present in 10. This segregation coincided with 3:1 ratio as indicated by the χ^2 test. Thus, the character of notching was confirmed to be controlled by two pairs of complementary dominant genes.

RELATION OF NOTCHING TO OTHER GRAIN CHARACTERS

Relation of notching to grain weight. In 1980, the 1,000-grain weight was determined for the notched and normal grain plants among the F_2 of Zhou yun nu/Zheng rong 13. The ratio of plant number of notched grain to plant number of normal grain increased as the grain weight increased, as shown in Figure 4. The frequency of notched grain plants should thus increase in breeding programs for increased grain weight. There were certain relations between grain weight and notch, although they are different grain characters. Thus there are barriers to increasing grain weight in rice breeding programs.

Relation of notching to grain shape. As shown in Table 5 in the F_2 population, the grain length, width, and thickness of normal grain plants were less than those of notched grain plants. The maximum difference existed in grain width. For example, in Zhou yun nu/Zhou ke No. 2, grain lengths of normal plants and notched grain plants differed by 0.4%, widths by 3.5%, and thickness by 2.2%. Such differences varied in degree among combinations, but the tendencies were consistent.

Relation of notching to belly chalk. The occurrence of grain notch and belly chalk was investigated among germplasms in order to learn if there was pleiotropism of notching genes, and a close relationship was found between the two characters (Table 6).

DISCUSSION AND CONCLUSIONS

Opinions vary concerning the genetic analysis of notching. The difference could be caused by various experimental materials and standards of testing. In this paper, we used a notched kernel percentage above 90% as the standard of genetic notched kernel type to distinguish clearly the genetic factor from the influence of environmental and physiological factors.

Table 4. The segregation of F_2 populations from notched/normal crosses.

Year	Crosses	Plants observed			χ^2 9:7	P
		Total	Notched	Normal		
1978	Zhou yun nu/Zhou ke No. 2	140	87	53	1.957	0.20-0.10
	Zhou ke No. 2/Zhou yun nu	119	75	44	2.281	0.20-0.10
	Hong jie dao No. 2/Zhou yun nu	79	50	29	1.592	0.25-0.20
1979	Guang lu ai No. 4/Zhou yun nu	63	37	26	0.157	0.70-0.50
	Zhou yun nu/Zheng rong 13	77	40	37	0.578	0.50-0.30
1981	Yuan Feng zao/Lu te ke No. 3	199	101	98	2.256	0.20-0.10
	Guang lu ai No. 4/Zhou yun nu	197	121	76	1.997	0.20-0.10

Table 5. The relationship of notching to grain weight, length, width, and thickness in F_2 's of crosses between notched and normal varieties.

Cross	Normal ^a				Notched ^a			
	GW (g)	GL (mm)	GWD (mm)	GT (mm)	GW (g)	GL (mm)	GWD (mm)	GT (mm)
Zhou yun nu/Zhou ke No. 2	33.1	9.89	3.03	2.24	35.1	9.93	3.14	2.29
Zhou ke No. 2/Zhou yun nu	35.6	9.88	3.06	2.27	38.3	9.99	3.10	2.29
Zhou yun nu/Hang jie dao No. 2	35.7	9.94	3.09	2.07	35.2	10.10	3.25	2.14
Hang jie dao No. 2/Zhou yun nu	38.3	10.21	3.24	2.36	39.1	10.35	3.34	2.27

^aGW = 1000-grain weight, GL = grain length, GWD = grain width, GT = grain thickness.

Grain notching is induced by incomplete development of the grain or by excessive size of grain, causing incomplete grain filling. Our investigations showed that notching in our materials is controlled by two pairs of complementary dominant genes, which can be called *NK1* and *NK2*, and that environmental conditions may modify the degree of occurrence (Fig. 5). This, of course, will not exclude other inheritance modes in other materials. Notched grain plants should be discarded from early generations in breeding for extra-high yield.

Grain weight is correlated with notching with a correlation coefficient of 0.6195. It is possible, however, to increase grain weight without the occurrence of notching through selection, which would benefit breeding programs.

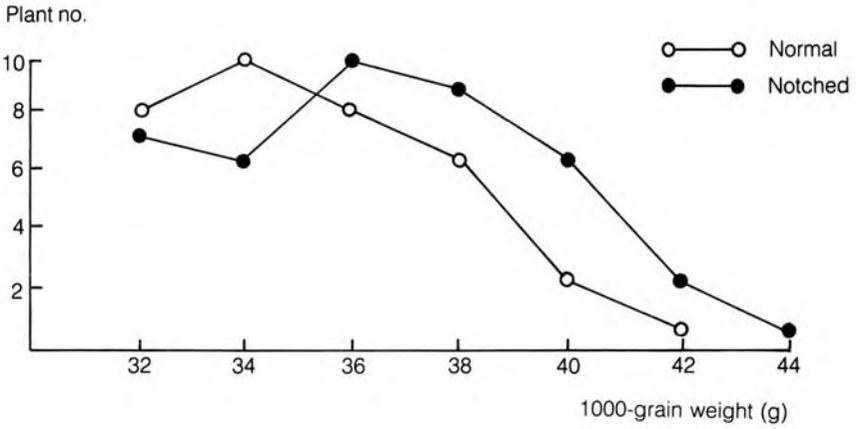


Fig. 4. The relation between notching and grain weight in the F₂ of Zhou yun nu/Zheng rong 13.

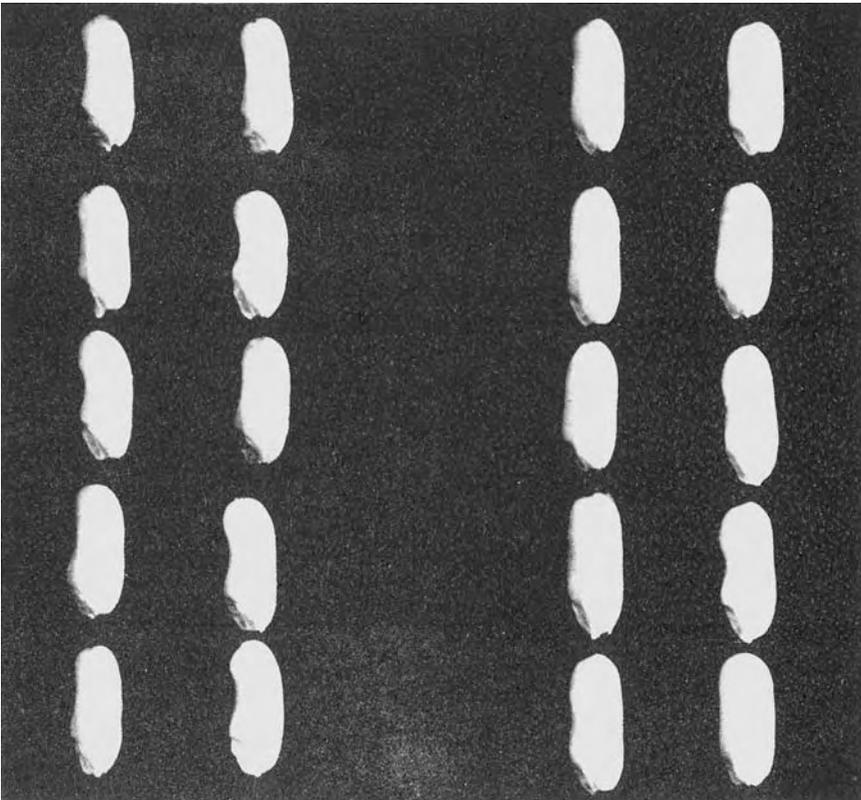


Fig. 5. The influence of season on the appearance of notching (planting date of right April-July, of left July-October).

Table 6. The relationship of notching to belly chalk.

Original	Type	No.	Belly chalk (%)		
			0	<10	>10
Semidwarf germplasm	notched	50	10.0	42.0	48.0
	normal	50	20.0	38.0	42.0
Breeding lines	notched	30	10.0	57.0	33.0
	normal	30	27.0	60.0	13.0
Yun-nan germplasm	notched	20	40.0	25.0	35.0
	normal	20	85.0	15.0	0.0

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DEVELOPMENTAL MECHANISM AND INHERITANCE OF GRAIN NOTCHING IN RICE

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Several major genes concerning grain notching have been described, and notched grain percentage shows continuous variation; the feature may be controlled by polygenes in most cases. Grain notching is caused primarily by imbalanced growth between the caryopsis and floral glumes. The caryopsis is naturally cast in the mold of the floral glumes. However, when the tip of the floral glumes is clipped soon after anthesis for allowing full elongation of the caryopsis to its hereditary limit, the caryopsis is capable of elongating beyond the scope of the floral glumes. Varieties with high imbalance between the length of the caryopsis and that of the floral glumes tend to develop notched grains. Grain notching is induced by low temperatures during the early grain filling period, because the growth duration of the caryopsis is expanded under low temperature conditions, resulting in a longer caryopsis and higher imbalance between its length and that of the floral glumes.

Rice grains with a notch or notches on the kernel occur in some varieties from China, Europe, India, Japan, Nepal, Southeast Asia, the US, and West Africa. Although this is a rather rare malformation of rice, it has attracted researchers' attention for a long time. Thus, there exist many studies from both the practical and the scientific points of view. This paper reviews work published since Gammie in 1908 (3).

NOTCHED GRAIN FEATURE

Many notched grains have a single deep constriction on the ventral side of the caryopsis, slightly above the embryo, although the position, direction, and number of notches on the caryopsis are variable (9,11,14). The notched grains scatter almost at random on a panicle, but when the notched grain percentage is low they are found more frequently on the second rachis than on the primary rachis (6,9,11). The notched grain percentage of underdeveloped kernels is higher than that of fully developed ones (5,11). When the percentage of notched grains is low, the inferior spikelets tend to develop notched grains, and thus the size of the notched grain is smaller than that of the normal one (9,11,29). However, when the

majority of the spikelets developed notched grains, the difference in grain size between notched grains and normal ones is small (17). The notched grain is easily broken while hulling and milling (14,15). For this reason the hardness of notched grains is significantly lower than that of normal ones (11).

The lemma and the palea encasing the notched grain are normal in appearance and their inner surface is quite smooth, as with normal grain. Portéres (15) observed that the ventral side of notched grain grows more slowly than its dorsal side and surmised that such growth is hormonally controlled. Nagato and Kobayashi (9) concluded that the occurrence of notched grains is due to effects of such environmental factors as low temperature or shading during the grain filling period. On the other hand, although the caryopsis is naturally cast in the mold of the floral glumes, when the tip of the floral glumes is clipped soon after anthesis, the caryopsis elongates, exceeding the scope of the floral glumes (8).

Takahashi and Takeda (18) and Takeda and Takahashi (29) investigated the potential length of the caryopsis by clipping the tip of the floral glumes and revealed that the potential length of the caryopsis always exceeds the scope of the floral glumes. A ratio of the length of the glume-clipped caryopsis (Tl) to the length of the caryopsis developed naturally encased in the floral glumes (Cl) was calculated as an index of imbalance between the potential length of the caryopsis and the capacity of the floral glumes. Varieties with a Tl/Cl ratio developed notched grains frequently, whereas varieties with a Tl/Cl ratio less than around 1.20 do not develop notched grains. When the tip of the floral glumes is clipped soon after anthesis, the caryopsis never shows grain notching. In some F₂ plants the Tl/Cl ratio is as high as 1.7, and plants with high Tl/Cl ratios frequently develop notched grains (Fig. 1) (20,22,30).

Hybrid populations of normal varieties often segregate plants that develop notched grains (21). These transgressive segregants for grain notching have a high Tl/Cl ratio. This imbalance between Tl and Cl is considered to be caused by the recombination of genes for long caryopsis and short floral glumes.

Takeda (22) conducted a selection experiment to examine the correlated response between notched grain percentage and Tl/Cl ratio. Heritability of Tl/Cl ratio was estimated from the selection response to be 0.57–0.61, and the genetic correlation coefficient between notched grain percentage and Tl/Cl ratio was estimated from the correlated response to be 0.41–0.58. This indicates that the Tl/Cl ratio is under genetic control and that it correlates with notched grain percentage.

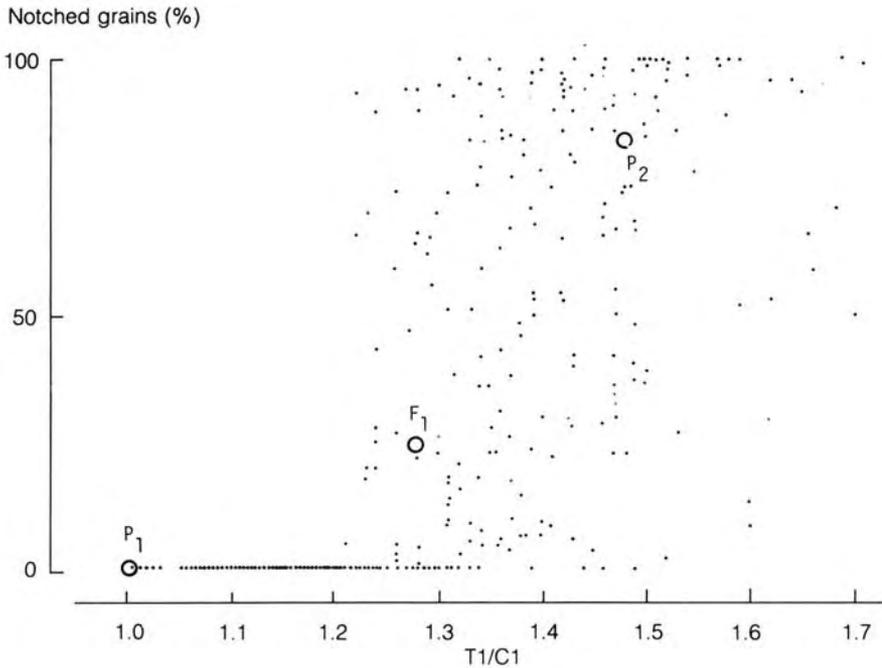


Fig. 1. Relationship between notched grain percentage and degree of imbalance between the length of the caryopsis and that of the floral glumes (T1/C1) in an F_2 population from H-347/H-346.

The phenotypic correlation coefficient between T1/C1 ratio and notched grain percentage has been estimated to be about 0.5 in varieties (29) and about 0.7 in F_2 plants (20).

Morimura and Honma (6) confirmed that varieties with high notched grain percentage have a high T1/C1 ratio and, when the tip of the floral glumes is clipped, those varieties never develop notched grains. Pavithran (13) also studied the developmental cause and inheritance of notched grain in India and reported that the glume-clipped caryopsis was longer than the floral glumes in grain notching varieties and the occurrence of notching might be attributable to a mutation that leads to the combination of a long kernel and a short hull.

Takeda (19) observed the growing process of notched grains utilizing soft (long-waved) X-rays. The growing caryopsis reached the ceiling of the floral glumes around 6 days after anthesis. The glume-clipped caryopsis continued elongation to its own hereditary limit, without being restricted by the floral glumes. If a notched grain developed, the caryopsis was forced to bend to the ventral side immediately after the top of the

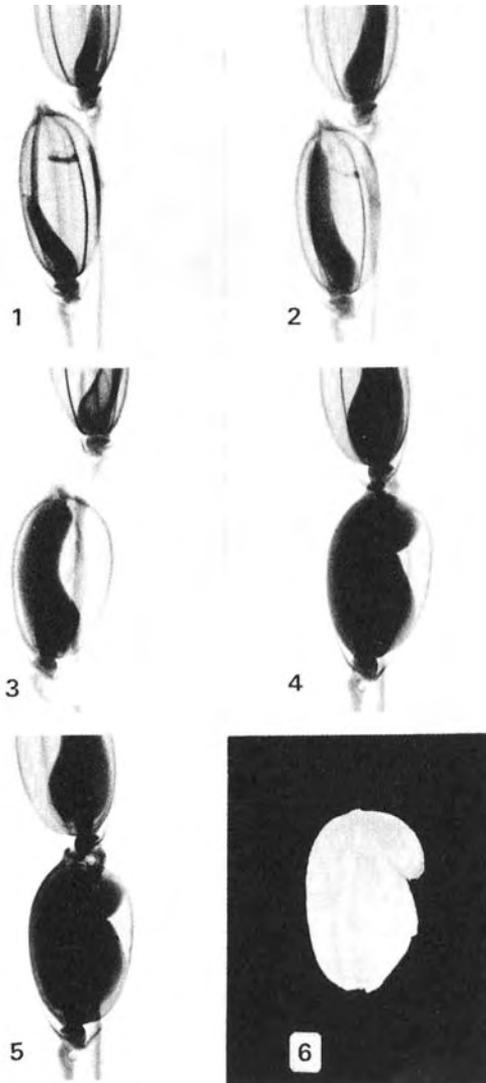


Fig. 2. X-ray photograph showing the growth process of notched kernel (1-5 = 2-10 days after anthesis, 6 = maturity).

caryopsis reached the ceiling of the floral glumes, and a pronounced indentation was formed by 9 days after anthesis (Fig. 2).

The author has concluded that grain notching is neither environmental in its origin nor attributable to hormonal control but is simply due to

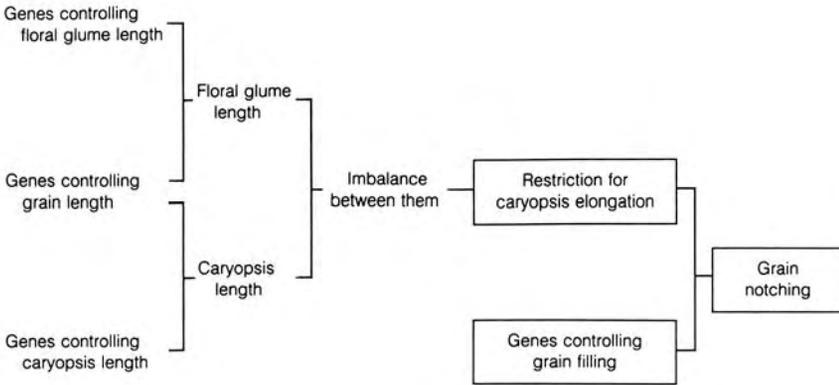


Fig. 3. The genetic mechanism of grain notching.

mechanical restriction of the growth of the caryopsis by the scope of the floral glumes. The genetic mechanism of grain notching is shown in Figure 3 (20).

VARIETAL VARIATION

Varietal variation in notched grain percentage is very large. Okamura (11), using a total of 582 varieties grown under ordinary conditions, found that notched grain percentage varied from 0 to 92%. Pavithran (13) discovered that varietal variation ranged from 0 to 100%. Varietal variation is expanded under low temperature conditions (10,28).

Portéres (15) and Pavithran (13) reported that varieties with small grains tend to develop grains frequently. However, Okamura (11) found no correlation between grain size and notched grain percentage. Takeda (22) estimated the genetic correlation coefficient between kernel length and notched grain percentage to be from -0.14 to -0.31 in F_2 populations. The correlation is so loose that various combinations between kernel length and grain notching may exist. Actually, a notched variety with large grains (> 30 mg) has been collected at Yunnan, China (Z. M. Xiong, personal communication), while the author has found some small-grained (< 10 mg) notched varieties.

Grain shape seems to affect grain notching. Many notched varieties have round grains (10). Pavithran (13) reported a correlation between grain index (length/breadth \times thickness) and notched grain percentage, indicating that varieties with round grains tend to develop notched grain.

Takeda (23) analyzed the relationship between grain shape index (length/breadth) and notched grain percentage and found that it is curvilinear and that the optimal grain shape index to develop notched grains is around 1.3.

ENVIRONMENTAL VARIATION

Many environmental factors that affect grain filling such as fertilizer, soil type, soil moisture, light intensity, and temperature have an influence on grain notching (9,11,13).

It has often been reported that low temperatures during the early grain filling period are liable to induce notching (2,9,10,17,27,28,31). Many researchers have reported that the critical temperature to develop notched grain is 15–20 °C. However, Takeda (26) recognized that notched grain percentage varies with temperature in the early ripening period without any threshold in the range of 18–20°C. The maximum temperature is dominant over the minimum one to induce notching (26). Continuous high temperature after anthesis reduces grain notching, whereas temporal exposure to high temperature increases it (13).

Takeda (26) found a remarkable variation in the notched grain percentage of materials grown at 12 locations between 26°N and 41°N in Japan due almost completely to the temperature condition; the correlation coefficient between notched grain percentage and mean temperature during 15 days after anthesis was as high as 0.9.

Nagato and Kobayashi (9) explained that when nutrient translocation is checked temporarily by low temperature, starch cells of the middle portion of the kernel nearly stop growing in the dorso-ventral direction, but those of the other portion continue growth after the recovery of nutrient translocation. Even though they could not explain why only the middle portion of the kernel is affected by low temperatures, this became a widely accepted interpretation of how low temperatures induce notching.

Contrary to this, Takeda (24) found that the length of the glume-clipped caryopsis is affected by temperature after anthesis, with notched grain percentage being affected as well, while the length of the naturally grown caryopsis encased in floral glumes is not. This finding suggests that low temperatures promote elongation of the caryopsis, resulting in high imbalance between the length of the caryopsis and that of the floral glumes and causing the kernel to notch. Takeda (25) investigated the growing process of the glume-clipped caryopsis under controlled temper-

atures and clarified that under low temperature conditions the growth duration is considerably lengthened; the final length of the glume-clipped caryopsis was the longest, while the length of the caryopsis developed in the floral glumes followed the size of the floral glumes and did not change with temperature after anthesis (Table 1).

Table 1. Elongation and notching of rice kernels developed under low (18 ± 5 °C), medium (23 ± 5 °C), and high (28 ± 5 °C) temperature conditions after anthesis,^a average of seven varieties with two replications.

Temperature (°C)	Growth rate at maximum (mm/d)		Growth duration (d)		Kernel length (mm)		T1/C1	Notched grain (%)
	T1	C1	T1	C1	T1	C1		
18 ± 5	0.85	0.70	11-13	9-11	6.79	5.03	1.35	45.8
23 ± 5	1.05	0.88	9-11	7-9	6.35	5.00	1.27	3.2
28 ± 5	1.06	1.09	7-9	5-7	5.12	4.94	1.24	0.2

^aT1 = glume-clipped caryopsis, C1 = caryopsis encased in floral glumes.

INHERITANCE OF GRAIN NOTCHING

Morinaga and Fukushima (7) reported a single recessive gene controlling notched grain. Ghose and Butany (4) and Seetharaman (16) observed a segregation ratio of 13:3 normal to notched. Pavithran (12) reported a segregation ratio of 7:9. Seetharaman (16) described ratios of 37:27 and 55:9. A more complex segregation ratio of 229:27 was reported by Bhattacharya (1). Most of the genes controlling grain size may affect grain notching. Takeda (20) demonstrated that such short grain genes as *Mi*, *d-1*, and *d-7* pleiotropically control grain notching, because these genes remarkably reduce floral glume length and cause high imbalance between the lengths of the caryopsis and the floral glumes. When the length of the caryopsis and/or floral glumes is under the control of a major gene(s), Pavithran (13) suggested that segregation of notching might vary with number, dominance, and linkage of the gene(s) concerned. However, as the notched grain percentage shows continuous variation in most cases, it should be seen as a quantitative character controlled by polygenes.

Takeda (22) conducted a selection experiment for notched grain percentage up to the F_5 generation. The heritability of notched grain

percentage was estimated to be 0.63–0.77 from the selection response. Eleven F_5 lines selected for high grain notching developed more than 90% notched grains in both field and glasshouse conditions, and their T1/C1 ratio was more than 1.33. Contrary to this, another 11 F_5 lines selected for low grain notching developed almost no notched grains, and their T1/C1 ratio was less than 1.27. The results of this selection experiment indicate that notched grain percentage is under genetic control and correlates with the degree of imbalance between potential length of the caryopsis and the scope of the floral glumes.

CONCLUSION

The length of the floral glumes and that of the caryopsis are controlled by different genetic paths. Genetic imbalance between them results in grain notching; there is no gene directly controlling grain notching. Thus, notched grain is an example of a pseudo-character that is determined by the interaction among more than two distinguishable characters controlled by particular genes.

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GENETIC ANALYSIS OF SHEATHED PANICLE IN A NEPALESE RICE CULTIVAR GAMADI

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Gamadi, a native rice cultivar from Nepal in which the panicle remains enclosed within its flag leaf sheath up to maturity, was crossed with genetic marker testers, and the linkage relationships between "gamadiness" (the panicle enclosing character) and the marker characters were examined. Gamadiness is controlled by two complementary dominant genes with segregation ratio of 9:7 gamadiness to normal types. These genes have been temporarily proposed as "*Ga*" and "*Gb*." *Ga* was found to be linked with the neckleaf gene (*nl*) of Takahashi's linkage group VI + IX, with a crossover value of 0.3733 ± 0.0027 . *Gb* appears to be associated with the brittle culm gene (*bc*) of Takahashi's linkage group XI, with a crossover value of 0.2725 ± 0.0061 .

Gamadi is a peculiar rice cultivar from the western Terai of Nepal in which the panicle is enclosed within the sheath until maturity and cleistogamic pollination takes place within the sheath of the flag leaf.

Ramiah (7) reported that poor panicle exertion is controlled by polygenes. Sethi et al (8) postulated three complementary genes for "sheathed ear" in variety Sathi. "Gamadiness" (sheathed panicle) of Gamadi rice showed a different segregation pattern from those reported previously.

This paper reports the linkage relationships among the gamadiness character and the known marker characters of the identified linkage groups in rice.

MATERIALS AND METHODS

Gamadi was crossed with normal panicle exerting genetic marker testers of semidwarf type (9) with at least one identified marker character of 11 known linkage groups each (Table 1). The F_1 was grown in the field in summer 1982, and the F_2 was seeded on 21 April and transplanted on 5

June 1983. Observations on different genetic marker characters were taken when they appeared, and the gamadiness segregation was observed at maturity.

The F_1 plants showed a slight exsertion on the tip of the sheath of the flag of the primary tillers, but never complete exsertion, and the side tillers showed mostly gamadiness.

Table 1. Marker testers used in the gamadiness gene location.

Designation	Linkage group	Marker character
wx124-730-14-3-1	I	wx
HP907-B-1-1-6-1	II	lg
HP849-32-1-1-B-1	III,V	Pn, Ps
HP748-1-23-B-1	IV	g
HP748-1-7-2-B-1	VI+IX	gh
HP914-3-2-1	VII	Lh
HP904-B-1-1-B-1	VIII	la
HP887-1-1-1-B-1	VI+IX, XI	nl, bc
HP745-2-1-B-1	X	bl ₂
HP907-B-2-1-B-1	XII	gl
Gamadi	?	

RESULTS AND DISCUSSION

Marker character segregation

The results obtained from the experiment regarding the marker character segregation are given in Table 2. All of the marker characters used in this study segregated into expected ratios, with clear indications that these marker testers are eligible ones as reported previously (1, 6, 9, 10). It also implies that the genetic constitution of Gamadi rice is essentially the same as that of the marker testers.

Segregation of gamadiness character

The segregation patterns of gamadiness type and normal type were calculated and are given in Table 3. All the crosses involved with Gamadi parent and genetic marker testers showed the segregation ratio of 9:7 gamadiness to normal type.

Table 2. Segregation of marker genes in F₂ populations of Gamadi/marker crosses.

Cross	Normal	Marker	χ^2	P
Garnadi/wx	399	114	2.1111	0.25–0.10
Gamadi/Ig	406	140	0.1196	0.75–0.50
Gamadi/Pn	135	355	1.7007	0.25–0.10
Gamadi/g	370	107	1.6779	0.25–0.10
Gamadi/Ps	213	657	0.1241	0.75–0.50
Gamadi/gh	360	135	1.3636	0.25–0.10
Gamadi/Lh	110	353	0.3808	0.75–0.50
Gamadi/la	359	126	0.2481	0.75–0.50
Gamadi/nl	364	136	1.2907	0.50–0.25
Gamadi/bl ₂	369	117	0.2223	0.75–0.50
Gamadi/bc	367	133	0.6827	0.50–0.25
Garnadi/gl	363	132	0.7333	0.50–0.25

Table 3. Segregation pattern of gamadiness (panicle enclosing) character in the F₂ population of Gamadi/marker tester cross combinations.

Cross combination	Segregation ratio		Total	χ^2 (9:7)	P
	Gamadi	Normal			
Garnadi/Wx124-730-14-3	267	228	495	1.074	0.50–0.25
Garnadi/HP907-6-1-1-6-1	306	241	547	0.021	0.95–0.75
Garnadi/HP849-32-1-1-6-1	226	149	375	2.458	0.25–0.10
Garnadi/HP748-1-2-1-8-1	261	216	477	0.455	0.50–0.25
Garnadi/HP748-1-7-2-8-1	267	228	495	0.530	0.50–0.25
Garnadi/HP914-3-2-1	246	219	465	2.116	0.25–0.10
Garnadi/HP904-6-1-1-6-1	267	224	491	0.699	0.50–0.25
Garnadi/HP887-1-1-1-6-1	286	199	485	1.458	0.25–0.10
Garnadi/HP745-2-1-6-1	279	207	486	0.264	0.75–0.50
Garnadi/HP907-6-2-1-6-1	267	228	495	0.530	0.50–0.25

As early as 1932, Ramiah reported that poor panicle exertion is under polygenic control due to transgressive segregation (7). Sethi et al (8) postulated that the panicle enclosing “sheathed ear” in variety Sathi is controlled by at least three complementary genes, and their conclusion for inheritance appeared complicated and confusing. They also proposed three complementary dominant genes, viz., “P” for partial emerged, “T” for tip emerged, and “E” for enclosed panicle. Kadam and Ramiah (3) proposed “Shp” as the symbol for the panicle enclosing gene in reference to the original work of inheritance of sheathed ear in rice by Sethi et al (8). Kinoshita et al (4) used the gene symbol “Ex” for exerted panicle on the basis of the similar work of Sethi et al.

Both “*Shp*” (for sheathed panicle) and “*Ex*” genes for the same character of sheathed ear are single dominant genes, whereas Sethi et al (8) proposed three different complementary genes “*P*,” “*T*,” and “*E*.” The gamadiness gene segregation in this experiment clearly shows control by two dominant complementary genes, which does not agree with Sethi et al (8) and suggests that the Gamadi cultivar from western Nepal might be different from that used by Sethi et al.

On the basis of the results and discussions on panicle exertion, we temporarily proposed the gene symbols “*Ga*” and “*Gb*” for gamadiness (panicle enclosing nature) on the basis of 9:7 gamadi to normal type panicle segregation.

Linkage relationships between marker character and gamadiness

The linkage relationships between marker characters and gamadiness are shown in Table 4. The characters controlled by the genes *wx*, *lg*, *Pn*, *g*, *Ps*, *gh*, *Lh*, *la*, *bl*₂, and *gl* of linkage groups I, II, III, IV, V, VI+IX, VII, VIII, X, and XII, respectively, were found to segregate independently of gamadiness as shown in Table 4. The remaining two characters, viz., neck leaf of linkage group VI+IV and brittle culm of linkage group XI, segregated in different ways. On the basis of the dihybrid segregation analysis of neck leaf and brittle culm with gamadiness character, the

Table 4. Dihybrid segregation of gamadiness and marker characters in F₂ populations of Gamadi/marker crosses.

Cross	Gamadi		Normal		χ^2 for linkage	P
	Normal	Marker	Normal	Marker		
Gamadi/ <i>wx</i>	226	68	173	46	2.613	0.50–0.25
Gamadi/ <i>lg</i>	220	86	186	54	2.375	0.50–0.25
Garnadi/ <i>Pn</i>	75	195	60	160	1.973	0.75–0.50
Gamadi/ <i>g</i>	195	66	175	41	4.784	0.25–0.10
Gamadi/ <i>Ps</i>	125	376	88	281	0.886	0.90–0.75
Gamadi/ <i>gh</i>	192	75	168	60	2.654	0.50–0.25
Garnadi/ <i>Lh</i>	66	180	46	173	4.536	0.25–0.10
Gamadi/ <i>la</i>	190	74	169	52	2.168	0.75–0.50
Gamadi/ <i>nl</i>	233	64	131	72	11.707	<0.001
Gamadi/ <i>bl</i> ₂	197	74	172	43	3.710	0.50–0.25
Gamadi/ <i>bc</i>	199	95	168	38	12.487	<0.001
Gamadi/ <i>gl</i>	191	76	172	56	2.738	0.50–0.25

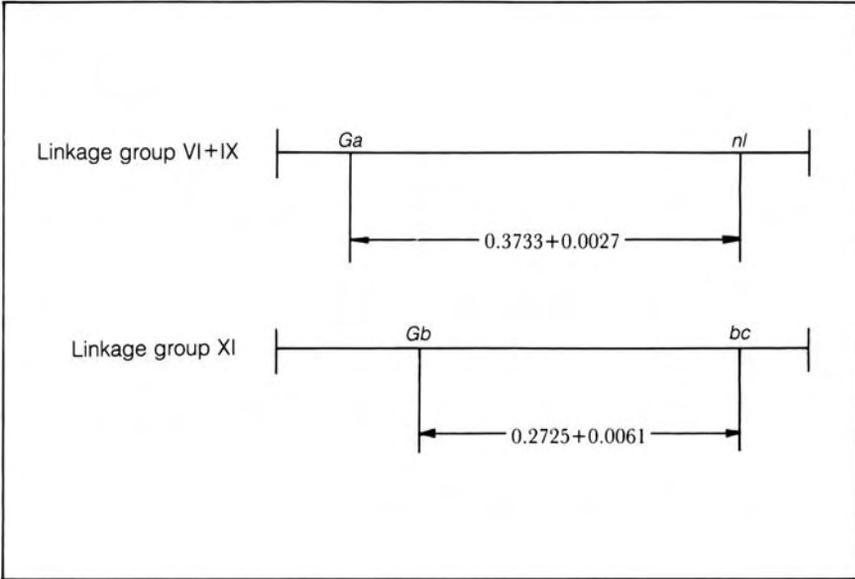


Fig. 1. Association of gamadiness genes (*Ga* and *Gb*) with marker genes (*nl* and *bc*) in linkage groups VI+IX and XI.

linkage intensities were calculated according to Immer (2) and Mather (5). The crossover value calculated between *nl* and *Ga* was 0.3733 ± 0.0027 , and the crossover value between *bc* and *Gb* was 0.2725 ± 0.0061 , as shown in Figure 1.

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TR-5 DWARFING GENE IN RICE*

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Inheritance was studied in a morphologically distinct, moderately short height mutant, TR-5 showing cigar-shaped panicles and concurrent changes in several other characters. The mutant was crossed with a tall stock K8 and three semidwarf reference stocks: Dee-geo-woo-gen, I-geo-tze, and Cheng-chu-ai-11. F₁ plants were tall; progenies in the F₂ segregated into either two or four phenotypes; and genotypic and phenotypic segregations in the F₂ families confirmed mono- or digenic inheritance patterns. The mutant trait is thus governed by a single recessive gene that is tentatively designated as d_1 . The gene has a pleiotropic effect on panicle nature and is influenced by modifiers. It is nonallelic and not linked to the sd_1 gene of Dee-geo-woo-gen and I-geo-tze nor the semidwarfing gene of Cheng-chu-ai-11.

The induced mutation method in developing alternative sources of reduced plant height is of considerable interest (18). During the course of induced mutation studies on rice carried out at the Bhabha Atomic Research Centre, several mutants of academic and economic importance have been isolated. One of them is TR-5, a short statured mutant induced by fast neutron treatment of the saline resistant variety SR-26-B. The mutant shows 45% reduction in plant height due to shortening of all internodes (9). It has a distinct morphology and exhibits concurrent changes in a number of other characters: The plant habit is erect, stiff, and nonlodging; the leaves are dark green, upright, rather short, broad, thick, and rigid; the panicles are short, very compact, and erect even after ripening; and the grains are short, slender, and numerous (Fig. 1). The mutant shows well condensed short chromosomes at the pachytene stage (9, 10). TR-5 closely resembles Nadula dwarf of IRRI collection. At Trombay it showed a yield potential up to 5 t/ha, but at other locations its yield did not exceed the parent variety or check (12) or was only 77% that of IR8(1). Nevertheless, the mutant has been used in crossing with IR8 to impart long slender grain, translucent kernel, and absence of white belly characters (14). In order to understand the inheritance of the dwarfing character complex of TR-5 and its allelic relationship with other sources of dwarfing genes in rice, a series of crosses

was made. The results obtained in the F_1 , F_2 and F_3 of four such crosses are reported in this paper.

MATERIALS AND METHODS

Three dwarf reference stocks — Dee-geo-woo-pen (= Di-jiao-wu-jian = Ti-chio-wu-chien), I-geo-tze (= Ai-chiao-tze), and Cheng-chu-ai-11 — and a tall stock K8 obtained from IRRI were crossed with TR-5 in the dry season of 1981–82. Twenty-one F_1 plants together with the respective parents were grown in the 1982 wet season, and plant height and other characters were studied. In the F_2 a limited seedling population was transplanted. Plants were observed periodically at various stages of growth. Phenotypic classification was made on the basis of height, typical appearance, and panicle shape of the segregants. At maturity, plant height from ground level up to the tip of the panicle (or up to the junctura of the flag leaf in double dwarf plants showing nonexsertion of panicles) was recorded on surviving plants in one of two of the progenies of each cross. Plants in each phenotypic category in such progenies were selected. F_3 studies were completed in the 1984 wet season. Five to six week old seedlings were grouped according to their respective height and type, and counts were taken. Most of the clearly identifiable tall in the majority of families were, however, discarded before transplanting. Plants in the field were again observed periodically to confirm the phenotypes. Allelic relationships among TR-5 dwarf and dwarfs of other stocks were determined on the basis of F_1 and F_2 population behavior, while the mode of inheritance was determined through c^2 analyses of F_2 and F_3 segregation data.

RESULTS

All the hybrid plants in the F_1 were more vigorous in growth than their respective parents. While ear emergence in the three dwarf reference stocks was within 77–88 days, their hybrids with TR-5 showed panicle emergence between 103 and 110 days; the duration of ear emergence in K8 was 111 days, while its hybrid with TR-5 showed 116 days, which is similar to that of TR-5. At maturity all the hybrids were far taller with longer panicles, more productive tillers, and higher yields than their respective parents. Plant height in TR-5 was 88 cm, while it was 106.5 cm in Dee-geo-woo-pen, 98.1 cm in I-geo-tze, 91.6 cm in Cheng-chu-ai-11,

and 135.6 cm in K8. The respective F_1 's were 171.0, 161.5, 160.9, and 168.2 cm. In the F_2 generation, the three crosses involving dwarf reference stocks, viz., Dee-geo-woo-gen, I-geo-tze, and Cheng-chu-ai-11, showed, particularly at the post-flowering stages, four distinct types: tall, other parental dwarf with normal panicle, TR-5 dwarf with cigar-shaped panicle, and double dwarf with cigar-shaped panicle. In the K8/TR-5 cross, only tall and Tr-5-types segregated. Plant height in the F_2 of all the crosses showed transgressive segregation ranging between 21.5 and 214.3 cm (Fig. 2). The height of TR-5 and the other dwarfs overlapped to some extent. Some weak tall plants were similar in height to dwarf types. Plants with less than 60 cm height were classified as double dwarfs. Among them those with less than 40 cm showed no emergence or only partial exsertion of panicles. Unintentional bias for transplanting tall seedlings in F_2 , segregation of plants for the duration to ear emergence, leaf width recombinants among dwarfs, and occurrence of gaps all complicated the studies to some extent. Yet, because of repeated observations at different growth stages, it was possible to assign almost all the segregants to their respective classes. The F_2 data on phenotypic segregants are presented in Table 1. While the data fit fairly well to a 9:3:3:1 ratio in the case of TR-5/Cheng-chu-ai-11, in two other crosses, viz., Dee-geo-woo-gen/TR-5 and I-geo-tze/TR-5, the c^2 values for digenic ratios are rather high. In the case of K8/TR-5 the data show good agreement with the monogenic ratio.

F_3 data on genotypic segregation are presented in Table 2 and data on phenotypic segregation in the segregating F_2 families in Table 3. All the double dwarf selections in crosses among dwarfs and the single dwarfs in the cross K8/TR5 bred true to type, except occasional natural crosses in a few families. Genotypic segregation ratios agreed with the expected ratios. Similarly, mono- and digenic segregation ratios in each of the segregating F_2 families were confirmed. The c^2 for linkage was not significant.

DISCUSSION

In the present studies, single and double dwarf plants of TR-5 type varied in height from 21.5 to 126 cm. They probably belong to the Daikoku group of Japanese dwarfs, all of which have been reported to possess similar plant stature but to have quantitative differences in height (20–77 cm) and other characters (13). The rice mutant investigated by Parnell et al (15), the Caloro mutant described by Jones (7, 8), T 436



Fig. 1. TR-5 plant (foreground) and its cigar-shaped panicle (inset).

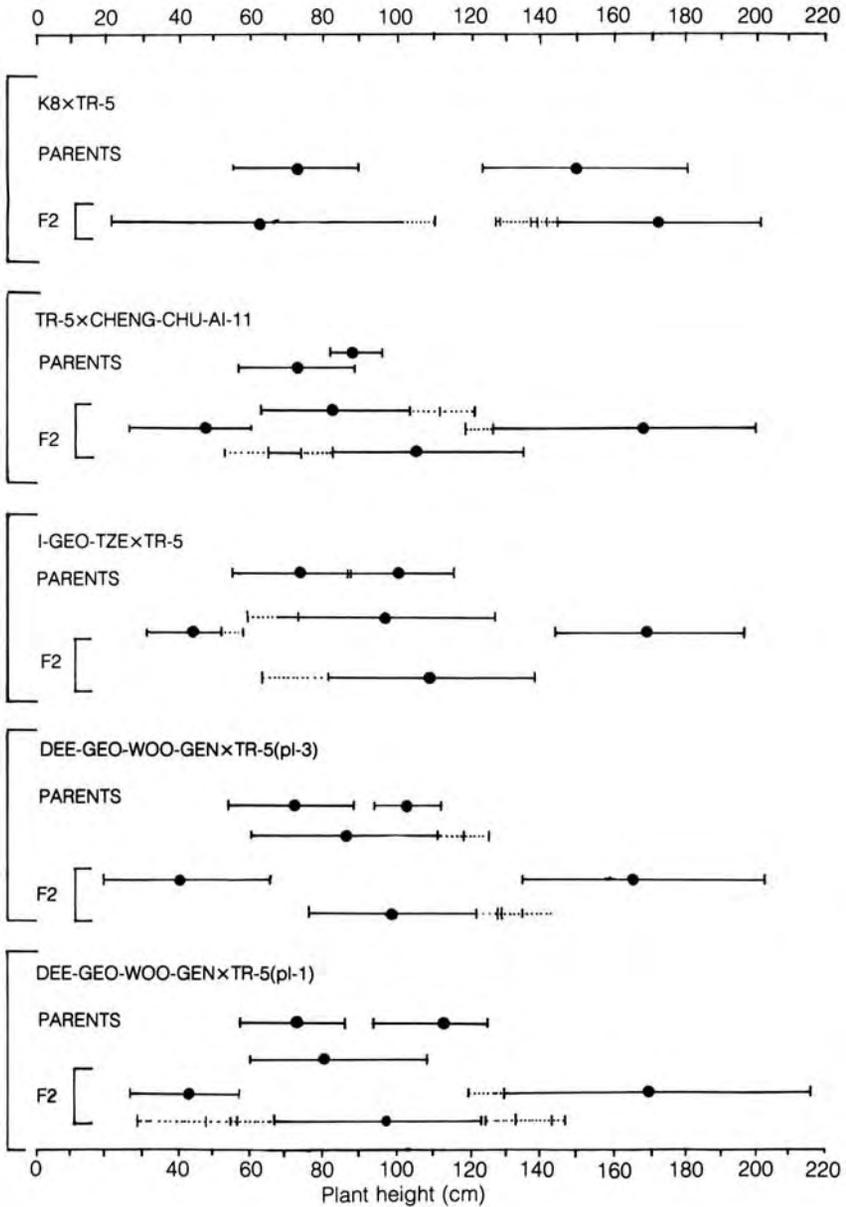


Fig. 2. Mean and range of plant height in parents and F₂ progeny. ● = mean, /—/ = continuous range, |...| = discontinuous range. Parents = TR-5 (left) and other parent (right). F₂ progeny = (left to right) two phenotypes: TR-5 and tall; four phenotypes: double dwarf, TR-5 dwarf, other dwarf, and tall.

Table 1. F₂ segregation in crosses with TR-5, 1983 wet season.

F ₁ plant no.	F ₂ segregant type				Total	c ² (9:3:3:1) or (3:1)	P
	Tall	Other dwarf	TR-5 dwarf	Double dwarf			
TR-5/Cheng-chu-ai-11							
5	334	104	116	38	592	0.94	>0.80
6	348	113	106	23	590	6.25	>0.10
7	359	91	120	28	598	8.41	>0.02
Subtotal	1041	308	342	89	1780	8.22	>0.02
Dee-geo-woo-gen/TR-5							
1	430	107	93	31	661	21.72	<0.001
3	387	69	114	29	599	26.09	<0.001
4	417	72	105	5	599	62.08	<0.001
Subtotal	1234	240	312	65	1859	89.31	<0.001
I-geo-tze/TR-5							
1	350	119	107	21	597	8.39	>0.02
3	341	100	133	19	593	14.37	>0.001
5	369	108	100	19	596	13.70	>0.001
Subtotal	1060	327	340	59	1786	28.13	<0.001
K8/TR-5							
	1273	—	415	—	1688	0.16	>0.50

investigated by Seetharaman and Srivastava (16), and Jaribala Mutant described by Siddiq et al (17), all seem to correspond to the TR-5 type. Mutants isolated by Khadiikar (11) in the variety Dodka and by Misra in Tainan-3 (16) seem to be similar to the double dwarf of the TR-5 type.

A monogenic inheritance of the trait, as obtained here in the cross K8/TR-5 was reported by several workers from Japan (13), India (15, 16, 17), and America (7, 8). Nagai (13) further cited references of Japanese workers who found the double recessive nature of the Daikoku dwarf or who observed digenic segregations in Bonsai/Daikoku and Ebisu/Daikoku crosses. Recently Siddiq et al (17) also reported a dihybrid mode of inheritance in the cross Jaribala Mutant/CRM-8-5711 (Basmathi-370 mutant). The patterns of segregation in the F₂ and F₃ of the three crosses among dwarfs observed in the present studies indicate

Table 2. Genotypic segregation in F₂ families of crosses with TR-5.

Genotypic class ^a	Expected ratio	TR-5/ Cheng-chu-ai-11			Dee-geo-woo-gen/ TR-5			I-geo-tze/IR 5			K8/TR-5		
		Obs. freq.	χ^2	P>	Obs. freq.	χ^2	P>	Obs. freq.	χ^2	P>	Obs. freq.	χ^2	P>
Tall/homozygote	1	5	0.17		4	0.67		10	2.67		13	1.39	
Tall/heterozygote I	2	9	0.75		11	0.08		6	3.00		–	–	
Tall/heterozygote II	2	17	2.08		20	5.33		16	1.33		41	0.69	
Tall/heterozygote I & II	4	23	0.04		19	1.04		22	0.17		–	–	
Subtotal	9	54	3.04	0.30	54	7.12	0.05	54	7.17	0.05	54	2.08	0.10
Other dwarf homozygote	1	4	0.51		8	0.67		5	0.17		8 ^b	0.02	
Other dwarf heterozygote	2	13	0.26		10	0.33		13	0.08		11 ^b	0.01	
Subtotal		18	0.77	0.30	18	1.00	0.30	18	0.25	0.50	17	0.03	0.80
TR-5 dwarf homozygote	1	5	0.17		9	1.50		10	2.17		18 ^c (10)	–	
TR-5 dwarf heterozygote	2	13	0.08		9	0.75		9	1.08		Nil	–	
Subtotal	3	18	0.25	0.50	18	2.25	0.10	19	3.25	0.05	–	–	–
Double dwarf	all	5	–	–	5	–	–	10	–	–	–	–	–

^aI = other dwarf, II = TR-5 dwarf. ^bK8 height selections. ^cDouble dwarf height selections.

Table 3. Phenotypic segregation in F₂ families of crosses with TR-5.^a

Genotypic class ^a	Seg. F ₂ families (no.)	Observed frequency					χ^2 (9:3:3:1) or (3:1)	Linkage		
		Tall	Other dwarf	TR-5 dwarf	Double dwarf	Total		P >	χ^2	P >
<i>TR-5/Cheng-chua-ai-11</i>										
Tall heterozygote I & II	5	405	134	124	48	711	1.003	0.80	0.62	0.30
<i>Dee-geo-woo-gen/TR-5</i>										
Tall heterozygote I & II	16	1984	631	597	227	3439	5.788	0.10	3.80	0.05
<i>I-geo-tze/TR-5</i>										
Tall heterozygote I & II	18	2219	708	668	243	3838	5.607	0.10	2.24	0.10

^aSegregation in all other tall and dwarf families heterozygous for one or the other dwarf type also showed a good fit to the respective monogenic ratios.

^bI = other dwarf, II = TR-5 dwarf.

that TR-5 has a pair of recessive genes that are different from those in the other dwarf parents.

In the single and the double dwarf plants of the TR-5 type, the character syndrome is inherited together. This could be attributed to the pleiotropic effect of the gene governing the trait. The characteristic cigar-shaped panicle results from an extreme reduction not only in the length of the main axis but also in the rachis and rachilae of branches, which additionally form acute angles at their origin. Segregants in the present studies occasionally showed a tendency to open out slightly, particularly at the later stages of maturity. This phenomenon could be explained on the basis of incomplete expressivity of the gene or a modifier effect in the residual genetic milieu (16).

The complementary behavior of TR-5 for tall height in the F_1 of the crosses studied, the transgressive height behavior in the F_2 populations, and the dihybrid mode of segregation in the F_2 and F_3 with varying probabilities, all suggest that the dwarfing genes of TR-5 are nonallelic and not linked to the dwarfing genes of Dee-geo-woo-gen, I-geo-tze, and Cheng-chu-ai-11 dwarfs. Epistatic interaction of modifiers is involved in altering the heights of the F_2 segregants as has also been reported by Chang et al (4), Aquino and Jennings (2), and Singh et al (18). Dee-geo-woo-gen semidwarf is assumed to have originated as a spontaneous mutation in a Chinese tall strain, Woo-gen (3). The same single partially recessive gene (sd_1) was found responsible for short height in both Dee-geo-woo-gen and I-geo-tze (5, 6, 18). The semidwarfing gene in Cheng-chu-ai-11 is believed to be different from sd_1 . Dwarfing genes governing the TR-5 type character complex in the Daikoku group have been assigned the symbols d_1 , d_8 , and d_7 (13). Seetharaman and Srivastava (16) suggested that the recessive gene in the homozygous condition acts as a "suppressor" for height expression and accordingly assigned the gene symbol $su-T$ for the trait in T436. The dwarfing gene in Jaribala Mutant, with a similar phenotype, was designated sd_1 by Siddiq et al (17). The TR-5 dwarfing gene is now tentatively designated d_t . This gene could be useful in finding the linkage relationships between the marker genes and useful agronomic characters.

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DISCUSSION

SESSION 5: GENETICS OF MORPHOLOGICAL TRAITS

Q – Kinoshita: In your study, the hybrids between *sd-1* and *eui* showed semi-dwarfness. Would you please explain the gene scheme? Was the *sd-1* gene common to both parents?

A – Rutger: Yes, the *sd-1* gene was present in both parents. In the studies (see Table 1) we found that *eui* is inherited independently of the *sd-1* locus.

Q – Morishima: Among a large number of dwarf mutants, almost all agronomically useful mutants of different origins were found to be allelic. Could you give your comments about this?

A – Rutger: There appears to be something very special about the *sd₁* locus. To date the nonallelic (to *sd₁*) semidwarfs have not shown a yield advantage over lines with the *sd₁* locus, although some approach it. We have speculated that there might be some association between the productivity of the *sd₁* locus and endogenous GA₁, but to date we have not been able to confirm this hypothesis.

Q – Virmani: We have observed at IRRI that “*eui*” gene, when transferred to high tillering varieties such as IR36 or IR50, does not express in all the tillers of a plant. Do you think the expression of this trait is related to the tillering ability of a genotype?

A – Rutger: In our environments, we deal with broadcast seeded rice, in which only one or two panicles are produced per plant. Since there are usually 90–180 plants/m², yields can still be very high. Thus, we have not evaluated the expression of the *eui* gene under widely spaced conditions.

Q – Narahari: Chlorina mutants generally show yellowish panicles in the initial stages. How different is your yellow panicle mutant from the Chlorina types?

A – Rutger: I have not compared it with Chlorina mutants. However, it is my understanding that Chlorina mutants have reduced productivity, which is not the case with the yellow panicle mutant, and it was for this reason that we included the latter among “useful” mutants.

Q – Abifarin: Do the endosperm mutants have any effect on protein and lysine content? Have you analyzed the endosperm mutants for these traits?

A – Rutger: Their main effect is on physical appearance, with some reduction in amylose content. We have not analyzed the protein or lysine content of the opaque mutants.

Q – Lin: Is apomixis commonly found in rice? Is it found in wild relatives of rice? Is apomixis in rice influenced by environmental conditions? Do you have a specific procedure to screen for apomictic mutants of rice?

A – Rutger: To my knowledge, apomixis has not yet been reported in either rice or its wild relatives. Judging from reports that apomixis is influenced by environment in other grasses, I assume it also would be in rice if we could find it. Initially, we are using phenotypic screening procedures, i.e., looking for the appearance of maternal plants among or instead of F_1 's in crosses, and for abnormal F_1 segregations characterized by the appearance of maternal (F_1) plants. Regarding screening in wild relatives, we are using the cleared pistil technique described by Young et al (1979).

Q – HilleRisLambers: Is the opaque kernel opaque throughout or only in the outer layers?

A – Rutger: It appears to be opaque throughout, similar to waxy mutants.

Q – Brar: Were any of the opaque mutants associated with high protein/high lysine characteristics? If not, do you have any information on high lysine mutants in rice similar to those of maize and barley?

A – Rutger: We have not made such determinations. I think high lysine mutants are unlikely to occur in rice, because rice already has a very low prolamine fraction, 3-4% as I recall. The prolamine fraction is a lysine-poor protein. In maize and barley, the high lysine mutants resulted when a large prolamine fraction (35-50%) was reduced to low levels (5-10%). The reduction in prolamine was accompanied by a corresponding increase in lysine-rich fractions, such as the glutelins.

Q – Kumar: What was the range of amylose content in the opaque mutants?

A – Rutger: Apparently 12-14%.

Q – Badino: Have you found any correlation between shattering and the semidwarf genes you induced by radiation?

A – Rutger: No. We have observed shattering in progenies of crosses between the Dgwg source of semidwarfism and japonicas, but not in crosses between our induced semidwarf japonica mutants and other japonicas.

Q – Kumar: The dwarfing genes in rice in Japan have been designated from d_1 to more than d_{50} . Have the allelic relationships among these been worked out?

A – Futsuhara: As to the 50 kinds of dwarf genes that were proposed by the Japanese Committee on Gene Nomenclature and Linkage Groups of Rice, all except d_{36} - d_{41} and d_{43} - d_{48} were identified by crossing and morphological experiments.

Q – Kumar: How many dwarfing genes have been used commercially in Japanese varieties?

A – Kikuchi: Several semidwarf varieties, of spontaneous origin or naturally induced with mutagenic treatments have been used as the parents for crossbreeding in Japan. However, gene analysis has been carried out only in the varieties that I reported today and in a few other lines such as Fukei 71 and Hokiyuku 100.

Q – Morishima: Among a number of dwarf mutants, agronomically useful dwarfs of different origin were found to be controlled by the same gene. Could you comment on this?

A – Kikuchi: It seems to me that the *Dgwg* dwarfing locus (*Sd-1*) is liable to mutation and to induction of the semidwarfing genes that have the least deleterious effects on the other agronomic traits, compared with other dwarfing loci. Mainly due to these characteristics the mutants at the *Sd-1* locus might have been selected consciously or unconsciously.

Q – Bollich: Other things being equal, the larger the grain, the higher the yield. However, my experience is that very large grains are associated with a reduced number of spikelets per panicle. Have you noted the same relationship between very large grain size and spikelet number per panicle?

A – Xiong: This is a serious problem in rice breeding. In general, there was a negative correlation between these two characters. But, if you increase the population size, some promising plants with large grains and with normal number of spikelets may become available. However, we have not succeeded in this objective up to now.

Q – Chaudhary: Since you concluded that there are no genes for kernel notching, should they not be assigned to linkage maps?

A – Takeda: Some *nk* genes have been assigned to linkage map, but most of them must be genes controlling grain size. Therefore, they should not be assigned to the linkage map as *nk* genes, but as grain size genes.

Q – HilleRisLambers: You presented kernel notching as an imbalance in development. Do you see it as a source/sink imbalance, and if so, have you any indication that notching can be reduced by shading or leaf pruning?

A – Takeda: The imbalance between the lengths of caryopsis and floral glumes is not primarily due to sink source imbalance, because they are not remarkably affected by treatments such as spikelet thinning or leaf blade cutting.

Q – Oka: How did you identify genes for floral length and caryopsis length? Did you use isogenic lines? Are these characters quantitative?

A – Takeda: Genotypic correlation analysis gives some information on the relationship between them. In some cases where major genes control the grain size, clear segregation may occur independently for floral glume length or caryopsis length.

Q – Gupta: How do you define a pseudocharacter, which you have referred to in relation to notching in rice? There are many characters where genes of plants (or even of plants + parasites) interact to produce a phenotype. Would all these be pseudocharacters by your definition?

A – Takeda: A pseudocharacter is defined as a character that is not controlled directly by any gene but is conditioned by the interaction among more than two distinguishable characters controlled by respective genes. Contrarily to this, disease resistance is a real genetic character that is controlled by the resistant gene, even though it is detectable only in the presence of parasites.

C – Wang, X.M.: I think the report of Dr. Takeda is very interesting in the sense that it shows that almost any of the agronomic characters of the rice plant can be studied from the standpoint of developmental genetics.

C – Pavithran: Notched kernel is formed due to the physical interactions of glumes and kernel during kernel development, and not by genetic factors. As such it is a pseudotype or pseudocharacter. The gene *nk* used for notching in the linkage map should be understood to be responsible for kernel length in relation to lemma and palea length.

GENETICS OF PHYSIOLOGICAL TRAITS

SESSION 6

GENE LOCI AND ALLELES CONTROLLING THE DURATION OF BASIC VEGETATIVE GROWTH OF RICE

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The use of isogenic lines is necessary for precise and subtle analysis of genes controlling heading time, since it is a quantitative trait. By using isogenic lines of Taichung 65 obtained by recurrent backcrossing, three independent gene loci with different alleles that control the basic vegetative period were identified, viz., *Ef-1* (abbreviated *E*), its dominant alleles advancing heading time for about 10 days; *m-Ef-1* (abbr. *m*), its recessive alleles emphasizing the effect of *E* alleles; and *lf-1* (abbr. *lf*), its recessive allele retarding heading for about 20 days. At the *E* locus, several isoalleles were obtained from two donor parents and radiation-induced mutants. The magnitude of *E-m* epistasis differed according to the *E* alleles. New *E* alleles were also obtained from offtypes occurring in certain crosses, and a compound structure of the locus is suggested. Furthermore, various unusual segregation patterns were observed in hybrids segregating for the *m* and *E* alleles. The data suggest that some transposable elements could be incorporated in these loci.

The genetic control of heading time in rice involves two different categories — photoperiod response and basic vegetative period. The author has been engaged mainly in the study of genes controlling basic vegetative period in photoperiod-insensitive materials. The data so far accumulated are bulky, with some evidencing strange behavior not explainable by Mendelian segregation. In this paper, the results of genetic analysis are briefly outlined, and the data for strange segregation patterns are presented for discussion.

MATERIALS AND METHODS

All experimental lines dealt with in this paper have the genetic background of Taichung 65 (T65), a famous Ponlai or japonica variety of Taiwan nearly insensitive to photoperiod. The experiments were con-

ducted at Taichung (24°N) twice a year: in the winter or the first-crop season (seeded in January) and in the summer or the second-crop season (seeded in July).

Genes for early heading were introduced into T65 from two donors—early maturing cultivars from northern China and Japan—by recurrent backcrossing. Those genes and a late heading gene were also obtained from radiation-induced mutants of T65. Isogenic lines of T65 carrying these different genes were the basic material of the present study (Table 1).

For establishing an isogenic line, in principle, backcrosses were repeated seven times when the gene donor was a cultivar. It is expected that the larger the number of backcrosses, the shorter will be the chromosome segment accompanied by a target locus ($1/n$ of an arm length after n backcrosses on the average) (3). The maximum number of backcrosses carried out was 20. The repetition of backcrosses is called purification in this paper.

GENE LOCI AND ALLELES IDENTIFIED

Three independent loci—*Ef-1* (abbreviated *E*), *m-Ef-1* (abbr. *m*), and *lf-1* (abbr. *lf*)—were worked out. The *E* gene (dominant) moves the time of heading about 10 days ahead of that of T65. The *m* gene (recessive) is a modifier that intensifies the effect of *E*. The *lf* gene (recessive) delays the time of heading for about 20 days.

The chromosomes carrying these three loci are, when numbered according to Khush et al (4), as follows: *Ef-1* is most probably located on chromosome 10; according to S. Sato (personal communication), *Ef-1* is at the same locus as *Ef-2*, which is on the same chromosome. *m-Ef-1* is located on chromosome 7 as it is linked with *Rc* (red pericarp; a 23% recombination) (13). *lf-1* is probably located on chromosome 3, as it appears to be linked with *d-4* (one of the genes expressing tillering dwarf) (unpublished data).

Ef-1

At this locus, at least one recessive and five dominant alleles have been detected, viz., *e* (carried by T65), *E^a* and *E^{al}* (from donor A), *E^b* (from donor B), *E^g* (from an *g*-ray mutant of T65). The allelism among them was confirmed by crossing experiments (8). The variance in heading time of an F_2 between an *E* line and T65 was in many cases smaller than the value expected from the differences among P_1 , P_2 , and F_1 plus the environmental variance, although the F_2 heading dates were distributed over the parental range (11).

The differences in heading advancing effect among the five *E* alleles, being a few days, were in many cases statistically significant when enough replications were made (Table 1) (11). Also, the F₂ populations between lines with different *E* alleles generated an amount of genetic variance making the F₁ variance significantly larger than the parental environmental variance (11). Therefore, the *E* alleles may be considered as isoalleles as defined by Allard (1).

With repetition of backcrosses—purification of the locus—the heading advancing effect of *E^a* was reduced while that of *E^b* was increased significantly (Table 1) (10). Allele *E^{al}*, similar to *E^a*, was obtained in a sib-line of (7) *E^a* (backcrossed 7 times; 8). Its heading advancing effect in line (7) *E^{al}* was significantly greater than that of *E^a* in (7) *E^a*, but the effect was reduced with purification in the same manner as in *E^a*.

The *E* alleles appeared to be arranged in decreasing order of their effects as (7) *E^{al}* > (7) *E^a* ≥ (0) *E^x* > (0) *E^g* > (7) *E^b* when isolated first, but the order changed to (13) *E^{al}* ≥ (5) *E^x* ≥ (20) *E^b* ≥ (20) *E^a* ≥ *E^y* after purifica-

Table 1. Isogenic lines of T65 with single alleles, used for crosses.

Line	Genotype ^a	Mean heading time ^b (difference from T65)		Source
		Summer	Winter	
T65	<i>e m⁺ lf⁺</i>	0 (124.9)	0 (88.6)	A pure line of Taichung 65 (recurrent parent)
Donor A	(<i>E^a m^a lf⁺</i>)	-40.5	-34.0	Tatung-tsailai, north China
Donor B	(<i>E^b m^b lf⁺</i>)	-21.4	-23.0	Bozu 5, northern Japan
(7) <i>E^a</i>	<i>E^a m⁺ lf⁺</i>	-13.7a	-15.6a	T65/NIT65 ⁷
(20) <i>E^a</i>	<i>E^a m⁺ lf⁺</i>	-13.2ab	-13.6 c	T65/A//T65 ²⁰
(7) <i>E^{al}</i>	<i>E^{al} m⁺ lf⁺</i>	-15.8	-16.7	A sib line of (7) <i>E^a</i>
(13) <i>E^{al}</i>	<i>E^{al} m⁺ lf⁺</i>	-14.0	-15.7	(7) <i>E^{al}</i> /T65 ⁶
(7) <i>E^b</i>	<i>E^b m⁺ lf⁺</i>	-11.1 c	-13.3 c	T65/B//T65 ⁷
(20) <i>E^b</i>	<i>E^b m⁺ lf⁺</i>	-13.2ab	-14.1 bc	T65/B//T65 ²⁰
(0) <i>E^x</i>	<i>E^x m⁺ lf⁺</i>	-14.3a	-13.8 c	T65 M ₃ , X-rayed
(5) <i>E^x</i>	<i>E^x m⁺ lf⁺</i>	-13.7a	-15.4ab	T65// <i>E^x</i> /T65 ⁵
(0) <i>E^g</i>	<i>E^y m⁺ lf⁺</i>	-12.4 b	-14.6abc	T65 M ₃ g-rayed
(5) <i>E^g</i>	<i>E^y m⁺ lf⁺</i>	-12.5 b	-13.2 c	T65// <i>E^y</i> /T65 ⁵
(7) <i>E^{ab}</i>	<i>E^{ab} m⁺ lf⁺</i>	-13.7	-15.3	An offtype, (7) <i>e^a</i> /(15) <i>e^b</i>
(7) <i>m^a</i>	<i>e m^a lf⁺</i>	- 4.3	1.6	T65/A//T65 ⁷
(7) <i>m^b</i>	<i>e m^b lf⁺</i>	- 5.4	1.8	T65/B//T65 ⁷
(0)lf	<i>e m⁺ lf</i>	18.5	25.2	T65 M ₃ , X-rayed

^a*E* = *Ef*-1, *e* = *ef*-1, *m* = *m-Ef*-1. *lf* = *lf*-1.

^bValues followed by a common letter differ at the 5% level of significance. Values followed by a letter and those for T65 are means for 5 years, 1974-1978; other values are from different years and are adjusted to be comparable with the means for 5 years. The values for T65 in parentheses are days from seeding to heading.

tion. Probably some genes with minor effect, linked with the *E* alleles and reinforcing or mitigating their effects, would have been replaced by other genes during backcrossing.

m-Ef-1

At this locus a recessive (*m*) and a dominant (m^+) allele are known. The recessive alleles were obtained from both donors A and B and were symbolized as m^a and m^b , respectively (17). Since they showed no significant differences in effect, they were later considered identical (11). The m^+ allele is carried by T65. It is incompletely dominant over *m*, while the dominance relation differs to some extent according to growing season (10). Generally, *m* may be treated as a recessive allele.

The main effect of *m* is to emphasize the heading advancing effect of the *E* alleles. The heading time of plants with genotype *E m* is 10–20 days earlier than that of *E m*⁺ plants. This difference is called the *E-m* epistasis in this paper. The heading time of *e m* plants is a few days earlier than that of *e m*⁺ plants in the winter season, but there is no difference in the summer season; *m* is almost silent if not combined with *E*.

The *E-m* epistasis differs according to *E* alleles. When the *E* alleles were purified, the *E-m* epistasis tended to be weakened with E^a and E^x , while it was intensified with E^b and E^y (Table 2). The *E* alleles were arranged in decreasing order of *E-m* epistasis as (7) $E^a > (0)E^x \geq (7)E^b \geq (0)E^y$ in an early generation of backcrosses, and after purification as (20) $E^b \geq (20)E^a \geq (5)E^x \geq (5)E^x$. The changing pattern with purification was similar to that in the effect of *E* alleles themselves (combined with m^+).

Table 2. Magnitude of *E-m* epistasis compared among different *E* alleles.

Line	Mean heading time, shown by difference from the mean for corresponding <i>E</i> line ^a		
	Winter	Summer	Average
(7) $E^a m$	-16.4a	-17.2a	-16.8a
(20) $E^a m$	-12.4ab	-16.6a	-14.5ab
(7) $E^b m$	-9.5 bc	-11.0 bc	-10.3 cd
(20) $E^b m$	-16.1a	-16.1a	-16.1a
(0) $E^y m$	-8.2 c	-8.9 c	-8.6 d
(5) $E^y m$	-10.0 bc	-16.0a	-13.0abc
(0) $E^x m$	-7.8 c	-13.9 bc	-10.9 bc
(5) $E^x m$	-8.4 bc	-9.5 c	-9.0 d

^a Values followed by a common letter differ at the 5% level of significance. Mean values for 1974–1978 are given.

When lines (20) $E^b m^+$ and (7) $E^b m$ were crossed, the F_2 produced transgressive segregants heading about 1 week earlier than (7) $E^b m$ with a $\frac{3}{16}$ frequency, and the F_3 lines from the transgressive segregants were either fixed as the same type or segregated into the (20) $E^b m$ or (7) $E^b m$ types (14). This also indicated that the m allele responds to the E alleles differently.

lf-1

A recessive allele *lf* was obtained from a late heading mutant of T65 induced with 40KR X-rays (unpublished data). It delayed heading time for about 20 days but had no other deleterious effects. Its dominant counterpart *lf* is carried by T65. When an *lf* line (with *lf e m*⁺) was crossed with (7) E^a (with *lf*⁺ $E^a m^+$), the F_1 showed an early heading date as with the E line, and the F_2 segregated into 137:113:12 E type to T65 type to late type, giving a good fit to a 9:6:1 ratio (unpublished data). This suggests that the phenotype of E/E *lf/lf* and E/e *lf/lf* plants is similar to that of e/e *lf*⁺/*lf*⁺ (T65) plants. When a line with *lf E*⁺ m^+ derived from the above cross was crossed with line (20) $E^a m$ (with *lf*⁺ $E^a m$), the F_2 segregated into 4:9:3 $E-m$ to $E-m^+$ to *lfE* (T65) types. This indicates that the *lf* locus is independent of the E and m loci, and its effect is concealed by (hypostatic to) the effect of m when E is present.

The *lf* line (*lf e m*⁺) appeared to be sensitive to daylength to some extent, since its heading was 36 days later than that of T65 (*lf*⁺ $e m^+$) when seeded in May, and it reached a growth stage capable of floral initiation under the long days in June–July. T65 shows a slight response (delay of a few days) to the long days. Probably *lf* interacts with a gene for the slight response of T65 to reinforce its action.

OCCURRENCE OF OFFTYPES

The F_2 population between an E line and T65 segregates into 3:1 E to e types but has a smaller genetic variance in heading time than expected. From such F_2 populations, certain e type plants whose heading dates were slightly earlier than that of T65 were selected, and their progeny lines, designated e lines, were established. For instance, line (7) e^a was obtained as a sib of (7) E^a , and (20) e^a as a sib of (20) E^a . When these T65-like lines were intercrossed, early heading offtypes like an E plant were found in the F_2 with low frequency, although care was taken to prevent contamination (Table 3) (10). Their F_3 lines segregated in a complicated manner, some segregants being very early (like $E-m$ plants)

Table 3. Occurrence of early heading offtypes in the F_2 between two T65-like sib-lines obtained from ee/EE crosses, and their heading behavior in F_3 progeny.

Cross	F_2 offtype frequency	Intralocus recombination (%)	F_3 segregation for heading types (phenotype)					New E allele fixed
			Em	E	T65	Late	Total	
1. $(7)e^a/(20)e^a$	1/255	0.39		38	32	17	87	
2. $(7)e^a/(15)e^b$	7/2455	0.27		160	11		171	E^{ab-1}
			50	13			63	E^{ab-2}
			32	68			100	E^{ab-3}
				38	18	8	64	E^{ab-4}
				173	2		175	
				178	3		181	
				76	24		100	
3. $(7)e^a/(20)e^b$	2/255	0.78	14	57			71	
			19	53			72	
4. $(7)e^b/(15)e^b$	1/339	0.30		61	18		79	E^{bb-1}
5. $(7)e^b/(5)e^x$	2/337	0.60		161	35	1	197	E^{bx-1}
				31	35	4	70	E^{bx-2}
6. $(15)e^b/(5)e^x$	1/205	0.49		93	56	18	167	E^{bx-3}

Table 4. Unusual segregation patterns for heading time observed in crosses between different $E-m$ lines.

Cross	Generation	Phenotypic segregation			Offtype frequency (%)
		Em -type	E -type	T65-type	
$(7)E^{a_m a}/(7)E^{a_m a-2}$	F_2	274	6		2.1
	F_3 , 6 lines pooled	17	98		14.8
$(7)E^{a_m a}/(7)E^{a_m b}$	F_2	254	3		1.2
	F_3 , 3 lines pooled	60	102		37.0
$(7)E^{a_m b}/(20)E^{a_m b-1}$	F_2	342	8		2.3
	F_3 , 5 lines pooled	18	60	3	25.9
	F_3 , 2 lines pooled	93	4		95.9
$(7)E^{a_m+}/(7)E^{a_m a-1}$	F_2	28	121		0
	F_3 , 22 lines from Em	Fixed			
	F_3 , 6 lines from Em	80	10		11.1
	F_4 , 25 lines from Em	Fixed			
	F_4 , 5 lines from E	21	60		25.9

and others being much later (like *lf* plants). Yet, fixed *E*-like lines were obtained in the F_4 , which appeared to carry different *E* alleles symbolized as E^{ab-1} , E^{bb-1} , etc. Their heading dates differed among themselves slightly, but sometimes significantly (10, 11).

When the *E*-like lines were crossed with *E* lines, some of the F_2 populations released variances significantly larger than the parental environmental variance. When they were crossed with an *m* line) with *e* *m*), the *E*-*m* epistasis occurred in varying degrees, and the *E*-*m* plants were generally smaller than those due to true *E* alleles (11).

The very early segregants (like *E*-*m* plants) also produced fixed progeny lines when selfed. When two of them (designated $E^{ab}m-1$ and $E^{ab}m-2$) were test crossed with line (7) E^am^a , the results suggested that they could have an *m* allele that was not involved in the original crosses. Experiments with the *lf*-type segregants will be mentioned later.

These observations strongly suggest that the *E* locus has a compound structure and that different isoalleles are produced through intralocus recombination, particularly by unequal crossing over. The recombination values between supposed subsites estimated from the frequency of F_2 offtypes ranged from 0.27% to 0.78%. Probably, when different subsites or domains of the locus are recombined in *cis*, a new *E* allele will be born. This process might stimulate further structural changes of the locus to bring about very early or late heading offtypes in the selfed progeny of offtypes until homozygosity is attained. It may also be supposed that some changes in the *E* locus provoke mutations at other loci.

UNUSUAL SEGREGATION PATTERNS

Crosses between two similar lines both having an *Em* genotype occasionally produced F_2 plants like Em^+ (*E*-type) with a frequency that ranged from 1.2% to 2.3% (Table 4). Then, their F_3 progeny showed strange segregation patterns. Several such cases were observed. For instance, the F_2 between line (7) E^am^a and (7) E^am^a-2 produced 6 *E*-type plants out of 280 observed. Their F_3 lines each segregated into early (Em^+) and very early (*E m*) types, the ratio being 17:98 when pooled. Of three F_4 lines derived from the very early F_3 plants, one was fixed as *E m*, while the other two segregated into 1:3 *E m* to Em^+ types. Also, of 16 F_4 lines from the Em^+ type F_3 plants, 6 were fixed as Em^+ while 10 showed a 1:3 *E m* to Em^+ segregation, suggesting that the parental F_3 plants had been heterozygous for the *m* locus, although no m^+ allele was involved in the original cross (Table 4).

A similar case was also found in the cross between lines (7) $E^a m^a$ and (7) $E^a m^b$, where alleles m^a and m^b did not differ in effect. Another case of unusual segregation was found in (7) $E^a m^b$ /(20) $E^a m^b -1$; 8 of 350 F_2 plants were $E m^+$ types. The F_3 lines from seven of them segregated either into $E m : E m^+ : e$ types or into $E m : E m^+$ types (Table 4). The behavior of F_4 lines was similar to that in the first case. The occurrence of e type plants must be noted, since there was no e allele in the parental genotypes.

Furthermore, the eight late heading (*lf* type) offtypes found in the F_3 of (7) e^a /(15) e^b (Table 3, line 2) produced four fixed late heading F_4 lines (designated AB-60-1, -2, -3, and -4). They were short statured (8-25 cm shorter) compared with T65. When they were crossed with T65, the F_2 segregated into 3:1 normal (T65 type) to late short type. When one of them was crossed with an *lf* line (*lf e m^+*), the F_1 was a late heading type, the F_2 showed a wide range of continuous variation in heading time, and 1 early heading plant out of 200 was recovered; it was 19 days earlier than T65. Its F_3 progeny showed a complicated segregation pattern. Of 102 plants observed, 17 were weak and chlorophyll deficient and had few tillers, while the other 85 were normal. The normal plants showed a range of heading time from $E m$ to e types, mostly being E type (unpublished data)

It may be inferred that some alleles at the E and m loci are mutable in certain heterozygous states.

GENETIC EFFECTS ON CHARACTERS OTHER THAN HEADING TIME

The E alleles move up the time of floral initiation about 7 days and shorten the period from floral initiation to heading by about 3 days (16). They then reduce to some extent the size of organs that develop after floral initiation and grain yield (9). They markedly shorten the fourth and fifth internodes from the top, reducing plant height by about 5 cm. However, the spikelet size and grain test weight, as well as the growth of plants before floral initiation, are not influenced by the E alleles. The m gene emphasizes these effects of E alleles (11). The *lf* gene has shown no effect on plant characters except for a delay in heading time.

The effects of E alleles and E - m epistasis on heading time and those on other plant characters were found to be strongly correlated. For instance, when the effects of five different E alleles on heading time and on plant height were compared, the time-height correlation was 0.929 in the winter and 0.940 in the summer season and was 0.935 when averaged after z transformation ($df = 6$). High time-height correlations were also

found in the *E-m* epistasis, differing with the five *E* alleles with $r = 0.982$ ($df = 6$, averaged for winter and summer seasons). This suggests that the reduction in plant height in early maturing plants is a direct outcome of the hastening of floral initiation and heading.

These observations hold true in the genetic background of T65. A somewhat different pattern was observed in the genetic background of a tall variety, Chianung 242; the E^b allele introduced into this variety reduced plant height more strikingly and increased grain yield significantly (12). There might be a particular gene that interacts with *E* in this variety.

DISCUSSION

It was suggested that the *E* locus has a compound structure. This hypothesis is supported by the occurrence of offtypes with an *E* allele in crosses of different *e* type plants. The locus would contain a domain responsible for the *E-m* epistasis, as the magnitude of the epistasis differed according to *E* allele. Two *E* alleles were induced by irradiation as dominant mutations. This is most easily explained by assuming the presence of a suppressing subsite in the *e* allele that was inactivated by irradiation. However, it does not seem possible to explain by this hypothesis all of unusual behavior of the genes observed.

A favorable explanation would be the assumption that certain lines used for the experiments had some transposable elements in or nearby the loci concerned, as was demonstrated by McClintock (2, 5). In view of the high mutability of the *m* locus, it may be tentatively assumed that an autonomous transposon is attached to the *E* locus and a nonautonomous transposon is attached to the *m* locus. The site of incorporation of the transposons would differ according to cross, giving rise to a variety of phenotypic expressions. The occurrence of weak and abnormal plants in certain crosses of the progeny of late heading offtypes would serve as support for this working hypothesis. At this moment, however, the data are not complete enough to detect which of the parental lines have had and which have not had the assumed transposons.

A slender kernel gene was found by Yamagata (18) in a γ -ray progeny of a rice cultivar (Ginbozu), and it provoked mutations at different loci when introduced into other plants. But it must be admitted that precise location of transposons is not an easy task in higher plants, in which controlled pollination is laborious.

The *E* alleles are commonly distributed in the different rice cultivars of Japan and the Ryukyus (7). An *E* allele was also found in a Japanese

photoperiod-sensitive cultivar, Kisshin (15). Moreover, some other japonica cultivars—Awned Asahi, Yachikogane, Kanto 5—and a linkage tester, Ebisu (6), also commonly have the same *E* allele (8). While the *Ef-2* gene was found to be allelic, the *e* allele is commonly distributed in the japonica cultivars of Taiwan (12). Most probably, the *E* locus is a basic one controlling the growth duration of rice cultivars. The distributions of *m* and *lf* alleles remain unknown. Genes similar to *m* are widely distributed in cultivars of northern Japan (7). Genes like *lf* may be found in tropical japonica cultivars (unpublished data). The high mutability of alleles at these loci, as pointed out in this paper, could have played an important role in the evolution of rice cultivars, helping them to extend from south to north.

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ANALYSIS OF GENES CONTROLLING HEADING TIME IN JAPANESE RICE

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Gene actions of E_1 , E_2 , and E_3 controlling heading time are discussed based on data from seven lines having different genotypes for these genes. From growth cabinet experiments, these genes were shown to control photoperiod sensitivity but not to affect basic vegetative growth, and the intensity of action was recognized as $E_1 \gg E_3 > E_2$. These genes respond to night break treatment, delaying the differentiation of panicle primordia. The response of E_1 is far greater than those of E_2 and E_3 . From an experiment on regional variation, it was recognized that E_1 is a necessary gene for rice cultivation in southwest Japan, although it significantly delayed heading time in northern Japan. Through an allelism test, E_1 proved to be different from Lm , although an allele of $Lm-Lm^u$ —manifested photoperiod sensitivity similar to E_1 . Experimental results suggest that E_1 is sometimes alone and sometimes combined with E_2 , E_3 , or some early maturing gene.

Heading time is not infrequently a decisive factor in the regional adaptability of a rice cultivar. This holds true particularly in Japan, which has a wide range of climatic conditions. Thus, in Japan heading time has always been treated as one of the most important selection criteria in rice breeding programs, and since 1915 (1) quite a few studies have been carried out on its genetics with the aim of increasing breeding efficiency. Various genes responsible for heading time have been revealed, and several of them — E_1 , E_2 , E_3 , Lm , E , and Ef_2 — have been subjected to intensive analysis to elucidate the genetic mechanism of heading time (5,7,9,10). Of these six genes, three — E_1 , E_2 , and E_3 — that are independent of one another and delay heading time are discussed in this paper with regard to their actions on both basic vegetative growth (BVG) and photoperiod sensitivity (PS), their responses to night break, their responses to different environments, and the allelic relationship of E_1 to Lm . Then their roles in the regional distribution of Japanese cultivars are discussed.

MATERIALS AND METHODS

E_1 , E_2 , and E_3 were detected through intercrosses among the Japanese cultivars Gimbozu, Aikoku, and Kyotoasahi (4,6). Seven lines possessing

different genotypes for the three genes, viz., EG 1 through EG 7, were then developed (Table 1). These seven lines, referred to as EG lines, were used as the principal materials in this study, which comprised four experiments. Throughout the study, heading date was recorded for every plant as the day of first panicle emergence.

Experiment 1. To estimate the gene actions upon BVG and PS, the 7 EG lines and 14 representative rice cultivars (Table 1) were grown in two growth cabinets whose inner conditions of temperature and day length were adjusted to 30 °C and 14 h (long-day treatment, LT) and 30 °C and 10 h (short-day treatment, ST). BVG was expressed by number of days from sowing to heading under ST conditions for convenience, while PS was estimated by the difference in number of days from sowing to heading between the LT and ST conditions.

Experiment 2. To clarify the effect of night break on gene actions, seedlings of the seven EG lines were placed under 24 h day length for 50 days after sowing; then for a further 50 days they were subjected to 10 h day length with one of three night break treatments — early (E), middle (M), or late (L) — as shown in Figure 1. Each treatment was conducted for 1 h with an intensity of more than 300 lux. At 50 days after the start of the treatment, the developmental stage of the young panicle was determined (Table 2).

Experiment 3. To evaluate the response of the genes to the environment, the seven EG lines and two isogenic lines with the *Lm* locus — ER, which carries *Lm^e*, and LR, which carries *Lm^u* (9) — were grown at seven places throughout Japan (Table 3). These places cover five ecologically different regions: cold (Hokkaido District), cool (Tohoku and Hokuriku Districts), mild (Kanto, Chubu, Kinki, Chugoku, and Shikoku Districts), warm (Kyushu District), and subtropical (Okinawa District). Seeding was done on either 1 May or 16 May.

Experiment 4. To explain the differences between E_1 and *Lm^u*, which has similar action to E_p , the linkage relationship was analyzed for E_1 and *Pi-z^t*, a blast-resistant gene that tightly links with *Lm* of linkage group I (9). The seven EG lines were crossed with a cultivar carrying *Pi-z^t*, Toride 1, and clearcut monofactorial heading time segregation due to E_1 was observed in the F₂ generation following EG 2/Toride 1. Then the F₃ progeny of this cross were submitted to a test for resistance to blast

Table 1. Basic vegetative growth, photoperiod sensitivity, and days to heading under natural conditions of 7 lines having different genotypes for heading time (EG lines) and 14 representative cultivars in Japan.

Line or cultivar	Genotype ^a or cultivated region ^b	Basic vegetative growth ^c	Photoperiod ^d sensitivity	Days to heading ^e in natural condition
EG1	$E_1e_2e_3$	37	58	112
EG2	$e_1E_2e_3$	38	46	98
EG3	$e_1e_2E_3$	38	52	101
EG4	$E_1E_2e_3$	40	60	118
EG5	$e_1E_2E_3$	39	54	105
EG6	$E_1e_2E_3$	37	81	121
EG7	$E_1E_2E_3$	41	92	131
Kiyonishiki	Cool	50	44	95
Fujiminori		49	14	95
Koshihikari		46	50	103
Norin 8	Mild	40	59	113
Norin 22		43	60	114
Nipponbare		38	60	112
Manryo		38	55	107
Kinmaze		40	68	117
Nakateshinsenbon		39	70	114
Akebono		38	77	125
Hoyoku	Warm	38	74	123
Shiranui		40	74	121
Zuiho		41	76	125
Shinriki		40	74	121

^aGenotype for E_1 , E_2 , and E_3 , and all lines being homozygous.

^bSee Table 3.

^cDays from sowing to heading under short day (10 h) at 30 °C.

^dDifference in days to heading between a long day (14 h) and a short day (10 h) at 30 °C.

^eSown on 6 May 1980.

fungus strain Ken 53–33, and the relationship between heading time and blast resistance was checked by their segregation.

RESULTS

Basic vegetative growth and photoperiod sensitivity. With the seven EG lines, no significant differences in BVG were observed, but PS

clearly differed with different lines, its magnitude being in the order of EG 7 > EG 6 > EG 4 > EG 1 > EG 5 > EG 3 > EG 2. It was concluded that E_1 , E_2 , and E_3 are all the genes controlling PS and that the intensity of gene action is in the order of $E_1 \gg E_3 > E_2$.

Of the 14 cultivars, those that are cultivated mainly in cool regions and exhibit a relatively few number of days to heading under natural conditions generally showed long BVG and weak PS compared with the EG lines. The cultivars grown in mild regions showed similar BVG and PS to those of EG 1 or EG 4, while the cultivars from warm regions showed similar BVG and PS to those of EG 6. These facts suggest that BVG and PS — consequently the genotypes of E_1 , E_2 , and E_3 — bear a close relationship to region.

Response to night break. As clearly seen in Figure 2, the response of young panicle development to night break treatment depended upon both the method (time) of treatment and the line examined. Middle time break (M) caused far greater suppression of young panicle development than early (E) or late (L) time break. In each treatment, on the other hand, EG lines could be distinctly classified into two groups: highly sensitive and slightly sensitive to night break. Highly sensitive lines all had E_1 and, responding to the degree of sensitivity observed at treatments E and L, could be arranged in the order EG 7 > EG 6 > EG 4 > EG 1, while slightly sensitive lines were devoid of E_1 and responded slightly only to treatment M. These results suggest that E_1 , E_2 , and E_3 are all sensitive to night break, and that the sensitivity of E_1 is extremely high compared with the other two and is considerably enhanced by the coexistence of E_3 and especially by that of both E_2 and E_3 .

Response to cultivation environment. Table 3 shows the days to heading of nine lines at seven places. Sowing at Kyoto was done twice to provide comparisons with all other places.

Comparing the three places where seeds were sown on 1 May at Sapporo (cold region), the highest latitude in this experiment, the heading time of each line was extremely delayed compared with that at the other two locations. Besides, no heading was observed in all five lines having E_1 or Lm^u . At Kashimadai in the cool region, all lines except ER showed apparently later heading than at Kyoto in the mild region.

Comparing the five places where seeds were sown on 16 May, heading time was excessively advanced in all lines at Naha in the subtropical region, the lowest latitude in this experiment. Among Tsukuba, Kyoto, and Kurashiki in the mild region, however, only slight differences were

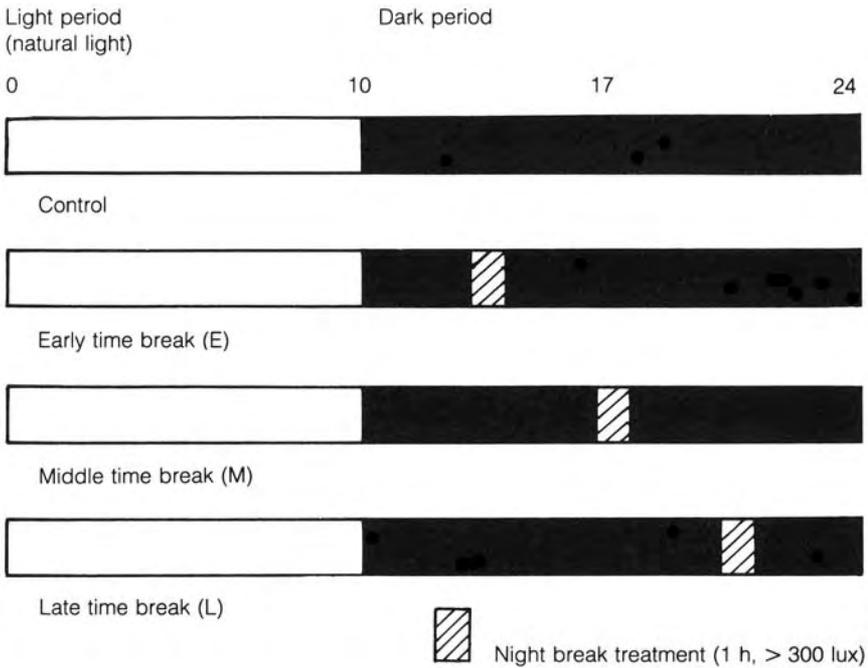


Fig. 1. Scheme of night break treatment.

observed. At Fukuoka in the warm region, all lines tended to head earlier than that in the mild region.

Allelic relation between E_1 and Lm . As shown in Table 4, the blast resistance test applied to the F_3 progeny of EG 2 (E_2)/Toride 1 (E_1E_2) revealed a monogenic line segregation (1:2:1) due to the gene $Pi-z^t$. Based on this result, F_2 parents were classified into three genotypes of dominant homozygous, heterozygous, and recessive homozygous for $Pi-z^t$, and frequency distribution of heading date in each of those three genotypes was examined. As a result, the segregation of heading date by E_1 showed a 1:3 ratio irrespective of the genotype for $Pi-z^t$, indicating that E_1 is transmitted independently of $Pi-z^t$. Lm is thus a different gene from E_1 , because Lm tightly links with $Pi-z^t$ as mentioned before.

DISCUSSION

An extensive analysis of the genes responsible for heading time of various Japanese representative cultivars (8) revealed that most of the cultivars

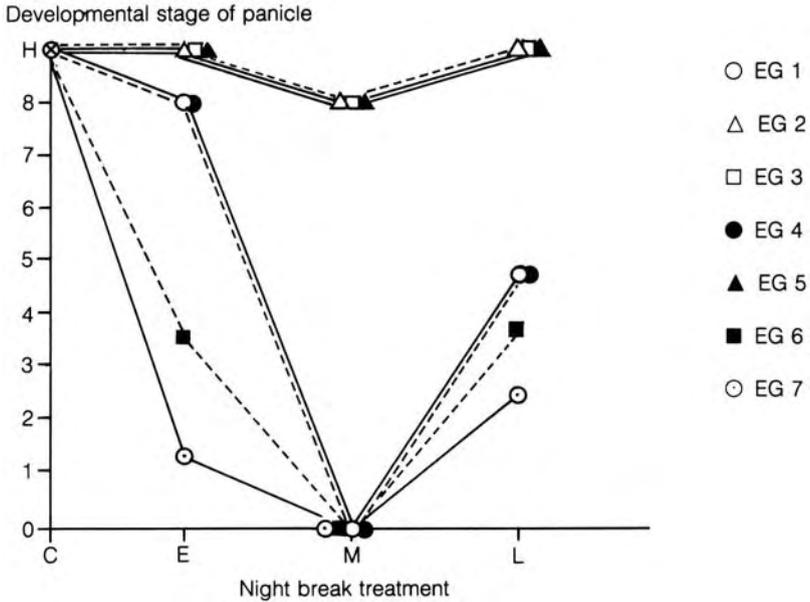


Fig. 2. Response of young panicle development to night break treatment in seven different genotypes for E_1 , E_2 , and E_3 (see Table 2). C = control, E = early time break, M = middle time break, L = late time break.

Table 2. Developmental stages of young panicle (partly after 3).

Developmental stage	Situation of panicle	Panicle length (mm)
0	Vegetative stem primordia, before neck node differentiation	—
1	Differentiating stage of primary rachis branch primordia	—
2	Differentiating stage of secondary rachis branch primordia	0.5–0.9
3	Early differentiating stage of flower primordia	1–3
4	Late differentiating stage of flower primordia	3–15
5	Differentiating stage of pollen mother cell	15–50
6	Early stage of reduction division of pollen mother cell	50–100
7	Late stage of reduction division of pollen mother cell	100
8	Completing stage of pollen	ca.200
H	(Heading finished)	ca.200

grown south of Tohoku District have the E_1 gene, suggesting the great importance of E_1 in Japan. From the present study, it became clear that E_1 controls PS but not BVG, and its effect is far greater than those of E_2 and E_3 (Table 1, Fig. 2). This means that under LT conditions, E_1 firmly

inhibits the differentiation of panicle primordia and thus ensures a sufficient vegetative growth, leading to high yield. Moreover, though the critical day length where E_1 starts to induce panicle differentiation is not yet evident, and though it is quite natural that the action of E_1 varies widely with genetic background, it is apparent that a cultivar possessing E_1 commences panicle formation more sharply as a response to the shift from long day to short day than a cultivar without E_1 ; hence a cultivar with E_1 produces less variation year-to-year in heading time and less variation in yield unless it undergoes particular stresses such as unusually low temperatures. These may be the reasons why most cultivars grown south of Tohoku District have come to have the E_1 gene.

Heading time in cool regions was much delayed by E_1 compared with that in mild regions (Table 3). On the other hand, cultivars grown in cool regions showed longer BVG and weaker PS than those in mild regions (Table 1). These facts suggest that most of the cultivars grown in cool regions contain not only E_1 but also some gene(s) that inhibits the effects of E_1 and prolongs BVG.

It is known that the cultivars grown in Hokkaido District have an extremely low PS compared with those in other districts (2). Besides, the four EG lines with E_1 did not show any heading at Sapporo (Table 3). These facts suggest that if the cultivars of Hokkaido have E_1 they also have another gene that completely suppresses the effect of E_1 .

Table 3. Days from sowing to heading of 9 lines having different genotypes for heading time at 7 places, 1984.

Line	Sown on 1 May				Sown on 16 May			
	Sapporo ^a (ca.43°N)	Kashimadai ^b (ca.38°N)	Kyoto ^c (ca.35°N)	Tsukuba ^c (ca.36°N)	Kyoto ^c (ca.35°N)	Kurashiki ^c (ca.35°N)	Fukuoka ^d (ca.34°N)	Naha ^e (ca.26°N)
EG 1	NH	125.9	112.3	103.9	104.4	101.7	97.3	71.6
EG 2	126.9	108.8	95.8	94.7	91.1	87.0	88.8	67.5
EG 3	134.1	110.8	101.8	96.9	97.4	92.0	92.6	72.8
EG 4	NH	130.5	116.0	108.6	107.6	104.4	101.6	74.0
EG 5	138.4	114.2	103.5	98.5	101.5	92.5	94.9	74.6
EG 6	NH	139.4	123.1	118.1	119.8	115.8	111.8	88.0
EG 7	NH	153.9	133.2	128.3	130.2	123.3	119.6	95.7
ER	109.9	98.7	97.8	87.5	97.2	90.9	90.5	65.9
LR	NH	131.1	121.0	111.8	113.7	109.2	105.9	82.2

^a Cold region (Hokkaidō District); NH = nonheading.

^b Cool region (Tōhoku and Hokuiku Districts).

^c Mild region (Kantō, Chūbu, Kinki, Chūgoku, and Shikoku Districts).

^d Warm region (Kyūshū District).

^e Subtropical region (Okinawar District).

As to genes E_2 and E_3 , it was proved that they control PS but do not affect BVG as E_1 does, and their actions are not as strong as that of E_1 (Table 1, Fig. 2).

However, E_3 induced a remarkable delay of heading time when it coexisted with E_1 (Table 1, Fig. 2). This synergistic effect seems to have resulted not from the shortening of critical day length but from the increase of delaying effect under excessive day length. Most later maturing cultivars grown in regions south of Tohoku District such as Akebono, Hoyoku, Shiranui, and Zuiho (Table 1) are expected to have both E_1 and E_3 (8). E_2 , having the smallest action among the relevant three genes, also showed a remarkable synergistic effect when it coexisted with both E_1 and E_3 (Table 1, Fig. 2). In Japan, however, it appears that there exist no cultivars possessing E_1 , E_2 , and E_3 like EG 7, because even a cultivar extremely sensitive to photoperiod, Zuiho, has proved to be less sensitive than EG 7 (8).

Yokoo and Kikuchi (9) reported that the Lm locus has played an important role in the ecological distribution of Japanese rice cultivars. The relationship between this indication and the authors' finding that most rice cultivars grown south of Tohoku District have E_1 appears to be an interesting problem deserving further study, because E_1 and Lm were proved to be different genes (Table 4).

Table 4. Linkage analysis for E_1 and $Pi-z^1$ based on the segregations in F_2 and F_3 following the cross between EG 2 and Toride 1.

Group for heading time (genotypes in F_2 plants)	Resistance to Ken 53-33 (F_3 lines)			
	Resistant	Segregated	Susceptible	Total
Early (e_1e_1)	17	43	20	80
Late (E_1E_1, E_1e_1)	58	125	63	246
Total	75	168	83	326

Goodness of fit (F_2 plants)				
Item	Ratio	d.f.	χ^2	P
Total	1:2:1:3:6:3	5	0.956	0.975-0.950
$Pi-z^1$	1:2:1	2	0.699	0.750-0.500
E_1	1:3	1	0.036	0.900-0.750
Independence		2	0.250	0.900-0.750

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PHENOL COLOR REACTION OF SEED INTEGUMENTS IN RICE

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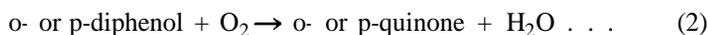
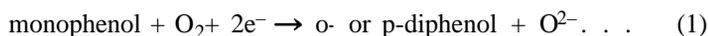
The phenol color reaction of rice grains can be clearly and quantitatively determined by measuring the colorations of both the grain surface and the phenol solution after soaking the grains in 2% phenol for 3–5 days. This method is more effective in clarifying the phenol reaction of cultivars with colored hulls and brown rice than the ordinary method in which only the coloration of the grain surface is observed. Hulls and brown rice separated from the grain showed different patterns of the color reaction with phenol. F₃ hybrid seeds of the cross between wild (positive phenol reaction) and cultivated (negative phenol reaction) rice segregated into four combinations of hulls and brown rice regarding their color reaction with phenol, i.e., both positive, positive and negative, negative and positive, and both negative. Therefore, when testing the phenol reaction of rice seeds, hulls and brown rice should be examined separately. The phenolase activity test suggested that the rice cultivars showing negative reaction with phenol are unable to further hydroxylate the aromatic ring of phenol due to a lack of hydroxylating enzyme and/or a shortage of electron donors.

Oka and Chang (8) proposed the following discriminant formula for classifying indica and japonica types:

$$X = -K + 0.75Lt - 0.22Hr + 0.86Ph$$

where K = concentration of KClO₃ solution, which causes a certain degree of damage; Lt = index number for damage to seedlings treated at 0–1 °C; Hr = apiculus hair length; and Ph = phenol reaction. This discriminant formula is known to be the most useful and highly available for classifying indicas and japonicas (7). So far, we have studied these four functions in the discriminant formula from the physiological viewpoint.

In this study the ordinary method of phenol reaction (3) was reexamined to find a suitable way to classify rice ecotypes collected from different parts of the world. The cultivars that showed negative phenol reaction were examined from the viewpoint of the monophenol oxidation system above. Kondo and Kasahara (3) showed that coloration of wheat grains with phenol is caused by phenol oxidase, which is contained in living organs. The monophenol oxidation is conducted by the following two chemical reactions (2):



MATERIALS AND METHODS

The rice cultivars used belonged to the different ecotypes described by Morinaga (5) such as bulu and tjereh from Indonesia; aman, aus, and boro from the Bengal-Assam region in India; and upland and lowland cultivars from Japan. In addition, several cultivars from Korea, Taiwan, China, Nepal, Europe, Brazil, the US, the USSR, and mountainous parts of Thailand (upland rice) were used.

Phenol oxidase activity has been investigated only in grains. In this experiment, the color reactions of ears, nodes, and internodes were examined using a 2% phenol solution. These plant parts were longitudinally divided into halves with a razor. One part was soaked in 2% phenol solution for 3 days and then dried, while the other part was kept at 5 °C as the control to compare with the treated half.

The effects of soaking time in phenol solution and of drying in air after soaking on the color expression of hulls and brown rice were investigated by observing the changes of coloration after 5 days at 30 °C. The color of the phenol solution was measured by spectrophotometer (Hitachi model 101) at 500 nm. The method used in this experiment was compared with the ordinary method in which the change of color is observed during air drying after soaking in phenol solution.

To clarify the cause of the negative reaction in grains of japonicas and of some cultivars of other ecotypes, hulls and brown rice were soaked in 2% phenol, 1% catechol, or 1% hydroquinon solution under dark conditions at 30 °C for 5 days. Solutions of these reagents without grains were the controls. After 5 days, the color reactions of hulls, brown rice, and the phenol solutions were observed, and absorbance was measured by spectrometer at 500 nm.

RESULTS

When ears, leaves, nodes, and internodes of indica cultivars were soaked in 2% phenol solution for 3 days at 30 °C, only the grains showed a positive reaction with phenol, i.e., monophenol oxidase was exclusively localized in the grains and not in the rachis and branches, leaves, nodes, and internodes.

Table 1. Relationships between phenol reaction of grains and geographical distribution.

Color reaction	Number of cultivars										
	Japan ^a		Korea	China	Taiwan	India	Nepal	Europe	USSR	US	Total
	L	U									
+	6 ^b	13	0	4	2	17	17	7	4	1	71
-	94	3 ^c	4	4	4	3	15	6 ^d	11	9	152
Total	100	16	4	8	6	20	32	13	15	9	223

^a L = lowland rice, U = upland rice.

^b Kyuu shuu-8, -58, Tuyokuni-32, Senich, Ginbuzu, and Toyukuni.

^c Daisen, N-I, Urasan.

^d All Italian cultivars.

Soaking time clearly affected the color reaction on the grain surface. A difference in phenol reaction was observed between soaking for 6 and 18 h. Hulls and brown rice separated from grains were easily colored even in the treatment for 6 h as compared with intact grains for the same period.

The method that measures the coloration of phenol solution after soaking grains was more effective to distinguish among cultivars than the ordinary one because it was available to identify the enzyme activity even in the grains with colored hulls and/or brown rice.

Table 1 shows the phenol reaction (negative or positive) of rice cultivars from various countries. Although the number of cultivars is not sufficient to be definitive, it can be concluded that most of the Japanese, Italian, and bulu cultivars expressed a negative color reaction with phenol.

Half of the Nepal cultivars were negative and the other half positive. Most upland japonica cultivars were positive, but Thai cultivars such as the bulu ecotype, whose grains are large, showed a negative reaction. Italian cultivars showed a negative color reaction. Table 2 shows clearly that the bulu ecotype is negative, but not boro. While most aman and tjereh showed positive reactions as reported by Morinaga and Kuriyama (6), the color reaction with phenol did not always coincide between hulls and brown rice, e.g., in Dular (aus), Marich-bati (aus), and Bendang Putih (tjereh).

F₃ progeny of the cross between wild rice (*O. sativa* f. *spontanea*), whose hulls and brown rice are positive to phenol reaction, and a Japanese cultivar Norin No. 20, whose hulls and brown rice are negative, segregated into four combinations of hulls and brown rice regarding phenol reaction, i.e., both positive, positive and negative, negative and positive, and both negative, as shown in Figure 1 and Table 3. A total of 112 F₃

Table 2. Color reaction^a with phenol in rice ecotypes in Southeast Asia.

Ecotype	Cultivar	Grain	Hull	Brown rice
Aus	Kele	3	3	3
	Dular	3	3	1
	Pusur	2	3	3
	Kataktara	0	0	0
	Marich-bati	0	0	1
Aman	Bhasamanik	2	3	3
	Bashabhog	0	0	0
	Blue Stick	3	3	3
	Daudkhani	2	3	3
	Patnai-23	3	3	3
	Daudin	3	3	3
	Hatishail	3	3	3
Tjereh	Peta	3	3	3
	Salak	3	3	3
	Skrivimankoti	3	3	3
	Lati Sail	3	3	3
	Ketan Serang	3	3	3
	Bendang Putih	0	1	0
Bulu	Benong 130	0	0	0
	Baok	0	0	0
	Ketan Gadjih	0	0	0
	Sukannandi	0	0	0
	Gemdjah Beton	0	0	0
Boro	Bhumuri 36	3	3	3
	Chinsurah Boro I (L)	3	3	3
	Chinsurah Boro I (D)	3	3	3
	Assam IV	3	3	3
	Tepa I (L)	3	3	3
	Tepa I (D)	3	3	3

^aFigure shows the degree of coloration of phenol solution in which rice grains were soaked. OD values of phenol solution containing 10 grains:

Range	Index
0-0.010	0
0.050	1
0.100	2
0.200	3

^b(L) = immature seeds, (D) = mature seeds.

seeds were classified into these four types by the phenol reaction. Of 88 F₃ seeds classified as positive hulls, 32 were of brown rice with negative phenol color. Of 24 F₃ seeds classified as negative hulls, 8 were of brown rice with a positive phenol reaction. The F₃ seeds that led to the discord in color reaction with phenol were about 36% in total number of seeds.

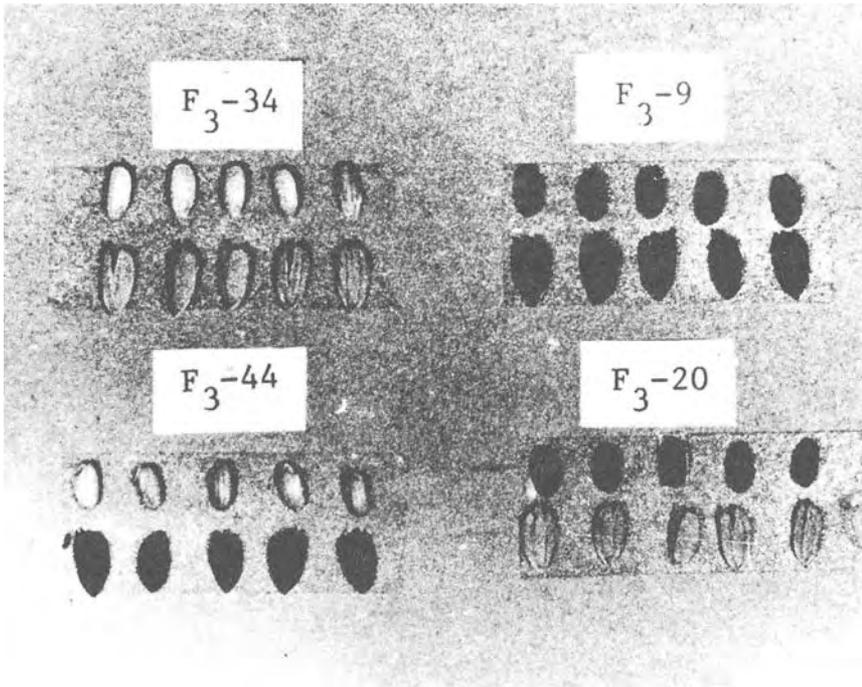


Fig. 1. Phenol color reaction in progeny of wild and Japanese rice. Progeny of F_3 seeds segregated into four groups regarding coloration of hull and brown rice: (F_3-34) neither hull nor brown rice reacted, (F_3-9) both reacted, (F_3-44) only hull reacted, (F_3-20) only brown rice reacted.

DISCUSSION

Takahashi and Hamza (9) reported on the color reaction with phenol in plant parts, e.g., ears, leaves, nodes, and internodes. They showed monophenol oxidase to be extremely localized in the grains, although monophenol must be commonly present in other parts of plants (2).

The method of soaking in diluted phenol solution for 3–5 days is most effective for classifying rice cultivars. The results of this experiment roughly agreed with Morinaga and Kuriyama's (9) and Kuriyama and Kudo's (4) data. However, our method, which measures the coloration of phenol solution after soaking the grains, makes it easier to identify cultivars than with the ordinary method, because it can be done even in grains with colored hulls or in brown rice. Moreover, the color reaction with phenol did not show the same tendency in hulls and brown rice. We

Table 3. Color reaction with phenol in hull and brown rice of F_3 seeds from a cross between japonica (Norin No. 20)³ and wild rice (*O. sativa* f. *spontanea*).

A. Observed frequencies

Phenol reaction of brown rice	Phenol reaction of hull		Total
	Positive	Negative	
Positive	56	8	64
Negative	32	16	48
Total	88	24	112

B. Probabilities calculated border total

Item	Phenol reaction			χ^2	P
	Positive	Negative	Total		
Hull	88 (84) ^a	24 (28) ^a	112	0.7619	0.4
Brown rice	64 (48) ^a (63) ^b	48 (28) ^a (49) ^b	112 112	11.1111 0.0363	0.01 0.95–0.90

^a Expected ratio = 3:1, expected numbers in parentheses.

^b Expected ratio = 9:7.

confirmed the four combinations of hulls and brown rice regarding phenol reaction. The phenomenon may occur frequently in natural conditions and in the breeding process. The difference in the phenol reactions of hulls and brown rice seems to be due to the difference in genetic backgrounds, presumably concerning enzyme systems.

In the examination concerning phenol oxidation of rice grains, the negative phenol reaction detected in some cultivars was due to the lack of the first step of the phenol oxidation system. The fact was also recognized in other parts of plants, e.g., leaves, nodes, and internodes. It has been confirmed in living organs that in the first step the aromatic ring of phenol can be further hydroxylated to quinol (1). Therefore, it is suggested that the cultivars that show negative reaction are unable to hydroxylate further the aromatic ring of phenol because of lack of hydroxylating enzyme and/or a shortage of electron donors.

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GENETIC STUDIES ON SEED DORMANCY IN RICE

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Genetic studies on seed dormancy in rice were made under greenhouse conditions in crosses involving two dormant parents, N22 and IR13429-287-3, and two nondormant parents, Mahsuri and IR19735-5-2. Germination tests were conducted at 30 °C with 10 light hours per day in a growth chamber. Both hull and pericarp were involved in the dormancy of N22, whereas hull was the primary factor in IR13429-287-3. Kernel dormancy (due to pericarp) in N22 disappeared 3 weeks before grain dormancy (due to hull). Dormancy behaved as dominant in all the crosses (N22/Mahsuri, N22/IR19735-5-2, and IR13429-287-3/Mahsuri) studied. At 30 days after harvest (DAH) — when kernel dormancy was not evident in the dormant parents or the F₁ hybrids — the F₂ segregations for hull factors conformed to a 3:1 monogenic ratio in the two crosses involving N22 as a parent and a 9:7 digenic complementary ratio in the cross with IR13429-287-3 as parent. The distribution in all three crosses is suggestive of modifier genes. Segregations at 10 DAH in the N22 crosses had confounding effects on kernel dormancy. Dormancy due to hull and pericarp may be independently controlled. Results from earlier studies conducted under natural field conditions suggesting polygenic control of dormancy might have been due to the confounding effects of environmental factors and kernel dormancy. Reciprocal crosses between N22 and Mahsuri indicated that cytoplasmic factors are not involved in the control of dormancy. The intensity and duration of dormancy are considered to be either independently controlled or both regulated by modifier genes. Resistance to preharvest sprouting in N22 was dominant and appeared to be controlled by one major gene and some modifiers. Preharvest sprouting and period of dormancy upon maturity were found to be negatively correlated ($r = -0.88^{**}$) in the F₂ of N22/Mahsuri. In a study of 22 cultivars of diverse origin, preharvest sprouting was observed to be positively correlated with germinability at low temperature of 14 °C ($r = 0.92^{**}$) and with germinability under osmotic pressure of polyethylene glycol solution at -12 ($r = 0.84^{**}$).

The failure of the fully mature and viable seed to germinate to an appreciable degree under favorable environmental conditions is termed dormancy, and the rest period refers to the duration of dormancy.

Seed dormancy in rice (*Oryza sativa* L.) is both useful as well as disadvantageous. It serves as a mechanism for protecting the grain from germinating in the field during wet weather or when the crop lodges in standing water. However, it proves undesirable in raising two to three crops a year of the same cultivar by using seed from one crop to raise the succeeding crop. It is also problematic to plant breeders for growing breeding material in quick succession and to seed technologists for the tests that are performed upon harvest.

Rice varieties differ significantly in their degree of dormancy. The japonica subspecies generally have shorter periods of dormancy than the tropical indica subspecies (3, 29). Most javanica varieties have a moderate degree of dormancy. The African cultivated rice *Oryza glaberrima* Steud. possesses strong seed dormancy (16). The wild species of rice, in general, have strong dormancy extending even beyond 6 months. Although earlier observations indicated association of long growth duration or photoperiod sensitivity with higher degree of dormancy (4, 7, 22), subsequent studies identified early maturing varieties possessing varying degrees of dormancy (17, 18, 23). Tang and Chiang (29) considered that varieties with red pericarp have a longer dormancy period than those with normal pericarp.

Although genetically controlled, the expression of dormancy in rice is influenced by environmental factors such as temperature, moisture, and oxygen supply (8, 10, 24). High temperature and low humidity at the grain ripening stage appear to reduce the dormancy period.

Various factors have been recognized as causing seed dormancy: rudimentary or physiologically immature embryos, mechanically resistant or impermeable seed coats, environmental factors, and the presence of endogenous germination inhibitors. Grain dormancy in rice is not considered as true embryo dormancy, because excised embryos germinate readily (14). Experimental evidence is lacking to indicate that mechanical or impermeability factors are involved in dormancy (12). Roberts (23) considered that the seed covering structures may have a germination inhibiting effect by acting as a barrier to the diffusion of O₂, which may possibly be needed in a dormancy-breaking oxidation mechanism. Navasero et al (21) observed that reduction by the hull of the level of O₂ available to the embryo was mainly responsible for grain dormancy in rice. They found that a decrease in grain dormancy is accompanied by a decrease in the peroxidase activity of the hull. Baun (2) suggested the possibility that peroxidases in the outer coverings may be competing with the embryo for O₂. Takahashi and Oka (28) reported that dormancy can be broken under anaerobic conditions. The occur-

rence of endogenous germination inhibitors in the covering structures of the rice grain has been reported by several workers (11, 13, 14, 15). Hayashi (9) concluded that the main inhibitor was abscissic acid. Takahashi (27) and Mikkelsen (14) observed that substances extracted from dormant seeds inhibited germination of nondormant seeds. Amen (1) proposed a model of seed dormancy based on the principle that dormancy onset, control, and termination are regulated by a balance of growth inhibitors and promoters.

Although dormancy in rice is a valuable agricultural trait, only limited studies have been carried out to understand its inheritance (5, 18, 20, 25, 26, 30). It is this concern that prompted the present study. There are three genetically different tissues involved in the grain: (1) diploid embryo produced by fertilization of the ovum; (2) triploid endosperm containing one set of paternal genes and two sets of maternal genes; and (3) diploid testa, pericarp, glumes, paleas, and lemmas — all of maternal genetic constitution. In planning the genetic studies, it is important to understand which tissue is associated with dormancy, because the embryo and endosperm in hybrid populations are one generation in advance of the hull and bran layer. Also, variation due to external environmental factors needs to be minimized.

MATERIALS AND METHODS

A major part of the study was carried out in the facilities of Cornell University, Ithaca, N.Y., U.S.A. during the sabbatic leave of the senior author. Four crosses involving two dormant and two nondormant parents were studied. The dormant parents were N22, an early maturing traditional tall cultivar from India, and IR13429-287-3, an early maturing semidwarf breeding line from IRRI. The nondormant parents were Mahsuri, a moderately photoperiod sensitive tall cultivar derived from a japonica/indica hybrid, and IR19735-5-2, an early maturing semidwarf breeding line from IRRI. Mahsuri is susceptible to preharvest sprouting. The crosses and the generations studied were as follows:

- N22/Mahsuri (F_1 , F_2 , F_3)
- Mahsuri/N22 (F_1)
- N22/IR19735-5-2 (F_1 , F_2 , F_3)
- IR13429-287-3/IR19735-5-2 (F_1 , F_2)

The test material was grown in pots (five plants per pot) in the greenhouse with the temperature maintained between 25 °C and 28 °C and the relative humidity between 75% and 80%. Panicles were tagged

as they emerged from the boots and were harvested 30 days after flowering. Seeds were air dried for 3 days in the laboratory (26 ± 1 °C). Germination tests were carried out in a growth chamber maintained at 30 °C with 10 hours light period per day. All tests included 2 replications of 30 seeds each, and all values indicated in the results represent an average over the 2 replications. The seeds were germinated in 9-cm petri dishes lined at the bottom with one piece of 9-cm Whatman No. 1 filter paper and irrigated with 10 ml of distilled water. Germination counts were taken 1 week after soaking. Germination tests were conducted at 10, 20, 30, 40, 50, and 60 days after harvest (DAH) for the parents and F_1 hybrids; at 10, 30, and 60 DAH for the F_2 segregants; and at 30 DAH for the F_3 progenies. (The stage of testing for F_2 and F_3 was decided after obtaining the results from parents and F_1 hybrids.) Dehulling (parents and F_1 hybrids) was done by removing lemma and palea carefully by hand. Pericarp tissue was removed where required by scraping with a razor blade with utmost caution.

For the preharvest sprouting studies, panicles were harvested 25 days after emergence, and the test seeds were collected from the central part of the panicles and wrapped in moist paper towels. Germination counts were made 5 days later.

Twenty-two cultivars were included in a study to determine the association of preharvest sprouting with germinability under low temperature and osmotic pressure. The cultivars included both japonicas and indicas originating from Japan, the US, Hungary, tropical Asia, Africa, and Latin America. Some among them were adapted to upland conditions. The low temperature test was conducted at 14 °C with a control treatment at 30 °C. For germination tests under osmotic pressure, polyethylene glycol (PEG 6000) solution was used with two treatments of $-\bar{2}$ and $-\bar{12}$ at 25 °C under light.

RESULTS AND DISCUSSION

The dormant parents showed 75% germination at 50 DAH for N22 and 40 DAH for IR13429-287-3, and the nondormant parents IR19735-5-2 and Mahsuri showed 75% germination at 10 DAH (Table 1). Although Mahsuri gave more than 75% germination at 10 DAH and is also susceptible to preharvest sprouting, the fully mature grains did not germinate immediately upon harvest, and germination was negligible even at 5 DAH. It is possible that germination inhibitors appear in this variety at maturity and remain active for a very short period. Dehulling

Table 1. Duration of seed dormancy in the rice cultivars included in the genetic studies.

Cultivar	Treatment	Germination %					
		10	20	30	40	50	60
N22	hull intact	0	10	20	46	78	98
N22	dehulled	0	15	76	98		
N22	pericarp scraped	96	100				
N22	pericarp scraped and enclosed in hull	0	21	48	66	86	98
IR13429-287-3	hull intact	0	16	24	74	100	96
IR13429-287-3	dehulled	38	82	98			
Mahsuri	hull intact	81	100				
IR19735-5-2	hull intact	77	99				

did not improve germination in N22 at 10 and 20 DAH but resulted in more than 70% germination at 30 DAH. IR13429-187-3 on dehulling showed improved germination at 10 DAH and more than 80% germination at 20 DAH. When the pericarp of N22 was scraped, 96% germination was obtained at 10 DAH. However, when the scraped kernel was enclosed in the hull, the pattern of germination at different DAH was similar to that with intact hulls (control), although the percentages were relatively higher than with the latter. The duration of dormancy is longer in N22 than in IR13429-287-3 by about 10 days. Dehulling did not improve germination in N22 until 30 DAH, and the fact that full germination was secured at 10 DAH when the pericarp was scraped indicates that both hull and pericarp are involved in dormancy in this cultivar. The duration of kernel dormancy, however, appears to be shorter by about 3 weeks than that of hull dormancy. In other words, at 30 DAH dormancy appears to be primarily due to the hull. Nair and Sahadevan (19) and Baun (2) demonstrated that the bran layer, which differs widely in rice varieties, may also have an inhibitory influence. Kernel dormancy in IR1313429-287-3 did not appear to be significant, because more than 80% germination was obtained on dehulling at 20 DAH.

The germination of the cross seeds obtained from reciprocal crosses between N22 and Mahsuri was compared. At 10 DAH cross seeds with N22 as the female parent failed to germinate, but the ones with Mahsuri as the female parent showed 56% germination. At 30 DAH there was

Table 2. Seed dormancy in three F_1 rice hybrids.

Cross	Treatment	Germination %					
		10	20	30	40	50	60
		(days after harvest)					
N22/Mahsuri	hull intact	0	8	28	62	98	96
N22/Mahsuri	dehulled	42	76	96			
Mahsuri/N22	hull intact	8	12	34	60	100	94
Mahsuri/N22	dehulled	36	78	98			
N22AR19735-5-2	hull intact	0	15	32	78	100	100
N22AR19735-5-2	dehulled	18	38	78	92	96	100
IR13429-287-3/Mahsuri	hull intact	4	22	38	76	100	98
IR13429-287-3/Mahsuri	dehulled	63	100				

22% germination in the former and 94% in the latter. This suggested that the covering structures play the major role in controlling dormancy. Thus, F_1 observations were made on the seeds borne on F_1 plants, and F_2 observations on the seeds borne on F_2 plants. If the covering structures have no role and the factors for dormancy are located in the embryo or endosperm, F_1 studies should be made on the cross seeds and F_2 studies on the seeds borne on F_1 plants.

The results of germination tests with seeds harvested from F_1 plants indicate that seed dormancy is dominant in all four crosses (Table 2). The reciprocal crosses N22/Mahsuri and Mahsuri/N22 showed similar patterns of germination, suggesting that cytoplasmic factors are not involved. Dehulling nearly removed dormancy in the F_1 of IR13429-287-3/Mahsuri at 10 DAH, which confirms that the hull is the primary factor for dormancy in IR13429-287-3. Dehulling the seed of the F_1 of N22/Mahsuri and its reciprocal resulted in moderate improvement in germination at 10 DAH, over 70% germination at 20 DAH, and complete germination at 30 DAH. Improvement of germination due to dehulling was relatively low at 10 and 20 DAH in the F_1 of N22/IR19735-5-2. Unlike grain dormancy, kernel dormancy did not show complete dominance at 10 DAH. Although N22/Mahsuri and N22AR19735-5-2 showed similar patterns of dormancy with intact hull, expression of kernel dormancy due to factors in the hull and in the kernel (in this case, pericarp) may be governed by independent sets of genes. F_1 hybrids involving N22 as the dormant parent showed full germination at 50 DAH, unlike the parent, which required 60 DAH for complete germination.

Table 3. Segregation for seed dormancy in F_2 generations of three rice hybrids.

Cross	Days after harvest	Frequency of F_2 segregants under diff. classes of germination %				Ratio of 0-25:26-100 germination %	χ^2	P
		0-25	26-50	51-75	76-100			
N22/Mahsuri	10	248	28	13	15	3:1	2.96	0.10-0.05
	30	215	9	16	64			
	60	1	2	4	298			
N22/IR19735-5-2	10	262	12	9	9	3:1	1.83	0.25-0.10
	30	209	6	19	58			
	60	0	3	6	283			
IR13429-287-3 Mahsuri	10	198	42	32	48	9:7	4.11	0.05-0.01
	30	191	8	20	101	9:7	1.53	0.25-0.10
	60	1	4	9	312			

Seed dormancy in the segregating F_2 populations of N22/Mahsuri, N22/IR19735-5-2, and IR13429-287-3/IR19735-5-2 was studied at 10, 30, and 60 DAH. The results are tabulated under four classes of segregation: 0-25%, 26-50%, 51-75%, and 76-100% (Table 3). At 10 DAH, most segregants in N22/Mahsuri and N22/IR19735 came under the category of 0-25%, with no second peak. However, at 30 DAH the segregation in these two crosses with a cutoff for dormancy at 25% germination followed a monogenic ratio of 3:1 dormant to nondormant, with a second peak in the 76-100% class. The two peaks indicate the control of dormancy by major genes (in this case one dominant gene), and the spread over different classes of germination indicates the presence of modifier genes, as can be expected in such quantitative physiological traits. Dormancy factors located in the hull as well as in the pericarp were active at 10 DAH, with the segregation pattern reflecting cumulative effects, whereas at 30 DAH the segregation was primarily due to the hull. It would thus be appropriate to say that dormancy due to hull in the above two crosses is controlled by a single dominant gene along with some modifier genes. Almost all segregants in both the crosses showed above 75% germination at 60 DAH, with very few giving transgressive reactions. The F_2 segregations in IR13429-287-3/Mahsuri at 10 DAH as well as 30 DAH are suggestive of a digenic complementary ratio of 9:7, with dormancy being dominant. Kernel dormancy being less significant in IR13429-287-3, the ratio was perhaps evident at 10 DAH as

well, although the c^2 value at 10 DAH was higher (4.11, $P = 0.05-0.01$) than the one at 30 DAH (1.53, $P = 0.25-0.10$). In all the above three crosses, segregation at 30 DAH appeared to be more reliable for studying the genetic ratios pertaining to the hull factors, because at that stage the dormant parents N22 and IR13429-287-3 and the three F_1 hybrids involving them tended to be dormant when the hull was intact and showed 70–100% germination when dehulled.

F_3 studies were carried out only in the two crosses involving N22 as the dormant parent (Table 4). Each F_3 family consisted of 35 plants. The results of germination tests at 30 DAH from 45 families of N22/Mahsuri and 36 families of N22/IR19735-5-2 confirmed the monogenic control of dormancy. The percentage of germination in the true breeding families ranged from 0% to 38% in the 11 dormant progeny of N22/Mahsuri; from 0% to 44% in the 7 dormant progeny of N22/IR19735-5-2; from 62% to 100% in the 10 nondormant progeny of N22/Mahsuri; and from 68% to 100% in the 10 nondormant progeny of N22/IR19735-5-2.

The patterns of segregation for the intensity and duration of dormancy of selected F_2 segregants of the cross N22/IR19735-5-2 based on germinations at 10, 30, and 60 DAH are shown in Table 5. Plant #132 was similar in response to the dormant parent N22, and plant #115 was similar to the nondormant parent IR19735-5-2. Plant #87 had only 5% germination at 10 DAH as compared with 47% germination for plant #63. However, plant #87 showed a shorter duration of dormancy than plant #63. Percentages of germination for plant #45 at 10, 30, and 60 DAH were intermediate between those of #87 and #63. These results suggest that possibly the intensity and duration of dormancy are controlled independently. Alternatively, the modifier genes may have a role in regulating both the intensity and duration.

Most earlier genetic studies on seed dormancy in rice indicated that this trait is dominant, as observed in the present studies. However, the reports differed on the nature of genetic control, suggesting control either by one or more major genes and modifiers (25, 30), or by multiple genes (18, 20). Chang and Tagumpay (5) considered that dormancy in the crosses they studied is controlled by a varying number of partially dominant genes that have cumulative and unequal effects. Segregating populations in most of these earlier studies were grown under natural field conditions, and the confounding effects of the environment as well as of kernel dormancy were not considered. This might have resulted in the observation in some cases that dormancy is polygenically controlled. It is possible that dormancy as attributable to individual parts of the grain may be simply inherited, but the combined effects may produce continu-

Table 4. Segregation for seed dormancy in F₃ progenies of two rice hybrids.

Cross	No. of F ₃ families	Source	F ₃ behavior of dormancy		Ratio (A:B)	c ²	P
			No. families bred true (A)	No. families segregating 3:1 D:NDa (B)			
N22/Mahsuri	45	35 dormant (from 0–25% germination class of F ₂ at 30 DAH)	11	24	1:2	0.06	0.90–0.75
		10 nondormant (from 76–100% germination class of F ₂ at 10 DAH)	10	0			
N2211R19735-5-2	36	26 dormant (from 0–25% germination class of F ₂ at 30 DAH)	7	19	1.2	0.48	0.50–0.25
		10 nondormant (from 76–100% germination class of F ₂ at 10 DAH)	10	0			

^aD = dormant, ND = nondormant.

Table 5. Intensity and duration of dormancy in selected F₂ segregants of N22/IR19735-5-2.

F ₂ segregant/parents	Germination %		
	10	30 (days after harvest)	60
F ₂ #132	0	38	96
F ₂ #45	22	45	86
F ₂ #63	47	58	72
F ₂ #87	5	75	100
F ₂ #115	84	100	
N22	0	20	98
IR19735-5-2	77	100	

ous variation. The present studies minimized the variation due to external environmental factors by growing the test material under controlled conditions in the greenhouse. Segregation counts at 30 DAH as explained above steered clear of the effects of kernel dormancy, thus permitting a reliable analysis of the nature of inheritance of dormancy associated with the major factor, viz., hull. Takahashi (26) evaluated the role of the cytoplasm and nucleus of coats, embryo, and endosperm in the dormancy of inbred lines, reciprocal crosses, and F₂ offspring of two varieties and considered that only the genotypes of the embryo and the coat were effective. Present studies did not show evidence of the role of the embryo in dormancy, and the lack of differences between the reciprocal crosses of N22 and Mahsuri suggests the noninvolvement of cytoplasmic factors.

Preharvest sprouting and duration of dormancy

The nondormant parent Mahsuri included in the present studies is known to be susceptible to preharvest sprouting. The pattern of segregation for this trait and its association with duration of dormancy upon maturity were studied in the cross N22/Mahsuri. F₁ seeds behaved like those of the dormant parent N22, with only a negligible amount of sprouting. The F₂ distribution was studied with 86 plants that indicated higher frequency of segregants in the 0–10% and 11–20% sprouting percentage classes (Fig. 1). The F₁ expression and F₂ segregation indicate that resistance to preharvest sprouting is dominant, and from the limited number of F₂ plants the pattern of segregation suggests that resistance is controlled by a single dominant gene along with some modifier genes.

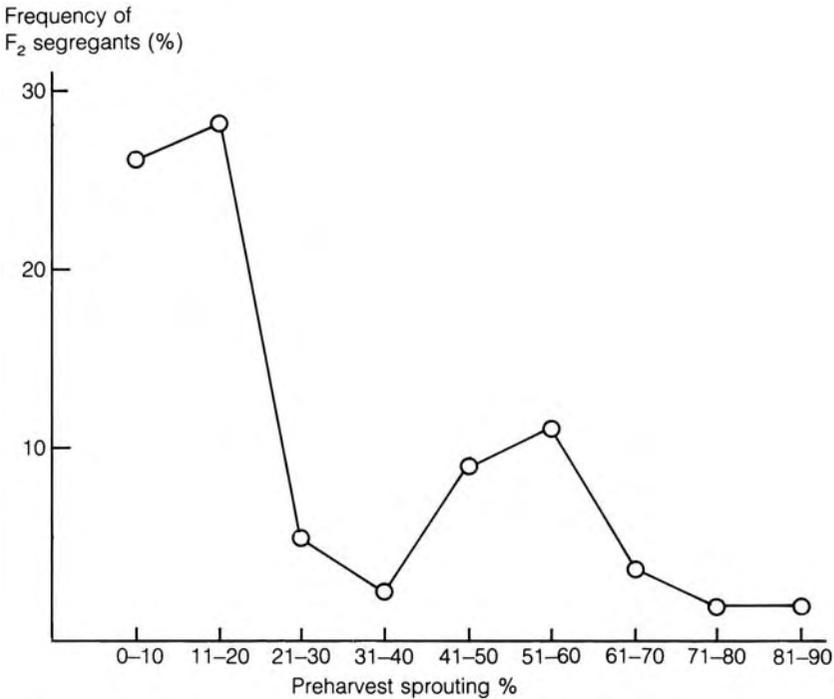


Fig. 1. Distribution of preharvest sprouting percentage in F₂ of N22/Mahsuri. (Mean values were 0% for N22, 61% for Mahsuri, and 5% for F₁ hybrid.)

Among the above 86 F₂ plants, study of the association between the percentage of preharvest sprouting and days required upon maturity to secure 75% germination indicated a significant negative correlation ($r = -0.88^{**}$) between the two traits. This observation is in conformity with the findings of Chen et al (6).

Preharvest sprouting and germination under stress

The 22 cultivars included in the study ranged from 0% to 68% for preharvest sprouting, from 3% to 85% for germination of mature grains at 14 °C, and 0% to 75% for germination under -12 osmotic pressure (Table 6). Germination in the control treatment for low temperature (30 °C) ranged from 89% to 100% and for osmotic pressures ($-\bar{2}$) from 88% to 100%. Significant positive correlations were evident for the association between preharvest sprouting and ability to germinate under

Table 6. Association between preharvest sprouting and germinability under low temperature (14 °C) and osmotic stress (-12) in 44 rice cultivars.

Cultivar	Preharvest sprouting (%)	Germination % at		Germination % under osmotic pressure of	
		14 °C	30 °C	-12 PEG	-2 PEG
Mahsuri	62	80	98	75	96
Caloro	68	85	100	60	93
Dawn	60	62	96		
Blue Bonnet	48	69	100	46	98
Belle Patna	56	60	96	58	100
Zenith	38	58	96		
Fujiminori	70	86	100	68	100
IR8	1	15	92	3	96
IR20	8	28	96	47	95
IR36	2	31	94	33	92
Dular	0	3	89	17	90
Jodo	24	60	98	53	96
P33-C-30	36	68	100	65	95
Szegedi Szekallas	50	52	100	48	100
N22	0	6	98	0	94
IRAT13	48	51	89	60	96
IR19743-46-2-3	42	72	98	85	99
C22	0	15	100	23	100
BG34-8	2	14	100	16	98
IAC25	10	42	92	30	88
BPI 76	0	13	100	7	95
IR13429-287-3	0	20	96	14	97

low temperature ($r = 0.92^{**}$), between preharvest sprouting and germination under osmotic pressure ($r = 0.84^{**}$), and between germination under low temperature and osmotic stress ($r = 0.90^{**}$). Similar observations were made by Yasue and Asai (31), who also found a significant positive correlation between viviparous germination percentage and heading date. The correlation between tolerance to low temperature stress and osmotic stress is probably because of the involvement of a similar mechanism. The semidwarf line IR19743-46-2-3 appears promising for use in cold tolerance breeding programs.

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DISCUSSION

SESSION 6: GENETICS OF PHYSIOLOGICAL TRAITS

Q – Oka: You reported the occurrence of offtypes that carried a gene not present in the parental lines. And you suggested very briefly that a transposable element is involved. Could you give more details on this point?

A – Tsai: Some of the experimental results show strange behavior of genes that are hardly explainable by Mendelian segregation. A favorable explanation would be the assumption that certain lines used for the experiment had inserted some transposable elements in or near the loci concerned. However, I do not know at this moment which of the parental lines have had the assumed transposons. From the viewpoint of the high mutability of the *m* locus, it may be tentatively assumed that an autonomous transposon is attached to the *E* locus and a nonautonomous transposon is attached to the *m* locus. The occurrence of weak and abnormal plants found in progeny of a cross between two late heading lines would serve as support for the working hypothesis.

Q – Seetharaman: You have mentioned that the *E* gene is for late heading and controls photoperiod response. Why do you then symbolize it as *E*?

A – Yamagata: The use of the symbol *E*, such as in *E1*, *E2*, and *E3* genes, dates back to the designation given more than 30 years ago by Syakudo and Kawase. It is supposed that they took up “earliness” in an abstract sense as the most fitting word symbolizing heading time irrespective of earliness or lateness. We have inherited the relevant materials from them together with the symbol *E*.

Q – Second: Phenol reaction is a very useful tool to characterize cultivars during field collection. I would like to know whether the quantitative phenol reaction is influenced by growing conditions and degree of maturity or whether it is purely a genetically determined trait.

A – Takahashi: Our results show that the phenol reaction is controlled by enzymes in the phenol oxidase system. The enzymatic activity is affected by some environmental factors, but the phenol reaction is extremely stable compared with other traits. Strictly speaking, all traits are affected by environmental conditions. The optimum expression of a gene is expected under the right environmental conditions. Phenol reaction is a very useful tool to characterize cultivars, but the examination should be completed within 3 years after harvest because of the reduction of

enzymatic activity. The degree of maturity is not considered an important factor.

Q – Hsieh: Phenol reaction is often used for differentiating indica and japonica types. You showed that a positive reaction occurred in one japonica variety. My question is whether this japonica variety used in your experiment was derived from an indica/japonica cross.

A – Takahashi: The phenol reaction is one of the most important criteria for identifying indica and japonica varieties. The japonica cultivar used in this study is a typical japonica. The results suggest that there is no correlation between seed shape and phenol reaction. In addition, it appears that the seed shape is not so important a factor in identifying indica and japonica cultivars.

Q – Rutger: Are the phenol reactions associated with resistance to any diseases or insects, or with grain dormancy?

A – Takahashi: I think that the phenol reaction is not associated with grain dormancy, because many rice cultivars with strong dormancy show negative phenol reaction. It has been suggested that in rice, resistance to some diseases or insects may be related to some oxidation system.

C – Morishima: Spikelets taken from young panicles do not respond or respond only weakly to phenol even though they carry the *Ph* gene.

Q – Oka: I agree with your method of analyzing dormancy. But there may be several genes for the strong dormancy found in wild rices. Do you agree?

A – Seshu: It is possible that more genes may be involved in strongly dormant wild rices, and that the cultivated dormant varieties may also be expected to differ in respect to the number of genes. Even the present studies indicate that, as far as hull factors in N22 are concerned, dormancy is controlled by a single dominant gene. But there could be other genes associated with kernel dormancy, because our results indicate that dormancy due to hull and kernel dormancy are independently controlled. Our method permits looking at the components of grain dormancy separately rather than considering the grain as a whole as one unit in studying dormancy in segregating populations.

Q – Sahu: It was observed that the late duration indica varieties with high dormancy have longer viability. Can you comment on the relationship between dormancy and viability?

A – Seshu: There is no clear-cut evidence in rice suggestive of the association between duration of dormancy and duration of viability. On the other hand, some studies indicate that varieties differing in duration of dormancy, when stored under identical conditions, do not show significant differences in the period of viability.

GENETICS OF STRESS TOLERANCE

SESSION 7

GENETIC STUDIES ON THE COMPONENTS OF DROUGHT RESISTANCE IN RICE (*ORYZA SATIVA* L.)

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We used a variety of experiments and techniques to investigate the genetic control of component traits that contribute to drought escape and avoidance, and the genetic relationship among three rice germplasm sources: upland, bulu, and aus cultivars. Earliness, which contributes to drought escape in aus rices, was shown to be controlled by a few anisomeric genes. Low to moderate tillering ability in the three variety groups was dominant over high tillering in lowland varieties. Plant height showed multigenic control and the presence of modifying genes. Inheritance of the root system was facilitated by the aeroponic culture technique. Multigenic control of root number was indicated by F_2 data, with high root number slightly dominant over low. Dominant alleles controlled long roots in most upland varieties, while dominant alleles conferred short roots in two semidwarfs. Small degrees of dominance controlled thick roots in upland parents, whereas dominant alleles governed thin roots and low root-shoot ratio in semidwarfs. Strong character association existed among plant stature, tiller number, root length, and root thickness. The three variety groups were closely related to each other as shown by their F_1 pollen fertility, meiotic behavior in F_1 hybrids, and electrophoretic analyses. The upland rice varieties showed more advanced features in seed protein bands as well as in plant morphology than the other two. Implications of the findings in relation to breeding for drought resistance in upland rice are discussed; actual constraints encountered in wide crosses are enumerated; and areas for future research are proposed.

Among crop production factors, drought is one of the primary constraints depressing rice yield in chronic areas and destabilizing crop production in drought-prone areas. It is a yield-limiting factor common to all types of rainfed rice cultures: wetland, upland, and deep water. Breeding for drought resistance was initiated in the early 1970s as one of the major components of IRRI's Genetic Evaluation and Utilization (GEU) Program (3, 11).

The rich genetic diversity present in the two rice cultigens *Oryza sativa* L. and *O. glaberrima* Steud. has provided a continuous spectrum of varietal variations in plant responses to drought at different growth stages. Indeed, the scope of variability in the plant's reactions to drought

is remarkable for a tropically-based semi-aquatic grass (5, 6, 9, 26).

Physiological mechanisms contributing to drought resistance in rice cultigens are:

- escape — mainly due to early maturity,
- avoidance — mainly due to a deep and thick root system and plasticity in leaf rolling and unrolling behavior,
- tolerance — attributable to less understood physiological adjustments in plant tissues, and
- recovery — partly related to vegetative growth vigor.

These terms were defined and discussed by Sullivan et al (31), Chang et al (11), and Loresto et al (21).

Our genetic studies on the drought component focus on escape and avoidance mechanisms, and genetic affinity among diverse sources of drought-resistant germplasm. Our experiments were designed primarily to assist upland rice breeding programs.

GENETIC CONTROL OF GROWTH DURATION

Most traditional upland (or hill) rices of mainland and insular Southeast Asia have a growth duration of 115–130 days. The aus varieties of West Bengal State and the north central region of India (also known as the Gorai varieties) and of Bangladesh are slightly earlier in maturity, ranging from 95 to 105 days. Both variety groups are drought-resistant. Morphologically similar to the hill rices are the bulu and gundil varieties of Indonesia, which are known as the javanica eco-geographic race. However, the javanica varieties are generally late in maturity (150–160 days), having a long basic vegetative phase (BVP), and are irrigated.

Crosses between aus varieties and upland rices showed a unimodal F_2 distribution, markedly skewed in the positive direction. The cross of Aus 252/Khao Lo showed such a segregation pattern (Fig. 1a), which is typical of anisomeric genes — the *Ef* series. The F_2 distribution pattern is identical to the crosses of photoperiod-sensitive/insensitive varieties grown under short daylength (7) where two or three dominant genes having cumulative action conferred the short BVP. The anisomeric genes may differ in the magnitude of their individual effect on the trait (15).

In crosses between upland and bulu varieties where the parents differed more in maturity, the F_2 distributions showed a broader range. However, the distributions were also unimodal, and early-maturing plants predominated (Fig. 1b). The data suggest that the parents differed in a larger number of *Ef* genes than the aus/upland crosses.

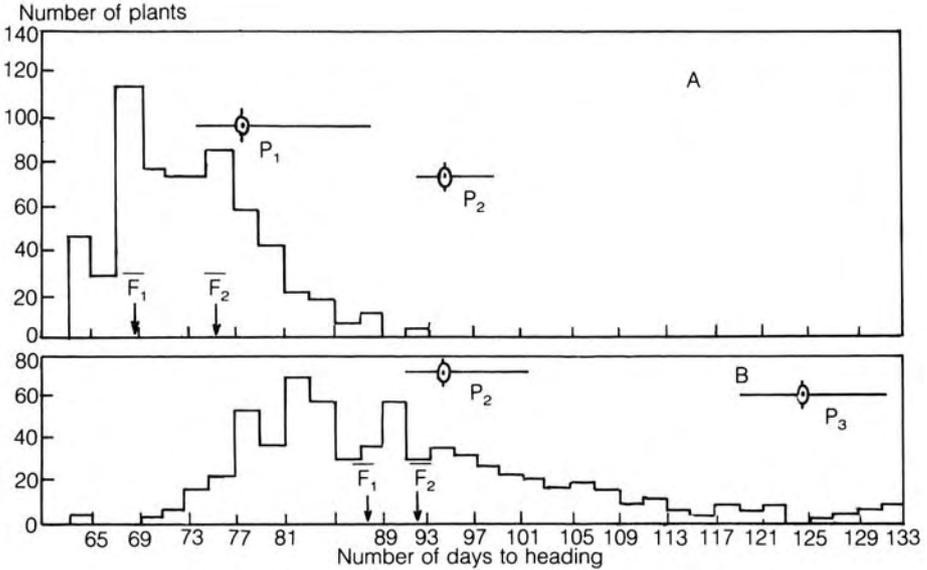


Fig. 1. Distribution and means of parents, F_1 , and F_2 plants by number of days to head classes in the crosses (A) Aus 252 (P_1)/Khao Lo (P_2) and (B) Hawara Batu (P_3)/Khao Lo (P_2). Solid horizontal lines show the range of parents about the mean (dotted circle); arrows show the means of the F_1 and F_2 populations.

The F_2 distributions of the above series of crosses were different from those obtained in an earlier diallel set involving four contrasting parents of low photoperiod sensitivity where the distributions were unimodal and essentially normal. The estimated number of effective gene groups in the diallel set was estimated to be at least four (17). In another set of 5×5 diallel crosses, the heading dates of F_1 plants indicated that dominant alleles exceeded recessive ones by 2.2 times. The effective gene groups were postulated at three (32). The *Ef* genes are more potent than those indicated in the diallel cross.

GENETIC CONTROL OF TILLERING ABILITY

The upland varieties were characterized by their rigidly low tillering habit (10). The aus and bulu varieties have tiller numbers intermediate between the upland and traditional indica varieties of the lowland group.

In both the upland/aus and bulu/upland crosses, low tillering F_2 plants formed the majority, while the F_2 distributions were unimodal and positively skewed (Fig. 2a, 2b). Only a few F_2 plants exceeded the higher parent in tiller number, indicating the dominant nature of low tillering ability.

The segregation patterns of these crosses are somewhat different from those obtained in the diallel set of lowland varieties mentioned earlier, where the distribution of panicle number was essentially normal and transgressive segregants for higher tiller numbers were found in each of the six crosses (17). High tiller number also showed dominance in F_1 plants of a 5×5 diallel set of lowland varieties (33). Our data indicate that the three variety groups have accumulated genes for low tillering.

GENETIC CONTROL OF PLANT STATURE

Among the three variety groups, the aus varieties have the shorter plant stature, while both upland and bulu varieties are tall. The F_2 segregations of culm length in two sets of crosses showed essentially normal distribution, slight negative skewness, and transgressive segregation on both ends. The data indicate multiple genes, and modifying genes were probably involved. Our findings are similar to those obtained in diallel crosses (17, 20, 32).

GENETIC CONTROL OF THE ROOT SYSTEM

The traditional upland varieties of Southeast Asia and of West Africa (also belonging to *O. sativa*) are marked by a system of low root number, a high proportion of long and thick roots, and a high ratio of root-shoot weights (1, 10, 19, 25). On the other extreme, the semidwarf lowland varieties have numerous, thin, and shallow roots (10, 29). The aus and bulu varieties were intermediate in the above respects, while the aus varieties were superior in root thickness and inferior in root number to the bulu rices (30). We have used the aeroponic culture technique extensively to study rice roots (2). The technique has been shown to be efficient and gives reproducible data within a cycle of 50 days (22).

In a set of 8×8 diallel crosses involving three upland, one traditional lowland, and four semidwarf parents, the following findings were obtained from the F_1 plants (1):

- Maximum root length: Deep roots are controlled by an excess of dominant alleles in three African upland parents and by recessive alleles in two parents (one lowland and one semidwarf). On the other hand, an excess of dominant alleles control shallow roots in two semidwarf parents (IR8 and IR20).

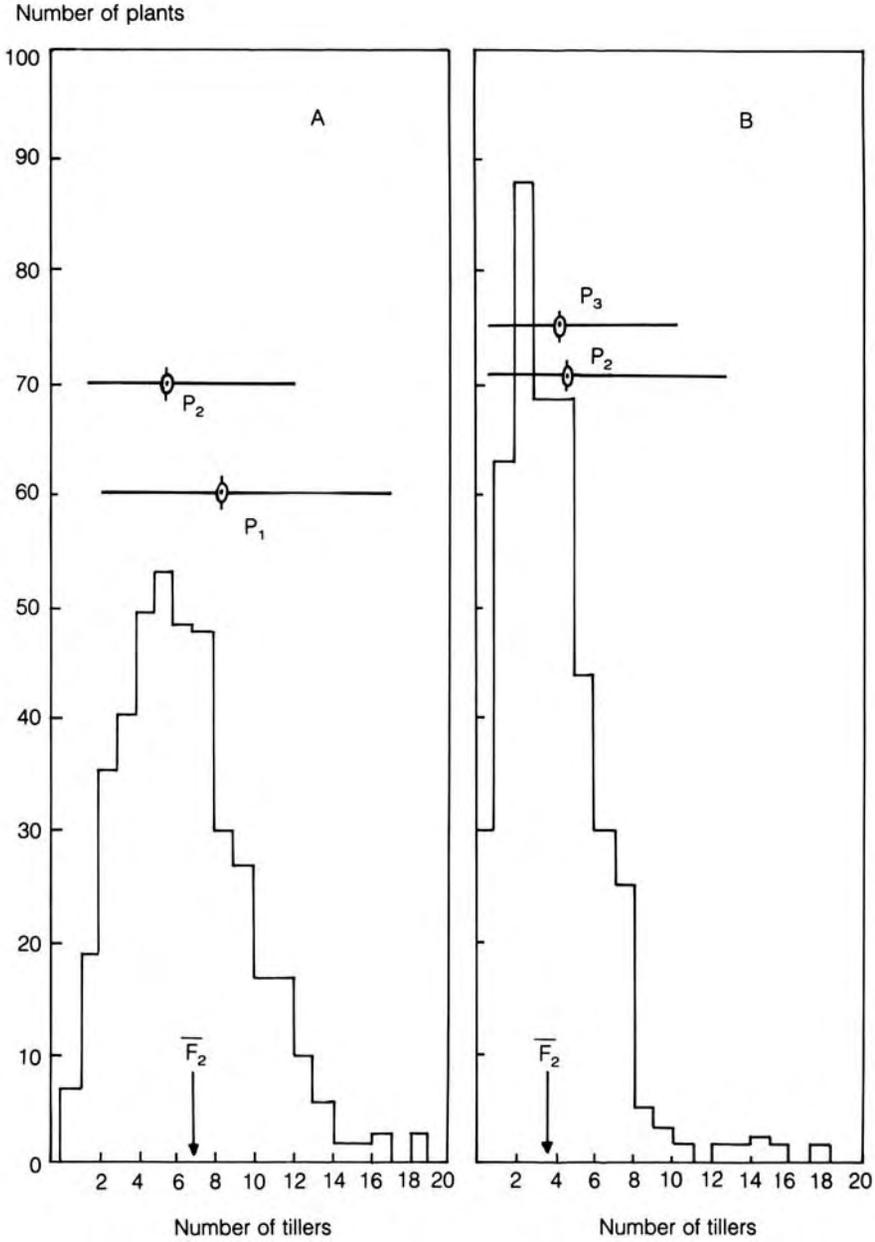


Fig. 2. Distribution and means of parents and F_2 plants by tiller number class in the crosses (A) Aus 252 (P_1)/Khao Lo (P_2) and (B) Hawara Batu (P_3)/Khao Lo (P_2).

- Root thickness: Thick roots in two African parents are controlled by an excess of recessive alleles and by dominant alleles in another African upland (OS4). Two thin-rooted semidwarfs and a lowland variety from India (MGL-2) carry dominant alleles for fine roots, while IR8 has recessive alleles for thin roots.
- Root number: Dominant alleles control high root number in the semidwarfs and MGL-2, while the African upland parents show a combination of low root number and recessive alleles.
- Root-shoot weight ratio: An excess of recessive alleles confer high root-shoot ratios in most parents, whereas Moroberekan (African upland) has a combination of dominant alleles and a high ratio. Dominant alleles are associated with low ratios (1).

Data on F_2 distribution in eight crosses were also obtained. Results from two sets of experiments (1, 23) are summarized below:

- Maximum root length: In upland/semidwarf crosses, multiple genes with both dominance and additive effects were indicated. Overdominance of long roots was frequently observed (Fig. 3).
- Root thickness: Segregation for root thickness showed considerable variation among crosses. In four crosses between African upland varieties and IR20, the F_2 distributions suggest slight dominance in IR20/20A, very small or no dominance of thick roots in OS4/IR20, Moroberekan/IR20, and Kinandang Patong/IR20. In the above crosses, the parents differed by 0.4–0.7 mm in thickness, whereas in the cross IR20/MGL-2 (differing by 0.3 mm in root thickness) the distribution was essentially normal (23). On the other hand, thin roots showed partial dominance in OS4/IR8 (1).
- Root number: The F_2 distributions were generally normal, and many transgressive segregants frequently appeared on the upper end. Smaller numbers of F_2 plants inferior to the lower parents were observed in all six crosses.
- Dry root weight: A positive skewness with low root weight predominating the F_2 distributions was generally observed, although a small number of F_2 plants had greater root weight than the high parent (Fig. 4).

We also studied character association among F_2 plants in 18 crosses. The general trends obtained from crosses involving contrasting parents are as follows (1, 30):

- Root length was positively and significantly correlated with root thickness, root weight, root-shoot weight ratio, and plant height.
- Root thickness was positively and significantly correlated with root weight and plant height.

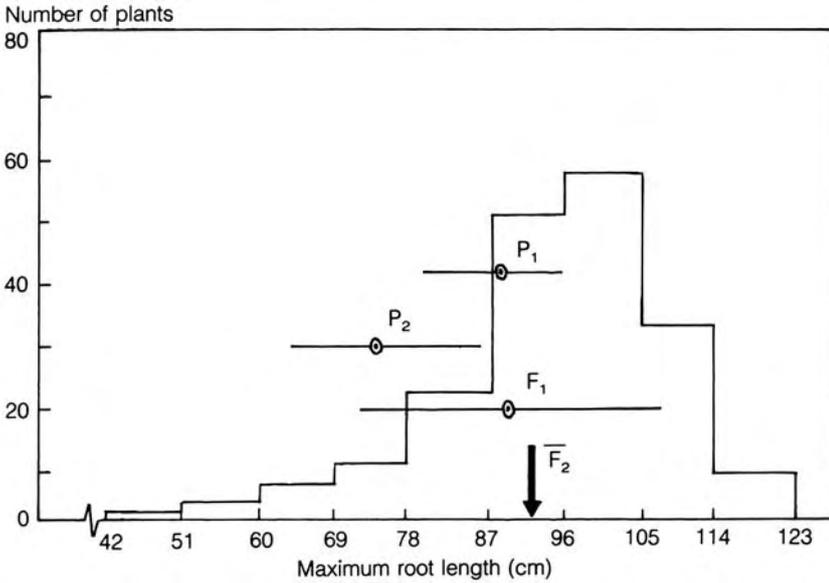


Fig. 3. Distribution and means of parents and F₁ and F₂ plants by maximum root length class in the cross OS4 (P₁)/IR8 (P₂).

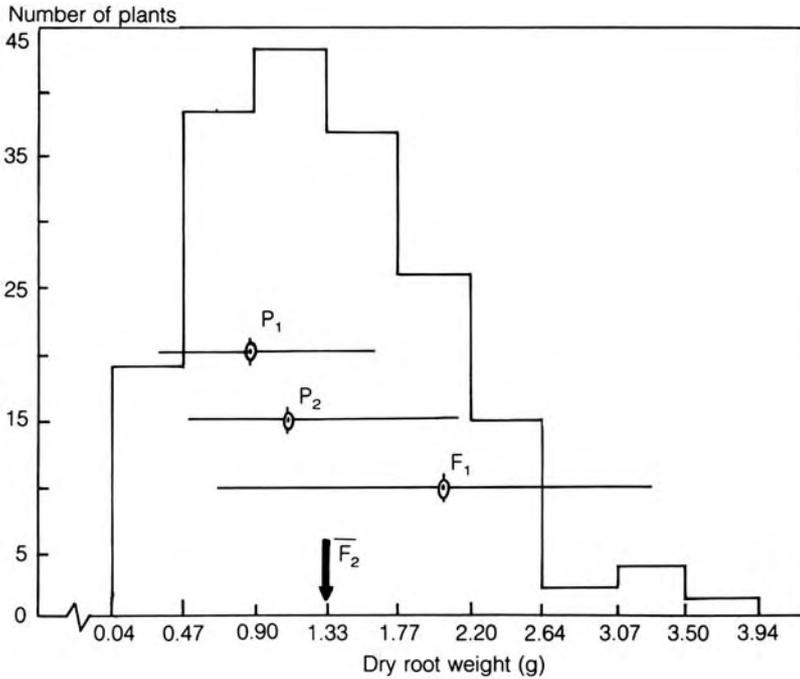


Fig. 4. Distribution and means of parents and F₁ and F₂ plants by dry-root weight class in the cross OS4 (P₁)/IR8 (P₂).

- Root thickness was negatively correlated with tiller number and root number.
- Association among root length, root number, and tiller number varied among different crosses.

GENETIC AFFINITY AMONG UPLAND, AUS, AND BULU VARIETIES

Intercrosses among upland, aus, and bulu varieties produced highly fertile F_1 hybrids, ranging from 83% to 85% in pollen fertility (Fig. 5). The upland varieties showed a slightly higher pollen fertility (85.50% and 85.34%) in their crosses with the other two groups, while aus/bulu crosses gave a slightly lower (83.20%) level. Low frequencies of minor chromosomal aberrations (loose pairing, univalents, chain-of-four, laggards, and bridge and fragments) were observed in the pollen mother cells of 146 hybrids (12). The frequency of chromosomal aberrations was lower than those identified in indica/japonica crosses (13) and in indica/javanica crosses (14). The above findings show that there is a close genetic relationship among the three varietal groups and that the upland rices are closer to the bulu rices than to the aus rices. Our findings are in agreement with the earlier postulate of Morinaga and Kuriyama (24) that the aus and bulu varieties form the intermediate type between indica and japonica.

Electrophoretic analyses have been made on three selected varieties each from the three groups, and on the F_1 hybrids of 145 intercrosses (28). Among seed proteins, esterases, and peroxidases, the tris-HC1 soluble seed proteins gave a better differentiation among parents than the two isozymes (Fig. 6). The upland varieties appeared to be more advanced than the other two groups in terms of the number of protein bands, confirming a point stressed earlier by Chang (4) on the basis of leaf, root, panicle, and grain characteristics.

When the distribution frequencies of all esterase or peroxidase bands in reciprocal parent arrays of 138 F_1 hybrids were averaged and arranged in a two-way diagram, the hybrid populations of the 13 parents separated into four clusters:

- all hybrids of the four aus varieties,
- hybrids from three bulu parents and one upland parent,
- hybrids of one bulu and three upland varieties, and
- hybrids derived from the Philippine upland variety Kinandang Patong, which has shown an intermediate position between aus and bulu varieties in esterase and peroxidase data (27).

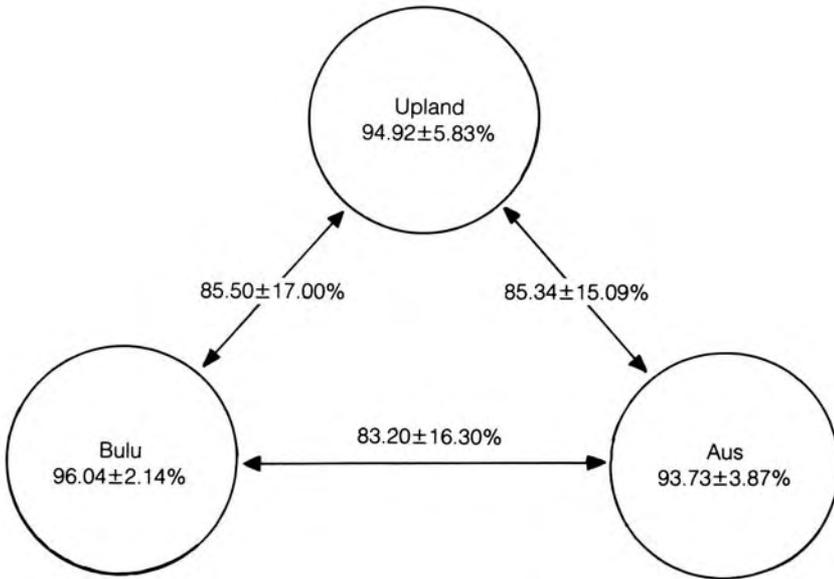


Fig. 5. Pooled mean pollen fertility of intra- and inter-group hybrids of bulu, aus, and upland rices (12).

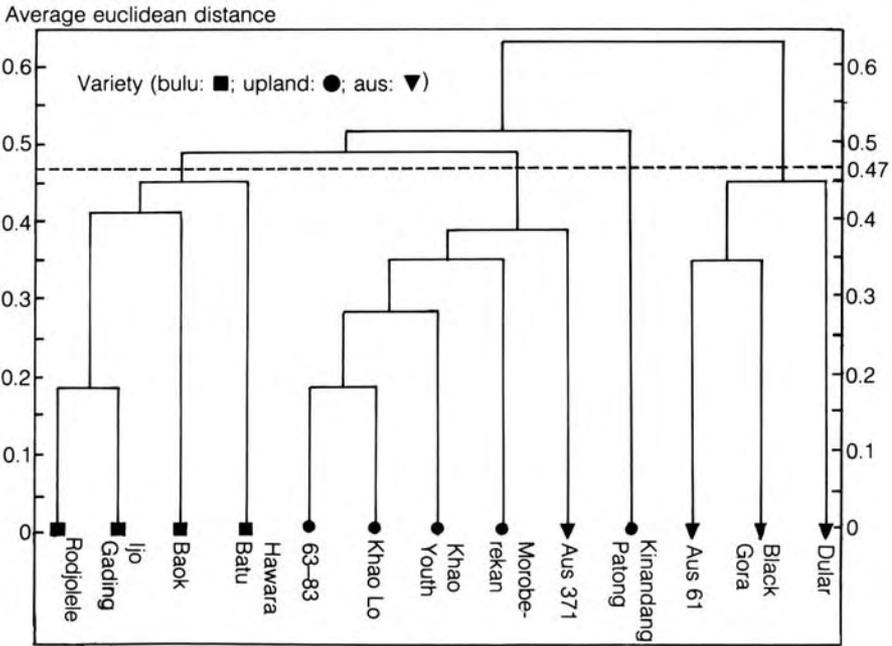


Fig. 6. Dendrogram showing intervarietal relationships obtained by weighted pair group method clustering of seed protein electrophoretic data (27).

CONCLUSIONS

The following concluding remarks may be made:

- Our search for useful sources of drought resistance has led to the identification of the aus and bulu rices as additional donors. The aus varieties can contribute early maturity and extensive root system, while the bulu rices have desirable panicle and grain characteristics and a root system superior to many lowland rices.
- The finding of a deep and thick root system in conferring drought avoidance has stimulated an intensive search for such a desirable feature and has motivated the use of different methods in evaluating root systems (8). Adoption of the aeroponic culture technique has greatly aided in the genetic studies of the rice root system in relation to shoot growth. Hitherto, there was a paucity of information about varietal differences in rice roots and their genetic controlling mechanisms.
- Our genetic studies have shown the ease of incorporating high root number and long roots into an agronomically desirable background, but a lower feasibility in recombining thick roots, intermediate plant height, and high tiller number when the traditional upland rice varieties are used in crosses. Such constraints have been experienced in our breeding activities. Meanwhile, drought avoidance and recovery ability are negatively correlated (9).
- Genetic affinity among the upland, bulu, and aus varieties is high enough to facilitate large-scale crossing and selection, whereas the genetic barriers existing in crosses between the upland and lowland rices (both traditional and semidwarfs) still pose a constraint in hybridizing the two cultural groups. Genetic evidence on such barriers has been demonstrated (16, 18).
- We need to investigate further the genetic relationship between the tropical upland varieties and aus varieties on the one hand and the drought-resistant upland varieties of Japanese origin on the other. The drought tolerance and recovery ability found in many cultivars adapted to rainfed wetland culture also need to be studied.

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GENETICS OF SALT TOLERANCE IN RICE

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Salt tolerance studies were conducted under controlled conditions by using solution and pot culture techniques at the International Rice Research Institute. Varieties Nona Bokra and Pokkali (tolerant), Damodar and Jhona 349 (moderately tolerant), and IR28 and IR35657-33-2 (sensitive) were selected for genetic analysis of traits under salinization (EC 12 dS/m) at the seedling and reproductive stages. Both additive and dominance effects were important in the inheritance of all the traits studied. Shoot length, Na and Ca content in the shoots, and dry weight of shoots and roots showed significant additive effects with a high degree of heritability. Genes controlling Na and Ca levels in shoots were found to be partially dominant. At least three groups of genes were found to be involved in the inheritance of Na and Ca levels at the seedling stage. Highly significant additive effects were also observed for plant height and yield/plant, with high heritability values. Selection on the basis of shoot length, Na level in the shoots, dry weight of shoots and roots, plant height, and yield/plant showing predominance of additive effects and high heritability values could lead to the development of salt-tolerant cultivars.

Recently much emphasis has been placed on the utilization of the saline soils of South and Southeast Asia for rice cultivation (1). Rice production in saline soils could be increased considerably if salt-tolerant varieties were developed. One of the limitations in the development of tolerant rices is our inadequate knowledge of the genetics of salt tolerance. Only a few studies on the inheritance of salt tolerance have been reported (3, 4); they indicate that resistance to salinity-induced panicle sterility in rice is dominant and controlled by at least three genes. The present studies were conducted to gather more information on the mode of inheritance of salt tolerance by diallel cross analysis.

MATERIALS AND METHODS

Since tolerance in rice varies with the stage of development, two experiments were conducted at IRRI in 1984, one at the seedling stage and the other from seedling to maturity.

Genetic analysis of traits under salinization of seedling stage

The experimental material for Experiment I consisted of a diallel set of six varieties. Two tolerant (Nona Bokra and Pokkali), two moderately tolerant (Damodar and Jhona 349), and two sensitive (IR28 and IR5657-33-2) varieties were crossed in all possible combinations, excluding reciprocals. The 15 F_1 's and their parents were evaluated at 6800 ppm (EC 12 dS/m) at the seedling stage in the glasshouse of the phytotron, where temperatures of 29°C and 21°C (day/night) and a relative humidity of 70% were maintained.

Seeds were sterilized with 0.1% $HgCl_2$ for 2–3 min. They were then soaked in water for 24 h and incubated for 48 h at 30°C. Pregerminated seeds were sown one seed per hole on a styrofoam sheet with 60 holes and a nylon net bottom. The nutrient solution (8) was provided for 14 days, then seedlings were subjected to salinization (EC 12 dS/m) by adding a 1:1 mixture of NaCl and $CaCl_2$ to the nutrient solution. The pH of the solution was maintained at 5.0. The nutrient solution was renewed once a week, while pH and water level were adjusted daily by either 1N H_2SO_4 or 1N KOH. Salinization continued for one month. A randomized complete block design with three replications was used. Twenty plants for each parent/ F_1 were grown in each replication. Data were recorded for various morphological characters on 10 plants selected randomly from each replication. Plant analysis for Na and Ca was done by atomic absorption (7) and was expressed on a percentage dry weight basis. The procedure suggested by Hayman (5) was used for genetic analysis. Narrow sense heritability was calculated as

$$\frac{0.5 D}{0.5 D + 0.25 H + E}$$

Genetic analysis of traits under salinization at maturity

A pot culture experiment (Experiment II) was conducted in the greenhouse. All possible crosses excluding reciprocals were made among the four varieties Jhona 349, Nona Bokra, Pokkali, and IR28. Seedlings of the parents and the six F_1 's were raised in nutrient solution in the glasshouse

of the phytotron under controlled temperatures of 29°C and 21°C (day/night) and a relative humidity of 70%.

Thirty-day-old seedlings were transplanted to pots having 14 kg of Maahas clay soil. A glass tube wrapped with glass wool at the bottom of each pot resulted in recycling of the solution. Each pot with four seedlings was placed in a tank filled with water to minimize temperature fluctuations. Salinization (EC 12 dS/m) was done 1 week after transplanting by using a 1:1 mixture of NaCl and CaCl₂. The soil was kept submerged up to 2.5 cm throughout the experiment by adding demineralized water. The soil solution in each pot was recycled every week to minimize stratification of salts. All other normal agronomic practices were followed. A randomized complete block design with three replications was used. The mean maximum temperature during the experimental period (August-early December) ranged from 29.9 °C to 31.8 °C, and the mean minimum temperature ranged from 23.4 °C to 23.9 °C, while the relative humidity ranged from 77% to 81%. Radiation ranged from 315 to 490 m Whr/cm². Data were recorded for various characters. Plant and diallel analyses were done as described in Experiment I.

RESULTS

Highly significant differences were observed at the seedling stage for salt tolerance among the parents. Differences among hybrids and between parents and hybrids were highly significant for most characters. At maturity highly significant differences were also observed for the parents in five of the six traits studied. Differences among hybrids were significant for all characters studied except Ca content in straw. The comparisons of parents vs hybrids were significant in three traits (Tables 1, 2).

Genetic analysis of traits under salinization at seedling stage

The assumptions of diallel analysis were met due to the nonsignificance of t^2 and the nonsignificant deviation of the regression coefficient b from zero for all characters. However, the regression coefficient b deviated significantly from unity for root length, denoting the failure of at least one of the basic assumptions, and this suggested the presence of genic interaction.

The statistics representing additive (D) and dominance (H_1) effects were highly significant for all characters (Table 3). The h^2 was also highly significant for all characters except Na content in roots. Both

Table 1. Analysis of variance for various characters in a 6 × 6 diallel cross in rice at the seedling stage.^a

Genotype	Shoot length (cm)	Root length (cm)	Dry wt. of shoots (g)	Dry wt. of roots (g)	Shoot content		Root content	
					Na (%)	Ca (%)	Na (%)	Ca (%)
Parents (P)	293.06**	47.92**	50.87**	42.82**	23.31**	26.97**	9.92**	40.87**
Hybrids (H)	91.74**	70.13**	28.52**	11.66**	1.05	3.96**	10.57**	0.31
P vs H	173.73**	296.01**	111.31**	84.23**	31.55**	28.88**	4.07	41.73**

^a** = significant at the 1% level.

Table 2. Analysis of variance in a 4 × 4 diallel cross in rice at maturity.^a

Genotype	Height (cm)	Productive tillers/ plant	Seed set (%)	Yield/ plant (g)	Straw content	
					Na (%)	Ca (%)
Parents (P)	177.35**	1.97	12.73**	9.96**	34.20**	15.49**
Hybrids (H)	42.37**	7.82**	49.05**	5.99**	2.91*	0.98
P vs H	0.14	2.87	42.62**	2.97	12.98**	5.45*

^a * = significant at the 5% level, ** = significant at the 1% level.

additive and dominance effects appear to govern the inheritance of the characters studied. However, a preponderance of additive gene action was revealed in the inheritance of shoot length and Na and Ca content in shoots. A predominance of dominant gene action was revealed in the determination of root length and Na and Ca content in roots. The relatively low value of narrow sense heritability for root length (36.2%) and Na content in roots (35.9%) also suggested that the additive genetic variation contributed only a small portion of the phenotypic variation for these characters. The high level of significance of D for shoot length and Na and Ca content in shoots, and the high values of heritability estimates ranging from 72.5% for Na content in shoots to 81.2% for shoot length also indicated that a major part of the variance was additive and/or additive × additive in nature. High narrow sense heritability values were also obtained for dry weight of roots and shoots and Ca content in roots. The significant but lower values for H₂ in relation to H₁ indicated asymmetry of positive and negative effects of genes for all traits.

The overall degree of dominance as indicated by the ratio $(H_1/D)^{1/2}$ was between zero and unity for shoot length, dry weight of shoots and roots, and Na and Ca content in shoots, suggesting partial dominance of genes. The values suggested overdominance of genes for root length and Na content in roots, and slight overdominance to complete dominance of genes for Ca content in roots.

The average frequency of positive and negative alleles in the parents indicated some asymmetry for all characters, since the ratio $H_2/4H_1$ was less than 0.25, suggesting that positive and negative alleles of genes do not appear to be distributed equally among the parents. The value $[(4DH_1)^{1/2} + F/(4DH_1)^{1/2} - F]$ provides an estimate of the proportion of the total number of dominant to recessive genes in all the parents. Asymmetry and an excess of dominant genes was indicated for all the traits. The ratio for shoot length, Na and Ca content in shoots, and Ca content in roots was more than unity, and the positive and significant F

value also suggested that the positive alleles for these characters may be slightly in excess.

The negative correlation between the order of dominance of the parents ($W_r + V_r$) and the parental measurement Y_r indicated that higher shoot length, root length, and dry weight of shoots and roots were dominant. The correlation for root length and dry weight of roots was negative and nonsignificant, suggesting that some of the genes showed dominance in the positive direction (greater number), while some acted in a negative direction. The $r(W_r + V_r, Y_r)$ indicated dominance for low Na and Ca content in both roots and shoots. The ratio h^2/H_2 estimates the number of groups of genes that control the character and exhibit some dominance. Approximately three groups of genes were involved in Na and Ca content in the shoots, while two groups of genes were noted for root length and dry weight of shoots and roots. If the positive and negative effects cancel each other the ratio of dominance will be underestimated. However, the dominance was significantly unidirectional to cause h^2 to be highly significant in all but one trait. Therefore, in all probability this estimate of the number of groups of genes in the present study may approximate the actual number.

Genetic analysis of traits under salinization at maturity

The t^2 was nonsignificant for all the traits measured for salt tolerance at EC 12 dS/m, suggesting fulfillment of the assumptions. Failure of at least one of the basic assumptions and the presence of genic interaction was revealed for productive tillers and seed set percentage as the regression coefficient b was not significantly different from zero. The regression coefficient b was, however, not significantly different from unity for all characters. Plant height, yield/plant, and Na and Ca content in straw fulfilled all the assumptions of the diallel analysis.

The genetic components representing additive (D) and dominance effects (H_1) were all highly significant, suggesting the importance of both additive and dominance components in the inheritance of all the characters (Table 4). The h^2 was significant, however, only for yield/plant. The value of component D was higher for the characters plant height and yield/plant, suggesting the greater importance of additive gene action in the inheritance of these traits. The relatively high values of heritability for plant height and yield/plant, and the high to moderately high values for Ca and Na content in straw and seed set percentage indicated that the proportion of the genetic portion of variation to the total variation was high.

Table 3. Estimates of genetic parameters in a 6 × 6 diallel cross in rice under salinization.^a

Components of variation	Shoot length (cm)	Root length (cm)	Dry wt. of shoots (g)	Dry wt. of roots (g)	Shoot content		Root content	
					Na (%)	Ca (%)	Na (%)	Ca (%)
D	490.513**	9.071**	22.364**	.899**	.152**	.088*	.012**	.004**
F	154.607*	-4.278	-.013	.392	.136**	.049**	.004	.006**
H ₁	219.279**	31.135**	22.041**	.705**	.088**	.034**	.037**	.005**
H ₂	190.070**	21.239**	19.336**	.572**	.058**	.025**	.033**	.002**
h ²	148.962**	26.276**	28.462**	.951**	.141**	.062**	.007	.002**
(H ₁ /D) ^{1/2}	0.669	1.853	993	.886	.762	.620	1.791	1.080
H ₂ /4H ₁	.217	.171	.219	.203	.165	.187	.224	.122
K _D /K _R ^b	1.615	.773	.994	1.638	3.828	2.638	1.200	5.546
r(Wr + Vr, Yr)	-.81	-.35	-.76	-.46	.96	.97	.79	.75
h ² /H ₂	.784	1.237	1.422	1.662	2.415	2.480	.223	.880
Heritability	81.2	36.2	64.0	67.7	72.5	78.7	35.9	60.0

^a** = significant at the 5% level, * = significant at 1% level.

^bK_D/K_R = (4DH₁)^{1/2} + F/(4DH₁)^{1/2} - F.

Table 4. Estimates of genetic parameters in a 4 × 4 diallel cross in rice under salinization.^a

Components of variation	Height (cm)	Productive tillers	Seed set (%)	Yield/plant (g)	Straw content	
					Na (%)	Ca (%)
D	5276.307**	12.074**	1694.209**	36.359**	.239**	.248**
F	6858.379**	20.855**	2794.763**	3.919	.310**	.409**
H ₁	3631.148**	23.533**	2992.621**	24.180**	.313**	.261**
H ₂	358.057	9.681**	1172.057	14.644**	.200**	.078**
h ²	-4.481	1.624	652.061	8.839*	.069	.032
(H ₁ /D) ^{1/2}	.688	1.949	1.766	1.283	1.310	1.052
H ₂ /4H ₁	.025	103	.098	.151	.160	.075
K _D /K _R ^b	8.235	1.619	4.271	1.202	3.616	9.194
r(Wr + Vr, Yr)	-.13	-.45	.47	.99	.96	.91
h ² /H ₂	-.013	.168	.556	.604	.345	.410
Heritability	74.2	47.8	52.7	66.0	58.6	63.3

^a* = significant at the 5% level, ** = significant at the 1% level.

^bK_D/K_R = (4DH₁)^{1/2} + F/(4DH₁)^{1/2} - F.

The average degree of dominance given by the ratio $(H_1/D)^{1/2}$ suggested partial dominance of genes for plant height, complete dominance or slight overdominance for Ca content in straw, and overdominance for productive tillers, seed set percentage, yield/plant, and Na content of straw.

The proportion of h^2/H_2 suggested a minimum of one gene group for all the traits under study. The average frequency of positive and negative alleles in the parents as revealed by the ratio $H_2/4H_1$ indicated that positive and negative alleles of genes were not in equal proportion. Asymmetry of the number of dominant and recessive genes in the parents and an excess of dominant genes for all the traits was indicated by the proportion $[(4DH_1)^{1/2} + F/(4DH_1)^{1/2} - F]$, which was found to be greater than unity. The positive correlation between the order of dominance between the parents ($W_r + V_r$) and the parental measurement (Y_r) indicated lower yield/plant, seed set percentage, and Na and Ca content in straw to be dominant. The correlation for plant height and productive tillers was negative and nonsignificant, suggesting that some of the genes show dominance in a positive direction while some act in a negative direction.

DISCUSSION

For the development of salt-tolerant rice varieties, the donors are usually selected on the basis of seedling survival or leaf injury symptoms, and these criteria may or may not hold true at later stages of plant development. In such a situation the understanding of the genetic basis governing salt tolerance through diallel cross analysis would be of great help. In the present study the genetic components of variation at the seedling and maturity stages indicated both additive and dominance effects to be important in the inheritance of all characters studied. At the seedling stage characters like shoot length, Na and Ca content in the shoots, and dry weight of shoots and roots showed significant and high magnitude of additive effects. The estimates of heritability in these characters were also high. The high heritability values observed in the present study could have been possible because of controlled environmental conditions, as salt tolerance is highly modified by various environmental factors. These characters as such appear to have high predictive value and may be used as criteria for selection of salt-tolerant lines at the seedling stage. Selection for salt tolerance based on such characters can also be done in early generations because of the high additive effects governing their inheri-

tance. Selection based on root length, or Na and Ca content in the roots may not prove effective, as these traits have shown low heritability values and predominance of dominant gene action. From the present study it appears that at least three groups of genes are involved in the inheritance of Na and Ca content in the shoots at the seedling stage. Resistance to salinity-induced panicle sterility has earlier been reported to be a dominant character, controlled by at least three pairs of genes (4).

The present results also indicate high additive effects for plant height and yield/plant at maturity. The heritability values for these characters were also high, ranging from 63% to 74%. Unlike at the seedling stage, the Na and Ca content in the straw showed predominance of dominant gene effects. This suggests that selection for Na and Ca content can best be practiced at the seedling stage. The high dominance effects and low heritability for productive tillers and seed set percentage suggest postponement of selection for these traits to later generations, when dominance effects will have dissipated. The high additive effects for yield, however, suggest that better yielding genotypes can be developed for saline conditions. It has been demonstrated that salt-tolerant lines can be obtained from tolerant/sensitive and tolerant/tolerant crosses (2, 6). Selection based on the criteria discussed above both at the seedling and the adult plant stage thus appears to have potential in the development of salt-tolerant cultivars.

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GENETIC ANALYSIS OF SALT TOLERANCE IN MANGROVE SWAMP RICE

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Salinity tolerance was determined by a comparative measurement of root growth in saline and in nonsaline conditions. There was good evidence that tolerance differences are largely genetic in origin. Biometrical analysis of the data showed additive genetic variation and the dispersion of genes determining the maternal effect. Some evidence of dominance was found. Heritability was moderately high for seedling tolerance ratio, indicating the possibility of early selection for salt tolerant individuals. Transgressive segregation in the direction of high tolerance was observed.

Salinity is one of the main factors limiting rice production and the expansion of cultivable areas in the mangrove swamps of Gambia, Guinea, Guinea Bissau, Nigeria, Senegal, and Sierra Leone in West Africa. An estimated 1.5 million ha of cultivable mangrove swamps are affected by salinity in these countries. Therefore, the development of highly salt tolerant rice varieties could be of considerable benefit to rice production.

Work on the adaptation of rice to concentrations of salt has shown that considerable variability exists within the most widely cultivated rice species *Oryza sativa* (1,6,11). The variability in salinity tolerance of existing rice varieties has not been used in the past by plant breeders because of insufficient knowledge about the genetics of salt tolerance in rice. Studies on the genetics of salt tolerance are needed to assist breeders in the incorporation of this character into improved varieties.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the WARDA Mangrove Swamp Rice Research Station in Rokupr. The following generations were used:

- P₁ = the higher scoring parent
- P₂ = the lower scoring parent

$$\begin{aligned} F_1 &= P_1/P_2 \\ F_s &= F_2 \text{ selfed} \\ BC_1 &= P_1/F_1 \\ BC_2 &= P_2/F_1 \end{aligned}$$

The data were analyzed with the following assumption, of which only (e) was untestable.

- (a) No genotype-environment interaction
- (b) Diploid segregation
- (c) Homozygous parents
- (d) No linkage and no nonallelic interaction
- (e) No multiple allele
- (f) Uncorrelated gene distribution in the parents

Four rice varieties — Pokkali, Pa Merr 108A, Djabon, and BG 90–2 — were selected on the basis of degree of salt tolerance and other characters, as indicated in Table 1.

Seeds of each variety were pregerminated for 48 h and raised in buckets for 1 week. Four healthy seedlings of each variety were then transplanted in a plastic tray measuring 35 × 35 × 20 cm. Six weeks after transplanting, they were taken into a dark room at 1700 h and taken out the next day at 900 h. This was done for 15 days to synchronize flowering, as the parents involved photosensitive or weakly sensitive varieties. Two weeks later flowering started in the varieties.

The parental varieties along with population of F_1 , F_2 , BC_1 , and BC_2 of each cross were tested for salt tolerance by the root growth technique. Carefully selected filled seeds of each variety or cross, pregerminated for 48 h, were placed through a drilled hole on the mesh screen bottom of a plywood tray. The seeds were allowed to develop for 7 days in normal culture solution. The root length of the seedlings was measured 7 and 11 days after germination. Then they were transferred and allowed to grow in 80 mM NaCl culture solutions. Root length was measured again 13 and 17 days after germination. Salinity tolerance was calculated as the tolerance ratio (TR) of root growth after 4 days in saline solution to root growth after 4 days in nonsaline solution. The plants most tolerant of salt had the highest tolerance ratio.

Heritabilities were calculated as:

$$\begin{aligned} h_b^2 \quad (\text{broad sense heritability}) &= (\frac{1}{2}D + \frac{1}{4}H)/(\frac{1}{2}D + E) \\ h_n^2 \quad (\text{narrow sense heritability}) &= \frac{1}{2}D/(\frac{1}{2}D + \frac{1}{4}H + E) \end{aligned}$$

RESULTS

Biometrical analysis

The observed values of all the generation means and their weights, calculated as the reciprocal of the variance of the mean and the number of plants on which the means and variance were based, are shown in Table 2. The mean tolerance ratios of the F_1 plants from both crosses generally fell between the parental means, but the mean TR of the F_2 in the Pokkali/Djabon cross was higher than the value obtained from Pokkali, the higher scoring parent. This heterosis in the F_2 may suggest the presence of a maternal effect on the progeny. In this study, the lower scoring parents, Djabon and BG 90-2, were used as the female parents in the crosses. Therefore, in the presence of a maternal effect, the F_1 would show greater resemblance to the maternal parent while contributing to the performance of the progeny.

It was possible to assess whether the variation observed in the generation means can be explained on an additive-dominance basis or whether the interaction between genes at different loci is important. This was achieved by using the scaling test developed by Cavalli (4) and used by Jinks (7, 8, 9) for the detection of nonallelic interaction. The test consists of estimating three parameters — m , $[d]$, and $[h]$ — by weighted least square, taking as weight the reciprocal of the variance of each generation mean. The terminology represented is that of Fisher et al (5), where individuals with genotype AA have the quantitative phenotype of da , genotype Aa phenotype ha , and genotype aa has phenotype $-da$. The midparent is the origin m used in measuring the effect of various combinations of genes at a single locus; $[d]$ represents the balance of the additive gene effects either in the positive or negative direction from the origin; and $[h]$ indicates the balance of the dominance effects (10).

It was possible to compare the observed and expected generation means as the sum of squares minimized in the fitting process — SL_i ($gi - Egi$)², where L_i is the amount of information about the generation mean and is distributed as c^2 with a degree of freedom equal to the number of generation means minus the number of parameters estimated.

The estimated values of the parameters m , $[d]$, and $[h]$ and the results of the scaling test are as shown in Table 3. It is obvious from the c^2 test that the model failed to fit the tolerance ratio in the Pokkali/Djabon cross, while it just adequately described the variation observed in the Pa

Table 1. Some characteristics of four parental varieties.

Variety	Origin	Type	Parent	Photoperiod sensitivity	Salt tolerance	
					Score	Reaction
Pokkali	India	traditional	male	nonsensitive	0.52	high tolerance
Pa Merr 108A	Sierra Leone	traditional	male	sensitive	0.51	high tolerance
Djabon	Indonesia	improved	female	weakly sensitive	0.26	low tolerance
BG 90-2	Sri Lanka	improved	female	nonsensitive	0.23	low tolerance

Table 2. Mean, weight, and number of plants tested in each family in the crosses Pokkali/Djabon and Pa Merr 108A/BG 90-2.

Generation	Pokkali/Djabon			PaMerr108A/BG90-2		
	Mean	Weight	Number	Mean	Weight	Number
P ₁	0.510	2,016.807	47	0.505	2,181.818	47
P ₂	0.306	4,363.636	47	0.264	1,118.012	17
F ₁	0.433	1,322.314	15	0.414	828.402	13
F ₂	0.515	1,458.967	47	0.480	1,450.151	47
BC ₁	0.521	788.044	28	0.473	1,441.860	30
BC ₂	0.343	558.376	10	0.369	1,228.070	20

Table 3. Scaling test in the two crosses.

Parameter	Pokkali/Djabon			Pa Merr 108A/BG 90–2		
	Estimate	Standard error	Probability	Estimate	Standard error	Probability
m	= 0.418	0.013	<0.001	0.397	0.017	<0.001
[d]	= 0.019	0.013	<0.001	0.114	0.017	<0.001
[h]	= 0.052	0.023	ns ^a	0.057	0.035	ns
	$c^2_{(3)} = 11.495$			$c^2_{(3)} = 6.313$		
	P < 0.01			P = 0.01 – 0.05		

^ans = not significant.**Table 4. Weighted least square estimates of gene effects in the six parameter model for Pokkali/Djabon and Pa Merr 108A/BG 90–2.**

Parameter	Pokkali/Djabon			Pa Merr 108A/BG 90–2		
	Estimate	Standard error	Probability	Estimate	Standard error	Probability
m	= 0.743	0.153	<0.001	0.622	0.131	<0.001
[d]	= 0.102	0.013	<0.001	0.122	0.081	<0.001
[h]	= –0.599	0.396	ns ^a	–0.357	0.320	ns
[i]	= –0.355	0.152	<0.05	–0.237	0.131	ns
[j]	= 0.152	0.114	ns	0.324	0.859	ns
[l]	= 0.290	0.252	ns	0.149	0.203	ns

^ans = not significant.

Merr 108A/BG 90-2 cross, in which the additive effects of the homozygous loci [d] were highly significant while the dominance effects [h] were not significant. There was no evidence of nonallelic interaction.

The significant deviations of the observed generation means from the expected means in the Pokkali/Djabon cross suggested that nonallelic interactions were present. For a pair of loci, Mather and Jinks (12), using the definitions of the interactions described by Van der Veen (14), recognized three sorts of first order interactions: those between additive properties of the genes symbolized by [i], interaction between additive and dominance components ($d \times h$) symbolized by [j], and interaction involving the dominance effects ($h \times h$) denoted by [l]. These parameters m, [d], [h], [i], [j] and [l] were estimated using the weighted least square procedure discussed earlier. The results are shown in Table 4.

No evidence of nonallelic interaction was observed in the Pa Merr 108A/BG 90-2 cross, agreeing with the conclusions from the joint scaling test in Table 3. In the Pokkali/Djabon cross, many terms were still nonsignificant, [d] was still highly significant in both crosses, and [i] was the only important interaction parameter suggesting homozygous interaction. A consistent pattern emerged when terms were dropped out so that the remaining terms were all significant, as indicated in Table 5. For example, the term [d] in both crosses was always highly significant (<0.001), indicating a significant additive effect. The dominance effect [h] was apparently a major component in the genetic variation of salt tolerance in the Pokkali/Djabon cross.

Among the three types of epistatic gene effects, [i] (additive \times additive) was the only significant effect. The sign of [i] was generally negative, indicating its diminishing effects. This result should be viewed with caution, as the interaction observed may have been due to the presence of a maternal effect as explained earlier. For this reason it seemed appropriate to fit a simple additive-dominance model with a maternal effect based on five parameters: m, [d], [h], [dm], and [hm]. The symbol [dm] represents the additive components of the maternal genotype, and [hm] symbolizes the dominant component of the maternal genotype.

The expected components with the results of the scaling test are shown in Table 6. Only m, [d], and [hm] were significant, and the model was satisfactory. Since most of the parameters were nonsignificant, a more appropriate model was fitted by the progressive removal of nonsignificant items. The final model is shown in Table 7. It can be observed that the same three terms m, [d], and [hm] emerged as the most important parameters. The highly significant [d] confirmed that the variation observed in the generation means was due mainly to additive genetic

Table 5. Minimum model for each cross.

Parameter		Pokkali/Djabon			Pa Merr 108A/BG 90-2		
		Estimate	Standard error	Probability	Estimate	Standard error	Probability
m	=	0.582	0.057	<0.001	0.447	0.016	<0.001
[d]	=	0.105	0.013	<0.001	0.118	0.017	<0.001
[h]	=	-0.155	0.075	<0.05			
[i]	=	-0.175	0.059	<0.01	-0.064	0.026	<0.05
		$c^2_{(3)} = 2.729$			$c^2_{(3)} = 2.994$		
		P = 0.50 - 0.25			P = 0.50 - 0.25		

Table 6. Estimate of generation means in the presence of a maternal effect.

Parameter	Pokkali/Djabon			Pa Merr 108A/BG 90-2			
	Estimate	Standard error	Probability	Estimate	Standard error	Probability	
m	=	0.407	0.013	<0.001	0.390	0.017	<0.001
[d]	=	0.051	0.044	ns ^a	0.108	0.041	<0.01
[h]	=	0.073	0.045	ns	0.045	0.041	ns
[dm]	=	0.052	0.040	ns	0.007	0.032	ns
[hm]	=	0.072	0.033	<0.05	0.068	0.31	<0.05
$\chi^2_{(1)} = 0.3486$ $P = 0.75 - 0.50$				$\chi^2_{(1)} = 0.678$ $P = 0.75 - 0.50$			

^a = not significant.

Table 7. Minimum model in the presence of a maternal effect.

Parameter	Pokkali/Djabon			Pa Merr 108A/BG 90-2			
	Estimate	Standard error	Probability	Estimate	Standard error	Probability	
m	=	0.418	0.011	<0.001	0.404	0.012	<0.001
[d]	=	0.110	0.013	<0.001	0.113	0.016	<0.001
[hm]	=	0.098	0.028	<0.001	0.077	0.029	<0.05
$\chi^2_{(3)} = 3.8171$ $P^{(3)} = 0.50 - 0.25$				$\chi^2_{(3)} = 1.985$ $P^{(3)} = 0.50 - 0.25$			

effects for salt tolerance and that [hm] represents a dominant maternal effect of F_1 mothers, which increased the tolerance of the progeny. In all cases the c^2 is nonsignificant, showing that there were no nonallelic interactions.

Analysis of F_2 data

The F_2 distribution of tolerance ratios in both crosses was continuous and appeared to be slightly bimodal, with major groupings in the classes of 0.45 and 0.85 as shown in Figure 1.

The bimodal distribution suggests the operation of more than one and probably a small number of genes. In both crosses, the F_2 showed transgressive segregation, which appeared in the high tolerance direction. Thus, few plants had tolerance ratios beyond the higher parental range. Analysis of the population means indicated that this variation is largely attributable to additive effects and to the dominant component of the maternal genotype. The variation also seemed to be partly affected by environmental influence, which is indicated by the wide range in the ratios of the parents and the F_1 plants.

Estimate of heritability

The genetic heritabilities for TRs in both crosses are shown in Table 8. It is clear from the results that environmental effects contributed partly to the variation of tolerance ratios in both the Pokkali/Djabon and Pa Merr 108A/BG 90-2 crosses. The broad sense heritabilities were intermediate in the Pokkali/Djabon cross ($h^2b = 49.27$) but fairly high in the Pa Merr 108A/BG 90-2 cross ($h^2b = 83.34$). A low narrow sense heritability of 28.72 was observed in the Pokkali/Djabon cross.

DISCUSSION

Seedling tolerance ratios in *O. sativa* are controlled mostly by additive gene effects. In both the Pokkali/Djabon and Pa Merr 108A/BG 90-2 crosses, a highly significant [d] was obtained in all the models, suggesting that the genetic variation for salt tolerance is principally due to the additive gene effect.

In both crosses the F_1 means were mainly intermediate between their parental means. This can be explained by the presence of a number of genes that have additive effects.

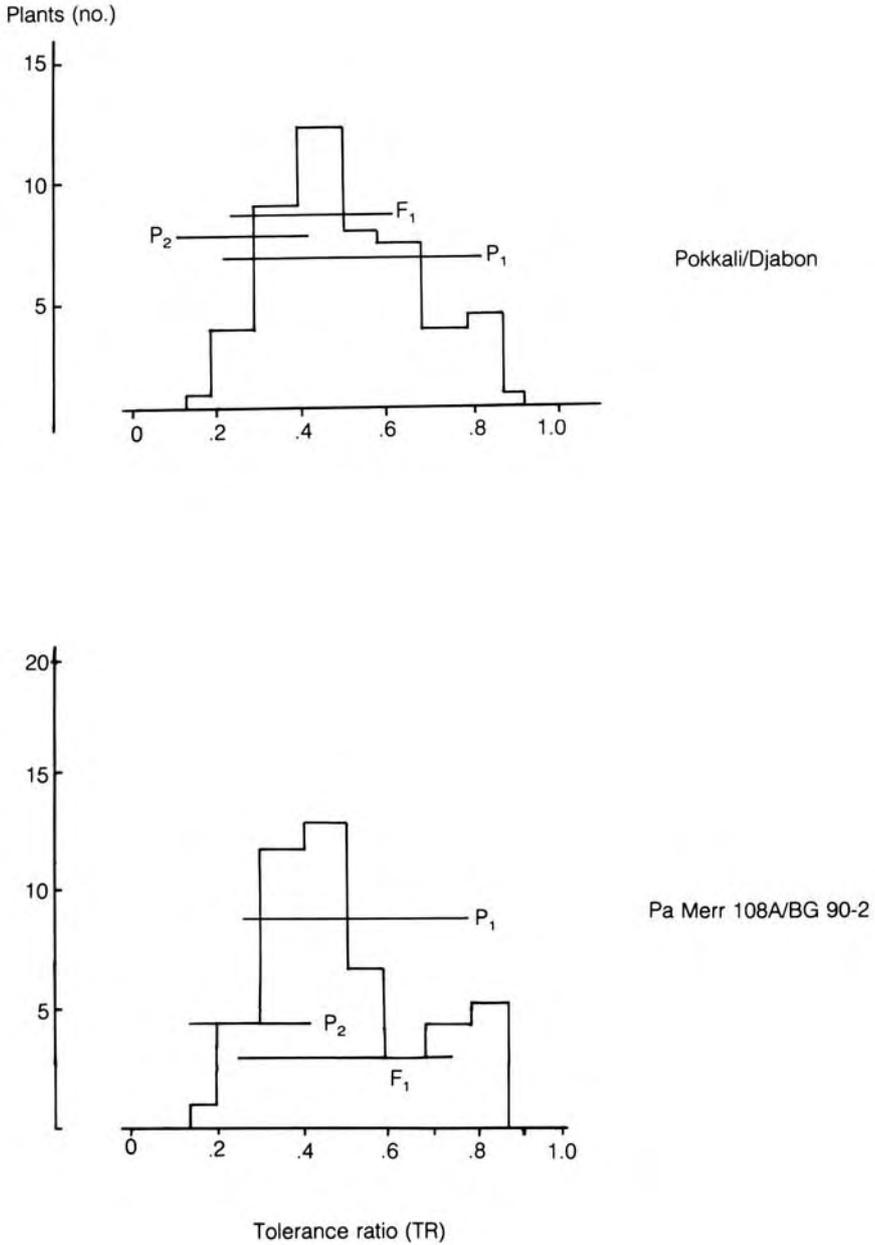


Fig. 1. Distribution and mean of parents, F_1 , and F_2 plants. Solid horizontal lines show the range of the parents and F_1 plants about their mean.

Table 8. Estimate of broad and narrow sense heritabilities.

Cross	Heritability (%)	
	Broad h^2_b	Narrow h^2_n
Pokkali/Djabon	49.27	28.27
Pa Merr 108A/BG 90-2	83.34	—

A simple additive-dominance model with maternal components was found to describe adequately the variation of tolerance ratios observed in the generation means. Barnes (3) reported two types of heterosis: (a) F_1 superiority over the parents, which is associated with the genotype of the progeny, and (b) F_2 superiority over the F_1 , which is a property of both the progeny's own genotype and the genotype of the F_1 mother. In this study, it was clear that the heterosis determined by the F_1 maternal genotype was important. The five parameter model with maternal effect was found to fit at the 50% probability level as implied in Table 6. No evidence of nonallelic interaction was therefore observed.

Analyses of the seedling TR of the generation means in Table 2 and the F_2 frequency distribution in both crosses (Figure 1) show that salinity tolerance in rice varieties is inherited. The broad sense heritabilities range from 49.2% in Pokkali/Djabon to 83.3% in Pa Merr 108A/BG 90-2. The results also suggest the operation of more than one type of gene action as was reported by Akbar and Yabuno (2). The F_2 distribution for salt tolerance exceeded the higher limit of the tolerant parents in both crosses. This transgressive segregation has been reported for rice by several workers. For example, Akbar and Yabuno (2) and Moeljopawiro and Iekhashi (13) found more tolerance in progeny from F_2 to F_4 compared with the tolerance of the parents. The slightly bimodal distribution of the F_2 generation with a big peak at 0.40–0.49 TR and a minor peak at 0.80–0.89 TR suggests the operation of only a small number of genes in the expression of salt tolerance determined by the root growth technique.

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INHERITANCE OF TOLERANCE TO IRON TOXICITY IN TWO RICE CULTIVARS*

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The primary objective of the study was to determine if the genes for resistance to Fe toxicity in Suakoko 8, a tolerant cultivar, and those in Gissi 27, a highly tolerant cultivar, are different or allelic. Determining the mode of inheritance of tolerance to Fe toxicity was the secondary objective. The reactions of the F₁ and F₂ populations and their parents were determined under Fe toxic field conditions using 0–9 scores. Lines scoring 0–4 were classified as tolerant and those scoring 5–9 as susceptible. It was found that tolerance in Suakoko 8 is controlled by a dominant gene and in Gissi 27 by a recessive gene.

There are many inland swamps and valleys in West Africa where Fe toxicity is a great problem for rice production. Tolerant varieties have been identified for these conditions, examples being Suakoko 8 and Gissi 27 in Liberia. Many new tolerant lines have also been identified in Liberia (1) and Nigeria (3, 6). However, there are not many reports on the inheritance of tolerance to the toxicity problem, especially in Africa. The only published study found (5) concerns the F₃ families of a cross of MRC 172–9 and LAC 23, which is an upland variety; it was found that the expression of tolerance to Fe toxicity at the maximum tillering stage is probably controlled by three genes. Two appeared to be dominant and complementary in action, but the third gene was inhibitory over them. The study concluded that, in the absence of inhibitory genes at the maximum tillering stage, the trait should behave as dominant and should show 9:7 segregation in the F₂; the author of the study stated, however, that this hypothesis needed confirmation, and that investigation of the segregation behavior of more crosses was necessary.

In order to make more rapid progress in the development of improved tolerance to Fe toxicity combined with good agronomic traits, it is essential to have more genetic knowledge of the plants, such as the identification of new genes for resistance, the mode of inheritance of different tolerant cultivars, the number of genes responsible, and the linkages between some traits and Fe tolerance. As more tolerant genes are identified, the faster the development of tolerant varieties will be,

since many of the genes will be incorporated systematically in our breeding work as has been done for pest resistance (2).

Therefore, the main objectives of the present study were to determine the mode of inheritance of tolerance to Fe toxicity in two tolerant cultivars and to determine the allelic relationships of these genes.

MATERIALS AND METHODS

Four cultivars — Gissi 27, Suakoko 8, IR5, and IR26 — were crossed in various combinations in late 1983. The F_1 seeds were raised under nontoxic soil conditions, and 4-week-old seedlings were transplanted into a moderately toxic soil. The female parents were also grown under the same field conditions. The female parents were used to distinguish self from true crosses within the F_1 plants. The field was continuously flooded, and Fe toxicity scores based on leaf symptoms and plant vigor were made on all of the plants. The scores of 0–9 based on the Standard Evaluation System for Rice (4) were taken at 4, 6, and 10 weeks after transplanting (WAT).

Seeds harvested from true F_1 plants were sown in 1984 in nontoxic soil and transplanted into toxic soil when the seedlings were about 4 weeks old. Toxicity scores were taken as done for the F_1 plants at 2, 4, 6, 8, and 10 WAT. The scores of 8 WAT were used in the genetic analyses based on our previous studies (unpublished data) that the reaction at 8 WAT is the most reliable.

Plants with scores of 0–4 were regarded as tolerant and those with 5–9 as susceptible. Susceptible (IR26) and resistant (Suakoko 8) checks were grown perpendicular to the plots to indicate whether or not there was sufficient or too high a level of toxicity in any given row. Any row with IR26 growing luxuriously (or Suakoko 8 with a score of 8–9) was discarded or the plants close to them were disregarded. This meant either that there was insufficient stress or that the stress was too high and might have been due to other factors (when Suakoko 8 scored 8–9).

Some of the crosses, e.g., Gissi 27/Suakoko 8, IR5/Gissi 27, were not analyzed in this study due to either high level of rat damage or high day length sensitivity.

RESULTS

Inheritance of tolerance

Table 1 shows the reactions of the four cultivars and those of the F_1 's to the moderately Fe toxic conditions. All the F_1 plants were tolerant or highly tolerant.

The analyses of the F_2 plants from the crosses of IR26/Suakoko 8 and IR5/Suakoko 8 did not fit any of the F_2 ratios expected (Table 2). The calculated χ^2 's for 1:3, 1:15, 55:9, or 37:27 were highly significant, indicating no goodness of fit. On the other hand, F_2 plants from a cross of Gissi 27/IR5 produced 295 tolerant plants and 922 susceptible plants. A good fit was obtained for a 1:3 ratio. This indicates that only one recessive gene confers tolerance in Gissi 27.

Allele tests

Suakoko 8 and Gissi 27 are both tolerant cultivars. The F_1 plants, as expected, were highly tolerant. However, the F_2 populations produced 407 plants that were tolerant and 1,771 that were susceptible, or a ratio of 3:13 tolerant to susceptible. This indicates that two genes—one recessive and one dominant—segregated in this cross. Since Gissi 27 has a recessive gene, Suakoko 8 must have a dominant gene. The data further show that these genes are independent.

DISCUSSION AND CONCLUSION

In general, it is important to note that conclusions made from this type of study can be affected by the level of Fe toxicity, age of the plants, the susceptible and resistant checks, the uniformity of the soil, other soil mineral deficiencies and toxicity, and the diseases and insects that may affect the number of plants classified as tolerant or susceptible. Therefore, there is a need for further studies with these and other crosses. The development of repeatable screening conditions is also essential.

There are two different genes that control Fe tolerance in Suakoko 8 and Gissi 27. Suakoko 8 has a dominant gene while Gissi 27 has a recessive gene for tolerance to Fe toxicity. The practical importance of this finding is that a cross of the two cultivars should produce lines that combine the two genes for tolerance, barring the presence of modifying or inhibitory genes, and that such lines should be more tolerant than either of the parents.

Table 1. Means of Fe toxicity scores on F₁'s grown under moderate Fe toxic conditions in Suakoko, Liberia, 1984.

F ₁ and parents	Fe toxicity scores (WAT) ^a			F ₁ plants (no.)	Level of resistance or susceptibility
	4	6	10		
IR26/Suakoko 8	2.6	1.6	1.6	9	highly tolerant
IR26	6.0	8.0	9.0		highly susceptible
Gissi 27/Suakoko 8	3.7	1.5	1.8	6	highly tolerant
Gissi 27	2.5	1.0	1.0		highly tolerant
IR5/Gissi 27	3.8	1.9	2.2	13	moderately tolerant
IR5	4.9	2.0	3.8		moderately tolerant
IR5/Suakoko 8	4.2	3.3	3.3	13	moderately tolerant
IR5	2.0	2.0	4.0		moderately tolerant
Suakoko 8/Gissi 27	1.8	1.5	1.7	19	highly tolerant
Suakoko 8	0.5	1.0	2.0		tolerant
Gissi 27/IR5	2.1	1.6	1.6	14	highly tolerant
Gissi 27	2.0	2.0	2.0		tolerant

^aWAT = weeks after transplanting.

Table 2. Reactions to Fe toxicity in F₁ and F₂ rice populations from crosses of four cultivars scored at 8 weeks after transplanting at Suakoko, Liberia.

Cross	F ₁ reaction	F ₁ plants				χ^2 (df = 1)	
		(no.)		(%)		3:13	1:3
		Toler-ant	Suscep-tible	Toler-ant	Suscep-tible		
Suakoko 8/Gissi 27	highly tolerant	407	1771	18.67	81.31	0.0023	
IR26/Suakoko8	tolerant	115	1084	9.59	90.41		
IR5/Suakoko8	tolerant	229	121	65.43	34.57		
Gissi 27/IR5	tolerant	295	922	24.24	75.76	0.3355	

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GENETIC ANALYSIS OF COLD TOLERANCE IN RICE

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One hundred thirty-four rice varieties were examined for cold tolerance. Seedling tolerance was scored by growing seedlings in the phytotron in Yoshida's culture medium at 10 °C for 30 days and applying IRRI's Standard Evaluation System of Rice. Similarly, adventitious root regeneration was scored by growing seedlings in Yoshida's culture medium at 10 °C for 10 days. Rice varieties could be grouped into four categories according to reactions for cold tolerance at two stages of growth. Inheritance of cold tolerance was examined in seven hybrids between tolerant and nontolerant parents. Cold tolerance as measured by leaf yellowing is governed by a single dominant gene. A single dominant gene also controls cold tolerance as measured by root regeneration ability.

The rice plant grows in different ecological environments. One of the environments is subtropical and high altitude areas, where low temperature becomes the limiting factor for rice production. It is estimated that about 7 million ha of rice are grown in cold areas in South and Southeast Asia alone. In the subtropics, when more than one crop of rice is cultivated, the second crop grown during the late winter period suffers from low temperature during the seedling and tillering stage. In a rice variety, cold tolerance may be needed in the seedling and tillering stage, in the booting and flowering stage, or at both stages, depending on altitude and latitude. In the present study it was found that the cold tolerance of roots and that of panicles are independent of each other, and both play a vital role in the cold tolerance mechanism of the rice plant.

Genetic studies on rice varietal tolerance to low temperature are few and have been conducted mostly under natural conditions. Toriyama and Futsuhara (8) and Futsuhara and Toriyama (3, 4, 5) reported that cold tolerance at the flowering stage is a quantitative character controlled by four or more genes. Sasaki (7) reported that four or more genes are responsible for germination at low temperature. Amamiya (1) and Chung (2) reported monogenic control of leaf and seedling

yellowing at low temperature. Li and Rutger (6) reported that five or more genes are responsible for seedling vigor at low temperature.

MATERIALS AND METHODS

A total of 134 entries from Japan, Korea, the Philippines, Nepal, India, and Pakistan were tested for cold tolerance at IRRI in the phytotron at 10 °C for 30 days at the seedling stage and at 15 °C for 4 days at the booting stage. The scores were recorded for seedling tolerance and for spikelet sterility. The materials included japonica, indica, and intermediate type varieties. For the seedling tolerance study, the seed samples were surface-sterilized for 1 min with a 0.1% HgCl₂ solution and then washed thoroughly with several changes of demineralized water. The seed samples were allowed to soak for 24 hours in a beaker of demineralized water. The seeds were spread on a nylon net stretched over a piece of Styrofoam floating in a tray containing Yoshida's complete culture solution at pH 5.0 as outlined by Yoshida et al (9). The pH of the culture solution was adjusted to 5.0 after the addition of demineralized water to the tray every morning. The culture solution was changed once a week at an early growth stage (up to 3 weeks) and twice a week later on. The seedlings were then transferred from the glasshouse (29/21 °C) to the phytotron.

About 200 different crosses were made, and F₁ progenies were grown in the screenhouse and in the field. Since the material used in the crosses belonged to different varietal groups, we grew the F₁'s at the IRRI farm to estimate F₁ sterility under natural field conditions. Based on the absence of F₁ sterility and root regeneration capacity at 10 °C, seven parents—namely Mansara, JC 24, Barkat, IR9758K₂, IR20654B, Ratomarshi, and Shenei—were selected for genetic analysis. After 10 days, all seedlings of the parental, F₁, and F₂ populations of the seven crosses were transferred to the phytotron at 10 °C and scored for leaf discoloration after 30 days. The seedlings were classified as green or yellow depending upon the degree of discoloration. For the study of root regeneration capacity of adventitious roots at the seedling stage, roots were cut 2 cm below the base of the seedlings, which had been raised in culture solution as mentioned earlier. Three-week-old seedlings, after the roots were cut, were transferred to the phytotron at 10 °C and scored for root regeneration after 10 days. The parental, F₁, and F₂ populations of the seven hybrids were so examined.

Table 1. Cold tolerance scores^a— at the seedling and booting stages of some of the known cold tolerant entries.

Entry	Score at seedling stage	Spikelet fertility (%)
Akihikari	2	88.27 ± 5.94
Tatsumi Mochi	2	89.71 ± 8.43
Shenei	2	75.96 ± 22.67
K-143	2	71.99 ± 13.01
Kalamarshi-3	1	44.56 ± 9.55
Ratomarshi	1	30.95 ± 6.88
Setomarshi	1	40.16 ± 7.60
J.P.-5 (local)	2	44.49 ± 26.12
JC-24	9	89.66 ± 6.55
IR9758K ₂	9	79.66 ± 9.13
IR20654B	9	82.31 ± 17.38
IR15429	9	78.74 ± 3.86
Ghaiya	9	44.00 ± 10.21
Mansara	9	36.68 ± 12.01
K-31	9	49.45 ± 6.97
IR9224-K ₁	9	43.92 ± 14.09

^aFor cold tolerance reaction at the seedling stage, 3-week-old plants were placed at 10 °C for 30 days. For determining spikelet sterility, plants at the booting stage were exposed to 15 °C temperature for 4 days.

RESULTS

The varieties tested could be classified into four categories based on their adventitious root regeneration ability and spikelet sterility under low temperatures:

1. High root regeneration ability and high spikelet fertility
2. High root regeneration ability but low spikelet fertility
3. Poor root regeneration ability but high spikelet fertility
4. Poor root regeneration ability and low spikelet fertility

Some of the entries with these characteristics are given in Table 1. Varieties belonging to category 1 (Akihari, Tatsumi Mochi, and Shenei) are primarily japonicas from northern Japan. Varieties belonging to category 2 (Kalamarshi-3, Ratomarshi, Setomarshi, and J.P.-5) are from Nepal and the Swat region of Pakistan, where the rice crop is planted during low temperature but the temperatures at flowering time are optimum. Entries belonging to the third category are primarily breeding lines that have been selected (at Banaue, Philippines, elevation c. 1200 m)

to tolerate low temperatures at the flowering stage. The varieties belonging to the fourth category are tropical indicas.

The inheritance of cold tolerance at the seedling stage was investigated in seven intervarietal crosses. Cold tolerance was measured by two criteria: leaf yellowing after treatment at 10 °C for 30 days and root regeneration ability after treatment at 10 °C for 10 days.

The segregation data for leaf yellowing in seven crosses are given in Table 2. In all crosses, green and yellow seedlings segregated in a 3:1 ratio, indicating that tolerance as measured by the green color is governed by a single dominant gene. Similarly, the data in Table 3 indicate that tolerance as measured by root regeneration ability (Fig. 1) is under single dominant gene control.

DISCUSSION

The results of this study clearly show that cold tolerance at the seedling stage as measured by the absence of leaf yellowing is governed by a single dominant gene. Similar results were obtained by Amamiya (1) and Chung (2). However, cold tolerance as measured by root regeneration ability at low temperature was investigated for the first time in this study. The results show that this trait is also governed by a single dominant gene. Whether the same gene is responsible for lack of leaf yellowing and root regeneration ability under low temperature in these parents is not known. For this purpose, F_3 lines from a cross between a tolerant and a susceptible parent should be evaluated for tolerance to leaf yellowing and root regeneration ability. The two-way classification should yield information whether the same gene or different genes govern these two traits.

Cold tolerance at the flowering stage as measured by panicle sterility appears to be a quantitative trait (3, 4, 5, 8). However, as the results of this study show, cold tolerance at the seedling stage and cold tolerance at the flowering stage are independent traits. Many parents used in the breeding program for cold tolerance at IRRI are resistant at both stages. However, most of these are japonicas, which are not suitable for growing in the high altitude areas of the tropics. Therefore, efforts are being made to develop improved plant type indicas with cold tolerance at the seedling as well as the flowering stage.

Table 2. Segregation for cold tolerance as estimated by the degree of leaf discoloration at 10 °C for 30 days.

Cross	Reaction of populations ^a							
	P ₁	P ₂	F ₁	F ₂				P
				Green (no.)	Yellow (no.)	Total	c ² 3:1	
Goode/K-143	S	R	R	225	67	292	0.657	0.55–0.30
JC-24/Ratomarshi	S	R	R	210	80	290	1.034	0.40–0.30
IR9758K ₂ /Tatsumi Mochi	S	R	R	278	97	375	0.150	0.70–0.50
IR9758K ₂ /Ratomarshi	S	R	R	280	96	376	0.056	0.90–0.80
JC-24/Shenei	S	R	R	204	80	284	1.521	0.30–0.20
Barkat/Ratomarshi	S	R	R	191	83	274	4.092	0.05–0.02
IR2298/Ratcmarshi	S	R	R	215	84	299	1.526	0.30–0.20

^aS = yellow, R = green.

Table 3. Segregation for cold tolerance as estimated by regeneration of adventitious roots at 10 °C for 10 days.

Cross	Reaction of populations ^a							
	P ₁	P ₂	F ₁	F ₂				P
				R (no.)	S (no.)	c ² 3:1		
Goode/K-143		S	R	R	221	81	0.5577	0.55–0.30
JC-24/Ratomanhi		S	R	R	144	86	0.0154	0.95–0.90
IR9758K ₂ /Tatsumi Mochi		S	R	R	209	88	1.4848	0.30–0.20
IR9758K ₂ /Ratomanhi		S	R	R	217	82	0.3914	0.70–0.50
JC-24/Shenei		S	R	R	208	98	3.3024	0.10–0.05
Barkat/Ratomanhi		S	R	R	192	106	1.1913	0.30–0.20
IR2298/Ratomanhi		S	R	R	202	88	0.6197	0.50–0.30

^aS = no regeneration of roots, R = regeneration.

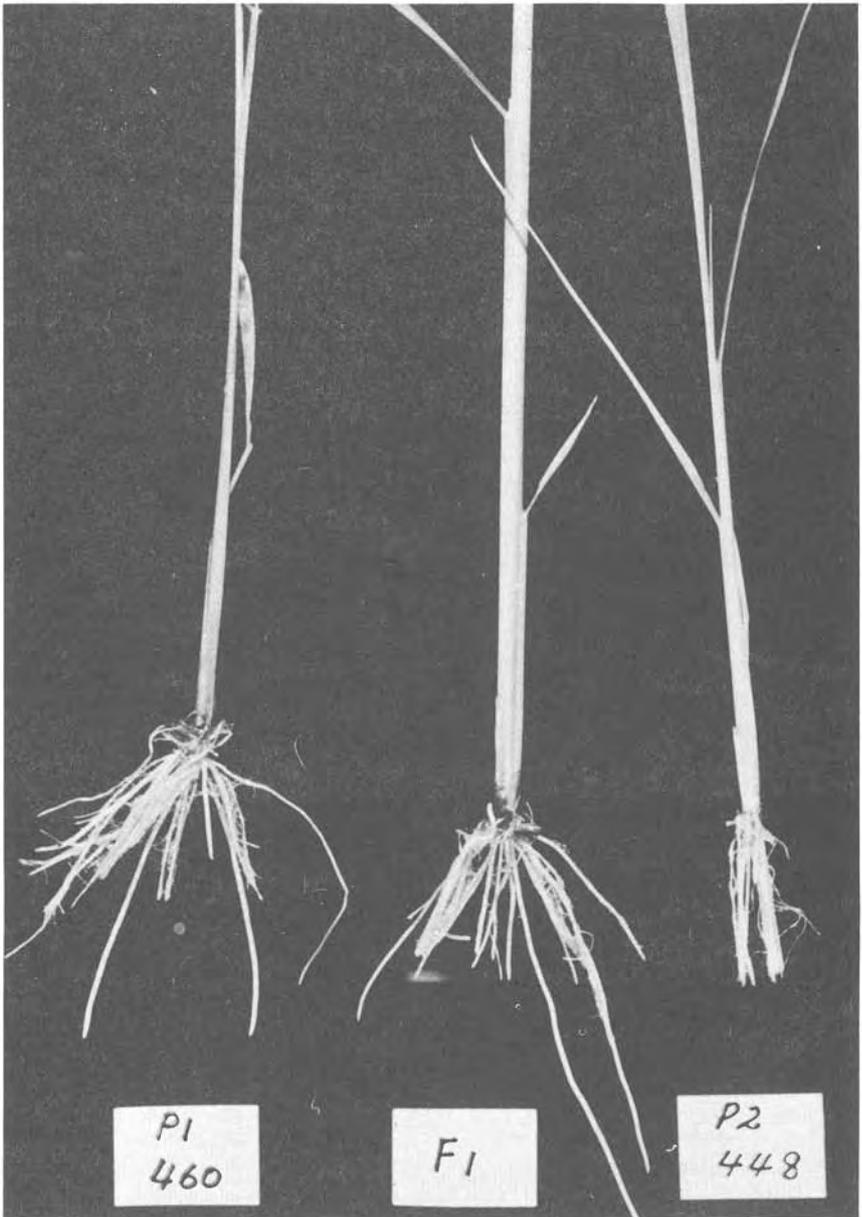


Fig. 1. Differences in root regeneration ability of P_1 (Ratomarshi), P_2 (JC-24), and their F_1 hybrid.

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DISCUSSION

SESSION 7: GENETICS OF STRESS TOLERANCE

Q – Riley: Is it possible to select for root length on the basis of the positive correlation between plant height and root length? Further, are you aware of any difference in the efficiency of genotypes in extracting nitrogen?

A – Chang: It is possible to select for root length on the basis of plant height and leaf rolling behavior, especially during a stress period. Any plant that does not show marked stunting or leaf death during stress probably has an extensive root system involving thick roots. Thick roots are more effective in penetrating into the lower soil horizon. We have no direct evidence on genotypic differences in nitrogen extraction. But both our plant physiologist and crop physiologist have obtained data showing that a deep root system enables a variety to extract more water and nutrients than a shallow rooted variety during a stress period.

C – Ikehashi: In applying diallel analysis, the magnitude of variance should be independent of the mean value for each measure of the trait. Under stress, affected varieties show a low mean and a small range of variance. This limits the applicability of diallel analysis for stress tolerance.

Q – Rutger: Are there different genetic mechanisms for salt tolerance at the seedling stage than at the reproductive stage?

A – Akbar: We think that different genetic mechanisms are involved for tolerance at the seedling and reproductive stages because the variety that is tolerant at the seedling stage is affected by salinity at the flowering stage.

Q – Chang: Your data in Table 2 do not strongly suggest simple Mendelian control of the trait. I suggest you try alternative hypotheses, such as a multigenetic pattern.

A – Abifarin: As I explained during my presentation, there are many factors that can affect data on toxicity, so it is possible that classification into different groups is affected. We shall repeat the experiment and investigate the possibility of alternate hypotheses. I was also surprised that we had such a few tolerant F_2 plants in the IR26/Suakoko 8 cross.

Q – Chelliah: Is there overlapping of symptoms at your center between iron toxicity and rice tungro virus?

A – Abifarin: Fortunately, we do not have tungro virus in Africa. We have yellow mottle virus, but the symptoms are different from those of iron toxicity. Earlier work by Dr. Virmani showed that the observed symptoms are those of iron toxicity.

Q – Ikehashi: The allelism test of tolerant source varieties for problem soils is a very good idea. It may be more valuable if you test more varieties from Thailand and Malaysia. What do you think?

A – Abifarin: Your comment is well taken. Having developed good screening methods, we shall next screen many materials from other countries. Contacts have been made to receive materials from Asia. Tolerant varieties will be analyzed for allelism or mode of inheritance.

Q – Wang, X. M.: Are the local varieties of *O. glaberrima* in West Africa more tolerant to iron toxicity than *O. sativa* cultivars?

A – Abifarin: We have in the past screened many *O. glaberrima* cultivars and have found some to be tolerant, but we have not found many that are more tolerant than *O. sativa* cultivars. We have found few that are just as tolerant as indicas. We shall use our refined screening methods to screen more *O. glaberrima* cultivars for tolerance to iron toxicity.

Q – Rutger: Did you investigate inheritance of cold tolerance at the flowering stage? If so, was it in the field or in the growth chamber?

A – Shahi: We studied F_1 , F_2 , and F_3 populations for root regeneration ability at low temperature in the growth chambers.

C – Mahadevappa: You must have noticed in the Poster Session (Poster 11) that there are some indica genotypes (CT. 19, CT103, S. 317, CT1351, S705, Cold Adokkan, Cold Kodandan) that are tolerant at all growth stages. Our results also indicate that early stage and late stage cold tolerance are independently inherited.

GENETICS OF DISEASE RESISTANCE

SESSION 8

A METHOD FOR ESTIMATING TRUE RESISTANCE GENES TO BLAST IN RICE VARIETIES

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We propose a new method for estimating the true resistance genotype to blast disease by testing backcross progeny for specific reactions. Variety A, having true resistance gene(s), is crossed with Variety B, having no true resistance genes. BC_1F_2 lines backcrossed to Variety B are inoculated with a blast fungal strain belonging to the basic race that is virulent to Variety B but avirulent to any one of the known true resistance genes. BC_1F_2 lines that show monogenic segregation are randomly taken, and ten sets of them are inoculated with ten primary fungal strains, each virulent in a match with a known true resistance gene. When susceptible fixed lines appear in any set, Variety A proves to have the true resistance gene(s) corresponding to the fungal strain(s) with which it was inoculated. When any lines show monogenic segregation against all ten primary fungal strains, Variety A proves to have a new true resistance gene. A modified testing procedure that utilizes the available fungal strains is proposed for practical use.

A number of strategies have been proposed for developing rice varieties resistant to blast disease caused by *Pyricularia oryzae* Cavara (2,6): pyramiding true resistance genes in a single variety, strengthening a level of field resistance, combining true resistance with field resistance, constructing

multiline varieties, rotating varieties with different true resistance genes, etc. Information concerning the genotypes of true resistance to blast disease in the parental varieties is essential to employ true resistance gene(s) in rice breeding.

Genetic analyses for true resistance of rice varieties to blast disease have been made using several fungal races from Japan (1). To date, the true resistance genes *Pi-a*, *Pi-i*, and *Pi-k^s* have been found in Japanese indigenous varieties, and *Pi-b*, *Pi-t*, and the allelic genes on the loci of *Pi-k*, *Pi-ta*, and *Pi-z* have been found in nonindigenous varieties (4). Of these true resistance genes, *Pi-k^s* is ineffective against common Japanese fungal strains, though it was found effective against some Philippine fungal strains; it is therefore not considered here.

A rapid method for estimating true resistance genotypes of rice varieties has been developed by analysis of specific reaction patterns of rice varieties against several selected fungal races standardized by Kiyosawa (4). The method is inapplicable to introduced varieties having unknown true resistance gene(s), because they are mostly resistant to all races. The conventional method of gene analysis using F_2 and F_3 generations requires laborious work to estimate the genotypes for resistance of those introduced varieties.

We propose a new method to estimate genotype by analysis of the reaction patterns of backcrossed progeny to several blast fungus strains (7).

BASIC TESTING PROCEDURE

The blast fungal strains employed for this new method are selected by the following rules,

- Rule 1. One basic fungal strain is selected from the race avirulent to any true resistance genes identified so far (except *Pi-k^s*).
- Rule 2. Ten primary fungal strains are selected from those races, each of which is virulent to any one of the true resistance genes listed in Table 1 and is avirulent to the others.

The code numbers for naming blast fungal races follow the proposals by Yamada et al (8) and Kiyosawa et al (5).

The basic procedure in the new method consists of nine steps, as follows:

1. Variety A, which has the unknown genotype for true resistance, is crossed with Variety B, having none of the true resistance genes (except *Pi-k^s*). F_1 plants are backcrossed to Variety B to obtain BC_1F_1 plants. The number of BC_1F_1 plants required for estimating the

Table 1. True resistance genes and their corresponding code numbers for naming races of *Pyricularia oryzae* (after 5, 8).

Gene	Code no.	Gene	Code no.	Gene	Code no.	Gene	Code no.
Pi-ks	1	<i>Pi-k</i>	10	<i>Pi-ta</i>	100		
Pi-a	2	<i>Pi-k^m</i>	20	<i>Pi-ta²</i>	200	<i>Pi-b</i>	.2
Pi-i	4	<i>Pi-z</i>	40	<i>Pi-z^t</i>	400	<i>Pi-t</i>	.4

number of true resistance genes involved in Variety A is shown in Table 2 on the hypothesis that resistance is completely dominant. One hundred BC₁F₁ plants are enough for genetic analysis including less than five genes with a probability of 90%.

2. BC₁F₂ lines, including 20–30 individuals, are inoculated with the basic fungal strain (Rule 1) belonging to race 001, which is avirulent to any of the known true resistance genes (except *Pi-k^s*).
3. BC₁F₂ lines are grouped into the following categories:

- (a) homozygous susceptible lines
- (b) segregating lines with resistant plants

The number of true resistance genes involved in Variety A is roughly estimated from the ratio of the number of lines in group (a) and the number of lines in group (b) as shown in Table 3.

4. BC₁F₂ lines, which show a monogenic segregation ratio of 3:1 resistant to susceptible, are randomly taken in accordance with the number of true resistance genes indicated in Table 4.
5. Ten sets of monogenically segregating lines are inoculated with ten primary fungal strains belonging to the races shown in Table 5. Each set includes 7–10 plants. Races 031 and 301 are listed as the primary fungal races in Table 5 instead of races 021 and 201 because *Pi-k^m* has an epistatic effect over the gene *Pi-k*, and *Pi-ta²* over *Pi-ta*, and the fungal strains belonging to races 021 and 201 have not yet been identified.
6. When susceptible fixed lines appear in any set, Variety A proves to have the true resistance gene(s) corresponding to the fungal strain(s) with which it was inoculated. For example, when the number of true resistance genes is estimated to be three and any susceptible fixed lines are observed in the sets inoculated with the fungal strains belonging to races 003, 041, and 101, Variety A is considered to have *Pi-a*, *Pi-z*, and *Pi-ta* and does not have any new true resistance gene. When the set includes the lines susceptible to the fungal strain

Table 2. The number of BC₁F₁ plants required for identifying the different number of true resistance genes involved when inoculated with the basic fungus strain.

Number of genes involved		1	2	3	4	5	6	7
Number of BC ₁ F ₁ plants in the probability of	99%	7	16	35	72	145	295	588
	90%	4	8	18	36	72	145	293

Table 3. The expected ratios of the number of BC₁F₂ lines fixed for susceptibility to that of lines including resistant plants for different numbers of true resistance genes involved.

Number of genes involved	1	2	3	4	5
Ratio of homozygous susceptible lines to segregating lines with resistant plants	1:1	1:3	1:7	1:15	1:31

Table 4. The number of monogenically segregating BC₁F₂ lines needed for the next step of the procedure.

Number of genes involved		1	2	3	4	5	6
Number of lines needed for next step in the probability of	99%	1	7	12	16	21	26
	90%	1	4	6	8	11	13

Table 5. Races of *Pyricularia oryzae* necessary for the fundamental gene analysis procedure and the corresponding resistance genes.

Race number	003	005	011	031	041	101	301	401	001.2	001.4
Matching genes for true resistance	<i>Pi-a</i>	<i>Pi-i</i>	<i>Pi-k</i>	<i>Pi-k</i> <i>Pi-k^m</i>	<i>Pi-z</i>	<i>Pi-ta</i>	<i>Pi-ta</i> <i>Pi-ta²</i>	<i>Pi-z^t</i>	<i>Pi-b</i>	<i>Pi-t</i>

Note: *Pi-k^s* corresponding to the race 001 is not effective against common Japanese fungus strains.

belonging to race 031, and these lines show segregation to the fungal strain of race 011, Variety A proves to have $Pi-k^m$. On the other hand, when lines susceptible to both fungal strains appear, Variety A proves to have $Pi-k$ because of an epistatic effect of $Pi-k^m$ over $Pi-k$. The relation between $Pi-ta^2$ and $Pi-ta$ is the same.

7. When some lines show monogenic segregation against all ten primary fungal strains, Variety A proves to have a new true resistance gene(s). Resistant plants are selected from these BC_1F_2 lines, and homozygous resistant lines are selected from the BC_1F_3 generation.
8. Homozygous resistant BC_1F_3 lines possessing a new resistance gene(s) are crossed with the tester lines, each of which has a respective single known true resistance gene.
9. The F_2 population of these crosses, including more than 100 individuals, is inoculated with the basic fungal strain belonging to race 001. When susceptible plants appear, the new true resistance gene proves to be located at a different site from the known true resistance gene in that tester line. When susceptible plants do not appear, the new true resistance gene may be located on the same locus as the tested gene and may have an epistatic effect over that gene. In that case, we recommend inoculation of more than 500 F_2 plants of the same cross again to check whether the new gene is closely linked to the tested gene or not. Figure 1 shows the outline of this procedure.

MODIFIED TESTING PROCEDURE

This basic testing procedure presupposes the existence of a complete set of primary fungal strains, each virulent in a match with a known true resistance gene. It would be difficult to obtain such a complete set. Available fungal strains are, therefore, selected by the following rules for the modified testing procedure:

- Rule 3. When a complete set of primary fungal strains indicated in Rule 2 is not available, secondary fungal strains are selected from the races that have virulence matching two true resistance genes, and it is desirable that one of the two genes is common.
- Rule 4. When a set of fungal strains is not completed by primary and secondary races, tertiary fungal strains are selected from the races that have virulence matching three true resistance genes.

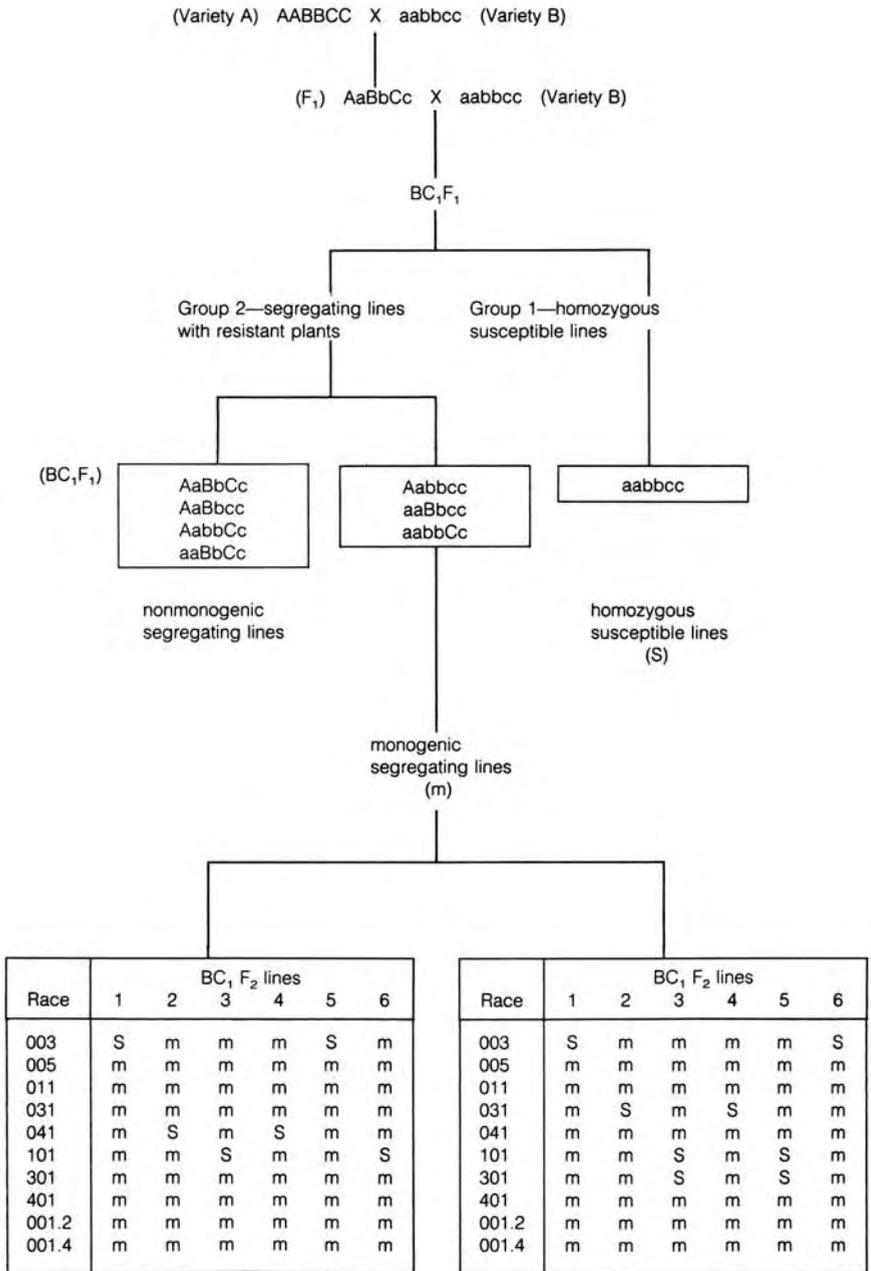


Fig. 1. Fundamental procedure and examples of estimating true resistance genes in new rice variety (Variety A).

Table 6. Races of *Pyricularia oryzae* necessary for the modified gene analysis procedure and the corresponding resistance genes.

Race number	003	007	033	043	303	403	003.2
Matching genes for true resistance	<i>Pi-a</i>	<i>Pi-a</i> <i>Pi-i</i>	<i>Pi-a</i> <i>Pi-k</i> <i>Pi-k^m</i>	<i>Pi-a</i> <i>Pi-z</i>	<i>Pi-a</i> <i>Pi-ta</i> <i>Pi-ta²</i>	<i>Pi-a</i> <i>Pi-z^t</i>	<i>Pi-a</i> <i>Pi-b</i>

The modified testing procedure, using fungal strains available in Japan belonging to the races shown in Table 6, is proposed for practical use. Steps 1–4 of the modified testing procedure are the same as those of the basic procedure, and the subsequent steps are as follows:

5. One set of BC₁F₂ lines selected from Step 4 is inoculated with the fungal strain belonging to the primary race 003. When susceptible fixed lines appear, Variety A proves to have *Pi-a*. When any lines show the halo lesions that are known to be specific to the interaction between *Pi-t* and an avirulent strain, Variety A proves to have *Pi-t*. BC₁F₂ lines having *Pi-a* and/or *Pi-t* are discarded for the next step.
6. Six sets of remaining BC₁F₂ lines are inoculated with the fungal strains belonging to races 007, 033, 043, 303, 403, and 003.2, indicated in Table 6. When susceptible fixed lines appear in any set(s), Variety A proves to have the true resistance gene(s) corresponding to the fungal strains inoculated. When there appear some fixed lines susceptible to the fungal strain belonging to races 033 or 303, additional samples of these susceptible BC₁F₂ lines are inoculated with the fungal strains belonging to races 013 or 103 to examine the true resistance gene on the *Pi-k* or *Pi-ta* locus.

Steps 7–9 are the same as those of the basic testing procedure.

FURTHER CONSIDERATIONS

When Variety A to be tested for resistance belongs to the indica type of rice and the check Variety B belongs to the japonica type, a sufficient amount of BC₁F₁ seeds may not be available because of the high sterility of F₁ plants, and/or segregation distraction may be observed in the segregating population due to gametic lethality or certation of the gametophyte. In such cases, the check Variety B should be selected from the indica or javanica (bulu type) varieties that are susceptible to the fungal

strains of race 001 and show a high sexual compatibility with Variety A belonging to the indica type.

When a recessive segregation for true resistance is observed in any BC_1F_2 lines, the true resistance gene in this cross proves to be new, because all the true resistance genes known so far are dominant.

This method could be applied to F_3 lines instead of BC_1F_2 lines, but it would be laborious, because the number of F_3 lines needed for checking is much larger than for BC_1F_2 lines when the related genes are more than three.

When the objective of testing is limited to finding a new true resistance gene without getting any information about the known resistance genes in Variety A, it is possible to use the fungus strains possessing the widest range of pathogenicity in the inoculation test to Step 2 for selecting monogenically segregating BC_1F_2 lines. For example, the fungal strain belonging to race 777.6, which is virulent to $Pi-k^s$, $Pi-a$, $Pi-i$, $Pi-k$, $Pi-k^m$, $Pi-z$, $Pi-ta$, $Pi-ta^2$, $Pi-z^!$, $Pi-b$, and $Pi-t$, may be employed for this purpose.

The idea of basic and modified testing procedures proposed here may be applied generally to genetic analyses for true resistance to diseases and insect pests fitting the "gene for gene" hypothesis.

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GENETIC ANALYSIS OF MINOR GENE RESISTANCE TO BLAST IN JAPONICA RICE

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Rice cultivars with major gene resistance to blast usually become susceptible after a short duration of cultivation in an epidemic area. Thus breeding rice for durable resistance is economically important. In 1982–1984 several experiments were carried out in Donggou District, Liaonin Province to discover highly field resistant japonica varieties to be the starting points in rice breeding and to clarify the inheritance of field resistance. It was found that Homare-nishiki, Ginga, and Reimei are highly resistant to leaf blast, the trait being highly heritable and controlled by polygenes with additive and partially dominant effects. The parent-offspring correlation for blast resistance of F_2 plants and F_3 families is also high. The least number of operative genes controlling resistance for Homare-nishiki is estimated to be five pairs, for Ginga three pairs, and for Reimei six pairs. Some traits concerning leaf and panicle blast were investigated in other japonica rice crosses, and their simple correlations and multiple regression equations were studied. A combination of major and minor gene resistance may be the most effective goal in breeding rice varieties with durable resistance to blast.

The resistance of rice cultivars to blast caused by *Pyricularia oryzae* has been classified into two categories, one of which is controlled by 13 major dominant genes, the other by some minor genes (2,3,4,7). Breeding rice for blast resistance in China has generally dealt with the utilization of major genes with broad spectrum resistance to the pathogen. In most cases the breeding has been successful and the cultivars released have proved to be highly resistant, but not long after their popularization in an epidemic area they inevitably become susceptible due to the appearance of a certain virulent race (2,4,7). An early indica rice variety Hong 410 became severely susceptible to blast in Fujian Province in 1981, and a medium japonica rice Zhongdan 2 was terribly infected by the newly emerged physiologic race ZA 61 in Donggou District, Liaonin Province in 1982. These varieties were believed to have major genes for blast resistance, but they underwent a similar process of rise and decline in production. These disastrous facts impel us to pay more attention to comprehensive studies and practical applications of minor gene resistance to this disease.

Although breeding rice for blast resistance is considered to be of high priority in China, little work has been done in the genetic analysis of minor gene resistance. To fill this gap, several experiments were carried out in the epidemic area in Donggou District in 1982–1984. Preliminary results are presented in this paper.

DISEASE PROGRESS IN BLAST AREA

Investigations of blast are always carried out among rice varieties with different levels of field resistance (6). Varieties with major gene resistance (except with *Pi-a* or *Pi-i* alone) are not suitable for this purpose. Some field resistant rice varieties possess a nonspecific reaction to physiologic races of *P. oryzae* and slow blasting character, so that the severity of yield fluctuations due to blast damage can be reduced to a certain extent. Selection for rice cultivars with field resistance thus has practical importance.

In 1982 in Donggou District, leaf blast progress was investigated under field conditions with blast-induced seedlings interplanted randomly along the experimental miniplots. Twenty japonica varieties were used in the experiment. Observations of leaf blast were made once a week after the appearance of blast lesions on the leaves. Data on eight varieties representing different levels of resistance are presented in Table 1 and Figure 1. From the disease progress curves (Fig. 1) and grades of resistance (Table 1), it is clear that the blast resistance of the eight varieties is different and only concerns major genes (*Pi-a*) in two varieties. Thus the apparent difference in blast resistance lies in the minor genes. The difference between Homare-nishiki, Ginga, and Reimei on one hand and Mokoto on the other is outstanding, with Chiyohikari and Jingyue 1 included in the resistant group and Jiefangzu in the susceptible.

In Table 1 the grade of field resistance for Jingyue 1 is r, indicating that its dilatory blast resistance is lower than those of Homare-nishiki, Ginga, and Reimei. Jingyue 1 is a widespread variety in Donggou District and has a relatively long history of cultivation (about 18 years). Certainly, cultivars with field resistance of r grade are not good enough to withstand attack from the pathogen in epidemic areas like Donggou and the surrounding districts. From the viewpoint of breeding rice for durable blast resistance, to develop a variety with rr grade of field resistance would be valuable.

Table 1. Genotypes and grades of field resistance of eight japonica varieties.

Variety	Origin	Genotype	Grades of field resistance ^a	
			Determined in Japan ^b	Determined by the author
Homare-nishiki	Japan	<i>Pi-a</i>	rr-r-m	rr
Ginga	Japan	+	rr-r-m	rr
Reimei	Japan	<i>Pi-a</i>	rr-r-m-s	rr
Chiyohikari	Japan	+	rr-r-m	r
Jingyue 1	China	+	—	r
Xintuanheigu	China	+	—	m
Jiefangzu	DPRK	+	—	s
Mokoto	Japan	+	ss	ss

^arr = highly resistant, r = resistant, m = moderately resistant, s = susceptible, ss = highly susceptible.

^bLetter(s) underlined indicate mode of grade.

INHERITANCE OF MINOR GENE RESISTANCE TO BLAST

In order to clarify the quantitative nature of field resistance, we crossed the highly susceptible variety Mokoto with the highly resistant ones Homare-nishiki, Ginga, and Reimei to study the segregation behavior of resistance in the F₁, F₂, and F₃ generations along with parents.

The frequency distributions of diseased leaf area (DLA %) for P₁, P₂, F₁, F₂, and F₃ of the three crosses are shown in Figures 2-4. The difference between the two parents is very significant. The DLA for the F₁ population lies between P₁ and P₂. The F₂ population exhibits a continuous skewed distribution toward the resistant side, with its peak located on the position of F₁ and the two tails overlapping the positions of the two parents. The DLA distribution for F₃ families (10.6–14.2% of F₂) has a similar skewed tendency.

From the data it is obvious that the inheritance of field resistance to rice blast is quantitative, with additive and partially dominant effects of minor genes.

Since the DLA data are expressed in percentage and the distribution has a strong deviation from normality, they have to be transformed. In this study we adopted the relative resistance index (RRI) (8) to represent the minor gene resistance to leaf blast:

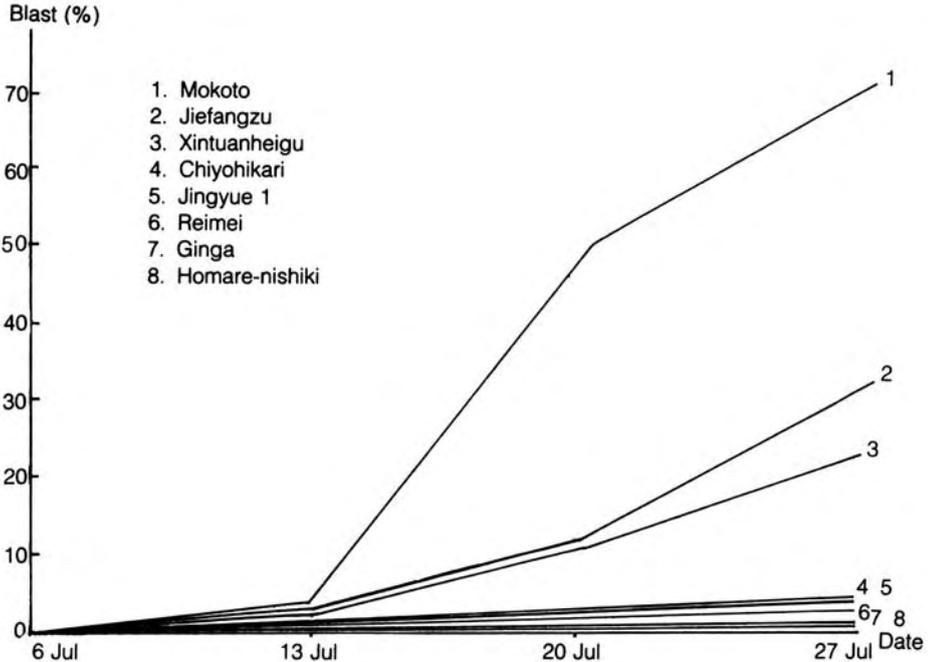


Fig. 1. Disease progress curves of diseased leaf area (%) of leaf blast in eight rice varieties.

$$RRI = \ln \frac{y_i}{1-y_i} - \ln \frac{x_i}{1-x_i}$$

where y_i denotes the percentage DLA of each stage for the susceptible check, and x_i stands for the variety tested.

Through transformation, the F_2 frequency distributions of the three crosses attained normal distributions (being fit in a χ^2 test). The F_2 frequency distribution of RRI for Homare-nishiki/Mokoto is shown in Figure 5. The broad sense heritability h_b^2 estimated for RRI of Homare-nishiki/Mokoto is 76.09%, for Mokoto/Ginga 82.86% and for Mokoto/Reimei 66.26%. Interrelations between the F_2 and F_3 generations for the three crosses showed positive correlations, with correlation coefficients of 0.6483 for Homare-nishiki/Mokoto, 0.8054 for Mokoto/Ginga, and 0.6357 for Mokoto/Reimei, all significant at the 1% level. Genes controlling field resistance to blast for these three crosses are estimated to be at least five pairs for Homare-nishiki, three pairs for Ginga, and six pairs for Reimei.

F₂ DISTRIBUTION AND CORRELATION OF TRAITS

Some investigators consider the number of blast lesions as an index of resistance to the invasion by the pathogen and the rate of lesion expansion as an index of resistance to the expansion of DLA. But the points of invasion are not necessarily the points of parasitism (e.g., hypersensitivity of host plants), so we chose to observe points of parasitism. The data from Ginga/Mokoto are presented.

Number of scale 4 blast lesions and large S lesions

A scale 4 lesion is typically confined to the two main veins; a large S lesion typically exceeds the two main veins. The F₂ distribution for these two traits was skewed toward the resistant (Fig. 6). For the first trait, there was only a small difference between the mean value of F₂ population (24.8/100 cm²) and the midparent value (29.1/100 cm²), and the mean value for the F₁ (12.1/100 cm²) was much lower. For the second trait, the difference in mean values between the F₁ and the F₂ was not great, but they both showed significant differences from the midparent. This may be the basis of partial dominance of minor gene resistance. Heritabilities were estimated at only 49.9% for scale 4 lesions and 38.4% for large S lesions. The correlation coefficient between these traits was 0.842 (significant at the 1% level).

Length, width, and length × width of lesions

The F₂ populations of lesion length (Fig. 7), lesion width, and length × width (Fig. 8) exhibit the normal distribution, with their mean values very near or slightly toward the resistant side of the midparent value. This means the additive effect is the main effect of the genes. Heritabilities were estimated at 56.4% for lesion length, 59.8% for lesion width, and 35.3% for length × width. Lesion length shows a very high correlation with lesion area, with a coefficient of 0.918, and lesion width correlates with area at 0.875, both being significant at the 1% level.

Diseased leaf area

This trait was studied in three crosses and was reported earlier in this paper. Additional data from a cross of Ginga/Mokoto are presented in

Table 2. Simple correlations among leaf and panicle blast traits in Ginga/Mokoto F₂^a.

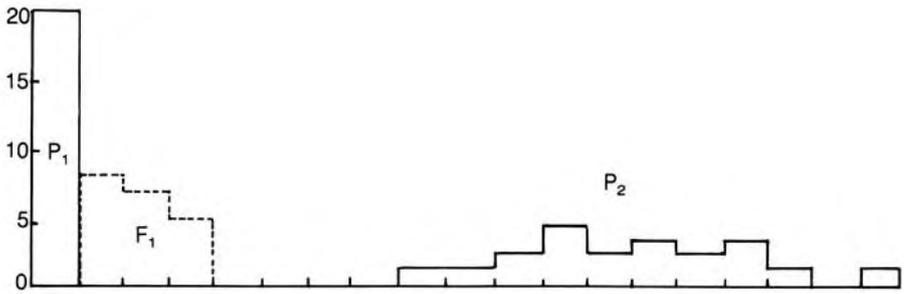
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Number of scale (1) 4 lesions	** 0.842	** 0.382	** 0.257	** 0.363	** 0.670	** 0.233	** 0.273	** 0.227
Number of large (2) S lesions		** 0.393	** 0.371	** 0.426	** 0.731	** 0.214	** 0.331	** 0.311
Lesion length (3)			0.630	** 0.918	** 0.357	** 0.098	0.111	0.012
Lesion width (4)				** 0.875	** 0.370	0.114	0.098	0.053
Lesion length × (5) lesion width					** 0.408	0.121	0.114	0.029
Diseased leaf (6) area (%)						** 0.218	** 0.347	** 0.293
Rachilla blast (7) (%)							** 0.677	** 0.723
Neck blast (%) (8)								** 0.870
Neck blast + (9) diseased panicle branch								

^aN = 172, ** = significant at the 1% level.

Fig. 9. Comparing the reciprocal crosses between Ginga and Mokoto (Fig. 3, 9) there is no essential difference in the tendencies of DLA distribution. This suggests that there may be no cytoplasmic influence on the inheritance.

Interrelations among resistance to invasion, expansion rate, and DLA

Although the simple correlations between traits of resistance to invasion (scale 4 of lesion) and resistance to expansion (length, width, length × width) are very significant, the values are quite low. This means that there may be some independence of inheritance between these two traits. Therefore, a combination of high resistance to invasion and high resistance to expansion rate is theoretically possible.



Mean value
P₁ = 0.28 %
P₂ = 51.83
F₁ = 9.66
F₂ = 14.19
F₃ = 6.35
MP = 26.06

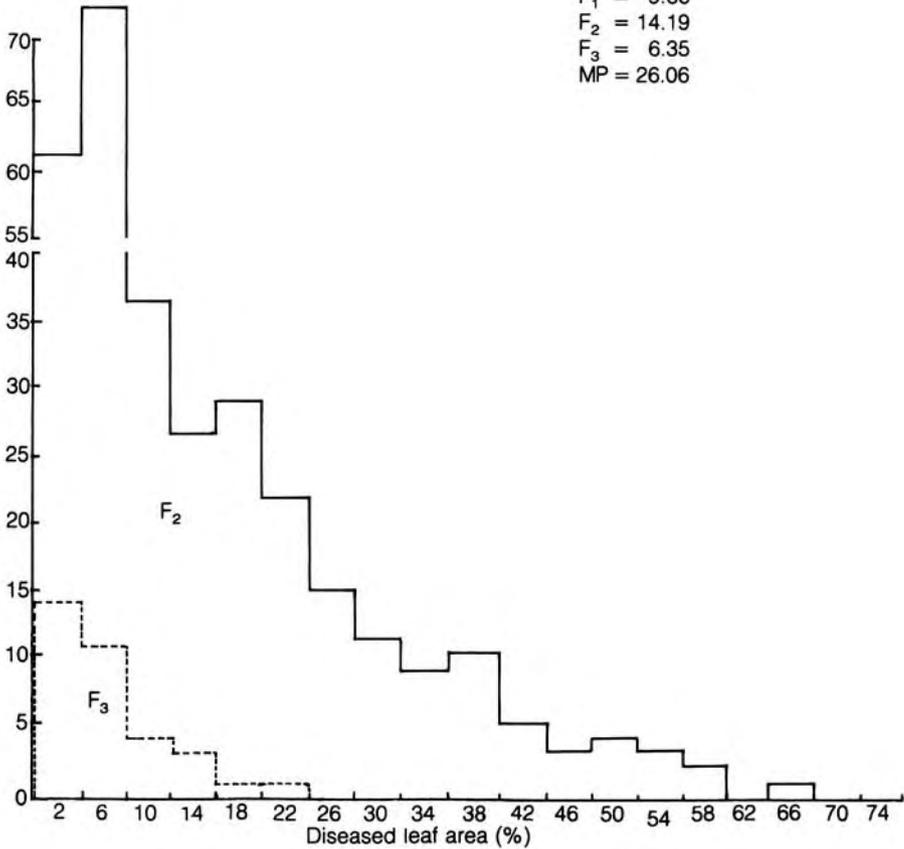
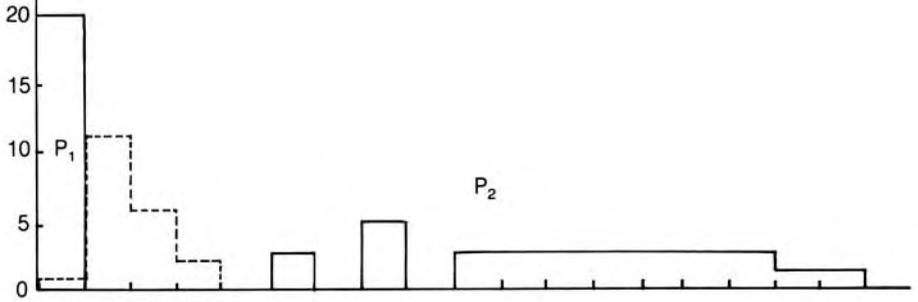


Fig. 2. Frequency distribution of diseased leaf area in Homare-nishiki/Mokoto.



Mean value
P₁ = 0.51 %
P₂ = 47.78
F₁ = 7.61
F₂ = 10.07
F₃ = 3.86
MP = 24.14

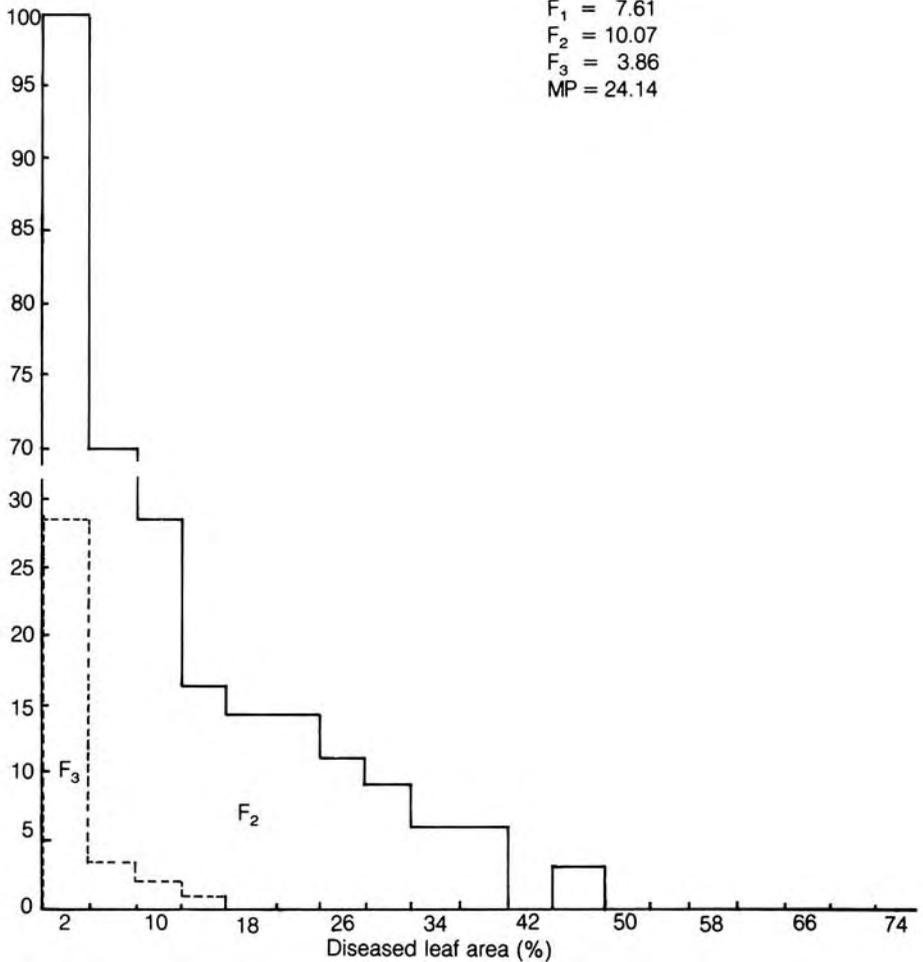


Fig. 3. Frequency distribution of diseased leaf area (%) in Mokoto/Ginga.

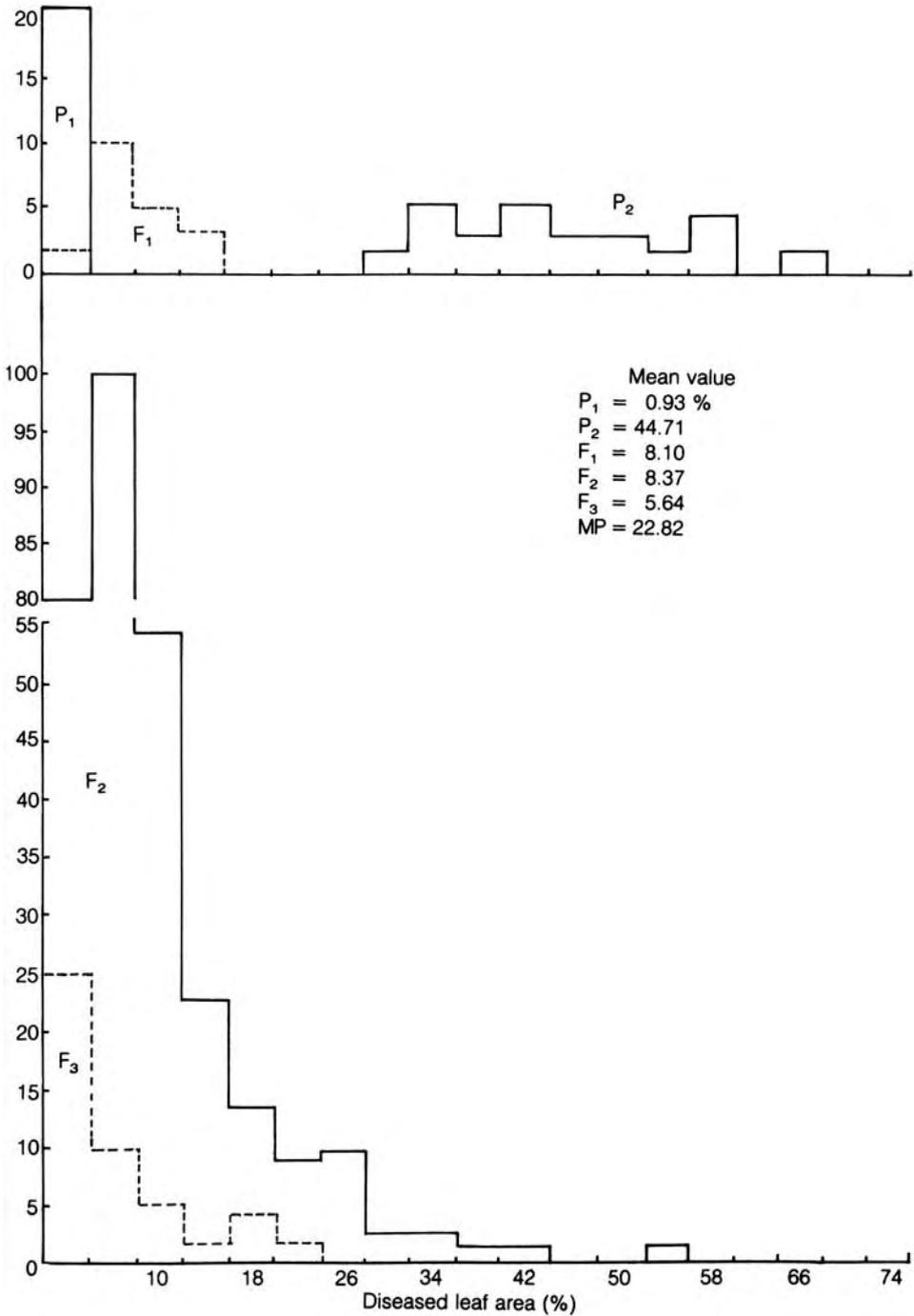


Fig. 4. Frequency distribution of diseased leaf area (%) in Mokoto/Reimei.

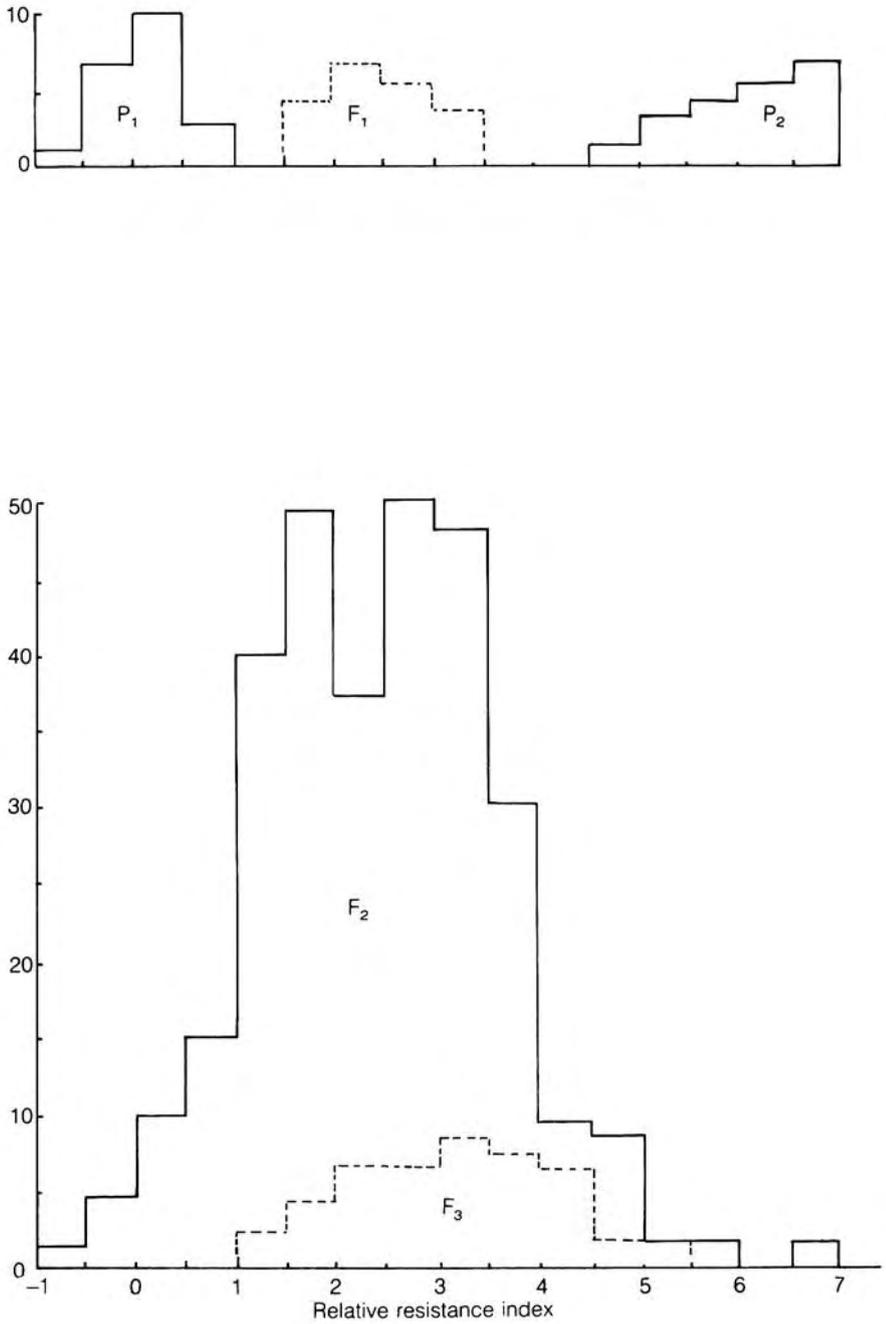
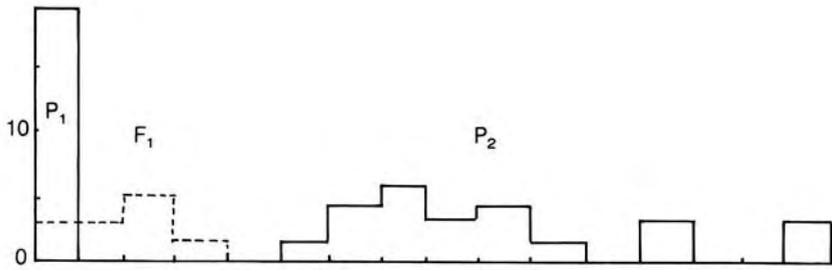


Fig. 5. Frequency distribution of relative resistance index of P₁, P₂, F₁, F₂, and F₃ of Ginga/Mokoto.



Mean value
 P₁ = 0.2 no/100 cm²
 P₂ = 17.8
 F₁ = 3.6
 F₂ = 5.8
 MP = 9.0

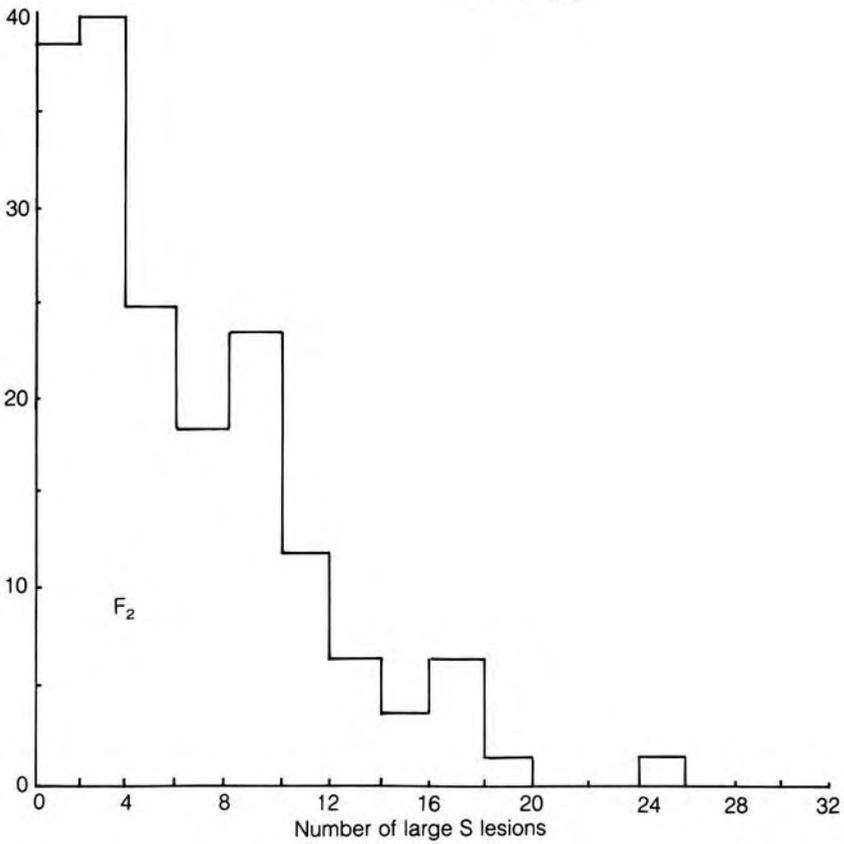
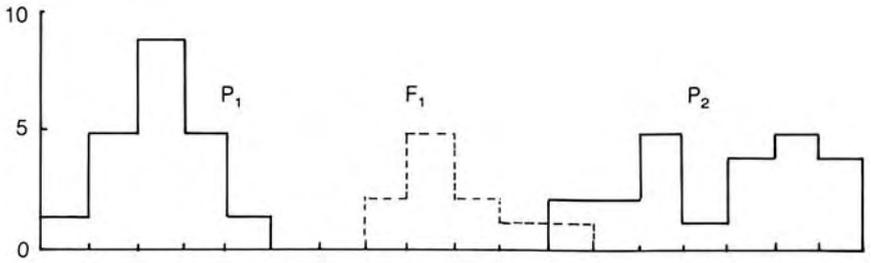


Fig. 6. Frequency distribution of number of large S lesions per 100 cm² of leaf area.



Mean value
P₁ = 4.7 mm
P₂ = 17.0
F₁ = 11.3
F₂ = 10.4
MP = 10.9

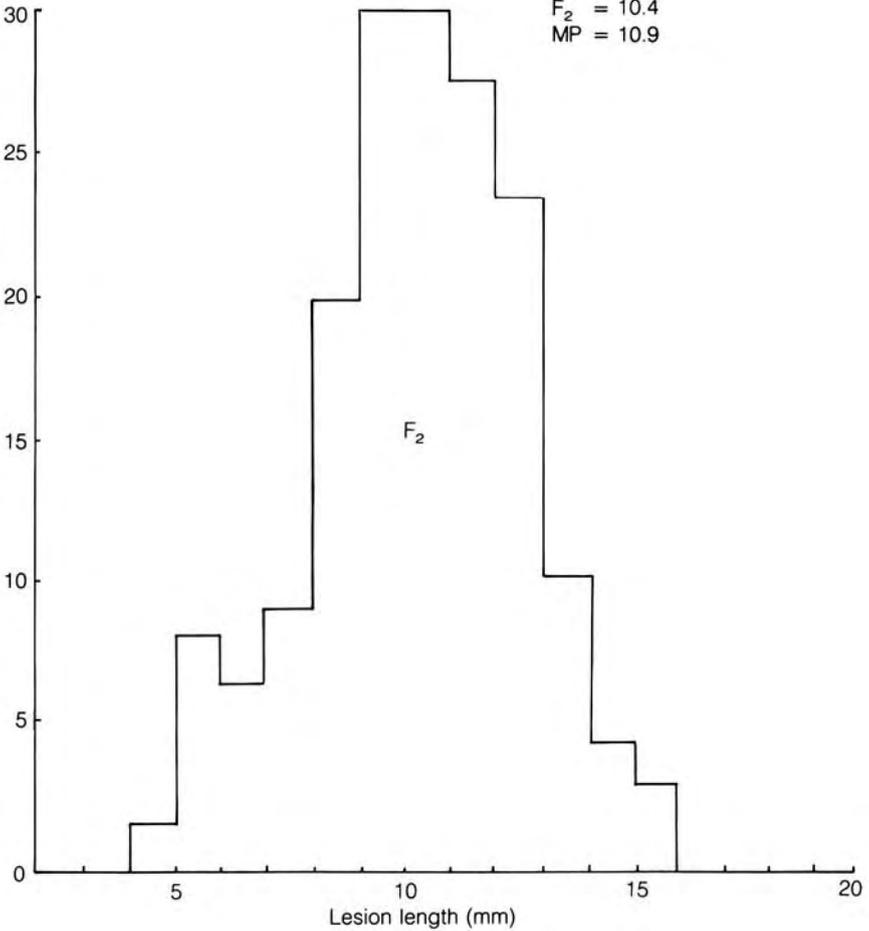


Fig. 7. Frequency distribution of lesion length of leaf blast in Ginga/Mokoto.

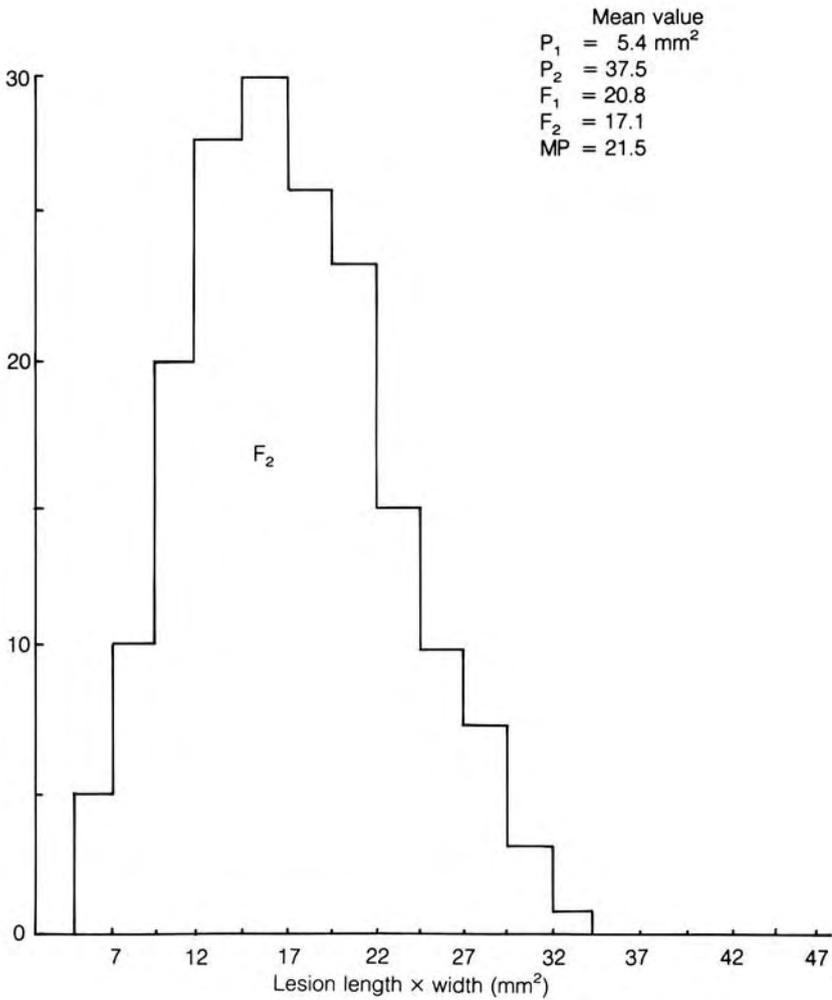
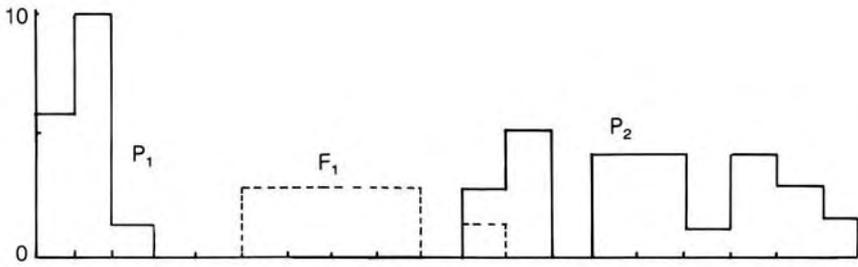


Fig. 8. Frequency distribution of lesion length x width of leaf blast in Ginga/Mokoto.

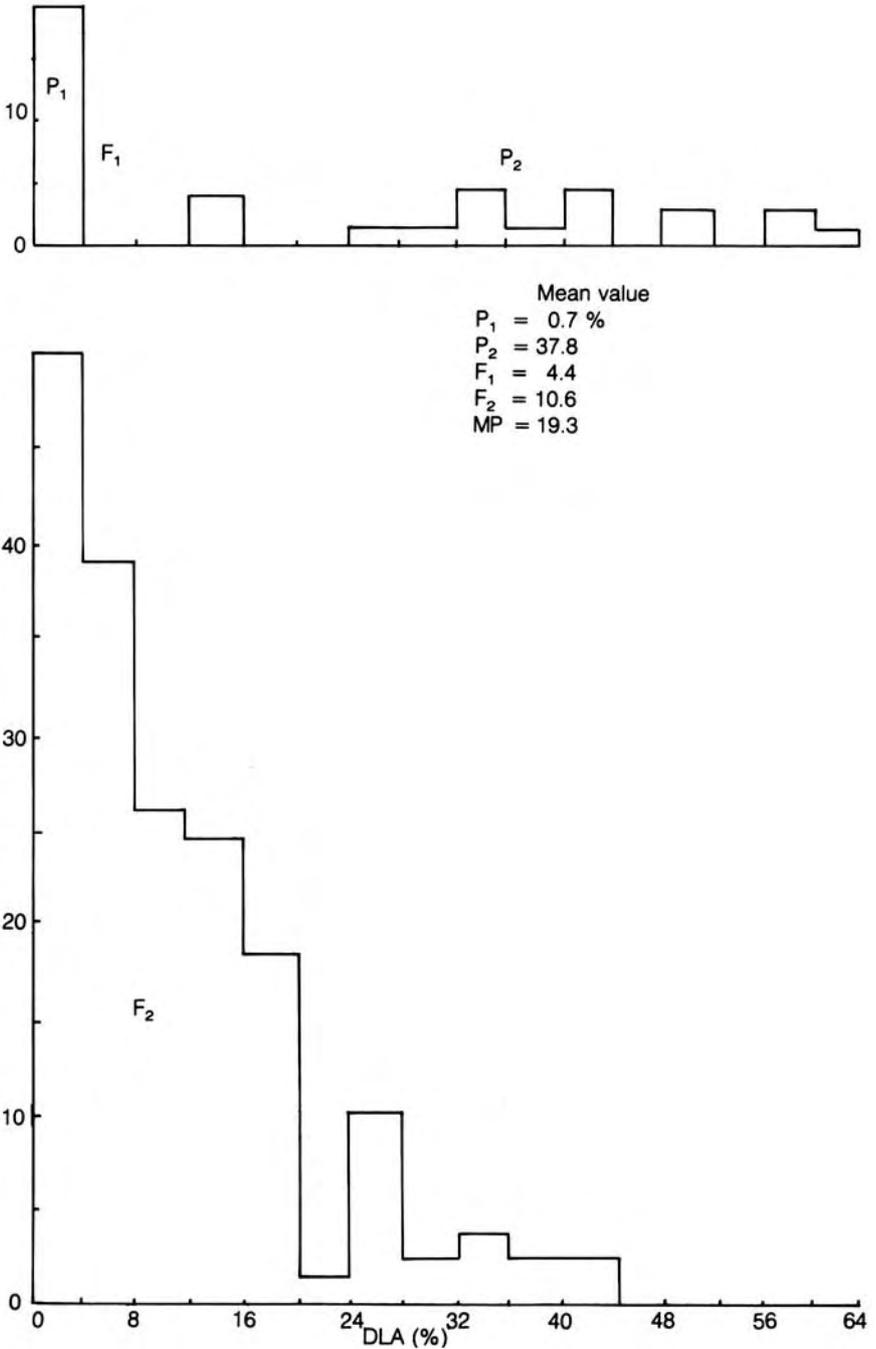


Fig. 9. Frequency distribution of diseased leaf area of leaf blast in Ginga/Mokoto.

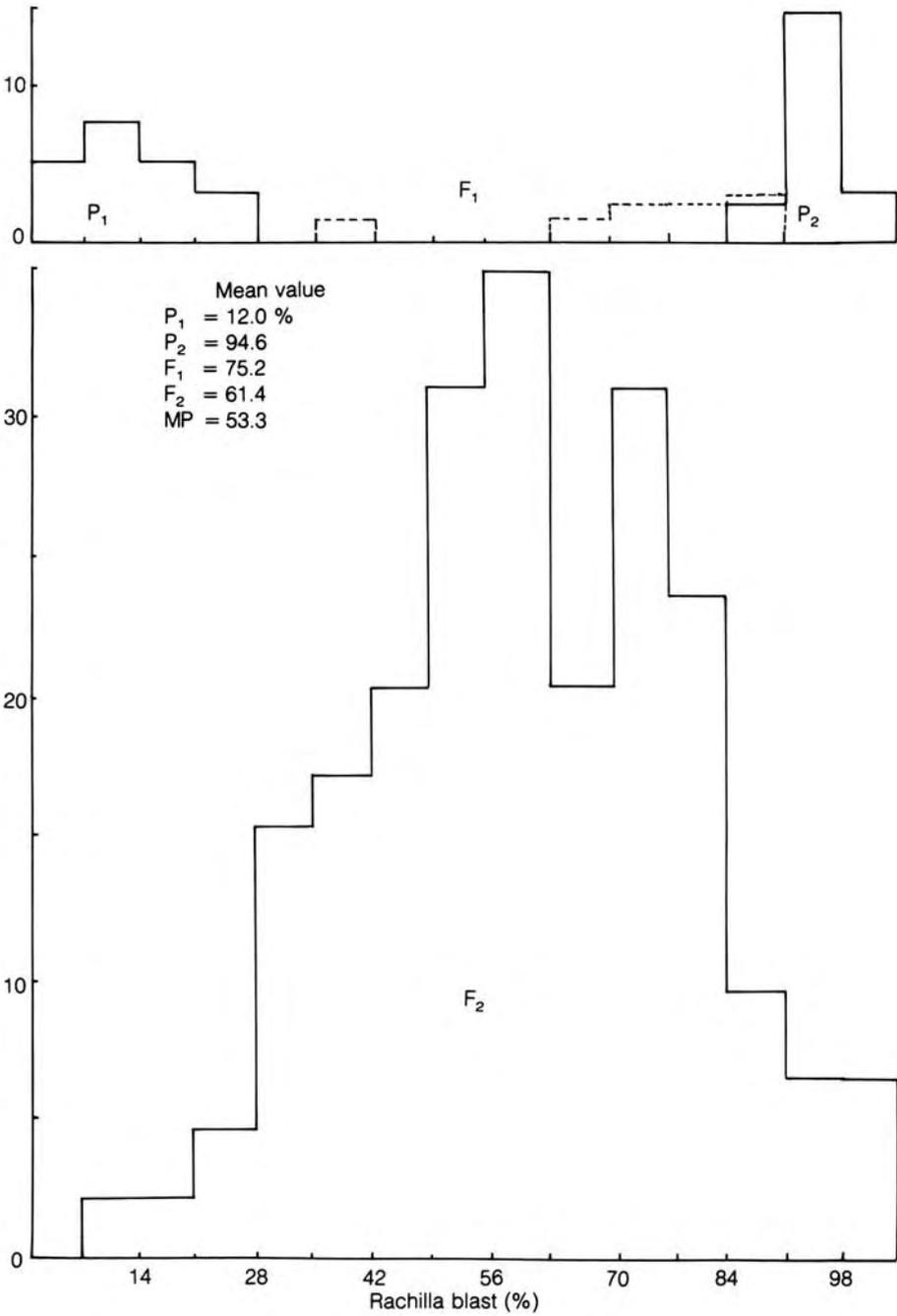


Fig. 10. Frequency distribution of diseased rachilla (%) of panicle blast in Ginga/Mokoto.

Large S lesions on a rice leaf manifest not only the invasion point of the pathogen but a higher expansion rate. A large S lesion has been determined by the combined action of the two traits. The multiple regression equation of large S lesion (Y) on scale 4 lesion (x_1) and lesion length \times width (x_2) is:

$$Y = 0.2245 x_1 + 0.1131 x_2 - 1.70$$

$$R^2 = 0.7260$$

But, to improve diseased leaf area, scale 4 lesion (x_1) and length \times width (x_2) are much less important than large S lesion (x_3). The regression equation and R^2 are as follows:

$$Y = 0.0986 x_1 + 0.1778 x_2 + 0.9855 x_3 - 0.6055$$

$$R^2 = 0.5557$$

Improvement of three variables, especially the large S lesion, would improve DLA to a certain extent (55.6%), and the rest (44.4%) would be influenced by environmental factors.

PANICLE BLAST AND LEAF BLAST

There are three traits involving panicle blast: diseased rachilla (%), neck blast (%), and neck blast + diseased panicle branches (%).

Owing to strong negative correlations between heading date and panicle blast trait, the original data were submitted to a rectification treatment with the same heading date. The frequency distributions of the rectified date for each trait are presented in Figures 10 and 11. The distribution of these traits is approximately normal to slightly skewed. The F_1 and F_2 mean values for diseased rachilla and for neck blast + diseased panicle branch are slightly susceptible in comparison with the midparent value. The reason for the slight susceptibility in the F_1 may be the earlier date of heading.

The genetic variations coefficient of diseased rachilla is 24.4%, of neck blast and of neck blast + diseased panicle branches 36.2%. The heritability of diseased rachilla is 62.4%, of neck blast 52.2%, and of neck blast + diseased panicle branches 48.8%. Correlations among them are quite high (Table 2).

Scale 4 lesion number shows a very significant correlation with three traits of panicle blast (Table 2), meaning there exist some common genetic factors controlling the mechanism of resistance. But the correlation coefficient is generally low. Therefore it is impossible to improve one character by improving the other.

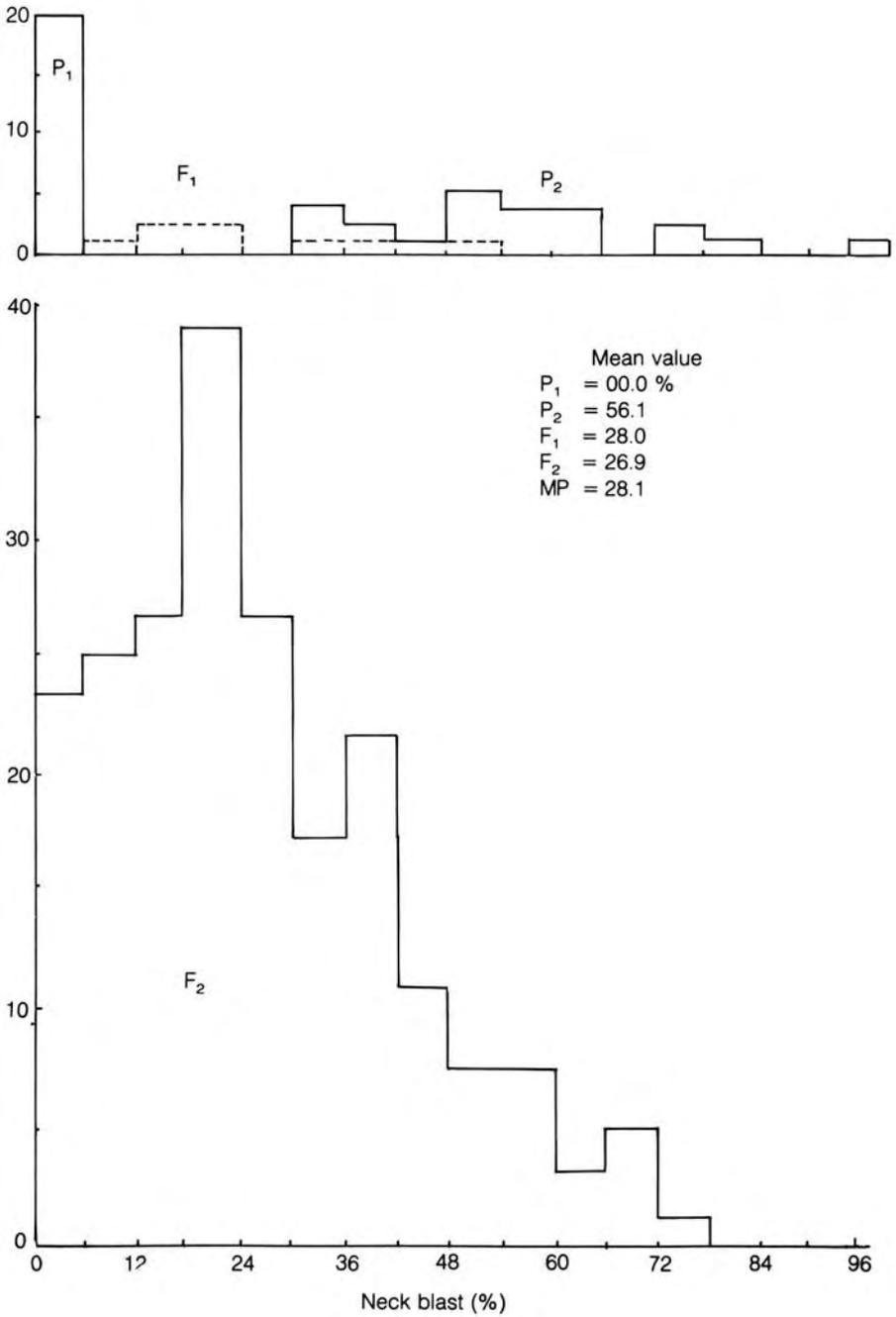


Fig. 11. Frequency distribution of neck blast in Ginga/Mokoto.

It is very interesting that all correlation coefficients in Table 2 are very significant except those between resistance to expansion of leaf blast and panicle blast traits. This means that the mechanism of this resistance to blast is completely different, but the reason is not clear yet.

SIGNIFICANCE OF MINOR GENE RESISTANCE TO BLAST

On growing resistant varieties as a measure for rice blast control in epidemic areas, there are two successive challenges. One arises from the constant attack of local physiological races of the pathogen, the other from new virulent races of mutagenic or latent origin or both. For counteracting the first challenge we have adopted certain major genes to raise the initial resistance to blast, from which we found that the *Pi-Z'* gene might be the most effective because of its broad spectrum of resistance to races in northern China. Nevertheless, we have seen in southern China that certain japonica cultivars that have successfully inherited the *Pi-Z'* gene develop typical leaf blast lesions. This gives us an indication that the high resistance expressed by the *Pi-Z'* gene might not be durable. It is repeatedly confirmed in rice production practice that the effective approach to blast control should lie in integrated measures. The experimental data provided in this paper suggest that there may be complementary effects between major and minor gene resistance in rice blast control. As the first step in resistance, a major gene with broad spectrum resistance would be superior in checking virulent races. This situation may continue for several years until the inevitable appearance of a new virulent race that renders the resistant variety susceptible. Once the major gene loses its resistance, minor genes in the same variety would become responsible for further resistance.

In breeding rice for durable resistance to blast, one should make every effort to combine major and minor gene resistance and other characters such as good grain quality, high yielding ability, and multiple resistance to other harmful factors. To combine the major and minor gene resistances the difficulty comes in how to identify minor genes for resistance in the presence of a major gene or genes that cover up the expression of the minor. Therefore, the breeding process should be composed of two steps: First, introduce most of the minor genes for rice blast resistance to a cultivar with economic superiority. Second, cross the semi-improved variety with a donor possessing major gene resistance and then back-cross the hybrid to the semi-improved recurrent parent until resistance

from both origins is combined. For this purpose suggestions made by Asaga and Higashi (1) are also a feasible way of breeding rice for blast resistance.

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INHERITANCE OF RESISTANCE TO BACTERIAL BLIGHT IN RICE

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Three breeding lines of rice — IR1529-680-3-2, IR944-102-2-3, and RP9-3 — were genetically analyzed for resistance to bacterial leaf blight using Japanese races of *Xanthomonas campestris* pv. *oryzae*. From the data on the reaction of F₂ populations from the crosses between the three cultivars and Japanese differentials, it was concluded that IR1529-680-3-2 and IR944-102-2-3 each have two dominant genes that convey resistance to Japanese races. One gene of IR944-102-2-3 that conveys resistance to Japanese races IA, II, and IIIA is either the same as *Xa-3* of Chugoku 45 or very closely linked to it. The second dominant gene of IR944-102-2-3 conveying resistance to Japanese races II and IIIA is independent of *Xa-1* of Kogyoku and *Xa-3* of Chugoku 45 and was designated *Xa-11*. RP9-3 and IR8 are also postulated to have this *Xa-11* gene from their pedigree records and reaction patterns. Furthermore, the one dominant gene of IR1529-680-3-2 conveying resistance to Japanese races II and IIIA appears to be identical to *Xa-11*.

Bacterial leaf blight caused by *Xanthomonas campestris* pv. *oryzae*, which prevails in the rice-growing countries of Asia, causes severe damage to rice. There is a considerably high reliance on the use of resistant varieties for controlling the disease due to the scarcity of effective bactericidal agents.

Since pathogenic specialization in the causal bacterium of rice bacterial leaf blight was first reported in Japan by Kuhara et al (4), a number of reports have been published on the variability of pathogenicity in the bacterium, as well as on the variability of resistance in rice varieties.

Genetic studies have been carried out mainly in Japan and at IRRI using isolates of *X. campestris* pv. *oryzae* collected in each country. As a result, four dominant genes for resistance to Japanese isolate(s) — *Xa-1*, *Xa-2*, *Xa-3*, and *Xa-kg* — were identified in Japan (1, 7, 10). Recently, Yamada and Horino (14) reported two genes — *Xa-1^h*, which is allelic to

Xa-1, and *Xa-kg^h*, which is allelic to *Xa-kg*. Seven genes — *Xa-4*, *xa-5*, *Xa-6*, *Xa-7*, *xa-8*, *xa-9*, and *Xa-10* — for resistance to Philippine isolates were identified at IRRI (8, 9, 11, 12, 13, 15). Furthermore, *Xa-4^b* was identified as allelic to *Xa-4* (*Xa-4^a*) (5).

However, since the resistant varieties and races used were different, it was difficult to distinguish the resistance genes identified by the two groups of investigators. It was desirable to compare and analyze the results of the studies carried out in Japan and at IRRI to devise effective methods of control. We thus initiated genetic analyses of the resistant varieties identified at IRRI using Japanese isolates. This paper deals with the relationship between the genes (*Xa-1* and *Xa-3*) identified in Japan and those (*Xa-4* and *Xa-6*) identified at IRRI, using Japanese isolates. Furthermore, we studied the inheritance of resistance in varieties having reaction patterns similar to that of IR8.

MATERIALS AND METHODS

For analysis of the genes identified in Japan and at IRRI, two IR lines (IR1529-680-3-2 and IR944-102-2-3) and three Japanese differentials (Kinmaze, Kogyoku, and Chugoku 45) were crossed in six cross combinations. F₂ plants were tested for their resistance to representative isolates of four races (IA, II, IIIA, and IV). The isolates of *X. campestris* pv. *oryzae* used for screening were T7174 (IA), T7147 (II), T7133 (IIIA), and IVA7505 (IV). These isolates were used for screening after they were shown to have the typical pathogenicity of races IA, II, IIIA, and IV. They were cultured on potato semisynthetic agar medium for 2 days at 25°C, suspended in distilled water at a concentration of about 10⁸ cells/ml, and used for inoculation.

The F₂ plants were transplanted individually to experimental plots after they had been grown in an upland nursery bed for 45 days. Their tillers were separated into four groups one month after transplanting, and then they were retransplanted to experimental plots. Fertilizer was applied according to recommended practices.

At the time of full heading, the four plants originating for the same F₂ plant were inoculated by the clipping method (3), each with a different isolate of the four races IA, II, IIIA, and IV. All F₂ plants were inoculated at the same time.

Three weeks after inoculation, the reaction of each individual to each isolate was examined and evaluated as R (resistant) or S (susceptible) according to the degree of lesion development. The reaction pattern to

the four races was designated RRRR, SRRS, etc., where the letters (R = resistant, S = susceptible) stand for the reaction to races IA, II, IIIA, and IV, respectively, from left to right. This experiment was carried out at the Chugoku National Agriculture Experiment Station in Japan.

The three hybrids IR1529-680-3-2/IR944-102-2-3, IR944-102-2-3/RP9-3, and RP9-3/Chugoku 45 were analyzed to study inheritance in resistant varieties that have reaction patterns to Japanese races similar to that of IR8. The experiment was carried out at the Tropical Agriculture Research Center in Japan.

The isolate of *X. campestris* pv. *oryzae* used for the experiment was T7133 (IIIA). F₂ plants were transplanted at two plants/pot (200 cm²) after they were grown in seedling boxes for 30 days. Between the booting and flowering stages they were inoculated by the clipping method with an isolate of race IIIA.

RESULTS AND DISCUSSION

Inheritance of resistance in IR1529-680-3-2 and IR944-102-2-3 to Japanese races

The results of F₂ analysis of the resistance of six rice hybrids to four Japanese races of *X. campestris* pv. *oryzae* are summarized in Table 1.

The F₂ population of Kinmaze/IR1529-680-3-2 showed a segregation ratio of 12:3:1 RRRR to SRRS to SSSS, suggesting that resistance in IR1529-680-3-2 is controlled by two dominant genes that are independent of each other. One of the genes conveys resistance to Japanese races IA, II, IIIA, and IV, and the other to Japanese races III and IIIA.

The F₂ population of Kogyoku/IR1529-680-3-2 showed a segregation ratio of 48:9:3:3:1 RRRR to RRRS to SRRS to RSSS to SSSS, indicating that there is no linkage between the dominant gene *Xa-1* of Kogyoku and the two dominant genes of IR1529-680-3-2.

On the other hand, the F₂ data of IR1529-680-3-2/Chugoku 45 showed a segregation ratio of 3:1 RRRR to RRRS, thereby showing that the dominant gene *Xa-3* of Chugoku 45 is completely or very closely linked to the one dominant gene of IR1529-680-3-2 that conveys resistance to Japanese races IA, II, IIIA, and IV.

From the reaction of F₂ populations of crosses between IR1529-680-3-2 and three Japanese differentials, IR1529-680-3-2 was found to have two dominant genes for resistance to Japanese races, and one of these genes is completely or very closely linked to *Xa-3* of Chugoku 45.

Table 1. F₂ data on the resistance of six rice hybrids to four Japanese races.

Cross combination		Number of plants for each reaction pattern ^a					Total	Expected ratio	χ^2	P
		RRRR	RRRS	SRRS	RSSS	SSSS				
Kinmaze/ IR1529-680-3-2	observed	144.		49		9	202	12:3:1	4.651	0.1–0.05
	expected	151.5		37.9		12.6				
Kogyoku/ IR1529-680-3-2	observed	155	27	8	6	3	199	48:9:3:3:1	1.609	0.9–0.8
	expected	149.3	28.0	9.3	9.3	3.1				
Chugoku 45/ IR1529-680-3-2	observed	146	56				202	3:1	0.799	0.5–0.3
	expected	151.5	50.5							
Kinmaze/ IR944-102-2-3	observed		145	48		12	205	12:3:1	2.984	0.3–0.2
	expected		153.8	38.4		12.8				
Kogyoku/ IR944-102-2-3	observed		185	3	6	2	196	57:3:3:1	5.905	0.2-0.1
	expected		175	9	9	3				
Chugoku 45/ IR944-102-2-3	observed		194				194	1:0	0	1
	expected		194							

^aThe combined four capitals stand for the reactions to Japanese races IA, II, IIIA, and IV, respectively, from left to right. R = resistant, S = susceptible.

IR1529-680-3-2 was earlier reported to have the dominant gene *Xa-4* that conveys resistance to a Philippine isolate at IRR1 (2). Thus, it is also clear that the *Xa-4* gene of IR1529-680-3-2 is completely or very closely linked to *Xa-3* of Chugoku 45, and it has one additional gene for resistance to Japanese races. Thus IR1529-680-3-2 appears to have two genes for resistance to Japanese races in addition to *Xa-4*.

The F₂ population of Kinmaze/IR944-102-2-3 showed a segregation ratio of 12:3:1 RRRS to SRRS to SSSS, showing that IR944-102-2-3 has two dominant genes that are independent of each other. On the other hand, there was no susceptible plant in the F₂ population of IR944-102-2-3/Chugoku 45; and all F₂ plants showed the reaction pattern RRRS. Therefore, it appears that the one dominant gene of IR944-102-2-3 is the same as *Xa-3* of Chugoku 45.

IR944-102-2-3 was identified as having one dominant gene *Xa-6* that conveys resistance to a Philippine isolate (8, 11). Thus it appears that the *Xa-6* gene of IR944-102-2-3 is the same gene as *Xa-3* of Chugoku 45, and that IR944-102-2-3 has an additional dominant gene for resistance to Japanese races; or perhaps IR944-102-2-3 has two additional genes for resistance to Japanese races besides *Xa-6*.

Inheritance of resistance in varieties having reaction patterns similar to that of IR8

We showed that IR1529-680-3-2 and IR944-102-2-3 each have one dominant gene for resistance to Japanese races II and IIIA, but these are susceptible to Japanese races IA and IV. The reaction pattern to Japanese races is very similar to that of IR8 (6). Table 2 presents some varieties showing this reaction pattern.

In F₂ populations of IR1529-680-3-2/IR944-102-2-3 and IR944-102-2-3/IR944-102-2-31 RP9-3 there were no susceptible plants. On the other hand, the F₂ population of RP9-3/Chugoku 45 showed a segregation ratio of 15:1 R to S (Table 3). These results indicate that the dominant gene of RP9-3 is the same as or allelic to one dominant gene of IR944-102-2-3, and that the dominant gene of RP9-3 is independent of the dominant gene *Xa-3* of Chugoku 45. Furthermore, one dominant gene of IR1529-680-3-2 is the same as or allelic to one dominant gene of IR944-102-2-3.

From the pedigree records (Table 4) of the IR cultivars used in this study, it is evident that the same dominant gene has been introduced into IR944-102-2-3, IR1529-680-3-2, and RP9-3, and that this gene came through IR8 from Peta. We have designated this dominant gene *Xa-11*. It

Table 2. Varieties that showed IR8 pattern of the reaction to bacterial leaf blight.

Variety	Japanese races					
	IA T7174	II T7147	IIIA T7133	IV IVA7505	IB T7156	IIIB Q6809
IR8	S	R	R	S	R	S
IR1414-67-3-2	S	R	R	S	R	S
IR1416-131-5-2-3	S	R	R	S	R	S
RP9-3	S	R	R	S	R	S
Dee-geo-woo-gen	S	S	S	S	S	S
TN 1	R	R	S	S	R	S

Table 3. F₂ data on the resistance of three hybrids to Japanese race IIIA.

Cross-combination		Number of plants for each reaction pattern ^a		Total	Expected ratio	c ²	P
		R	S				
IR1529-680-3-2/ IR944-102-2-3	observed	200	0	200	1:0	0	1
	expected	200	0				
IR944-102-2-3/ RP9-3	observed	197	0	197	1:0	0	1
	expected	197	0				
RP9-3/ Chugoku 45	observed	174	13	187	15:1	0.130	0.75–0.5
	expected	175.3	11.7				

^aR = resistant, S = susceptible.

Table 4. The pedigree records of IR1529-680-3-2, IR944-102-2-3, and some other IR lines

Variety	Cross
IR1529-680-3-2	Sigadis × 2/TN1/4/IR8//CR231/SLO-17//Sigadis
IR944-102-2-3	TN1/Malagkit Sungsong//IR8
IR8	Peta/Dee-geo-woo-gen
IR1414-67-3-2	Mudgo/IR8*2
IR1416-131-5-2-3	Peta*4/TN1//Te-tep
RP9-3	IR8/W1251

conveys resistance to Japanese races II and IIIA. *Xa-11* is independent of *Xa-1*, *Xa-3*, and *Xa-4*.

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GENETICS OF BACTERIAL BLIGHT RESISTANCE IN RICE

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The reaction to the Punjab isolate of bacterial blight caused by *Xanthomonas campestris* pv. *oryzae* of differential rice varieties from Japan and the Philippines revealed that the Punjab isolate is different in pathogenicity from the Japanese and Philippine races. None of the resistance genes *Xa 1*, *Xa 2*, *Xa 3*, *Xa-kg*, *Xa 4*, *xa 5*, *Xa 6*, *Xa 7*, *xa 8*, *xa 9*, and *Xa 10* convey resistance to the Punjab isolate. Single dominant genes that are allelic convey resistance to the Punjab isolate in rice cultivars Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32. Single recessive genes that are also allelic confer resistance to this isolate in DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129. These dominant and recessive genes for resistance to the Punjab isolate of bacterial blight are independently inherited and may or may not be allelic to some of the resistance genes for the disease already identified.

The genetics of resistance to bacterial blight caused by *Xanthomonas campestris* pv. *oryzae* (Ishyama) Dye has been extensively investigated in Japan and at IRRI in several rice varieties resistant to the Japanese and the Philippines pathotypes. Eleven genes for resistance to the disease have so far been identified. The genes conveying resistance to the Japanese pathotypes have been designated *Xa 1*, *Xa 2*, *Xa 3*, and *Xa-kg* (1, 6, 8), while the resistance genes for the Philippine pathotypes have been designated *Xa 4*, *xa 5*, *Xa 6*, *Xa 7*, *xa 8*, *xa 9*, and *Xa 10* (7, 9, 10, 11). *Xa 1* and *Xa 2* are closely linked and are located on chromosome 11, as are *Xa 2* and *Xa-kg* (1, 6). *Xa 6*, *xa 9*, and *Xa 10* are linked to *Xa 4* (10, 11, 12). *Xa 6* confers resistance at the booting and flowering stages of plants to race 1 of the bacterium from the Philippines and shows reversal of dominance at the booting stage (9).

Two alleles have been reported at the *Xa 4* locus: *Xa 4*^a governs resistance at the early as well as later stages of plants to race 1 of the bacterium from the Philippines and *Xa 4*^b confers resistance to this race at the booting and post-flowering stages (5). *Xa 4* confers resistance only to race 1 and *Xa 10* only to race 2, while *xa 5* governs resistance to races 1, 2, and 3 from the Philippines and is located on chromosome 2 (13).

We have recently initiated studies on the genetics of resistance to bacterial blight in rice at the Rice Research Station of the Punjab Agricultural University at Kapurthala by using the virulent isolate of *X. campestris* pv. *oryzae* prevalent in the Punjab. The results of our preliminary investigation are reported in this paper.

MATERIALS AND METHODS

The pathotype of *X. campestris* pv. *oryzae* used in the study was isolated from naturally infected leaves of rice in the Punjab. Leaves with young, developing lesions were taken for isolation of the bacterial pathogen, which was purified by using Wakimoto medium and standard pathological techniques. The pathogenicity of the purified bacterial isolate was tested by inoculating it in the susceptible rice variety Taichung Native 1 (TN 1). The pathogenic bacterial isolate was maintained for further studies.

Rice differential varieties of Japan and the Philippines listed in Table 1 were inoculated with the Punjab isolate, and their reaction to this isolate was compared with their reaction to the Japanese and the Philippine races (2). The differential varieties were inoculated at the maximum tillering, booting, and post-flowering stages to determine their reaction at different stages to the Punjab isolate. Plants were inoculated by the clipping technique with a bacterial suspension of about 10^9 cells/ml (4). The disease score was recorded 2 weeks after inoculation as percentage of leaf area affected (3).

In addition to the varieties listed in Table 1, rice varieties possessing different genes for resistance to bacterial blight were also inoculated with the Punjab isolate to identify donors resistant to this isolate (Table 2). Resistant varieties, viz., Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, Patong 32, DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129, were crossed with the susceptible variety TN 1. The reactions to the Punjab isolate of the F_1 , F_2 , and F_3 generations of these crosses were evaluated to study the inheritance of resistance. The varieties possessing dominant genes for resistance were crossed in all possible combinations, and the reactions of the F_1 , F_2 , and F_3 progenies were evaluated to determine allelic relationships among dominant resistance genes. All possible crosses of varieties with recessive resistance genes were also attempted, and the reactions to the Punjab isolate of the F_1 , F_2 , and F_3 progenies were evaluated to study allelic relationships among recessive resistance genes. In addition, rice variety DV 85 was crossed with Patong

Table 1. Comparison of pathogenicity of the Punjab isolate of *X. campestris* pv. *oryzae* with the Japanese and the Philippine pathotypes.

Variety	Resistance gene	Reaction to ^a								Reaction to Punjab isolate	
		Japanese pathotypes					Philippine pathotypes				
		1	2	3	4	5	1	2	3		4
Kinmaze	none	S	S	S	S	S	S	S	S	S	S
Kogyoku	<i>Xa 1</i>										
	<i>Xa-kg</i>	R	S	S	S	R	S	S	S	S	S
Tetep	<i>Xa 1</i>										
	<i>Xa 2</i>	R	R	S	S	R	S	S	S	S	S
Wase											
Aikoku 3	<i>Xa 3</i>	R	R	R	S	S	R	R	R	R	S
Java 14	<i>Xa 1</i> , <i>Xa 3</i> , <i>Xa-kg</i>	R	R	R	S	R	R	R	R	R	S
IR 8		S	R	R	S	R	S	S	S	S	S
IR20	<i>Xa 4</i>	R	S	S	S	R	R	S	S	S	S
IR1545-339	<i>xa 5</i>	R	R	R	R	R	R	R	R	S	S
DV 85	<i>xa 5</i> , <i>Xa 7</i>	R	R	R	R	R	R	R	R	R	R
Cas 209	<i>Xa 10</i>	S	S	S	S	S	S	R	S	S	S

^aReaction reported in 2; R = resistant; S = susceptible.

32 and the reactions of the F₁, F₂, and F₃ progenies were evaluated to study the linkage relationship of the recessive resistance gene to the dominant resistance gene. Inoculation and scoring of plants in hybrid progeny was done by the methods described above (3, 4). TN 1 and resistant parents were grown with hybrid populations as susceptible and resistant checks. The F₁ and F₂ populations were scored on an individual plant basis. In the F₃ families, individual plants were scored, and each family was then classified as resistant, segregating, or susceptible. The expected segregation ratios for resistance versus susceptibility in the F₂ and F₃ populations were tested by calculating χ^2 values.

RESULTS

Pathogenicity test

The reactions of the Japanese and the Philippine differential varieties to the Punjab isolate indicated that the isolate of *X. campestris* pv. *oryzae*

Table 2. Reaction of some rice varieties with different genes for resistance to the Punjab isolate of bacterial blight.

Variety	Country of origin	Reaction ^a	Resistance gene	Resistance gene reported by
Bomba	Spain	S	<i>Xa 1</i>	8
Rantai-emas	Japan	S	<i>Xa 1, Xa 2</i>	8
Wase Aikoku 3	Japan	S	<i>Xa 3</i>	1
Lead Rice	USA	S	<i>Xa 3</i>	1
Bilekagga 36	India	S	<i>Xa 4</i>	10
Bajong	Indonesia	S	<i>Xa 4</i>	10
Aus 32	Bangladesh	S	<i>xa 5</i>	10
Malagkit Sungsong	Philippines	S	<i>Xa 6</i>	9
1160-8-6-1 ^b	Philippines	S	<i>Xa 7</i>	—
PI 231129	USA	R	<i>xa 8</i>	10
Sateng	Laos	S	<i>xa 9</i>	11
Cas 209	Senegal	S	<i>Xa 10</i>	12
Lua Ngu	Vietnam	R	<i>Xa 4</i>	10
Nagane Tia	Laos	R	<i>Xa 4</i>	10
Nam Sagui	Thailand	R	<i>Xa 4</i>	10
Nam Sakouy	Laos	R	<i>Xa 4</i>	10
Patong 32	Malaysia	R	<i>Xa 4</i>	10
BF 1	Bangladesh	MR	<i>xa 5</i>	10
DV 29	Bangladesh	MR	<i>xa 5</i>	10
DV 85	Bangladesh	R	<i>xa 5, Xa 7</i>	10
DV 86	Bangladesh	R	<i>xa 5, Xa 7</i>	10
DZ 78	Bangladesh	R	<i>xa 5, Xa 7</i>	10
BJ 1	India	R	<i>xa 5</i>	7

^a S = susceptible, R = resistant, MR = moderately resistant.

^b = 1160 is an F₁ line derived from the cross TN 1/DZ 78. It is susceptible to the PXO 61 isolate at the seedling stage but resistant at the adult stage. It is likely to have inherited *Xa 7* from DZ 78.

used in the present study is different in pathogenicity from the races from Japan and the Philippines (Table 1). Only DV 85 was resistant to the Punjab isolate, while all other differential varieties were susceptible to this isolate at the early as well as adult plant stages. However, at least two differential varieties are resistant to each of the Japanese and the Philippine races of the pathogen. The results given in Table 1 and Table 2 show that, except for *xa 8* present in rice variety PI 231129, no other known genes for resistance to bacterial blight convey resistance to the Punjab isolate. Though rice varieties Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32 are known to carry *Xa 4* (10), this gene does not confer resistance to the Punjab isolate in these varieties, as IR20 with the *Xa 4* gene is susceptible. Similarly, resistance to the Punjab isolate in BJ 1, DF 1, and DV 29 with *xa 5* and DV 85, DV 86, and DZ 78 with *xa 5*

Table 3. Reaction^a to the Punjab isolate of bacterial blight of F₁ and F₂ plants and F₃ lines from crosses of TN 1 with resistant varieties.

TN 1 crossed with	F ₁ reaction	F ₂ plants (no.)			F ₃ lines (no.)			
		R	S	χ^2	R	Se	s	χ^2
				3:1/1:3				1:2:1
Lua Ngu	R	423	114	4.0575	75	171	84	0.927
Nagane Tia	R	340	98	1.6103	41	78	32	1.238
Nam Sagui	R	325	96	1.0839	92	171	73	2.256
Nam Sakouy	R	420	126	1.0769	38	58	26	2.656
Patong 32	R	286	117	3.4946	74	137	75	0.369
DF1	S	108	316	0.0503	26	62	32	0.733
DV 29	S	75	310	6.255	28	51	32	1.018
DV 85	S	95	275	0.090	30	65	30	0.20
DV 86	S	91	242	0.962	24	60	30	0.947
DZ 78	S	129	348	1.063	23	62	31	1.655
BJ1	S	107	323	0.003	26	54	28	0.074
PI231129	S	122	310	2.420	38	93	39	1.518

^a R = resistant; S = susceptible; Se = segregating.

and *Xa 7* is not governed by these genes because rice varieties IR1545-339, which possesses *xa 5*, and 1160-8-6-1, which has *Xa 7*, are susceptible (Table 2).

Genetics of resistance to the Punjab isolate

The results presented in Table 3 show that the F₁ progeny of the crosses of TN 1 with Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32 are resistant. The plants in F₂ populations of these crosses segregated in the expected ratio of 3:1 resistant to susceptible, indicating that resistance to the Punjab isolate of bacterial blight is governed by single dominant genes in Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32. The reaction of the F₃ families confirmed the results obtained on the basis of the reaction of the F₂ populations. The F₃ families segregated in the expected ratio of 1:2:1 resistant to segregating to susceptible (Table 3), which confirmed that resistance in Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32 is governed by single dominant genes.

The F₁ plants of the crosses of TN 1 with DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129 were susceptible. The F₂ populations of these

Table 4. Reaction^a of F₁ and F₂ plants and F₃ lines to the Punjab isolate of bacterial blight from the crosses of varieties possessing dominant resistance genes.

Cross	F ₁ reaction	F ₂ plants (no.)		F ₃ lines (no.)		
		R	S	R	Se	S
Nagane Tia/Lua Ngu	R	412	0	113	0	0
Nagane Tia/Nam Sagui	R	481	0	112	0	0
Nagane Tia/Nam Sakouy	R	413	0	114	0	0
Nagane Tia/Patong 32	R	406	0	120	0	0
Nam Sakouy/Nam Sagui	R	424	0	124	0	0
Nam Sakouy/Patong 32	R	401	0	109	0	0
Nam Sakouy/Lua Ngu	R	420	0	116	0	0
Nam Sagui/Patong 32	R	407	0	117	0	0
Nam Sagui/Lua Ngu	R	494	0	101	0	0
Lua Ngu/Patong 32	R	461	0	118	0	0

^aR = resistant; S = susceptible; Se = segregating.

crosses segregated in the expected ratio of 1:3 resistant to susceptible plants (Table 3). These results show that resistance to the Punjab isolate of bacterial blight in DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129 is governed by single recessive genes, which was also confirmed by the reaction of F₃ families. The F₃ families segregated in the expected ratio of 1:2:1 resistant to segregating to susceptible (Table 3).

The data on the reaction of F₁, F₂, and F₃ populations of the crosses among varieties with dominant resistance genes revealed that single dominant resistance genes in Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32 are allelic. All F₁ and F₂ plants and F₃ families were resistant (Table 4). The data on the reaction of F₁, F₂, and F₃ populations of the crosses among varieties with single recessive resistance genes showed that all F₁ and F₂ plants and all F₃ families were resistant (Table 5). These results therefore reveal that single recessive genes governing resistance to the Punjab isolate of bacterial blight in DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129 are also allelic.

The linkage relationship of the dominant resistance gene with the recessive resistance gene was determined from the reaction of the F₁, F₂, and F₃ populations of the cross DV 85/Patong 32. As expected, the F₁ plants were resistant. The F₂ population segregated in the expected ratio of 13:3 resistant to susceptible plants. Out of 628 plants, there were 502 resistant and 126 susceptible ones ($\chi^2 = 0.71$). The F₃ population segregated in the expected ratio of 7:8:1 resistant to segregating to

Table 5. Reactiona of F₁ and F₂ plants and F₃ lines to the Punjab isolate of bacterial blight from the crosses of varieties possessing recessive resistance genes.

Cross	F ₁ reaction	F ₂ plants (no.)		F ₃ lines (no.)		
		R	S	R	Se	S
DF 1/DV 29	R	488	0	112	0	0
DF 1/DV 85	R	486	0	122	0	0
DF 1/DV 86	R	481	0	121	0	0
DF 1/DZ 78	R	406	0	124	0	0
DF 1/BJ 1	R	510	0	114	0	0
DF 1/PI 231129	R	498	0	113	0	0
DV 29/DV 85	R	477	0	119	0	0
DV 29/DV 86	R	412	0	122	0	0
DV 29/DZ 78	R	431	0	119	0	0
DV 29/BJ 1	R	467	0	112	0	0
DV 29/PI 231129	R	446	0	121	0	0
DV 85/DV 86	R	410	0	124	0	0
DV 85/DZ 78	R	447	0	114	0	0
DV 85/BJ 1	R	410	0	108	0	0
DV 85/PI 231129	R	414	0	119	0	0
DV 86/DZ 78	R	437	0	117	0	0
DV 86/BJ 1	R	506	0	114	0	0
DV 86/PI 231129	R	444	0	116	0	0
DZ 78/BJ 1	R	410	0	124	0	0
DZ 78/PI 231129	R	437	0	116	0	0
BJ 1/PI 231129	R	474	0	123	0	0

^aR = resistant; S = susceptible; Se = segregating.

susceptible families ($c^2 = 0.92$). Of 117 F₃ families there were 46 resistant, 63 segregating, and 8 susceptible. These results suggest that dominant and recessive resistance genes conferring resistance to the Punjab isolate of bacterial blight identified in this study are independently inherited.

DISCUSSION

The data on the reaction of differential rice varieties of Japan and the Philippines to the Punjab isolate of bacterial blight suggest that the isolate used in the present study represents a different race of the pathogen than the races prevalent in Japan and the Philippines. Rice varieties Kogyoku with *Xa 1* and *Xa-kg*; Rantai-emas with *Xa 1* and *Xa 2*;

Wase Aikoku 3 with *Xa 3*; Java 14 with *Xa 1*, *Xa 3*, and *Xa-kg*; IR20 with *Xa 4*; IR1545-339 with *xa 5*; Malagkit Sungsong with *Xa 6*; 1160-8-6-1 with *Xa 7*; Sateng with *xa 9*; and Cas 209 with *Xa 10* genes for resistance were susceptible to the Punjab isolate at the maximum tillering, booting, and post-flowering stages. Investigation of the genetics of resistance revealed that resistance to the Punjab isolate in rice varieties Lua Ngu, Nagane Tia, Nam Sagui, Nam Sakouy, and Patong 32 is governed by the same single dominant gene, while resistance to this isolate in DF 1, DV 29, DV 85, DV 86, DZ 78, BJ 1, and PI 231129 is conferred by the same single recessive gene. These two genes are independently inherited. Except for PI 231129, which carries *xa 8*, rice varieties with other known genes are susceptible to the Punjab isolate. Though PI 231129 is resistant to the Punjab isolate, *xa 8* does not seem to govern resistance to the isolate used in this study. By using the PXO 61 isolate, which is representative of race 1 of the pathogen from the Philippines, it has been demonstrated (7) that rice cultivar BJ 1 has only the *xa 5* gene effective against the PXO 61 isolate. Furthermore, *xa 8*, present in PI 231129 and effective against the PXO 61 isolate, is inherited independently of *xa 5* (10). The results obtained in the present study clearly demonstrate that the same recessive resistance gene conditions resistance to the Punjab isolate in BJ 1 and PI 231129, which suggests that *xa 8* is not effective against the Punjab isolate. If *xa 8* were effective against the Punjab isolate, BJ 1 should have two independently inherited genes effective against the PXO 61 isolate, which is not the case. Thus none of the eleven genes for resistance to bacterial blight so far identified appears to convey resistance to the Punjab isolate of the pathogen. Studies are in progress to determine allelic relationships of the resistance genes effective against the Punjab isolate with those of resistance genes already identified.

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GENETIC AND CYTOLOGICAL CHARACTERIZATION OF THE RICE BLAST FUNGUS, *PYRICULARIA ORYZAE*

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Pyricularia oryzae parasitizes a variety of gramineous hosts and causes the rice blast disease worldwide. Although a high degree of pathogenic variation has been reported in the fungus, little is known about the nature of this variability. Genetic and cytological studies were therefore made to facilitate further investigation of pathogenic variation. Polymorphism in 12 enzymes was examined among 350 isolates from 12 rice growing regions. All isolates were monomorphic at 16 of the 18 enzyme putative loci. In contrast, a sample of isolates from several gramineous hosts displayed more allelic diversity. Through matings among isolates from rice, finger millet, and weeping lovegrass, monogenic control of six enzyme variants was demonstrated. Ascospores derived from rice isolates were either nonpathogenic or only partially pathogenic on rice. A light microscopic survey of nuclear number in living hyphal cells from geographic isolates showed that 91.1% of cells were uninucleate. Six chromosomes were observed at pachytene, diakinesis, and the metaphase of ascospore mitosis. Chromosome movement at meiotic and mitotic anaphase was asynchronous, which might account for the variable chromosome numbers reported previously.

Rice blast caused by *Pyricularia oryzae* is one of the most destructive and widespread diseases in rice. Deployment of host resistance has been the major control measure for blast in most rice growing regions of the world, but breeding for blast resistance has not been entirely effective due to the high degree of pathogenic variability exhibited by the fungus. Although a number of cytological and genetic mechanisms, including heterokaryosis, parasexuality, and aneuploidy (4), have been proposed to account for pathogenic variation, the genetic basis of variability in *P. oryzae* remains unknown.

In recent years, the successful induction of the perfect state in *P. oryzae* isolates from various gramineous hosts has provided opportunities for genetic analysis of the fungus. The perfect state, *Magnaporthe grisea* (Hebert) Barr comb. nov., is a bipolar, heterothallic ascomycete that produces eight-spore asci (2). Using fungicide resistance and auxotrophic

markers, considerable genetic characterization has been made among isolates from finger millet [*Eleusine coracana* (L.) Gaertn.] (1,5). However, due to the low fertility of crosses involving rice isolates, relatively little is known about the genetic behavior of isolates from rice.

In order to further the genetic analysis of *P. oryzae* there is a need to develop strains that are genetically and cytologically characterized. This research was initiated to examine the genetic and cytological characters of a large collection of *P. oryzae* isolates collected from major rice growing regions of the world. Isozyme analysis was used to survey the genetic variability of the global population of *P. oryzae*. From this survey, electrophoretic markers were identified that permit genetic analysis among selected mating strains. As cytological abnormality has often been invoked to account for pathogenic variation in *P. oryzae*, cytological studies were conducted to provide a detailed description of the nuclear number in the hyphal cells of geographic isolates and the chromosomal behavior during ascosporeogenesis in fertile strains.

GENETIC CHARACTERIZATION

Enzyme polymorphism among geographic isolates

Between 1975 and 1983 we acquired 350 rice isolates of *P. oryzae* from 12 major rice growing regions of the world. In addition, 34 isolates were obtained from a variety of other gramineous hosts (Table 1). Most samples were received by mail in the form of dry diseased leaves, from which either hyphal-tip or single-spore cultures were obtained. Mycelial pellets from individual isolates were produced in potato sucrose broth, dried, and homogenized to obtain a crude extract for starch gel electrophoresis. Twelve enzymes were analyzed [aconitase (Aco), aspartate aminotransferase (Aat), α -esterase (α -Est), fumarase (Fum), glycerate-2-dehydrogenase (G2dh), hexokinase (Hk), glutamic-pyruvic transaminase (Gpt), lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh), phosphoglucose isomerase (Pgi), phosphoglucomutase (Pgm), and xanthine dehydrogenase (Xdh)], from which 18 putative enzyme loci were identified. The amount of variability expressed as gene diversity in the rice and non-rice isolate populations of *P. oryzae* is summarized in Table 2. Gene diversity for a locus is defined as $H = 1 - \sum X_i^2$, where X_i is the frequency of the i -th allele (3). This provides a useful measure of genetic variability by taking into account the number and frequency of alleles at a locus.

Table 1. Geographic origin, sample size, and years of collection of *Pyricularia oryzae* isolates.

Origin	No. of isolates	Type of isolation ^a	Years collected
United States	30	S	1975–1981
Colombia	22	H	1979
Brazil	9	H	1980
India	56	H	1983
Nigeria	38	H	1983
Japan	10	S	unknown
Korea	34	H	1980
North China	25	H	1980
Central China	28	H	1980
South China	35	H	1980
Taiwan, China	7	H	1980
Philippines	56	H	1979–1980
Non-rice hosts ^b	34	S	1977–1983

^aS = single conidial culture, H = hyphal tip culture.

^b13 from finger millet, 2 from goosegrass, 2 from weeping lovegrass, 8 from crabgrass, and 9 from unknown species.

Table 2. Gene diversity^a at 10 enzyme loci of rice and non-rice isolates of *Pyricularia oryzae*.

Locus	Population	
	Rice isolate	Non-rice isolate
<i>Hk1</i>	0.00	0.39
<i>Hk2</i>	0.00	0.39
<i>Aco</i>	0.00	0.39
<i>Pgm</i>	0.00	0.39
<i>Pgi</i>	0.00	0.43
<i>Mdh3</i>	0.01	0.43
<i>G2dh</i>	0.08	0.43
<i>Ldh1</i>	0.49	0.44
<i>Ldh2</i>	0.01	0.00
<i>Ldh3</i>	0.01	0.53

^a Gene diversity = $1 - \sum X_i^2$ where X_i is the frequency of the i -th allele at a locus (3).

In contrast to the assumed high degree of genetic diversity conditioning pathogenicity in *P. oryzae*, relatively little variability in enzyme loci was detected. Among the 18 loci tested, only 2, *Ldh1* and *G2dh*, exhibited allelic diversity (Fig. 1). On a global basis, 6 of the 12 regional populations were monomorphic for all loci. Populations from the United States, Colombia, Korea, Nigeria, and South China were polymorphic for *Ldh1*, and a population from India was polymorphic for *G2dh*. However, this

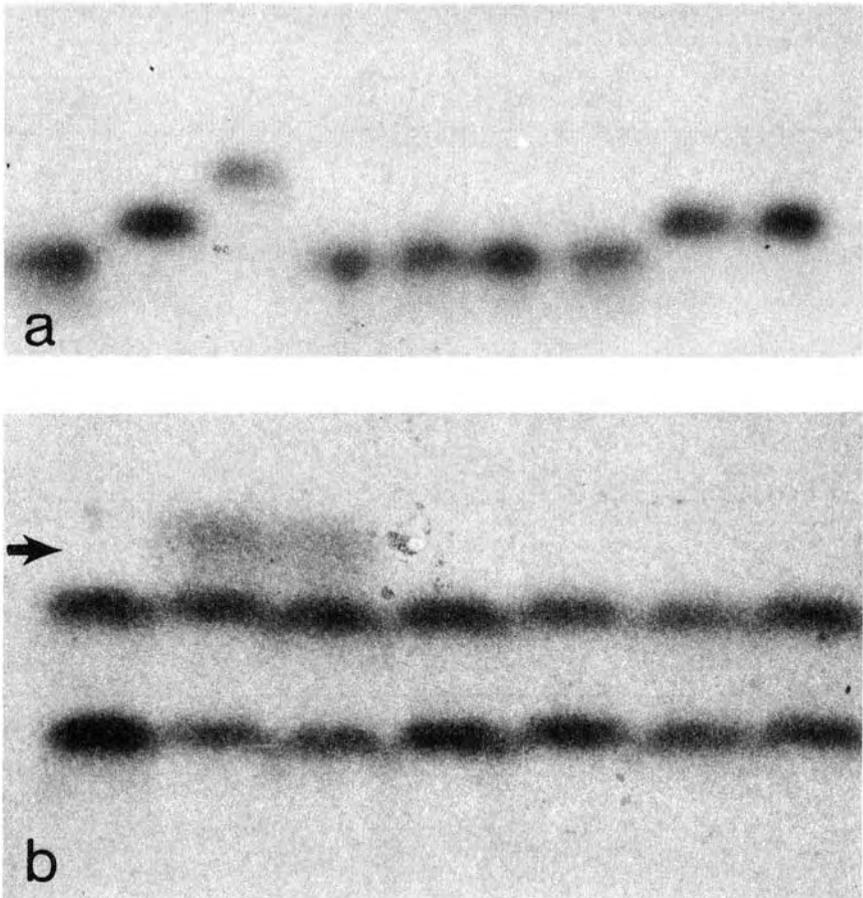


Fig. 1. Electrophoretic variation in enzyme loci of *Pyricularia oryzae* detected by starch gel electrophoresis. A = variation at the glycerate-2-dehydrogenase locus. b = variation at the lactate dehydrogenase-1 locus (arrow) showing the functional and the null alleles.

low variability is not characteristic of *P. oryzae*. In the limited number of isolates taken from non-rice hosts, nine loci (*Mdh3*, *Pgi*, *Ldh1*, *Ldh3*, *Aco*, *Pgm*, *Hk1*, *Hk2*, and *G2dh*) were polymorphic, with gene diversities ranging from 0.39 to 0.53. This higher level of variability in the non-rice isolates probably reflects the greater diversity of the hosts from which these isolates were derived.

The unusually high degree of homogeneity observed among the rice isolates raises the question of how such genetic uniformity is maintained over a broad geographic range. One possible explanation is that individual pathogen populations were established by a few founder isolates

that originated from a common ancestral population and hence carried only a fraction of the genetic variability that existed in the species. It is also possible that most electrophoretic variants are deleterious or that a set of coadapted enzyme loci in *P. oryzae* has been selected under the influence of the rice hosts.

Genetic analysis

From the enzyme survey, fertile isolates from weeping lovegrass [*Eragrostis curvula* (Schrad.) Ness] and finger millet that carry electrophoretic variants were crossed to rice isolates in order to determine the mode of inheritance and linkage relationships among the enzyme variants. Among 33 crosses between rice (R) and non-rice (NR) isolates, only 8 produced a small number of asci with a few viable ascospores. However, fertility was improved in the F_2 progeny of the F_1 's derived from R/NR crosses, with ascospore germination ranging from 20% to 50%. The segregation data for six electrophoretic markers are summarized in Table 3. Despite the low viability of ascospores in many crosses derived from rice isolates, the segregation ratios for *Pgm*, *Pgi*, *Ldh1*, and *G2dh*, conform to the 1:1 expectation, indicating that these loci are not associated with major viability factors. In contrast, there were significant deviations at the *Mdh3* and *Ldh3* loci, where excesses of the variant alleles ($Mdh3^n$ and $Ldh3^{83}$) were found. The fact that $Mdh3^n$ and $Ldh3^{83}$ were both derived from the weeping lovegrass isolates suggests the possibility of preferential transmission of alleles from the genomes of the weeping lovegrass isolates. When enzyme loci were tested for independent assortment, loose linkage was detected between *Pgm* and *G2dh*, with a recombination frequency of 43.0%. Furthermore, among approximately 500 ascospores assayed for the segregation of electrophoretic markers, none was found to exhibit disomic or nullisomic patterns, suggesting that, at least for the chromosomes bearing these markers, the incidence of aneuploidy is less than 0.2%.

When the pathogenicity of F_1 ascospores from crosses between rice and weeping lovegrass isolates was tested on rice differential hosts, no simple segregation patterns were observed. No F_1 progeny were found to show the same level of pathogenicity on rice differentials as the parental rice isolates. As shown in Figure 2, the lesions caused by the hybrid progeny were restricted in size and exhibited more pronounced brownish margins. Further understanding of such pathogenic changes during sexual hybridization may provide some clues to pathogenic variation observed in the asexual stage of the fungus.

Table 3. Goodness-of-fit tests for single locus segregation of six electrophoretic variants in *Pyricularia oryzae*.

Locus	Parental genotype ^a a/b	Ascospore segregant a:b	c ²	P
<i>Pgm</i>	100/107	318 : 322	0.30	0.5–0.3
<i>Pgi</i>	100/113	229 : 218	0.27	0.7–0.5
<i>Mdh3</i>	100/null	218 : 281	7.95**	<0.005
<i>Ldh3</i>	100/83	149 : 204	8.57**	<0.005
<i>Ldh1</i>	100/null	211 : 226	0.51	0.7–0.5
<i>G2dh</i>	100/63	245 : 274	1.62	0.3–0.2

^aNumbers designate relative electrophoretic mobility.

CYTOLOGICAL CHARACTERIZATION

Nuclear condition in geographic isolates

To examine the potential significance of nuclear variation in conditioning pathogenic variation, 89 isolates from 10 rice growing regions were surveyed for the number of nuclei in living hyphal cells using phase contrast optics. Of 8,460 cells examined, 91.1% were uninucleate, 8.3% binucleate, and 0.3% multinucleate. The binucleate condition was more prevalent in the tip cells (12.2%) and intercalary cells (10.6%) than in the penultimate cells (2.3%). No striking differences were found in the frequency of binucleate cells among isolates of diverse geographic origins. The only exception to this were the Brazilian isolates, which had a significantly higher average nuclear number per cell than other regional isolates (Table 4). In view of this lack of regional variation in nuclear condition among geographic isolates, it seems unlikely that variation in nuclear number is responsible for the differences in pathogenic variability observed among regional isolates.

Chromosome behavior during ascosporeogenesis in fertile isolates

Meiosis and mitosis during ascosporeogenesis in fertile mating strains were studied using a propionic-Fe-hematoxylin procedure that stained chromosomes, nucleolus, and spindle pole bodies. Meiosis and mitosis in *P. oryzae* resembled those in other ascomycetes. After nuclear fusion, the ascus initial began to enlarge up to 20–30 μm long. The diploid nucleus entered prophase as the chromatin began to condense around the nucleolus. Zygotene chromosomes were highly contracted, followed by elonga-

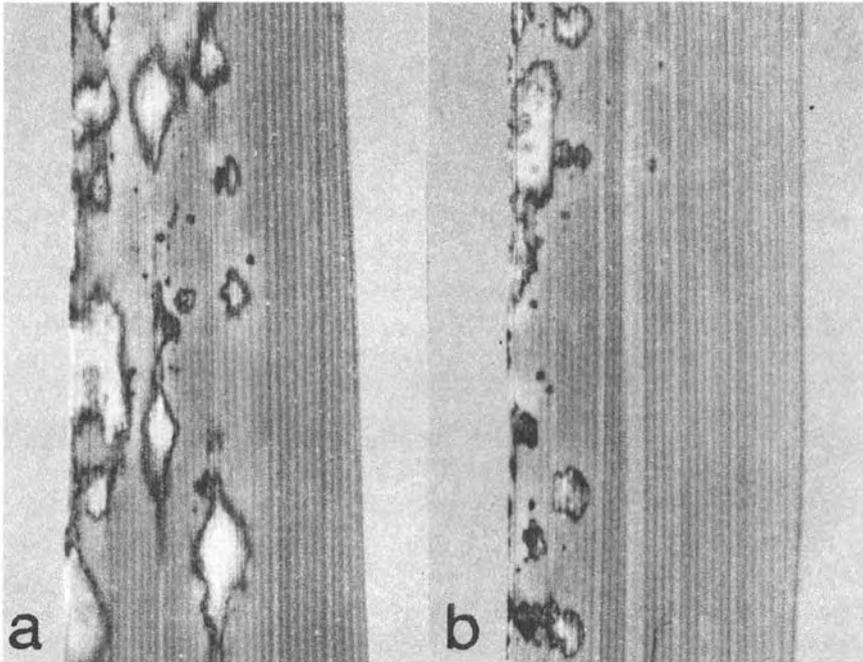


Fig. 2. Disease reaction of rice differential She-tiao-tsao to *Pyricularia oryzae* rice isolate Naga 71-127 (a) and its F_1 progeny strain 807 (b) derived from a cross with a weeping lovegrass isolate.

tion at the pachytene stage, when close pairings of homologous chromosomes were observed (Fig. 3a). The nucleolus attained a maximum size of 3.8 μm in diameter during pachytene. Chromosome lengths varied among pachytene cells, with the longest chromosome averaging 8.5 μm and the smallest 2.9 μm . Six chromosomes were observed at pachytene, diakinesis (Fig. 3b), and during metaphase of ascospore mitosis, confirming the observation of Yeagashi and Hebert (7) and Tanaka et al (6).

Table 4. Average number of nuclei per cell in the hyphal tip cells, penultimate cells, and intercalary cells of regional isolates of *Pyricularia oryzae*.

Region	Number of isolates	Average number of nuclei per cell ^a		
		Tip cell	Penultimate cell	Intercalary cell
Brazil	6	1.13 a	1.08 a	1.25 a
Taiwan, China	8	1.11 a	1.00 a	1.17 b
Japan	10	1.10 a	1.03 a	1.15 b
North China	10	1.14 a	1.00 a	1.13 bc
United States	10	1.12 a	1.05 a	1.12 bc
Central China	10	1.13 a	1.03 a	1.09 bc
Korea	10	1.09 a	1.00 a	1.08 c
Colombia	10	1.16 a	1.02 a	1.07 c
South China	10	1.14 a	1.02 a	1.07 c
Philippines	10	1.14 a	1.03 a	1.06 c

^aMeans followed by a common letter are not significantly different at the 5% level.

Since the strains used for the study of meiosis resulted from highly fertile matings, it was assumed that the documented chromosome behavior would provide a basis for interpreting cytological variations reported in the fungus. Earlier studies of chromosome number in the somatic cells of *P. oryzae* had yielded chromosome counts ranging from 2 to 12, and, more recently, Tanaka et al (6) reported varying frequencies of lagging chromosomes during meiotic and mitotic anaphase in crosses between rice and non-rice isolates. Such observations led Ou (4) to suggest aneuploidy as a potential mechanism for pathogenic variation. We have observed that asynchronous disjunction at anaphase can often give the appearance of variable chromosome number and lagging chromosomes (Fig. 3c, d) and might be misinterpreted as a cytological anomaly. It is therefore important that such cytological observations be complemented with genetic analysis in determining the potential role of chromosomal variation in pathogenic variability.

CONCLUSION

Despite the high degree of pathogenic variation reported in *P. oryzae* the enzyme and nuclear phenotypes observed in this study did not reveal an unusual level of variability. It appears that whatever factors condition pathogenic variation affect only certain regions of the genome. The

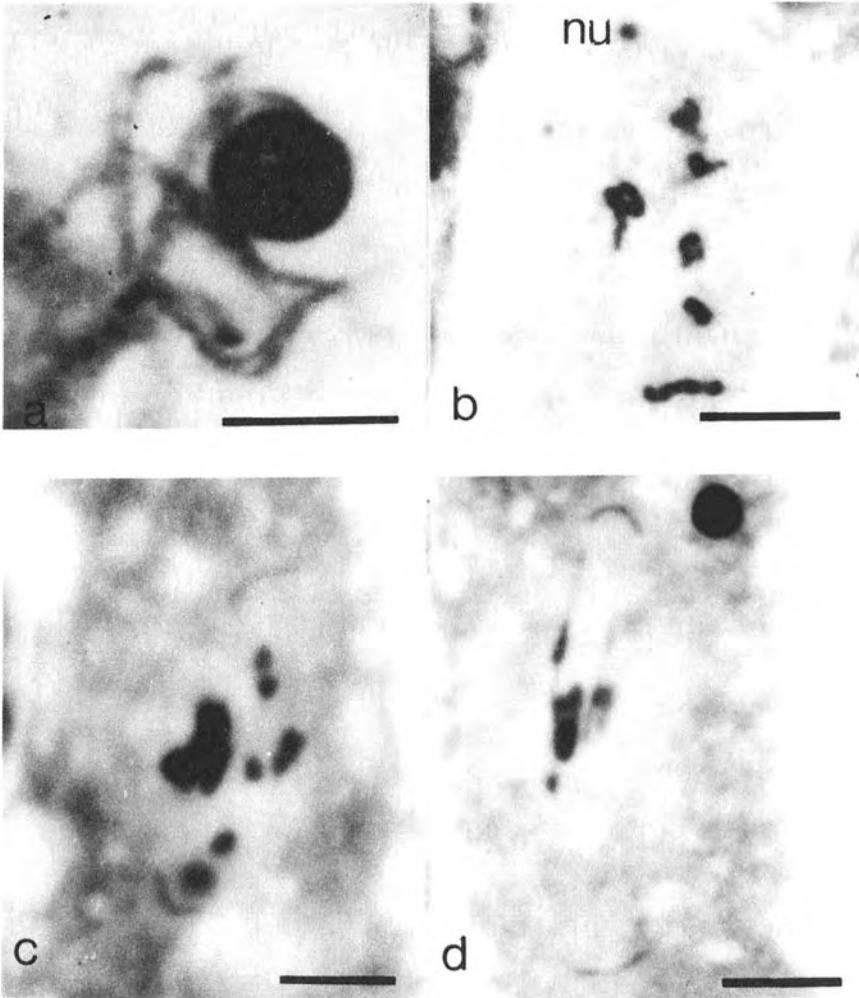


Fig. 3. (a) Pachytene chromosomes in *Pyricularia oryzae* with the nucleolus attaining its maximum size of 3.8 μm in diameter. (b) Six bivalents at diakinesis with homologous chromosomes in repulsion. Nu = nucleolus. (c) and (d) Two views of anaphase I when homologous chromosomes are migrating to the opposite poles. Note that the movement of chromosomes is asynchronous, resulting in many staining bodies lying between the two poles. Scale bar = 5 μm .

availability of fertile genetic strains should make the search for such factors in the genome an achievable goal in the near future.

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DISCUSSION

SESSION 8: GENETICS OF DISEASE RESISTANCE

Q – Mackill: Is there sufficient variability in the Japanese isolates of *P. oryzae* to characterize fully the resistance genes from indica rices? The data I have seen indicate that indica rices may have several resistance genes for the Japanese isolates.

A – Toriyama: Japanese isolates of *P. oryzae* are just enough to distinguish the true resistance genes identified so far in Japan. I suppose a new set of isolates from tropical areas should be prepared for genetic analysis of resistance genes from indica varieties at IRRI or elsewhere.

Q – Shahi: What do you mean by “basic fungal strain”?

A – Toriyama: We can get some fungal strains stable for virulence that can be used for genetic analysis for resistance. The basic fungal strain is the strain virulent to any true resistance genes identified so far.

Q – Jacquot: You have observed different levels of durable resistance to blast in a sample of japonica varieties. Do you think we can find good levels of durable resistance to blast in indica varieties, too? Can a high level of durable resistance to blast be negatively correlated with a high level of grain productivity?

Q – Lin: Most of the materials used in our work were japonica varieties from northern China. I think it is possible to find good levels of durable resistance to blast in indica varieties, too. We know some local indica varieties that have high level of durable resistance to blast. A negative correlation between durable resistance to blast and grain productivity may exist, but I think it should be possible to produce cultivars with durable resistance to blast and with good grain productivity.

Q – Toriyama: You have used both systems of designating *Xa* genes, i.e., *Xa-1* to *Xa-10* and *Xa-Kg*, *Xa-Pt*, etc. Is it very difficult to evolve a common system of designating genes?

A – Ogawa: I do not use both systems of designating *Xa* genes. I designated *Xa-Kg* and *Xa-pt* only.

Q – Kinoshita: Your colleagues are using gene symbols from both the *Xa*-number and *Xa*-shortened name systems. Please give me the reason. I suggest you use a uniform system such as *Xa*-number.

A – Ogawa: If we use the *Xa*-number system and assign a number, there is the possibility that the same number may be assigned to a different gene discovered at the same time in another country. Therefore, I think we

should not use the *Xa*-number system immediately after a new gene is found.

C – Khush: This is an important issue that will be discussed separately in the gene nomenclature committee.

Q – Wu, H. K.: How do you test horizontal resistance in rice varieties?

A – Ogawa: By using the clipping method, you may evaluate the horizontal resistance of rice varieties by measuring lesion length 2 or 3 weeks after inoculation and by observing secondary infection of these varieties 1 or 2 months after inoculation under field conditions and at high fertilizer levels.

GENETICS OF INSECT RESISTANCE

SESSION 9

GENETIC ANALYSIS OF RESISTANCE TO BROWN PLANTHOPPER IN RICE

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A method is proposed to estimate the genotypes of rice cultivars resistant to BPH, consisting of two procedures: reaction to BPH biotypes and test crosses with cultivars of known genotype. From reactions to biotypes 2 and 3, it is determined that the resistant gene in the cultivar is either *Bph 1*, *bph 2*, or another/others. The cultivars resistant to both biotypes are crossed with a susceptible check, either Rathu Heenati (*Bph 3*) or Babawee (*bph 4*). If the resistance gene is dominant, the Rathu Heenati cross is used for an allelism test, and if the gene is recessive, the Babawee cross is used. When two resistance genes are concerned, biotypes 2 and 3 are used in the cross with a susceptible check to decide whether *Bph 1* or *bph 2* is contained in those two genes or not. Using this method, 11 cultivars were analyzed. It was estimated that Balamawee (70-518), Kaharamana, and Pokkali had an unknown dominant gene; Col. 5 Thailand, Col. 11 Thailand, and Chin saba had an unknown recessive gene; and Balamawee (70-164), PTB 7, PTB 12, and PTB 20 had one dominant and one recessive gene.

The brown planthopper (BPH) *Nilaparvata lugens* Stal. is one of the most serious pests throughout tropical and temperate Asia. In Japan, the breeding of japonica rice cultivars with BPH resistance has been proceeding since 1968 (3).

Inheritance studies of resistance to BPH were initiated by Athwal et al (1), who identified two genes for resistance, *Bph 1* and *bph 2*, and reported that *Bph 1* is closely linked or allelic to *bph 2*. Lakshminarayana and Khush (4) identified *Bph 3* and *bph 4*, which are independent of *Bph 1* and *bph 2*. Sidhu and Khush (7) reported that *Bph 3* is closely linked or allelic to *bph 4* and disclosed the relation between *Bph 1* and *bph 2*.

With a view to extending the genetic base so as to enable the reliable use of BPH resistance, the identification of a large number of cultivars with BPH resistance along with the characterization of their genotypes is desirable. However, if the resistance gene is not clarified in the resistant

cultivars, the value of utilizing the cultivars as donor parents of the resistance gene is still low. The usual method that identifies the gene by test crosses with known cultivars is very time- and labor-consuming. Whereas there are only three biotypes of BPH, resistance genes other than *Bph 1* or *bph 2* cannot be identified by reactions to BPH biotypes only.

In this paper, we propose a new method to estimate the genotypes of BPH resistance cultivars and then report on the identification of the resistance gene in 11 unclassified resistant cultivars by using the method.

A NEW ANALYTICAL METHOD

The procedure for identifying the resistance gene(s) in unclassified resistant cultivars is shown in Figure 1. First, the resistant cultivars screened by BPH biotype 1 (wild type) are retested by using biotypes 2 (infests cultivars with the gene *Bph 1*) and 3 (infests cultivars with the gene *bph 2*). From the patterns of biotype reactions, the cultivars are classified into three genetic groups, viz., *Bph 1* (susceptible to biotype 2 and resistant to biotype 3), *bph 2* (resistant to 2, susceptible to 3), and the other group (resistant to both biotypes). Resistance in the cultivars belonging to the third group must be controlled by either *Bph 3*, *bph 4*, a single unknown gene, or two or more resistance genes. Accordingly, to decide which gene or genes are concerned, it is necessary to cross the cultivars with a susceptible check, Rathu Heenati carrying *Bph 3* or Babawee carrying *bph 4*. By testing for resistance in the F_2 of the cross between a resistant cultivar and a susceptible check using biotype 1, it is clarified how many resistance genes are concerned and whether they are dominant or recessive.

Now, supposing that the number of resistance genes in an unclassified cultivar is two and that they are independent of each other, the segregation ratio in the F_2 population from the cross with a susceptible check should be either 3:1 resistant to susceptible, 1:3 resistant to susceptible, 15:1 resistant to susceptible, 13:3 resistant to susceptible, or 7:9 resistant to susceptible.

On the cultivars estimated to carry a single dominant gene, F_2 populations from the crosses with Rathu Heenati (*Bph 3*) are tested for allelism to *Bph 3*. Similarly, on those carrying a single recessive gene, F_2 populations from the cross with Babawee (*bph 4*) are analyzed.

If two resistance genes are concerned, one of them may be *Bph 1* or *bph 2*. In that case, F_2 populations from the crosses between the cultivars and

biotype 3 whether the cultivars have *bph* 2 or not. On the cultivars carrying one dominant and one recessive gene, it is necessary to clarify whether they have *Bph* 1, *bph* 2, or not by using biotypes 2 and 3.

Thus it is possible to identify the resistance gene(s) in unclassified resistant cultivars by three test crosses and three biotype reactions. Moreover, this procedure does not always need all F_2 populations from three test crosses, or all reactions to three biotypes. As shown in Figure 1, if we analyze systematically according to the procedure mentioned above, we can identify the resistance gene(s) in unclassified resistant cultivars alternatively.

This analyzing method, therefore, is efficient for identification of the resistance gene(s) in the cultivars.

IDENTIFICATION OF RESISTANCE

The inheritance of resistance to BPH of 11 cultivars was studied.

Materials and methods

The 11 cultivars investigated are listed in Table 1. These cultivars are resistant to both biotypes 2 and 3. All were crossed in 1981 with Milyang 23, which is susceptible to BPH; Rathu Heenati, which carries *Bph* 3; and Babawee, which carries *bph* 4. Panicles of F_1 plants were covered with glassine bags to avoid outcrossing and were self-pollinated. The test for resistance in the F_2 populations from each cross was carried out according to the method discussed in the preceding section.

The bulk seedling test, modified from the one described by Pathak et al (6), was used to test plants for BPH resistance (2).

Results and discussion

The reactions of the F_2 progeny from the crosses between Milyang 23 and the resistant cultivars are shown in Table 2. The F_2 populations from the crosses of Milyang 23 with Hondarawala 378, Balamawee (70-518), Kaharamana, and Pokkali segregated in the ratios of 3:1 resistant to susceptible seedlings, indicating that resistance in these cultivars is conditioned by single dominant genes.

The F_2 populations from the crosses of Milyang 23 with Col. 5 Thailand, Col. 11 Thailand, and Chin saba segregated in the ratio of 1:3

Table 1. Cultivars resistant to BPH.

Cultivar	Seed source	Acc. No.	Origin
PTB 7	7th Lab. Genet NIAS	67-112	India
PTB 12	"	67-116	"
PTB 20	"	67-120	"
Balamawee	"	70-164	Sri Lanka
Balamawee	"	70-518	"
Hondarawala 378	"	70- 43	"
Kaharamana	"	70-505	"
Pokkali	"	70-189	"
Col. 5 Thailand	"	69-5	Thailand
Col. 11 Thailand	"	69-11	"
Chin saba	Seed Stor. Lab. NIAS	190004	Burma

Table 2. F₂ segregations for resistance to BPH in the crosses between Milyang 23 (susceptible) and unclassified resistant cultivars.

Cross	Segregation in F ₂			c ²			P
	Res.	Sus.	Total	3:1	1:3	13:3	
Milyang 23/Hondarawala 378	169	69	268	2.203			.10-.20
Milyang 23/Balamawee (70-518)	190	71	261	0.676			.30-.50
Milyang 23/Kaharamana	234	77	311	0.009			.90-.95
Milyang 23/Pokkali	140	42	182	0.359			.50-.70
Milyang 23/Col. 5 Thailand	42	118	160		0.133		.70-.80
Milyang 23/Col. 11 Thailand	73	221	294		0.004		.90-.95
Milyang 23/Chin saba	142	387	529		0.959		.30-.50
Milyang 23/Balamawee (70-164)	302	73	375			0.127	.70-.80
Milyang 23/PTB 7	388	93	481			0.108	.70-.80
Milyang 23/PTB 12	211	39	250			1.628	.20-.30
Milyang 23/PTB 20	238	47	285			0.955	.30-.50

resistant to susceptible, indicating that resistance in these cultivars is conditioned by single recessive genes.

The proportion of resistant and susceptible seedlings in the F₂ populations of Milyang 23/Balamawee (70-164), Milyang 23/PTB 7, Milyang 23/PTB 12, and Milyang 23/PTB 20 agreed with the ratio of 13:3 expected for independent segregation of one dominant and one recessive gene.

The F₂ populations from the crosses of Rathu Heenati (*Bph* 3) or Babawee (*bph* 4) with four cultivars carrying a single dominant gene for

resistance were studied. These results are shown in Table 3. In the F_2 population of Rathu Heenati/Hondarawala 378, two seedlings were classified as susceptible, while in the F_2 population of Babawee/Hondarawala 378, no F_2 seedlings were susceptible. These results show that resistance in Hondarawala 378 is conditioned by *Bph 3*, because *Bph 3* is closely linked or allelic to *bph 4*.

On the other hand, the F_2 populations of Rathu Heenati/Balamawee (70–518), Rathu Heenati/Kaharamana, and Rathu Heenati/Pokkali segregated in the ratio of 15:1 resistant to susceptible, expected on the basis of independent segregation of two dominant genes. Moreover, the F_2 populations of Babawee/Kaharamana and Babawee/Pokkali segregated in the ratio of 13:3, expected on the basis of independent segregation of one dominant and one recessive gene. From these results, it is estimated that resistance in Balamawee (70–518), Kaharamana, and Pokkali is controlled by an unknown single dominant gene.

As shown in Table 4, the F_2 populations from the crosses of Babawee with Col. 5 Thailand, Col. 11 Thailand, and Chin saba segregated in the ratio of 7:9 resistant to susceptible, expected on the basis of independent segregation of two recessive genes. Consequently, these allelism tests revealed that resistance in Col. 5 Thailand, Col. 11 Thailand, and Chin saba is conditioned by an unknown single recessive gene.

The F_2 populations from the crosses of Milyang 23 with the four cultivars found to carry one dominant and one recessive gene were retested by using biotype 2 or 3, and the F_2 populations from the crosses of Rathu Heenati (*Bph 3*) or Babawee (*bph 4*) with these cultivars were tested by using biotype 1, as shown in Table 5. The F_2 population of Milyang 23/Balamawee (70–164) segregated in the ratio of 1:3 resistant to susceptible to biotype 2, indicating that the one gene is *Bph 1*. The F_2 population of Babawee/Balamawee (70–164) segregated in the ratio of 55:9, expected on the basis of independent segregation of one dominant and two recessive genes. These results indicate that Balamawee (70–164) has *Bph 1* and one unknown recessive gene, that is independent of *Bph 1* and *bph 4*.

Because with regard to both biotypes 1 and 2 the F_2 population of Milyang 23/PTB 7 segregated in the ratio of 13:3 resistant to susceptible, PTB 7 cannot have *Bph 1*. Moreover, there were no susceptible seedlings in the F_2 population of Babawee/PTB 7, thereby suggesting that one of the genes is allelic to either *Bph 3* or *bph 4*. These results suggest that PTB 7 has either *bph 2* and *Bph 3*, *bph 4* and an unknown dominant gene, or *Bph 3* and an unknown recessive gene.

With regard to biotype 3, the F_2 populations of Milyang 23/PTB 12 segregated in the ratio of 13:3 resistant to susceptible. With regard to

Table 3. F₂ segregations for resistance to BPH in crosses between Rathu Heenati (*Bph 3*) or Babawee (*bph 4*) and four resistant cultivars that have one dominant gene.

Cross	Segregation in F ₂			c ²		P
	Res.	Sus.	Total	15:1	13:3	
Rathu Heenati/Hondarawala 378	478	2	480			
Rathu Heenati/Balamawee (70-518)	384	33	417	1.969		.10-.20
Rathu Heenati/Kaharamana	491	27	518	0.951		.30-.50
Rathu Heenati/Pokkali	242	9	251	3.041		.05-.10
Babawee/Hondarawala 378	403	0	403			
Babawee/Kaharamana	256	50	306		1.167	.20-.30
Babawee/Pokkali	216	57	273		0.812	.30-.50

Table 4. F₂ segregations for resistance to BPH in the crosses between Babawee (*bph 4*) and three resistant cultivars that have one recessive gene.

Cross	Segregation in F ₂			c ²		P
	Res.	Sus.	Total	7:9		
Babawee/Col. 5 Thailand	118	157	275	0.079		.70-.80
Babawee/Col. 11 Thailand	122	135	257	1.446		.20-.30
Babawee/Chin saba	204	224	428	2.663		.10-.20

Table 5. F₂ segregations for resistance to the biotypes of BPH in the crosses between testers and four resistant cultivars that have one dominant and one recessive gene.

Cross	Bio-type used	Segregation in F ₂			c ²			P
		Res.	Sus.	Total	1:3	13:3	61:3	
Milyang 23/Balamawee (70-164)	2	133	363	496	0.871			30-.50
Babawee/Balamawee (70-164)	1	263	39	302			0.329	.50-.70
Milyang 23/PTB 7	2	318	85	403		3.283		.05-.10
Babawee/PTB 7	1	515	0	515				
Milyang 23/PTB 12	3	128	34	162		0.533		.30-.50
Rathu Heenati/PTB 12	1	307	12	319		0.612		.30-.50
Babawee/PTB 12	1	318	37	355			3.892	.02-.05
Milyang 23/PTB 20	2	129	39	168		2.198		.10-.20
Rathu Heenati/PTB 20	1	293	16	309		0.167		.50-.70
Babawee/PTB 20	1	297	36	333			2.914	.05-.10

biotype 2, the F_2 populations of Milyang 23/PTB 20 segregated in the ratio of 13:3 resistant to susceptible. Moreover, the F_2 populations from the crosses of Rathu Heenati with these cultivars segregated in the ratio of 61:3 and the F_2 populations from crosses with Babawee segregated in the ratio of 55:9, expected on the basis of independent segregation of a trihybrid.

PTB 21, PTB 33, Sudu Hondarawala, and Sinna Sivappu had already been found to carry two resistance genes (4, 7). Resistance in these four cultivars is conditioned by one dominant and one recessive gene. In addition, four cultivars found in the present study have one dominant and one recessive gene. A cultivar carrying two dominant genes or two recessive genes has never been found.

Recently, Nemoto et al (5) clarified that resistance in Col. 5 Thailand, Col. 11 Thailand, and Chin saba is conditioned by an identical recessive gene. In the near future, they will clarify the allelic relationships between the resistance genes in Balamawee (70–518). Kaharamana, and Pokkali.

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GENETICS OF RESISTANCE IN RICE TO PLANTHOPPERS AND LEAFHOPPERS

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The genetics of resistance in rice to three important rice hoppers has been investigated in detail. Four major genes, *Bph 1*, *bph 2*, *Bph 3*, and *bph 4*, have been identified as controlling resistance to BPH. There are indications that *Bph 3* and *bph 4* are either allelic or closely linked. Five dominant genes, *Wbph 1*, *2*, *3*, *4*, and *5*, have been identified as contributing resistance to WBPH. Although a number of new genetic sources of resistance to BPH and WBPH have been identified in India, the genes are yet to be designated. Seven resistance genes, *Glh 1*, *Glh 2*, *Glh 3*, *glh 4*, *Glh 5*, *Glh 6*, and *Glh 7*, have been identified for GLH resistance. Testing rice varieties with known genes for resistance through international cooperation leads to the discovery of promising gene sources.

In pest management, plant resistance forms an important component on which several other methods of pest suppression can be superimposed with a high degree of complementarity. Plant resistance is of two types: ecological resistance and genetic resistance. Genetic resistance is more dependable since it is inheritable. For utilizing genetic resistance in crop plants, information on the genetic mechanisms of resistance is of great importance in breeding programs in the identification of various sources of resistance, orienting the program of crossings and progeny selection, improving the genetic base of resistant varieties, and developing isogenic lines that can be used in the study of mechanisms of resistance. This paper reviews the genetics of resistance in rice to planthoppers and leafhoppers.

PLANTHOPPERS

Brown planthopper *Nilaparvata lugens* (Stal)

Genetic resistance to the brown planthopper (BPH) was first noted by Athwal et al (3), who reported that resistance in Mudgo, Co 22, and MTU 15 was controlled by single dominant genes that appeared to be

allelomorphic. The cultivar ASD 7 was reported to possess a single recessive gene that was either allelic or closely linked to the locus that conditioned resistance in the three cultivars. Athwal and Pathak (2) reported that varieties with dominant genes and those with recessive genes reacted differently to different BPH biotypes, and it was concluded that the two genes were different. The common gene for resistance in Mudgo, Co 22, and MTU 15 was designated as *Bph 1* and the recessive gene in ASD 7 as *bph 2* (2).

Martinez and Khush (13) showed that resistance in IR747 B2-6 is conditioned by a single dominant gene that is allelic to the dominant gene of Mudgo (*Bph 1*). Resistance in IR1154-243 and IR4-93 is governed by a recessive gene that is allelic to the recessive gene conditioning resistance in ASD 7 (*bph 2*). They also showed that TKM 6 is homozygous for *Bph 1* but is also homozygous for *I-Bph 1*, which inhibits *Bph 1*. They also thought that the variety Zenith possesses a similar inhibitor gene. Prasad Rao et al (16) reported that resistance in Leb Mue Nahng is governed by a recessive gene, but the allelic relationships of this gene with known resistance genes are unknown (Table 1).

Table 1. Resistance to brown planthopper in Lab Mue Nahng^a (16).

Cross	F ₁ reaction	F ₂ reaction (no. of seedlings)			F ₃ reaction			
		R	S	χ^2 3:1	R	Se	s	χ^2 1:2:1
RP31-49-2/ Leb Mue Nahng	S	536	1492	2.20	476	1041	556	6.18

^aR = resistant, S = susceptible, Se = segregating.

Lakshminarayana and Khush (12) stated that the identified 9 varieties possess single dominant genes for resistance that are allelic to *Bph 1*, while 16 other varieties possess single recessive genes for resistance that are allelic to *bph 2*. A single dominant gene was also reported to govern resistance in Rathu Heenati, which segregates independently of *Bph 1*. This gene was designated *Bph 3*. Similarly, a single recessive gene was found to govern resistance in Babawee, which segregated independently of *bph 2*, and this gene was designated as *bph 4*. Lakshminarayana and Khush (12) also reported that resistance in PTB 21 is controlled by one dominant and one recessive gene. Ikeda and Kaneda (7) thought the

resistance genes present in PTB 21 to be *bph 2* and *Bph 3*. They also reported that resistance in Andaragahawewa is controlled by *Bph 1* and resistance in PTB 34 by *bph 2*.

Sidhu and Khush (18) stated that the single dominant genes that were allelic to *Bph 3* conditioned resistance in seven cultivars and that single recessive genes that were allelic to *bph 4* governed resistance in nine other cultivars. Resistance in PTB 33, Sudu Honsarawala, and Sinna Sivappu was reported by them to be governed by one dominant and one recessive gene that segregate independently of each other. They also reported that the dominant genes in those cultivars are allelic to either *Bph 1* or *Bph 3*, and, similarly, the recessive genes in those cultivars appear allelic to either *bph 2* or *bph 4*. Sidhu and Khush (19) reported that there is no segregation for susceptibility in F₃ families of crosses between varieties having *Bph 3* and *bph 4* genes for resistance; thus *Bph 3* and *bph 4* are either allelic or closely linked.

Studies at Hyderabad, India, on the genetics of resistance indicated that, while resistance in PTB 33, ARC 6650, ARC 14636 B, and ARC 7080 is governed by dominant genes, recessive genes control resistance in six other sources (9). Investigations carried out at Coimbatore, India, involving Vazhaipoo (V.P.) Samba and other resistant donors with Vaigai and IR20 indicated the dominance of resistance (Table 2). Detailed studies of the progeny indicated that resistance in V.P. Samba is governed by a pair of dominant genes (23).

Conferring of resistance to BPH by genes identified in one country to the population existing in another country was investigated by Velusamy (23), who found that, while the genes *Bph 1* and *bph 2* identified at IRRI,

Table 2. Reaction to brown planthopper of F₁, F₂, and F₃, progeny of the cross between Vaigai and resistant accessions (23).^a

Cross	F ₁ reaction	Reaction to BPH							
		F ₂ seedlings (no.)				F ₃ families (no.)			
		R	S	c ²	3:1	R	Se	S	c ² 1:2:1
Vaigai/V.P. Samba	R	236	83	0.234	53	106	41	2.16	
IR20/V.P. Samba	R	255	79	0.161	57	95	48	1.31	
Vaigai/ASD 11	R	238	85	0.297	Not tested (NT)				
Vaigai/IET 5741	R	198	85	3.826	NT				
Vaigai/IET 6315	R	241	92	1.225	NT				
Vaigai/T7	R	207	76	0.518	NT				

^aR = resistant, S = susceptible, Se = segregating.

Philippines, did not contribute to resistance to the BPH population existing at Coimbatore, India, *Bph 3* and *bph 4* contributed to resistance to the BPH population at Coimbatore (Table 3). Furthermore, there were varieties like ARC 6650 and Sinna Sivappu possessing gene(s) for resistance to BPH populations in India and the Philippines. This investigation can lead to the appropriate choice of BPH-resistance varieties with varied genetic backgrounds for introduction in a country.

Table 3. Reaction of rice accessions to brown planthopper at Coimbatore, India, and the Philippines (23).

Accession	Gene for resistance	Damage rating (BPH population from)				Damage rating (BPH biotypes)		
		Aduthurai	Coimbatore	Madurai	Pondicherry	1	2	3
Mudgo	<i>Bph 1</i>	9	9	9	9	1	9	1
ASD 7	<i>bph 2</i>	9	9	9	9	1	3	9
Rathu Heenati	<i>Bph 3</i>	3	3	3	3	1	3	1
Babawee	<i>bph 4</i>	3	3	3	3	1	3	1
Sinna Sivappu	* ^a	1	1	1	1	1	3	1
ARC 6650	*	1	1	1	1	1	1	1
ASD 11	*	3	3	3	3	9	9	9
IET 5741	*	3	3	3	3	9	9	9
TN1	no gene	9	9	9	9	9	9	9

^a* =gene(s) not known

Whitebacked planthopper *Sogatella furcifera* (Horvath)

The inheritance of resistance to the whitebacked planthopper (WBPH) was first investigated in the resistant variety N 22 by Sidhu et al (20), who reported that a single dominant gene, designated *Wbph 1*, governs resistance in this variety. Angeles et al (1) analyzed the genetics of resistance in 12 more resistant varieties, among which six have *Wbph 1*. A new dominant gene *Wbph 2* was identified in ARC 10239. In addition, the variety Colombo was found to possess *Wbph 2* and another recessive gene. *Wbph 1* and a recessive gene were found to convey resistance in WC 1240 and 368. Angeles et al also observed that the genes *Wbph 1* and *Wbph 2* govern resistance in IR2035-117-3.

Nair et al (15) investigated the inheritance of resistance and allelic relationships of the genes for resistance in 21 rice varieties; *Wbph 1* was found to confer resistance in all the varieties studied. However, in varieties 65 and 274 A, resistance is governed by *Wbph 1* and an addi-

tional recessive gene. Hernandez and Khush (6) reported a new dominant gene for resistance, *Wbph 3*, in ADR 52 and a new recessive gene, *wbph 4*, in Podiwi-A8.

Saini et al (17) analyzed the inheritance of resistance in 13 rice varieties. Resistance in three was found to be conditioned by a single dominant gene *Wbph 1*. *Wbph 2* governs resistance in two others. Resistance in Hornamawee is governed by a single dominant gene that differs from *Wbph 1* and *Wbph 2* and is yet to be designated.

Studies at Hyderabad, India, revealed that single dominant genes confer resistance in PTB 33, ARC 14636, and ARC 14766. Single recessive genes govern resistance in ARC 6650 and ARC 14394 (11). At the same center, resistance in IET 6288 was reported to be controlled by a single dominant gene (14) see Table 4. At Coimbatore, India, Gunathilagaraj and Chelliah (5) studied the genetics of resistance in IET 5741 and described its dominant, monogenic resistance (Table 5).

Singh et al (21) studied the genetics of resistance in two resistant varieties, Balamawee and ARC 10464, and reported that in both resistance is governed by a single, recessive gene. They further reported that the recessive genes for resistance in both varieties are nonallelic (Table 6).

Recent studies indicate that two independent dominant genes govern resistance in four accessions. The single dominant gene in nine accessions and two independent dominant genes in four other accessions are allelic to *Wbph 1* and the second gene in one accession is allelic to *Wbph 3*. The dominant genes in two accessions segregate independently of *Wbph 1*, *Wbph 2*, and *Wbph 3*. The dominant resistant gene in N Diang marie was designated *Wbph 5*, but the allelic relationship to *Wbph 5* of the dominant gene of Manggar is unknown (8).

LEAFHOPPER

The genetics of resistance to the green leafhopper [GLH *Nephotettix virescens* (Distant)] was first reported in Pankari 203, ASD 7, and IR8 to be controlled by single genes that are nonallelic and dominant (3). The genes were designated *Glh 1* (Pankari 203), *Glh 2* (ASD 7), and *Glh 3* (IR8) (2). Athwal and Pathak (2) also reported that the variety PTB 18 possesses two genes for resistance to GLH, and that one of the two genes in PTB 18 is the same as *Glh 1* present in Pankari 203, while the other gene was not characterized.

Table 4. Segregation for resistance to whitebacked planthopper in the progeny of IET 6288 (resistant) / RP 2149 (susceptible) (14).

Lot	Plants ^a (no.)		c^2 3:1	P Value
	R	S		
1	57	19	0.00	0.995 – 1.00
2	112	34	1.29	0.50 – 0.75
3	306	116	1.31	0.25 – 0.50
Total	475	169	0.51	0.25 – 0.50

^aR = resistant, S = susceptible.

Table 5. Resistance to whitebacked planthopper in IET 5741 (5).^a

Cross	F ₁ reaction	Reaction to WBPH						
		F ₂ seedlings (no.)			F ₃ families (no.)			
		R	S	c^2 3:1	R	Se	S	c^2 1:2:1
IET5741/IR36	R	171	63	0.462	44	105	51	0.99

^aR = resistant, S = susceptible, Se = segregating.

Table 6. Resistance to whitebacked planthopper in Balamawee and ARC 10464 (21).^a

Cross	F ₁ reaction	F ₂ reaction (no. of seedlings)		Assumed genetic ratio	c^2	P value
		R	S			
		TN1/Balamawee	S			
TN1/ARC 10464	S	40	96	1:3	1.41	0.30–0.20
Balamawee/ARC10464	S	77	112	7:9	0.56	0.50–0.30

^aR = resistant, S = susceptible.

Siwi and Khush (22) stated that a dominant gene that is allelic to the gene *Glh 1* originally identified in Pankari 203 conveys resistance to GLH in Jhingasail, and that in three other cultivars a single dominant gene for resistance is located at the *Glh 2* locus, originally identified in ASD 7. Resistance in Betong, DNJ 97, and H5 was reported to be conditioned by a single dominant gene that is allelic to *Glh 3* originally found in IR8.

Five accessions were found to possess single dominant genes that are independent of *Glh 1*, *Glh 2* and *Glh 3* (22). A single recessive gene that is independent of *Glh 1*, *Glh 2* and *Glh 3* was found to condition resistance to GLH in PTB 8, and this recessive gene was designated *glh 4*, while the single dominant gene conditioning resistance in ASD 8 was designated *Glh 5*. The allelic relationships of the dominant genes that convey resistance to GLH in ARC 6602, DM 77, DS 1, and Khama 49/8 to *glh 4* and *Glh 5* are unknown.

At Coimbatore, India, Chelliah and Hanifa (4) evaluated 11 rice varieties with known resistance genes to GLH and found that varieties possessing genes *glh 4*, *Glh 6*, and *Glh 7* contributed resistance to the population of GLH. This finding is of practical utility in introducing to this region resistant varieties with the above genes (Table 7).

Karim and Pathak (10) found that the variety Kosatawee possesses a single recessive gene for resistance to GLH. Eleven accessions were reported to possess single dominant genes for resistance, which were different from *Glh 1*, *Glh 2*, *Glh 3* and *Glh 5*. The single dominant genes conveying resistance in three accessions were stated to be allelic to each other but nonallelic to the dominant resistance gene of Maddai Karuppan. The resistance gene in TAPL 796 was designated as *Glh 6* and that in Maddai Karuppan as *Glh 7* (10). The dominant gene in Sulai was found to be independent of *Glh 6*, but its allelic relationship to *Glh 7* is unknown. The allelic relationships of the dominant genes for resistance in six accessions to *Glh 6* and *Glh 7* are yet to be investigated.

Table 7. Resistance rating to green leafhopper of rice varieties with known genes at Coimbatore, India (adapted from 4).

Variety	Resistance gene	Damage rating ^a (days after infestation)	
		7	10
Pankhari 203	<i>Glh 1</i>	9.0 c	9.0 d
ASD 7	<i>Glh 2</i>	3.0 ab	7.0 cd
IR8	<i>Glh 3</i>	3.7 b	7.0 cd
PTB 8	<i>glh 4</i>	2.3 ab	3.7 ab
ASD 8	<i>Glh 5</i>	3.0 ab	5.7 bc
TAPL 796	<i>Glh 6</i>	1.7 ab	3.7 ab
Maddai Karuppan	<i>Glh 7</i>	1.0 a	1.7 a

^aIn a column, means followed by a common letter are not significantly different at the 5% level (DMRT).

RELATIONSHIPS BETWEEN BPH AND GLH RESISTANCE GENES

Mudgo was reported to be resistant only to BPH. Pankari 203 and IR8 were reported to be resistant only to GLH, while ASD 7 was found to have resistance to both insects. Athwal et al (3) showed that the ASD 7 genes for resistance to the two insects (*bph 2* and *Glh 2*) are independently inherited and also that Pankari gene for resistance to GLH (*Glh 1*) is inherited independently for *Bph 1* present in Mudgo. Athwal and Pathak (2) stated that recombination between *Bph 1* and *bph 2* is rare or absent and that each gene is inherited independently of the three genes for resistance to GLH. With available genetic information, they thought that several recombinations of one or more of the three genes for GLH resistance, viz., *Glh 1*, *Glh 2*, and *Glh 3*, with any of the two genes for BPH resistance, viz., *Bph 1* and *bph 2*, might be possible in future varieties. This may enable the breeding of varieties with broad spectrum resistance to different pests.

CONCLUSION

Resistance in rice cultivars to planthoppers and leafhoppers reported so far is due to major genes. Our experience with vertical resistant sources, particularly in the context of development of biotypes of rice hoppers, emphasizes the need to identify sources of resistance with polygenes. Sustained efforts should lead to the identification of such sources that will have extended commercial life when incorporated into acceptable rice varieties. The performance of hopper-resistant rice varieties developed in one country/region, with known gene(s), in another country/region will lead to judicious utilization and exchange of such varieties through international cooperation.

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INHERITANCE OF RESISTANCE TO GALL MIDGE IN SOME CULTIVARS OF RICE

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Inheritance of resistance to gall midge in five resistant rice cultivars was studied under conditions of natural infestation. All five cultivars were found to have a single dominant gene for resistance. Allele tests revealed that Usha, Samridhi, and Bd 6-1 have the same gene for resistance; this gene was designated *Gm-1*. Surekha and IET 6286 have the same gene for resistance, which is nonallelic to and independent of *Gm-1*; this gene was designated *Gm-2*.

Rice gall midge, *Orseolia oryzae* Wood-Mason, is an endemic pest of rice, *Oryza sativa* L. It occurs in several countries of Asia and Africa (3,9) and causes considerable damage to the crop. Clear cases of varietal resistance to this insect were reported by Bhatt et al (1), Ou and Kanjansoon (7), and Israel et al (5). In the post-IR8 era, a large number of varietal collections have been screened for resistance to this insect, and several hundred resistant varieties have been identified (2). Using resistant varieties as donors, several improved varieties with resistance to this insect have been developed in India, Thailand, Sri Lanka and the Philippines (6). However, due to different biotypes of the insect, varieties resistant in one area are not necessarily resistant in another.

Inheritance of resistance was investigated by Shastry et al (13) in two crosses involving resistant varieties W1263 and Ptb 21 and susceptible variety IR8. They postulated the existence of three complementary recessive genes for resistance and a dominant inhibitory gene. Satyanarayaniah and Reddy (12) also investigated the inheritance of resistance in the cross IR8/W1263 at the same station and location as Shastry et al (13) and clearly showed that one dominant gene conferred resistance in

W1263. The results of Sastry and Prakasa Rao (10) and Sastry et al (11) are at variance with the above studies.

This study was undertaken to investigate the inheritance of resistance to the Raipur biotype of gall midge.

MATERIALS AND METHODS

Five resistant varieties were used. Four of these—Usha, Samridhi, Surekha, and IET 6286—are improved plant type varieties derived from different donor parents as shown in Table 1. Bd 6-1 is a pure line selection from the tall traditional variety Assamchudi. They have consistently shown a resistant reaction at Raipur, Madhya Pradesh, India for several years. These were crossed with several susceptible varieties such as TN 1, Jagriti, Kranti, and IR1552, which have been consistently susceptible at Raipur (Table 1).

Crosses were made in the greenhouse during the wet season of 1982. The F_1 progenies were grown during the dry season of 1983. The F_1 and F_2 populations were screened under field conditions with a naturally occurring population of the insect during the 1983 wet season. The planting of the materials was delayed to 30 July to coincide the active tillering phase of the plants with the peak population of the insect in the field. No insect control measures were followed.

The F_1 rows were flanked on both sides by one row of a highly susceptible purple-leaved cultivar R259-WR37-2. This cultivar was also planted all around the screening plots to build up and maintain the insect population. Two rows of this cultivar were planted between different F_2 populations. Respective parents were planted next to each F_2 population. Parents, F_1 , F_2 , and F_3 populations were scored on an individual plant basis, and the presence of a single silver shoot per plant was taken as an index of susceptibility.

For obtaining F_3 progenies, part of the F_2 seed was planted at optimum planting time and full insecticide protection was given to minimize the insect damage. A random sample of 100 F_2 plants was harvested and planted in F_3 progeny rows (20 plants each) during the wet season of 1984. Planting was delayed as during 1983, and no insect protection was given. Two rows of purple-leaved susceptible cultivar were planted after every 10 rows. Each F_3 row was classified as homozygous resistant, segregating, or susceptible. Some of the F_1 and F_2 populations were also evaluated during the 1984 wet season to verify the previous year's results.

Table 1. Rice cultivars used in the study and their reaction to gall midge.

Cultivar	Parentage	Year	No. of plants studied			Reaction ^a
			Resistant	Susceptible	Infestation (%)	
Usha	IR22/W1263	1983	118	0	0	R
Samridhi	IR22/W1263	1983	120	0	0	R
Surekha	IR8/Siam-29	1983	105	0	0	R
IET6286	Leuang 152/IR8	1983	110	0	0	R
Bd 6-1	Sel. from Asamchudi	1983	45	0	0	R
TN-1	DGWG/Tsaiyuan-Chung	1983	0	160	100	S
Jagriti	Rikuu/R4	1983	0	60	100	S
Kranti	Cross 115/IR8	1984	0	95	100	S
IR1552	IR160-25/Crosa 2	1984	0	60	100	S

^a R = resistant, S = susceptible.

The F₁ and F₂ populations of the crosses between resistant parents were handled in the same manner.

RESULTS

Inheritance of resistance

The natural infestation of the gall midge in the wet seasons of 1983 and 1984 was very high. All the plants of the susceptible checks showed 100% infestation. No damage was observed in the resistant checks. Thus the field scores were considered highly reliable for classifying the segregating populations.

The reactions of the F₁, F₂, and F₃ progenies from the crosses of susceptible and resistant parents are given in Table 2. All the F₁ progenies were resistant, thereby showing that resistance in these cultivars is governed by dominant genes. All the populations segregated into a ratio of 3:1 resistant to susceptible, indicating that a single dominant gene confers resistance in these cultivars. These conclusions were verified from the reactions of F₃ progenies of the crosses of TN 1/Usha, Jagriti/Usha, TN 1/Surekha and TN 1/IET 6286. The F₃ families of these crosses segregated in the ratio of 1:2:1 resistant to segregating to susceptible expected for monogenic control of resistance. F₃ families from the cross TN 1/Bd 6-1 were not available.

Table 2. Reaction^a to gall midge of F₁, F₂, and F₃ progenies of the crosses between resistant and susceptible parents.

Cross	F ₁ reaction	Reaction of F ₂ plants			Reaction of F ₃ families			
		Res.	Sus.	c ² 3:1	Res.	Seg.	Sus.	c ² 1:2:1
TN 1/Usha	res.	293	102	0.14	31	49	20	2.46
Jagriti/Usha	res.	337	109	0.07	26	57	17	3.59
IR1552/Samridhi	res.	300	116	1.85	—	—	—	—
TN 1/Bd 6-1	res.	121	46	0.58	—	—	—	—
TN 1/Surekha	res.	179	71	1.54	26	55	17	3.10
Jagriti/Surekha	res.	239	79	0.00	—	—	—	—
TN 1/IET 6285	res.	331	91	2.66	17	57	25	3.93

^aRes. = resistant, Seg. = segregating, Sus. = susceptible.

Allele tests

The allelic relationships of the resistance genes were investigated from the reaction of F₁ and F₂ populations of the crosses between resistant parents (Table 3). As expected, all the F₁ progenies were resistant. The F₂ progenies of the crosses Samridhi/Usha, Usha/Bd 6-1, and Surekha/IET 6286 did not show any susceptibility. An occasional susceptible seedling was observed in these three crosses. These were probably volunteers or a result of seed mixture. These results show that Usha, Samridhi, and Bd 6-1 have the same gene for resistance. Similarly Surekha and IET 6286 have the same gene for resistance. The F₂ populations of the crosses Samridhi/Surekha, Surekha/Bd 6-1 and JET 6286/Bd 6-1 segregated into a 15:1 resistant to susceptible ratio, thus showing that the dominant resistance gene of Surekha, Samridhi, and Bd 6-1 is nonallelic to and independent of the dominant resistance gene of Surekha and IET 6286.

DISCUSSION

The results of this study clearly show that cultivars Usha, Samridhi, and Bd 6-1 have the same dominant gene for resistance. Usha and Samridhi are derived from crosses of W1263. Cultivar W1263 was shown to have a single dominant gene for resistance in three earlier studies (8, 12, 14). Thus Usha and Samridhi have inherited the same resistance gene from a

Table 3. Reaction to gall midge of F₁ and F₂ progenies of the crosses between resistant parents.

Cross	F ₁ reaction	Reaction of F ₂ plants				c ² 15:1
		Year	Resistant (no.)	Susceptible (no.)	Segregation	
Samridhi/Usha	resistant	1983	499	1	no	—
		1984	160	0	no	—
Usha/Bd 6-1	resistant	1983	464	6	no	—
Surekha/IET 6286	resistant	1983	495	5	no	—
		1984	1305	2	no	—
Samridhi/Surekha	resistant	1984	1506	99	yes	0.02
Surekha/Bd 6-1	resistant	1983	235	15	yes	0.03
IET 6286/Bd 6-1	resistant	1983	419	22	yes	0.03

common resistant parent. Other reports on the multigene control of resistance in W1263 (10, 11, 13) could not be confirmed.

Cultivar Surekha (a derivative of Siam 29) and IET 6286 (a derivative of Leuang 152) were found to have the same dominant gene for resistance. It may be concluded that Siam 29 and Leuang 152 have the same dominant gene. The gene in these cultivars is nonallelic to and independent of the dominant gene of W1263 and its derivatives. According to the international rules for gene nomenclature (4) the dominant gene of W1263 is designated *Gm-1* and the dominant gene of Surekha is designated *Gm-2*.

As discussed by Heinrichs and Pathak (2) at least five biotypes of gall midge are known. *Gm-1* confers resistance to the Thailand biotype as well as to the Andhra Pradesh (India) biotype, but not to the Indonesia, Sri Lanka, and Orissa (India) biotypes. On the other hand, *Gm-2* confers resistance to the Indonesia, Orissa, Andhra Pradesh, and Sri Lanka biotypes but not to the Thailand biotype. Since numerous other resistant donors are now available, efforts should be made to identify additional genes for resistance.

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SOURCES AND INHERITANCE OF RESISTANCE TO WHITEBACKED PLANTHOPPER *SOGATELLA* *FURCIFERA* IN RICE

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A collection of 887 cultivars/genetic stocks was tested for reaction to the local population of *Sogatella furcifera* (Horv.) using the modified seed box technique and the Standard Evaluation System proposed by IRRI. Only 34 stocks were found to be resistant. Observations on these resistant stocks and some selected moderately resistant stocks are presented and compared regarding their reactions at IRRI and at other centers in India. Out of 21 stocks having the gene *Wph-1* that were tested, only 1 stock, Sufaida-172, was resistant. Two stocks, Chempan and Cheriya Chittari, which have *Wph-2*, were resistant. ADR 52 possessing *Wph-3* and one accession of Podiwi-A8 having *wph-4* were resistant. IRRI's resistant check, IR2035-117-3, was heterogenous, with resistant and susceptible plants occurring in almost equal proportions (average rating 5.4). Twenty-seven more stocks with resistant to moderately resistant ratings are listed. Genetic characterization of selected stocks is in progress. A single recessive gene appears to control resistance in IET4695, IET6288, and NCS212. Resistance in 274-A appears to be controlled by two genes. Cross 274-A/TN 1 segregated in a ratio of 3:13 resistant to susceptible seedlings, indicating some additional gene(s) in this stock.

In recent years the whitebacked planthopper *Sogatella furcifera* (Horv.) has caused extensive damage to rice crops in the Punjab, India. While screening to identify resistant cultivars/genetic stocks, it was observed that most of the stocks obtained from different centers including IRRI showed susceptible in our reactions in our tests (2). Some of the stocks with known *Wph* genes have been tested, and genetic characterization of the stocks showing resistant reactions is being done at Punjab Agricultural University (PAU). Observations on the effectiveness of genes *Wph-1* to *wph-4* and the genetic nature of resistance in some selected resistant stocks are presented here.

MATERIALS AND METHODS

A total collection of 887 cultivars/genetic stocks comprising 110 stocks from IRRI, Philippines; 261 from AICRIP, Hyderabad; 239 from CRRRI, Cuttack; and 277 obtained from other breeding centers was tested for reaction to *S. furcifera*. Insects collected from farmers' fields around Ludhiana were reared in the laboratory as per the procedure of Shukla and Gupta (9). This insect population was used for testing the reaction of different rice stocks using the modified seed box technique recommended by Kalode et al (5). Seven-day-old seedlings were infested with second instar nymphs of *S. furcifera* at an approximate level of 7–8 nymphs/seedling. Reactions were scored 6–8 days after infestation using the Standard Evaluation System (SES) recommended by Pathak and Saxena (8). Observations were recorded on a single plant basis on different stocks as well as F₂ and F₃ populations. Seedlings were classified as resistant (rating 1 to 3), moderately resistant (rating 5 to 7), or susceptible (rating above 7). Chi-square tests were applied to confirm segregation in expected ratios in the F₂ and F₃ populations of different crosses.

RESULTS AND DISCUSSION

Out of 887 stocks tested, only 34 were found to be resistant. The reactions of 21 cultivars/stocks obtained from IRRI that are known to carry the gene *Wph-1* are summarized in Table 1. While all 21 stocks were either resistant (R) or moderately resistant (MR) at IRRI, only one, Sufaida-172, was resistant and six were moderately resistant at PAU. These observations indicate that the gene *Wph-1* is not very effective in conferring resistance to *S. furcifera* in Punjab. Some of the stocks carrying *Wph-2*, *Wph-3*, *wph-4*, or a combination of two genes were also tested. The reactions of these stocks compared with those at IRRI are summarized in Table 2. One stock with *Wph-2*, ARC 10239, which was moderately resistant at IRRI, was highly susceptible at PAU, while Cheriya Chittari, carrying the same gene, was resistant at PAU as well as at IRRI. Another stock, ADR 52, reported to have *Wph-3*, was resistant at both PAU and IRRI. Two accessions of Podiwi-A-8 (known to carry *Wph-2*) were tested; the one obtained from Coimbatore was highly resistant, while the one obtained from IRRI (Acc. No. 15201) was moderately resistant. Other stocks with genes *Wph-1* + *Wph-2* or either of these two genes along with another recessive gene showed moderate resistance at PAU. However, stock 274-A was observed to be resistant at PAU as well as at IRRI.

Table 1. Reaction of rice stocks with *Wph-1* gene to whitebacked planthopper *Sogatella furcifera* (Horv.).

Stock	IRRI acc. no.	Damage rating ^a at PAU		Rating at IRRI
		Range	Mean	
CI 6037-4	3667	5-9	8.2	3.0
Bansphul	28813	4-9	8.0	3.7
293	28442	7-9	8.3	3.7
Siah Nakidar 195	28265	9	9.0	4.3
Latighawar	23964	4-9	8.1	4.3
76 S	28399	5-9	7.9	4.3
180	28424	3-9	8.4	4.7
39	28377	9	9.0	5.0
Tiri Surkh 251	28310	4-9	8.4	5.0
S39 JKW	6836	1-9	8.4	5.0
24A	28358	9	9.0	5.7
Jhinuwa	23910	3-9	7.5	5.0
N32	3717	5-9	8.4	6.3
N 22	4819	3-9	7.9	6.3
213 B	28428	5-9	6.9	3.7
Sonpattar 45	28285	3-9	6.2	3.0
78	28404	3-9	6.9	6.3
P 580	28098	1-9	6.7	5.0
Sathra 267	28237	1-9	6.7	-
NP 97	3700	1-9	5.4	2.7
Sufaida 172	28298	1-9	2.9	3.7
ARC 6248	(local res. check)	1-3	1.5	R
TN 1	(local sus. check)	7-9	9.0	9.0

^aAverage of 2-12 replications and 20-25 plants/replication; 1-3 = resistant (R), 5-7 = moderately resistant (MR), above 7 = susceptible (S); scored 6-8 days after infestation.

Other differential reactions to *S. furcifera* have been reported (4). Out of 118 cultivars compared, 49 showed different reactions at IRRI and in India. However, 22 stocks were reported to be resistant both at IRRI as well as in tests done in India. Out of these 22 we tested 17 stocks at PAU, and only 6 were found to be resistant, 3 moderately resistant, and 8—ARC 14342, Chittari, Ptb 12, Ptb 19, Ptb 21, Ptb 33, T 1471, and Velutha Chera—were highly susceptible. Differential reactions between varieties showing resistance at IRRI and susceptibility in India have also been reported by Vaidya and Kalode (10). Our observations indicate that the *S. furcifera* prevalent in Punjab is able to infest those lines, e.g., Ptb 19, Ptb 21, and Ptb 33, that are resistant at other centers in India (6) as well as at IRRI. Four selected resistant lines from our collection, namely

Table 2. Reaction of rice stocks with other *Wph* genes to whitebacked planthopper *Sogatella furcifera* (Horv.).

Stock	Source/ IRRI acc. no.	Gene(s) for resistance	Damage rating ^a PAU		Rating at IRRI
			Range	Mean	
ARC 10239	IRRI-20803	Wph-2	2–9	8.6	4.3
Cheriyā Chittari	IRRI-534424	Wph-2	1–9	3.2	2.3
Cheriyā Chittari	AICRIP, Hyd.	Wph-2 ?	3–9	8.8	–
Chempan	AICRIP, Hyd.	Wph-2 ?	1–9	3.3	–
ADR 52	IRRI-40638	Wph-3	1–5	1.9	R
Podiwi-A-8	Coimbatore	wph-4 ?	0–9	1.7	–
Podiwi-A-8	IRRI-15201	wph-4	1–9	6.8	R
NP 130	IRRI-3702	Wph-1+ Wph-2	5–9	7.6	S
Colombo	IRRI-6662	Wph-2+ 1 rec. gene	2–9	7.1	4.0
368	IRRI-28451	Wph-1+ 1 rec. gene	1–9	4.6	MR
WC 1240	IRRI-I3742	1 rec. gene	1–9	3.9	R
274-A	IRRI-28440	1 rec. gene	1–9	2.7	R
IR2035-117-3	(IRRI's res. check)	Wph-1+ Wph-2	1–9	5.4 ^b	R
ARC 6248	(local res. check)	–	1–3	1.5	R
TN 1	(lucal sus. check)	–	7–9	9.0	9.0

^aAverage of 2–12 replications and 20–25 plants/replication; 1–3 = resistant (R), 5–7 = moderately resistant (MR), above 7 = susceptible (S); scored 6–8 days after infestation.

^bAverage of 584 seedlings tested, out of which 273 had scored 1–3, 85 had 4–7, and 226 had 8–9.

NCS212, ARC 6248, IET 6288, and IET 4695, have been found to be resistant at IRRI (G.S. Khush, personal communication).

Breeding line IR2035-117-3, which has been used as a resistant check in tests at IRRI, is reported to carry two genes *Wph-1* + *Wph-2* (1). This line, identified as most resistant in tests at IRRI (3), was observed to be heterogenous and only moderately resistant (average rating 5.4), having susceptible and resistant plants in almost equal proportion (Table 2). It appears that different plants in this line may not carry the same genes. Single plant progeny from this line have been developed, and lines with pure resistant/susceptible reactions are being selected for further genetic analysis.

Twenty-seven more stocks showed resistance in our tests; 19 were highly resistant and 8 moderately resistant (Table 3). This included 9

Table 3. Some other rice stocks with rating R to MR against whitebacked planthopper *Sogatella furcifera* (Horv.) at PAU, Ludhiana.

Stock	Source/IRRI acc. no.	No. of seedlings tested	Average rating ^a at PAU
ARC 6650	IRRI-55429	40	6.0
ARC 6564	IRRI-53428	17	5.8
T 1426	IRRI-53426	33	3.5
T 1421	IRRI-53425	32	3.2
S 2204	IRRI-3702	58	2.4
Boegi Boera	IRRI-4165	45	2.0
Desi Basmati	IRRI-56978	63	1.8
Bohai	IRRI-17048	35	1.7
ARC 5752	IRRI-12119	66	1.2
IET 4695	AICRIP, Hyd.	77	4.1 ^b
ARC 10912	AICRIP, Hyd.	78	3.5
IET 6288	AICRIP, Hyd.	359	3.4 ^b
Vellathil Cheera	AICRIP, Hyd.	101	3.4 ^b
Kula Peruvela	AICRIP, Hyd.	57	2.7
Eswaramangalam	AICRIP, Hyd.	23	2.5
Vellai Langayan	AICRIP, Hyd.	85	2.3 ^b
Chemban	AICRIP, Hyd.	61	2.2 ^b
Valsara Champara	AICRIP, Hyd.	24	2.0
ARC 11367	AICRIP, Hyd.	167	1.8 ^b
Pandi	AICRIP, Hyd.	75	1.8
ARC 6248	AICRIP, Hyd.	57	1.5
Mudgo	CRRI, Cuttack	148	2.7
NCS 37	CRRI, Cuttack	21	2.6
NCS 212	CRRI, Cuttack	36	2.2
NCS 67	CRRI, Cuttack	19	2.0
NCS 211	CRRI, Cuttack	18	1.2
Vytilla-2	KAU, Cochin	87	1.1

^a 1–3 = resistant (R), 4–7 = moderately resistant (MR), above 7 = susceptible (S).

^b Rating score varied for the same stock received in different years from 1979 to 1982.

stocks obtained from IRRI (4 moderately resistant with a rating of 3.2–6.0 and 5 highly resistant); 12 stocks from AICRIP, Hyderabad (4 moderately resistant with a rating of 3.4–4.1 and 8 highly resistant); and 5 stocks received from CRRI, Cuttack (all highly resistant). A high degree of resistance (rating 1.1) was observed in the cultivar Vytilla-2 received from the Rice Research Station at Cochin, Kerala, India.

Eleven selected stocks that have shown good resistance in tests at PAU are being characterized for their resistance genes. Four stocks are pre-

Table 4. Reactions of F₂ populations from the crosses of TN 1 with four resistant cultivars to whitebacked planthopper *Sogatella furcifera* (Horv.).

Cross	F ₂ seedlings				c ²	
	Total (no.)	Resistant (no.)	Susceptible (no.)	(%)	1:3	3:13
NCS 212/TN 1	1018	282	736	72.30	3.96	53.54
274A/TN 1	926	151	775	83.69	28.32	3.63
IET 6288/TN 1	1131	277	854	75.51	0.16	24.44
IET 4695/TN 1	1145	329	816	71.27	8.51	74.91

sently in advanced tests (Table 4). Resistance in NCS 212, IET 6288, and IET 4695 appears to be monogenic recessive. In the F₂ of 274-A/TN 1 a ratio of 3:13 resistant to susceptible plants was observed. One dominant gene (*Wph-1*) and another recessive gene are reported to be present in 274-A, giving a ratio of 13:3 resistant to susceptible plants in the F₂ of 274-A/TN 1 (7). Since *Wph-1* is not effective at PAU, it appears that the recessive gene, in addition to some other gene that is yet to be identified, controls resistance in 274-A.

These observations indicate that only a few of the available resistant stocks are effective against whitebacked planthopper in the Punjab, and it may be possible to identify additional and more effective sources of resistance using local populations of this insect.

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INHERITANCE OF RESISTANCE TO PLANTHOPPERS AND LEAFHOPPERS IN RICE

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The genetics of resistance to zigzag leafhopper (ZLH), whitebacked planthopper (WBPH), brown planthopper (BPH), and green leafhopper (GLH) in rice cultivars Rathu Heenati, Ptb 21, and Ptb 33 was studied. Single dominant genes that segregate independently of each other were found to convey resistance to ZLH in Rathu Heenati (*Zlh-1*), Ptb 21 (*Zlh-2*), and Ptb 33 (*Zlh-3*). Resistance to WBPH in the three cultivars is conditioned by single dominant genes that allelic tests showed to be allelic to *Wbph-3* originally found in ADR 52. The digenic *bph-2* and *Bph-3* control of resistance to BPH in Ptb 21 and Ptb 33 was confirmed. GLH resistance in Ptb 21 was found to be controlled by a single recessive gene; the allelic relationships of this gene to *glh-4*, which conditions resistance to GLH in Ptb 8, are, however, not known. Tests for independence of the various genes for resistance to planthoppers and leafhoppers in the test cultivars revealed that *Zlh-1*, *Zlh-2*, and *Zlh-3* are independently inherited of *Wbph-3*. Both *Zlh-2* and *Zlh-3* also segregate independently of *bph-2* and *Bph-3*. *Zlh-2* was similarly found to be independent of the recessive gene governing resistance to GLH in Ptb 21. *Wbph-3* segregated independently of *bph-2* and *Bph-3*. The recessive gene for GLH resistance in Ptb 21 segregated independently of *Wbph-3*, *bph-2*, and *Bph-3*. The independent segregation of the genes for resistance to planthoppers and leafhoppers indicates the possibility of incorporating them in a single variety of good agronomic background.

The use of resistant varieties is the most logical and economical way of reducing planthopper and leafhopper damage in rice. Therefore, one of the major objectives of the rice improvement program at IRRI is the development of improved germplasm with resistance to these pests. At present, a number of varieties and breeding lines with resistance to the more important planthoppers and leafhoppers have already been developed. These varieties are widely planted in major rice growing countries of the world. Concurrently with the development of improved breeding materials, we are studying the inheritance of resistance to identify diverse genes for resistance.

Four genes for resistance to brown planthopper (BPH) have been identified. One dominant gene, *Bph-1*, and one recessive gene, *bph-2* — which are closely linked — were identified by Athwal et al (2). Another dominant gene, *Bph-3*, and a recessive gene, *bph-4*, were identified by Lakshminarayana and Khush (6) and were found to be closely linked (4,8).

So far, seven genes for resistance to green leafhopper (GLH) have been identified. The dominant genes *Glh-1*, *Glh-2*, *Glh-3*, (2), and *Glh-5* (10) and the recessive gene *glh-4* (10) govern resistance to GLH populations in the Philippines. Two dominant genes, *Glh-6* and *Glh-7*, confer resistance to the Bangladesh population of GLH (7).

Four genes for resistance to whitebacked planthopper (WBPH) are known. The dominant gene *Wbph-1*, which conveys resistance in N22, was identified by Sidhu et al (9), while the dominant gene *Wbph-2*, which governs resistance in ARC 10239, was reported by Angeles et al (1). Two additional genes were identified by Hernandez and Khush (3): the dominant *Wbph-3*, which confers resistance in ADR 52, and the recessive *wbph-4*, which confers resistance in Podiwi-A8.

Planthoppers and leafhoppers are capable of developing new biotypes that can overcome the resistance conveyed by major genes in improved varieties. It is therefore important to identify additional genes for resistance to these insects. This paper presents the results of genetic analyses for resistance to planthoppers and leafhoppers in three resistant rice cultivars.

MATERIALS AND METHODS

Three rice cultivars that are resistant to zigzag leafhopper (ZLH), WBPH, BPH, and GLH were used (Table 1). They were crossed to Taichung Native 1 (TN 1), a variety highly susceptible to planthoppers and leafhoppers. The F_1 , F_2 , and F_3 progenies of these crosses were evaluated for resistance to ZLH, WBPH, BPH, and GLH to determine the mode of inheritance of resistance and the linkage relationships of the genes for resistance in these cultivars.

Crosses among the three resistant cultivars were also made. The F_1 , F_2 , and F_3 progenies of these crosses were similarly tested for their reaction to the insect pests to establish the allelic relationships of the resistant genes.

The bulk seedling test (2) was used to test the reaction of the hybrid materials to the insects. This is done by seeding the test materials in seedboxes and uniformly infesting them with second to third instar

Table 1. Reaction to planthoppers and leafhoppers of three rice cultivars.

Cultivar	IRRI accession no.	Reaction ^a					
		BPH biotype			WBPH	GLH	ZLH
		1	2	3			
Rathu Heenati	11730	R	R	R	R	S	R
Ptb 21	6113	R	R	R	R	R	R
Ptb 33	19325	R	R	R	R	S	R

^aBPH = brown planthopper. WBPH = whitebacked planthopper. GLH = green leafhopper. ZLH = zigzag leafhopper. R = resistant, S = susceptible.

nymphs of the insect reared on TN 1 plants at the rate of five to seven insects per seedling. Infestation of the materials is done at the one-leaf stage or 6–8 days from sowing.

The reaction of the test materials was recorded when the susceptible checks (TN 1) were completely killed by the insects. The reactions of the F_1 and F_3 progenies were scored on a row basis, while F_2 populations were scored on an individual plant basis. F_3 lines were classified as either homozygous resistant, segregating, or homozygous susceptible.

RESULTS AND DISCUSSION

Inheritance of resistance and allelic relationships of genes for resistance

Zigzag leafhopper. The reactions to ZLH of the F_1 , F_2 , and F_3 progenies from the crosses of the three rice cultivars with TN 1 are shown in Table 2. The F_1 hybrids from all the crosses showed a resistant reaction, indicating that resistance to this insect in Rathu Heenati, Ptb 21, and Ptb 33 is dominant.

The F_2 progenies from these crosses segregated into 3:1 resistant to susceptible, suggesting that a dominant gene controls resistance to ZLH in the test varieties. This was confirmed by the reaction of F_3 progenies, which segregated into 1:2:1 homozygous resistant to segregating to homozygous susceptible. As shown in Table 2, two F_3 populations of each cross were tested.

Table 2. Reaction^a to zigzag leafhopper of F₁, F₂ populations, and F₃ lines from the crosses of resistant rice cultivars with TN 1.

Cross	F ₁	F ₂ seedlings			F ₃ lines			
		Res.	Susc.	c ² 3:1	Res.	Seg.	Susc.	c ² 1:2:1
TN 1/Rathu Heenati	Res.	562	206	1.36	35	82	33	1.3
					39	78	37	0.08
TN 1/Ptb 21	Res.	527	199	2.25	39	68	34	0.53
					41	77	36	0.33
TN 1/Ptb 33	Res.	820	276	0.02	44	80	30	2.78
					41	81	31	1.85

^aRes. = resistant, Seg. = segregating, Susc. = susceptible.

Table 3. Reaction^a to zigzag leafhopper of F₁, F₂ populations, and F₃ lines from the crosses of resistant rice cultivars.

cross	F ₁	F ₂ seedlings			F ₃ lines				
		Res.	Susc.	c ² 15:1	Res.	Seg.	Susc.	c ² 7:8:1 15:1	
Rathu Heenati/ Ptb 21	Res.	779	48	0.28	82	55	8	9.78	0.1
					57	75	10	0.80	-
Rathu Heenati/ Ptb 33	Res.	753	49	0.03	69	72	10	0.32	-
					68	79	7	0.78	-
Ptb 21/Ptb 33	Res.	740	61	2.61	64	80	10	0.32	-
					57	85	12	3.02	-

^aRes. = resistant, Seg. = segregating, Susc. = susceptible.

The data on the reaction to ZLH of F₁ hybrids, F₂ populations, and F₃ lines of crosses among the resistant varieties are presented in Table 3. As expected, the F₁'s from all the crosses showed a resistant reaction.

The F₂ populations from the crosses Rathu Heenati/Ptb 21 and Rathu Heenati/Ptb 33 segregated into 15:1 resistant to susceptible, while the F₃ lines approximated a segregation ratio of 7:8:1 resistant to segregating to susceptible; this becomes a 15:1 ratio if the resistant and segregating lines

Table 4. Reaction^a to whitebacked planthopper of F₁, F₂ populations, and F₃ lines from the crosses of resistant rice cultivars with TN 1.

Cross	F ₁	F ₂ seedlings			F ₃ lines			
		Res.	Susc.	c ² 3:1	Res.	Seg.	Susc.	c ² 1:2:1
TN 1/Rathu Heenati	Res.	535	160	1.45	38	81	31	1.61
TN 1/Ptb 21	Res.	285	128	7.91	36	74	31	1.21
TN 1/Ptb 33	Res.	612	213	0.29	37	85	32	1.99

^a Res. = resistant, Seg. = segregating, Susc. = susceptible.

are pooled. These data show that the gene governing resistance to ZLH in Rathu Heenati is different and independent of the Ptb 21 and Ptb 33 genes. Similar reactions of the F₂ and F₃ progenies were observed in the cross Ptb 21/Ptb 33, indicating that the genes of Ptb 21 and Ptb 33 are also nonallelic. These dominant genes for resistance to ZLH in Rathu Heenati, Ptb 21, and Ptb 33 are designated *Zlh-1*, *Zlh-2*, and *Zlh-3*.

Whitebacked planthopper. The F₁, F₂, and F₃ progenies from the crosses of Rathu Heenati, Ptb 21, and Ptb 33 with TN 1 were evaluated for resistance to WBPH to determine the mode of inheritance in these varieties. The results are presented in Table 4. The F₁ hybrids of all the crosses showed a resistant reaction, suggesting that dominant genes convey resistance to WBPH in these cultivars.

The F₂ populations from the crosses TN 1/Rathu Heenati and TN 1/Ptb 33 segregated in the ratio of 3:1 resistant to susceptible, and the F₃ lines from the same crosses segregated in the ratio of 1:2:1 resistant to segregating to susceptible. These results show that resistance to WBPH in Rathu Heenati and Ptb 33 is controlled by a single dominant gene. A somewhat higher proportion of susceptible seedlings was observed in the F₂ population of the cross TN 1/Ptb 21, but its F₃ progenies showed a clear 1:2:1 resistant to segregating to susceptible segregation ratio, indicating that resistance in Ptb 21 is also governed by a dominant gene.

The reactions to WBPH of F₁ hybrids, F₂ populations, and F₃ lines from the crosses Rathu Heenati/Ptb 21, Rathu Heenati/Ptb 33, and Ptb 21/Ptb 33 are shown in Table 5. The resistant reaction of the F₁ hybrids in all the crosses was expected, as resistance to WBPH in the test cultivars is governed by a dominant gene. A few susceptible seedlings were observed in the F₂'s of the above-mentioned crosses, but the number

Table 5. Reaction^a to whitebacked planthopper of F₁, F₂ populations, and F₃ lines from the crosses of resistant rice cultivars.

Cross	F ₁	F ₂ seedlings			F ₃ lines		
		Res.	Susc.	χ^2 15:1	Res.	Seg.	Susc.
Rathu Heenati/Ptb 21	Res.	854	9	—	145	0	0
Rathu Heenati/Ptb 33	Res.	836	18	—	154	0	0
Ptb 21/Rb 33	Res.	818	10	—	154	0	0
Rathu Heenati/ADR 52	Res.	793	5	—	154	0	0

^aRes. = resistant, Seg. = segregating, Susc. = susceptible.

Table 6. Reaction^a to brown planthopper of F₁, F₂ populations, and F₃ lines from the crosses of resistant rice cultivars with TN 1.

Cross	F ₁	F ₂ seedlings			F ₃ lines			
		Res.	Susc.	χ^2 13:3	Res.	Seg.	Susc.	χ^2 7:8:1
TN 1/Ptb 21	Res.	335	88	1.18	57	75	9	0.66
TN 1/Ptb 33	Res.	614	123	2.06	64	82	7	1.21

^aRes. = resistant, Seg. = segregating, Susc. = susceptible.

was so small that genetic segregation was considered unlikely. Moreover, the F₃ progenies of these crosses showed no segregation for susceptibility, indicating that Rathu Heenati, Ptb 21, and Ptb 33 have the same gene for resistance to WBPH.

To determine the allelic relationships of the dominant gene for resistance to WBPH in Rathu Heenati with *Wbph-3*, the F₁, F₂, and F₃ progenies from the cross Rathu Heenati/ADR 52 were studied. As shown in Table 5, the F₁ of the cross was resistant and the F₂ populations and F₃ lines showed no segregation for susceptibility. These results indicate that the dominant gene controlling resistance to WBPH in Rathu Heenati is allelic to *Wbph-3*. Ptb 21 and Ptb 33 must, therefore, also have *Wbph-3*.

Brown planthopper. The reactions to BPH of F₁, F₂, and F₃ progenies from the crosses TN 1/Ptb 21 and TN 1/Ptb 33 are shown in Table 6.

Table 7. Reaction^a to green leafhopper of F₁, F₂ populations, and F₃ lines from the cross TN 1/Ptb 21.

Cross	F ₁	F ₂ seedlings			F ₃ lines			
		Res.	Susc.	c ² 1:3	Res.	Seg.	Susc.	c ² 1:2:1
TN 1/Ptb 21	Susc.	254	657	4.04	36	73	32	0.41
					44	76	34	1.34

^aRes. = resistant, Seg. = segregating, Susc. = susceptible.

The F₁ hybrids from the crosses were resistant and the F₂ populations segregated in the ratio of 13:3 resistant to susceptible. These results indicate that resistance in the two cultivars is conditioned by a dominant and a recessive gene. F₃ families from the crosses segregated in the ratio of 7:8:1 resistant to segregating to susceptible, confirming the digenic control of resistance in these cultivars.

The data obtained from the above tests confirm the results of earlier genetic investigations made on these varieties. Lakshminarayana and Khush (6) reported that a dominant gene, designated *Bph-3*, conditions resistance to BPH in Rathu Heenati, while a dominant and a recessive gene convey resistance in Ptb 21. These genes were later identified to be *bph-2* and *Bph-3* (4). Resistance in Ptb 33 was similarly found to be governed by a dominant and a recessive gene (8). Our unpublished data show that Ptb 33 also has *bph-2* and *Bph-3*.

Green leafhopper. Among the three test cultivars, only Ptb 21 is resistant to GLH. The reactions of the F₁, F₂, and F₃ progenies from the cross of this variety with TN 1 are shown in Table 7. The F₁ progenies were susceptible, and the F₂ populations segregated in the ratio of 1:3 resistant to susceptible. These results suggest that resistance to GLH in Ptb 21 is controlled by a single recessive gene. F₃ lines of this cross segregated in the ratio of 1:2:1 resistant to segregating to susceptible, thus confirming the monogenic recessive control of resistance in Ptb 21.

Linkage relationships of the resistance genes

The data from the two-way classification of the reactions of F₃ families to ZLH, WBPH, BPH, and GLH in the crosses TN 1/Rathu Heenati, TN

Table 8. Reaction to zigzag leafhopper and whitebacked planthopper of F₃ lines from the cross TN 1/Rathu Heenati.

Reaction to ZLH	Reaction to WBPH			
	Resistant	Segregating	Susceptible	Total
Resistant	4	22	9	35
Segregating	23	41	18	82
Susceptible	11	18	4	33
Total	38	81	31	150

c^2 for independence of two traits = 6.20^{ns}^a

c^2 for 1:2:1:2:4:2:1:2:1 = 8.39^{ns}

^ans = nonsignificant at the 5% level.

Table 9. Reaction to zigzag leafhopper and whitebacked planthopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to ZLH	Reaction to WBPH			
	Resistant	Segregating	Susceptible	Total
Resistant	10	22	7	39
Segregating	20	36	12	68
Susceptible	6	16	12	34
Total	36	74	31	141

c^2 for independence of two traits = 4.84^{ns}^a

c^2 for 1:2:1:2:4:2:1:2:1 = 6.72^{ns}

^ans = nonsignificant at the 5% level.

1/Ptb 21, and TN 1/Ptb 33 were used to calculate c^2 values for testing the independence of the resistance genes in the cultivars.

The analysis of F₃ data for reaction to ZLH and WBPH from the cross TN 1/Rathu Heenati is presented in Table 8. The c^2 values for the independence of two traits, and for a 1:2:1:2:4:2:1:2:1 ratio were nonsignificant, thereby showing that *Zlh-1* and *Wbph-3* are independent of each other. Similar results were obtained from the crosses TN 1/Ptb 21 and TN 1/Ptb 33 (Tables 9, 10), indicating that *Zlh-2* and *Zlh-3* also segregate independently of *Wbph 3*.

Tables 11 and 12 present the data for reaction to ZLH and BPH of F₃ lines from the crosses TN 1/Ptb 21 and TN 1/Ptb 33. The c^2 for the

Table 10. Reaction to zigzag leafhopper and whitebacked planthopper of F₃ lines from the cross TN 1/Ptb 33.

Reaction to ZLH	Reaction to WBPH			Total
	Resistant	Segregating	Susceptible	
Resistant	13	23	8	44
Segregating	20	43	17	80
Susceptible	4	19	7	30
Total	37	85	32	154

c^2 for independence of two traits = 4.27^{ns}^a

c^2 for 1:2:1:2:4:2:1:2:1 = 7.01^{ns}

^a ns = nonsignificant at the 5% level.

Table 11. Reaction to zigzag leafhopper and brown planthopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to ZLH	Reaction to BPH			Total
	Resistant	Segregating	Susceptible	
Resistant	16	23	0	39
Segregating	29	32	7	68
Susceptible	12	20	2	34
Total	57	75	9	141

c^2 for independence of two traits = 5.42^{ns}^a

c^2 for 7:8:1:14:16:2:7:8:1 = 6.93^{ns}

^a ns = nonsignificant at the 5% level.

independence of the two traits and the c^2 for the 7:8:1:14:16:2:7:8:1 ratio expected for the independent segregation of three genes in both crosses were nonsignificant. It appears, therefore, that both *Zlh-2* and *Zlh-3* are inherited independently of *bph-2* and *Bph-3*.

The two-way classification for the reactions of F₃ lines to ZLH and GLH in the cross TN 1/Ptb 21 also gave nonsignificant c^2 values for the independence of the two traits and for a 1:2:1:2:4:2:1:2:1 ratio (Table 13). These data suggest the independence of *Zlh-2* and the recessive gene for resistance to GLH in Ptb 21.

The reactions to WBPH and BPH of F₃ lines from the cross TN 1/Ptb 21 are presented in Table 14. The c^2 values calculated from the data show that *Wbph-3* also segregates independently of *bph-2* and *Bph-3*.

Table 12. Reaction to zigzag leafhopper and brown planthopper of F₃ lines from the cross TN 1/Ptb 33.

Reaction to ZLH	Reaction to BPH			
	Resistant	Segregating	Susceptible	Total
Resistant	18	21	2	41
Segregating	37	43	1	81
Susceptible	9	18	4	31
Total	64	82	7	153

c^2 for independence of two traits = 8.39^{ns}^a

c^2 for 7:8:1:14:16:2:7:8:1 = 8.69^{ns}

^ans = nonsignificant at the 5% level.

Table 13. Reaction to zigzag leafhopper and green leafhopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to ZLH	Reaction to GLH			
	Resistant	Segregating	Susceptible	Total
Resistant	12	18	9	39
Segregating	17	36	15	68
Susceptible	7	19	8	34
Total	36	73	32	141

c^2 for independence of two traits = 1.17^{ns}^a

c^2 for 1:2:1:2:4:2:1:2:1 = 2.19^{ns}

^ans = nonsignificant at the 5% level.

Similar results were obtained when the reactions of F₃ families to WBPH, BPH, and GLH in the cross were analyzed (Tables 15, 16). These analyses indicate that *Wbph-3*, *bph-2*, and *Bph-3* are also inherited independently of the recessive gene governing resistance to GLH in Ptb 21.

SUMMARY AND CONCLUSIONS

The mode of inheritance of resistance to ZLH, WBPH, BPH, and GLH in rice cultivars Rathu Heenati, Ptb 21, and Ptb 33 was studied. The results revealed that resistance to ZLH in these varieties is conditioned

Table 14. Reaction to whitebacked planthopper and brown planthopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to WBPH	Reaction to BPH			Total
	Resistant	Segregating	Susceptible	
Resistant	17	18	1	36
Segregating	31	40	3	74
Susceptible	9	17	5	31
Total	57	75	9	141

χ^2 for independence of two traits = 7.54^{ns}^a

χ^2 for 7:8:1:14:16:2:7:8:1 = 8.18^{ns}

^ans = nonsignificant at the 5% level.

Table 15. Reaction to whitebacked planthopper and green leafhopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to WBPH	Reaction to GLH			Total
	Resistant	Segregating	Susceptible	
Resistant	9	18	9	36
Segregating	20	38	16	74
Susceptible	7	17	7	31
Total	36	73	32	141

χ^2 for independence of two traits = 0.43^{ns}^a

χ^2 for 1:2:1:2:4:2:1:2:2:1 = 1.51^{ns}

^ans = nonsignificant at the 5% level.

by single dominant genes. Tests for allelism showed that these genes are nonallelic and segregate independently of each other. In accordance with the international rules on gene nomenclature (5), the genes for ZLH resistance in Rathu Heenati, Ptb 21, and Ptb 33 were designated *Zlh-1*, *Zlh-2*, and *Zlh-3*, respectively.

A single dominant gene was found to confer resistance to WBPH in each of the three cultivars. Allelism tests showed that these genes are allelic to *Wbph-3*.

The digenic control of resistance to BPH in Ptb 21 and Ptb 33 was reconfirmed in this study. The results also confirmed that a dominant and a recessive gene govern resistance in these varieties. GLH resistance

Table 16. Reaction to brown planthopper and green leafhopper of F₃ lines from the cross TN 1/Ptb 21.

Reaction to BPH	Reaction to GLH			
	Resistant	Segregating	Susceptible	Total
Resistant	19	27	11	57
Segregating	16	41	18	75
Susceptible	1	5	3	9
Total	36	73	32	141

χ^2 for independence of two traits = 3.81^{ns}^a

χ^2 for 7:8:1:14:16:2:7:8:1 = 4.73^{ns}

^ans = nonsignificant at the 5% level.

Table 17. Summary of information on the genes for resistance to planthoppers and leafhoppers in three cultivars of rice.

Cultivar	Genes for resistance			
	BPH	WBPH	ZLH	GLH
Rathu Heenati	<i>Bph-3</i>	<i>Wbph-3</i>	<i>Zlh-1</i>	none
Ptb 21	<i>bph-2</i> + <i>Bph-3</i>	<i>Wbph-3</i>	<i>Zlh-2</i>	1 recessive
Ptb 33	<i>bph-2</i> + <i>Bph-3</i>	<i>Wbph-3</i>	<i>Zlh-3</i>	none

in Ptb 21 was found to be conditioned by a recessive gene. The allelic relationship of this gene to *glh-4*, which conveys resistance to GLH in Ptb 8, however, is not known and therefore needs to be investigated.

Tests for independence of the various genes for resistance to planthoppers and leafhoppers in the test cultivars (Table 17) were also undertaken. Analyses showed that *Zlh-1*, *Zlh-2*, and *Zlh-3* are inherited independently of *Wph-3*. *Zlh-2* and *Zlh-3* also segregated independently of *bph-2* and *Bph-3*. *Zlh-2* was similarly found to be independent of the recessive gene governing resistance to GLH in Ptb 21.

Wbph-3 is also inherited independently of *bph-2* and *Bph-3*. These three genes segregated independently of the recessive gene for GLH resistance in Ptb 21.

The information obtained in this study is useful for rice breeders in the task of developing improved varieties with multiple resistance to rice planthoppers and leafhoppers. Any one of the newly identified genes for resistance to ZLH can serve as a source of resistance to this insect. Because various genes for resistance to ZLH, WBPH, BPH and GLH are independent, they can be combined in cultivars of improved agronomic background.

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DISCUSSION

SESSION 9: GENETICS OF INSECT RESISTANCE

Q – Oka: Do you think there are new genes for BPH resistance?

A – Ikeda: Yes, I think so. Khush et al recently identified *bph-5* and *Bph-6*. Accordingly, we need to make the allelism test of our new dominant resistance gene to *Bph-6* and of our new recessive resistance gene to *bph-5*.

Q – Siddiq: Several sources of resistance to various insect pests and their biotypes have been reported from the Assam rice collection. Geographically, this is a relatively small region located on the periphery of the center of the origin of cultivated rice. What could be the reason for the concentration of several resistance genes in this area? Could it be that the varieties here have never had the opportunity of being subjected to natural selection pressure against such stresses and now, because of artificial selection/screening pressure, we are able to find such genes in this collection?

A – Khush: Many sources of resistance to rice pests have been isolated from the southern states of India. Similar sources of resistance have been found in the Assam rice collection. There is no high insect pressure in the Assam region as in the southern states of India. However, it is possible that, in the past, the insect pressure in the Assam region may have been high.

Q – Chaudhary: To identify new genes, the simple checkerboard reaction of biotypes can be used. This technique, used in wheat, barley, etc., does not involve hybridization work. Can a rice geneticist use it?

A – Khush: For checkerboard studies, it is necessary to have all biotypes in one place. Since such collections have not been assembled at one place, it is not possible to conduct checkerboard studies.

Q – Dalmacio: What could be the reason why some cultivars showed differential reactions to whitebacked planthopper in different places, e.g., resistance at IRRI but susceptibility in India?

A – Chelliah: The reason was probable difference in biotype.

GENETICS OF QUANTITATIVE TRAITS

SESSION 10

DETECTION OF ADDITIVE AND NONADDITIVE VARIATION IN RICE*

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A modified triple test cross analysis was done on the results of two experiments involving 11 quantitative traits in rice, viz., heading date, maturity date, culm height, plant height, panicle length, tiller number, straw weight, panicle weight, spikelet number, 1,000-grain weight, and grain yield. Epistasis did not affect any of the traits studied, while additive gene action was predominant in both experiments for all traits. Dominance was not detected for heading date, maturity date, culm height, panicle length, plant height, tiller number, or straw weight in Experiment 1, nor for tiller number and straw weight in Experiment 2. Dominance contributed to the variation of heading date, maturity date, culm height, panicle length, plant height, panicle weight, spikelet number, 1,000-grain weight, and grain yield in Experiment 2.

Determination of gene action on quantitative traits has been done in rice using diallel methods (1, 2, 7, 12). Shaalan and Aly (12) observed that additive gene effects were significant for grain yield in the cross Giza 159/IR8, although in another cross dominance and epistatic gene action were significant for grain yield. Li (7) concluded that both additive and dominance inheritance were important in determining higher panicle number, heavier panicles, spikelets/panicle, and panicle length.

Knowledge of the genetic system controlling quantitative traits is important for devising an efficient selection program. Through the use of an appropriate mating design, estimates of additive and nonadditive components of genetic variation could be worked out. Kearsey and Jinks (5) suggested a triple test cross method that has general validity for investigating any kind of population, irrespective of both gene frequencies or mating system. This design has several advantages over other multiple mating designs. It provides a test for epistasis and dominance as long as the testers (denoted as L_1 and L_2) are extreme selections from the population. It also provides an estimate of additive and dominance

variation with equal precision. Since each male parent is crossed to three testers only, it allows one to investigate a larger sample from the population for the same experimental effort. The three testers are inbred lines or F_1 and hence can be replicated many times, thus avoiding the necessity of multiple matings to the same female.

Jinks et al (4) described a simplified version of the triple test cross in which the L_3 families or hybrid tester are replaced by P families (the selfed population that was under test). Jinks and Perkins (3) proposed a modification of the analysis of Kearsey and Jinks (5) using F_2 and backcross populations derived from two crosses between inbred varieties of *Nicotiana rustica*. Pooni et al (11) suggest that for plant species where cross pollination is difficult and self pollination is the norm, the families of the rather demanding triple test cross design be replaced by their progeny families produced from selfing. Ketata et al (6) suggested a similar model in which the testers L_1 , L_2 , and L_3 are crossed to a number of varieties instead of random F_2 individuals as suggested by Kearsey and Jinks.

In the present study, the detection of epistasis, additive, and dominance components was carried out using the method of Kearsey and Jinks (5) as modified by Perkins and Jinks (9) for their population of inbred lines. The main objective was to investigate whether epistasis was involved in determining the inheritance of 11 agronomic traits in rice and, in its absence, to detect the extent of additive and dominance variance.

MATERIALS AND METHODS

The study consisted of two experiments, the first carried out during the main rice season and the second in the off season. In Experiment 1, two inbred lines, Y460 and RU-1378, were designated tester $L_{1,1}$ and $L_{1,2}$, respectively. The hybrid of the cross Y460/RU-1378 was designated tester $L_{1,3}$. These three testers were then crossed to ten other inbred lines. In Experiment 2, two other inbreds, RU-1318-17-2-1 and RU-1315-33-2-4, were chosen as tester $L_{2,1}$, and $L_{2,2}$, respectively. Their F_1 hybrid became tester $L_{2,3}$. These three testers were also crossed with the 10 previous inbred lines. Each experiment consisted of 12 inbred lines (2 testers and 10 inbred lines), 20 single crosses, and 11 three-way crosses. The parental materials used as testers are presented in Table 1 and the characteristics of the 10 inbred lines in Table 2.

Each experiment was arranged in three randomized complete blocks

Table 1. Parental materials used as testers.

Source	Pedigree	Tester
<i>Experiment 1</i>		
Y 460	MR 13/MR 16	$L_{1,1}$
RU 1378	IRB/Sigadis/T(N) 1//Tjina /// SM 2	$L_{1,2}$
F_1 (Y 460/RU 1378)		$L_{1,3}$
<i>Experiment 2</i>		
RU 1318-17-2-1	Mahsuri/Pongsu Seribu Dua/Mahsuri	$L_{2,1}$
RU 1315-33-2-4	MR 1/Pongsu Seribu Dua/MR 1	$L_{2,2}$
F_1 (RU 1318-17-2-1/RU 1315-33)		$L_{2,3}$

with one plot/entry per replicate. Each plot consisted of 8 x 8 plants at a spacing of 25 cm between and within plots.

The data collected were as follows: heading date, maturity date, culm height, plant height, panicle length, tiller number, straw weight, panicle weight, spikelet number, 1,000-grain weight, and grain yield.

For the detection of epistasis, the method adopted by Ketata et al (6) was employed. Where epistasis was not significant, an additive-dominance model was fitted as outlined by Kearsey and Jinks (5) and Jinks et al (4). This method employs a set of cultivars crossed to three testers (two inbred lines and the F_1 hybrid of the two inbred lines). The various phenotypes were described by the following model:

$$L_{ijk} = \mu + G_{ij} + r_k + e_{ijk}$$

where L_{ijk} denotes the phenotypic value in replication k of the cross between the tester L_1 and cultivar j , μ denotes the mean of all single and three-way crosses, G_{ij} is the genotypic value of the cross between tester L_1 and cultivar j , r_k represents the effect of replication k , and e_{ijk} is the error associated with that particular cross in replication k . It then follows that

$$L_{1jk} + L_{2jk} - 2L_{3jk} = G_{1j} + G_{2j} + 2G_{3j} + (e_{1jk} + e_{2jk} - 2e_{3jk}).$$

Deviations $L_{1jk} + L_{2jk} - 2L_{3jk}$ and their means over replications (de-

Table 2. Characteristics of parental lines selected for crossing with testers.

Parental line	Drought score	Tillering	Stature	Time of flowering	1,000-grain weight
IR442-2-58	resistant	medium to low	short	early	heavy
Pongso Seribu Dua	moderately resistant	medium to low	tall	late	medium
Pulut Hitam Siam	moderately susceptible	low	tall	late	heavy
MR 1	susceptible	medium	medium	medium	heavy
H-8	susceptible	medium to high	tall	medium	medium
Y 353	susceptible	medium	tall	medium	medium
Ria	moderately resistant	medium to low	short	early	heavy
IR20	susceptible	high	medium	medium	medium
T(N)1	susceptible	medium	short	early	heavy
IR36	susceptible	high	short	early	heavy

noted $L_{1j} + L_{2j} - 2L_{3jk}$) were computed for each cultivar. The variation of the deviations $L_{1jk} + L_{2jk} - 2L_{3jk}$ was also computed and pooled over cultivars to provide an error term. For the j th cultivar and for any number of loci, the expectation of $G_{1j} + G_{2j} - 2G_{3j}$ depends on epistatic gene effects because of the cancelling of additive and dominance effects involved in the expression. It follows that if the mean squares for the deviations $L_{1j} + L_{2j} - 2L_{3j}$ are significantly greater than the pooled error, as evaluated by an F-test, evidence of epistasis is indicated. The F-test indicates whether cultivars differed in their contribution to the expression of epistasis for a particular trait.

The linear correlation coefficient ($r_{s,d}$) between the sum ($L_{1j} + L_{2j}$) and the corresponding difference ($L_{1j} - L_{2j}$) for all cultivars determined the direction of dominance. Positive and negative correlations would indicate a predominant direction of dominance towards decreasing and increasing values of the trait, respectively (4).

RESULTS AND DISCUSSION

Test for epistasis

The mean square values for epistatic deviation of the 11 traits in Experiments 1 and 2 are given in Table 3. The variance due to epistasis in both experiments is nonsignificant for all traits studied, implying that epistasis was absent in the crosses and the parents. These results are in agreement with those found by Nanda et al (8) working with the triple test cross in wheat. They postulated that the testers may have some common loci that do not segregate in the population. In the present study, the two testers $L_{1,1}$ and $L_{1,2}$ were significantly different ($P \leq 0.01$) for culm height, panicle length, and plant height in Experiment 1, while in Experiment 2, the two testers $L_{2,1}$ and $L_{2,2}$ differed significantly ($P \leq 0.01$) for heading date, culm height, plant height, and ($P \leq 0.05$) for straw weight. In spite of these differences there is still a possibility that the testers may not be divergent enough to enable detection of epistasis by this method. As observed in Table 1, some common parentage of testers $L_{2,1}$ and $L_{2,2}$ is evident. Pooni and Jinks (10) suggested that the failure to detect nonallelic interaction for certain traits was probably due to the size of the experiments, and suggested 200 individuals as the minimum requirement. However, the present study is well within the optimum requirement for an efficient detection of epistasis in a modified triple test cross, and this factor can therefore be ruled out.

Table 3. Mean square values for epistatic deviation for the 11 traits of Experiments 1 and 2.^a

Source	df	Expt. no.	Heading date	Maturity date	Culm height	Panicle length	Plant height	Tiller no.	Straw wt.	Panicle wt.	Spikelet no.	1,000-grain wt.	Grain yield
Cultivars	9	1	172.1ns	168.4ns	127.3ns	2.3ns	111.7ns	15.5ns	37454.3ns	475.1ns	2610.0ns	10.0ns	316643.0ns
		2	61.0ns	2483.5ns	221.3ns	8.5ns	221.6ns	42.9ns	12754.5ns	91.1ns	4051.3ns	8.9ns	198253.0ns
Error (sum of mean square of all lines)	20	1	587.0	2535.7	635.8	41.0	680.6	98.1	73026.9	1114.3	33109.4	25.0	5285407.0
		2	166.3	6623.7	1393.2	65.7	957.7	406.2	55530.7	659.3	28343.9	33.8	1610820.0

^a ns = nonsignificant.

Additive dominance model

In the absence of epistasis, analysis of variance for sums ($L_{1i} + L_{2i}$) and differences ($L_{1i} - L_{2i}$) provides a direct means of estimating the additive and dominance components, respectively (Tables 4 and 5). These estimates may be confounded with location effects, since the experiments were conducted at only one location. In other environments or with other sets of lines, the traits studied may show epistatic effects.

In both experiments, additive gene effects accounted for a significant amount of the genetic variation (Tables 4, 5) in all traits analyzed. The magnitude of additive variance was generally higher in Experiment 1 than in Experiment 2. For Experiment 1, the inheritance of all traits, with the exception of panicle weight, 1,000-grain weight, and grain yield, appeared to be controlled by additive genes. However, according to Kearsy and Jinks (5), nonsignificance of variance does not necessarily indicate absence of dominance. Some measure of dominance (H_1/D)^{1/2} was observed: this ranged from 0.04 for culm height to 0.59 for grain yield in Experiment 1. Only maturity date and tiller number were free from dominance. The preceding two traits are agronomically important in rice, and the absence of nonadditive effects would facilitate breeding, since they could be subjected to standard selection procedures. This is particularly true for tiller number in these materials, since even in Experiment 2 only additive variance was significant. However, additive and dominance gene effects are observed for maturity date in Experiment 2, suggesting that nonadditive factors are not totally irrelevant in the inheritance of this character.

Dominance contributed to the variation of heading date, maturity date, culm height, panicle length, plant height, panicle weight, spikelet number, 1,000-grain weight, and grain yield in Experiment 2. Partial dominance, at varying levels, coefficients ($r_{s,d}$) were only significant for maturity date ($r_{s,d} = 0.97$, significant at 0.01), 1,000-grain weight ($r_{s,d} = 0.61$, significant at 0.05), and grain yield ($r_{s,d} = 0.79$, significant at 0.01). As observed earlier, positive correlations indicate a predominant direction of dominance towards the decreasing value of each trait, and vice-versa.

With respect to the additive and dominance components, the two experiments gave consistent results for the following traits: tiller number, straw weight, panicle weight, 1,000-grain weight and grain yield, and contrasting results for the remaining traits. As pointed out by Ketata et al (6), to obtain more reliable estimates of genetic variance components, the

Table 4. Analysis of variance to detect additive and dominant components for the 12 characters in Experiments 1 and 2.^a

	Analysis of sums				Analysis of differences			
	Replicates		Sums (additive component)		Replicates		Differences (dominance component)	
	<i>Expt 1</i>	<i>Expt 2</i>	<i>Expt 1</i>	<i>Expt 2</i>	<i>Expt 1</i>	<i>Expt 2</i>	<i>Expt 1</i>	<i>Expt 2</i>
df	2	2	9	9	2	2	9	9
Heading date	77.2	36.1	215.9**	207.1**	112.2	4.9	60.2ns	55.3*
Maturity date	134.5	425.8	379.0**	2953.7**	25.2	704.4	59.8ns	2123.5**
Culm height	45.0	11.3	5095.5**	2938.6**	16.2	184.5	21.9ns	156.8**
Panicle length	0.2	5.8	41.6**	40.5**	0.8	2.7	3.0ns	5.6**
Plant height	46.0	3.2	59314**	3462.6**	17.3	62.3	32.1ns	213.9**
Tiller number	10.1	2.8	23.3**	21.2**	1.4	2.6	4.0ns	3.0ns
Straw weight	13147.6	1307.6	64674.8**	34279.6**	1499.6	62.6	4903.8ns	2877.1ns
Panicle weight	59.3	12.1	483.9**	298.2**	6.2	3.6	151.1*	95.8*
Spikelet number	2365.1	300.3	14734.6**	11617.8**	636.4	240.4	1518.5ns	4348.1**
1,000-grain wt.	1.8	0.5	79.9**	57.2*	0.1	1.1	2.4**	7.3**
Yield	521278.6	17768.5	1034930.6**	895567.7**	15619.9	43612.8	416267.8**	409618.7**

^a* = significant at the 5% level, ** = significant at the 1% level. ns = nonsignificant.

Table 5. Estimates of additive (D) and dominance (H_1) variance components, and degree of dominance ($(H_1/D)^{1/2}$) for traits not showing significant epistasis.^a

	Experiment 1			Experiment 2		
	D	H_1	$(H_1/D)^{1/2}$	D	H_1	$(H_1/D)^{1/2}$
Heading date	218.0*	16.5	0.08	261.6**	51.5**	0.44
Maturity date	418.7**	-17.5+	-	3153.8**	2016.4**	0.80
Culm height	6969.4**	9.6	0.04	3880.8**	154.3**	0.20
Panicle length	53.9**	2.3	0.21	51.1**	5.9**	0.34
Plant height	7884.4**	21.4	0.05	4588.9**	251.1**	0.23
Tiller no.	26.0**	-0.7+	-	24.0**	-0.2+	-
Straw weight	80534.1**	3763.3	0.22	13962.6**	1803.6	0.20
Panicle weight	559.8**	125.1**	0.47	253.2**	87.1*	0.50
Spikelet no.	18248.4**	973.0	0.23	12235.0**	4240.9**	0.59
1,000-grain wt.	105.6**	2.5**	0.15	74.3	8.2**	0.33
Grain yield	1317848.4**	463132.3**	0.59	1120701.5**	513348.4**	0.68

^a* = significant at the 5% level, ** = significant at the 1% level. + = negative values that may be interpreted as zero.

testers L_1 and L_2 should comprise high vs. low selections for the trait under consideration, even though this would be difficult when many traits are being analyzed at the same time. Nevertheless, the above approach enables the plant breeder at least to investigate the type of gene action involved in agronomic traits important to his breeding strategy.

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GENETIC ARCHITECTURE OF ECONOMICALLY IMPORTANT CHARACTERS AND PREDICTION OF PERFORMANCE OF RECOMBINANT INBRED LINES IN RICE*

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It is possible to predict the properties of the recombinant inbred lines that can be extracted from an F_2 of a cross between two inbred lines with a high degree of accuracy, provided that an appropriate estimate of the additive genetic variance of the F_2 generation is available. Among several experimental designs that can be used to obtain an estimate of this genetic parameter, the triple test cross design is the best. The results of a completely randomized triple test cross experiment are presented, discussed, and used to predict the proportion of superior recombinant inbred lines that can be extracted from this cross with respect to four characters of economic interest. The results indicate that all four characters are controlled by genes with additive, dominance, and epistatic effects of the duplicate type; and that at least some of these genes are linked in the repulsion phase. The predictions of the proportions of inbred lines that can be extracted from this cross indicate that it should be fairly easy to obtain superior lines for three of these characters.

In order to exploit the variation in his source material as efficiently as possible, the breeder needs to have a knowledge of the genetic architecture of the characters he is seeking to improve. The experimental designs used by most breeders, however, rarely allow more than the detection of additive genetic variation and the estimation of heritability. The detection and estimation of the magnitudes of the full range of genetic effects — additive, dominance, and epistatic — require experimental designs that are more complex than those ordinarily used by breeders. The best design for this purpose is the triple test cross design, which allows the detection of all three genetic effects and, in the absence of epistasis, also provides independent estimates of the additive genetic and dominance components of variation that are of equal precision (3, 4). The first of two chief purposes of this paper is to present the results obtained from a triple

test cross analysis of an F_2 family derived from a cross between two varieties of rice with respect to four characters of interest to the breeder.

The chief method of exploiting the variation in the source material of self-pollinating crop plant species is the widely used pedigree method. While the use of this method has led to the introduction of a very large number of varieties, it suffers from the disadvantage that the breeder is usually unable to determine the likely success of any pedigree much before the F_5 or F_6 generation. It is necessary, therefore, to make a very large number of crosses in the hope that a few will ultimately turn out to yield recombinant inbred lines of commercial interest. This can hardly be regarded as a very efficient procedure, for the great majority of crosses are ultimately discarded. It would clearly be desirable, therefore, for the breeder to be able to recognize the most promising crosses at the F_2 or F_3 stage of each pedigree so that attention and effort could be concentrated on these at an earlier stage. This is now possible, for Jinks and Pooni (2,5) have described a method of predicting the mean and variance of the distribution of recombinant inbred lines that can be derived from the F_2 of a cross between two inbred parents by single seed descent. The second purpose of this paper is to apply this procedure to the cross whose properties have been investigated in the triple test cross experiment performed.

MATERIALS AND METHODS

The F_2 family used in the triple test cross experiment originated from a cross between varieties Bg 380-2 (P_1) and Bg 34-6 (P_2), which were bred at the Central Rice Breeding Station at Batalogoda. Both varieties are currently recommended for use in Sri Lanka, Bg 380-2 being a high yielding variety and Bg 34-6 a red pericarp one. This cross was chosen for investigation because it had been made by the breeders at Batalogoda in order to obtain a new high yielding red pericarp variety.

Each of a randomly chosen sample of 30 plants from this F_2 family was backcrossed to each of the parental varieties and also to their F_1 , giving a total of $30 \times 3 = 90$ crosses in all. Five plants were raised from the seed of each of these 90 crosses. In addition, for reasons that will presently be apparent, the experiment also included the families of the basic generations P_1 , P_2 , F_1 , F_2 , BC_1 (F_1/P_1), and BC_2 (F_1/P_2), 10 plants being raised from the seed of the nonsegregating families and 30 from that of the segregat-

ing ones. The experiment also included a random sample of 30 F_3 families, 5 plants being raised from the seed of each family. The total number of plants that were raised from all of the families that were derived from the cross was thus 720.

Because both the age and quality of seed can affect the performance of plants raised from it, all seed for this experiment was produced in the same round of crossing 2 months prior to starting the present experiment; and all seed except that used to raise the F_3 families was produced by hand-crossing or, in the case of the P_1 , P_2 , and F_2 material, by hand-selfing in an attempt to ensure that all seed was of a similar quality. The seed from each of the 126 crosses was germinated on moist filter paper in Petri dishes before being sown, 3 days later, in 11 cm clay pots, one seed being sown in each pot. Despite the fact that most of this seed (all except that used to raise the F_3 families) had been produced by hand, the rate of germination was close to 100%. After they had been sown, the pots were moved to a glasshouse, where they were arranged in a single, completely randomized block. Three weeks later, the experiment was transplanted into the field, where the plants were again arranged in a single, completely randomized block at a spacing of 40 cm \times 40 cm. This spacing, which is a little greater than that ordinarily used, was chosen to allow access to the plants during the course of the experiment without causing excessive disturbance to their root systems. The entire experiment was surrounded by three rows of guard plants of one of the parental varieties, Bg 34-6. The management of the experiment was similar to that of others at Batalagoda except that all weeding was done by hand in order to avoid any damage by herbicide. No plants were lost during the course of the experiment.

Though each of the 720 plants in the experiment was scored for 16 characters in all, we shall confine our attention to just 4 of these in the present paper, namely number of tillers, scored at maturity (NT); days to heading, reckoned from the date on which the experiment was sown (DH); number of full grains per panicle (GP); and number of panicles per plant (PP).

In addition to the material derived from the cross with which we are concerned in this paper, the experiment also included the triple test cross progenies, the basic generations, and the F_3 families of four other crosses, all of which were chosen for investigation because they were of current interest to the breeders at Batalagoda. A full account of this experiment will appear elsewhere.

Table 1. Mean performance of the parents and their F₁s and results of tests for heterosis.

Family mean	Character			
	NT	DH	GP	PP
\bar{P}_1	22.7±2.09	98.3±2.16	171.7±37.45	18.2±1.06
\bar{P}_2	20.5±1.25	85.7±1.42	123.5±32.43	16.6±0.49
\bar{F}_1	29.8±0.40	99.3±0.29	175.6±131.36	24.4±0.12
Heterosis ^a	***	ns	ns	***

^a*** = significant at the 0.5% level, ns = not significant.

RESULTS

Genetic architecture of characters

There are two points worth making about the performance of the parental varieties and their F₁ (Table 1). First, although Bg 380-2 (P₁) is consistently the highest scoring parent, the difference between the parents is generally small and insignificant except for the character DH, where this difference is highly significant. Second, while the performance of the F₁ is always superior to that of the higher scoring parent P₁, this heterosis is not significant for the characters DH and GP. For NT and the dependent character PP, on the other hand, heterosis is substantial, the F₁ mean being more than 30% higher than that of P₁.

For the triple test cross experiment, each of a random sample of F₂ plants, used as females, was backcrossed to P₁, P₂ and their F₁. Each of these F₂ plants was therefore the maternal parent of three families in the present experiment, referred to as L₁ (F₂/P₁), L₂ (F₂/P₂), and L₃ (F₂/F₁), so that in all we are concerned with 30 sets of such families. Three orthogonal comparisons may be made between the family means of each set:

$$\begin{aligned} C_1 &= \bar{L}_1 + \bar{L}_2 + \bar{L}_3 \\ C_2 &= \bar{L}_1 - \bar{L}_2 \\ C_3 &= \bar{L}_1 + \bar{L}_2 - 2\bar{L}_3 \end{aligned}$$

The variance of the C_1 comparison over the 30 sets of crosses detects and estimates the additive component of genetic variation (D) in the absence of epistasis. Similarly, the variance of C_2 over sets detects and estimates the dominance component of variation (H) in the absence of epistasis; and that of the C_3 comparison provides a general test for epistasis that can be partitioned into an item with 1 degree of freedom that detects additive \times additive interaction (I) and an item with 29 degrees of freedom (in the present case) that detects the combined effects of additive \times dominance (J) and dominance \times dominance (L) epistasis (1,3,4).

The results obtained from the analysis of the triple test cross progeny of the present experiment (Table 2) leave little doubt that P_1 and P_2 differ genetically, despite the fact that their means were similar for three out of the four characters scored, for there is clear evidence of genetic variation for all characters in these families. In terms of their genetic architecture, these characters appear to be controlled by genes with additive, dominance, and epistatic effects. Since the epistasis (I) item is not significant for any character, epistasis in these families appears to be confined to the J and L type of nonallelic interaction.

Estimates of the genetic (D and H) and the environmental (E) components of variation that can be obtained from the variance components of these triple test cross analyses are shown in Table 3. These estimates suggest that the narrow-sense heritability of three of these characters (DH, GP, and PP) is quite high and that dominance for all characters is an important source of variation, since their dominance ratios are close to unity — which, taken at face value, suggests that dominance is complete. However, in the presence of epistasis, the tests for additive and dominance variance in the triple test cross analysis of variance are no longer unambiguous tests of their respective sources of variance, for both contain an epistatic component. It follows, therefore, that estimates of D and H obtained from the additive and dominance mean squares of this analysis, and hence the estimates of heritability that are derived from them, are also biased. We thus need to consider the direction and magnitude of the bias on these estimates in order to obtain a better idea of the genetic architecture of these characters.

The best source of information about the question of bias on these estimates is that derived by fitting a model to the means of the basic generations (Table 4). Estimates of the genetic components of these means can be obtained by the weighted least squares procedure, and the adequacy of the model can be assessed by calculating a goodness of fit χ^2 , which tests for agreement between the observed and expected means (4). There are two conclusions of relevance to the question of bias on D and H

Table 2. Analyses of variance of the triple test cross data.

Comparison	Item	df	Character ^a			
			NT	DH	GP	PP
C ₁	additive	29	42.583**	239.594***	2623.234***	37.832***
	error	360	19.468	65.470	461.217	9.904
C ₂	dominance	29	37.852**	102.099**	2005.267**	26.301***
	error	240	18.532	57.222	493.872	11.238
C ₃	epistasis (I)	1	11.111 ns	507.751 ns	4151.654 ns	47.151 ns
	epistasis (J+L)	29	45.842***	177.002***	2608.604***	33.121***
	error	360	20.404	73.718	428.562	8.571

^aEntries are mean squares. ns = nonsignificance; **p = 0.01–0.001; ***p < 0.001. The expected mean squares (MS), variance components, and the genetic expectations of these variance components are:

Item	df	MS		EMS
additive	n-2	MS ₁	$\sigma_{w1}^2 + 3r\sigma_{sums}^2$	$\sigma_{sums}^2 = 1/8D$
dominance	n-1	MS ₃	$\sigma_{w2}^2 + 2r\sigma_{diffs}^2$	$\sigma_{w2}^2 = 1/8D + 1/8H + E$

Table 3. Estimates of the genetic components of variation (D and H) and the environmental component (E) that can be estimated from the variance components of the analysis of variance of the triple test cross progenies.

Estimate ^a	Character			
	NT	DH	GP	PP
D	12.33	92.87	1153.08	14.89
H	15.46	35.90	1209.12	12.05
E	15.06	41.12	198.60	7.87
(H/D) ^{1/2}	1.12	0.62	1.02	0.90
h^2_N (%)	25	48	54	41
h^2_B (%)	40	57	82	57

^a(H/D)^{1/2} = dominance ratio:

h^2_N = narrow heritability appropriate to an F₂ family.

h^2_B = broad heritability appropriate to an F₂ family.

that can be drawn from these estimates. First, there is no doubt that variation between these generation means is due in part to genes that display epistatic effects, for [l] is large and significantly different from zero for all characters. Furthermore, since [l] is always large and positive and [h] large and negative, this nonallelic interaction is of the duplicate type and is a property of genes whose net dominance is in the decreasing direction. Second, in contrast to [h] and [l], the estimates of [d], [i], and [j] are either small or not significantly different from zero. Now, the second group of parameters is sensitive to variation in the distribution of genes between the parents, whereas [h] and [l] are not (which is why estimates of these parameters have been used to infer the type of epistasis that is present in these data). In particular, estimates of [d], [i], and [j] are expected to be large only when genes of like effect are associated in the parents, i.e., when most or all of the genes of increasing effect enter the cross from one parent, those of decreasing effect entering from the other parent. Conversely, if genes of like effect are dispersed in the parents, [d], [i], and [j] are expected to be small or not significantly different from zero, even if the genetic effect they measure is large. Hence, the fact that the absolute magnitudes of [h] and [l] are much larger than those of [d], [i], and [j] strongly suggests that genes of like effect are dispersed in the parents of the present cross.

Table 4. Means of the basis generations (upper half) and estimates of the genetic components of these means (lower half).

Generation	Character ^a			
	NT	DH	GP	PP
P ₁	22.7±2.09	98.3±2.16	171.7± 37.45	18.2±1.06
P ₂	20.5±1.25	85.7±7.42	123.5± 32.43	16.6±0.49
F ₁	29.8±0.40	99.3±0.29	175.6±131.36	24.4±0.12
F ₂	20.6±1.25	87.5±3.57	129.6± 27.05	17.7±0.80
BC ₁	22.1±0.61	95.7±0.99	141.9± 19.15	17.8±0.34
BC ₂	18.7±0.22	81.6±0.35	117.7± 6.67	16.1±0.19
Estimate				
m	21.9	92.0	147.6	17.6
[d]	2.3	6.3	24.1	1.2
[h]	-14.8	-21.0	-99.4	-9.1
[i]				
[j]				
[l]	22.7	28.3	127.4	15.9
c ²	3.497	0.338	0.002	1.606
(df)	(2)	(1)	(2)	(2)

^aOnly those estimates that are significantly greater than zero are shown. The genetic model used is as follows:

$$\bar{P}_1 = m + [d]$$

$$\bar{P}_2 = m - [d]$$

$$\bar{F}_1 = m + [h] + [l]$$

$$\bar{F}_2 = m + \frac{1}{2}[h] + \frac{1}{4}[l]$$

$$\overline{BC}_1 = m + \frac{1}{2}[d] + \frac{1}{2}[h] + \frac{1}{4}[i] + \frac{1}{4}[j] + \frac{1}{4}[l]$$

$$\overline{BC}_2 = m - \frac{1}{2}[d] + \frac{1}{2}[h] + \frac{1}{4}[i] - \frac{1}{4}[j] - \frac{1}{4}[l]$$

Armed with this information about the type of epistasis that is present in these families and the distribution of genes between the parents, we can now return to the question of bias on the estimates of D and H that have been obtained from the variance components of the triple test cross analysis. The effect of the duplicate type of epistasis on the estimate of D is to deflate it relative to its value in the absence of epistasis; and its effect on H is to inflate it. Furthermore, there is good evidence that there is an additional source of bias in the present data that reinforces that caused by epistasis. Thus, while dispersion alone has no effect on the estimates of second degree statistics (e.g., D and H), linkage of dispersed genes does. The best test for linkage in the present experiment is that provided by a comparison between the grand means of the triple test cross families— \bar{L}_1 , \bar{L}_2 , and \bar{L}_3 —and those of the basic generations— \overline{BC}_1 , \overline{BC}_2 , and \bar{F}_2 (4). Thus, in general \bar{L}_1 is expected to be equal to \overline{BC}_1 sampling variation apart; similarly, $\bar{L}_3 = \overline{BC}_2$ and $\bar{L}_2 = \bar{F}_2$. These family means

Table 5. The results obtained by testing for equality between the means of the triple test cross families and those of the basic generations BC₁, BC₂, and F₂.

Test	Character ^a			
	NT	DH	GP	PP
$\bar{L}_1 - \bar{B}_1$	ns	*	ns	***
$\bar{L}_2 - \bar{B}_2$	***	ns	*	***
$\bar{L}_2 - \bar{F}_2$	*	ns	ns	***

^a n.s. = not significant,
 * = significant at the 5% level,
 *** = significant at the 0.5% level.

are not expected to be equal, however, when part of the variation of the character in question is determined by genes with epistatic properties that are linked in their inheritance (though neither linkage nor epistasis on their own disturb these equalities). Hence these equalities provide simple and sensitive tests for the presence of linked epistatic genes. Since there is clear evidence of epistasis in the present data, these tests provide an appropriate test for linkage.

The results obtained by testing these equalities (Table 5) leave little doubt about the presence of linkage for two of the characters (NT and PP). Though the evidence for the remaining two characters, DH and GP, is less convincing, the expectations of these tests in terms of a linked digenic interaction model are not the same; hence each comparison tests for a particular combination of products between the linkage and epistatic parameters, the summed effects of which are expected to vary from one test to another according to the relative magnitudes of *i*, *j*, and *l*. It is possible, therefore, that part of the variation for the characters DH and GP is also determined by linked epistatic genes, though the evidence in the case of this pair of characters is, of course, less compelling than that for the others.

Since the estimates of the parameters obtained from the basic generations suggest that for all characters genes of like effect are dispersed in the parents, this linkage must be predominantly in the repulsion phase. But linkage in the repulsion phase deflates *D* relative to its value in the absence of linkage and inflates *H*. Hence the effect of epistasis of the

duplicate type will be reinforced by that of linkage in the repulsion phase; their joint effect, therefore, is likely to cause a serious downward bias on D and a corresponding upward bias on H. This bias has two consequential effects on the other estimates shown in Table 3. First, though three of the estimates of the dominance ratio suggest that dominance is approximately complete (for characters NT, GP, and PP), this is unlikely to be the case, for estimates of this ratio will also be biased upwards; that is, it is likely that dominance is incomplete for all characters. Second, since D has been underestimated by \hat{D} , it follows that the estimates of heritability are also biased downwards.

The final point worth making about the genetic architecture of these characters concerns the genetic basis of the heterosis displayed by the F_1 generation for two of the characters under consideration (Table 1). Thus, the most likely cause of this heterosis is that it is due to dispersed dominant and interacting genes; or, to put this another way, that it is due to genes with dominance and epistatic properties that are in linkage disequilibrium.

Prediction of the properties of inbred lines

In the absence of epistasis, the mean (m) and genetic variance of the distribution (D) of the recombinant inbred lines that can be derived from an F_2 family by single seed descent are assumed to show a normal distribution. The proportion of these inbred lines whose means equal or exceed (or are equal to or less than) a desired value, x say, can then be found from

$$z = \frac{x-m}{\sqrt{D}}$$

where z is the one-tailed normal deviate. If $x = \bar{P}_1$ or \bar{P}_2 , $z = [d]/\sqrt{D}$; and if $x = \bar{F}_1$, $z = [h]/\sqrt{D}$ (2,5). In the presence of nonallelic interaction, however, this prediction equation is more complex, because, whereas the expected mean of the distribution of inbred lines is still m , the genetic variance of this distribution is now $D + I$, where $I = \sum i^2$. In addition, because in the presence of epistasis $\bar{P}_1 = m + [d] + [i]$, $\bar{P}_2 = m - [d] + [i]$, and $\bar{F}_1 = m + [h] + [l]$, the numerators of z become $[d] + [i]$, $-[d] + [i]$, and $[h] + [l]$, respectively. While estimates of m , $[d]$, $[h]$, and $[i]$ are available for each of the characters with which we are concerned (Table 4), we cannot estimate the quantity $D + I$. In practice, however, it has been found that the estimate of D obtained from a triple test cross analysis approximates $D + I$ more closely than that obtained, for example, from the basic generations.

The proportions of superior inbred lines that are expected for each of the characters in the present experiment are NT, 1%; DH, 26%; GP, 20%; and PP, 4%. In making these predictions, we have assumed that the breeder will be interested in greater expression for the characters NT, GP, and PP; we have accordingly calculated the proportion of lines that are expected to equal or exceed \bar{F}_1 . For DH, on the other hand, we have supposed that the breeder will be interested in early flowering and have therefore calculated the proportion of lines whose values are expected to be equal to or less than \bar{P}_2 . The proportions given above suggest that it should be relatively easy to obtain recombinant inbred lines from this cross that would head earlier than P_2 and produce more grains per panicle than the F_1 . On the other hand, it would be necessary to raise several hundred lines by single seed descent to be reasonably sure of obtaining at least some lines that produced more tillers and more panicles than the F_1 . In practice, however, it has nearly always been the case that, because of linkage in the repulsion phase, the observed proportion of desired lines exceeds the expected proportion. Since we have evidence of such linkage in the present data, it is likely that the above proportions underestimate the proportion of superior lines that will be derived from this cross in practice. It will obviously be a matter of some interest to test these predictions when the inbred lines from this cross are available.

DISCUSSION

While the triple test cross design is the best available for inferring the genetic architecture of characters in that it independently tests for additive, dominance, and epistatic effects and, in the absence of epistasis, is also capable of yielding independent estimates of D and H that are of equal precision, in practice it can be a rather demanding design. Thus, in the present experiment it was necessary to make 90 crosses in order to produce the triple test cross families and 6 more to produce the basic generations, making a total of 96 crosses in all. Since this experiment also included the families of four other pedigrees, it was necessary to make over 400 crosses by hand in order to produce the material required for analysis. With some species this would not be a particularly demanding task; but with rice it is possible to justify the investment of time and effort required for a crossing program of this size only in rather unusual circumstances. It is worth asking, therefore, whether it is possible to obtain the information that is required for the prediction of the properties

of recombinant inbred lines in a less demanding way.

In principle, it is possible to obtain approximate estimates of m and D from F_3 families that have been produced by allowing a random sample of F_2 plants to set seed by self-pollination in the usual way. Thus, in the absence of epistasis, the grand mean of a set of F_3 families produced in this way is

$$\bar{F}_3 = m + 1/4 [h]$$

and the expected variance components that can be estimated from the analysis of variance of these families are

$$s_b^2 = 1/2D + 1/6H \text{ and}$$

$$s_w^2 = 1/4D + 1/8H + E$$

where s_b^2 is the true variance of F_3 family means and s_w^2 the average variance within these families. While, with only two equations available for their estimation, it is clearly not possible to obtain estimates of D , H , and E , since the coefficient of H in s_b^2 is small; D can be estimated approximately from $2s_b^2$ and m from \bar{F}_3 by neglecting the term $1/4 [h]$.

Estimates of m and of D that have been obtained from the 30 F_3 families that were included in the present experiment and the predictions based on these estimates are shown in Table 6. The estimates of m shown in these tables are similar to those obtained from the basic generations (Table 4); and the estimates of D obtained from these F_3 families are also similar to those obtained from the triple test cross families (Table 3). In consequence, the rank order of the proportions shown in Table 6 (DH > GP > PP > NT) is the same as that calculated in the previous section. While agreement between the F_3 and triple test cross predictions is very good for the characters NGP and NPP, it is less close for NT and DH. Thus, the prediction based on the F_3 estimates suggests that it is likely to be more difficult to obtain a recombinant inbred line of the desired level of performance for the character NT than the prediction based on the triple test cross and basic generation estimates (1%); conversely, the F_3 prediction suggests that it should be even easier to obtain an early flowering line than the triple test cross prediction (26%). Nevertheless, taken as a whole, it is clear that there is reasonable agreement between these two sets of predictions.

In self-pollinating species like rice it is obviously easier and hence less costly to raise F_3 than triple test cross families. Indeed, since F_3 families are raised in conventional breeding programs anyway, it would require

Table 6. Estimates of m and D obtained from F_3 families and the proportion of inbred lines that can be extracted from the F_3 generation of the cross between Bg 380-2 and Bg 34.6, calculated from these estimates.

	Character ^a			
	NT	DH	GP	PP
m	22.2	83.9	137.4	19.7
D	5.94	114.15	1507.33	5.73
Prediction	0.09%	57%	16%	2.5%

^a For NT, GP, and PP, the proportion shown is that which is expected to equal or exceed F_1 ; For DH, the proportion indicated is that expected to be equal to or less than P_2 .

only a small modification of the standard procedure to produce the information necessary for predicting the proportion of inbred lines that could be derived from any cross. Indeed, it is not even necessary to raise the F_3 families in a completely randomized experiment, for any of a number of experimental designs could be used for this purpose provided that they yield unbiased estimates of the true variance between family means σ_b^2 and m . However, while F_3 families may be adequate for this purpose, they are not capable of providing much information about the genetic architecture of characters of interest; if information of this kind is also required, it will be necessary to raise other families, among which those of the triple test cross design are the most suitable.

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INHERITANCE OF SOME QUANTITATIVE CHARACTERS INCLUDING HETEROSIS IN RICE BY COMBINING ABILITY ANALYSIS

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Twelve early varieties—nine semidwarfs plus three traditional tall— and all their possible F_1 's without reciprocals were analyzed for combining ability in 30 different characters. The study also included heterosis in grain yield and traits influencing yield. Both general combining ability (GCA) and specific combining ability (SCA) were highly significant in almost all the characters. Genes with additive action were predominant in the inheritance of 21 traits, including such important ones as days to flowering, number of spikelets, and grains/panicle. On the other hand spikelet fertility and harvest index showed a predominance of genes with nonadditive effects. Characters like biological yield and grain yield showed more or less equal importance for both types of gene action. With regard to grain yield and some of its components, varieties OR79-21, IR36, Blackgora, and CR143-2-2 were found to be good general combiners, in that order. Heterosis over midparent in grain yield varied greatly and was closely associated with heterosis in biological yield and to a lesser extent harvest index. The four parents showing high GCA also exhibited high variety heterosis, thus indicating the importance of genes with additive action in heterosis. The significance of SCA in some of the heterotic hybrids showed the importance of nonadditive gene action. However, heterotic hybrids appeared more frequently in crosses involving parents with high x low (HL) rather than HH or LL for GCA, thus indicating the importance of genetic diversity in GCA effects for realizing heterosis.

In recent years there has been increased emphasis on developing early maturing rice varieties for irrigated lands in the wet as well as dry seasons. Further breeding projects have been initiated at a number of centers to evolve early maturing hybrids for irrigated rice. This study was carried out to assess the genetic potential of a number of early rice cultivars to produce high yielding recombinants in the homozygous condition or highly heterotic hybrids.

Table 1. Rice cultivars and breeding lines used in the study.

No.	Parental line	Origin	Plant height	Maturity	Grain type ^a
1.	Annapurna	Ptb 10/TN 1	short	early	MB
2.	Parijat	TN 1/TKM 6	short	early	MS
3.	Suphala	T 141/TN 1	short	early	SS
4.	Blackgora	Traditional upland rice, Bihar, India	tall	early	MB
5.	Kalakeri	Traditional upland rice, Orissa, India	tall	early	MB
6.	CR143-2-2	N 22/TN 1//Lalnakanda	short	mid-early	SB
7.	OR165-18-8	Parijat///N 22/TN 1//T90/IR8	short	ultra-early	MS
8.	OR79-21	T 90/IR8//W 1263	short	mid-early	SB
9.	Keshari	T 90/IR8//Jagannath	short	early	SS
10.	N 22	Traditional upland rice, UP, India	tall	early	SB
11.	IET 1444	TN 1/Co 29	short	mid-early	SB
12.	IR36	IR1561-228// ⁴ IR24/ <i>O. nivara</i> ///CR94-13	short	mid-early	LS

^aMB = medium bold; MS = medium slender; SS = short slender; SB = short bold; LS = long slender.

MATERIALS AND METHODS

Twelve rice varieties—three traditional upland cultivars, six modern short cultivars, and three short breeding lines, were crossed in all possible combinations without reciprocals to produce 66 hybrids (Table 1). The hybrids and the parents—constituting a 12×12 half-diallel set—were raised under field conditions in a randomized complete block design with three replications in the wet season of 1982 at the Rice Research Station, Bhubaneswar, India. Each plot consisted of one row of 12 plants with spacing of 40×20 cm. Observations were recorded on 10 sample plants for 30 quantitative traits (Table 2). The sample means were used for combining ability analysis following Method II and Model I of Griffing (4). The variance ratio $2s^2_{gca}/(2s^2_{gca} + s^2_{sca})$ was computed from the expected components of the mean square assuming a fixed model to assess the relative importance of additive and nonadditive gene effects in predicting progeny performance (1). Analysis of heterotic effects was carried out after Gardner and Eberhart (3). Heterosis was measured as the percentage increase of the F_1 over the midparent.

Table 2. Analysis of variance for combining ability effects in 31 characters in a 12 x 12 diallel set of crosses in rice by Griffing's Method II.

Characters	Mean sum of squares (df) ^a			Predictability factor $\left(\frac{2 s^2_{gca}}{2 s^2_{gca} + s^2_{sca}} \right)$
	GCA (11)	SCA (66)	Error (154)	
<i>Plant traits</i>				
Days to heading	112.382**	6.425**	0.493	0.73
Plant height (cm)	2751.455**	119.847**	0.377	0.77
Flag leaf length (cm)	125.960**	5.863**	1.587	0.81
Flag leaf breadth (cm)	0.275**	0.010**	0.002	0.84
Flag leaf area(cm ²)	1089.923**	46.893**	9.172	0.80
Penultimate leaf length (cm)	197.888**	7.241**	3.427	0.88
Penultimate leaf breadth (cm)	0.180**	0.010**	0.003	0.79
Penultimate leaf area (cm ²)	1123.532**	51.334**	10.507	0.80
<i>Panicle traits</i>				
Panicle length (cm)	14.450**	1.281**	0.180	0.65
Panicle exertion (cm)	16.521**	1.986**	0.294	0.58
Panicle number	25.494**	2.539**	1.257	0.57
Spikelets/panicle	5748.162**	194.223**	51.919	0.85
Grains/panicle	5536.356**	364.946**	52.044	0.71
Panicle density	7.642**	0.241**	0.088	0.87
Spikelet fertility	310.459**	129.679**	3.998	0.26
<i>Grain traits</i>				
1000-grain weight (g)	69.191**	1.028**	0.291	0.93
1000-kernel weight (g)	45.912**	0.752**	0.223	0.91
Grain length (mm)	1.219**	0.039**	0.013	0.86
Kernel length (mm)	0.655**	0.022**	0.007	0.85
Grain breadth (mm)	0.289**	0.004 ^{ns}	0.003	0.98
Kernel breadth (mm)	0.194**	0.004 ^{ns}	0.002	0.93
Grains L/B ratio	0.252**	0.008**	0.005	0.91
Kernel L/B ratio	0.207**	0.006**	0.003	0.89
Grain thickness (mm)	0.059**	0.003**	0.001	0.87
Kernel thickness (mm)	0.044	0.002**	0.001	0.87
<i>Yield</i>				
Biological yield (g/plant)	951.432**	112.525**	31.837	0.62
Straw yield (g/plant)	264.359**	37.516**	9.950	0.57
Grain yield (g/plant)	303.586**	50.948**	8.168	0.50
Panicle yield (g/plant)	298.547**	67.948**	9.706	0.41
Harvest index	0.010**	0.004**	0.0003	0.27

^a** = significant at the 1% level; ns = not significant.

RESULTS

Combining ability effects

Both general combining ability (GCA) and specific combining ability (SCA) effects were highly significant for the characters studied with the exception of grain and kernel length, in which SCA effects were not significant (Table 2). Thus there was considerable genetic variability in the population in spite of the fact that 9 out of the 12 cultivars were semidwarfs, having in common the same dwarfing gene as AGWG. This further shows that sustained breeding efforts at different centers during the past two decades considerably broadened the genetic base of the semidwarf cultivars. The general predictability factor was high (above 0.70) in all 8 plant traits, all 10 grain traits, and 3 out of 7 panicle traits, thus indicating that additive gene action was more important than nonadditive gene action in the inheritance of 21 out of 30 characters included in the present study. In spikelet fertility and harvest index nonadditive gene effects appeared to be more important than additive gene effects. In the remaining seven characters—biological yield, straw yield, panicle yield, grain yield, panicle length, panicle exertion, and panicle number—the predictability factors were about 0.50, thus showing the more or less equal importance of both types of gene action in the inheritance of these traits.

It is further seen that related traits such as length, breadth, and area of flag leaves versus penultimate leaves; different features of grains versus kernels; and yields of total biomass, straw, panicle, and grain behaved more or less similarly regarding the relative importance of **GCA** and **SCA** effects. In the present study, correlation of grain yield with some of the traits influencing it was found highly significant with the exception of panicle number, where the correlation coefficient (-0.181) was not significant. The magnitudes of the other correlation coefficients were: 0.843 (biological yield), 0.778 (grains/panicle), 0.540 (spikelets/panicle), 0.444 (harvest index), and 0.258 (1000-grain weight). Subsequent analyses were limited to grain yield and the traits influencing it, with the exception of spikelets/panicle, whose behavior was very similar to grains/panicle.

The merits of the parents were evaluated on the basis of GCA effects (Table 3). OR79-21 was the top general combiner for grain yield, number of grains/panicle, and biological yield and second best in harvest index, but a low general combiner for panicle number and 1000-grain weight. Next in order of merit were IR36, Blackgora, OR165-18-18, and

CR143-2-2, showing positive and significant GCA effects in four, three, three, and two out of six characters, respectively. It is further seen that IR36 was the top general combiner in panicle number, Kalakeri in 1000-grain weight, and OR165-18-8 in harvest index. As expected, the GCA effects were in close agreement with the mean performances of the parents (Table 3).

SCA effects were studied in 10 top yielding hybrids for grain yield, biological yield, grains/panicle, and harvest index (Table 4). The best parent (OR79-21) showed a mean grain yield of 44.2 g/plant and the standard parent (Annapurna) 41.7 g/plant (Table 3). Thus the 10 hybrids were highly heterotic for grain yield and also for some other traits, particularly biological yield. The hybrids in general involve parents with high general combining ability: OR79-21 in five hybrids, IR36 and CR143-2-2 in three each, and Blackgora in two. SCA effects were significant and of high magnitude in seven hybrids in each of grain yield and biological yield; in four for grains/panicle; and in two for harvest index. This indicates the importance of nonadditive gene action for heterosis. The relationship between SCA effects, hence heterosis of the hybrids, and GCA effects of the parents involved in the cross was studied by scoring parents as high general combiners (H) when GCA effects were positive and significant, and otherwise as low combiners (L). Out of total of 40 SCA estimates (4 traits in 10 hybrids), 20 estimates were significant and more than zero, and out of these 20 significant SCAs, 16 were associated with HL, 3 with LL, and only one with HH parental combinations for GCA. In the remaining 20 estimates of SCA effects that were nonsignificant, 6 were associated with HH, 9 with HL, and five with LL parental combinations for GCA. This has been studied further under heterosis.

Heterosis

Heterosis was computed as the percentage increase in the F_1 over the midparent value, while overall heterosis of F_1 's in an array was computed as the percentage increase of the F_1 mean over the mean of all the parents. Heterosis in grain yield varied from -48.7% in Parijat/Kalakeri to 72.6% in CR143-2-2/OR79-21. Overall heterosis was high in the OR79-21 array, followed by the Blackgora, CR143-2-2, and IR36 arrays (Table 5). Thus, parents showing high GCA produced heterotic hybrids more frequently (Tables 3,5). The association between GCA effects and heterosis is further attested by the occurrence of low overall heterosis in the hybrids involving low general combiners like Parijat, Suphala, Kalakeri,

Table 3. Mean performance of the parents and their GCA effects for grain yield and five traits influencing yield in rice.^a

Parent	Grain yield (g)		Biological yield (g)		Harvest index		Panicle number		Grains/panicle		1000-grain weight (g)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Annapurna	41.7	0.13	69.1	0.29	0.60	-0.003	18.8	0.29	137.5	-5.97	24.6	0.75**
Parijat	32.1	-5.79**	60.2	-8.13**	0.53	-0.020	23.7	2.26**	88.7	-26.80**	20.4	-0.95**
Suphala	29.8	-5.80**	49.4	-12.36**	0.60	0.011	19.7	1.09**	137.8	0.07	17.0	-4.08**
Blackgora	36.3	4.99**	74.5	10.63**	0.49	0.008	15.9	-1.14**	109.9	-0.17	27.5	3.41**
Kalakeri	38.5	-2.93**	72.6	3.13	0.53	-0.064**	16.3	-0.33**	104.3	-27.42**	29.6	4.11**
CRI43-2-2	28.8	2.28*	60.8	3.04	0.47	0.004	12.5	-2.42**	146.3	19.27**	21.5	0.01
OR165-18-8	27.5	-2.39*	47.0	-10.01**	0.58	0.047**	20.1	0.77**	87.6	-18.34**	25.0	0.96**
OR79-21	44.2	10.04**	82.4	-14.63**	0.54	0.024**	14.5	-1.09**	207.2	45.50**	21.7	-0.37**
Keshari	32.3	-3.75**	53.4	-7.57**	0.61	0.012	17.0	-0.63**	151.6	3.85	17.6	-2.49**
N 22	29.1	0.44	56.3	1.04	0.51	-0.001	15.6	-0.87**	140.6	9.80**	20.1	-0.84**
IET1444	34.7	0.61	64.9	0.43	0.53	-0.012	17.9	0.21**	133.7	-2.22	23.2	-0.12**
IR36	36.9	3.39**	72.2	4.89**	0.51	0.010*	20.1	1.85**	131.9	2.46	20.8	-0.41**
Mean	34.3		63.6		0.54		17.7		131.4		22.3	
Standard error	1.5	0.53	3.2	1.44	0.01	0.005	0.9	0.08	9.3	3.41	1.1	0.02

^a* = significant at the 5% level, ** = significant at the 1% level.

Table 4. Mean performance, SCA effects, and high (H) and low (L) ratings for GCA effects in four characters in 10 top yielding hybrids.^a

Cross ^b	Grain yield			Biological yield			Grains/panicle			Harvest index		
	Mean	SCA	GCA rank	Mean	SCA	GCA rank	Mean	SCA	GCA rank	Mean	SCA	GCA rank
1/8	58.9	8.79**	LH	104.7	13.13**	LH	190.6	22.18**	LH	0.56	0.016	LH
3/8	51.2	6.99*	LH	90.8	12.27**	LH	192.9	19.97**	LH	0.56	0.002	LH
4/11	52.3	8.04**	HL	104.5	17.19**	HL	150.1	24.16**	LL	0.50	0.003	LL
4/12	50.4	2.13	HH	91.9	0.14	HH	127.6	-3.01	LL	0.57	0.022	LL
5/8	56.3	9.21**	LH	99.7	5.78	LH	142.0	-4.47	LH	0.57	0.081**	LH
6/8	63.0	10.77**	HH	111.3	19.37**	LH	199.2	6.07	HH	0.56	0.016	LH
6/11	54.0	12.37**	HL	96.4	16.68**	LL	148.8	3.38	HL	0.56	0.045*	LL
6/12	50.2	4.63	HH	94.4	10.23**	LH	147.8	-2.19	HL	0.54	-0.004	LL
8/12	58.9	5.51	HH	100.6	4.21	HH	200.6	24.32**	HL	0.59	0.027	HL
10/12	52.9	9.19**	LH	103.6	21.42**	LH	146.4	5.81	HL	0.51	-0.025	LL
Mean	39.9			76.3			127.9			0.53		
Standard error	1.8	2.66		3.3	5.25		4.2	6.72		0.01	0.017	

^a* = significant at the 5% level, ** = significant at the 1% level.

^bFor identification see Table 1.

and Keshari. However, among the F_1 's involving the high general combiner OR79-21, there was only one heterotic hybrid with more than 50% heterosis, while in parents next in order for GCA and overall heterosis such as CR143-2-2 and IR36 there were three such hybrids each, and in OR165-18-8 and showing low GCA and low overall heterosis the number of such heterotic hybrids was two each. This indicates the importance of both GCA (additive plus additive \times additive epistasis) and/or SCA (nonadditive gene action) effects in heterosis. Thus there may be two types of heterotic hybrids—with low or with high SCA.

Gardner and Eberhart (3) partitioned heterosis (h_{ij}) into average heterosis (\bar{h}), variety heterosis (h_i/h_j), and specific heterosis (s_{ij}) as per the model, $h_{ij} = \bar{h} + h_i + h_j + s_{ij}$. Analysis of variance for heterosis on the basis of this model showed that all three components of heterosis are highly significant with the exception of average heterosis in grain number and variety heterosis in panicle number (Table 6). Average heterosis is the difference between the mean of the parents and the mean of the F_1 's ($\bar{F} - \bar{P}$); the significance of this component suggests unidirectional dominance, i.e., nonadditive gene action. In the present study grain yield, biological yield, and grains/panicle showed high average heterosis, while the values were low in panicle number and 1000-grain weight and negative in grain number and harvest index (Table 6). Variety heterosis indicates the overall contribution of a variety to the heterosis of all crosses where it enters as one of the parents. There appears to be a good correspondence between GCA and varietal heterosis, suggesting the importance of GCA in heterosis (Tables 3, 7). Exceptions, were observed to this generalization in N22, which showed significant variety heterosis in grain yield and biological yield although GCA effects were not significant for the two traits. On the other hand, IR36 showed significant variety heterosis only for harvest index against high and significant GCA in four different characters, including harvest index.

In Table 8, GCA and variety heterosis were scored as high (H) and low (L) for the parents involved in two selected hybrids in each of the three heterotic classes such as highly, moderately, and marginally heterotic hybrids. Table 8 also shows heterosis in each of the six hybrids for the six characters mentioned earlier. It is apparent that heterosis in grain yield was conditional to a considerable extent on expression of heterosis in biological yield. In the highly heterotic hybrids, heterosis in grain yield appeared to be the result of a more or less balanced rise in heterosis in the component characters, including biological yield. In the marginally

Table 5. Number of heterotic hybrids for grain yield (g/plant) in different parental arrays.

Array	Heterosis over midparent				Overall heterosis	Top heterotic hybrid (%)
	Marginal or negative (10% or less)	Moderate (11–30%)	Mod. high (31–50%)	High (above 50%)		
1. Annapurna	7	2	2	—	15.8	1/8 (37.1)
2. Parijat	7	4	—	—	–9.5	2/8 (23.0)
3. Suphala	6	1	4	—	0.2	3/4 (44.1)
4. Blackgora	—	4	7	—	36.8	4/11 (47.5)
5. Kalakeri	5	3	3	—	6.2	5/8 (36.1)
6. CR143-2-2	1	2	5	3	30.7	6/8 (72.0)
7. OR165-18-8	3	3	3	2	14.1	7/12 (54.4)
8. OR79-21	—	5	5	1	51.3	8/6 (72.0)
9. Keshari	5	4	2	—	6.5	9/4 (40.2)
10. N 22	—	4	5	2	23.7	10/12 (60.6)
11. IET 1444	4	4	2	1	16.9	11/6 (69.7)
12. IR36	4	2	2	3	30.5	12/11 (60.6)

heterotic hybrids, all the component traits showed severe lack of heterosis. A study of parental ranking for GCA plus variety heterosis (HH, HL, and LL) and heterosis showed that in highly as well as moderately heterotic hybrids, HL combinations were more frequent; however a sizable number of HH combinations did occur, particularly with regard to grain yield and biological yield. In the marginally heterotic hybrids the LL class was more frequent, followed by HL, and there was one case of HH for GCA only. Broadly, the analysis was in agreement with the findings for GCA and SCA (Table 4) that in heterotic hybrids, HL combinations of parents are more frequent than either HH or LL with regard to GCA and/or variety heterosis.

DISCUSSION

The existence of enormous genetic variability in the experimental population consisting of nine semidwarf and three traditional tall early rice varieties shows its merit as a base population for any varietal improvement program. The variations among the semidwarfs that were appar-

Table 6. Analysis of variance for heterosis in six characters.

Source		(df)Mean square ^a					
		Grain yield	Biological yield	Harvest index	Panicle number	Grains/panicle	1000-grain weight
Heterosis (h_{1j})	66	51.0**	112.5**	0.00	2.54**	365.0**	1.03**
Average heterosis (\bar{h})	1	445.9**	2277.7**	0.01**	25.68**	172.9 ns	15.37**
Variety heterosis (h_j)	11	82.8**	92.3**	0.05**	0.93 ns	367.8**	1.98**
Specific heterosis (s_{ij})	54	37.1**	76.6**	0.21**	2.45**	367.9**	0.57**
Error	154	8.2	31.8	0.00	1.26	52.0	0.29
Average heterosis (\bar{h})		6.62	14.92	-1.84	1.59	-4.13	1.33

^a** = significant at the 1% level, ns = not significant.

Table 7. Varietal heterosis (h_j) in grain yield and five traits influencing yield.

Parent	h_j^a					
	Grain yield	Biological yield	Harvest index	Panicle number	Grains/panicle	1000-grain weight
Annapurna	-4.99*	-3.46	-0.05**	-0.35	-12.06*	-0.57
Parijat	-6.54**	-9.02*	-0.02	-1.04	-7.05	0.07
Suphala	-4.94*	-7.38*	-0.03*	0.11	-3.83	-1.94**
Blackgora	5.60**	7.24*	0.03*	-0.37	15.38**	1.16**
Kalakeri	-7.01**	1.93	-0.08**	0.50	-18.83**	0.69*
CR143-2-2	7.07**	6.21*	0.06**	0.23	17.12**	0.63*
OR165-18-8	1.44	-2.41	0.04**	0.61	5.49	-0.51
OR79-21	7.16**	7.32*	0.03*	0.72	11.20**	-0.02
Keshari	-3.84	-3.47	-0.03*	-0.41	-11.50**	0.31
N 22	4.27*	6.42*	0.02	0.25	4.52	0.40
IET1444	-1.11	-0.33	-0.00	0.14	-4.14	-0.76*
IR36	2.94	0.80	0.04**	0.87	3.65	0.52
SE = $\sqrt{3/8} MS_e$	1.75	3.45	0.01	-	4.42	0.33

^a * = significant at the 5% level, ** = significant at the 1% level

ently alike in stature could possibly be due to the genetic diversity of the parents that entered into their ancestry and their selection under different environments.

A preponderance of genes with additive action for important characters like days to flowering, leaf area, number of spikelets, grains/panicle, 1000-grain weight, grain length, and length/breadth ratio for grains enhances the possibility of isolating recombinants with a desirable assemblage of genes governing the traits in the homozygous condition. However, direct selection for yield might not be that effective, considering the existence of a considerable amount of nonadditive gene effects. Several workers have emphasized the importance of indirect selection for yield through the use of component characters governed predominantly by genes with additive action and showing strong correlation with yield (2). The results obtained in this study show that it would be rewarding to use spikelets or grains/panicle and to a certain extent 1000-grain weight as the basis of selection for improving grain yield. Use of harvest index as a criterion for yield improvement did not appear to be that promising, considering the predominance of genes with nonadditive action in its inheritance.

The relative magnitude of GCA effects and variety heterosis (h_j) showed the merits of OR79-21, Blackgora, IR36, CR143-2-2, and

Table 8. Heterosis in grain yield and five other characters influencing yield in some selected hybrids with different levels of heterosis and its relationship with parental GCA and variety heterosis (h_j), each shown as high (H) or low (L).

Hybrids ^a	Grain yield	Biological yield	Harvest index	Panicle number	Grains/panicle	1000-grain weight
<i>Highly heterotic</i>						
6/8 : Heterosis	62.6	55.4	13.2	43.8	12.7	5.0
GCA	HH	LH	LH	LL	HH	LL
h_j	HH	HH	HH	—	HH	HL
10/12: Heterosis	60.6	61.0	-0.3	25.1	7.5	10.7
GCA	LH	LH	LH	LH	HL	LL
h_j	HL	HL	LH	—	LL	LL
<i>Moderately heterotic</i>						
8/12: Heterosis	45.2	30.1	12.1	14.1	18.3	1.1
GCA	HH	HH	HH	LH	HL	LL
h_j	HL	HL	HH	—	HL	LL
4/12: Heterosis	37.9	25.3	10.4	7.2	5.6	11.9
GCA	HH	HH	LH	LH	LL	HL
h_j	HL	HL	HH	—	HL	HL
<i>Marginally heterotic</i>						
2/3 : Heterosis	9.2	6.8	1.5	0	-1.5	-1.5
GCA	LL	LL	LL	HH	LL	LL
h_j	LL	LL	LL	—	LL	LL
2/6 : Heterosis	5.7	4.3	1.7	-7.9	-8.8	13.9
GCA	LH	LL	LL	HL	LH	LL
h_j	LH	LH	LH	—	LH	LH

^aFor identification see Table 1.

OR165-18-8 with regard to both recombination breeding and any hybrid rice programs. The close correspondence between GCA and h_j (Tables 3,7) showed the importance of genes with additive action in heterosis, further suggesting the potential of highly heterotic hybrids in pedigree breeding. This relationship could be tested by measuring the response to selection in the immediately succeeding generations of highly heterotic hybrids.

The analysis of heterosis in relation to parental GCA showed that hybrids involving parents of high (H) x low (L) GCA were more frequently heterotic than those having parents of HH or LL types. A similar observation was also recorded by Reddy and Arunachalam (5) in pearl millet, and they concluded that diversity in parental GCA is necessary in addition to genetic divergence for realizing heterosis.

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COMBINING ABILITY FOR LOW-TEMPERATURE TOLERANCE IN RICE

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One hundred and two F_1 rice hybrids and 23 parents were evaluated for low-temperature tolerance at the early seedling, seedling, booting, and flowering stages. The indica parents had higher mean values for emergence coefficient, flowering duration, number of fertile spikelets, depression in fertile spikelets, and depression in fertility. The japonica parents were high in seedling height, cold tolerance indices, and percent fertility. The japonica/indica hybrids showed high cold tolerance at the vegetative growth stage and low cold stability at the reproductive growth stage. Indica/indica hybrids had high mean values for number of fertile spikelets and percent fertility and were later flowering. The correlations of measures of cold hardiness and emergence ability at low temperature suggest that these responses are controlled differently, and only in part by the same genetic mechanism. Highly significant associations of seedling height with tolerance indices at the early seedling and seedling stages suggest similarity in the inheritance of these traits. Seedling height at the early seedling stage and tolerance indices at any stage appear to be highly effective indicators of cold hardiness at the vegetative growth stage. More productive indicas or indica/indica hybrids appear to be less cold hardy at the seedling stage. Seedling height, all cold tolerance indices, number of fertile spikelets at the booting stage, and percent fertility seem to be predominantly under additive gene action because of their higher magnitude of estimated general combining ability (gca) variances compared with specific combining ability variances. A preponderance of non-additive gene action was revealed in the inheritance of emergence coefficient, flowering duration at the booting stage, and number of fertile spikelets at the flowering stage. The parental performance per se was a good index of their gca effects for seedling height, cold tolerance indices, flowering duration, and number of fertile spikelets. The cultivar Barkat was identified to be the best general combiner for cold tolerance at the vegetative growth stage, followed by Stejaree 45, K332, SR 3044-78-3, and SR5204-91-4-1—all japonicas. For cold stability at the reproductive growth stage K39-96, China 988, Shoa-Nan-Tsan, Leng Kwang, and Suweon 287 were good combiners. High rank correlations indicated high concordance in the rankings based on gca effects and the array means.

More than 15 million ha of rice throughout the world suffer from cold damage at one or another stage of growth. In about 7 million ha in South and Southeast Asia alone, modern rice varieties cannot be planted

because of their susceptibility to low temperatures (3). China probably has the largest area where cold injury occurs to the rice crop — about 6 million ha (Li Taigui, personal communication). The amount of cold damage varies with the temperature and its duration, diurnal changes, physiological status of the crop, and of course the variety used.

The introduction of cold tolerance genes into the high yielding, good plant type indica varieties would extend their cultivation to low temperature areas, with the possibility of growing two rice crops a year in some others. The concept of choosing parents on the basis of their general combining ability (gca) and genetic divergence has been widely used in crop plants and has led to substantial genetic advances in many crops. Reports of combining ability studies in rice with regard to traits related to cold tolerance are, however, not available.

The present study is therefore an attempt to make use of line \times tester analysis to predict the prepotency of the parents and also to evaluate the type of gene action involved in low-temperature tolerance in rice.

MATERIALS AND METHODS

One hundred and two rice hybrids, produced by crossing 17 rice cultivars and elite lines having low-temperature tolerance at different growth stages with six high yielding and/or blast resistant elite IRRI lines, were evaluated for low-temperature tolerance along with their 23 parents.

The 17 female parents, comprised of 9 japonica (including 1 J/I) and 8 indica (including 2 I/J) types, were selected for ecogeographic diversity; they originated from India (Kasmir and Assam), China, Japan, Korea, Indonesia, and the USSR. These were Suweon 235 (Sangpungbyeo), SR5204-91-4-1, SR3044-78-3, Barkat (K78-13), K332, Shimokita, Stejaree 45, K84, and Anna — forming the japonica group—and Suweon 287 (Taebaegbyeo), Samgangbyeo, China 988, K39-96-3-1-1-1-2, Leng Kwang, Shea-Nan-Tsan, Silewah, and ARC 6000—forming the indica group. The six male parents crossed with each of the females (lines) in a line \times tester fashion were indica type elite IRRI lines IR8866-30-3-1-4, IR8455-K2, IR15889-32-1, IR7167-33-2-3, IR29506-60-3-3-2, and IR9202-10-2-1-5-1. Data pertaining to the early seedling, seedling, booting and flowering stages of evaluation in the controlled conditions are presented below. The relevant screening procedures were as follows:

Experiment I. Evaluation for cold hardiness at early seedling stage

The 102 F₁ and 23 parents were screened in the Koitotron KG cabinets of the phytotron for low-temperature tolerance from seedling emergence to the 3-leaf stage. Pre-sprouted seeds were sown in duplicate sets of soil-filled porcelain trays and transferred to artificially lighted cold cabinets at 15 °C for 22 days. Twelve pre-sprouted seeds were sown to each culture in each of the two replications. Each tray had 10 test cultures besides the constant resistant check variety Fujisaka 5 assigned at random.

Emergence data were recorded every 24 hours up to 12 days. Based on vigor of emergence (emergence percentage) and speed of emergence (the average number of days to emergence), an emergence coefficient was worked out. The cold tolerance score of the cultures and seedling height were recorded on the 8th, 15th, and 22nd day of treatment (only last two reported). Survival percentages of emerged seedlings were recorded on a 0–1 scale on the 15th and 22nd day of treatment as per Amirshahi and Patterson (2). The seedling height and percent survival were calculated as percent of the resistant check. A cold tolerance index was worked out (9) by combining cold tolerance score based on leaf discoloration and survival percent. This index allowed rating the genotypes for relative cold stability and quantifying the data on cold tolerance.

Experiment II. Evaluation for cold hardiness at seedling stage

Screening was conducted using 12 °C water on 10-day-old seedlings for 10 days. Pre-sprouted seeds of 125 entries were sown in porcelain trays filled with 3 kg of Maahas clay fertilized with 5 g (NH₄)₂ SO₄ and allowed to grow for 10 days in the greenhouse. Each tray contained a single row of about 12 plants of each of the 10 test cultures and the resistant check Fujisaka 5. There were three replications. Percent establishment was recorded in each entry before being subjected to cold water treatment.

The test cultures removed after 10 days of cold water stress were scored for cold tolerance as per the Standard Evaluation System (8) and allowed to revive in the glasshouse for 10 days. The revival score was made at 5 and 10 days after treatment on a 0–1 scale as in Experiment I. Most of the F₁ hybrids were observed to revive quickly and to appear green. For a

critical assessment of the resistance or susceptibility of the cultures to cold water, two of the three replications were subjected to a second cold water treatment of 10 days and rated for survival immediately after removal. The tolerance index was worked out based on revival/survival of plants and the cold tolerance score as already described.

Experiment III. Low temperature stress at booting stage

About 40–45 pre-sprouted seeds of each of the 102 F_1 hybrids and 23 parents were sown in 5 cm × 5 cm plastic pots, one seed per pot, in the greenhouse. About 30 normal looking, healthy, uniform single-culm plants per culture were transferred to the phytotron glasshouse under a temperature regime of 29/21 °C, 70/70 ± 1 relative humidity, and natural daylength conditions. Out of 30 plants, two sets of 10 plants each per entry were composited in two replications for being subjected to low temperature treatment. The remaining 10 plants were kept as a control.

Plants earmarked for treatment were transferred to the darkroom from 1700 h to 0700 h for 5 consecutive days at a temperature of 15 ± 1 °C at -5 to 0 auricle distance between auricles of the last two leaves on the main stem. The test cultures were allowed to flower and mature in the glasshouse at 29/21 °C after the expiry of the low-temperature treatment. The depression in number of fertile spikelets and percent fertility on account of low-temperature stress was used as a measure of cold stability of characters within the cultures at the reproductive growth stage (the lower the depression, the greater the stability). It was computed as the difference between control and treated plants, expressed as a percentage of the control.

Experiment IV. Low temperature stress at flowering stage

The test cultures were grown in the glasshouse chamber of the phytotron under natural daylength at 25/21 °C temperature regime as in Experiment III, except during the period of cold treatment. The plants were subjected to cold treatment for 5 days in the naturally lighted 3-SAL cabinets in the phytotron at 20 °C from 0600 h to 1800 h and at 15 °C from 1800 h to 0600 h at the first indication of panicle exertion. All computations were made as in the previous experiment.

The line × tester analyses of combining ability were based on a procedure developed by Kempthorne (10).

RESULTS AND DISCUSSION

Highly significant differences were observed among parents and F_1 's for all characters. The means of parents and hybrids are presented in Table 1. Averaged over 23 parents, the parents showed high mean values for seedling height on the 15th day, number of fertile spikelets, and fertility percent, besides recording low mean depression in both number of fertile spikelets and fertility. For all the remaining traits, the hybrids had higher overall mean values. The intergroup comparisons revealed high mean values for emergence coefficient, flowering duration, number of fertile spikelets, depression in percent fertility, and depression in number of fertile spikelets in the indica parents. On the contrary, japonica parents were high in seedling height, cold tolerance indices, and percent fertility. The japonica/indica hybrids similarly demonstrated high mean values for seedling height, cold tolerance indices, depression in percent fertility, and depression in number of fertile spikelets, exhibiting high cold tolerance at the vegetative growth stage (early seedling and seedling) and low cold stability at the reproductive growth stage (booting and flowering). The indica/indica hybrids, by comparison, had high mean values for flowering duration, number of fertile spikelets, and percent fertility, as well as low depression in both number of fertile spikelets and fertility.

Obviously the japonicas appear to be a good source of genes for seedling cold tolerance and earliness, and the indicas for number of fertile spikelets and percent fertility. Low-temperature tolerance at the vegetative growth stage and that at the reproductive growth stage appear to be different and governed by genetic mechanisms that act most likely in opposite directions. The higher cold tolerance of japonica cultivars at the vegetative growth stage has been reported by many workers (5,6,7).

Since tabulations of the mean performance of 125 entries for all the characters would be too voluminous, the mean performance of the hybrids grouped as lines for japonica/indica and indica/indica crosses averaged over the array of 6 common male parents for the 14 traits has been tabulated in Table 2. Based on array means, Barkat (K78-13), a japonica/indica cross (Shen-ei/China 931) appears to have the best potential as a donor of genes for seedling stage cold tolerance. Barkat has been released for high elevation areas in Kashmir (1,675–2,130 m). It had the tallest seedling height at low temperature (15 °C) on the 15th day and the second tallest on the 22nd day, besides having maximum cold tolerance at both the early seedling and seedling stages. Incidentally,

Table 1. Means of parents and F₁ hybrids grouped as japonica/indica and indica/indica crosses for 14 traits in cold tolerance evaluation studies at different growth stages.

Trait ^a	Mean values ^b					
	Parents			Hybrids		
	I	J	Overall	I/I	J/I	Overall
A. Early seedling stage:						
1. Emergence coefficient	22.9	21.9	22.5	23.5	23.5	23.5
2. Seedling height on 15th day	8.9	10.0	9.3	8.0	10.4	9.1
3. Seedling height on 22nd day	13.0	13.8	13.3	11.8	15.1	13.3
4. TI on 15 days survival	0.34	0.88	0.57	0.35	0.85	0.58
5. TI on 22 days survival	0.26	0.88	0.48	0.28	0.84	0.54
B. Seedling stage						
6. TI on revival, 5 DAT	0.40	1.07	0.66	0.56	0.78	0.67
7. TI on revival, 10 DAT	0.42	1.04	0.64	0.55	0.78	0.66
8. TI on final survival	0.17	0.89	0.42	0.34	0.95	0.64
C. Booting stage						
9. Flowering duration	76.0	55.1	68.7	78.9	66.6	73.1
10. No. of fertile spikelets	55.8	39.9	50.3	59.9	25.3	43.6
11. No. of fertile spikelets (depression rate)	10.7	12.3	11.3	13.9	26.6	19.9
11a. % fertility	66.7	80.0	71.3	63.5	27.8	46.7
11b. % fertility (depression rate)	7.8	4.8	6.8	12.0	20.4	16.0
D. Flowering stage						
12. Flowering duration	76.2	50.4	67.3	76.8	65.8	71.6
13a. No. of fertile spikelets	45.9	39.3	43.6	37.1	11.8	25.2
13b. No. of fertile spikelets (depression rate)	26.9	10.9	21.3	34.3	61.3	47.0
14a. % fertility	64.7	76.9	68.9	48.5	14.5	32.5
14b. % fertility (depression rate)	17.5	5.4	13.3	31.0	59.1	44.2

^aTI = tolerance index, DAT = days after treatment. ^bI = indica, J = japonica

Table 2. Mean performance of hybrids grouped as lines from japonica/indica and indica/indica crosses, averaged over common male parents for 14 traits.

Female parents	Early seedling stage ^a					Seedling stage			Booting stage					Flowering stage				
	1	2	3	4	5	6	7	8	9	10a	10b	11a	11b	12	13a	13b	14a	14b
<i>Japonica group</i>																		
Suweon 235	23.9	10.7	14.5	0.87	0.83	0.76	0.76	0.99	68.1	20.7	23.6	32.1	18.1	66.7	10.5	66.8	14.0	59.7
SR5204-91-4-1	23.4	10.2	14.7	0.86	0.73	0.80	0.80	1.05	69.6	33.6	30.2	33.7	19.1	69.7	15.4	61.1	19.0	54.7
SR3044-78-3	23.8	11.0	15.6	0.95	0.96	0.78	0.78	1.00	68.5	24.6	25.6	25.4	22.1	67.3	15.9	61.0	19.5	57.9
Barkat (K78-13)	25.0	11.8	16.4	0.93	1.11	0.89	0.89	1.12	65.1	20.6	23.2	23.3	21.9	63.6	10.3	52.5	13.1	57.3
K332	25.7	10.3	14.9	0.94	0.99	0.80	0.80	0.90	58.9	20.1	18.0	20.6	16.3	59.5	8.9	64.6	10.2	63.2
Shimokita	21.8	9.6	14.0	0.80	0.69	0.76	0.78	0.90	63.2	24.6	28.2	29.2	17.3	63.2	13.9	57.1	16.9	53.9
Stejaree 45	23.5	11.6	17.3	0.95	0.91	0.81	0.81	0.94	70.7	31.4	25.9	35.1	26.2	68.3	13.2	63.3	15.4	60.2
Anna	20.7	8.4	13.2	0.49	0.46	0.68	0.70	0.69	68.4	18.8	38.4	22.7	25.4	67.9	6.7	64.0	7.6	65.5
K84	22.9	11.2	14.9	0.94	0.79	0.79	0.79	0.90	77.0	41.4	28.1	42.3	22.8	73.0	15.5	58.1	20.6	48.8
<i>Indica group</i>																		
Suweon 287	26.8	6.6	9.1	0.15	0.10	0.24	0.25	0.32	71.0	60.0	14.8	71.5	7.1	73.6	38.8	22.9	61.7	21.1
Samgangbyeol	24.8	6.5	9.4	0.18	0.13	0.27	0.25	0.09	71.3	57.2	29.1	61.6	18.9	71.6	39.7	31.5	64.0	21.2
China 988	21.4	7.6	11.0	0.18	0.15	0.52	0.49	0.21	79.9	57.0	16.0	72.8	5.8	75.4	45.9	28.7	64.4	18.3
K39-96-3-1-1-1-2	20.2	6.3	10.5	0.15	0.14	0.56	0.52	0.27	83.4	67.9	4.8	70.7	10.0	76.8	56.3	17.6	63.5	19.0
Leng Kwang	21.4	7.0	11.4	0.15	0.12	0.64	0.63	0.27	79.8	72.9	11.1	73.5	8.3	77.9	41.2	29.7	48.9	32.2
Shoa-Nan-Tsan	22.5	6.5	10.5	0.16	0.09	0.54	0.53	0.19	82.5	69.0	5.1	75.9	8.6	76.3	49.4	26.3	61.1	22.6
Silewah	25.2	10.6	15.2	0.94	0.74	0.78	0.78	0.58	80.3	52.0	14.1	48.4	16.8	77.8	24.6	40.4	22.3	42.8
ARC 6000	26.2	9.4	14.3	0.31	0.22	0.68	0.67	0.28	84.9	60.1	12.5	55.1	10.1	88.6	22.1	53.9	29.7	52.8
LSD (5%)	1.39	0.49	0.94	0.08	0.23	0.06	0.06	0.15	0.41	2.08	3.8	1.3	2.8	0.4	1.5	2.6	0.9	1.5

^aTraits 1 to 14b are the same as in Table 1.

seedling height showed highly significant and positive association with cold tolerance indices, ranging from 0.62** to 0.84** (Table 3). Stejaree 45 from the USSR demonstrated the next best potential for producing hybrids with high cold tolerance indices and seedling height on the 15th day. It also produced hybrids with the tallest seedlings on the 22nd day of low-temperature treatment. K332 (Shen-ei-Norin II), another selection from Kashmir, produced the earliest flowering hybrids. Silewah from Indonesia appeared to have the capacity among the indicas to produce hybrids with tall seedlings at low temperature and possessed high to moderate levels of tolerance at the vegetative stage. Following Silewah were ARC 6000 (India) and Leng Kwang (China). Silewah and Leng Kwang had earlier been identified as 2 of the 11 outstanding entries at IRRI and are most commonly used in the IRRI breeding program (19). Regarding number of fertile spikelets and percent fertility, Leng Kwang, Shoa-Nan-Tsan (China), and K39-96 (Kashmir) appeared better, in that order, at the booting stage evaluation, and K39-96, China 988 (India), Shoa-Nan-Tsan, Samgangbyeon (Korea) were better at the flowering stage evaluation. These cultures also produced hybrids showing high cold tolerance at the reproductive growth stage by demonstrating low depressions due to low-temperature stress in number of fertile spikelets and fertility. The best emergence at low temperature (15 °C) was observed in hybrids of Suweon 287 (Korea), followed closely by ARC 6000, K332, Silewah, and Barkat, indicating their capacity for fast emergence under cold stress.

Intercharacter associations calculated among 10 characters for 102 hybrids and 23 parents are presented in Table 3. The correlations of measures of cold hardiness and emergence ability suggest that these responses are controlled differently, and only in part by the same genetic mechanism. Similar conclusions have been drawn in winter wheat (14). The only significant positive correlations shown by the emergence coefficient were with seedling height on the 15th day (0.31**) and seedling height on the 22nd day (0.19*). However, the selective value of these relationships appears limited. Tolerance index (TI) on revival 10 days after treatment was the character very closely associated with TI on revival 5 days after treatment (0.996**). The other characters most highly associated were TI at 22 days survival with TI at 15 days survival (0.91**), seedling height on the 15th day with that on the 22nd day (0.87**), and seedling height on 15th day with TI on 15 days survival (0.84**). All intercharacter relationships between tolerance indices and seedling height were in fact highly significantly and positively correlated. Presumably, therefore, the cold hardiness at the early seedling and

Table 3. Intercharacter associations^a among 10 characters related to cold tolerance at different stages, measured on 102 rice hybrids and 23 parents.

Correlated characters ^b	1	2	3	4	5	6	7	8	9a	9b	10a
1. Emergence coefficient											
2. Seedling height on 15th day	0.31**										
3. Seedling height on 22nd day	0.19*	0.87**									
4. TI on 15 days survival	0.14	0.84**	0.77**								
5. TI on 22 days survival	0.08	0.75**	0.74**	0.91**							
6. TI on revival, 5 DAT	0.01	0.68**	0.65**	0.75**	0.66**						
7. TI on revival, 10 DAT	0.03	0.68**	0.66**	0.76**	0.67**	0.996**					
8. TI on final survival	0.01	0.68**	0.62**	0.81**	0.77**	0.74**	0.75**				
9a. No. of fertile spikelets BS	0.01	-0.47**	-0.41**	-0.64**	-0.62**	-0.42**	-0.43**	-0.73**			
FS	-0.10	-0.48**	-0.45**	-0.59**	-0.51**	-0.36**	-0.39**	-0.65**			
9b. No. of fertile spikelets BS	-0.03	0.17	0.13	0.31**	0.29	0.07	0.09	0.36**	-0.52**		
(depression rate) FS	0.06	0.39**	0.37**	0.43**	0.36**	0.23*	0.26**	0.51**	-0.87**		
10a. % fertility BS	-0.10	-0.48**	-0.47**	-0.59**	-0.55**	-0.32**	-0.35**	-0.66**	0.81**	-0.43**	
FS	-0.10	-0.47**	-0.48**	-0.56**	-0.49**	-0.34**	-0.37**	-0.64**	0.90**	-0.89**	
10b. % fertility BS	0.03	0.21*	0.16	0.33**	0.29**	0.15	0.17	0.39**	-0.49**	0.65**	-0.58**
(depression rate) FS	0.10	0.39**	0.41**	0.45**	0.39**	0.28**	0.32**	0.55**	-0.87**	0.94**	-0.95**

γ fertile spikelets at booting, fertile spikelets at flowering = 0.77**

γ fertility at booting, fertility at flowering = 0.89**

γ depression in fertile spikelets at booting, flowering = 0.35**

γ depression in fertility at booting, flowering = 0.44**

^a* = significant at the 5% level, ** = significant at the 1% level.

^bTI = tolerance index, DAT = days after treatment, BS = booting stage evaluation, FS = flowering stage evaluation.

seedling stages in this material is governed by similar genetic mechanisms. Seedling height had earlier been shown to be a good indicator of cold-water tolerance and is used as a criterion for evaluating rice seedlings in the US (1, 4, 13). These results also suggest that seedling height at the early seedling stage or cold tolerance index at any stage, especially on revival 10 days after treatment at the seedling stage evaluation, would be highly effective indicators of cold hardiness at the vegetative growth stage. As expected, percent fertility showed a high positive relationship with number of fertile spikelets at both the booting (0.81**) and flowering (0.90**) stage evaluations, indicating a high predictive value.

Correlations of number of fertile spikelets and percent fertility with seedling height and various cold tolerance indices showed a highly significant and negative relationship among all the traits. This reflects a tendency for more productive types to be less cold hardy at the seedling stage and thus substantiates the results reported earlier. However, the coefficients were not large enough to suggest that selection for low-temperature tolerance simultaneously for both the vegetative and reproductive growth stages is not feasible. Figure 1 represents the relationship of TI based on 15 days survival with number of fertile spikelets. The constellation of points reveals that a majority of entries (representing mostly indica parents and indica/indica hybrids) having number of fertile spikelets per panicle in the range of 30–85 demonstrated a low early seedling cold TI (up to 0.4) at the booting stage evaluation. On the contrary, most of the entries with a high cold TI of more than 0.6 (representing mostly japonica parents and japonica/indica hybrids) produced 0–50 fertile spikelets per panicle. The same was true at the flowering stage evaluation as well. However, Figure 2, which represents the association of TI on revival 10 days after treatment with percent fertility, indicates that it should be possible to combine high seedling cold tolerance and high fertility by judicious planning of the crossing program and proper selection of the segregants. A highly significant and positive relationship was observed between the measure of tolerance at the booting stage and that at the flowering stage of evaluation. Lee (11) has suggested that large-scale screening of cold tolerant varieties may be done with low-temperature treatments at heading because of the positive correlation of sterility between plants treated with low temperature at the meiotic and heading stages. The present results are supportive of this view.

The analyses of variance for combining ability (Table 4) reveal highly significant variances due to females for all the characters. The variances

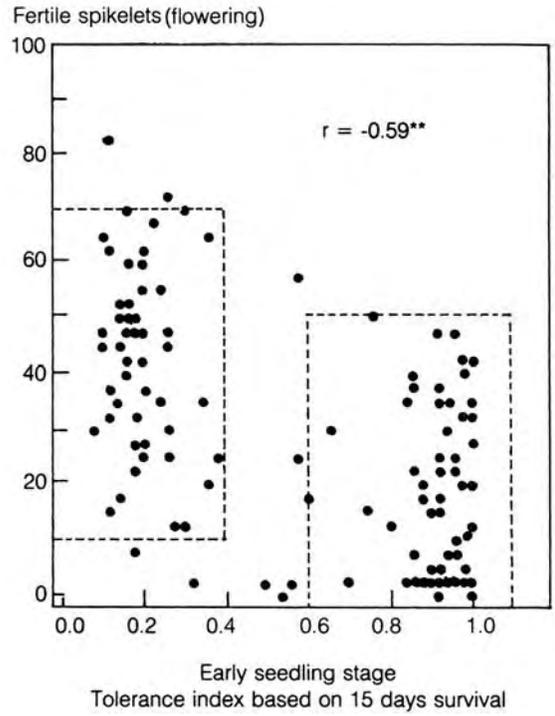
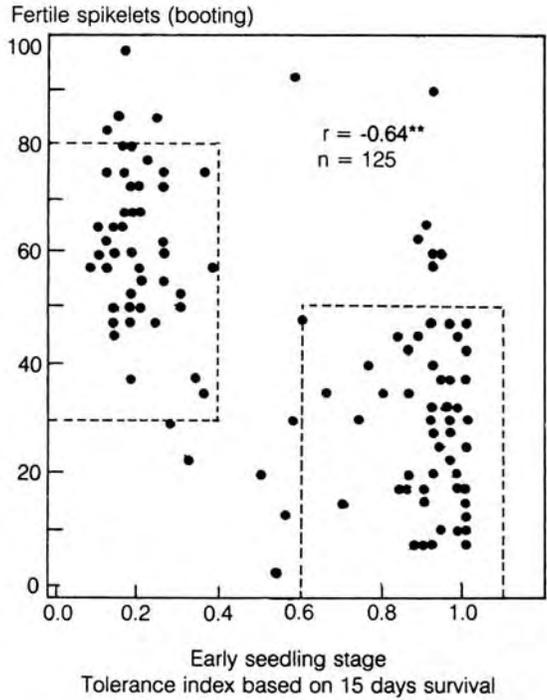


Fig. 1. Relationship between tolerance index based on 15 days survival (early seedling stage) and number of fertile spikelets at the booting and flowering stages of low-temperature treatment.

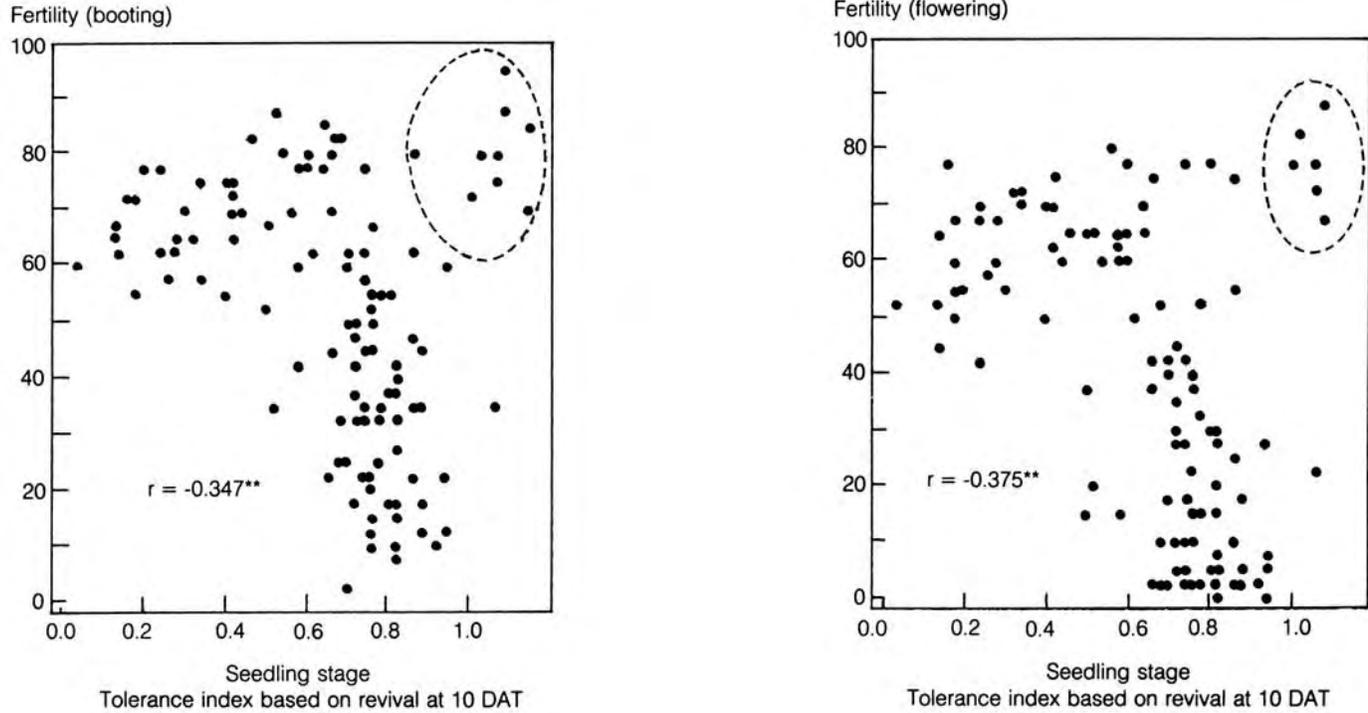


Fig. 2. Relationship between tolerance index based on revival 10 days after treatment (seedling stage) and percent fertility at the booting and flowering stages of low-temperature treatment.

Table 4. Analyses of variance for combining ability for cold tolerance traits at different growth stages.

Character ^a	Mean squares ^b				s ² gca	s ² sca
	Females	Males	Females x males	Error		
A. Early seedling stage (df)	16	5	80	101		
1. Emergence coefficient	46.17**	48.99**	11.62**	3.99	1.56	3.81
2. Seedling height on 15th day (cm)	48.24**	3.51**	1.96**	0.55	1.04	0.70
3. Seedling height on 22nd day (cm)	19.99**	5.02**	2.26	1.88	0.44	0.19
4. TI on 15 days survival	1.591**	0.044*	0.019*	0.014	0.035	0.003
5. TI on 22 days survival	1.687**	0.123	0.079	0.109	0.036	-0.015
B. Seedling stage (df)	16	5	80	202		
6. TI on revival, 5 DAT	0.617**	0.116**	0.016**	0.008	0.010	0.003
7. TI on revival, 10 DAT	0.655**	0.116**	0.016**	0.008	0.010	0.003
8. TI on final survival	1.680**	0.479**	0.051	0.065 ^c	0.045	0.007
C. Booting stage (df)	16	5	80	101		
9. Flowering duration	709.9**	774.5**	76.3**	0.33	28.95	38.01
10a. No. of fertile spikelets	4517.1**	3293.0**	284.5**	0.41	157.41	137.84
10b. No. of fertile spikelets (depression rate)	1254.3**	768.8**	408.7**	28.22	26.20	190.36
11a. % Fertility	5127.3**	2020.2**	221.6**	2.97	145.75	109.19
11b. % Fertility (depression rate)	519.9**	702.6**	293.3**	17.35	13.82	137.98
D. Flowering stage (df)	16	5	80	101		
12. Flowering duration	589.9**	389.4**	40.0**	0.41	19.55	19.82
13a. No. of fertile spikelets	3182.7**	1871.2**	277.3**	4.16	97.81	136.58
13b. No. of fertile spikelets (depression rate)	3562.8**	5227.4**	480.5**	12.65	170.20	233.92
14a. % Fertility	5914.7**	2855.0**	307.9**	1.42	177.26	153.25
14b. % Fertility (depression rate)	3720.1**	4495.4**	492.3**	5.08	157.19	243.63

^aTI = tolerance index, DAT = days after treatment.^b* = significant at the 5% level, ** = significant at the 1% level.^cMean square for error based on 101 df.

due to males and to females \times males interaction were also highly significant for most of the characters. Greater diversity among females was indicated because of the larger magnitude of mean squares due to females for most of the characters. Seedling height, all cold tolerance indices, number of fertile spikelets at the booting stage, and percent fertility seemed to be predominantly under additive gene action, as the estimated gca variances (s^2 gca) were more pronounced compared with the estimated specific combining ability (sca) variances (s^2 sca). These traits can as such be utilized in developing cold tolerant homozygous lines by the usual pedigree breeding methods that exploit additive and additive \times additive types of gene effects. A preponderance of nonadditive gene action was revealed in the inheritance of emergence coefficient, flowering duration at booting stage evaluation, number of fertile spikelets at flowering stage evaluation, and depression in both fertile spikelets and percent fertility at both the booting and flowering stages. Both additive and nonadditive gene action appeared to be equally important in the inheritance of flowering duration at flowering stage evaluation. While the importance of additive gene effects in the inheritance of cold tolerance has been emphasized (12), the significance of both additive and dominance effects with consistency of additive effects over both F_1 and F_2 generations in the inheritance of seedling vigor as measured by height of rice seedlings has also been reported (13). For number of spikelets per panicle, the unimportance of sca has been observed (15). Spikelet sterility has, however, been suggested to be conditioned by nonadditive gene action (16).

The gca effects of the parents and the correlation between per se performance and gca effects for various characters are given in Table 5. The performance of the parents per se was a good index of their gca for seedling height, cold tolerance indices, flowering duration, and number of fertile spikelets. Evidently the per se performance of the parents could be considered as a criterion for selection of parents for these characters. Similar reports are available for various other characters in rice.

The variety Barkat was revealed to be the best general combiner for cold tolerance at the vegetative growth stage. It had the highest gca for four of the five tolerance indices, showed maximum effects for seedling height on the 15th day, and ranked third best in earliness. The other entries showing promise for cold tolerance at the vegetative growth stage were Stejaree 45, K332, SR3044-78-3, and SR5204-91-4-1 — all from the japonica group. From the indica group only Silewah showed some promise. K332 was the best general combiner for earliness, followed by Shimokita. Suweon 287 was the best general combiner for emergence coefficient, followed by ARC 6000, IR15889-32-1, K332, Silewah, and

Barkat. For cold stability at the reproductive growth stage, K39-96, China 988, Shoa-Nan-Tsan, Leng Kwang, Suweon 287, and Samgang-byeo were good general combiners for number of fertile spikelets and fertility. K-39-96 (Ch.1039/IR580-19-2-3-3) is the most predominantly cultivated variety in the plains of Kashmir up to an altitude of 1,675 m. It has also been released in the hills of Utter Pradesh, India (as VLK Dhan 39) and very recently in the hill region of Nepal. ARC6000, Leng Kwang, Stejaree 45, and Shoa-Nan-Tsan have been reported to be excellent in at least three plant characters desired in low-temperature areas (18). Hybrids of Silewah, Shoa-Nan-Tsan, Leng Kwang, and ARC 6000 are apt to grow tall, are prone to lodging, and have delayed flowering.

Per se performance and sca effects were used to study the performance of lines in their specific combinations. Five best crosses with respect to 12 important traits were selected on the basis of per se performance and sca effects separately along with five best parents selected on the basis of per se performance and gca effects. Significant correlations between sca effects and per se performance of crosses indicate that the performance of the F_1 as such could be used to predict the effects of sca for the parents. However, it was observed that crosses selected on the basis of per se performance and sca effects were not necessarily the same. This means that the best cross combination selected on high sca basis may not necessarily be the high performing one.

Since sca effect is a measure of the deviation of F_1 performance over parental performance and may be high or low depending upon whether the parental performance is low or high, high sca would not necessarily mean high performance by the hybrid. The per se performance, on the other hand, is the actual realized value and appears to be more realistic for selecting the best cross combinations. Incidentally, per se performance of the crosses was in good agreement with the combining ability of their parents.

In order to facilitate comparisons and allow a definite choice of parents, the female parents involved in the crosses were first ranked on the basis of array means and second on the basis of gca effects for each character separately. The ranks over the various characters were then pooled for each of the lines separately. The rankings of parents based on gca were then compared with the rankings based on array means of parents (Table 6) as per Snedecor (17). High rank correlation coefficients (r_s) indicated high concordance in the rankings based on gca effects and array means. Thus, effective use of array means could be made in judging the combining ability effects of parental lines. According to the rankings, Barkat appears to be the best choice of parents for vegetative growth

Table 5. General combining ability (gca) effects and correlation^a between per se performance and gca effects.

Parents	Growth stage ^b							
	Early seedling					Seedling		
	1	2	3	4	5	6	7	8
<i>Males</i>								
IR8866-30-1-4	-0.17	-0.17	-0.94**	0.03	0.09	0.01	0.01	0.10*
IR8455-K2	-0.33	0.27*	-0.10	0.02	0.00	0.07**	0.07**	0.19**
IR15889-32-1	2.26**	0.45**	0.92**	0.02	-0.03	-0.02	-0.01	-0.09*
IR7167-33-2-3	-0.30	0.07	0.25	0.02	-0.01	0.03**	0.03**	-0.04
IR29506-60-3-3-2	-1.36**	-0.21	-0.81**	-0.04	-0.09	-0.01	-0.02	-0.05
IR9202-10-2-1-5-1	-0.10	-0.41**	0.69**	-0.05	0.04	-0.07**	-0.07**	-0.11**
SE (ĝi) ±	0.34	0.13	0.23	0.02	0.06	0.01	0.01	0.04
<i>Females (japonica group)</i>								
Suweon 235	0.39	1.56**	1.18**	0.28**	0.29**	0.09**	0.10**	0.37**
SR5204-91-4-1	-0.05	1.06**	1.35**	0.27**	0.19	0.13**	0.14**	0.42**
SR3044-78-3	0.34	1.86**	2.28**	0.37**	0.42**	0.12**	0.12**	0.37**
Barkat (K78-13)	1.54**	2.64**	3.07**	0.34**	0.57**	0.22**	0.23**	0.49**
K332	2.20**	1.21**	1.61**	0.35**	0.45**	0.14**	0.14**	0.27**
Shimokita	-1.71**	0.49	0.63	0.21**	0.15	0.10**	0.12**	0.28**
Stejaree 45	0.01	2.46**	3.92**	0.37**	0.37**	0.15**	0.15**	0.31**
Anna	-2.81**	-0.74**	-0.17	-0.10**	-0.08	0.02	0.01	0.07
K84	-0.57	2.11**	1.51**	0.35**	0.25**	0.12**	0.13**	0.27**
<i>Females (indica group)</i>								
Suweon 287	3.31**	-2.56**	-4.24**	-0.43**	-0.44**	-0.42**	-0.41**	-0.52**
Samgangbyeon	1.32*	-2.60**	-3.95**	-0.40**	-0.41**	-0.39**	-0.41**	-0.54**
China 988	-2.05**	-1.52**	-2.32**	-0.41**	-0.39**	-0.14**	-0.17**	-0.41**
K39-96-3-1-1-1-2	-3.27*	-2.87**	-2.82**	-0.44**	-0.40**	-0.11**	-0.13**	-0.35**
Leng Kwang	-2.06**	-2.13**	-1.97**	-0.43**	-0.42**	-0.02	-0.02	-0.18**
Shoa-Nan-Tsan	-0.99	-2.66**	-2.88**	-0.42**	-0.45**	-0.13**	-0.13**	-0.44**
Silewah	1.70**	1.42**	1.84**	0.35**	0.20*	0.12**	0.12**	-0.05
ARC 6000	2.69**	0.27	0.95**	-0.27**	-0.32**	0.01	0.01	-0.35**
SE (ĝj) ±	0.58	0.21	0.40	0.035	0.096	0.02	0.02	0.074
g (per se performance:gca)								
	0.030	0.413*	0.569**	0.773**	0.753**	0.724**	0.739**	0.791**

^a* = significant at the 5% level; ** = significant at the 1% level.

^bTraits 1 to 14b as in Table 1.

stage cold tolerance, followed by Stejaree 45, SR3044-78-3, K332, SR5204-91-4-1 and K84. Samgangbyeon is the poorest. On the other hand, K39-96 and Shoa-Nan-Tsan are the best with regard to reproductive growth stage cold stability, followed by China 988, Leng Kwang, Suweon 287, and Samgangbyeon.

Booting					Flowering				
9	10a	10b	11a	11b	12	13a	13b	14a	14b
-2.7**	-6.1**	6.6**	-10.4**	8.7**	2.1**	-5.2**	12.5**	-7.7**	8.2**
-5.2**	-10.4**	-3.6**	-12.0**	-3.0**	-3.5**	-11.5**	16.8**	-12.8**	15.5**
-4.9*	0.4	3.4**	-1.8**	-3.0**	-4.2**	1.3**	-1.5**	-2.0**	4.0**
-1.1**	-0.9	-5.4**	10.9**	11	-0.2	0.5	-2.3**	3.6**	-2.9**
7.3**	7.4**	-3.3**	9.8**	-14	4.6**	8.4**	-13.1**	9.5**	-15.5**
1.1**	9.7**	2.2**	3.5**	-2.4**	1.3**	6.5**	-12.4**	9.4**	-9.3**
0.10	0.51	0.91	0.31	0.71	0.11	0.35	0.61	0.20	0.39
-5.0**	-14.6**	3.6*	-14.9**	2.2	-4.9**	-14.7**	19.7**	-18.5**	15.5**
-3.6**	-13.0**	10.3**	-10.0**	-0.1	-2.0**	-9.8**	14.1**	-13.5**	10.5**
-4.6**	-21.3**	5.7**	-19.0**	6.1**	-4.3**	-9.3**	13.9**	-12.9**	13.7**
-8.0**	-23.3**	3.3*	-23.0**	5.9**	-8.0**	-14.9**	5.5**	-19.3**	13.1**
-14.1**	-26.1**	-1.9	-23.6**	0.3	-12.1**	-16.3**	17.6**	-22.3**	19.0**
-9.9**	-17.5**	8.3**	-19.0**	1.4	-8.3**	-11.3**	10.1**	-15.6**	9.7**
-2.4**	-11.6**	5.9**	-12.2**	10.3**	-3.3**	-12.0**	16.3**	-17.0**	16.0**
-4.6**	-24.0**	18.5**	-24.8**	9.4**	-3.7**	-18.5**	17.0**	-24.9**	21.3**
4.0**	4.4**	8.2**	-2.3**	6.8**	1.4**	-9.7**	11.0**	-11.9**	4.6**
-2.1**	24.8**	-5.1**	16.4**	-8.8**	1.96**	13.6**	-24.2**	29.3**	-23.0**
-1.7	14.9**	9.2**	13.6**	2.9**	0.01	14.5**	-15.5**	31.5**	-23.0**
6.8**	26.0**	-4.0**	14.2**	-10.1**	3.8**	20.7**	-18.4**	32.0**	-25.9**
10.2**	24.0**	-15.2**	24.3**	-6.1**	5.2**	31.1**	-29.5**	31.0**	-25.2**
6.7**	26.8**	-8.8**	29.3**	-7.6**	6.3**	16.0**	-17.3**	16.4**	-12.0**
9.4**	29.2**	-14.8**	26.2**	-7.4**	4.7**	24.2**	-20.7**	28.6**	-21.6**
7.2**	1.7	-5.8**	8.4**	0.8	6.2**	-0.6	-6.6**	-10.1**	1.4*
11.8**	8.4**	-17.5**	16.5**	-5.9**	17.0**	-3.1**	6.8**	-2.8**	8.6**
0.17	0.86	1.53	0.52	1.20	0.19	0.59	1.03	0.34	0.65
0.793**	0.688**	0.274	-0.366	-0.333	0.795**	0.555**	-0.122	-0.223	-0.161

The *gca* effects of the 23 parents for the 5 cold tolerance indices at the early seedling and seedling stages were correlated to find out if any association for the combining ability effects existed. Highly significant and positive relationships were observed between the *gca* values of all the traits. While the least *r* value of 0.841** was shown between the *gca*

Table 6. Ranking of female parents based on their array means and gca effects at vegetative (early seedling and seedling) and reproductive (booting and flowering) stage evaluations.

Female parent	Vegetative growth stage ^a		Reproductive growth stage ^b			
	Array mean	gca effects	Depression excluded		Depression included	
			Array mean	gca effects	Array mean	gca effects
<i>Japonica group</i>						
Suweon 235	5	8	13	12	12	13
SR5204-91-4-1	5	5	9	9	8	8
SR3044-78-3	2	4	10	10	9	10
Barkat	1	1	14	13	11	12
K332	4	3	15	14	13	14
Shimokita	6	9	12	11	9	9
Stejaree 45	3	2	11	10	10	11
K84	4	6	8	8	7	7
Anna	8	10	16	15	14	15
<i>Indica group</i>						
Suweon 287	13	17	4	4	3	3
Samgangbyeon	14	16	5	5	4	4
China 988	11	13	3	3	2	2
K39-96	12	14	2	2	1	1
Leng Kwang	10	12	3	3	3	3
Shoa-Nan-Tsan	12	15	1	1	2	2
Silewah	7	7	7	7	6	6
ARC 6000	9	11	6	6	5	5
r_s vegetative stage (array mean:gca effects) = 0.906 r_s reproductive stage excluding depression (array mean: gca effects) = 0.993 r_s reproductive stage including depression (array mean: gca effects) = 0.993						

^aBased on 5 tolerance indices.

^bBased on fertile spikelets and % fertility.

effects of tolerance indices at 22 days survival at the early seedling stage and at revival 5 days after treatment at the seedling stage, the highest r value of 0.998** was observed between tolerance indices at revival 5 and 10 days after treatment (seedling stage). The next most highly associated gca values were for tolerance indices at 15 and 22 days survival ($r = 0.996^{**}$). These associations are suggestive of the fact that computation of gca effects for one TI would give a fairly accurate idea of the parental potential.

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DISCUSSION

SESSION 10: GENETICS OF QUANTITATIVE TRAITS

Q – Virmani: If a trait such as yield is determined primarily by additive gene action, why have we not been able to develop inbred lines higher yielding than semidwarf varieties like IR8 and IR36?

If heterosis results from covering the effects of repulsion phase linkages, how do you propose to break these repulsion phase linkages through the pedigree method of breeding?

A – Perera: The inability to develop inbred lines with higher yields is because of inadequacies in the procedures of selection in the breeding programs. If recombined inbred lines are produced by the single seed descent procedure, it will be possible to extract high yielding inbred lines as predicted. Linkage disequilibrium breaks down on selfing.

Q – Glaszmann: It may be interesting to know that variety Silewah falls into the japonica group as far as isozymes are concerned. Regarding cold tolerance, it also seems to behave more like a japonica variety. Can you comment?

A – Kaw: Silewah is intermediate between the indicas and japonicas as far as cold tolerance at both the vegetative and reproductive stages is concerned. In plant type and other morphological features, it is more akin to indicas than to japonicas. It has been listed as an indica in the IRGC records.

CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION

SESSION 11

GENETIC ANALYSIS OF FERTILITY RESTORATION IN MALE STERILE LINES OF RICE

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The distribution of restoring (R) genes in the pedigree of IR24 has been revealed through test crosses of related parents. The genotypes of these parents are also determined by making statistical analyses of the fertility of F_2 plants of restored and semirestored combinations. Meanwhile, whether an additive effect of R genes in the partially restoring parents exists has been explored by crossing them and test crossing their F_1 with cytoplasmic male sterile lines. From the results obtained it is concluded that IR24 contains two pairs of major restoring genes ($R_1 R_1 R_2 R_2$) that are independently inherited. One pair of genes ($R_1 R_1$) comes from Cina, a late indica variety in China, while another pair ($R_2 R_2$) is from SLO 17, which has a relationship with some varieties in India. There is a difference in strength between these two pairs of genes, and an additive effect markedly exists between different restoring genes.

More than 95% of the hybrid rice varieties used now in production in China are derived from the WA cyto sterility system. In the past, the restorer lines of WA type were mainly screened by test crossing existing rice varieties. Now, in addition to the screening method, restorer line \times restorer line, maintainer line \times restorer line (or restorer line \times maintainer line), male sterile line \times restorer line, and multiple crosses are also made to develop new elite restorer lines. Although some good results have been obtained, the breeding work, as a whole, has many limitations, with the result that many of the materials selected are not as satisfactory as desired, and quite a few selections possessing good combining ability and strong heterosis have to be discarded due to their weak restoring ability. Therefore, studies on the inheritance of the fertility restoration of WA type cytoplasmic male steriles (CMS) are of vital importance for improving the efficiency and quality of restorer line breeding.

In general, IR24 is used as a representative variety in the study of the inheritance of the fertility restoration in WA type CMS. This is not only because IR24 is an excellent restorer line, discovered early and used very widely, but many derivative restorer lines have been developed by using IR24 as the parent. There have been some reports on the genetic study of

restoring ability of IR24 made in China. For instance, Gao Mingwei (1) pointed out that IR24 possesses two pairs of independent dominant restoring genes and there exists an obvious dosage effect between them. Yang Rencui (6) suggested that there may be a difference in strength between these two pairs of restoring genes but no additive effect. Li Zebing et al (3) reported that the two pairs of restoring genes in IR24 are linked and that the recombination frequency is 34%. Zhou Tianli (8) held that the restoring ability of IR24 is controlled by a pair of partially sterile genes and a pair of restoring genes, and that the latter is epistatic to the former. According to Young and Virmani's study (7) there are two independent restoring genes in IR54, one of them with a stronger fertility restoration ability than the other. Besides, Wang (5) considers that the restoring ability of IR24 is controlled by a pair of genes; or it is polygenically inherited (4). The present study explored the genetics of the restoring ability of IR24 as well as the source and evolution of these genes by analyzing the pedigree of IR24.

MATERIALS AND METHODS

Following the pedigree given by IRRI, 21 varieties related to IR24 such as IR8, Peta, and Cina (Fig. 1) were used to make test crosses with male sterile lines Er-Jiu-Nan 1A and Zhen Shan 97A.

According to the preliminary result of test crosses made in 1982, the seeds were collected from the restored combinations Er-Jiu-Nan 1A/IR24, -/Peta, -/Cina, -/SLO 17, and Zhen Shan 97A/IR24, -/Peta, -/Cina, -/SLO 17, and from the semirestored combinations Er-Jiu-Nan 1A/IR8, -/Latisail, -/CP-SLO, and Zhen Shan 97A/IR8, -/Latisail, -/CP-SLO. Seeds of topcross combinations were collected from Er-Jiu-Nan 1A/IR8/CP-SLO, IR8/Latisail, Latisail/CP-SLO, and Zhen Shan 97A/IR8/CP-SLO, IR8/Latisail, Latisail/CP-SLO. The seeds used for test crossing were sown in two groups on Hainan Island from December to April 1983 in accordance with their growth duration; the late maturing ones were sown earlier and the early maturing ones later so that each combination would flower as much as possible under similar climatic conditions. Twenty plants were grown for each cross at a spacing of 26 × 26 cm. One hundred to 150 plants were grown in paired rows for each F₂. The distance between each pair of rows was 60 cm.

At the heading stage, the size, color, and degree of dehiscence of the anthers was recorded for every plant. Completely sterile plants were distinguished from highly sterile plants by examining their pollen micro-

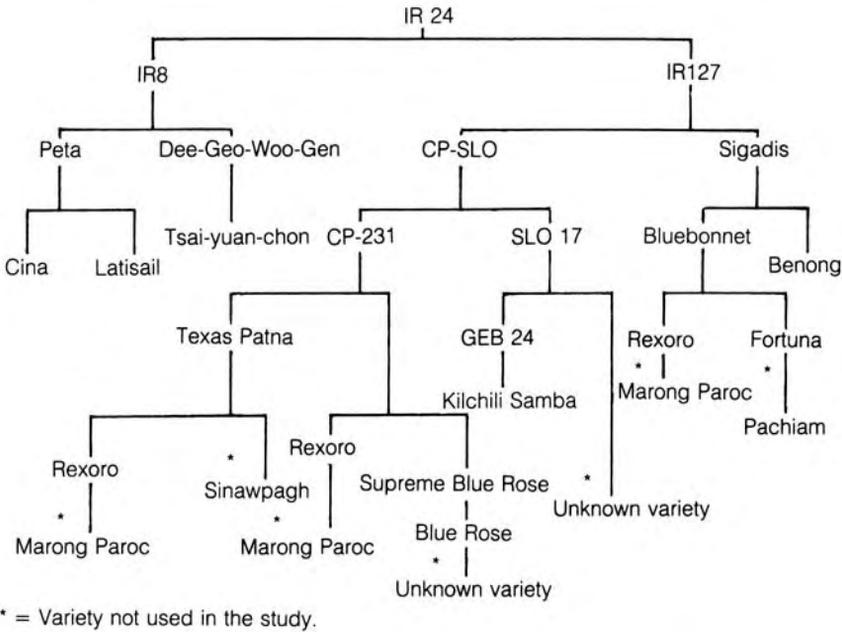


Fig. 1. The pedigree of IR24.

scopically. About 15–25 days after heading, three panicles were randomly collected from each plant to observe the seed set percentage. Every filled ovary was considered as seed set. The plants with varied fertility in the F₂ populations were classified and counted and the genotype of each parent was determined.

RESULT AND ANALYSES

Results of test crosses

The parents related to IR24 were test crossed with Er-Jiu-Nan 1A and Zhen Shan 97A (Table 1). When Peta, Cina, SLO 17, and IR24 were test crossed with Zhen Shan 97A the seed set percentage of the F₁ ranged from 81.3% to 86.9%, and when test crossed with Er-Jiu-Nan 1A the seed set percentage of the F₁ was 75% to 81.5%. Thus, Peta, Cina, SLO 17, and IR24 possessed similar restoring ability to the WA cytoplasm. The anthers of the hybrids dehisced normally, and more than 80% of the pollen was stained. When IR8, Latisail, and CP-SLO were test crossed, the seed set rate of F₁ ranged from 55.3% to 68.7%, indicating that their

Table 1. Spikelet fertility of the test cross F_1 involving WA type cytoplasmic male sterile lines and parental line in the development of the restorer IR24.

Male parent		Female parent		Anther and pollen characteristic of F_1	Remarks
Variety	Origin	Er-Jiu-Nan 1A	Zhen Shan 97A		
IR24	IRRI	81.5	86.9	anther dehiscent, stained pollen more than 80%	fertile
IR8	IRRI	63.3	68.7	anther partially dehiscent, about 60% stained pollen	partially fertile
Peta	Indonesia	75	81.3	anther dehiscent, stained pollen more than 80%	fertile
Cina	China	77.7	84.3	anther dehiscent, stained pollen more than 80%	fertile
Latisail	Bangladesh	57	61.2	anther dehiscent by pores, about 50% stained pollen	partially fertile
Dee-Geo-Woo-Gen	Taiwan	0 or 5–20		anther light yellow, a few dehiscent by pores and stained pollen	highly sterile
Tsai-Yuan-Chon	Taiwan	0 or 5–20		anther light yellow, a few dehiscent by pores and stained pollen	highly sterile
IR127-80-1-10	IRRI	0 or 10–50		anther light yellow, indehiscent; or yellow, dehiscent by pores, about 50% stained pollen	segregating in fertility
CP-SLO	USA	55.3	62.5	anther dehiscent by pores, about 50% stained pollen	partially fertile

Cont. Table 1.

CP 231		0	0	Typically abortive pollen, 0% stained pollen	sterile
Texas Patna	USA	0	0	anther light yellow, in- dehiscent, a few stained pollen	sterile
Rexoro	USA	0 or 1–15		anther light yellow, a few indehiscent by pores and stained pollen	highly sterile
Benong	Indonesia	0 or 5–20		anther light yellow, a few indehiscent by pores and stained pollen	highly sterile
Fortuna		0 or 5–20		anther light yellow, a few indehiscent by pores and stained pollen	highly sterile
Supreme Blue Rose	USA	0	0	typically abortive pollen, a few stained pollen	sterile
Blue Rose	USA	0 or 1–10		anther light yellow, a few dehiscent by pores and stained pollen	highly sterile
SLO 17		79	85.6	anther dehiscent, stained pollen more than 80%	fertile
GEB 24		0	0	anther light yellow, in- dehiscent, few small stained pollen	sterile
Kilchili Samba	Indonesia	0	0	typically abortive pollen	sterile
Sigadis	Indonesia	0	0	typically abortive pollen	sterile

restoring ability to the WA cytoplasm was not strong. The anthers of their hybrids were dehiscent by pores or partially dehiscent. When IR127 was test crossed, highly sterile plants and partially restored plants appeared in the F_1 . When the other varieties listed in Table 1 were test crossed, most of the F_1 plants were sterile or highly sterile. Hence, it might be concluded that the transmission of restoring genes to IR24 was mainly from IR8, Peta, Cina, Latisail, SLO 17, CP-SLO, and IR127 in the pedigree.

Fertility of F_2 and statistical analysis

Statistical analyses of the fertility of the F_2 plants of restored combinations and semirestored combinations were made to determine the genotype of each parent. In the parent experiment, the seed set percentage directly related to the fertility was used as the basis for classification of fertility while the expression of fertility in the field was also considered. The F_2 plants of restored combinations were grouped according to their seed set percentage (Table 2).

A plant whose seed set percentage was zero belonged to the completely sterile group, and a plant whose seed rate was higher than zero was classified as fertile. The sterile plants and fertile plants of each combination were approximately in the ratio of 1:15 (Table 2). Sterility/fertility has the characteristics of a qualitative character. Assuming that the male parents have two pairs of dominant restoring genes, the χ^2 was adopted to test the goodness of fit of the P value, which was >0.25 . This proved the hypothesis that fertility restoration is controlled by two pairs of genes.

Table 2 also suggests that the frequency distribution of the seed set percentage of fertile plants is continuous. Therefore, fertility also possesses the characteristics of a quantitative character. The seed set percentage of plants with varied fertility segregation from the F_2 was closely related to the color, size, and degree of dehiscence of their anthers. These plants were classified into four groups in accordance with the above characteristics:

- Completely sterile plants (S): anthers milky-white, thin, indehiscent, and containing typically abortive pollen or a few rounded abortive pollen grains and very few stained pollen grains. No self-fertilization occurred.
- Highly sterile plants (HS): anthers yellowish, rod-shaped, only a few anthers dehisced by pores, and 10–30% of pollen stained.

- Partially fertile plants (P): anthers yellow, smaller than normal dehiscent by pores or partially dehiscent, and about 50% of pollen stained.
- Normal fertile plants (F): anthers plumpy, bright yellow, dehiscent, and more than 80% of pollen normally stained.

The plants with varied fertility were arranged according to their seed set percentage. The frequency distribution of individual plants with varied fertility was counted. The results obtained were as follows:

- The fertile plants whose anthers dehisced normally had high seed set percentage.
- Most of the highly sterile plants whose anthers were dehiscent by pores had low seed set percentage.
- Most of the plants whose anthers were partially dehiscent were distributed between the above two.

The F_2 plants of restored combinations were grouped according to their seed set percentage and the expression of their fertility in the field (Table 3). The plants in each combination in a ratio of 1:3:3:9, and the χ^2 fitness test gave a P value of > 0.25 , which proved the hypothesis that IR24, Peta, Cina, and SLO 17 have two pairs of major restoring genes. Their genotype is $R_1 R_1 R_2 R_2$, and these two pairs of genes appear to be independent in inheritance.

The fertility segregation in F_2 plants of semirestored combinations was classified into sterile plants and partially fertile plants (including highly sterile plants). No plants with normally dehiscent anthers were found. The F_2 plants of these combinations were grouped according to their fertility (Table 4). The sterile plants and partially fertile plants were roughly in a ratio of 1:3. The χ^2 test for goodness of fit indicated $P > 0.25$ for each combination. Thus, the hypothesis that the partially restoring ability of IR8, Latisail, and CP-SLO is controlled by a pair of fertile genes was proved to be true.

Statistical analysis of topcross tests

IR8, Latisail, and CP-SLO had only partial restoring ability to WA cytoplasm. In order to explore whether there is an additive effect between their R genes, Zhen Shan 97A and Er-Jiu-Nan 1A were used to make topcrosses with the following three kinds of F_1 (IR127 was not used as a parent in the test due to its segregation for fertility): IR8/CP-SLO, IR8/Latisail, and Latisail/CP-SLO.

Table 2. F₂ segregation behavior of some test crosses with regard to spikelet fertility (%).

Cross	Grouping of seed set percentage												c ² 1:15	P value	
	0	0.1	10	20	30	40	50	60	70	80	90	100			
	Mid-value: No. of plants showing spikelet fertility											Total			
Er-jiu-nan 1 A/IR24	13	13	5	12	11	4	a	16	20	39	8	149	1.174	>	0.25
Zhen Shan 97A/IR24	12			7	3	2	5	6	16	40	55	146	0.675	>	0.25
Er-jiu-nan 1 A/Peta	8	10	9	3	6	4	10	11	17	15	2	95	0.438	>	0.5
Zhen Shan 97A/Peta	8	4	4	1	2	4	6	7	12	25	25	98	0.327	>	0.5
Er-jiu-nan 1 A/Cina	9	4	7	7	7	7	8	8	9	17	15	98	0.978	>	0.25
Zhen Shan 97A/Cina	4	1	1	4	3	6	4	6	10	31	27	97	0.430	>	0.5
Er-jiu-nan 1 A/SLO 17	3		1	1	1	3	5	6	21	29	29	99	1.246	>	0.25
Zhen Shan 97A/SLO 17	5		4	2	6	2	7	7	18	32	14	97	0.055	>	0.75

Table 3. F₂ segregation for spikelet fertility in some test crosses involving restorers.

Cross	Grouping of fertility of plants ^a				χ ² 1:3:3:9	P value
	Completely sterile (S)	Highly sterile (HS)	Partially fertile (P)	Fertile (F)		
Er-Jiu-nan 1 A/IR24	13	30	23	83	2.946	> 0.25
Zhen Shan 97A/IR24	12	23	30	81	1.870	> 0.5
Er-jiu-nan 1 A/Peta	8	22	20	45	3.302	> 0.25
Zhen Shan 97A/Peta	8	21	19	50	1.441	> 0.5
Er-jiu-Nan 1 A/Cina	9	18	22	49	2.747	> 0.25
Zhen Shan 97A/Cina	4	19	16	58	1.217	> 0.5
Er-jiu-Nan 1 A/SLO 17	4	16	21	58	1.545	> 0.5
Zhen Shan 97A/SLO 17	5	21	17	54	0.703	> 0.75

^aSeed set of S 0%, HS 1-30%, P 31-75%, F 76-100%.

Table 4. F₂ segregation for spikelet fertility in some test crosses involving partial restorers.

Combination	Sterile plant (S)	Partially fertile plant			c ² 1:3	P value
		(HS)	(P)	Total		
Er-jiu-Nan 1 A/IR8	30	13	57	70	1.080	> 0.25
Zhen Shan 97A/IR8	21	17	62	79	0.658	> 0.25
Er-jiu-Nan 1 A/Latisail	21	56	21	77	0.489	> 0.25
Zhen Shan 97A/Latisail	20	47	31	78	0.871	> 0.25
Er-jiu-Nan 1 A/CP-SLO	19	51	25	76	1.015	> 0.25
Zhen Shan 97/CP-CLO	22	49	29	78	0.333	> 0.5

Table 5. Segregation for spikelet fertility in some topcrosses involving cytoplasmic male sterile lines and intercrosses among partial restorer lines.

Grouping of seed set percentage												
	0	0.1	10	20	30	40	50	60	70	80	90	100
	Mid-value											
	5	15	25	35	45	55	65	75	85	95		
Cross	Number of plants											
Er-jui-nan 1A//IR8/CP-SLO				2	4	16	25	31	17	3		
Zhen Shan 97A//IR8/CP-SLO				1	4	15	24	25	22	6		
Er-jiu-nan 1 A//IR8/Latisail					4	16	17	25	6			
Zhen Shan 97A//IR8/Latisail						11	14	24	12	4		
Er-jiu-nan 1 A//Latisail/CP-SLO			4	6	18	13	13	7				
Zhen Shan 97N/ Latisail/CP-SLO			2	7	21	29	21	12				
Completely sterile	highly sterile			partially fertile					fertile			

The fertility of offspring of topcrosses was observed as follows: The anthers of most plants were partially dehiscent, and those of a few plants were dehiscent by pores. But some plants with normally dehiscent anthers were found in Er-Jiu-Nan 1A//IR8/CP-SLO, Zhen Shan 97A//IR8/CP-SLO, and Zhen Shan 97A//IR8/Latisail. Each plant of all the combinations was examined for its seed set percentage; the results are shown in Table 5.

It was observed that the three topcross combinations mentioned above had a certain number of progeny that had high seed set percentage, and their seed set percentage was significantly higher than that of the F_1 of their parents. This basically confirmed the earlier observation that some plants had normal dehiscent anthers. But no plants with high seed set percentage were found in the offspring of Er-Jiu-Nan 1A//Latisail/CP-SLO and Zhen Shan 97A//Latisail/CP-SLO, and the seed set percentage of most plants was only near to that of the F_1 of their parents.

Statistical analysis that the average seed set percentage of Zhen Shan 97A//IR8/CP-SLO was 71.3%, that of Zhen Shan 97A//IR8/Latisail, 72.5%, and that of Zhen Shan 97A//Latisail/CP-SLO only 55.4%. Similar results were obtained when Er-Jiu-Nan 1A was used for test crossing (Table 6).

The results obtained from topcrosses indicated that there was no additive effect in fertility between Latisail and CP-SLO; in other words, they may have the same restoring genes ($R_2 R_2$). But when they were respectively crossed with IR8, the additive effect in fertility was obvious. IR8 probably has a pair of restoring genes ($R_1 R_1$) different from the genes present in Latisail and CP-SLO ($R_2 R_2$). Thus, the F_1 plants of

Table 6. Statistical analysis of some characteristics of plants of topcrosses.

Cross	No. of plants	Mean value of seed set percentage	Standard deviation	Covariance
Er-jiu-nan 1A//IR8/CP-SLO	98	69.5	12.7	18.3
Zhen Shan 97A//IR8/CP-SLO	97	71.3	13.2	18.5
Zhen Shan 97A//IR8/CP-SLO	68	66.9	10.8	16.2
Zhen Shan 97A//IR8/Latisail	65	72.5	11.3	15.6
Er-jiu-nan 1A//Latisail/CP-SLO	61	52.5	13.3	25.4
Zhen Shan 97A//Latisail/CP-SLO	92	55.4	12.2	22.1

IR8/CP-SLO and IR8/Latisail could produce pollen containing (R_1R_2) genes through genetic recombination. It may be seen in Tables 1 and 3 that the restoring ability of R_1R_1 seemed to be stronger than that of R_2R_2 .

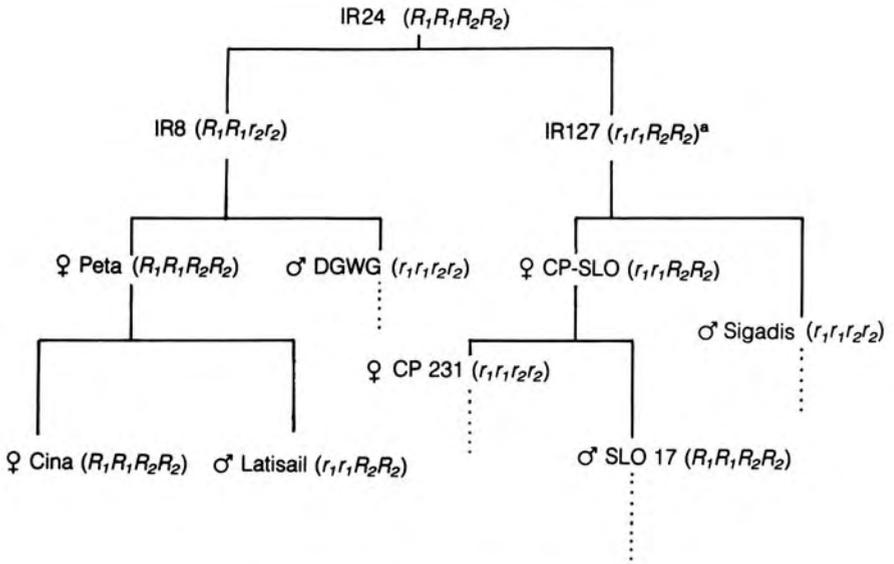
The genotypes of parents related to IR24 are shown in Figure 2. It is clear that one pair of restoring genes (R_1R_1) in IR24 comes from Cina, a late indica variety in China, and another pair of restoring genes (R_2R_2) is from SLO 17, which is related to some varieties in India. The restoring genes (R_1R_1) of Cina are inherited in IR8 through Peta, and the restoring genes (R_2R_2) of SLO 17 are inherited in IR127 through CP-SLO. R_1R_1 is combined with (R_2R_2) by crossing IR8 with IR127, and IR24 ($R_1R_1R_2R_2$), a strong restorer line possessing two pairs of restoring genes is developed. Both Cina and SLO 17 are also strong restorer lines possessing two pairs of restoring genes. While transferring R_1R_1 or R_2R_2 through hybridization, Cina or SLO 17 each gives up another pair of restoring genes. This process is shown in Figure 3.

DISCUSSION

Among the parents in the pedigree of IR24, the varieties with restoring ability may be divided into three kinds.

- Varieties with strong restoring ability: They possess two pairs of major genes ($R_1R_1R_2R_2$) and may also have modifier genes. Their restoring ability is rather stable.
- Varieties with semirestoring ability: They possess two pairs of major genes (R_1R_1 or R_2R_2), and modifier genes may also be present. Their restoring ability is easily affected by the environment, but when they are crossed with a male sterile line with good fertility restoration (such as Zhen Shan 97A) under optional climatic conditions, their F_1 hybrids may produce a normal seed set.
- Varieties with weak restoring ability: They do not possess major genes, but may have modifier genes for fertility. Among the above three kinds of varieties, the first kind is of great value and the second kind should not be used in production.

Our study has revealed the source and evolution of the two pairs of major restoring genes in IR24 through analysis of the parents in the pedigree of IR24. These two pairs of restoring genes are independently inherited. They can either be separated from each other or combined together. This basis may be utilized to select and breed restorer lines and expand the scope of the R-source used. At present, there are only a few



^a $r_1r_1R_2R_2$ and $r_1r_1r_2f_2$ probably exist in IR127.

Fig. 2. The pedigree of IR24 in relation to R genes.

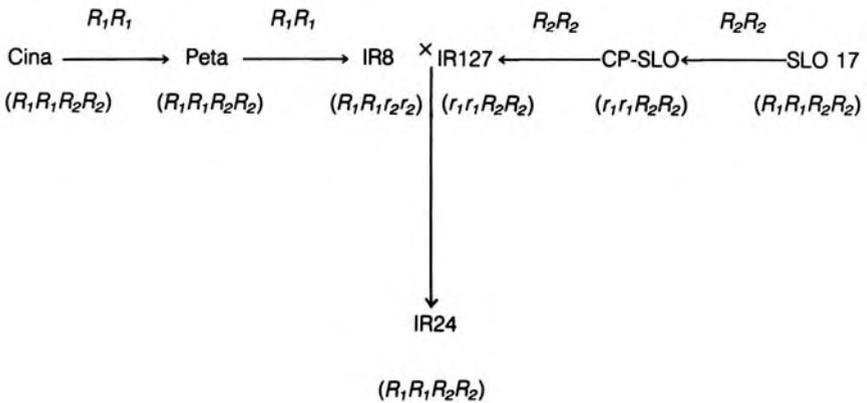


Fig. 3. Transmission pattern of two pairs of genes in IR24.

varieties that possess strong restoring ability to the WA cytoplasm. But many semirestoring varieties have been found. These semirestoring varieties may possess $R_1 R_1$ or $R_2 R_2$ genes and exhibit good agronomic characters and stress resistance. Therefore, selection of an R source should be made not only among a few strong restorer lines, but also among many semirestoring lines. When crosses are made between semirestoring lines it is possible to integrate the good agronomic characters with stress resistance and combine $R_1 R_1$ with $R_2 R_2$ for breeding strong restorer lines with various good characters. When a strong restorer line is crossed with a semirestoring variety with good characters, desirable results can also be obtained. But, in such crosses semirestoring lines may also appear in the offspring. Usually, the restoring ability is used as a main standard for screening of restorer lines. As a result, those advanced lines with good characters and better combining ability but poor restoring capacity have to be discarded. In fact, $R_1 R_1$ and $R_2 R_2$ in these promising lines may be combined by sib mating; thus strong restorer lines can be developed. And as the agronomic characters of these sister lines are very similar, stable characters can easily be obtained after sib mating. How to distinguish the genotype $R_1 R_1$ from the genotype $R_2 R_2$ and whether there are internal relationship between partially dehiscent anthers and $R_1 R_1$ as well as between dehiscent anthers by pores and $R_2 R_2$ are still problems to be studied further. Correct identification of the morphological characteristics of the genotypes $R_1 R_1$ and $R_2 R_2$ is a guide for selecting parents more efficiently.

It is necessary to understand correct methods for the identification of fertility in the study of inheritance of fertility of hybrid rice. A simple character (such as seed set percentage) is not enough to distinguish accurately the kinds of fertility. The seed set percentage and the morphological characteristics of anthers and pollen grains must be used together as the best indicators for estimations of fertility.

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CURRENT KNOWLEDGE OF AND OUTLOOK ON CYTOPLASMIC-GENETIC MALE STERILITY AND FERTILITY RESTORATION IN RICE

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Cyto-sterile rice lines developed so far have been classified into four different groups. However, only one system (WA or S_2) has been used to develop rice hybrids. The identification and the use of additional sources of cyto-sterility are considered very important to prevent vulnerability of hybrid rice to disease or insect epidemics. Cyto-sterility systems in rice are known to be gametophytic or sporophytic in nature. Their pollen abortion pattern is different cyto-histologically. The pollen abortion factor is believed to be present within the pollen grains in gametophytic cytoplasmic male sterile (CMS) lines, while in sporophytic CMS lines pollen abortion occurs due to the persistence and hypertrophy of tapetal cells. A number of effective restorers for some cyto-sterile lines are available. Fertility restoration appears to be under digenic control wherein one gene is stronger than the other. A sporophyte-gametophyte gene interaction model has been proposed to interpret some results obtained on the genetics of fertility restoration. Further studies aim to diversify the cyto-sterility systems and clarify the molecular basis of CMS and fertility restoration.

The identification and development of cytoplasmic genetic male sterile and restorer lines was a major step in the success of hybrid rice technology in China. The role of cytoplasm in causing male sterility in rice was first reported in 1954 (18, 28). Later Katsuo and Mizushima (12) also observed similar phenomenon in the progeny of the first backcross *Oryza sativa* f. *spontanea*/*O. sativa* cv. Fujisaka 5².

The first cytoplasmic male sterile (CMS) line (designated BT) in cultivated rice was developed by Shinjyo and Omura (23) from the cytoplasmic source of Chinsurah Boro II variety. Subsequently, a number of CMS lines of japonica rices were developed in the US (2, 7) from a cytoplasmic source of indica variety Birco (PI 297120).

Pankhari 203A, an indica CMS rice, was developed at IRRI by substituting the nuclear genes of Pankhari 203 into the cytoplasm of the

semidwarf indica variety Taichung Native 1. The first CMS line used to develop commercial F₁ rice hybrids was developed in China in 1973 from a male sterile plant occurring naturally in a wild rice population of *Oryza sativa* f. *spontanea* on Hainan island in 1970 (9, 32). This plant was designated wild rice with aborted pollen (WA). Since then, a number of CMS lines have been developed in China from various wild and cultivated rice accessions (14). Male sterility inducing cytoplasmic systems have also been identified in various geographical forms of *O. perennis* (17).

The practical use of cytoplasmic-genetic male sterility in developing hybrid varieties in grain crops is possible only when the effective restorer lines are identified and/or developed. Shinjyo (19, 20, 21) identified a restorer gene in the rice variety Chinsurah Boro II, the source of BT cytoplasm. Since then a number of effective restorer lines have been identified for different cyto-sterile lines 11, 14, 22).

The knowledge and understanding of cytoplasmic-genetic male sterility and the fertility restoration system help plant breeders to use these genetic tools more effectively to develop F₁ hybrids. This paper summarizes the current knowledge of and outlook on cytoplasmic-genetic male sterility and fertility restoration systems in rice.

AVAILABLE SOURCES OF CYTO-STERILITY IN RICE

Virmani and Edwards (25) listed 19 sources of cytoplasmic-genetic male sterility in rice reported in the literature. More than 100 CMS lines from indica and japonica backgrounds derived from five of these sources [naturally occurring wild rice with aborted pollen (WA), *O. sativa* f. *spontanea*, Chinsurah Boro II (BT type), Gambiaca (Gam type), and O-Shan-Tao-Bai] are currently available in China. However, only one source (viz., WA type) is extensively used, covering about 90% of the hybrid rice area in China (14). This situation makes hybrid rice in China potentially vulnerable to disease or insect epidemics.

CLASSIFICATION OF AVAILABLE CMS SOURCES

Lin and Yuan (14) classified the male sterile lines from China into three basic groups according to genetic properties and the relation between restorer and maintainer lines.

Among the various sources of cyto-sterility, CMS lines derived from the WA system have been found to be the most stable for their complete or nearly complete pollen sterility (14, 26).

At IRRI, we classified six CMS lines into three groups based on the shape and staining pattern of their pollen grains (3, 31). We related pollen staining pattern with the stage at which pollen abortion occurred.

Young et al (31) determined the cytogenic relationships among six CMS lines of rice, viz., Zhen Shan 97A, V20A (WA cytoplasm), Yar Ai Zhao A (Gambica cytoplasm), Pankhari 203A (Taichung Native 1 cytoplasm), Wu 10A (BT cytoplasm), and MS 577A (*O. sativa* f. *spontanea* cytoplasm). They classified the six CMS lines into four groups, and their cytoplasm have been designated tentatively as below:

Wu 10A/P 203A	S ₁ (earlier designated BT)
97A/V20A	S ₂ (earlier designated WA)
Yar Ai Zhao A	S ₃ (earlier designated Gam)
MS 577A	S ₄ (not designated earlier)

It would be useful to standardize the system of nomenclature of cytoplasm inducing male sterility in rice.

IDENTIFICATION OF ADDITIONAL CMS SOURCES

In order to diversify sources of CMS in rice, research is in progress in China, at IRRI, and elsewhere to identify additional CMS sources. At IRRI we have obtained a highly sterile BC₄F₁ progeny from the cross ARC 13829-26/IRI0179-2-3-1 (Table 1), suggesting that ARC 13829-26 does possess sterility inducing cytoplasm. However, we need to determine whether this source of cyto-sterility is the same as or different from those identified earlier. The breeding line IR10179-2-3-1 is also a maintainer of the WA (S₂) cyto-sterility system, and a CMS line in its genotype has been developed from cyto-sterile Zhen Shan 97A and designated IR46828A.

TRANSFER OF WA CMS SYSTEM IN ELITE RICE VARIETIES

In order to develop suitable CMS lines adapted to the tropics, we have been transferring the WA cyto-sterility system into elite rice varieties and breeding lines identified as maintainers using the backcross method of breeding. The experience to date (Fig. 1) has indicated that:

- certain genotypes tend to increase pollen sterility with successive backcrossing, with occasional decline in sterility in a BC generation, e.g., Iri 356, Suweon 310, IR19657-34-2-2-3-3, IR17492-18-2-2-2, and IR21845-90-3;
- certain genotypes (viz., BG 12-1, IET 3257, IR17525-278-1-1-2,

Table 1. Pollen fertility behavior of F₁ to BC₄F₁ generation plants from the cross ARC 13829-26/IR10179-2-3-1.

Generation	No. of plants with pollen fertility reaction ^a					Total
	HS	S	PS	PF	F	
F ₁	0	0	10	0	0	10
BC ₁ F ₁	0	3	5	10	0	18
BC ₂ F ₁	7	7	9	4	0	27
BC ₃ F ₁	44	1	0	0	0	45
BC ₄ F ₁	38	7	3	0	0	48

^aHS = highly sterile, S = sterile, PS = partially sterile, PF = partially fertile, F = fertile.

IR19661-3-2-2-3-1, Soekwang, BG 379-4) are difficult to sterilize because, even after seven to nine backcrosses, the frequency of completely pollen sterile plants is less than 100%; and

- certain genotypes, viz., PAU 269-1-8-4-1-1 and IR27301-62-2-2, are easy to sterilize, since completely pollen sterile plants occur at high frequency (90–100%) after one to three backcrosses.

The continuous occurrence of some incompletely pollen sterile plants even after five to nine backcrosses is not clearly understood. It may be due to the presence of some minor genes for fertility restoration present in the so-called maintainer genotypes.

During the past two years, we have developed some new CMS lines of rice that are better adapted to the tropics than the Chinese CMS lines V20A and Zhen Shan 97A. The new CMS lines are relatively less susceptible to major diseases and insects in the tropics compared with V20A and Zhen Shan 97A. Agronomic and floral traits of the new CMS and maintainer lines are summarized in Table 2.

ENVIRONMENTAL INFLUENCE ON MALE STERILITY

We planted a number of CMS lines at IRRI during different months and monitored their pollen sterility. The results (Table 3) indicate that pollen sterility of certain CMS lines, viz., IR46826A, IR46827A, IR46829A, and IR46831A, is affected by the date of seeding, while other lines, viz., IR46828A and IR46830A, are stable for complete pollen sterility and remain unaffected by environmental influence. The seasonal effect may also be the cause of variable expression of pollen sterility in backcross

Table 2. Agronomic and floral traits of Zhen Shan 97A and new CMS lines developed at IRRI, dry season 1984.

Line	Pedigree	Height at maturity (cm)	No. of panicles per hill	Total no. of spikelets/ panicle	Grain		1000 grain weight (g)	Anthesis (min)	Duration of floret opening (min)	Stigma exsertion (%)	Stigma length (mm)	Anther length (mm)
					Length (mm)	Width (mm)						
IR46826A	97A/8*IR10154-23-3-3	56	42	64				435	203	32	1.46	
IR468266	IR10154-23-3-3	66	27	70	8.31	3.14	21.5	77	72	21		1.94
IR46827A	97A/7*IR10176-24-6-2	56	46	49				306	153	15	1.52	
IR46827B	IR10176-24-6-2	64	31	47	8.84	3.05	23.1	74	52	20		2.10
IR46828A	97A/7*IR10179-2-3-1	53	37	91				426	190	31	1.72	
IR46828B	IR10179-2-3-1	67	25	74	9.31	2.56	17.1	92	68	51		1.91
IR46829A	V20A/IR19792-18-2-3-3	65	39	88				364	216	17	1.78	
IR46829B	IR19792-18-2-3-3	79	22	85	8.83	3.21	25.8	82	68	33		1.99
IR46830A	V20A/6*IR19807-21-2-2	62	39	89				397	223	23	1.45	
IR46830B	IR19807-21-2-2	75	32	81	9.25	2.69	23.5	67	87	60		2.12
IR46831A	V20A/6*Jikkoku Seranai	63	44	109				432	180	11	1.47	
IR46831B	Jikkoku Serania	74	42	81	8.57	2.80	19.4	114	65	4		2.15
Zhen Shan 97A		66	15	144				361	352	49	1.65	
Zhen Shan 97B		81	13	135	8.47	3.44	26.0	82	50	17		2.20

Table 3. Effect of planting date on the sterility of CMS lines.

Line	Parentage	Seeding date											
		11 June 1984				16 July 1984				12 December 1984			
		No. of plants observed	% of plants showing ^a			No. of plants observed	% of plants showing			No. of plants observed	% of plants showing		
HS	S		PS	HS	S		PS	HS	S		PS		
IR46826A	97A/10*IR10154-23-3-3	40	20	42	38	39	77	18	5	23	65	30	5
IR46827A	97A/9*IR10176-24-6-2	29	65	35	0	39	100	0	0	33	15	67	18
IR46828A	97A/9*IR10179-2-3-1	44	100	0	0	46	100	0	0	35	100	0	0
IR46829A	V20A/8*IR19792-15-2-3-3	30	67	27	6	23	100	0	0	27	93	7	0
IR46830A	V20A/8*IR19807-21-2-2	30	100	0	0	13	100	0	0	37	100	0	0
IR46831A	V20A/8*Jikkoku Seranai	29	97	3	0	27	96	4	0	43	60	30	10

^aHS = highly sterile, S = sterile, PS = partially sterile.

generations (Fig. 1). Athwal and Virmani (1) also reported the effect of time of planting on the expression of pollen sterility in rice.

AVAILABLE SOURCES OF FERTILITY RESTORATION

Effective restorer lines for WA, Gam and BT cyto-sterility systems have been identified among cultivated rice varieties and elite breeding lines (14, 19, 20, 21, 22, 25, 29). Effective restorer lines for cyto-steriles Pankhari 203A and MS 577A have not yet been identified.

A large number of effective restorer lines for WA cyto-sterile lines have been identified in China and at IRRI (9, 10, 14). The frequency of restorer lines is higher in China among rice varieties originating in lower latitudes than among varieties from the Yangtze Valley (L. P. Yuan, personal communication). The frequency of restorer lines was even less among varieties from Northern China, Eastern Europe, Japan, and Korea. About 20% of the rice varieties and breeding lines developed at IRRI have been found to possess effective restoration ability of WA cyto-sterile lines. This makes the task of developing F₁ hybrids in rice relatively easier than doing so in wheat. The frequency of restorer lines

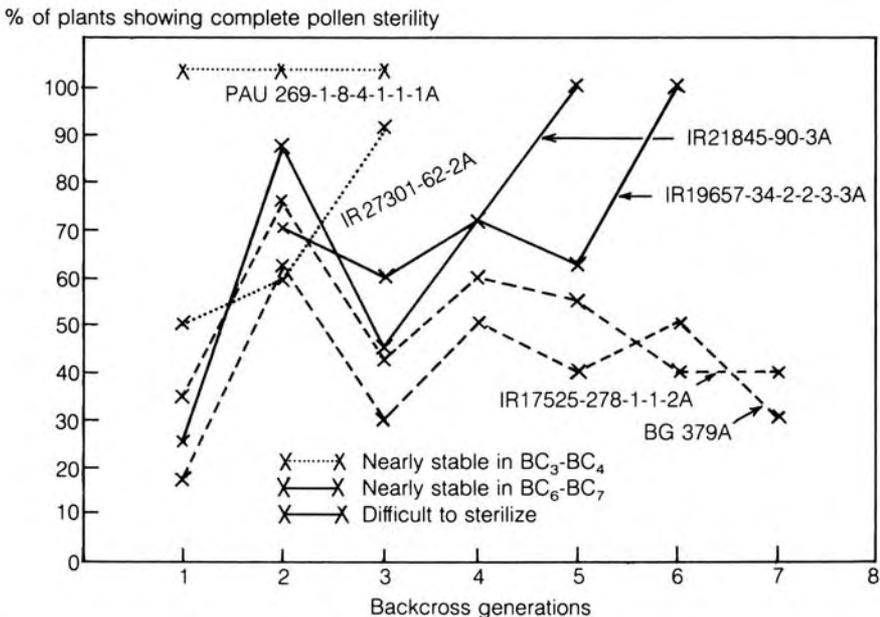


Fig. 1. Response of maintainer genotypes to sterilization using backcross method.

among japonica varieties is negligible (14, 22); consequently japonica F_1 rice hybrids in China have been bred by transferring restorer gene(s) into the male parents from indica rice. A number of Tongil rice varieties (indica/japonica derivatives) from South Korea have been found to be effective restorers. Their fertility restoration ability may be attributed to the gene(s) inherited from the indica parent.

The restorer lines used widely in the development of commercial rice hybrids in China are mostly introduced from IRRI (14, 25). Additional restorers are being selected from IRRI elite breeding lines that possess multiple disease and insect resistance to be used in developing hybrid rices for the tropics and subtropics.

EFFECTIVITY OF FERTILITY RESTORATION

The fertility restoration ability of some rice varieties and breeding lines was tested by crossing these to more than one WA CMS line. We observed that certain restorer lines, viz., IR13292-5-3-1, IR13299-96-2-2, IR13429-150-3-2-1-2, IR19058-107-13, IR36, IR54, and BR 10, are more effective than others, viz., IR13525-118-3-2-2-, IR42, IR56, Iri 360, IR18348-36-3-3, IR18350-93-2, BG 400-1, BPI Ri 4, and Suweon 318.

The lower effectivity of restorer lines in relation to CMS lines may be due to the presence of excess sterility nuclear genes in the female parent, which could act as inhibitors of pollen fertility restoration in the F_1 generation. The more effective restorer lines would, therefore, possess the whole spectrum of restorer genes required for complete fertility restoration. Besides, the CMS lines in those crosses may be providing some minor fertility genes that act in a complementary or additive fashion with restorer genes.

GENETICS OF FERTILITY RESTORATION

Shinjyo (19, 22) found that the restoration ability for BT cyto-sterile lines was controlled by one gene, and the action of this gene was gametophytic. Therefore, the hybrid between the BT male sterile line and its restorer showed 50% pollen fertility. Watanabe et al (27) reported a different restorer gene for a CMS line derived from the Burmese rice variety Lead. This restorer gene did not restore the fertility of CMS lines of BT and *spontanea* type. On the other hand, the restorer gene of BT cytoplasm was found to restore Lead CMS also, but not that of *spontanea* CMS.

Table 4. Segregation pattern for pollen fertility in some crosses involving WA cyto-sterile lines and their restorers.

Generation or genotype	Total no. of plants observed	Pollen fertility reaction ^a			Probability
		Fertile	Partially fertile	Sterile	
MR 365A (P ₁)	12			12	
IR54 (P ₂)	10	10			
MR 365A/IR54 (F ₁)	12	12			
MR 365A/F ₁ (BC)	40	20	10	10	P > 0.99 (2:1:1)
MR 365A/IR54 (F ₂)	259	191	53	15	P = 0.95–0.75 (12:3:1)
V20A (P ₁)	10			10	
IR9761-19-1 (P ₂)	10	10			
V20A/IR9761-19-1 (F ₁)	10	10			
V20A/F ₁ (BC)	68	18	35	15	P = 0.95–0.90 (1:2:1)
V20A/IR9761-19-1 (F ₂)	256	150	92	14	P = 0.99–0.95 (9:6:1)
97A (P ₁)	10			10	
IR42 (P ₂)	10	10			
97A/IR42 (F ₁)	10	10			
97A/F ₁ (BC)	52	11	15	26	P = 0.75–0.50 (1:1:2)
97A/IR42 (F ₂)	224	123	49	52	P = 0.50–0.25 (9:3:4)

^aFertile = 60–100%, partially fertile = 1–60%, sterile = 0%.

The mode of action of restorer genes with WA cytoplasmic type is sporophytic because the F₁ between the CMS line and the restorer shows high pollen fertility. Govinda Raj and Siddiq (8) reported monogenic, digenic, and trigenic segregation for fertility restoration in WA cyto-sterile lines. However, this study had limitations because the results were based on spikelet fertility only; pollen fertility was not monitored. Tiangli et al (24) found that the restoration ability of restorer lines IR24 and IR26 was controlled by two dominant genes. Similar conclusions were drawn by Ren-Cui and Hao-Ran (16) for IR24 crossed with another WA cyto-sterile line V41A. At IRRI, Young and Virmani (30) found that fertility restoration of IR54, an effective and stable restorer of WA cyto-sterile lines, was controlled by two dominant genes. One of the two genes appeared more effective than the other.

Recently, we studied the inheritance of fertility restoration on WA

cytoplasm in three different crosses involving restorers IR54, IR9761-19-1, and IR42. F_2 and backcross segregation data (Table 4) again indicated that the pollen fertility restoration ability of these restorers was governed by two independent and dominant genes. The mode of action of the two genes, however, varied in the three crosses. For example, the cross MR 365A/IR54 indicated dominant epistasis (BCF_1 ratio 2:1:1 and F_2 ratio 12:3:1), the cross Zhen Shan 97A/IR42 showed recessive epistasis (BCF_1 ratio 1:1:2 and F_2 ratio 9:3:4), and cross V20A/IR9761-19-1 showed semi-epistasis (BCF_1 ratio 1:2:1 and F_2 ratio 9:6:1). This variation in the behavior of fertility restoring genes could be due either to the presence of different restoring genes in the three restorers studied or to differential penetrance and expressivity of these restorer genes depending on the nuclear genotype of the female parent. Studies are in progress to ascertain the allelic relationships between the restoring genes in the three restorers.

In all the studies on genetics of fertility restoration in WA cyto-sterile lines, partially sterile and partially fertile plants are known to occur with high frequency in the F_2 and BC generations. This continuous variation with regard to pollen fertility is generally attributed to modifier genes and environmental influence. We hypothesize the occurrence of partially fertile/sterile plants as a result of sporophyte-gametophyte gene interaction (see next section).

PROPOSED SPOROPHYTE-GAMETOPHYTE GENE INTERACTION MODEL FOR FERTILITY RESTORATION

The cyto-histological studies have indicated abnormal behavior of the tapetum, a sporophytic tissue, as the cause for pollen abortion in male sterile plants of various crops (13). In rice, the cyto-histological basis of pollen abortion in sporophytic (e.g., WA and *O. rufipogon* type) and gametophytic (e.g., BT type) CMS lines is quite distinct (4,5,6,15). In sporophytic WA CMS lines, the pollen abortion occurred after meiosis on account of disorganization of tapetal cells (tapetal hypertrophy). In gametophytic CMS lines, the behavior of the tapetum and other anther wall tissues was normal, and factors within the developing pollen grains were responsible for pollen abortion (6).

Our model, therefore, assumes that some substance(s) essential for normal pollen development is produced in the anther wall tissue that, in interaction with the developing pollen grains, determines the fertility of the mature pollen grains. The gene(s) responsible for the production of the substance(s) is assumed to be active in the anther wall tissue only.

This substance(s), when it interacts with pollen grains carrying the appropriate gene(s), results in fertility of the pollen grains. It is a kind of interaction between sporophytic anther wall tissue and the gametophytic developing pollen grains. In the CMS plant the model assumes that the substance(s) is not produced, since it has an altered organelle genome and does not possess genes in the nucleus to correct the defect, while in the maintainer the substance(s) is produced because of the presence of the normal organelle genome, which probably is similar to the restorer genes in action.

This proposed model can be explained using the F_2 segregation ratio of 9:6:1 fertile to partially fertile to sterile obtained in the cross V20A/IR9761-19-1 as an example. Assuming that there are three genes involved in the fertility restoration of V20A/IR9761-19-1, with Rf_1 acting at the sporophytic level, being responsible for producing the substance(s) in the anther wall tissue, and interacting with the other two genes Rf_2 and Rf_3 , which act at the gametophytic level, the mode of action of these three genes is as follows: Rf_1 is assumed to be basic, as it produces the substance(s) responsible for normal pollen development that interacts with Rf_2 or Rf_3 present in the pollen grains to confer complete fertility. Partial fertility is conferred by the presence of Rf_1 alone or, in its absence, by the presence of either Rf_2 or Rf_3 . On the other hand, when all the three genes are recessive the plant would be completely sterile (Table 5). This model explains the occurrence of the partially fertile/sterile class and shows how the interaction of three genes (one sporophytic and two gametophytic in action) can result in a phenotypic segregation ratio similar to a digenic ratio such as 9:6:1. The hypothesis, however, needs to be tested experimentally.

OUTLOOK

More than 90% of the CMS lines of indica rice hybrids cultivated in China belong to the WA system. Although a direct relationship between an S cytoplasm and susceptibility to a major disease/insect has not been established so far, the extensive use of a single source of cytoplasm makes the hybrid rice in China potentially vulnerable to a disease or insect epidemic. It is therefore essential to diversify the cyto-sterility sources used in hybrid rice development. Both interspecific and intraspecific hybridization would be useful in this context. Research should also be intensified to determine any linkage relationship between a cyto-sterility factor and disease or insect susceptibility.

The cytoplasmic male sterility in some crops, viz., maize and sorg-

Table 5. Explanation of the F₂ ratio of 9:6:1 fertile to partially fertile to sterile obtained in the cross V20A/IR9761-19-1 based on proposed sporophyte-gametophyte gene interaction model.

Line/generation	Cytoplasm ^a	Genotype in		Phenotype
		Anther wall tissue	Pollen grains	
CMS line (V20A)	S	<i>rf₁rf₁</i>	<i>rf₂rf₃</i>	sterile
V20B	N	<i>rf₁rf₁</i>	<i>rf₂rf₃</i>	fertile
Restorer (IR9761-19-1)	S/N	<i>Rf₁Rf₁</i>	<i>Rf₂Rf₃</i>	fertile
V20A/IR9761-19-1 F ₁	S	<i>Rf₁rf₁</i>	<i>Rf₂Rf₃</i> <i>Rf₂rf₃</i> <i>rf₂Rf₃</i> <i>rf₂rf₃</i>	fertile
V20A/IR9761-19-1 F ₂	S	1 <i>Rf₁rf₁</i>	<i>Rf₂Rf₃</i>	1 fertile
			<i>Rf₂rf₃</i>	1 fertile
			<i>rf₂Rf₃</i>	1 fertile
		2 <i>Rf₁rf₁</i>	<i>rf₂rf₃</i>	1 partially fertile
			<i>Rf₂Rf₃</i>	2 fertile
			<i>Rf₂rf₃</i>	2 fertile
			<i>rf₂Rf₃</i>	2 fertile
		1 <i>rf₁rf₁</i>	<i>rf₂rf₃</i>	2 partially fertile
			<i>Rf₂Rf₃</i>	1 partially fertile
			<i>Rf₂rf₃</i>	1 partially fertile
		<i>rf₂Rf₃</i>	1 partially fertile	
		<i>rf₂rf₃</i>	1 sterile	

9F:6PF:1S

^aS = sterile, N = fertile.

hum, is known to be determined by the mitochondrial genome. Our understanding of the molecular basis of cytoplasmic genetic male sterility and fertility restoration in rice is very limited. With a clear understanding of the CMS mechanism, the search for diverse male sterile cytoplasm(s) and perhaps the donor(s) for their restorer genes would become expeditious. Characterization of the organellar genome of CMS lines may also lead to the identification of plasmid transposon-like elements. These findings may then be employed in the development of a vector system for the improvement of rice through directed gene transfer.

Developments in protoplast fusion and cell culture would also be helpful to transfer the available cyto-sterility system(s) expeditiously with different nuclear genotypes.

Genetic analysis of fertility restoration in BT and WA cyto-sterile lines has been accomplished to a certain extent. CMS lines involving both gametophytic and sporophytic systems are available in rice. The sporophytic-gametophytic gene interaction model we propose needs to be tested, and the genes involved in controlling these systems need to be identified and located on specific chromosome(s) using trisomic stocks. A clear understanding of the genetic mechanism of restoration ability in rice should enable incorporation of the restorer genes in the desired breeding lines more effectively.

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INHERITANCE OF FERTILITY RESTORATION OF CYTOPLASMIC MALE STERILITY IN INDICA RICE

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Two indica rice crosses between Zhen Shan 97 (WA)A and restorer lines were used in this study. The F_1 's that were fertile had a normal stainable pollen ratio. The spikelet fertility of the F_2 's segregated in a ratio of 3:1, indicating that the action of fertility restoration is sporophytic and that at least a single dominant gene is involved. Linkage between the fertility restorer and the color of the lemma and palea had a recombination value from 9.7% to 27.1%. No *Rf* plant or line that restored the Reimei (*rufipogon*) A cytoplasmic male sterility was recovered in the BC_1 , and F_5 of Reimei (*rufipogon*) A/IR5032-6B-13-1. The F_1 of this cross showed a degree of fertility restoration. When these F_5 lines were test crossed with Zhen Shan 97A, various degrees of fertility restoration occurred, including complete restoration.

Successful development of hybrid rice using the WA type cytoplasmic male sterility (cms) in China (6, 7) has created the possibility of utilizing hybrid vigor (11, 12). The fertility of WA type cms is generally restored by various IR varieties and strains (12), although the inheritance of fertility restoration remains to be investigated. In japonica rice, a fertility restoration gene (*Rf*) interacted with the Chinsurah Boro 2 cytoplasm and was found to be gametophytic in action. Hence the F_1 plant between cms and *Rf* lines had 50% pollen fertility with a spikelet fertility expected to be more than 90% (8). Another *Rfx* gene that interacted with the cytoplasm of Lead Rice also showed the gametophytic action of restoration (9). *Rfx* restored the fertility of Lead Rice male sterility but not those of boro and *spontanea*, while *Rf* restored all of them except *spontanea*. The *Rf* gene was found to be located on chromosome C designated by Iwata and Omura (5) for their trisomic stocks (10).

INHERITANCE OF FERTILITY RESTORATION

Zhen Shan 97A, having WA type cms, was test crossed with IR and other indica varieties or strains to identify the fertility restorer. A number of IR

Table 1. Spikelet fertilities of test cross F_1 and F_2 between Zhen-Shan 97A and Rf lines, TARI, 1983.

Generation (season) number	Total crosses or plants	Spikelet fertility (%)										χ^2 value ^a (1:3)
		5	15	25	35	45	55	65	75	85	95	
F_1 (I)	39	3	4	1	3	3	3	7	4	9	2	
F_1 (II)	125	12	8	9	2	1	7	35	53	8	0	
F_2 (II)-17 ^b	398	37	33	22	24	15	56	78	81	38	14	3.648 ^{ns}
F_2 (II)-21 ^c	392	(116)				(282)						0.87 ^{ns}
		(90)				(302)						

^ans = nonsignificant.

^bZhen Shan 97A/IR21931-78-2-2.

^cZhen Shan 97A/TNGSY A15 (Milyang 23/IR29).

or IR-related lines showed effective restoration, although the degree of restoration in the second crop reached only 65%–75% in most cases (Table 1). The seed set percentage of indica varieties is generally lower in the second crop than in the first crop in Taiwan. Microscopic observation revealed that most of these fertile F_1 's had a stainable pollen ratio as normal as the ordinary indica varieties.

The segregation pattern of spikelet fertility in 12 F_2 populations derived from the fertile test cross F_1 's varied considerably, including those showing continuous segregation. There were only two populations in which fertility segregation fit a 3:1 ratio (Table 1), indicating that at least a single dominant gene was involved.

Zhen Shan 97A, belonging to WA type cms, is stable in male sterility in the tropics (1) and also in Taiwan (3). The effect of its restorer gene(s) has been reported to be sporophytic (6, 12). In this study we reached the same conclusions—that the action of fertility restoration in the above-mentioned crosses is sporophytic and that at least a single dominant gene is involved. More precise F_2 and BC_1 data are needed to refine this conclusion.

RELATION BETWEEN FERTILITY RESTORATION AND OTHER CHARACTERS

Among 13 characters investigated in the test cross F_2 's between Zhen Shan 97A and IR21931-78-2-2 or F_2 (II)-17, 6 characters including spikelet fertility segregated in the expected ratio of 3:1 following the

description and scaling (4). A test for independent inheritance between these six characters revealed that linkage relationships exist between spikelet fertility and color of lemma and palea, and between spikelet fertility and basal leaf sheath color (Table 2). Linkage was also detected between basal leaf sheath color and stigma (apiculus) color, between days to heading and color of lemma and palea, and between days to heading and stigma color. Their recombination values presented in Table 2 gave figures in each segregation mode that were in reasonable agreement with the observed numbers as shown by the χ^2 test.

In another cross, Zhen Shan 97A/TNGSYA 15 or F_2 (II)-21, there was no detectable linkage between spikelet fertility and basal leaf sheath color (Table 3). The recombination value between spikelet fertility and color of lemma and palea was as close as 9.7%. A fairly consistent linkage was detected between basal leaf sheath color and stigma (apiculus) color in these two rice crosses. There thus exists a linkage relationship between fertility restorer and color of lemma and palea with a recombination value ranging between 9.7% and 27.1%.

TRANSFER OF FERTILITY RESTORER

Using the Reimei (*rufipogon*) A cms line from *Oryza rufipogon*/Reimei⁸ as the female parent, we test crossed 71 varieties for the fertility restorer in 1977 and found that none of them restored spikelet fertility except 2 varieties that restored it to about 50%. Reimei (*rufipogon*) A, developed by the authors was the most stable japonica cms line in Taiwan (2). One of the two restorer varieties was IR5032-6B-13-1, F_2 seeds of which were introduced from IRRI to Taiwan in 1974 and selected by us afterwards. The F_1 of Reimei (*rufipogon*) A/IR5032-6B-13-1 was backcrossed to Reimei using Reimei as the male parent in 1978. No *Rf* plants were recovered in the BC_1F_1 . Then we propagated the F_2 and later generations of this cross and practiced selection on them as breeding material for ideal plant type and good spikelet fertility. The selected lines in the F_5 were test crossed to Reimei (*rufipogon*) A and Zhen Shan 97(WA)A for the fertility restorer in 1980. None of these tested lines restored the fertility of Reimei (*rufipogon*) A (Table 4). However, various degrees of fertility restoration occurred when Zhen Shan 97(WA)A was used as the tester, with about a fourth of the tested lines showing complete restoration (Table 4). Since the two cms lines differ in cytoplasm and also in their nuclear background, it is difficult to determine the factors that are responsible for such a difference in fertility restoration. Effective restorers such as C55 and C57 for

Table 2. Linkage relationships among several characters in the F₂ of Zhen Shan 97A/IR21931-78-2-2 or F₂ (II)-17, 1983, second crop.

Character	Parent		Segregation mode				Test for indep. (χ^2) ^a	Recomb. value	Goodness of fit (χ^2) ^b
	♀	♂	AB	Ab	aB	ab			
Spikelet fertility (%)	msrf	<i>Rf</i> (AA)							
Lemma and palea color	brown spots on straw(BB)	straw	199	83	108	8	30.077**	0.271	3.952 ^{ns}
Spikelet fertility (%)	msrf	<i>Rf</i> (AA)							
Basal leaf sheath color	colored (BB)	green	212	70	78	38	7.994*	0.446	4.362 ^{ns}
Basal leaf sheath color	colored (AA)	green							
Stigma color	colored (BB)	white	282	8	11	97	337.950**	0.047	1.193 ^{ns}

^a* = significant at the 5% level, ** = significant at the 1% level.

^b_{ns} = nonsignificant.

Table 3. Recombination values between several characters in 2 crosses of rice related with Zhen-shan 97A.

Cross	Spikelet fertility		Basal leaf sheath color
	Lemma and palea color	Basal leaf sheath color	Stigma color
Zhen-shan 97A/ IR21841-140-3-2	27.1%	44.6%	4.7%
Zhen-shan 97A/ TNGSY15	9.7%	independent	2.8%

Table 4. Spikelet fertility of test crosses F₁ between *O. rufipogon* related lines or lines selected from IR5032 and two cytoplasmic male sterile varieties.

Female parent (cms)	Male parents	F ₁ spikelet fertility (%)										Total no. of test cross F ₁ 's	
		5	15	25	35	45	55	65	75	85	95		
	IR5032 F ₅ lines	22	11	2									35
Reimei (rufipogon) A	<i>O. rufipogon</i> related lines	17											17
	IR5032 F ₅ lines	4	8	8	8	8	10	3	11	2	2		64
Zhen Shan 97 (WA)A	<i>O. rufipogon</i> related lines	4											4

japonica rice hybrids cultivated in China have been developed by transferring restorer gene(s) from indica to japonica rices (12). However, the results of our breeding work indicate that it is not easy to transfer the *Rf* gene of sporophytic action from indica to japonica varieties.

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INTERSPECIFIC HYBRIDIZATION AND MALE STERILITY IN RICE

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In consideration of the good results obtained with hybrid varieties for irrigated rice in China, the same breeding method should be experienced for other types of rice growing and for different regions. Cytoplasmic and nuclear new male sterile lines necessary for the commercial production of hybrid seeds and composite populations may be found in the progeny of hybrids between cultivated rice and African species of *Oryza*. The cytological observation of pollen and ovules of the hybrids could facilitate their identification. A method based on the analysis of mitochondrial polypeptides and developed for other crop plants was tested for the characterization of male sterile cytoplasm in rice.

Rice breeding has been very successful during the last two decades, and yields have been raised dramatically in many parts of the world. The last important step was the production and utilization of hybrid varieties in China for exploitation of heterosis on a large scale. Nevertheless, several problems must be solved for more extensive application of hybrid technology: small number and poor quality of male sterile and restorer lines, and low efficiency of the available pollinators.

The most important challenge is probably the gaps persisting among different rice growing regions, cultural types, and farming methods. As Virmani et al (8) pointed out, the extra cost of hybrid seed must be paid for by increased yield, but the extra economic gain is correlated with the initial yield level.

In many African countries, a rapid increase in rice production is a vital objective, but the agricultural environment is very variable and rarely optimal. Hybrid vigor has been observed for nonirrigated rice, and positive results are expected from the application of hybrid technology in upland and rainfed conditions.

Plant vigor goes frequently with better root development and thus heterosis can increase the level of drought tolerance.

The use of interspecific hybridization is very limited in rice compared with most other important crops. However, *Oryza glaberrima* and some African wild rices can be sources of important characters for the cultivated varieties like tolerance to diseases and environmental stresses. Besides, they are a potential source of male sterile cytoplasm, of sterility and restoration genes, and of genes controlling the level of allogamy, which are necessary for the production of commercial hybrids.

INTERSPECIFIC HYBRID STERILITY

Artificial hybridization between *O. sativa* and related African species is not recent. *O. glaberrima* is the most frequently used species because of its broad genetic diversification stemming from its long history of domestication and cultivation in several parts of West Africa. *O. barthii* and *O. longistaminata* are less often involved in crossing programs. The level and nature of pollen and seed sterility, the chromosome pairing at meiosis, and the meiotic irregularities are rather similar in all these hybrids. The genomes of these cultivated and wild species are highly homologous, and transfers of genes are thus possible among them through introgression. Several experiments have further shown the possibility of recovering fertile lines after backcrossing.

The majority of the F_1 hybrids between *O. sativa* and the other three species are almost completely sterile when isolated from foreign pollinators, and their own pollen is sterile at the flowering time. But the presence of fertile plants in the vicinity of the hybrids and artificial pollination by one of the parents lead to a variable level of seed setting. Microscopic observation of the ovules shows that a high number of embryo sacs are well formed and, consequently, that sterility is much less important in the ovules than in the anthers. Meiotic irregularities are generally infrequent in the ovules and anthers, the failure of mega- and microgametophytes occurring after meiosis, a few days before flowering. Abortion is much more frequent in male than in female gametophytes.

The factors responsible for sterility in these interspecific hybrids are certainly complex and probably similar to those acting in indica/japonica hybrids. Japanese researchers postulated a "one locus sporogametophytic interaction" model with two sterility genes (7). More recently, Sano (6) proposed a third locus S_3 affecting pollen differentiation in heterozygous plants, but without influence on megaspore evolu-

tion, in order to explain the male sterility of a hybrid between *O. sativa* and *O. glaberrima*. Nevertheless, it seems difficult to present a general mechanism for all the observed cases; cytoplasmic factors are obviously involved, since large differences have been observed between reciprocal hybrids (1).

UTILIZATION OF MALE STERILITY

Hybrids between different species or unrelated varieties belonging to the same species are frequent sources of male sterile lines. In rice, male sterility has been obtained in japonica/indica hybrids, after hybridization between cultivated and wild rice ("*O. spontanea*"), and in *O. glaberrima*. Some of these male sterile lines have nuclear (or genic) control; in the others, sterile cytoplasm is also involved. Both types have or could have important applications in rice breeding.

In China, cytoplasmic male sterility was used for rice growing very quickly after the discovery of the first sterile lines in 1970 and of good restorer genes in 1973; since 1978 about 5 million ha of hybrid rice have been planted, giving 20–30% yield increases (4). Other male sterile cytoplasms and restorer genes have been isolated in several countries. These lines exhibit differences in agronomic characteristics and in the manifestation of sterility; some of them produce viable pollen grains at higher temperatures. Some characters could be modified by introgression from other varieties, but identification of new sterile cytoplasms should certainly be very useful, particularly for other tropical regions and for dryland rice.

Nuclear male sterility has been observed in the progeny of several hybrids or after mutagenesis. These lines are not useful for the commercial production of hybrid seeds and have no application yet in rice breeding. Nevertheless, the presence of some of them in a mixture of varieties could increase the frequency of recombinations between them and lead to the utilization of composite populations (5). While pure lines and F_1 hybrids between homozygous parents are desirable in many regions, their low flexibility is a limiting factor on small farms, particularly in Africa, where water control in the fields is rarely possible. Heterogenous and moderately heterozygous populations could adapt themselves to environmental conditions widely differing according to the seasons and from place to place.

IDENTIFICATION OF MALE STERILE CYTOPLASMS

To extend the exploitation of cytoplasmic male sterility and of cytoplasmic variation in general, there is a need to develop rapid biochemical tests to recognize and characterize these types of variations. Two biochemical methods are presently available. One is based on the analysis, by agarose gel electrophoresis, of mitochondrial DNA cleaved by restriction endonucleases; the other is based on the *in vitro* labeling of mitochondrial variant polypeptides analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

The method of DNA analysis may be simpler, but its relationship with the male sterile phenotype is unknown; the differences observed in the restriction pattern of DNA between sterile and fertile lines may not be transcribed or translated into protein and may, therefore, have no functional significance (3).

In our laboratory we have developed a micromethod for the analysis of mitochondrial polypeptides synthesized *in vitro* by mitochondria from leaves or etiolated shoots of male sterile lines of important crop plants such as maize, wheat, fava bean, and sugar beet (2). We are presently developing the method to characterize cytoplasmic male sterility in rice.

The correlation between the presence of the variant polypeptide and the male sterile phenotype seems to be well established, since the concentration of the variant polypeptide decreased in the lines restored to fertility. This method is rapid and needs only 0.5 g fresh weight of plant tissue. It can be applied at an early stage of growth without destroying the whole plant, and it allows the analysis of a large number of plants carrying different cytoplasms or different nucleo-cytoplasmic combinations. This method is particularly useful when very few seeds are available or when phenotypic observation is required at a later stage of the plant growth, or when seed production of the plant is expected. Therefore the variant polypeptides of the male sterile lines can be proposed as convenient biochemical markers in breeding programs involving cytoplasmic male sterility.

CONCLUSIONS

Identification of new male sterile lines of rice is an important goal for several reasons. A broader diversity of the cytoplasms and genotypes is necessary for the utilization of F_1 varieties in various countries and for different types of rice growing. In addition to cytoplasmic types, nuclear

male sterility could have applications under some conditions. On the other hand, the expression of cytoplasmic male sterility is variable; as in several other crops, the male sterility of some lines of rice is due to indehiscent anthers or is temperature dependent. These types would be useful, because they can be maintained through self-pollination.

Hybrids between *O. sativa* and African species of *Oryza* are a potential source of new cytoplasmic and nuclear male sterility. Cytological studies in the first hybrid generations may facilitate the identification of different types and the choice of the plants able to give new lines. The development of biochemical methods leading to the identification of male sterile cytoplasm would be a new and very interesting tool. If the observations performed on other species can be applied to rice, it will become possible to test a large number of plants in a short time.

On the other hand, some of the hybrids, namely those involving *O. longistaminata*, can be further used to increase the allogamy of varieties selected for the production of hybrid seeds or composite populations.

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HISTOLOGICAL OBSERVATIONS ON INDUCED GENETIC MALE STERILE MUTANTS IN RICE

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Representative lines of four genetic male sterile (GMS) types in rice, viz., stamable pollen abortion, partial pollen abortion, complete pollen abortion, and no pollen, and their original cultivar M-101 were investigated histologically by the method of plastic thin sectioning. Observations on tapetal behavior, pollen development, and stamen conducting tissue showed that the histology of the four types was essentially normal. Even if there was some abnormality present, it occurred after abnormality of the microspores appeared and thus was probably not the cause but rather the consequence of pollen abortion. The distinction of genetic behavior, cytology, and histology between GMS and cytoplasmic male sterile types in rice is also discussed.

Based on anther and pollen characteristics, 11 induced genetic male steriles (GMSs) were classified by the senior author into four types, viz., stainable pollen abortion type (SPA), partial pollen abortion type (PPA), complete pollen abortion type (CPA), and no pollen type (NP) (8). Cytological observations on representative lines of these four types revealed that apparent chromosomal aberration and genetic male sterility were closely related (9).

Unlike the GMSs, cytoplasmic male steriles (CMSs) in rice exhibit a meiotic chromosome behavior that is normal or basically so, the mechanism of pollen abortion being histological rather than cytological (2, 3, 10, 12). The abnormalities extensively present in the tapetum and stamen vasculature of angiosperm CMSs have been reviewed by Laser and Lersten (6). However, only rarely have histological studies on the genetic male sterility of plants been reported. Recently, Li and Deng (7) conducted studies on the Tai-Gu genetic male sterile line of wheat and concluded that abnormality of the tapetum and anther vasculature is one of the primary causes of pollen abortion. We are not aware of any report on histological studies of GMSs in rice. Histological observations implemented in the present study aimed at further elucidation of the mechanism of pollen abortion in four types of rice GMS.

MATERIALS AND METHODS

Materials used in this study were representative lines of four GMS types. They are line I-8 of SPA, line I-1 of PPA, line II-12 of CPA, and line I-15 of NP. All were derived from the M_5 generation of irradiated seeds of the cultivar M-101 (8), the original cultivar M-101 serving as the check.

The planting and sampling of the experimental materials were done at the University of California at Davis in 1981. The samples were fixed in FAA fluid, transferred to 70% ethyl alcohol, and stored in a refrigerator at 5°C.

The observations were carried out in 1984 at the South China Agricultural University in Guangzhou. Stamens of each type at six stages from the pollen mother cell (PMC) to engorged pollen were selected for study. From each spikelet three anther squashes were made to note the development of pollen grains as well as to measure their dimensions; the remaining three anthers of the same spikelet were sectioned to observe the development of the anther. The preparation of slides was done by the plastic thin sectioning method as described by Feder and O'Brien (4). The fixed anthers were dehydrated by serial transfer in graded concentrations of ethyl alcohol, then embedded in GMA (glycol methacrylate) and sectioned to a thickness of 2–3 μm . Staining was performed with PAS (periodic acid-Schiff's) and TBO (toluidine blue 0) dyes.

To study the vessels of filaments, the material was immersed in lactic acid and gently heated to boiling; then, after washing in water, it was stained for over half an hour in a saturated aqueous solution of basic fuchsin (adjusted to yolk color with NH_4OH) and, following another washing in water, mounted on a slide (11). Kodak Panatomic-X black and white film was used for taking photomicrographs.

RESULTS

Tapetal Behavior and Pollen Development

M-101 cultivar check. Disintegration of the tapetal cells and development of the pollen grains proceeded in a coordinated way. The radical thickness developed to its maximum at the late tetrad stage (Fig. 1.1). The tapetum commenced to disintegrate immediately following the microspores released from the tetrad, which formed primary exines (Fig. 1.2). Thereafter, the tapetum continued to become thinner with disintegration and the microspores increased in size, each forming an exine

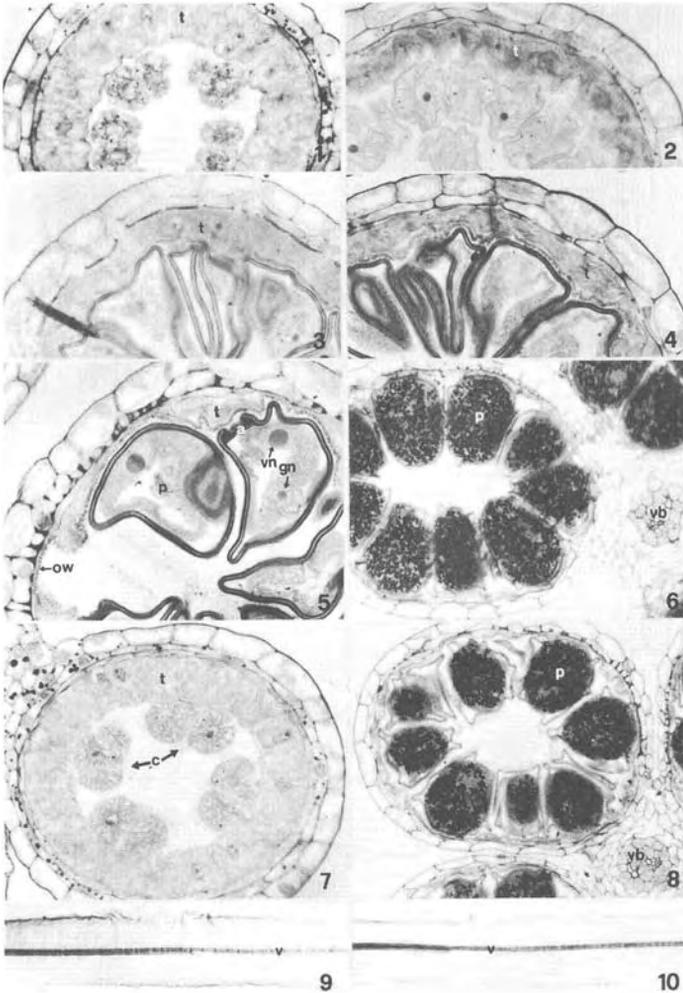


Fig. 1. Anther development of M-101 check and SPA type (cross section).

1. Microspores are just released from tetrads. Note intact tapetum (t). (700X).
2. Early microspore stage: tapetum (t) begins to disintegrate. (700X).
3. Middle microspore stage: note double layers of exine (t=tapetum) (700X).
4. Late microspore stage (t=tapetum) (700X).
5. Vacuolate pollen stage: note binucleate pollen (vn=vegetative nucleus, gn=generative nucleus, p=pollen, t=tapetum, ow=orbicular wall) (700X).
6. Mature anther: tapetum completely disappears and pollen grains filled with starch granules (p=pollen. vb=vascular bundle) (700X).
7. Tetrad stage (t=tapetum, c=callose) (700X).
8. Mature anther: pollen develop normally (p=pollen, vb=vascular bundle) (350X).
9. Filament of M-101 check (v=vessel) (150X).
10. Filament of SPA type (v=vessel) (150X).

comprised of sexine and nexine (Fig. 1.3–1.5). By the time the pollen grains became fully mature, complete disintegration of the tapetum had occurred, leaving only an orbicular wall appressed to the fibrillar layer. Pollen grains filled with starch granules (Fig. 1.6). At this point the diameter of the grains was approximately 41.7 μm (average of 60 measurements). These observations conformed to those of previous workers (2, 5, 10).

SPA type. The tapetum disintegration and pollen development of this type appeared similar to that of cultivar M-101. There was no apparent abnormality to be found (Fig. 1.7–1.8).

PPA type. Marked abnormality was evident during the development of the anther wall and the pollen grains. The first departure from normality occurred at the PMC stage. The surrounding of some PMCs lacked callose, some PMCs fused together to form pseudoplasmodia, and some of the anther locules had only one giant pseudoplasmodium each (Fig. 2.1). Some of the PMCs vacuolized and underwent breakdown and dissolution (Fig. 2.2).

From the late microspore stage on, some of the anther locules were found to be completely devoid of microspores (Fig. 2.3). About 11% of the 92 anther locules observed had no pollen grains, and as many as two locules per anther were without pollen. Therefore there were two kinds of anther locules to be found: those with pollen and those without pollen.

Of the anthers with pollen, those with little pollen exhibited abnormal phenomena such as marked thickening of the pollen exine, indistinct demarcation between sexine and nexine, roughened surface of the exine, and plugging of the aperture (Fig. 2.4). At maturation of the anther, the locules either contained empty pollen or had only a portion of the grains normal; but never were there locules with all the pollen grains normal (Fig. 2.5). A normal pollen ratio of 30.4% was judged from observation of the squashes (average value obtained from nine anthers of three spikelets). The disintegration of the tapetum in anthers with pollen was normal, except for the fact that at maturation of the anther and after complete disintegration of the tapetum the orbicular wall was not fully appressed to the fibrillar layer but instead a space was present between the two structures (Fig. 2.5).

No abnormality was seen in the tapetum of anther locules without pollen before the dissolution of the microspores, but the tapetum precociously disappeared after dissolution of the microspores. Numerous free

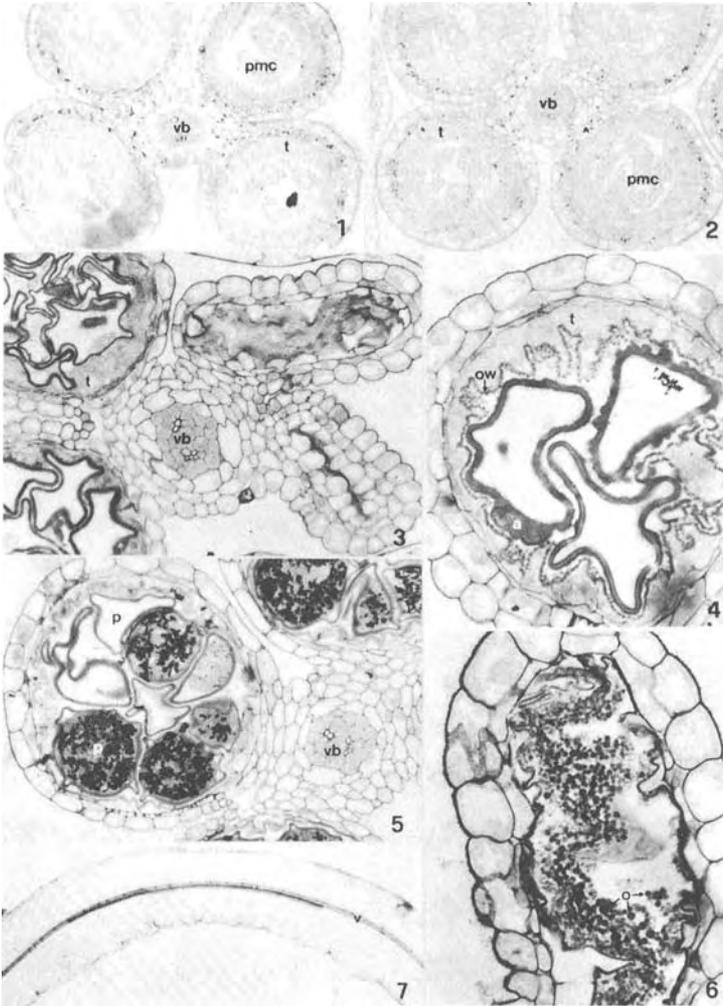


Fig. 2. Anther development of PPA type (cross section).

1. PMC stage: note PMCs fused together (pmc=pollen mother cell, vb=vascular bundle, t=tapetum) (300X).
2. PMC stage: note vacuolized and dissolving PMCs (vb=vascular bundle) (350X).
3. Late microspore stage: microspore and tapetum dissolution within two locules at right side (vb=vascular bundle, t=tapetum) (350X).
4. Late microspore stage: note pollen wall thickened and aperture plugged (a=aperture, ow=orbicular wall) (700X).
5. Mature anther: a portion of pollen becomes empty and vascular bundle of anther septum develops normally (p=pollen, vb=vascular bundle) (350X).
6. A locule of mature anther: note free orbicules within the locule (o=orbicule) (700X).
7. Filament and vessel develop normally (v=vessel) (170X).

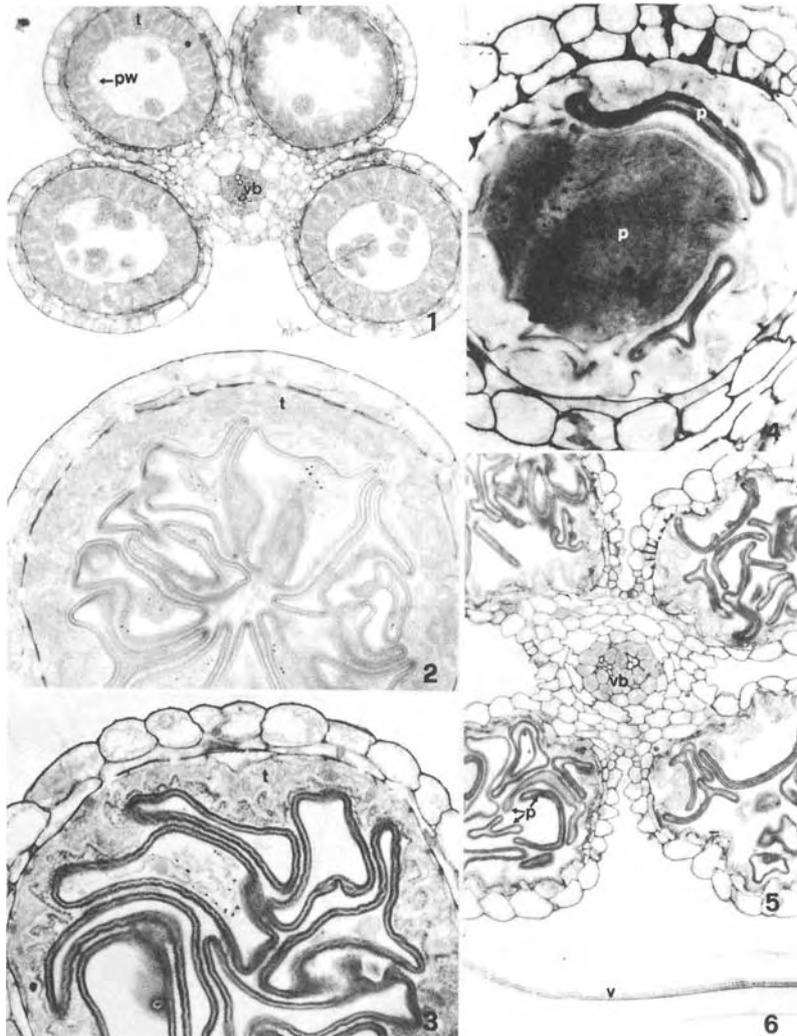


Fig. 3. Anther development of CPA type (cross section).

1. Early microspore stage: desynchronized disintegration of tapetum among locules and tapetal cells separated with inner tangential primary wall (pw=primary wall, t=tapetum, vb=vascular bundle) (350X).
2. Middle microspore stage (t=tapetum) (700X).
3. Late microspore stage: cytoplasm of microspore almost completely disappears and exine thickens abnormally (t=tapetum) (700X).
4. A giant mature pollen (p=pollen) (700X).
5. Mature anther: all pollen become empty and vascular bundle of anther septum develops normally (p=pollen, vb=vascular bundle) (350X).
6. Filament and vessel develop normally (v=vessel) (150X).

orbicules were present in some of the anther locules after disintegration of the tapetum (Fig. 2.6).

CPA type. A relatively uniform degree of pollen abortion was found existing among the individual anther locules. No abnormality was evident in the PMCs and early microspores (Fig. 3.1). However, after the middle microspore stage, it appeared that the cytoplasmic content of the microspores was always on the decrease, and the microspores tended to abort (Fig. 3.2). At the late microspore stage there was marked reduction in the cytoplasmic content of the microspores, which stopped enlarging but showed distinct thickening of their walls (Fig. 3.3). Thereafter, the pollen grains terminated development. At anther maturation, with the exception of a very few giant abnormal pollen grains (Fig. 3.4), virtually all the pollen grains were empty, leaving only double-layered exine (Fig. 3.5). Based on squash observations, the average diameter of the mature pollen was only 34.6 μm (average value for 60 measurements).

No unusual phenomena were encountered in the timing and process of the tapetal cell disintegration (Fig. 3.2–3.3), but before disintegration some degree of disassociation between the tapetal cells, as well as separation of the inner tangential primary wall of the tapetum and the tapetal cells, was seen. Desynchronism was noted between the disintegration of tapetal cells in different locules of the same anther (Fig. 3.1). At maturation of the anther, complete breakdown of the tapetum took place; however, the orbicular wall was not fully appressed to the fibrillar layer, and space was evident between the two structures (Fig. 3.5).

NP type. In this type the abortive process appeared very similar to that of the locules devoid of pollen in the PPA type. At the PMC stage, the PMCs lacked surrounding callose, were not uniform in size, and fused mutually to form pseudoplasmodia (Fig. 4.1). In some locules there could be seen only a single giant PMC (Fig. 4.2). Nothing unusual was discerned in the anther wall at this stage.

The microspores were either not uniform in size or undergoing dissolution at the early microspore stage (Fig. 4.3). Any of the following phenomena could be seen in the middle microspore stage: the anther locule contained rather numerous microspores with exine already formed; it included only a few microspores with markedly thickened exine; it contained many free orbicules; or all the substance and tapetal cells in the locules had dissolved (Fig. 4.4). The size of the free orbicules was usually larger than those on the tapetum, the maximum diameter reaching 7 μm (Fig. 4.5). In anthers that developed to the vacuolate pollen

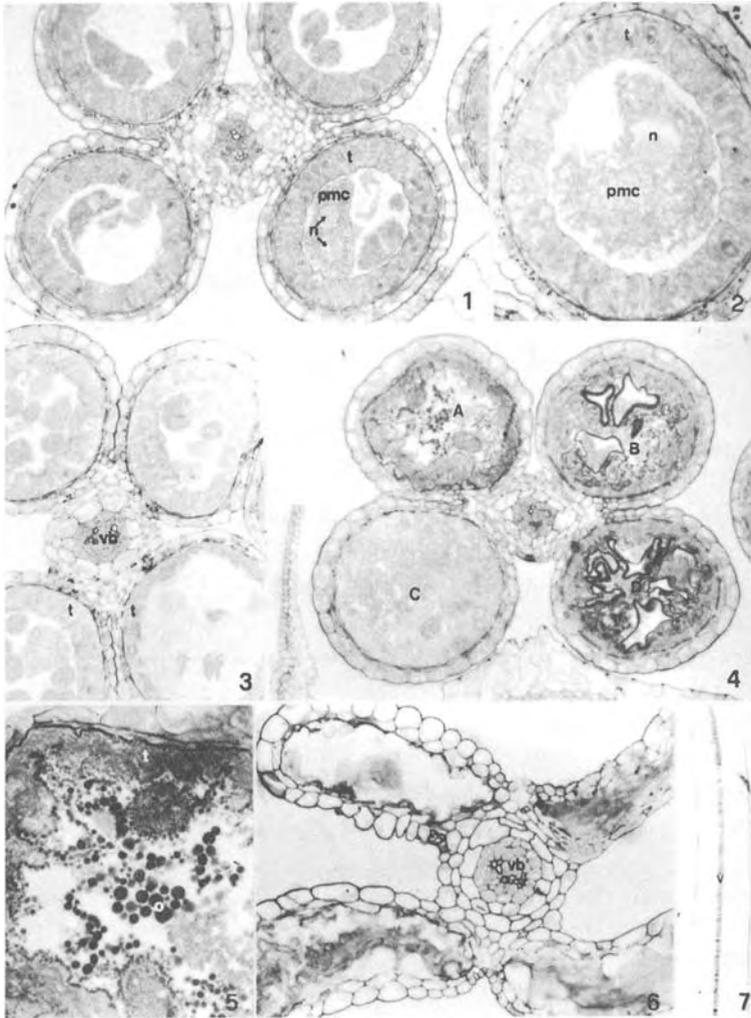


Fig. 4. Anther development of NP type (cross section).

1. PMC stage: note binucleate PMC (n=nucleus, t=tapetum) (350X).
2. A giant PMC (n=nucleus) (600X).
3. Early microspore stage: note microspores different in size and a locule undergoing dissolution (t=tapetum, vb=vascular bundle) (300X).
4. Middle microspore stage: four locules show different abnormalities (A=free orbicules, B=very few microspores with thickened exine, C=microspore and tapetum completely dissolved) (250X).
5. Free orbicules within locule (enlargement of Fig. 4.4; o=orbicule) (700X).
6. Mature anther: all pollen within four locules completely dissolved and vascular bundle of anther septum develops normally (vb=vascular bundle) (700X).
7. Filament and vessel develop normally (v=vessel) (150X).

stage, the microspores and tapetum in the locules had all dissolved, and free orbicules either had dissolved or were in the process of doing so. Finally, the substance in the locules became completely dissolved and, besides the vascular bundle of the anther septum, the entire anther was in an abnormal state (Fig. 4.6). Abnormality of the tapetum was closely associated with that of the microspores. The disintegration of the tapetum was relatively normal when a number of microspores were present. On complete dissolution of the microspores in the locules, disintegration of the tapetum was accelerated, with consequent precocious disappearance of the tapetum.

Stamen conducting tissue

The vascular bundles of anther septa of the four GMS types resembled those of the cultivar M-101 in being normal in development (Fig. 1.6, 1.8, 2.5, 3.5, 4.6), and no abnormality was apparent in the filament vessels (Fig. 1.9, 1.10, 2.7, 3.6, 4.7).

DISCUSSION

Taking into perspective the results of both cytological and histological observations should facilitate the comprehensive analysis of the mechanism of pollen abortion in the different types of male sterility in rice.

No abnormality could be detected in the anther tissues of the SPA type. Meiosis in the PMC was normal, yet the pollen lacked germination ability (8). Thus, the cause of pollen abortion most likely is the deficiency of certain enzymes like phosphorylase or reduced activity of the enzymes (1).

Meiosis in the PMC of the CPA type was found to be extremely abnormal; the frequency of abnormal chromosomal distribution at anaphase I attained, 36.7%, the occurrence of laggard chromosomes being 12.7% and that of the chromatin bridge being 7.4% (9). Microspore development almost completely ceased at the middle microspore stage. Although some abnormal phenomena were observed in the tapetal cells before disintegration, they apparently could not give rise to the severe abnormality encountered in the microspores. The primary cause of pollen abortion in the CPA type is again likely abnormality in meiotic behavior.

Pollen abortion in the NP type commenced early, the PMC generally being unable to accomplish meiosis (9). Since abnormalities of the anther

wall usually appear after dissolution of the microspores, pollen abortion may have been caused by certain factors present in the PMC.

The pollen abortion process in the PPA type appears to be more complex. The mechanism of pollen abortion in the anther locules lacking pollen may be like that in the NP type, and there may exist another genetic mechanism for pollen abortion in locules containing pollen. Abnormal meiotic behavior in a portion of the PMCs should be one of the major causes leading to pollen abortion (9).

Overt chromosomal aberration is closely associated with sterility in rice GMS (9). This study showed that the tapetum and stamen vascular bundle were normal or basically normal. Even if there was some abnormality present, it occurred after abnormality of the microspores appeared and thus was probably not the cause but rather the consequence of pollen abortion. Obviously, pollen abortion in four types of GMSs in rice was induced by abnormality of genetic behavior within the gametophyte itself.

It may be postulated that CMS and GMS in rice represent two categories of male sterility entirely different in properties. They differ not only in genetic behavior but also cytologically and histologically. The former belongs to maternal inheritance, the PMC meiosis being normal or basically normal, and abnormality of the tapetum and stamen vascular bundle being the main cause of pollen abortion. The latter is classified as Mendelian inheritance, the tapetum and stamen vascular bundle being normal or basically normal, and severe abnormality of PMC meiosis being the primary basis for pollen abortion.

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DISCUSSION

SESSION 11: CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION

Q – Rutger: Assuming a genetic vulnerability arose with the WA cytoplasm, what would be the next best CMS source to use?

A – Yuan: Besides the WA type, there are four other sources of cytoplasmic male sterility that are being used. We are still looking for new CMS sources, especially the gametophytic male sterility for indicas.

Q – Oka: (a) Please explain the origin of 29 Nan A. Does it have WA cytoplasm like Zhen-Shan 97A?

(b) Do you have evidence showing that R_1 and R_2 are restorers for the particular cytoplasm only?

(c) Do you consider that different cytoplasm require entirely different sets of restoring genes?

A – Yuan: (a) Both 29 Nan A and Zhen-Shan 97A have the WA type cytoplasm.

(b) Yes, we have. R_1 and R_2 are capable of restoring fertility of WA type CMS lines. For example, IR24 is the best restorer for any WA CMS line, but it cannot restore the fertility of the Hong-lian type CMS lines. The F_1 's of 29 Nan A/IR24, 29 Nan B/IR24, and IR24/29 Nan B were fertile.

(c) Yes.

C – Virmani: In response to Dr. Oka's question addressed to Mr. Yuan, I would like to add the following:

1. The confounding effect of intervarietal hybrid sterility can be ruled out in crosses where the F_1 of the cross CMS/restorer line shows normal fertility.
2. From the data reported in China and Japan, where restorers of one CMS line were found to be maintainers of other CMS lines and vice versa, it can be concluded that specific restorer genes exist for a specific cytoplasmic sterility system. However, it is still possible that certain restorer lines would restore the fertility of two different cytoplasmic sterile lines because they possess restorer genes for the two cytoplasmic sterility systems.

Q – Rutger: Have you observed any environmental conditions (or plant growth regulators) that will restore pollen fertility in "A" lines?

A – Yuan: For stable A lines, we have found none. For unstable A lines, there may be.

Q – Carlson: In maize, one can identify new restorer genes in CMS lines that give fertile revertants. Have you followed the inheritance of fertility in plants from your CMS lines that show environmental instability?

A – Virmani: We have observed fertile revertants in some CMS lines while still in the backcross nursery. We have not followed the inheritance of fertility in plants from the CMS lines showing environmental instability.

Q – Gupta: Do you have experimental evidence supporting your three gene model giving digenic expression? If not, could you separate lines carrying genes effective at the sporophytic and gametophytic stages and test their effects/expression?

A – Virmani: We do not have the experimental evidence yet to support the model. Theoretically, it is possible to separate lines carrying Rf genes effective at the sporophytic and gametophytic stages.

Q – Alzona: Economically speaking, hybrid rice seed would entail higher cost of production. What, then, do you think is the prospect of the success of hybrid rice in the Philippines or other tropical countries?

A – Virmani: Economic analysis of hybrid rice in China has indicated that the hybrids showing 15% or more yield advantage over the pure line varieties can be profitable if the paddy sale price is above US\$150 per ton. Therefore, the feasibility of hybrid rice outside China would depend on the cost of labor to produce hybrid seed and the price of paddy the farmer receives while selling his produce.

C – Ikehashi: There are some varieties, like CPSLO, that produce heterotic hybrids when crossed to indicas or to japonicas. If we introduce such a genetic background of wide compatibility into restorer lines, we may be able to get more pronounced hybrid vigor due to distant crosses.

Q – Wang, X. M.: Did you study the Hong-lien type of male sterile lines of rice for comparison?

A – Virmani: No. We do not have the Hong-lien type of CMS line in our collection. We would appreciate receiving it if you can supply it.

C – Wang, X. M.: I am interested in this because the Hong-lien type was developed in my laboratory, and the original plant of wild rice was brought by me from Hainan to Wahan in 1972. I think that it would be interesting to study all the different types comparatively. Prof. Huang Houzhe from Xiamen (Amoy) University and his colleagues have studied male sterile lines of rice in comparison with male sterile lines of maize, a rapeseed, etc. They have proposed as a model of origin of male sterility from the biochemical point of view that the imbalance of synthesis and degradation of auxins may be the reason for pollen abortion. Equilibrium of auxin metabolism seems to be important for microsporogenesis. We have observed that pollen abortion is the result of complicated biochemical and structural abnormalities. Therefore the progenies of test crosses and backcrosses usually manifest various gradations of abnormalities.

My colleagues have studied the F₂ progenies of hybrid rice and have established that segregation of sterility vs. fertility has a continuous range.

Q – Rutger: Is the same variant polypeptide involved in CMS lines of such diverse crops as maize, wheat, fava bean, sugar beet, and rice?

A – Bouharmont: Depending upon the species, male sterile and fertile lines can differ by addition, loss, or modification of a polypeptide. When several CMS lines are analyzed in the same species, we may expect to find some differences between them at the molecular level.

Q – Shao: Which species from Africa gives better male sterile lines?

A – Bouharmont: Our first objective is to find a method for identification of male sterile cytoplasms. The sorting of progenies will come later. I think *O. glaberrima* will be the most useful African species, because the number of available lines is higher than in the wild species.

Q – Shao: What is the potential of induced genetic male sterile mutant, and is there a correlation between dosage and 4-type sterility?

A – Lu: The potential use of rice GMS lines was reviewed by Rutger and Shinjo in 1980. At least GMS lines can be used in the production of hybrid seed (this will greatly depend upon improving the outcrossing rate of GMS) and population improvement (increasing outcrossing rate by introducing the *ms* gene into the rice population will lead to enhanced genetic recombination; the population may be improved by means of recurrent selection). Since the 11 rice GMS mutants used in the present study were derived from the same original cultivar and the same irradiation dosage, I never found and could not find any correlation between dosage and classification of 4-GMS types.

MUTAGENESIS

SESSION 12

INDUCED MUTAGENESIS IN RICE

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Studies on induced mutagenesis in rice from 1971 through 1982 at Himachal Pradesh Agricultural University indicated that the LD₅₀ for rice ranges from 25 to 40 Kr g-rays and averages 32.5 Kr g-rays. Differential responses of indica and japonica varieties and their respective mutants were observed. A new relationship among different panicle categories (main panicle, lateral I, and lateral II) with respect to the frequency of induced mutations in g-ray and DES-treated M₂ and M₃ populations was observed. The genetic background and the previous selection history of the materials under study, especially in the case of g-ray induced mutants or mutant varieties of g-ray origin, plays an important role in determining effectiveness and efficiency, and the most effective mutagen need not necessarily be the most efficient one. In the M₁, the means of quantitative characters were adversely affected, but the variance increased. In the M₂ and M₃, for the majority of characters, the mean did not change while the variance increased; for others the mean deviated in the positive or the negative direction while variance increased; and for still others an increased, decreased, or unchanged mean was accompanied by decreased variance. However, in different mutagen-treated populations of rice, in general, the mean either remained unchanged or deviated with equal frequency in both directions, but variance always increased. Some useful phenotypically constructive mutations, including those for blast resistance, have been induced and used for the development of mutant hill rice varieties.

Although techniques for the induction of mutations have been known for more than half a century, they have been recognized as a valuable tool for crop improvement only during the last 20 years or so. Up to 1964 only 31 mutant varieties had been released, out of which 23 had been evolved through the direct use of mutants and only 8 by cross breeding with induced mutants (13, 14). But during the next 20 years, 200 mutant varieties were evolved through direct use of mutants and 105 by cross breeding with induced mutants. Up to 1984, then, 336 mutant varieties of crops, belonging to 50 species, had been released for cultivation in 33 countries. Thus during the last two decades, not only the number of released mutant crop varieties increased greatly, but the proportion of mutant varieties developed through cross breeding with induced mutants also increased tremendously, indicating the recognition of induced mutants as parents by breeders. The released mutant varieties

have been further improved by cross breeding programs, implying the continuous impact of mutation breeding procedures for crop improvement. Perhaps the most important aspects of mutation breeding have been the quick mutational rectification of defects in varieties and advanced breeding lines, the induction of polygenic mutations, and the development of ideotypes for various agroclimatic conditions. The crop plant characters that have been improved by mutation breeding are generally those that have either not found favor with natural selection in the evolutionary process or were not achieved during previous plant breeding efforts. Induced mutagenesis in rice dates back to the early 1930s, when Ichijima (9) reported the production of the first early maturing mutants induced by x-ray treatment of seeds. Reports regarding the effects of various chemical mutagens on rice started appearing in the late 1950s (21). The first radiation-induced mutant variety of rice, Reimei, was released in Japan in 1966 as a national registered variety (6). But by 1984, 68 mutant rice varieties had been evolved, 44 of which were evolved through direct use of mutants and 24 by cross breeding with induced mutants.

The success achieved with mutation breeding techniques, especially for the improvement of the major crops of the world like rice, barley, and wheat, would indicate that it is no longer a controversial breeding method, but should be considered as an important technique to complement more conventional breeding technology. In 1971, therefore, mutation breeding studies were initiated for the improvement of several locally adapted indica and japonica varieties of hill rice at Himachal Pradesh Agricultural University (HPAU), Palampur. The major objectives of these studies were:

- to determine the relative sensitivity of various hill rice genotypes to different physical and chemical mutagens;
- to study the frequency and spectrum of induced mutations;
- to determine the effectiveness and efficiency of mutagenic treatments;
- to assess the comparative effects of different mutagens on various quantitative characters of indica and japonica varieties of rice in the M_1 , M_2 , and M_3 generations; and
- to explore the possibilities of isolating desirable phenotypic and physiological mutants for their utilization directly or indirectly in cross breeding programs for the quick improvement and rectification of the defects of adapted hill rice varieties.

The mutagenesis studies in rice conducted by postgraduate students are listed in Table 1 and will be referred to accordingly under various topics.

Table 1. Mutagenesis studies in rice conducted by postgraduate students at Himachal Pradesh Agricultural University, Palampur, India.

Years of study		Varieties		Seed treatments ^a and mutagens used	Generations studied by authors
1971–1973	indica	dwarf	Bala, Pusa 2–21, IR579	10, 20, 30, 40, & 50 Kr g-rays	M ₁ & M ₂ by 3
		tall	Basmati 370, Jhona 351, R 575, China 988		
	japonica	dwarf	Norin 18		M ₃ by 10
1973–1975	indica	tall	China 988, R 575, Mushkan Basmati	30 Kr g-ray and 0.3% DES for 2 h	M ₁ & M ₂ by 12
		japonica	dwarf		
1976	indica	tall	China 988	30 Kr g-rays 2 K-rad fast neutron 0.5% EMS for 8 h 0.3% DES for 2 h 0.02% NMU for 8 h	M ₃ by 18 (M ₁ & M ₂ grown in 1974 & 75)
1979–1980	indica	tall	China 988, China 988 (long slender) mutant	10, 20, & 30 Kr g-rays	M ₁ & M ₂ by 5
		dwarf	IR579, IR579 (tall & early) mutant		
	japonica	dwarf	Norin 18, Norin 18 (large glume) mutant		
1979–1980	indica	tall	Himdhan	20, & 40 Kr g-rays 0.5 & 1.0% EMS for 8 h	M ₁ & M ₂ by 20
		dwarf	Himalaya-1 (HPU 734). Himalaya-2 (HPU 71)		

^aSeed moisture stabilized at 13%–14% before treatment with g-rays, except in the first study, and chemical mutagen treatments given at 30°C after presoaking seeds in distilled water for 24 h.

RESULTS AND CONCLUSIONS

Sensitivity of hill rice genotypes to mutagens

In general, the response of rice varieties to different doses of γ -rays up to 50 Kr was curvilinear (3). The average LD₅₀ dose of γ -rays for rice was found to be 32.5 Kr, ranging from 25 Kr for China 988 to 40 Kr for Jhona 351. The LD₅₀ was 38 Kr for IR579; 32 Kr for Bala, Basmati 370, and Norin 18; 33 Kr for Pusa 2-21; and 38 Kr for R575, indicating the differential sensitivity of hill rice genotypes.

The lower doses of γ -rays up to 20 Kr had little effect on sprouting, except in the 20 Kr treatment of R575, where sprouting was increased, while the higher doses reduced sprouting highly significantly. Dogar (5) observed a differential response of the indica and japonica parent varieties and their respective mutants to γ -ray treatments, which generally increased the germination significantly in the M₁ of the mutants of indica varieties (IR579 and China 988) but decreased it in the mutant of a japonica variety (Norin 18), with little effect on the parent varieties. Although all treated populations exhibited reduction in seedling emergence and survival, only Norin 18 was found to be highly sensitive to both mutagens, viz., 30 Kr γ -rays and 0.3% DES, and China 988 only to 0.3% DES, where the reductions were significant (12). The other varieties (Reimei, R575, Mushkan Basmati) were not affected by any of the treatments. Differential sensitivity of various varieties to different doses of physical and chemical mutagens was also observed (20).

Frequency and spectrum of induced mutations

Frequency. The overall frequency of chlorophyll and viable mutations in the M₂ of five rice varieties (12) are given in Table 2, which leads to the following conclusions:

- In the M₂, the chlorophyll mutation frequency calculated on the basis of M₁ progeny was always highest (15.82%). This was followed by the frequency calculated on the basis of M₁ panicle progeny (8.50%), which in turn was always higher than that calculated on the basis of M₂ seedlings (1.16%). This suggests that all the tillers of a plant did not contain mutations, and only a few seedlings from different tillers contained them. This conclusion also holds true for viable mutations. Similar results were obtained in the M₂ (5) and in the M₃ (7, 18).

Table 2. Overall frequency of mutations observed in M₂ generation of five rice varieties (12).

Treatment	Percentage ^a								
	M ₁ plant progenies	M ₁ panicle progenies				M ₂ seedlings			
		MP	L ₁	L ₂	Total	MP	L ₁	L ₂	Total
<i>Chlorophyll mutations</i>									
Control	1.00 (100)	1.00 (100)	0.00 (72)	0.00 (62)	0.43 (243)	0.03 (3,297)	0.00 (2,782)	0.00 (2,110)	0.01 (8,189)
Kr	20.62 (97)	17.52 (97)	7.93 (63)	6.35 (48)	12.02 (208)	2.70 (2,145)	2.24 (1,024)	1.69 (710)	2.40 (3,879)
g-rays 0.3% DES	11.11 (99)	4.04 (99)	6.02 (83)	7.25 (69)	5.58 (251)	0.38 (3,110)	0.38 (2,363)	0.85 (1,752)	0.50 (7,225)
Total treated	15.82 (196)	10.71 (196)	6.85 (146)	6.83 (117)	8.50 (459)	1.33 (5,255)	0.94 (3,387)	1.10 (2,462)	1.16 (11,104)
<i>Viable mutations</i>									
Control	– (99)	0.00 (79)	0.00 (57)	0.00 (235)	0.00 (1,650)	0.00 (1,261)	0.00 (962)	0.00 (3873)	0.00
Kr	– (87)	22.9 (46)	32.61 (38)	21.05 (171)	25.15 (1,291)	3.48 (627)	3.19 (438)	2.74 (2,356)	3.27
γ-rays 0.3% DES	–	18.39 (87)	19.72 (71)	32.08 (53)	22.27 (211)	1.10 (1,538)	1.86 (1,119)	4.49 (780)	2.12 (3,437)
Total treated	–	20.69 (174)	24.79 (117)	27.47 (91)	23.56 (382)	2.19 (2,829)	2.35 (1,746)	3.86 (1,218)	2.59 (5,793)

^aMP = main panicle, L₁ = lateral I panicles, L₂ = lateral II panicles.

Figures in parentheses represent the numbers on the basis of which frequencies were calculated.

- For the induction of chlorophyll mutations in rice varieties, **g**-rays were twice as efficient as DES on the basis of M_1 plant progeny, as well as on an M_1 panicle progeny basis, and five times as efficient on the basis of M_2 seedlings. A somewhat similar situation, but in reduced proportions, was observed for viable mutations.
- Both on the basis of M_1 panicle progeny as well as M_2 seedlings, in general, the frequency of chlorophyll mutations induced by **g**-ray treatment was highest in the main panicle (MP), followed by that in the lateral I (L_1) and lateral II (L_2), in that order, while chlorophyll mutations induced by the DES treatment were highest in the L_2 panicles, followed by L_1 and MP, in that order. This brought out a new relationship between the panicle categories with respect to chlorophyll mutations and mutagen treatments. This relationship also holds true for viable phenotypic mutations.

Studies by Bhatia (3), Mehra (12), and Gupta (7) suggest that a high frequency of mutations (both chlorophyll and viable) in the M_2 and M_3 generations was obtained when a fairly large number of M_1/M_2 plants/panicles with high seed sterility were selected at random for raising the M_2 and M_3 populations.

The frequency of chlorophyll and viable phenotypic mutations varied from variety to variety and treatment to treatment. In this regard, irrespective of the indica and japonica subspecies, some varieties were more responsive to certain mutagens than others, while others like Reimei and R575 were quite resistant to mutagenic treatments. Thus the mutation frequencies in the M_2 and M_3 populations seem to depend upon genotype and previous selection history (3, 7, 10, 12, 18).

In the **g**-ray treated populations of M_2 , the chlorophyll mutation frequencies were almost equal in the parents and mutants of indica varieties (China 988 and IR579), whereas the same was very high in the japonica parent, Norin 18 (0.60%), as compared to the Norin 18 (LG) mutant (0.07%) (5). The same was true for viable mutation frequencies as well — 2.08% for Norin 18 and 1.24% for Norin 18 (LG) mutant. All the mutants studied were of **g**-ray origin. Similarly, Mehra (12) — in the M_2 of a different set of materials — and Gupta (7) — in the M_3 of the same (Mehra's) materials — found that, based on the chlorophyll mutation frequency, Norin 18 was more sensitive to **g**-rays (3.39% in the M_2 and 1.90% in the M_3), while Reimei, a mutant japonica variety of **g**-ray origin, was less sensitive to **g**-rays (1.85% in the M_2 and 0.19% in the M_3). The previous selection history of a rice variety, therefore, seems to play an important role in induced mutagenesis.

In the late sown (by 3 weeks) M_2 generation, the frequencies of albina, chlorina, and viridis chlorophyll mutations were lower, while those of xantha, albo-viridis, and virido-alba were higher than the same observed in the M_2 sown at the normal time, indicating the influence of environment (including temperature, light, and humidity) on the expression of mutations for chlorophyll (20). However, no marked differences in the spectrum of these mutations were observed in the two environments.

Spectrum. Although the spectrum of chlorophyll mutations in the M_2 and M_3 generations varied from variety to variety and treatment to treatment, in general, albinas were most frequent, followed by xantha, virescent, striata, and rare types like zebrina and tigrina. In the g -ray treated populations, albina, xantha, and striata were most frequent, while in DES treatments, virescent and rare types (zebrina and tigrina) were most common. The widest spectrum of chlorophyll mutations was induced by fast neutrons in China 988, while EMS and NMU did so in Norin 18 (18).

With regard to the spectrum of viable mutations in the M_2 and M_3 generations of different studies, in general, the highest frequencies of such mutations occurred for plant height (dwarf, semi-dwarf, tall), followed by those for maturity (early, late), grain type (shape, size, awned, beaked, extra glumed, large glumed, glabrous, reduced palea, palea-less spikelets), tillering ability (profuse tillering, unculms, branched tillers), plant habit (spreading, bushy), high yield, male sterility, and reverse mutations. Dwarf types occurred in almost all varieties, although more so in tall ones, tall types in dwarf varieties, early types in late varieties, and vice versa.

Reverse mutations. Dogar (5), in a study to determine whether some of the g -ray induced mutants in rice were the result of single gene mutations or chromosomal deletions, found two plants in an M_2 generation with normal coarse grains in the 30 Kr g -ray treatment of China 988 (long slender) mutant with a frequency of 0.229%. Similarly, she found five plants with normal glumes with a frequency of 0.612% in the M_2 of a 10 Kr g -ray treatment of Norin 18 (large glume) mutant. However, no reverse mutation was observed in any of the treatments of IR579 (tall and early) mutant. Ch 988 (LS) mutant was selected from the 20 Kr γ g -ray treatment of China 988 and N-18 (LG) mutant from the 10 Kr γ g -ray treatment of Norin 18 in 1974.

Effectiveness and efficiency of mutagenic treatments

Effectiveness. On the whole, DES was 23.22 times more effective than γ -rays — 56.09 times in China 988, 26.14 times in Norin 18, and 14.39 times in Reimei — but ineffective in R575 and Mushkan Basmati (12). However, the γ -ray treatment was most effective in Mushkan Basmati, followed by N-18 and Ch 988, and least effective in R575 and Reimei.

A 20 Kr γ -ray treatment was most effective in China 988 and its long slender mutant, whereas the 10 Kr treatment proved to be most effective in varieties Norin 18, IR579, and their respective mutants (5). This indicated that the genetic background of the material under study plays an important role in mutagenic effectiveness.

In the case of Himalaya-2 and Himalaya-1, the most effective dose was 40 Kr γ -rays, while in Himdhan the most effective dose was 20 Kr (20).

Efficiency. The mutagenic efficiency, calculated on the basis of lethality, was, in general, about five times higher in the γ -ray treatments than that calculated on the basis of seed sterility (0.7241 vs 0.1427) (12). However, the efficiency calculated on the basis of these two different criteria did not differ much in the DES treated populations (0.1965 vs 0.2098). On the whole, the γ -ray treatment was 2.13 times more efficient than the DES treatment — 3.10 times in China 988, 1.81 times in Reimei, and 1.77 times in Norin 18. However, γ -ray treatment was most efficient in China 988, followed by Mushkan Basmati, Norin 18, Reimei, and R575, while the DES treatment was efficient in China 988, Norin 18, and Reimei, in that order, and inefficient in R575 and Mushkan Basmati.

The efficiency of different doses of γ -rays, calculated on the basis of lethality and sterility in the parent varieties and their respective mutants, differed, but when the mutagenic efficiency, based on the average values of lethality and sterility, was considered, the 10 Kr treatment was found to be most efficient in varieties N-18 and Ch 988 and the 30 Kr treatment in their respective mutants, whereas it was just the reverse in IR579 and its tall and early mutant (5). Thus, in some varieties the parental materials were more sensitive to irradiations than their respective mutants, while the reverse was true for others, emphasizing the importance of the role of the genetic background of the materials under study in determining mutagenic efficiency.

When mutagenic efficiency was calculated on the basis of percentage lethality, the 1.0% EMS treatment was found to be most efficient in Himalaya-2 and Himdhan (20). However, in Himalaya-1 the 0.5% EMS treatment was most efficient. On the basis of sterility, 1.0% EMS

treatment was found to be most efficient in Himalaya-2 and Himalaya-1 and the 20 Kr γ -ray treatment in Himdhan. On the basis of the average of both lethality and sterility, the 1.0% EMS treatment was found to be most efficient in all three varieties tested.

These results indicate that the genetic background of the material under study plays an important role in determining the effectiveness and efficiency of the mutagens, and that the most effective mutagen may not necessarily be the most efficient one.

Effects of mutagens on quantitative characters in different generations

M₁ generation. In the M₁ generation of γ -ray irradiated populations in all studies, in general, the means of the vast majority of the quantitative characters were significantly adversely affected in different varieties, ultimately resulting in significant reduction in yield. However, in some treatments of certain varieties a stimulatory effect of γ -rays was evident on some of the characters like sprouting and survival percentage, plant height, leaf area index (LAI), tillers/plant, panicle length, and spikelets/panicle, especially in the early growth stages and at lower doses.

The 30 Kr γ -ray treatment had a drastic adverse effect on the population means of almost all the characters except LAI, while the effect of a 0.3% DES treatment was less drastic and varied in the negative or positive direction from variety to variety within the two subspecies (12). On the basis of various quantitative characters studied in the M₁, Norin 18, Reimei, and China 988 were most affected by the mutagens, indicating their high sensitivity to these mutagens compared with the other varieties tested. Variance for different characters in the M₁ of the mutagen-treated populations increased significantly in the majority of the cases. In some cases, however, it decreased significantly, especially for yield/plant.

M₂ generation. In the γ -ray treated M₂ populations, generally there was reduction in the survival percentage, plant height, and yield (3). The leaf area was increased and earing and maturity delayed in most of the varieties and treatments. No definite trend was evident with regard to the number of tillers/plant, panicle length, and spikelets/panicle. The character of sterility was not much affected. Thus, without applying any selection pressure, the means for various characters deviated in the positive and negative directions with equal frequency, while the variance increased in the vast majority of cases.

In the γ -ray treated M_2 populations of three parent varieties and their respective mutants, the mean values of the treated populations for different characters generally decreased while the variance increased when no selection pressure was applied (5). However, no definite trend was observed for LAI, number of tillers/plant, and panicle length.

In the absence of any selection pressure in the M_2 generation, the γ -ray treatment generally had a drastic adverse effect, i.e., the population means decreased significantly, whereas the DES treatment had a positive effect on the majority of the characters studied in five different rice varieties (12). The variance for different characters, however, increased or decreased equally frequently in both mutagen treatments.

Sood (20) observed in the M_2 significant changes in the mean values in only 9 of 72 cases of treated populations with respect to 8 quantitative characters. Of these, seven deviated in the plus direction and only two in the minus. The genetic variances for these traits were higher in many cases compared with their respective controls.

Heritability was low for LAI, but the genetic advance was quite high for this trait in all the three varieties tested. This indicated that, for the improvement of such low heritable characters, selection in later generations would be more rewarding. Though the mean value for gelatinization temperature in the case of Himdhan did not show any significant change, the range and genetic variance for this character increased in the treated populations. Hence, there was scope for improvement of GT in Himdhan. The mean value, range, and genetic variance for protein content increased in the 20 Kr γ -ray treatment for Himalaya-1, indicating a potential for its improvement.

M_3 generations. In the absence of any selection pressure in the M_3 , the frequency of decreased means was generally higher, while that of the increased or decreased variances was almost equal (10). However, the cases of significantly decreased means and variances were comparatively more. The maximum increase in variance for different characters in most of the varieties (with some exceptions) was induced by the 30 Kr and lower doses of γ -rays. In the 20 Kr γ -ray treatment of Bala, most of the characters were significantly affected in the negative direction, indicating the specificity of this dose for Bala.

In the absence of any selection pressure in the M_3 , the γ -ray treatment usually had a drastic adverse effect on means, whereas DES treatment had a positive effect on the majority of the characters studied in different rice varieties, resulting in skewed phenotypic variations (7). Variances for different characters in the γ -ray treated populations increased, while

in DES-treated populations they decreased more frequently. For some characters, the mean did not change while the variance increased; for others the mean deviated in the positive or negative direction while the variance increased; and for still others an increase or decrease in the mean was accompanied by decreased or unchanged variance. However, on the whole, for the rice crop treated with the two mutagens, the population mean remained almost unchanged, while the variance increased.

Sharma (18), investigating the M_3 generation of two rice varieties, China 988 (indica) and Norin 18 (japonica) — treated with 30 Kr γ -rays, 2K-rad fast neutrons, 0.5% EMS, 0.02% NMU, and 0.3% DES — found that in the absence of any selection pressure, the γ -ray treatment increased the population means most frequently among the physical mutagens, while among the chemical mutagens the EMS treatment was most effective in shifting the means more towards the positive direction. The variance increased more frequently for the majority of the characters of both varieties. For the majority of the characters, the mean did not change while the variance increased; for others the mean deviated in the positive or negative direction while the variance increased; and for still others an increased, decreased, or unchanged mean was accompanied by decreased variance. However, for the rice crop in general, treated with different mutagens, the population mean deviated with almost equal frequency in both directions while the variance increased. The randomly induced mutations resulted in skewed phenotypic variation in both varieties, because no attempt was made to eliminate the obvious phenotypic deviants from the randomly selected plants for taking observations on the quantitative characters.

Some of the treated populations exhibited moderate to high heritability associated with moderate to high genetic advance for various characters, indicating that improvement through selection is possible in segregating progeny.

Phenotypically constructive mutations

Late mutant of Bala. A true breeding, late maturing (10–13 days) mutant was isolated in the M_3 generation from the 20 Kr γ -ray treatment of variety Bala. The mutant had synchronous tillering and anthocyanin pigmentation at the plant base, in contrast to the nonsynchronous tillering and green base of the parent variety. The mutant showed significant increases in plant height, panicles/plant, panicle length, panicle weight, 1000-grain weight, and yield/plant, indicating its greater

efficiency than the parent Bala. One of the more efficient lines of this mutant, Bala late 6 (HPU 8020), has been extensively tested in the All India Coordinated Trials and in state trials in Himachal Pradesh. Based on its excellent performance it has been released for cultivation in Pondicherry, was identified during the AICRIP workshop in 1984 for trial in farmers' fields during the rabi season in the southern and eastern states, and is proposed for release in Himachal Pradesh. HPU 8020 (IET 5878) has been found to be highly resistant to blast and has been reported to possess multiple resistance to gall midge, stem borer, and green leafhopper.

Long slender mutant of China 988. A long, slender grain mutant was isolated from M_2 of a 20 KR γ -irradiated population of a widely cultivated variety China 988 in 1972. Except for grain size, no other character of this plant was phenotypically affected. In the M_3 , the progeny of this plant was true breeding. The grain length of the mutant was 6.59 mm, the width 2.02 mm, and the length/width ratio 3.27, compared with 6.00 mm, 2.46 mm, and 2.45, respectively, for the parent. Abdominal white was absent in the mutant. The mutant had 9.86% protein, compared with 8.34% for China 988. Except for a significant increase in the effective tillers per plant and LAI on the 90th day, and a significant decrease in days to flowering, no other character of the mutant differed from the parent. Its yield/plant was the same as that of the parent variety. However, since the mutant proved to be more susceptible to blast than the parent, it has been used in the hybridization program.

Large sterile glume mutant of Norin 18. A mutant plant with large sterile glumes and pale green leaves was isolated from the 10KR γ -ray treatment of Norin 18 in the M_2 generation in 1972. Progeny of this plant were true breeding in the M_3 . Compared to the control, the mutant had significantly higher LAI on the 90th day and at maturity, was earlier in flowering and maturity, and had more effective tillers, resulting in significantly higher yield and plant efficiency. There was no significant difference in milling percentage, and the mutant had 9.65% protein, compared with 8.13% in the parent.

Blast resistant mutants in rice. Bhardwaj (2) obtained blast resistant mutants of Mushkan Basmati from EMS- and DES-treated M_1 populations. The segregating pattern of M_2 progeny grown from the blast resistant M_1 plants in an epidemiological area indicated that in some of the segregating progeny, resistance to both leaf and neck blast is controlled by a dominant gene or genes. However, in most cases the number

of resistant plants fell short of the numbers expected from segregation due to a single dominant gene for leaf blast or neck blast. This was more pronounced in the case of segregation for resistance to neck blast. In almost all the M_2 progeny, many plants resistant to leaf blast were found susceptible to neck blast and vice versa. This indicated that resistance to leaf and neck blast is governed by different genetic factors.

Thirty-five blast resistant scented mutants in the M_3 showed wide variation in their agronomic characters, but most of the desirable semi-dwarf, blast resistant progeny were derived from a single plant selected in the M_1 from the EMS treatment. Wide variation for grain quality characters, like translucence, length/width ratio, protein percentage, gel consistency, gelatinization temperature, and scent, was also observed in these progeny. The grain yield of some of these blast resistant scented mutants in the M_3 generation was much higher than that of Mushkan (3.5 t/ha compared with 1.5 t/ha for Mushkan) and comparable to high yielding scented and nonscented varieties that yield 3.5 t/ha on an average.

Other mutants. Several other phenotypic mutants of different indica and japonica varieties of rice, obtained from different treatments of physical and chemical mutagens, are being maintained at HPAU, and some of these are being used in the hybridization program for the improvement of hill rice.

DISCUSSION

The LD_{50} appeared to be 32.5 Kr g-rays for rice in general, ranging from 25 Kr for China 988 to 40 Kr for Jhona 351. The LD_{50} was 32 Kr for Norin 18, a japonica variety. The radiation sensitivity of the indica varieties, therefore, varied to a great extent. According to Haq et al (8), 30 Kr g-rays was the most convenient working dose for all rice varieties. Ram (17) found that the effective dose range for rice varied from 30 to 35 Kr g-rays, and most of the viable and economic mutations were recorded in materials treated with 30 Kr. Differential responses of indica and japonica varieties and their respective g-ray induced mutants to g-rays were observed, and the same was true for mutant varieties of g-ray origin like Reimei. Siddiq and Swaminathan (19) found indica varieties to be more radiation resistant than the japonica and javanica varieties.

The frequency of induced mutations (chlorophyll and viable) was always found to be highest when calculated on the basis of M_1 plant progeny. This was followed by that calculated on an M_1 panicle progeny

basis, which in turn was always higher than that calculated on the basis of M_2 seedlings. This suggests that all the tillers of a plant did not contain mutations and only a few seedlings from different tillers contained mutations. Siddiq and Swaminathan (19) also reported that the frequency of chlorophyll mutations was always higher when calculated on the basis of plant progeny than when calculated on the basis of panicle categories in the M_2 generation. \mathbf{g} -rays were found to be several times more efficient than DES for the induction of mutations. The frequency of mutations induced by \mathbf{g} -rays was highest in the main panicle, followed by that in lateral I and lateral II panicles, in that order, while that induced by the DES treatment was in reverse order, i.e., L_2 had the highest frequency, followed by L_1 and MP. This brought out a new relationship among the panicle categories with respect to the frequency of mutations induced by different mutagens. A high frequency of mutations was obtained when a fairly large number of M_1/M_2 plants/panicles with high seed sterility was selected at random. Mutation frequencies seem to depend upon the genotype and previous selection history. For the induction of mutations, \mathbf{g} -ray induced mutants or mutant varieties of \mathbf{g} -ray origin were found to be resistant to \mathbf{g} -ray treatment, indicating the role of previous selection history for induced mutagenesis in rice. Environment (including light, temperature, and humidity) plays an important role in the expression of chlorophyll mutations and changes the frequencies of different chlorophyll mutations. A wide spectrum of chlorophyll and viable mutations was observed.

The occurrence of back mutations usually helps distinguish point mutations from large mutational effects such as deletions. Since a deletion involves a loss of genetic material, a reverse mutation restoring this exact portion of genetic material would be most unlikely. On the other hand, a point mutation caused by a small chemical change (nucleotide base substitution) without a significant gain or loss of genetic material would be more easily reversible. Reverse mutations, therefore, rule out deletions or insertions. Thus the reverse mutations occurring in M_2 populations of the \mathbf{g} -ray treated mutants indicated that the long slender mutant of China 988 and the large glume mutant of Norin 18 were the result of single gene mutations and not of deletions.

DES was found to be 23.22 times more effective than \mathbf{g} -rays, and the genetic background of the materials under study played an important role in determining mutagenic effectiveness. \mathbf{g} -rays were twice as efficient as DES, and efficiency varied from variety to variety and treatment to treatment. Thus a highly efficient mutagen need not necessarily be a highly effective one. A somewhat parallel conclusion was drawn by

Konzak et al (11), working on barley with a variety of alkylating agents. In some varieties the parental materials were found to be more sensitive to g -irradiation than their respective mutants, while the reverse was true for others. Thus the genetic background of the material under study plays an important role in determining the effectiveness and efficiency of the mutagens, and the most effective mutagen may not necessarily be the most efficient one. These results are in conformity with those of Mikaelson et al (15).

In the M_1 generation, the means of various quantitative characters were generally adversely affected, but the variances increased significantly in the majority of cases. However, in some treatments of certain varieties the stimulatory effect of g -rays was evident. In the M_2 and M_3 , the g -ray treatment had a drastic adverse effect on means, whereas DES treatment had a positive effect on the majority of characters. But the variances for different characters in the g -ray treated populations increased, while those for the DES treated populations decreased more frequently. For the majority of the characters, the mean did not change while the variance increased; for others the mean deviated in the positive or negative direction while the variance increased; and for still others an increased, decreased or unchanged mean was accompanied by decreased variance. Of these three categories, the first agreed with the results and conclusions of Oka et al (16), the second with those of Bateman (1) and/or Brock (4), and the third with none of them. In the third case, the shift in mean (increase or decrease) could have resulted from induced quantitative mutations with unequal effects, while the unchanged mean may have been due to the symmetrical induction of such mutations in both directions. The decreased variance in this case could perhaps be due to the elimination of the majority of the extreme types from the treated populations. However, for the rice crop treated with different mutagens in general, the population mean either remained unchanged or deviated with almost equal frequency in both directions, but the variance always increased.

The differential response of some rice varieties to different mutagens in the M_2 and M_3 could be attributed to differential sensitivity of different loci among the genotypes for the same character and within the genotype for different characters. The direction and frequency of micromutations seem to depend upon the genotype, the character under study, and the mutagen used.

Some of the M_2 and M_3 treated populations exhibited moderate to high heritability, associated with moderate to high genetic advance for various characters, including gelatinization temperature and protein content,

indicating that improvement through selection is possible in segregating progeny.

Some useful, phenotypically constructive mutations, including those for blast resistance and for efficient and desired plant type, have been induced in some hill rice varieties, resulting in mutant varieties through direct use of mutants or by cross breeding with induced mutants. Several phenotypic mutants of different origin are being maintained at HPAU.

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INDUCED MUTATIONS FOR GENETIC ANALYSIS IN RICE

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Induced mutants of basmati rice were used to investigate the genetic control of plant height and heading date in a 6×6 diallel set of crosses. For plant height V_r , W_r analysis revealed the complementary type of nonallelic interaction, and the slope of the regression line was also not in conformity with the estimates of genetic parameters. Omission of two interacting arrays resulted in a 4×4 subset of diallels that produced a V_r , W_r indicating the disappearance of nonallelic interaction and was in close conformity with the estimates of genetic parameters. The degree of dominance in plant height and days to heading were in the range of partial dominance. An allelism test with a dwarf mutant DM107-4 revealed that the dwarfing gene in the mutant is nonallelic to the tropical donor Dee-Geo-Woo-Gen. The mutants hold excellent potential for use in the improvement of plant type in basmati rice.

Genetic variability in rice can be generated by either hybridization or mutation. Induced genetic variations represent a more efficient source of genetic variability than gene pools conserved by nature (4). The use of induced mutation for rice improvement has gained considerable momentum during the last two decades. Several new rice varieties have been released through the propagation of superior mutants and the utilization of mutants as parents in cross breeding (15). Induced semi-dwarf mutants have played a vital role in California's rapid shift from nearly all tall varieties in 1978 to nearly all semidwarf varieties at present (19). In Pakistan, induced mutations have been utilized directly for varietal development (1) as well as for the creation of useful germplasm (3). This paper describes and discusses the results of some genetic studies on induced mutants and their possible use for the improvement of rice.

MATERIALS AND METHODS

The data used in this study were obtained in a 6×6 diallel crossing program, including reciprocals, involving a tall indica rice cultivar Basmati 370 and its five true breeding mutants (1 early, 4 semidwarf). The six parents and the resulting 30 F_1 hybrids were grown in a random-

ized complete block design with three replications during 1984. Inter- and intrarow distance was kept at 20 cm, with 15 plants/row. The experimental plots were fertilized with 67 kg N/ha and 13.2 kg P/ha. At maturity 10 competitive plants were selected from each row for recording data on plant height and days to heading. The data collected were subjected to diallel analysis to bring out the nature of genetic control in the two induced traits (9, 14). The gene action of these traits was estimated by V_r , W_r graph analysis (9).

An allelic relationship was studied in a cross of the induced dwarf mutant line DM107-4 (2) with a dwarf rice cultivar IR6 (DGWG), both about 100 cm tall. The cross was made in 1982, and in the subsequent seasons F_1 and F_2 populations along with parents were raised, keeping a single seedling/hill and 20 cm distance between and within rows. At maturity, data on plant height were recorded on 10 F_1 and parental plants and 464 F_2 plants. The c^2 method of analysis was used for testing goodness of fit for the segregation ratio (12).

RESULTS AND DISCUSSION

Plant height

Graphical analysis of the six parent diallel crosses for plant height is shown in Figure 1. The t^2 test showed that the assumptions underlying the diallel analysis had not been fulfilled. The slope of the V_r , W_r regression line ($b = 0.55 \pm 0.14$) deviates significantly from unity, indicating the presence of nonallelic interaction between the factors determining the expression of plant height. The position of all arrays gives a wide scatter of points, which also predicts epistatic effects among the parents. The regression line intercepts the W_r axis above the origin, suggesting partial dominance, whereas $(H_1/D)^{1/2} = 1.24$ (Table 1), showing overdominance of plant height. This discrepancy may also be due to nonallelic interaction. An attempt was thus made to identify the parent or parents giving nonallelic interaction by omitting some arrays (11). The elimination of arrays 4 (DM179-1) and 6 (DM15-4)—the arrays of highest variance—individually did not eliminate nonallelic interaction. However, when both these arrays were removed together, the regression coefficient (0.63 ± 0.34) did not differ significantly from unity (Fig. 2). In the absence of nonallelic interaction, the regression line

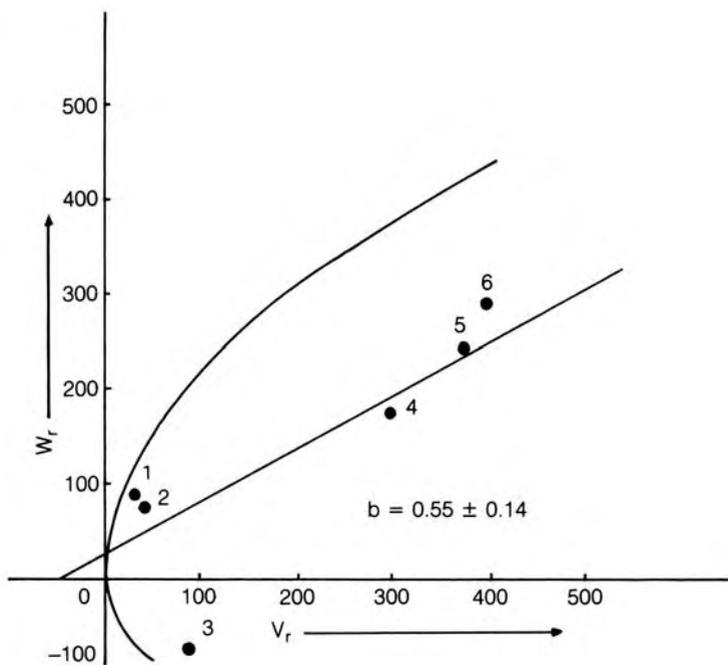


Fig. 1. V_p W_r graph for plant height in induced mutants of rice in 6×6 diallel cross. The points of intercept refer to (1) Basmati 370, (2) EF5-27-73-2, (3) DM-178-1, (4) DM-179-1, (5) DM-15-11, and (6) DM-15-4.

intercepts the W_r axis above the origin, showing partial dominance. The analysis also shows that array 4 (Mutant DM15-11) produced the largest V_p W_r values, indicating an excess of recessive alleles for plant height, whereas arrays 2 (Mutant EF-27-73-2), 1 (cultivar Basmati 370), and 3 (Mutant 178-1) belonged to the lowest V_p W_r groups, possessing an excess of dominant alleles.

The genetic components of variation were derived by unweighted least square estimation (14) and are presented in Table 1. In the 6×6 diallel cross, significant values of D , H_1 , H_2 , and h^2 showed the presence of additive and dominant effects. The relative magnitude of the additive component (D) was smaller than the dominance component (H_1), showing the role of dominant gene action. The positive and significant value of F (interaction of additive \times dominant effect) showed the presence of dominant genes in excess over the recessive ones. The measure of average degree of dominance [$(H_1/D)^{1/2} = 1.24$] indicates overdominance, which

Table 1. The components of variation and other parameters for plant height and days to heading in diallel analysis.^a

Parameter	Plant height		Days to heading
	6 × 6	4 × 4	6 × 6
D	483.78 ± 69.07**	542.08 ± 73.77**	61.50 ± 4.36**
F	402.66* ± 168.75*	365.32 ± 171.86*	18.82 ± 10.66
H ₁	747.89 ± 175.35**	413.62 ± 194.47*	44.88 ± 11.08**
H ₂	619.43 ± 156.64**	349.80 ± 179.80	40.86 ± 9.90**
h ²	1117.54 ± 105.43**	370.19 ± 121.76**	102.44 ± 6.66**
E	7.20 ± 26.11	8.10 ± 29.92	1.50 ± 1.65
(H ₁ /D) ^{1/2}	1.24	0.87	0.85
H ₂ /4H ₁	0.21	0.21	0.23
K _D /K _R	2.01	2.26	1.44
h ² /H ₂	1.80	1.06	2.51
h ² _(ns)	0.39	0.56	0.67
r _{yr} (V _r + W _r)	-0.75	-0.68	0.94

^a* = significant at the 5% level, ** = significant at the 1% level.

is not in conformity with the V_r, W_r graph analysis (Fig. 1) showing partial dominance. The value of H₁ was greater than H₂, suggesting unequal frequencies of positive and negative alleles exhibiting dominance for plant height.

In the 4 × 4 diallel cross, the value of H₂ was nonsignificant, whereas the relative magnitude of the additive component (D) was larger than the dominant component (H₁), indicating the more important role of additive gene action. Regarding the degree of dominance, the value of (H₁/D)^{1/2} = 0.87 shows partial dominance, which is in conformity with the V_r, W_r graph analysis (Fig. 2). Similar results on the interacting lines have been reported after eliminating arrays from the analysis (13, 20). Ceng (5) and Haque et al (8) reported greater and more important dominant and additive effects than nonadditive effects. The value of the ratio H₂/4H₁ was found to be 0.21, suggesting that the genes with positive and negative effects were in unequal proportion in the parents for this trait. The h²/H₂ ratio was greater than 1, suggesting that plant height is

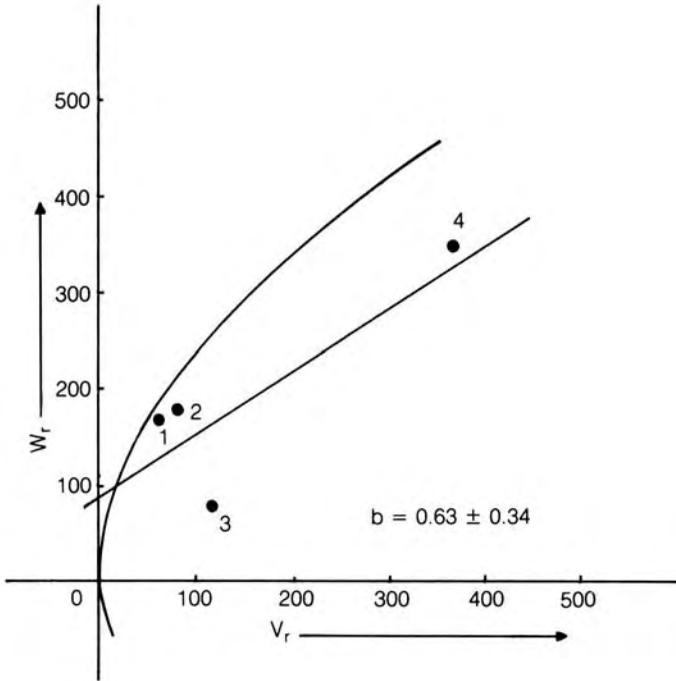


Fig. 2. V_p , W_r graph for plant height in induced mutants of rice in 4×4 diallel cross (arrays 4 and 6 omitted). The points of intercept refer to (1) Basmati 370, (2) EF-27-73-2, (3) DM-178-1, and (4) DM-15-11.

governed by at least one group of genes. The ratio of total number of dominant to recessive alleles (K_D/K_R) in all the parents included in the diallel analysis was more than 1, showing the presence of dominant genes in excess. The value of narrow sense heritability (h_{ns}^2) was 0.56, which shows that this trait is highly heritable and that successful selection could be practiced by the plant progeny method. The genes with positive effects were more dominant than recessive (r_{yT} , $V_r + W_r < 0$), which indicates that selection for semidwarfs is fairly rapid. In the present study, semidwarfism in mutant DM15-1 was controlled by recessive genes, whereas in DM178-1 it is influenced by dominant genes.

Days to heading

The V_r , W_r graph of days to heading is shown in Figure 3. The uniformity test of V_r , W_r was nonsignificant, indicating the validity of the assumptions (10). The regression line ($b = 0.820 \pm 0.22$) intercepts the W_r axis

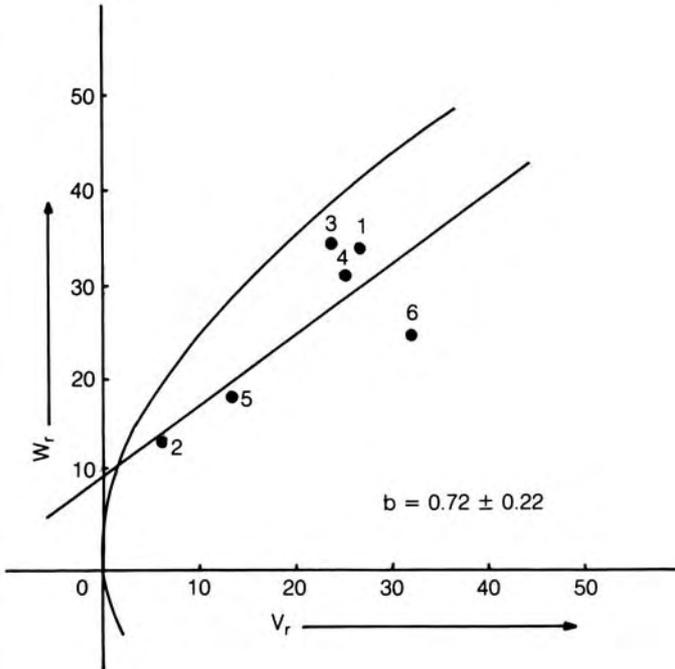


Fig. 3. V_r, W_r graph for days to heading in induced mutants of rice in 6×6 diallel cross. The points of intercept refer to (1) Basmati 370, (2) EF-27-73-2, (3) DM-176-1, (4) DM-179-1, (5) DM-15-11, and (6) DM-15-4.

above the point of origin, showing partial dominant gene action. Parental array 2 (EF-27-73-2), with lowest V_r, W_r value, indicates the presence of dominance, whereas arrays 1 (Basmati 370), 3 (dwarf mutant DM178-1), 4 (DM179-1), and 6 (DM15-4), with highest V_r, W_r values, show recessiveness for days to heading in the parents. Array 5 (Mutant DM15-11), with almost intermediate V_r, W_r values, seems to be controlled by both dominant and recessive genes, with a slight preponderance of dominance.

The presence of additive and dominant effects for days to heading was indicated by the highly significant values of D, H_1, H_2 and h^2 (Table 1). The relative magnitude of the additive component (D) was larger than the dominance component (H_1), suggesting the more important role of additive gene action. This may help in selection among lines in a

segregating population, since dominance starts declining with each generation of selfing. The positive value of F also indicates the presence of dominant increasing alleles. H_2 and H_1 values show equal frequencies of increasing and decreasing alleles. The environmental component (E) was nonsignificant.

Ratios computed from the genetic components show the degree, order, and direction of dominance of days to heading (Table 1). The value of the ratio $(H_1/D)^{1/2} = 0.85 < 1$ indicates partial dominance, which is also in conformity with the V_r , W_r graph analysis. The results of the present investigations are, therefore, in agreement with previous studies on days to heading (7, 16, 18). The genes with positive and negative effects were on the average in equal proportion in the parents ($H_2/4H_1 = 0.23$). There was an indication of more dominant genes than recessive ($K_D/K_R = 1.44 > 1$). The estimate of number of loci exhibiting dominance ($h^2/H_2 = 2.51$) suggests that at least three groups of genes control days to heading with dominant effect. According to Jinks (11), it underestimates the number of loci and provides no information about the number of loci exhibiting little or no dominance. The high estimate of narrow sense heritability (0.67) suggests that heading date is a highly heritable trait. Therefore, successful selection for this trait could be easily practiced in segregating generations. The genes with positive effect were recessive for days to heading (r_{yr} , $V_r + W_r > 0$). Such a relationship had also been reported earlier (6).

Allelic relationship

The data recorded on plant height of parents, F_1 , and F_2 in the form of frequency distributions are presented in Figure 4, showing that all the F_1 plants were tall (138 ± 6.19 cm); the bimodal curve of the segregating population ranged from 42 cm to 163 cm for plant height, resulting in two distinct groups of short and tall plants.

The total frequencies of short and tall plants in a cross of DM107-4/IR6 fit to a 7:9 ratio ($\chi^2 = 0.0087$, $P=0.93$), indicating that dwarfing gene loci in both the parents are nonallelic to each other. While making a genetic analysis of induced mutants in rice, similar results have been reported (17, 19). The dwarf mutant DM107-4 may prove an alternate source of dwarfism for the improvement of plant type in basmati rice.

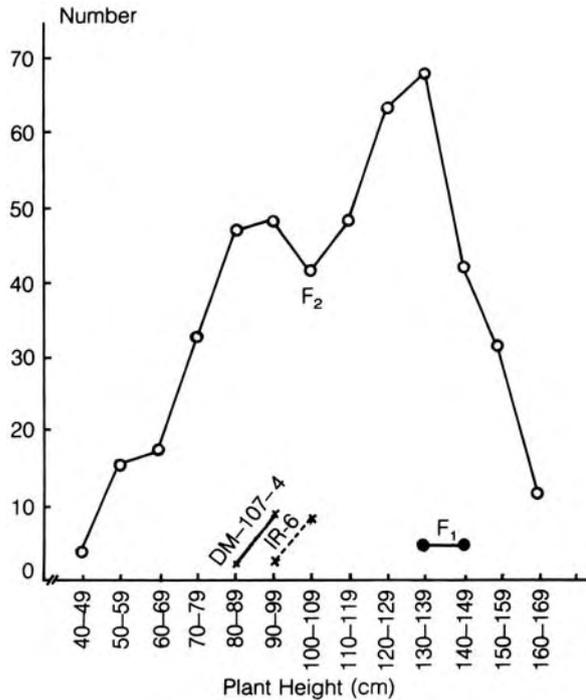


FIG. 4. Frequency distribution of plant height of parents, F_1 , and F_2 in DM-107-4/IR6.

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MUTAGENESIS IN RICE BY TREATING FERTILIZED EGG CELLS WITH NITROSO COMPOUNDS

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The mutagenic effects of nitroso compounds, mainly N-methyl-N-nitrosourea (MNU), on the fertilized rice egg cell at the single cell stage were compared with those on the developing embryo and the dry seed. The frequency of chlorophyll mutations in the M_2 progressively decreased with the development of the embryo. The efficiency of treatment in the fertilized egg cell was about twice that in the dry seed, although much higher dosage was employed in the latter treatment. The segregation ratio of the recessive iso-chlorophyll mutants in the M_1 decreased remarkably with embryonic development, caused perhaps by no chimera formation and no diplontic selection in the treatment of the fertilized egg cell with MNU. Panicle sterility in the M_1 plants showed a linear relationship with the frequency of the chlorophyll mutation in this treatment. The MNU treatment in the fertilized egg cells at various unicellular stages after flowering indicated that the frequency of mutation varied with intracellular condition and perhaps cell cycle. The mutagenicity of N-ethyl-N-nitrosourea was similar to MNU, but N-methyl-N¹-nitro-N-nitrosoguanide (MNG) was not. Several thousand rice mutants for endosperm, culm length, and heading date as well as chlorophyll were obtained by this method. The frequency of certain types of endosperm mutants seemed to differ according to the stage of treatment.

Since Muller (12) and Stadler (21) observed the artificial induction of mutations in drosophila and barley by X-rays, mutation breeding has been adopted in crop plants, and many varieties have been released by this method.

The important objects of mutation breeding are to increase mutation frequency and to control the mutation spectrum. Gaul (4) and Mericle and Mericle (11) expected that a high mutation frequency could be realized in the irradiation of zygotes at the single cell stage because of no chimera formation and no diplontic selection. Gaul (4) irradiated the zygotes and dry seed of barley with acute γ rays and observed that the former had about three times as many chlorophyll mutations as the latter.

On the other hand, many authors have pointed out that the chemical mutagens are more advantageous than ionizing radiations because of their higher frequency of gene mutations with few chromosomal aberrations (2, 5, 10).

We induced mutations by applying chemical mutagens to the fertilized egg cell in rice and achieved a high mutation frequency with N-methyl-N-nitrosourea (MNU) treatment (18, 19). We now maintain several thousand rice mutants obtained by this method. These mutants contribute not only to genetic research (8, 9, 15, 20, 24, 25) but also to the practical purposes of rice breeding.

TREATMENT OF FERTILIZED EGG CELL WITH MNU

In a series of experiments, the japonica variety Kinmaze was used. The MNU [$\text{CH}_3(\text{NO})\text{CONH}_2$, mol. wt. 103.08, obtained from Maruwaka Kagaku, Osaka, Japan] was dissolved in distilled water in the dark below 20 °C, and its pH was adjusted within the range 4.8–5.0 to prevent decomposition and inactivation of its mutagenic effects (17).

Fertilized egg cells were treated by immersing panicles in the solution. The procedure is as follows: At dusk, 1–2 days before treatment, rice plants were transplanted from the field to plastic pots and pollinated spikelets were removed. To synchronize the developmental stage of the fertilized egg cells as much as possible, the spikelets that opened from 1130 h to 1230 h were kept for MNU treatment and the rest were cut off. The panicles were exposed to various concentrations of MNU from 0.25 to 1.5 mM for 1–3 h at about 23 °C under dim light or in the dark. The panicles were rinsed several times immediately after treatment and then washed for 24 h in running tap water. More than 500 spikelets were used in each treatment. Progenies of M_1 plants were grown as M_2 lines and the segregation of mutants was investigated. Mutants in an M_2 line showing the same or quite similar phenotype were regarded as caused by a single mutation. Mutation frequency was expressed by the number of mutations per 100 M_2 lines (%).

MUTAGENIC EFFECTIVENESS OF MNU

The biological effects of MNU treatments on the fertilized egg cell at 6 h after flowering and on the mature seed are shown in Table 1. The physiological and mutational effects of MNU were markedly different in

Table 1. Mutagenic effects of MNU applied on the fertilized egg cell at 6 h after flowering and on the dry seed in rice.

Treatment			M ₁				M ₂	
Conc. (mM)	Period (h)	Seed setting ^a	Germination ^a	Seedling height ^a	Sterile plant (%)	Survivals (%) (a)	Chlorophyll mutations (%) (b)	Efficiency (%) (a × b)
<i>Fertilized egg cell stage</i>								
0.25	1	104	100	100	7	100	3	3
0.50	1	103	100	100	8	100	9	9
0.75	1	101	100	96	24	97	14	14
1.00	1	105	100	100	63	94	23	22
	2	107	100	81	69	61	49	30
	3	107	100	86	74	42	47	20
Water	3	100	100	100	0	100	0	0
<i>Dry seed stage</i>								
5	4		100	85	6	92	8	7
10	4		100	80	27	90	20	18
15	4		100	56	45	66	23	15
20	4		100	48	47	55	32	17
Water	4		100	100	0	100	0	0

^a % of control (water).

the two treatments. The inhibition of seedling height was greater in the treatment on dry seeds than that of fertilized egg cells. On the other hand, more sterile M_1 plants with fertility of less than 80% were produced by the latter treatment than by the former. While the difference in survival rate was slight between them, the causal factor for survival rate reduction was clearly different, being the growth inhibition due to poor growth of roots in the dry seeds and the increase in completely sterile M_1 plants in the fertilized egg cells. The frequency of M_1 plant progeny segregating chlorophyll mutations progressively increased from 3% to 49% with increase in MNU concentration or dosage (concentration \times duration) in the treatment of fertilized egg cells. The efficiency of treatment (frequency of chlorophyll mutations in $M_2 \times$ survival rate in M_1), as well as the mutation frequency, increased from 3% to 30% with increase in concentration or dosage. A dose of more than 3 mM·hr, however, was ineffective because of the drastic decrease in survival. Although the frequency of chlorophyll mutations in the treated dry seeds increased with increase in concentration, it was considerably lower than in the treated fertilized egg cells, being 8%–32%, despite the much higher concentration of MNU applied in the former. In addition, the efficiency of the treatment did not increase with concentrations of more than 10 mM because of the decrease in survival in the M_1 , in spite of the increase in the frequency of mutations. These results indicate that the fertilized egg cells are more sensitive to MNU than the shoot apex cells of dormant seeds. Therefore, the treatment of fertilized egg cells is preferable for mutation induction.

The advantage of treatment of the fertilized egg cell was strongly supported by the following experiments. When the embryos at different stages after flowering were treated with MNU, the frequency of chlorophyll mutations decreased remarkably with the advance in embryogenesis (Fig. 1). In addition, the frequency of recessive chlorophyll mutants in mutated M_1 plants fit well with the theoretical frequency of 25% in the treatment of fertilized egg cells at 6 h after flowering. This result indicates that each M_1 plant progeny in this treatment is derived from a single mutated cell. The mode of the segregation ratio of chlorophyll mutants steadily shifted downwards with the advance in development of the embryo and reached 1–5% in the treatment of dry seed. These results demonstrate that the elimination of mutated cells due to diplontic selection and the smaller size of the mutated sector are the principal reasons for low mutation frequency in the treatment of dry seed, and that the highest mutation frequency could be achieved by treating plant organs at the single cell level.

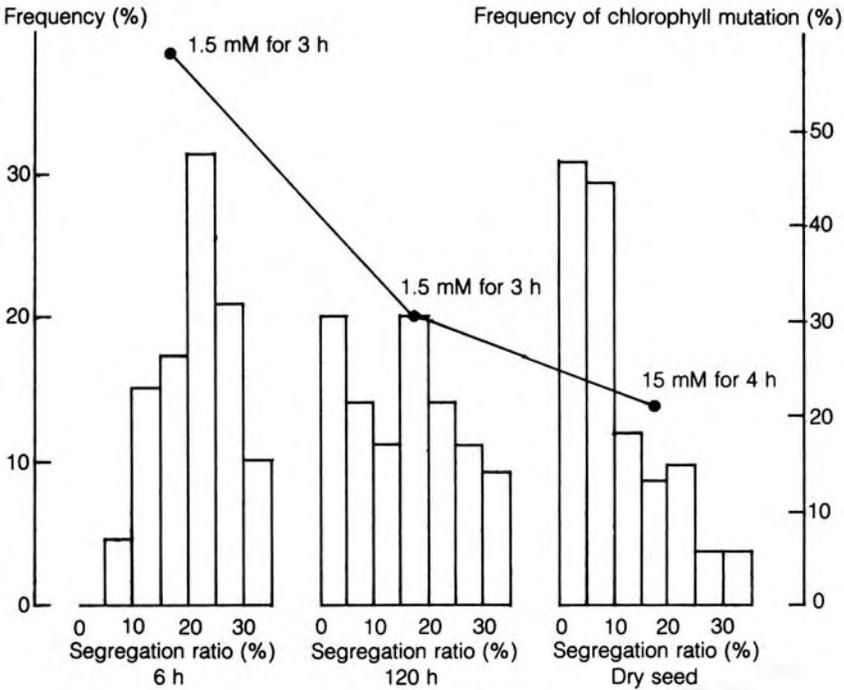


Fig. 1. Frequency of chlorophyll mutations and distribution of the segregation ratio of recessive chlorophyll mutants in M_1 plants mutated on the treatments of rice at different stages of embryonic development with MNU.

The frequency of chlorophyll mutations in M_1 plants with less than 20% panicle sterility was very low, about 18% (Table 2). However, it increased remarkably with increase in the panicle sterility of the M_1 plant. The maximum frequency of mutation was obtained with 81–90% panicle sterility, being about 70%. Veleminsky and Gichner (23) and Fujimoto and Yamagata (3) observed that panicle sterility in M_1 plants was closely related to mutation frequency with MNU treatment. Therefore, it is certain that mutants are able to be screened efficiently by harvesting partially sterile M_1 plants in the case of the treatment of fertilized egg cells with MNU.

The high mutation frequency realized in this experiment is thought to be attributed not only to the treatment of fertilized egg cells at the single cell stage but also to the mutagenesis of MNU itself. We also treated ethyl methane fertilized egg cells with ethyl methanesulfonate and ethylene imine, which are known as powerful mutagens of dry seed in higher

Table 2. Relationship of M₁ panicle sterility to the frequency of chlorophyll mutations in M₂.

	Sterility in M ₁									
	0	20	30	40	50	60	70	80	90	100
No. of M ₁ plants tested	3,587	154	278	378	532	446	386	418	272	
No. of M ₁ plants mutated	646	62	136	200	282	258	239	292	152	
Percent	18	40	48	53	53	63	67	70	56	

plants, but the mutation frequencies were lower than those of the respective dry seed treatments (18). Onozawa (16) reported that a chemical mutagen that has a strong mutagenic effect on dry seed is not always effective on the growing plant and observed that MNU is one of the powerful mutagens on developing embryos.

In a synchronized *Escherichia coli* culture mutations induced by MNU as well as N-methyl-N¹-nitro-N-nitrosoguanide (MNG) did not occur at random throughout the replicating chromosome but were preferentially localized in the region on the replicating fork of DNA (13). Hince and Neale (6, 7) observed in the treatment of a synchronized culture of *E. coli* with MNU that the number of mutations in each gene at the peak was five times the number at the basal level. If MNU acts on higher plants in the same manner as on microorganisms, the genes replicating DNA should mutate more than nonreplicating ones. We observed that the first cell division of the fertilized egg cell of Kinmaze was at about 20 h after flowering under field conditions in Fukuoka, though it varied considerably from year to year. As seen in Figure 2, the frequency of chlorophyll mutations was significantly different among treatment stages even at the single cell stage of fertilized egg cells, with a first peak at about 12 h after flowering and a second peak at about 17 h. The results shown in Figure 2 strongly suggest that the frequency of mutation relates to the cell cycle on treatment with MNU.

The mutagenicity of N-ethyl-N-nitrosourea (ENU) was similar to that of MNU, but MNG was ineffective in inducing mutation in fertilized egg cells.

We have so far obtained several thousand rice mutants for various characters including agronomic ones by treating fertilized egg cells with MNU. Mutants for endosperm property are remarkable because they have been little known in rice except for a waxy mutant. Recently, some

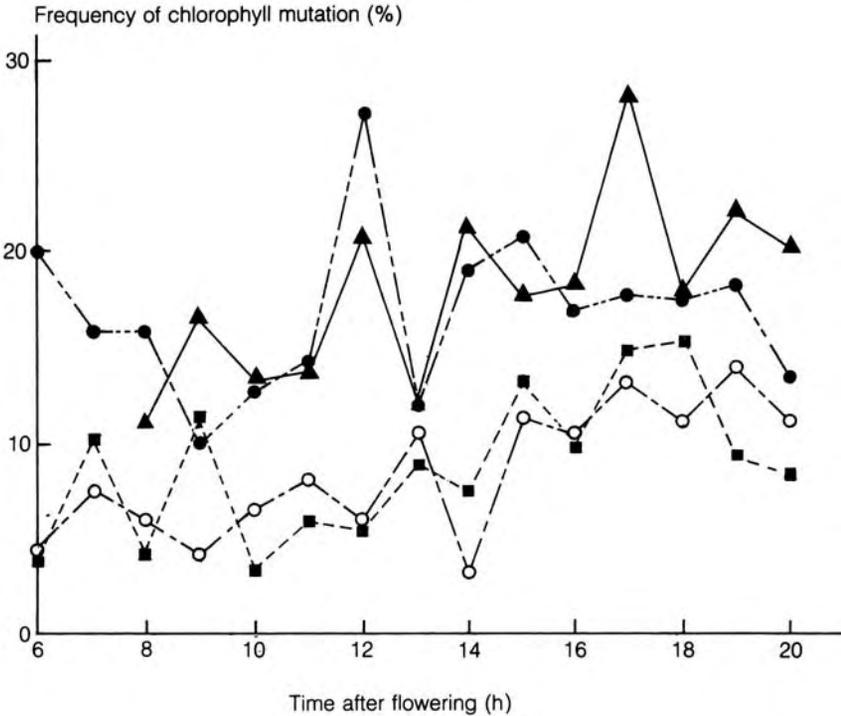


Fig. 2. Frequency of chlorophyll mutations on the treatment of fertilized egg cells at different stages from 6 h to 20 h after flowering with MNU. \blacktriangle = 0.75 mM MNU for 60 min in 1979, \blacksquare = 0.50 mM MNU for 60 min in 1980, \circ = 1.0 mM MNU for 45 min in 1981, \bullet = 1.0 mM MNU for 45 min in 1982.

different kinds of mutants have been obtained (1, 14, 22); we have also obtained various types of endosperm mutants for carbohydrate, lipid, or protein synthesis as shown in Table 3 (15, 20, 24, 25). The spectra and frequency of these mutants induced by the treatment of fertilized egg cells at different stages after flowering with 0.75 mM MNU for 1 h are given in Table 4. The frequency varied among traits, being highest in the white core mutant, possibly reflecting the number of genes involving each trait. The results of allelism tests supported this assumption. The mutation frequencies of certain types seemed to differ among the periods of treatment. The control of mutation spectra is the most important problem in mutation breeding, so it is very interesting to investigate whether the mutation spectra by the treatment of fertilized egg cells with MNU are different according to the cell cycle.

Table 3. Description of characteristics of endosperm mutation induced by the treatment of fertilized egg cells with MNU in rice.

Mutation	Endosperm color	Shape of brown rice	Hardiness of brown rice	Iodine test	Chemical properties	Genes
Kinmaze ^a	transparent	normal	hard	++	normal	
Waxy	white	normal	hard	–	less amylose	<i>wx, wx</i> ^a
Dull	translucent	normal	hard	+	low amylose	<i>du-1, du-2, du-3</i>
High amylose	white	slightly slender	soft	+++	high amylose	<i>ae</i>
Floury	white	almost normal	soft	++	normal or high protein	<i>flo</i>
White core	white at the center	normal	partially soft	++	almost normal	
Sugary	amber	wrinkled	hard	+	sucrose and WSP rich	<i>su</i>
Shrunken	white	wrinkled	soft	++	sugar rich	<i>shr-1^s, shr-1^a, shr-2</i>

^aOriginal variety, non-glutinous rice.

Table 4. Spectra and frequencies of endosperm mutations induced by the treatment of fertilized egg cells at different stages after flowering of rice with 0.75 mM MNU for 1 h.

Mutant	Treatment stage (h after flowering)																Total	%
	6	9	10	11	12	13	15	16	17	18	19	20	21	22	23	24		
Waxy				1			1	2		1		1	3	3			12	0.21
Dull	2	2	1		2	1	1		1		2		1				15	0.27
High amylose					1												1	0.02
Sugary							2			2		1		1	1		7	0.12
Shrunken-1 ^s						1							1		1		3	0.05
Shrunken-2		3	1	1										1		2	8	0.14
Floury		2		1	2		3	1	1	1	1	1	1	4	1	1	20	0.36
White core	3	5	4	3	2	3	3	8	6	4	5	1	5	7	1	1	61	1.10
Giant embryo					1	1	2	3	2		1		1	1	1		13	0.23
No. of M ₁	321	258	292	201	391	322	339	322	356	268	271	302	497	520	285	584	5619	

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INDUCED MUTAGENESIS IN NATIVE RICES

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Dry seeds of four upland rice varieties native to the drought prone plateau region of Bihar were irradiated with γ rays at different doses to induce early maturing, nonlodging, and high yielding mutants without affecting their drought resistance character. Ninety-eight early maturing mutants isolated in the M_3 were screened for drought resistance under field conditions in the M_4 and M_5 generations, and on that basis seven promising mutants were finally selected and evaluated for yield performance for three seasons. In addition to drought resistance, several good traits like early maturity, nonlodging, high yield, white kernel, and resistance to pests and diseases could be incorporated in Brown Gora Mutant-4 and Mutant-7. Both these mutants performed very well in trials in farmers' fields, especially because of their short duration (85 days), which helped the crop to escape drought. These two mutants can be used directly for commercial cultivation in the uplands of the plateau region of Bihar, where the rainy season is short but the growing conditions are relatively favorable.

In India nearly 5 million ha of rice area are upland (15%–20% of the total rice area), having various soil types, topography, and altitude with low or irregular and uncertain rainfall (13). In the absence of high yielding upland varieties, the farmers still grow well-adapted traditional tall varieties, which are poor yielding, weak statured, and coarse grained but are drought resistant. The improvement of upland rice is an urgent task, as the upland rice farmer is the poorest member of the farming community. Improvement of such specifically adapted native rices, particularly for earliness, plant height, and certain grain characteristics, is of great practical importance.

Induced mutagenesis, an effective tool for improving specific characters in well adapted native rices, has been successfully employed in recent rice improvement efforts (1, 2, 3, 4, 5, 6, 7, 9, 10, 11). Sigurbjornsson and Micke (15) listed only 13 rice mutants released for commercial cultivation up to 1974, but by now more than 30 have been released throughout the world. This clearly indicates that induced mutagenesis is proving increasingly useful in rice improvement programs (8).

The present study concerns improvement brought about in varieties native to the drought prone uplands of the plateau region of Bihar as a

result of induced mutagenesis. The performance of induced mutants with positive agronomic traits has also been evaluated.

MATERIALS AND METHODS

Four indica rice varieties of Gora (common name of local upland rices) — Karanga Gora, Bhura Gora (Brown Gora), Chain Gora, and Nawakhani Gora — which are popular upland varieties with wide adaptability to drought prone areas, were used. These Gora varieties, however, have the drawbacks of being low yielding, having the tendency to lodge even at low fertilizer levels, and having coarse grains with red kernels. It was therefore considered worthwhile to induce a wider range of variation through irradiation and to select desirable characters such as earliness, nonlodging, and better grain quality without disturbing the constellation of good characters that the local cultivators cherish in such native rices.

The seeds used for the present study were originally obtained from the genetic stock of local varieties maintained at the Birsa Agricultural University, Ranchi, Bihar. Five hundred dry, dormant, pure seeds of each of the four varieties with a moisture content of 12% were subjected to *g* irradiation treatments of 10, 20, 30, 40, 50, and 60 kR using the ⁶⁰CO source of the *g* cell at the Fertilizer Corporation of India, Sindri, Dhanbad, Bihar. The irradiated and control seeds were sown directly into the field on the second day after radiation treatment during kharif (June to December) 1978. To avoid cross pollination, individual inflorescences of M₁ plants were bagged and the seeds harvested. The M₂ progeny were raised from M₁ single plant seeds. Viable mutations were scored in the M₂ generation and were confirmed in the M₃ generation, after which the beneficial mutants were evaluated for yield and other desirable attributes. Preliminary station trials of the M₄ and M₅ generations were conducted in the dryland agriculture area of the University, and the mutants were screened for drought resistance and earliness. The selected drought resistant early mutants were direct seeded and tested for their performance for three seasons in the M₆, M₇ and M₈ generations in replicated yield trials with 60 kg N/ha, keeping the plot size 4 m × 3 m with a spacing of 20 cm. Simultaneously, the mutants were also evaluated for their reaction to major pests and diseases prevalent in the region. Finally, two promising mutants — Mutant-4 and Mutant-7 — were selected on the basis of earliness and high yield potential. They were evaluated in farmers' fields under direct seeded, rainfed upland conditions using a moderate level of 40 kg N/ha.

RESULTS

Early maturing mutants

All M_2 selections based on earliness and yield were carried over to the M_3 in progeny rows. A number of early maturing mutants observed in the M_3 were of no use, since, although they flowered more than 2 weeks earlier than the parent, they had reduced panicle length; these were rejected. However, 98 early maturing mutants with long panicles combined with high spikelet fertility were selected from the M_3 population. In preliminary station trials conducted for two seasons, these mutants were screened for drought resistance under field conditions. Observations were also recorded on plant height, maturity period, panicle length, ear-bearing tillers, 1000-grain weight, kernel size and shape, kernel color, husk color, and awning. Based on the above observations, seven early maturing, drought resistant mutants — all from Brown Gora — were finally selected in the M_5 generation and designated as Mutants-1 to -7 in order of maturity. Notable differences between promising Brown Gora mutants and their parent are given in Table 1.

All seven Brown Gora mutants (85-95 days duration) matured earlier than the parent (100 days). Among them, Mutants-4, -5, and -7 were the earliest, maturing 15 days earlier than the parent. The plant height of these seven mutants was reduced by about 34 cm (from 125 cm to about 91 cm), with only a slight decrease in panicle length except in Mutants-4 and -7, where a slight increase was observed. In addition, there was an improvement in the ear-bearing tillers, kernel size and shape, kernel color, hull color, and awning in all or most of the mutants (Table 1; Fig. 1, 2).

Performance of early maturing mutants

Yield trials conducted for three seasons during kharif, 1981–1983, utilizing the high yielding variety Bala as a standard check revealed that all the mutants performed better than the parent variety, as well as the standard check. Among the mutants, Mutants-4 and -7 (both 85 days duration) ranked first and second, respectively, and outyielded all other mutants (Table 2).

Observations on the incidence of major pests and diseases of rice recorded simultaneously with the yield trials for the three seasons indicated that Mutants-1, -4, -5, -6, and -7 were resistant to bacterial leaf

Table 1. Notable differences in ancillary characters between promising Brown Gora mutants and their parent.

Parent/mutant	Height (cm)	Duration (seed to seed)	Panicle length (cm)	Ear-bearing tillers	1000-grain weight (g)	Kernel size (mm)			Shape classification	Kernel color	Hull color	Awning
						Length (L)	Breadth (B)	L/B				
Brown Gora (parent)	125.3	100	20.2	6	28.9	6.10	2.60	2.35	medium	red	brown	short & partially awned
Mutant-1	90.5	95	18.8	12	30.8	6.68	3.10	2.16	medium	white	straw	absent
Mutant-2	85.4	95	19.8	10	27.2	6.00	3.20	1.88	bold	brown	light brown	long & fully awned
Mutant-3	86.8	90	18.0	14	19.2	4.67	2.51	1.86	short bold	white	light brown	absent
Mutant-4	99.7	85	20.6	14	23.1	5.01	2.98	1.68	short bold	white	straw	absent
Mutant-5	86.0	85	18.8	11	27.3	5.12	2.82	1.82	short bold	red	brown	absent
Mutant-6	88.3	85	19.2	12	24.8	5.83	2.70	2.17	medium	white	light brown	short & fully awned
Mutant-7	100.0	85	20.8	14	29.9	6.50	3.12	2.21	medium	white	straw	absent

blight and Mutants-2, -3, -4, -5, and -7 were resistant to brown leaf spot. Mutants-2, -3, -4, -5, and -7 were also resistant to gall midge. The check variety Brown Gora was susceptible to brown leaf spot and moderately susceptible to gall midge. The other check Bala was susceptible to bacterial leaf blight and also to gall midge (Table 3).

The performance of the mutants in trials conducted in about 700 farmers' fields under completely upland conditions during kharif, 1984 indicated that Mutant-4 yielded 51.7% and Mutant-7 61.8% more than the original parent (Table 4).

DISCUSSION

Early maturing mutants

Early maturing mutants for upland cultivation have been reported by various workers (2, 5, 14). Of the 98 mutants of 4 different Gora varieties examined during the present study, only Brown Gora produced 7 early maturing mutants while retaining the characteristic drought resistance of the parent variety. Mutants-4, -5, -6, and -7 were earlier than the parent in maturity by 15 days and also recorded significant increases in grain yield over the parent. In contrast to the coarse grain and red kernel of the parent variety Brown Gora, Mutants-4, -6, and -7 possessed improved grain size and shape and white kernel, which are positive attributes. Radiation-induced mutations for white rice from coarse red rices for uplands have also been reported (11, 12).

Another early maturing drought resistant mutant, Mutant-5, possessed desirable agronomic characters, especially reduction in height and better yielding ability than the parent, while retaining its characteristic red kernel, which is preferred by tribal farmers for specific purposes like preparation of rice wine.

Mutants-4 and -7 had intermediate plant height; the plant height was reduced by about 25 cm — from 125 cm to 100 cm. It is interesting to note that, because of the intermediate plant height, both mutants responded to up to 60 kg N/ha, still did not lodge at any stage of plant growth, and also sustained severe water stress in drought prone areas, resulting in high grain yield.

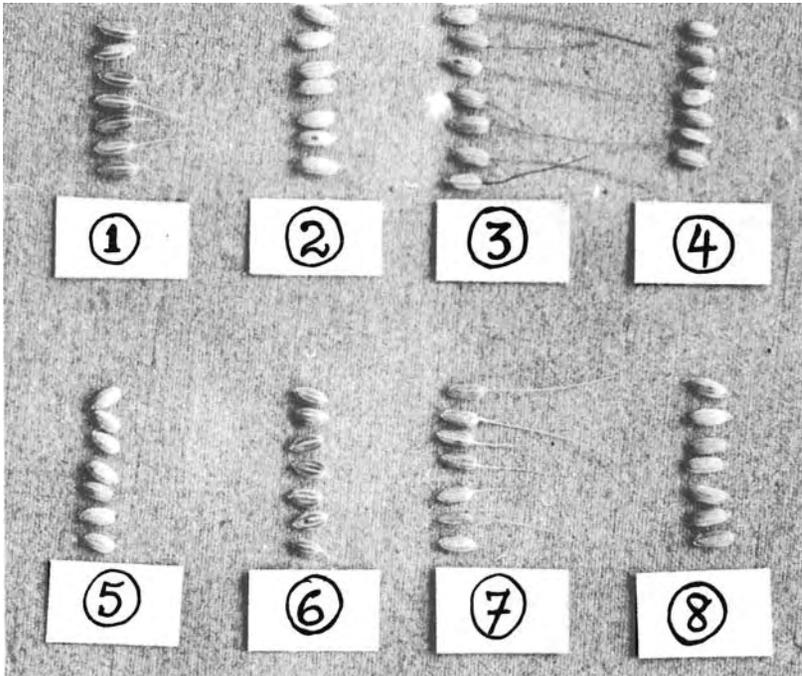


Fig. 1. Induced grain variation in Brown Gora mutants:

1. Brown Gora (control), 2. Mutant-1, 3. Mutant-2,
4. Mutant-3, 5. Mutant-4, 6. Mutant-5, 7. Mutant-6,
8. Mutant-7.

Performance of early maturing mutants

On the basis of combined mean and combined LSD obtained from 3 years' data, Mutants-4 and -7 were significantly superior to the parent and the standard check in yield and were at par with each other. Because of several good traits like drought resistance, early maturity, high yield, white kernel with good grain quality, and resistance to diseases and pests, Mutants-4 and -7 performed very well under rainfed upland conditions in farmers' fields. Thus the mutants seem to have a good future in the drought prone areas of the plateau region of Bihar, both as varieties and as parents for developing drought resistant, early maturing, high yielding varieties for upland conditions.

These results suggest that native rices can be improved through induced mutagenesis, keeping the integrity of their desirable combination of characters intact.

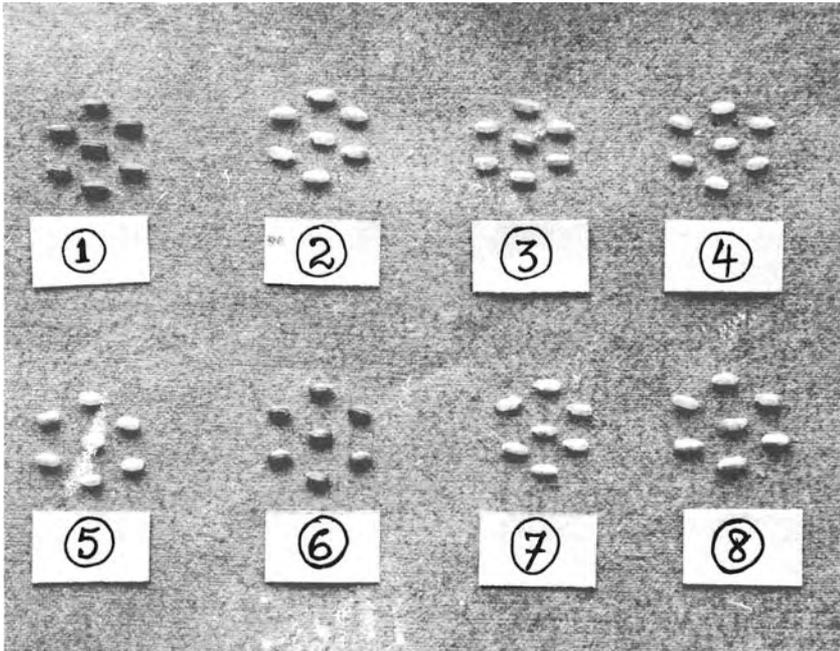


Fig. 2. Induced kernel variation in Brown Gora mutants:

- 1 Brown Gora (control), 2. Mutant-1, 3. Mutant-2,
4. Mutant-3, 5. Mutant-4, 6. Mutant-5, 7. Mutant-6,
8. Mutant-7.

Table 2. Grain yield (Kharif 1981-1983) of promising Brown Gora mutants.

Variety/mutant	Days to maturity	Grain yield (t/ha)			Mean
		Kharif 1981	Kharif 1982	Kharif 1983	
Brown Gora (parent)	100	2.39	2.13	1.98	2.17
Mutant-1	95	2.82	2.56	2.63	2.67
Mutant-2	95	2.72	2.32	2.45	2.50
Mutant-3	90	2.66	2.81	2.67	2.71
Mutant-4	85	3.26	3.21	3.02	3.16
Mutant-5	85	2.92	2.77	2.53	2.74
Mutant-6	85	2.88	2.82	2.63	2.78
Mutant-7	85	3.12	3.07	2.99	3.06
Bala (standard check)	92	2.03	1.78	2.07	1.96
LSD at 5%	-	-	-	-	0.11

Table 3. Reaction to important pests and diseases of Brown Gora mutants as observed during Kharif, 1981–1983^a.

Variety/mutant	Blast		Brown leaf spot	Bacterial leaf blight	Gall midge
	Leaf	Neck			
Brown Gora (parent)	3	—	7	3	5
Mutant-1	1	1	3	1	5
Mutant-2	1	3	1	3	1
Mutant-3	1	1	1	3	1
Mutant-4	1	—	1	1	1
Mutant-5	1	1	1	1	1
Mutant-6	1	1	3	1	3
Mutant-7	1	—	1	1	1
Bala ^b	3	3	1	7	7

^aGrading: IRRI system, 0–9 scale.^bSusceptible to bacterial leaf blight and gall midge.**Table 4. Performance of Brown Gora mutants-4 and -7 in farmers' fields during Kharif, 1984.**

Location	No. of tests	Grain yield (t/ha)		
		Mutant-4	Mutant-7	Brown Gora (check)
Tangarbashi	100	2.63	2.83	1.69
Girmi	100	2.56	3.12	1.75
Pungi	150	2.83	2.73	1.70
Banshjari	100	2.35	2.23	1.65
Katchacho	100	2.74	2.84	1.87
Burhakhukhra	150	2.73	3.14	1.78
Mean		2.64	2.82	1.74
Mean increase in grain yield over the parent Brown Gora		51.7%	61.8%	

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GENES FOR SEMIDWARFISM IN RICE INDUCED BY MUTAGENESIS

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Results of the application of mutation induction methods for rice improvement are given in terms of released mutant varieties. Then the usefulness of induced mutations is discussed. Information is presented on the number of released semi-dwarf varieties, on available stocks, and on mutant lines found in ongoing programs involving semi-dwarf mutants for rice improvement. Allelic relations between genes responsible for semi-dwarf characters of rice plants are discussed. The induced mutations technique could be most promising to avoid the genetic vulnerability of modern semi-dwarf rice varieties and could also improve local cultivars for higher production.

Twice in the history of plant breeding the introduction of one particular character has in a short time caused an economic revolution, both times involving the semi-dwarf character in cereals. Two genes of "Norin 10," a Japanese wheat, have been used in many "modern varieties" around the world. They have brought to a number of developing countries not only higher grain yields but also new agrotechniques and sociological changes. One can say the same about the rice revolution in Asia, which was initiated by the exploitation of a gene responsible for semi-dwarfism, the *Dgwg* gene. The economic success of semi-dwarf varieties developed by the International Rice Research Institute (IRRI) as well as by breeders in other countries has spread the *Dgwg* gene all over Asia and as a consequence has stimulated a search for other sources of semi-dwarfism. The first step was a germplasm collection. A great number of semi-dwarf stocks were identified, causing optimism and leading some people to conclude that natural variability for this character is completely sufficient for rice breeding purposes and that other approaches such as mutation induction are not needed (3). Only when it was found out at the International Rice Germplasm Center at IRRI that about half of the semi-dwarf stocks from the collection were allelic to the *Dgwg* gene (7) and that others possessed many negative agrobotanical

characters that limit their use in cross breeding programs did it become clear that the optimism was not justified.

The second step leading to alternative genetic sources for semi-dwarfism was undertaken by Futsuhara et al (2) and Rutger et al (15), who released short stature varieties, Reimei and Calrose 76, that were developed by mutation induction. These introductions had important consequences: First, it became obvious that by using mutation induction it is possible to obtain a new semi-dwarf rice variety well adapted to local environmental conditions that can be released as a new variety. Second, it was found that the new mutations are likewise allelic to the *Dgwg* gene. As similar results were reported by other laboratories, geneticists and breeders began to be concerned about the potential genetic vulnerability of semi-dwarf varieties of rice, even though such detrimental associations had not yet been reported. This concern stimulated investigations into the possibility of obtaining new gene sources for semi-dwarfness non-allelic to *Dgwg* and with acceptable agrobotanical characters. This paper presents a summary of achievements in rice mutation breeding and a status report on ongoing programs for obtaining new sources of semi-dwarfness in rice.

MUTANT VARIETIES OF RICE

During the last decade mutation breeding has become more popular, judged by the number of released new varieties developed by this method. Data presented here were collected by the Plant Breeding and Genetics Section of the Joint FAO/IAEA Division on the basis of information received from participants in our programs, from experts in the field, and from readers of the *Mutation breeding newsletter* (MBNL). Unfortunately, for self-evident reasons, information from commercial firms is not easily obtained. Therefore, the data herein should be treated as only a sample of achievements in the field.

MBNL mentioned official release of 689 varieties from 88 species through issue 25. This number included 430 crop plant and 259 decorative plant varieties. In comparison with other cereals, rice, with 103 varieties, seems to be a preferred material for application of mutation induction methods (Table 1). Even so, the real number of mutant rice varieties is much higher, considering that at least 20 varieties that are not listed, developed by a French group, were released in 1984 alone in West Africa. We are informed of about 10 newly released rice varieties per year, about the same number being reported for all other cereals together (Table 2).

Table 1. Number of released varieties of rice and other cereals developed through induced mutation.

Species	Common name	Direct release	Crossbred	Total
<i>Oryza sativa</i>	rice	75	28	103
<i>Hordeum vulgare</i>	barley	28	42	70
<i>Triticum aestivum</i>	bread wheat	46	10	56
<i>T. turgidum</i>	durum wheat	6	8	14
<i>Zea mays</i>	maize	3	11	14
<i>Avena sativa</i>	oats	4	7	11
<i>Secale cereale</i>	rye	3	—	3
<i>Sorghum bicolor</i>	sorghum	1	1	2
Total cereals		166	107	273

Table 2. Number of released mutant varieties of rice and other cereals, 1959–1982.

Period	Rice	Other cereals
1959–62	2	11
1963–66	6	13
1967–70	13	30
1971–74	16	29
1975–78	27	44
1979–82	38	42
Total	102	169

Mutant rice varieties are now growing on all continents with the exception of Australia. About 85% have been released in Asian and Pacific countries (Table 3). In China alone, during 30 years, 43 mutant varieties were released and 12 are now cultivated. In Japan, the famous variety Reimei, released by Futsuhara et al in 1966, has also been used as a parent variety in cross breeding programs with great success. This short straw variety, which is lodging resistant and also tolerant to lower temperatures, especially during the germination and seedling stages, led to seven new varieties in the period 1973–81 (Mutsuhonami, 1973; Hanahikari, 1975; Akihikari, 1976; Hayahikari, 1976; Houhai, 1976; Mutsukaori, 1981; and Mutsukomachi, 1981).

In Indonesia in 1977 the high yielding and good tasting local variety Pelita I/1, unfortunately susceptible to brown planthopper (BPH), was irradiated with 10 krad of gamma rays and its early mutant was irra-

Table 3. Number of released varieties of rice and other species developed through mutation breeding in different countries.

Country	Rice	Other cereals	Other species	Total
China	43	46	15	107
Japan	23	8	29	60
United States	10	15	26	51
India	8	8	95	111
France	3	2	15	20
Philippines	3	—	—	3
Thailand	3	—	—	3
Burma	2	—	2	4
Indonesia	2	—	1	3
Pakistan	2	1	3	6
Hungary	1	1	1	3
Italy	1	9	4	14
Ivory Coast	1	—	—	1
Korea	1	1	1	3
Other countries	—	91	222	303
Total	103	172	414	689

diated with 10 and 20 krad. After selection two new varieties were released — Atomita I in 1982 and Atomita II in 1983. These varieties maintain the genetic background of the original local variety and are therefore well adapted to particular environmental conditions and have high yield and other promising characters such as good taste and grain quality, which are extremely important for local marketing. With new characters obtained after mutation breeding like improved resistance to BPH biotypes 1 and 3 (11), resistance to green leafhopper and blast, early maturity, and high tolerance to salinity, these two varieties possess characteristics that make them very popular as parent varieties in cross breeding programs, not only in Indonesia but also in other countries.

A program that is now going on in Indonesia with irradiation of Atomita I has developed mutants with improved resistance to BPH biotype 2. If this mutant character is confirmed in the next several generations, a new stock with the genetic background of a good commercial variety and with resistance to BPH biotypes 1 and 2 may be released. The history of the breeding of Atomita varieties is a good practical example to confirm the views expressed in previous review papers on the topic of rice mutation breeding (8, 10).

Successes in rice breeding can also be noticed in the United States. The variety Calrose 76 released by Rutger and others in 1977 opened the way for a new generation of rice varieties in California and the southern

states. From ten released mutant varieties, three (Calrose 76, Calmochi-201, and M-401) were obtained as direct mutants, but six (M7, M-101, M-301, S201, M-302, and Calpearl) are progeny of Calrose 76, which was used as a parent in cross breeding (13). To its progeny Calrose 76 transferred short stature (ca. 95 cm as compared with 120 cm in Calrose), determined by the semi-dwarfness gene *sd₁*, allelic to the *Dgwg* gene, but with the gene complex needed for environmental adaptation, marketing quality, etc. in California.

Even in Europe, where rice is cultivated on a rather small scale, Italian, French, and Hungarian varieties were released with improved characters such as productivity (varieties Arlatan and Fulgente) and earliness (variety Nucleoryza) derived from mutation induction.

Results of rice mutation breeding (Table 4) indicate that practically all important characters for productivity of plants can be changed by mutation induction. Semi-dwarfness is a trait that has often changed rice plant morphology. This character was obtained by mutation (23 varieties) and then transferred by crossing to other genetic backgrounds (18 varieties). Increased effective tillering and other characters such as shape of leaf are traits, that, together with short stature, have drastically changed the architecture of mutant rice varieties.

Table 4. Improved characters in rice mutant varieties.

Character	Mutant varieties obtained		Total
	directly	by cross	
Plant morphology			
Reduced plant height	23	18	41
Other characters	16	3	19
Increased earliness	39	11	50
Increased yield	26	9	35
Seed characteristics			
Morphology	16	5	21
Quality	21	5	26
Resistance to disease caused by			
Microorganisms	28	3	31
Insects and nematodes	4	—	4
Other characters			
Adaptability	10	3	13
Threshability	2	—	2
Cold resistance	9	1	10

By using mutation induction it was possible as well to change seed morphology with respect to different consumer preferences in various regions. Bigger, thinner, finer, longer, and larger rice grains were found in mutants and introduced to new varieties. In addition, seed quality was often changed (about 25 varieties). After mutation breeding the content of amylose, protein (over 12%), and lysine (over 4%) was changed, as well as taste and glutinous endosperm. Forms suitable for sake brewing were found as well.

A lot of mutations were reported with changes in physiology important for improvement of adaptability of rice plants to different environmental conditions. By the use of mutagenic agents such important characters as earliness were very often obtained. A great number of early maturing varieties show very high grain productivity; from 39 varieties reported as early, more than 15 were described as highly productive. In about 10 cases earliness was induced together with other important traits such as short stature.

Better drought tolerance, fertilizer tolerance, improved suitability for ill-drained soils, improved tolerance to low as well as high temperatures, photoperiod insensitivity, and resistance or tolerance to diseases and pests are other examples of usefully altered characters (9).

For various reasons a gamma source was most frequently used for mutation induction (Table 5). More information on this topic as well as about practical methods of rice breeding with induced mutations and suitable selection procedures has been presented in other papers (1,4,5).

SEMIDWARF MUTANT COLLECTIONS

A list of semi-dwarf mutant stocks of cereal crops was published by IAEA in 1984 (6). It contains about 50 stocks obtained by mutation inductions in six countries that were involved in a previous program of mutation breeding of rice organized by the Joint FAO/IAEA Division. These 50 stocks were obtained after mutagenic treatment of 27 parent varieties. Gamma rays (38 stocks), chemical mutagens (6 stocks), and x-rays or fast neutrons (3 stocks each) were used as mutagenic agents. Short stature mutations were induced in 12 cases simultaneously with the trait of early maturity, in 14 cases with higher yield, and in 4 cases with both of these characters.

More than 200 promising semi-dwarf mutation lines of rice are being

Table 5. Number of released varieties of rice and other cereals developed by different mutagens.

Radiation type	Rice	All cereals
Gamma rays	60	116
X-rays	9	23
Neutrons	5	20
Chemical mutagens	2	14

evaluated for agrobotanical characters in ongoing coordinated research programs partially supported by IAEA. These 200 lines were selected in such countries as Bangladesh, India, Indonesia, Japan, Korea, Malaysia, the Philippines, Thailand, and the United States from a great number of plants obtained in M_2 or M_3 progeny after mutation treatment of 26 varieties. Participants involved in these programs have reported other important characters induced by mutagens. The next few years should reveal how relevant these mutants are.

ALLELIC RELATIONS OF SEMIDWARF MUTANTS

Allelic relations of different varieties of semi-dwarf rice were recently reviewed by Rutger (13). Only a few short stature mutant varieties have so far been tested for allelism with the *Dgwg* gene. It was often found that semi-dwarfness genes in such mutant varieties as Calrose 76, Calmochi-201, M-401, Jagannath, and Reimei are allelic to the *Dgwg* gene. Rather optimistic data in terms of the potential to avoid genetic vulnerability of modern rice varieties are presented in Table 6. The following promising stocks were described as non-allelic to the *Dgwg* gene (6):

DM-107-4 (from Basmati 370), reported by Awan, Pakistan

TR-5 (from SR-26B), reported by Narahari, India

T.K. (from Tellakathera), reported by Narahari, India

Short Labelle (from Labelle), reported by McKenzie and Rutger

Five new semidwarf lines obtained after mutagen treatment with agrobotanical characters very interesting for cross breeding were reported by Reddy (12) as non-allelic to the *Dgwg* gene:

Table 6. Allelic relations of semi-dwarf stocks and lines of rice developed by induced mutation.

Allelism	Stocks ^a until 1983 (no.)	Lines ^b (no.)
Allelic to <i>Dgwg</i> or <i>sd</i> ₁	9	6
Allelic to <i>sd</i> ₂	4	—
Allelic to <i>sd</i> ₄	3	—
Non-allelic to <i>Dgwg</i> and <i>sd</i> ₁ , <i>sd</i> ₂ , <i>sd</i> ₄	4	5
Not yet investigated	37	191

^aBased on list of semi-dwarf cereal stocks, IAEA, 1983.

^bM4 and following generations.

Bas 370 SD1	from Basmati 370
Bas 370 SD3	from Basmati 370
TCA 2(SD)	from Tilakchandan
TCA 3(SD)	from Tilakchandan
EMD	from IR8

The next three semi-dwarf mutant lines nonallelic to *sd*₁ were reported by Rutgers et al (14) during this symposium:

CI 11045	from M5
CI 11046	from M5
CI 11049	from Calrose

These preliminary results for our semi-dwarf rice breeding program give an optimistic picture, and it is hoped that in the near future more good sources of semi-dwarfness genes non-allelic to *Dgwg* will become available to rice breeders.

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EFFECTS OF DIMETHYL SULFATE, ETHYLENE IMINE, AND GAMMA RAYS ON RICE VARIABILITY IN M₂

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The mutagenic effects of dimethyl sulfate (0.03%, 0.05%, 0.1%, and 0.2%), ethylene imine (0.02%, 0.03%, 0.04%, and 0.06%), and gamma rays (5 kR, 10 kR, 20 kR, and 30 kR) on three introduced rice varieties—TCL, TQ 2, and IR8—were studied. Experimental and analytical findings are presented concerning the mutation rates and spectra observed in M₂, the specific effect of each mutagen used, the classification of the mutation types obtained, and the selection of some mutants of plant breeding value. The hereditary nature of some mutants is analyzed and confirmed.

Early research into the use of ionizing radiations for rice was conducted by Yamada (9), Nakamura (6), Komura (4), and Saeki (7). In 1934, Ichijima (3) was the first to succeed in creating mutants in rice by means of X-rays and ultraviolet irradiation. Chemical mutagens were used on rice plants sometime later; the research work of Sukanya Bai et al (8) on the effects of acenaphthene on rice was among the earliest. Following these pioneering efforts, research activity in experimental mutagenesis in rice accelerated to the point where in 1980 there were over 30 strains created by this method and put to use in production. These do not include hundreds of others that promise well and are being tested in many countries.

In Vietnam, research efforts along this line have been made since 1966 at the Department of Genetics of the University of Hanoi and later extended to other institutions, viz., the National Research Center of Vietnam, the Hanoi Pedagogical College No. 1, the Food Crop Research Institute, the Agricultural College No. 1, and the Institute of Agricultural Sciences. Using various physical and chemical mutagens, Vietnamese scientists have succeeded in creating quite a number of new rice strains that have been introduced into production, including strains with such advanced characters as high yield, early maturing (20–25 days earlier than the original materials), resistance to dried-leaf disease, and

high pest resistance. Besides, dozens of promising rice mutants are under test at the state level. Along with the use of separate ionizing or chemical factors, treatment with chemical mutagens combined or in conjunction with physical ones has been applied to definite phases in the cycle of the cell as well as to the early embryo or zygote stage.

This paper will communicate some research findings concerning the effects of dimethyl sulfate (DMS), ethylene imine (EI), and gamma rays on the variability of a number of rice strains observed in the M_2 generation.

MATERIALS AND METHODS

Our experiments have involved three strains of rice introduced into Vietnam during 1964–1970, viz., TCL, TQ 2, and IR8, the last two having been cultivated as a winter-spring crop on a considerable area in the north of the country. TCL was treated with DMS, TQ 2 with EI, and IR8 with gamma rays.

The dormant seeds of the TCL were immersed in 0.03%, 0.05%, 0.1%, and 0.2% DMS solutions and the dormant seeds of TQ 2 in 0.02%, 0.03%, 0.04% and 0.06% EI solutions. At each concentration a batch of 800 seeds was treated with a solution volume of 25 ml/100 seeds. The treatment lasted 24 h at 22°C, after which the seeds were washed in running water for 2 h. In the gamma ray experiments, IR8 dormant seeds were spread even into a layer and irradiated from a 75 R/min ^{60}Co source with doses of 5 kR, 10 kR, 20 kR, and 30 kR.

The M_1 generation was evaluated with regard to such indexes as germination rate, survival rate after the seedling stage, tillering, earing, chlorophyll mutation rate and spectrum, and morphological changes in vegetative and generative organs. The ears on the main stems of the M_1 plants were collected separately and were later sown as different families with respect to the M_2 generation in which a distinction was made as to family mutation, mutation rate and spectrum, and mutants of plant breeding and biological value.

To further clarify the specific effects of each mutagen and the mutability of the treated varieties, in addition to the general mutation rate for each concentration or dose (percentage of mutated plants to the total of treated plants), calculation was also made of the percentage of families exhibiting mutations for each concentration or dose and the number of mutation cases in every 100 families. The advantage of this approach lies in the fact that it does not exclude the case in which a mutagen can simultaneously induce mutations in different parts of one plant.

The selection and analysis of mutation families were continued for the M_3 generation, in which the stabilized mutants were crossed with the parent materials with a view to determining their hereditary nature.

RESULTS AND DISCUSSION

Mutation rate

Table 1 shows the mutation rate in M_2 as general mutation rate, i.e., the percentage of mutated plants to total plants treated with each concentration or dose, and as the number of mutation cases arising from every 100 families. For the DMS-treated TCL variety, the percentage of families exhibiting mutations increased with mutagen concentration, whereas the general mutation rate was higher at lower concentrations. On the other hand, both the lowest and highest concentrations proved to have engendered multiple mutations in one plant, which was almost the case as well with the EI-treated TQ 2 variety. The general mutation rate of TQ 2 also increased with EI concentration. IR8 also showed a steady rise in general mutation rate and in the percentage of families showing mutation when the gamma ray dose increased from 5 to 30 kR, whereas the doses of

Table 1. Mutation rate in M_2 .

Strain (Mutagen)	Concentration/dose (%)	(kR)	Families showing mutation (%)	Mutation rate (%)	Mutation cases/ 100 families
TCL (DMS)	0.03		50.01	62.52	403
	0.05		51.83	65.13	359
	0.10		56.34	56.36	317
	0.20		63.36	52.49	437
TQ 2 (EI)	0.02		57.64	34.74	411
	0.03		64.33	43.68	436
	0.04		55.25	50.27	278
	0.06		75.07	57.86	446
IR8 (gamma rays)		5	16.83	23.43	315
		10	18.56	27.22	216
		20	21.49	30.71	318
		30	24.62	38.36	252

5 kR and 20 kR seemed to induce more multiple mutations than the other doses.

In general, the respective mutagenic effects of DMS and EI on TCL and TQ 2 as observed in their M_2 generation were similar, although the concentrations of EI were about half those of DMS. It appears that the mutagenic effects of DMS and EI are by far higher than those of gamma rays.

Mutation types

Under the effect of DMS, EI, and gamma rays many mutation types were observed to appear in the three rice varieties. These can be divided into three major groups:

- Chlorophyll mutations. This group includes mutations related to changes in the chlorophyll content and distribution in the plant, ranging from albino to partial absence of chlorophyll known under various forms like xantha, viridis, or tigrina.
- Viable mutations of physio-morphological changes without special plant breeding value (possibly accompanied by sterility). Placed under this group are such mutations as narrow leaves, black spotted leaves, tawny lemma and palea, changes in growth and development rate, dense or sparse ears, long awns (Fig. 1), and anthocyanin pigmentation.
- Productive mutations of plant breeding value. This group comprises mutations characterized by higher yield, good grain quality, short and stiff culm, lodging resistance, dwarfing or semi-dwarfing, long ears with big grains, profuse tillering, and early maturing. A mutation in this group may involve one of these advanced characters or a complex of several.

Table 2 gives the percentage of each mutation type to the total mutations observed in each variety when treated with different mutagens at various concentrations and doses. For all three mutagens the proportion of mutations concerned with physio-morphological changes but without plant breeding value was the highest. Next to it was the proportion of mutations of plant breeding value, and the lowest proportion is accounted for by the chlorophyll mutation group.

With respect to the mutation spectrum, however, this index also varies with the concentration or dose of the mutagen. For instance, the 0.1% DMS concentration gave rise to the widest spectrum, whereas the narrowest spectrum appeared at 0.05%. For EI, the widest spectrum was

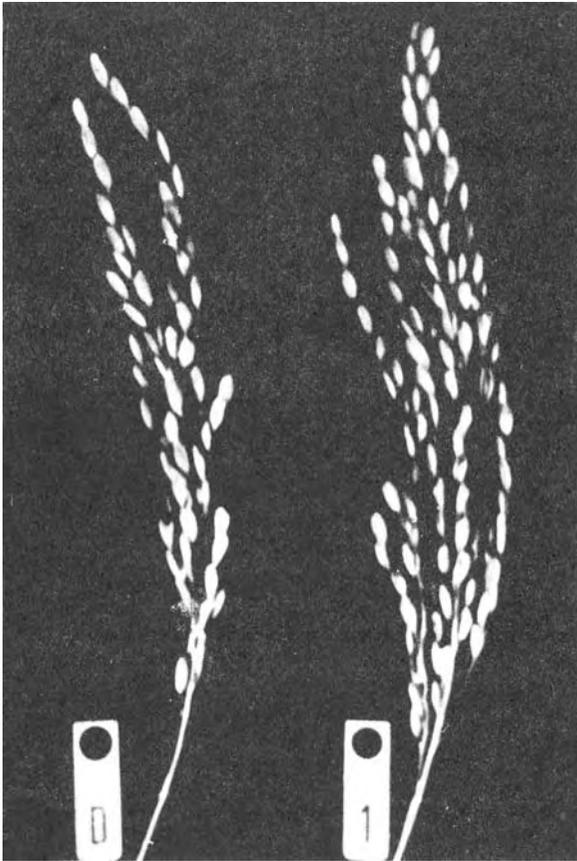


Fig. 1. 0 = check, 1 = mutant with larger ear and a larger number of spikelets and seeds.

observed at 0.03% and the narrowest at 0.06%. In the case of gamma rays the widest mutation spectrum was observed at the dose of 20 kR. Moreover, for all three mutagens, there was no proportional relationship between the mutation rate and spectrum.

As far as the specific effect of the mutagens is concerned, it can be noted that each of them is capable of inducing a mutation characteristic of itself. For instance, when treated with DMS the TCL variety generally exhibits such mutations as high culm, long ear with dense or sparse grains (Fig. 2) and three-four segmented husks (Fig. 3). Meanwhile, the mutations relating to the TQ 2 variety treated with EI involve, as a rule, short and stiff culm, round grains, and high fertility. Typical of the IR8 variety treated with gamma rays were mutants characterized by large

Table 2. Proportion of each mutation type to total mutants (%).

Strain Mutagen Concentration (%)/Dose (kR)	TCL DMS				TQ 2 EI				IR8 Gamma rays			
	0.03	0.05	0.10	0.20	0.01	0.03	0.04	0.06	5	10	20	30
Chlorophyll mutations	0.21	0.87	0.21	0.21	1.92	1.44	0.96	4.08	0	0.3	0.3	0.7
	(sum = 1.52)				(sum = 8.41)				(sum = 1.33)			
Physio-morpho- logical mutations	23.90	14.10	20.43	24.56	17.0	23.0	11.3	18.50	7.6	15.7	24.0	21.3
	(sum = 83.90)				(sum = 69.95)				(sum = 68.66)			
Productive mutations	1.08	5.68	3.26	4.34	6.73	5.30	5.52	4.08	6.3	8.7	5.6	9.33
	(sum = 14.76)				(sum = 21.60)				(sum = 30.00)			



Fig. 2. From left 1 = check, 2 = mutant with larger ear and dense arrangement of seeds (DMS 0.03%), 3 = mutant with accompanying palea to panicle (DMS 0.03%), 4 = mutant with larger but shrunken ear (DMS 0.03%), 5, 6, 7, 8 = other types of mutation with shrunken ears (DMS 0.03%).

ears, early maturing, and grains rotated at 180°. Moreover, even for a mutagen, there are mutations that appear often at certain concentrations but rarely or never at other concentrations. For instance, DMS concentrations of 0.03% and 0.05% were generally conducive to mutants with three-four segmented husks, while EI at 0.03% often gave rise to mutants with round grains and stiff culm.

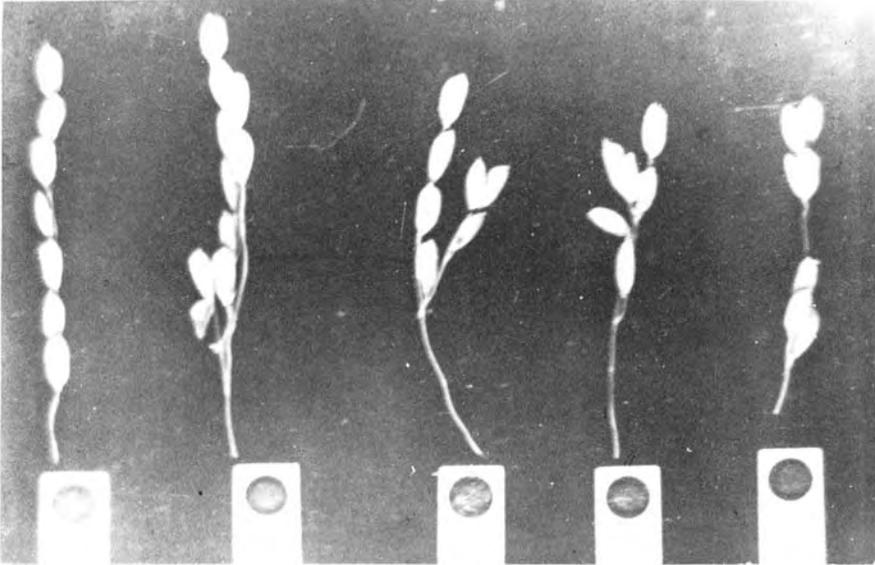


Fig. 3. From left 1 =check, 2, 3, 4, 5=mutants with 3-4 segmented husks (DMS 0.03%).

We have succeeded in singling out a number of mutants having high plant breeding value. There appeared from the TQ 2 variety treated with 0.03% EI solution a mutant that is nine days earlier than the check. Two mutants with large, dense ears and numerous grains (280-330 as against 135-140 in the check) were selected from the TCL variety treated with 0.05% DMS (Fig. 4 and 5) and from the IR8 variety treated with 30 kR of gamma rays. Besides, gamma irradiation of 10 kR brought about an IR8 dwarf mutant measuring only 40 cm in height (compared with 101 cm in the check).

Hereditary nature of some mutants

Some of the mutations with plant breeding value were isolated to be cultivated afterwards as different lines, the stability and segregation of which were observed in the M_3 generation. Those that appeared to be stable in this generation were backcrossed with the parent material. M_3 lines showing segregation continued to be separated through successive

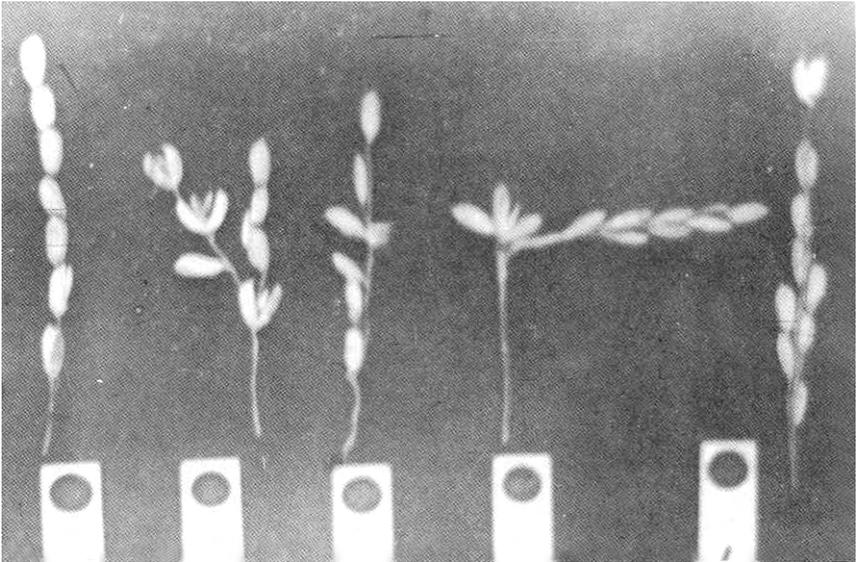


Fig. 4. From left 1 =check, 2, 3, 4, 5 =various types of mutation with two or more seeds emanating from one stalk (DMS 0.03%).

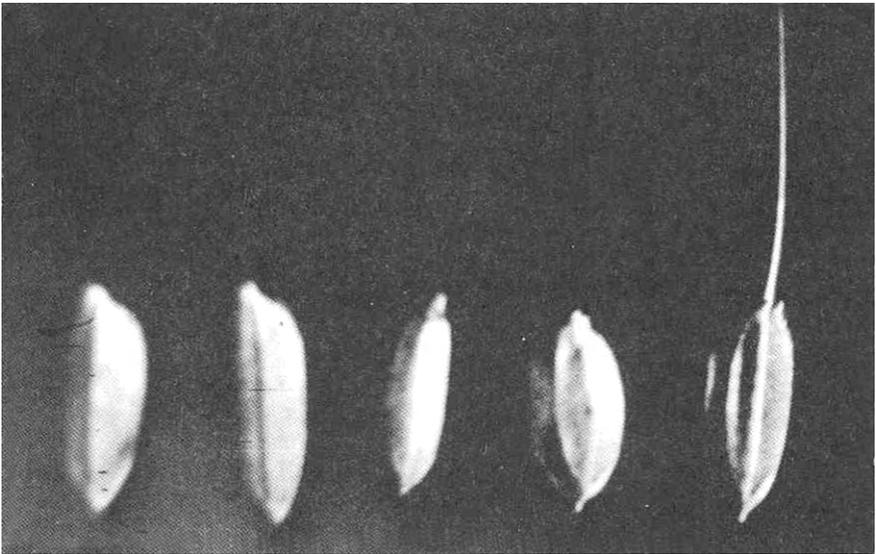


Fig. 5. From left 1, 2, 4 = various types of mutation with larger seeds, 5 = mutant with long awn, 3 = check.

generations. The analytical results obtained lead to the following remarks on the hereditary nature of some mutants.

Under the effects of gamma rays (20 kR) the IR8 variety produced a narrow-leaved mutant from which stable lines were isolated after the M_2 . The offspring of the F_2 cross of this mutant and the parental material showed segregation at the rate of 3:1 plants with normal leaves to plants with narrow leaves. This mutant is thus of the monogenic recessive type, which has been dealt with by Hsien (2).

In addition, we discovered a mutant with dark violet stem and leaf-sheath from the TQ 2 variety treated with 0.02% EI right in the M_1 generation. Segregation of this mutant was found in the generations following M_2 . The crossing of this stabilized line with the original material gave birth to F_1 individuals with dark violet stem and leafsheath. This result implies that this is a mutant of the dominant type. Similarly, a mutant showing anthocyanin pigmentation with purple leafsheath, stigma, lemma, etc. was mentioned by Basu and Choudhury (1), who realized that this pigmentation is dominant in segregation and crossing.

CONCLUSIONS

DMS, EI, and gamma rays are relatively highly effective mutagens for rice. With regard to the three rice varieties used in this study, DMS and EI appear to bring about a higher mutagenic effect than gamma rays.

As far as these mutagens and rice varieties are concerned, and taking into account the mutation rates observed in M_2 as well as the proportion of mutations of plant breeding value, it is recommended that DMS be used at concentrations ranging between 0.05% and 0.1%, EI between 0.03% and 0.04%, and gamma rays at a dose of 30 kR.

Although different mutagens can induce more or less identical mutants, each one as a rule has a specific effect of its own: EI tends to give rise to early-tillering mutants with short and stiff culm and round grains; DMS to ones with high culm, long ears, and sparse grains; and gamma rays to mutants characterized by early maturing, large ears, and 180°-rotated grains.

While DMS and EI ensure a higher mutation rate than gamma rays, the latter can engender a variety of small mutations of plant breeding value.

An analysis of the segregation observed in successive generations resulting from the backcrossing of the mutant lines with their respective parent materials has led to the conclusion that the dark violet stem

mutation is of a dominant type and the narrow leaf mutation is of a monogenic recessive type.

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DISCUSSION

SESSION 12: MUTAGENESIS

Q – Brar: MNU was found to give a high frequency of chlorophyll and endosperm mutants. Did you notice strong pleiotropy associated with mutants derived from fertilized egg cells vs. dry-seed derived mutants?

A – Satoh: I think that strong pleiotropy does not occur.

Q – Brar: MNU treatment of fertilized egg cells resulted in high mutation frequency, probably due to elimination of chimera formation and diplontic selection. However, with EMS and EI, the mutation frequency after treatment of fertilized egg cells has been reported to be lower than with the dry seed treatment. How do you account for such variation and elimination of diplontic selection?

A – Satoh: EMS (also EI) is very toxic to growing cells. A high concentration of EMS causes cell death, and low concentration induces a few mutations.

Q – Hsieh: Your data show that mutagen-treated progenies had a wide range of variation in amylose content (from low to high). It is understood that indica type rice has a high amylose content and japonica type rice has a low amylose content. Do you think your mutants with high amylose content can be classified as indica type mutant? Or do you think it is possible to derive indica type mutants from japonica rices?

A – Satoh: Our high amylose mutants may not be indica type mutants, because their amylose content is higher than that of indica and other characteristics are significantly different from those of indica rices. However, I think that some characteristics of indica rices can be induced in japonica rices.

Q – Rutger: Two mutants that would be very useful as breeding tools are dominant genetic male sterile and dominant gene for extremely early maturity. Have you ever observed either of these mutants?

A – Satoh: I have not obtained a dominant genetic male sterile mutant, though some recessive ones have been obtained. Several kinds of dominant or partially dominant as well as recessive mutants for earliness have been obtained.

Q – Wu, H. K.: How did you test the neck and/or leaf blast resistance of your mutants? By inoculation with known races or under natural field infection?

A – Singh: The reactions to blast disease of the mutants were recorded simultaneously with the yield trials for three seasons under field conditions.

Q – Abifarin: Do the upland mutants 4 and 7 possess deep and thick root systems?

A – Singh: I cannot say anything about this as this type of work has not been done with mutants 4 and 7.

C – Narahari: The number of mutants and mutant-derived varieties in rice, particularly those from India, needs updating. So far, 15 such varieties from India have been listed and not 8 as mentioned. Information up to 1984 in this regard completed by me has recently been sent to IAEA.

TISSUE AND CELL CULTURE

SESSION 13

SOMAACLONAL VARIATION IN RICE

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A review of the phenomenon of somaclonal variation, its features, and its possible mechanisms in rice is presented. Somaclonal variation is ubiquitous in tissue culture, and several cases have been documented in rice for agronomically important characters that have been recovered through this approach. Its significance to rice breeding is discussed.

Genetic variability provides the background upon which recombination and genotypic selection operate to help the plant breeder produce better adapted cultivars. At the moment, in addition to the genetic variability generated by conventional plant breeding methodology, another new avenue for creating variability is through genetic modifications occurring during the process of tissue culture, a phenomenon termed somaclonal variation (11). It appears that its occurrence covers a wide range of economically important species (3, 7, 11, 13, 15, 28, 32, 36); this paper discusses its possibilities in rice.

NOMENCLATURE

The nomenclature for the plant regenerant arising from tissue culture and its successive generations has been as varied and numerous as the authors (Table 1). Sibi (37) used P_0 for the primary regenerated plant and P_1 , P_2 , etc. for the subsequent generations. Chaleff (4) suggested the symbol R for the regenerant, with R_1 as the progeny of the primary regenerated plant and R_2 , R_3 , etc. as the successive selfed generations; this system has been used by several workers (9, 33). Schaeffer et al (30) used the symbol R_0 for the regenerant plant but S_1 , S_2 , etc. for the ensuing generations. Oono (22) proposed D_1 for the regenerant, followed by D_2 , D_3 , etc. to denote subsequent generations. In view of all these differences in nomenclature, it has been proposed (10) that the symbol SC_1 be adopted for the primary regenerant plant and SC_2 , SC_3 , etc. for the successive generations. This notation would correspond with conventional genetic nomenclature, assuming that the primary regenerant contains a mutation in the heterozygous state and would thus segregate in

Table 1. Various notations used for regenerated plants and their progenies.

Generation	Author				
	Chaleff (4)	Larkin et al (10)	Oono (22)	Schaeffer et al (30)	Sibi (37)
Regenerant (first generation)	R	SC ₁ ^a	D ₁	R ₀	P ₀
Primary regenerated plant (second generation)	R ₁	SC ₂	D ₂	S ₁	P ₁
Secondary regenerated plant (third generation)	R ₂	SC ₃	D ₃	S ₂	P ₂

^aCorresponds to F₁ and/or MF₁.

the SC₂ generation (i.e., F₂ from a conventional cross and M₂ from mutation induction). Thus an SC₂ family originates from selfed seed of a single SC₁ plant, and an SC₃ family originates from selfed seed of a single SC₂ plant.

GENERAL FEATURES OF SOMACLONAL VARIATION

Extensive research in wheat (10, 32) has revealed several features of somaclonal variation:

- Variation has been observed for both morphological and biochemical traits.
- Both traits under simple genetic control and quantitatively inherited characters are subject to variation.
- A single somaclone can be variant for a number of traits that appear to assort independently in preliminary progeny analysis.
- Both heterozygous and homozygous mutants have been recovered in the primary regenerant. A single somaclone could contain both states at different loci.
- Chromosome loss or addition is not evident as the primary cause of the variation.
- In analyses involving gliadin proteins, seed contamination or cross pollination has been excluded as the source of variation.
- The variation does not appear among seed progeny of the parent.

None of the variant phenotypes has been observed among more than 400 plants grown from the original seed source or its progeny.

- Mutations affect characters for which major gene loci are known to be located on all seven homeologous groups.
- Somaclonal mutants can be recessive, dominant, or codominant.

SOMACLONAL VARIATION IN RICE

Phenotypic variants among primary regenerant plants have been reported by Nishi et al (20), Henke et al (8), and Nakano and Maeda (16). Variation was observed in traits such as number of fertile tillers per plant, average panicle length, number of fertile seeds, plant height, and flag leaf length. Niizeki and Oono (17) observed the occurrence of albino plants from regenerants, while polyploidy has also been reported (18, 19).

Bajaj et al (2) and Bajaj and Bidani (1) reported that regenerated plants from embryos and mature and immature endosperms show a wide range of genetic variability. Apart from diploids ($2n = 24$), haploids, polyploids, and aneuploids were also observed, the chromosome number ranging from 11 to 60. Oono (25) suggested that phenotypic variations of regenerated plants may be induced by genomic, chromosomal, genic, and cytoplasmic mutations or by physiological effects. He added that it is possible to evaluate the quantity and quality of genetic variability in cultured cells through genetic analysis of regenerated plants and their offspring.

Oono (24, 25) investigated this genetic variability through several experiments using diploid seed callus of rice from a homozygous line. The progeny from 1121 SC_1 were examined for variation in seed fertility, plant height, heading date, morphology, and chlorophyll deficiency. About 72% of the regenerated plants carried some mutated characters (Table 2). F_2 analysis of several SC_3 mutants isolated showed the presence of single recessive mutations (22, 23). Oono (25) also studied the quantity and quality of genetic variability induced in haploid somatic tissues of rice. From 155 SC_1 plants comprising 74 haploids, 80 diploids, and a tetraploid, a great degree of variation was observed for heading date and plant height in both haploids and diploids, while seed fertility was most variable in diploids. An analysis of the SC_2 generation showed a wide variation of several agronomic traits such as heading date, plant height, number of grains, and seed fertility (Fig. 1). Oono (25) suggested

Table 2. Changes of characters of mutants in SC₂ lines derived from panicles of SC₁ plants regenerated from rice callus (data from 25).

Mutated characters ^a	No. of lines	%
Normal	214	28.1
Ploidy (4x)	12	1.6
Fer	273	35.8
Ht	19	2.5
Hd	3	0.4
Mor	1	0.1
Ch	27	3.5
Fer and Ht	74	9.7
Fer and Hd	14	1.8
Fer and Mor	7	0.9
Fer and Ch	59	7.7
Ht and Ch	3	0.4
Hd and Ch	1	0.1
Mor and Ch	1	0.1
Fer, Ht, and Hd	11	1.4
Fer, Ht, and Mor	2	0.3
Fer, Ht, and Ch	27	3.5
Fer, Hd, and Ch	2	0.3
Fer, Mor, and Ch	1	0.1
Fer, Ht, Hd, and Mor	1	0.1
Fer, Ht, Hd, and Ch	9	1.2
Fer, Ht, Mor, and Ch	1	0.1
Total	762	100

^aFer = seed fertility, Ht = plant height, Hd = heading date, Mor = morphological traits, Ch = chlorophyll deficiency.

that the genetic variability of the regenerated plants from haploid somatic tissues appears to be larger than the variability resulting from chronic radiation.

Schaeffer (28) demonstrated the recovery of genetic variation for short stature among anther-derived doubled haploid rice. Some haploids recovered from the parental Calrose 76 were shorter than the original cultivar. When the double haploid dwarfs were analyzed in the SC₂ generation it was found that from 15% to 30% were shorter than Calrose 76. In addition, seed and leaf size and seed weight were significantly lower in the SC₃ plants than in Calrose 76. In another study, Schaeffer et al (30) found significant variation among anther-derived doubled haploids when analyzed after several seed generations (Table 3). Plants with larger seeds, higher levels of seed protein, shorter stature, and more tillers than Calrose 76 were obtained from selfed anther-derived plants (Table 4).

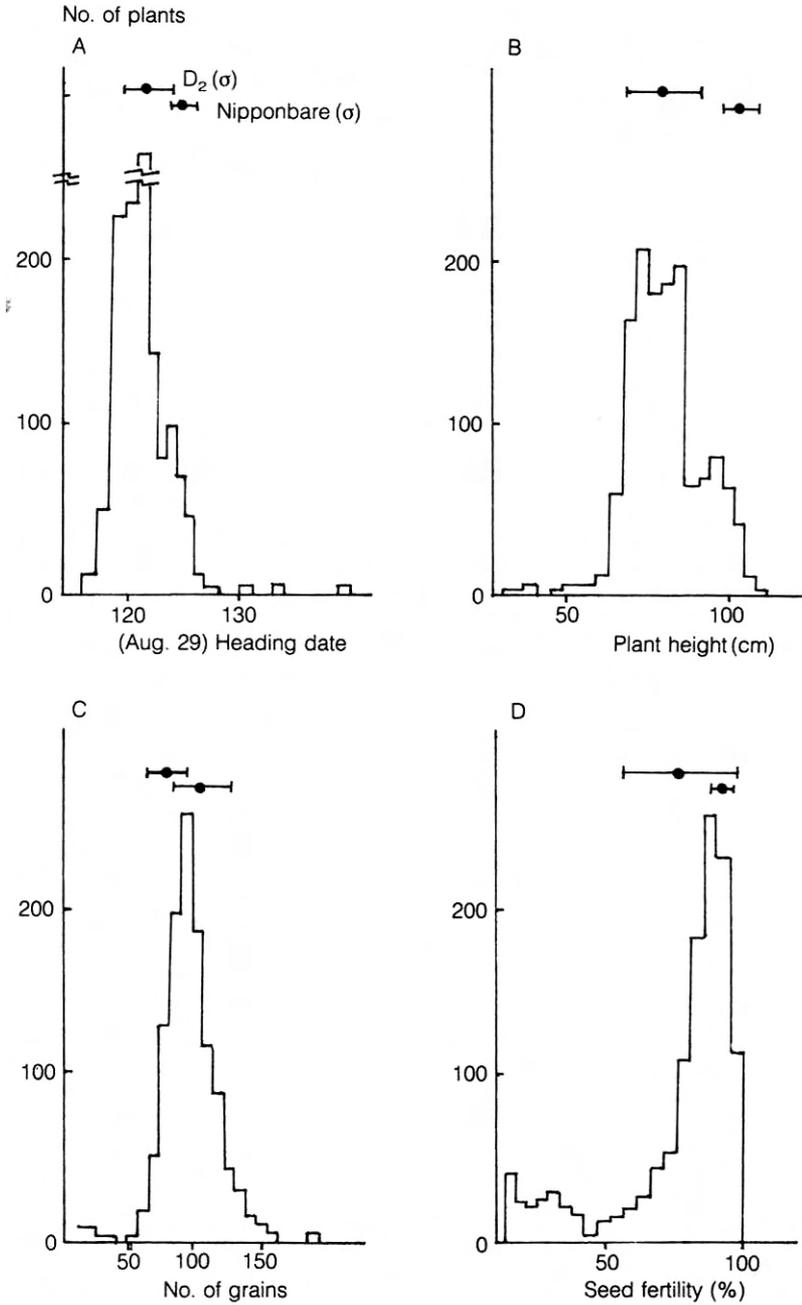


Fig. 1. Variation in four agronomic characters of SC_2 plants obtained from somatic callus of haploid rice (after 25).

Variations in dwarfism, sterility, heading date, brown leaf spots, chlorophyll mutations, and various morphological irregularities were observed in the SC₂ of a study involving seed-derived callus culture (38).

Table 3. Range of plant traits of third generation plants derived from anther culture (data from 30).

Trait	Population range					
	Control			Anther culture group		
	Lowest	Highest	Mean	Lowest	Highest	Mean
Seeds/plant (no.)	564	854	747	627	941	774
Seed wt. (mg)	21.4	23.7	22.8	20.3	22.3	21.6
Yield (g/plant)	12.1	20.2	17.0	12.7	21.0	16.7
Seed protein (%)	7.3	9.9	8.9	8.1	11.0	10.0
Protein (mg/seed)	1.56	2.25	2.04	1.73	2.45	2.16
Seed protein (g/plant)	1.26	1.85	1.53	1.30	1.88	1.66
Lysine (% of total amino acids)	3.38	3.60	3.45	2.94	3.60	3.25
Tillers (no./plant)	9	12	10.3	13	18	15.1
Spike height (cm) ^a	75	81	79	45	69	52
Lysine (mg/seed)	0.064	0.079	0.071	0.059	0.088	0.071
Total lysine (mg/plant)	43	63	54	43	66	54

^aMean of control significantly different from anther culture group mean at P = 0.05.

Table 4. Mean plant traits of Calrose 76 and several fourth generation anther-derived lines (data from 30).

Trait	Rice lines and means ^a				
	Control	AC-11	AC-7	AC-14	CD-28-3
Seeds/plant (no.)	1782	1703	1560*	1665	1634*
Seed wt. (mg)	20.7	19.5*	20.6	20.7	21.0
Seed protein (%)	8.16	8.36	8.34	8.55*	8.25
Protein (mg/seed)	1.69	1.63	1.71	1.77	1.74
Seed protein (g/plant)	3.0	2.8	2.7*	3.0	2.9
Spike height (cm)	95	68*	72*	70*	71*
Tillers (no./plant)	28	35**	34**	32**	33**
Grain/straw weight ratio ^b	0.31	0.32	0.32	0.34	0.33

^a* = significantly lower than control at P=0.05, ** = significantly higher than control at P=0.05.

^bStraw weight not used in statistical analyses.

MECHANISMS FOR SOMACLONAL VARIATION

The possible underlying causes of somaclonal variation in crop species have recently been reviewed (32), and according to the authors, apart from karyotypic abnormalities, there is little hard evidence to favor any one of several possible mechanisms to account for somaclonal variation.

The tissue culture system seems to generate a high frequency of chromosomal aberrations (14, 27). Even through aberrations that would give rise to lethality or sterility may not be observed when somaclonal progeny are analyzed (32), meiotic chromosomes of regenerated plants (21) show chromosomal rearrangements such as partial chromosome deletions, reciprocal and nonreciprocal translocations, paracentric inversions, or subchromatic aberrations. Attempts to discern a causal relationship between gross chromosomal aberration and genetic changes in somaclones have not been conclusive, as exemplified by the work on genetic changes at the phosphoglucomutase locus and their relationship to chromosome loss in celery callus cultures (27). It is possible, as suggested by Scowcroft and Ryan (32), that the two events may be under the same mechanism, but our limited powers of resolution are not yet able to determine it.

Another possible source of somaclonal variation that has been proposed is DNA amplification (11, 32), wherein the number of copies of a specific haploid genome increases during differentiation or as a result of environmental limitations. Both quantitative and qualitative changes in the DNA of tissue cultures and regenerated plants have been reported (5, 6). Research with animal cells has shown that genes coding for a specific functional product can be amplified many times when cell lines are selected for antimetabolite resistance (31, 39).

Genetic elements that transpose from one location to another in the genome (12) have been suggested as another mechanism for somaclonal variation. Shapiro and Cordell (35) observed that the occurrence of transposable elements in eucaryotes is widespread. However, direct evidence to show that transposable elements are somewhat responsible for somaclonal variation in plants is still not available; but circumstantial data such as the variability found in protoplast-derived potato clones (34) and the concurrent variation of wheat somaclones (10) seem to suggest underlying transpositional events. Recent data by Oono (26), however, imply that gene inactivation induced by tissue culture may be responsible for the distorted segregation pattern of the putative homozygous mutations in regenerated rice plants.

CONCLUSION

The phenomenon of somaclonal variation in plants has been adequately documented (11, 14, 32). The feasibility of using such variability, especially in selecting for agronomically important traits in rice at the cellular level, has already been demonstrated (25, 29, 30, 40). It remains to be seen whether an already established cultivar can be improved in a stepwise manner without risk to its basic genetic constitution by using somaclonal variation. Perhaps the euphoria over somaclonal variation today may not be dissimilar to the excitement generated by induced mutation several decades ago.

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EFFECTS OF PLANT GROWTH SUBSTANCES ON CALLUS FORMATION AND PLANT REGENERATION IN ANTHER CULTURE OF RICE*

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Anthers of a pure line of rice (*Oryza sativa* L.) at the midunicellular microspore stage were cultured on N6 medium supplemented with various combinations of kinetin, 2,4-D, and NAA. The calli formed were then transferred to MS medium containing 0.5 mg/litre NAA and 2 mg/litre kinetin for plant regeneration. NAA and 2,4-D were about equally effective in inducing callus formation, but calli formed in the presence of 2,4-D were less capable of plant regeneration compared to those formed on medium supplemented with NAA. In general, addition of kinetin slightly increased callus formation and markedly improved the morphogenetic potential of the callus. Histological examination revealed that embryogenesis was the main pathway to plant formation from callus; organogenesis also occurred but was rare.

Plant formation in rice anther culture is a two-stage process. The microspores first develop into unorganized tissues known as callus, and then the callus gives rise to plants through organogenesis and/or embryogenesis (19). Both stages require proper manipulation of plant growth substances in the culture medium.

The auxins and cytokinins used in rice anther culture media have varied considerably. In early studies, 2,4-dichlorophenoxyacetic acid (2,4-D) was thought to be required for in vitro microspore development, and it was always included in the callus induction medium, either alone (20,24,25) or in combination with other growth substances (10,11,14,19). Cytokinin was considered by some investigators (7,21) to be nonessential for plant regeneration. However, Chen and colleagues (4,6,15) maintained that 2,4-D is inhibitory to organ formation and could be substituted for by α -naphthaleneacetic acid (NAA), which was equally effective as 2,4-D in inducing callus formation. They also held the view that for obtaining callus of high morphogenetic potential, cytokinin should also be present in the induction medium.

Although plant regeneration in anther culture of rice does not seem to be a problem now, at least in cultivars belonging to the japonica subspecies, how the callus differentiates into plants is still not known for certain. Chang et al (1) observed that shoot and root primordia initiate independently in the callus tissues, thus indicating organogenesis in nature. However, a more recent study by Genovesi and Magill (9) showed that organogenesis and embryogenesis both occur.

In this paper we present a comparative study of the effects of 2,4-D and NAA in the presence and absence of kinetin on callus formation and plant regeneration, and histological observation of callus during plant regeneration.

MATERIALS AND METHODS

The plant material used in this study was a pure line of *Oryza sativa* L. cv. Tainan 5 that was obtained through self-fertilization of a doubled haploid plant (16).

The anther culture method described by Chen (2,3) was followed. Anthers at the midunicellular microspore stage were excised and cultured on N6 basal medium (8) containing 6% sucrose and variable amounts of 2,4-D, NAA, and kinetin. Fifty anthers were inoculated into one culture tube, and for each treatment 250 anthers were cultured. After 20–25 days of incubation, anthers were removed from culture tubes and opened with two fine-tip hooked needles under a dissecting microscope. Calli of about 0.3 mm in diameter and of presumably single microspore origin were dissected and transferred onto the same medium, where they were initiated for further proliferation. When calli reached approximately 2 mm in diameter, they were transferred individually to single culture tubes containing Murashige and Skoog's (17) basal medium, 0.5 mg/litre NAA, and 2 mg/litre kinetin for plant regeneration.

Only the callus initiated on medium with 2 mg/litre NAA and 1 mg/litre kinetin was used for histological study. Callus tissues were collected at various intervals after transfer to the regeneration medium. They were either (a) fixed in formalin-acetic acid-ethyl alcohol, dehydrated through a tertiary butyl alcohol series, embedded in paraffin, cut 8–10 μm thick, and stained in Delafield's hematoxylin, or (2) fixed in a mixture of 4% glutaraldehyde and 10% acrolein, post-fixed in 1% osmium tetroxide, dehydrated in a graded acetone series, infiltrated with Spurr's resin, cut 1 μm thick, and stained in 0.05% toluidine blue O in 1% sodium borate.

RESULTS

Effects of plant growth substances

Callus formation was very low when there was no auxin in the culture medium, and the frequency increased with the addition of kinetin (Table 1). All calli formed on the auxin-free media became necrotic after they were surgically removed from the anther and transferred to identical media for proliferation.

Addition of 2,4-D or NAA, singly or in combination with kinetin, to the basal medium resulted in significant increases in callus formation. Although the frequency of callusing anthers seemed to be higher on media supplemented with 2,4-D than on medium with NAA, the differences were not statistically significant. Similarly, the differences in callus formation between the two concentrations of the same auxin were not significant. In the presence of NAA, the frequency of callus formation tended to increase as the concentration of kinetin increased from 0 to 1 to 2 mg/litre. In combination with 2,4-D, however, the optimal concentration of kinetin was 1 mg/litre.

Plant regeneration of callus formed on media with various growth

Table 1. Callus formation from rice anthers cultured on N6 medium supplemented with various combinations of kinetin, 2,4-D, and NAA.

Combination of plant growth substances			Anthers producing callus ^a (%)	Calli per responding anther (no.)	
NAA	2,4-D (mg/litre)	Kinetin		Mean	Range
0	0	0	4.8 ^a	2.0	1-5
0	0	1	13.2 ^b	1.9	1-6
0	0	2	14.8 ^b	2.0	1-4
2	0	0	29.2 ^{cde}	3.3	1-8
2	0	1	33.2 ^{def}	3.7	1-10
2	0	2	36.8 ^{ef}	4.5	1-13
4	0	0	22.4 ^c	4.2	1-15
4	0	1	27.6 ^{cd}	4.0	1-19
4	0	2	33.2 ^{def}	3.9	1-12
0	2	0	34.4 ^{def}	4.7	1-19
0	2	1	38.8 ^f	3.6	1-9
0	2	2	33.2 ^{def}	3.9	1-15
0	4	0	28.4 ^{cd}	3.2	1-9
0	4	1	32.8 ^{def}	4.2	1-11
0	4	2	27.2 ^{cd}	3.6	1-6

^a Means followed by a common letter are not significantly different.

Table 2. Plant regeneration from rice microspore callus initiated on N6 medium with various combinations of kinetin, 2,4-D, and NAA.

Growth substances in callus induction medium			Calli cultured on regeneration medium (no.)	Calli producing plants (%)
NAA	2,4-D (mg/litre)	Kinetin		
2	0	0	94	33.0
2	0	1	81	74.0
2	0	2	126	72.2
4	0	0	89	41.6
4	0	1	80	70.0
4	0	2	92	73.9
0	2	0	108	7.4
0	2	1	121	10.7
0	2	2	111	15.3
0	4	0	95	14.7
0	4	1	113	2.7
0	4	2	95	2.1

substances is shown in Table 2. It can be seen that calli formed in the presence of NAA were more capable of plant regeneration than those initiated on media with 2,4-D. Addition of kinetin to medium containing 4 mg/litre 2,4-D was deleterious to morphogenesis of the callus. However, in all other cases the presence of 1 or 2 mg/litre kinetin in the induction medium was beneficial to callus differentiation.

Histology of plant formation

The microspore callus of rice produced under the culture conditions used in this study was compact and light yellow in color and had a smooth surface (Fig. 1.1). In addition to increase in size, the earliest change observed after transfer to the regeneration medium was the appearance of nodules on the surface of the callus (Fig. 1.2). Histological examination of callus revealed the presence of trichomes and vascular tissues in the nodular areas (Figs. 2.1, 2.2). The nodules increased in size as culture proceeded and eventually developed into leaf-like structures that varied in shape, size, and presence or absence of chlorophyll (Fig. 1.3). Subsequently, embryoids with a clearly defined shoot-coleorhiza axis (Figs. 1.4, 1.5) and shoots (Fig. 1.3) appeared at the base of the leaf-like structures. One piece of callus could give rise to both green and albino shoots, but green shoots were always associated with colored leaf-like

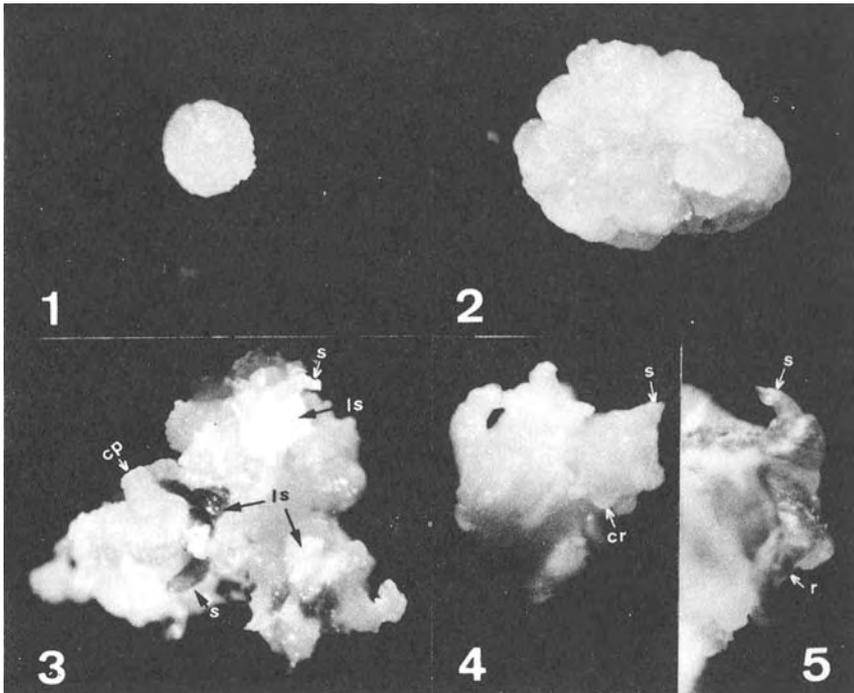


Fig. 1. Development of embryoids and shoots from microspore callus of rice. 1.1. Early stage of callus with a smooth surface. 1.2. Nodular callus. 1.3. Callus showing leaf-like structures, coleoptile, and shoots. 1.4. An embryoid (arrow) showing the shoot-coleorhiza axis. 1.5. Germination of an embryoid (arrow). cp = coleoptile, cr = coleorhiza, ls = leaf-like structure, r = root, s = shoot.

structures and albino shoot with white leaf-like structures.

Sections of embryoids showed that many of them possessed a coleoptile, shoot apex, root primodium, and a closed vascular system (Figs. 2.3, 2.4). The attachment of embryoids to leaf-like structures seemed to be rather loose because it could be broken easily by histological procedures (Fig. 2.4). Attempts to determine the origin of the embryoids by observation of sections of younger leaf-like structures were not successful. Some epidermal cells in the meristematic regions of the leaf-like structures were seen to be densely cytoplasmic with a prominent nucleolus and to be delimited from adjacent cells by a thick wall (Fig. 2.5), but whether these cells would form an embryoid is not known. In addition to embryoids, adventitious shoot apices (Fig. 2.6) and structures intermediate between shoot apex and embryoid were also observed.

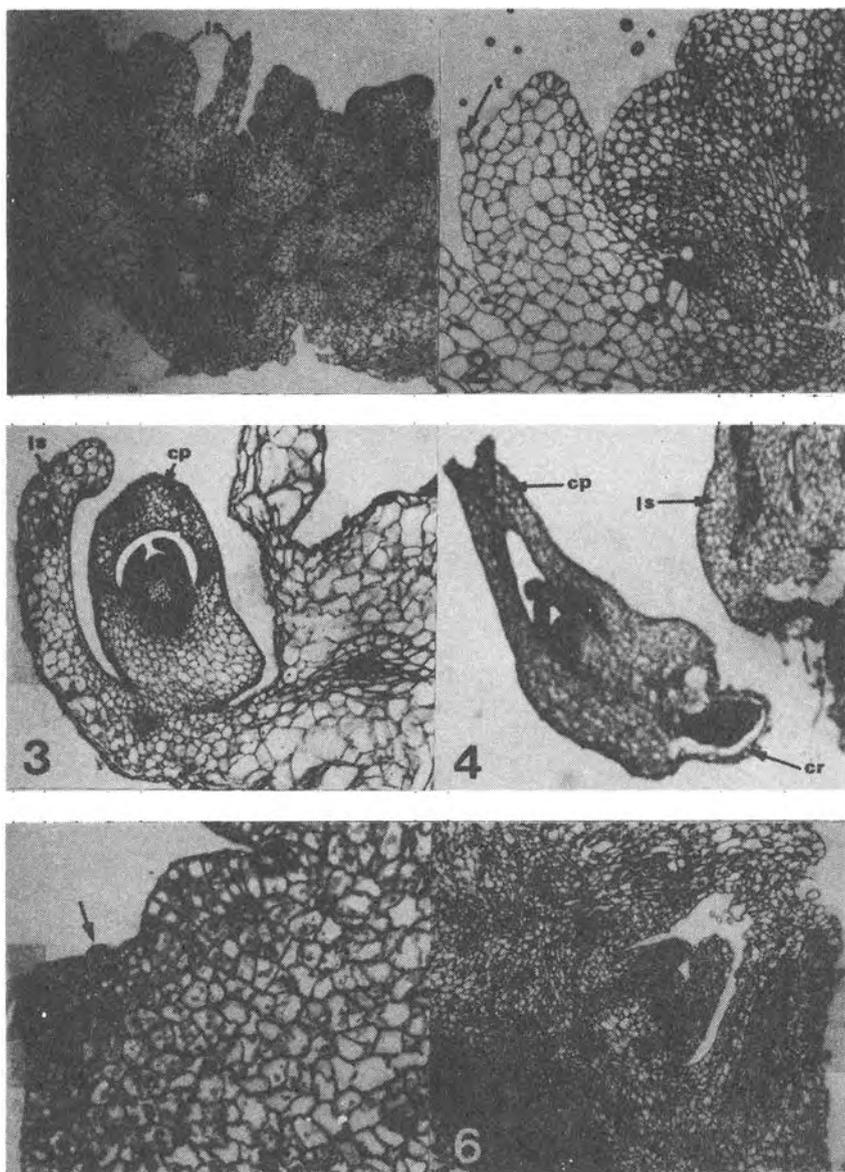


Fig. 2. Histology of embryoid and shoot formation in microspore callus of rice. 2.1. Initiation of leaf-like structures. 2.2. Leaf-like structures showing trichomes and internal vascular tissues. 2.3. An embryoid attached to the leaf-like structure. 2.4. An embryoid detached from the leaf-like structure. 2.5. Part of a leaf-like structure showing two epidermal cells with dense cytoplasm and a prominent nucleolus (arrow). 2.6. A shoot apex and leaf primordia. cp = coleoptile, ls = leaf-like structure, t = trichome, v = vascular tissues.

DISCUSSION

The plant material used in this study was a pure line; therefore genotypic effects on callus formation and plant regeneration (5, 10) were eliminated. Because in anther culture of rice usually more than 1 and sometimes up to 19 microspores in an anther can develop into callus, while testing morphogenetic potential it becomes necessary to use callus of single microspore origin rather than that derived from the whole anther, as a unit. This was what we did in this study.

Callus formation was very low and growth of callus was impaired when there was no auxin in the anther culture medium. Addition of 2,4-D or NAA resulted in significant increases in callus formation and growth, but regeneration potential of the callus was low if kinetin at appropriate concentrations was not simultaneously present. This indicates that auxin is required for callus induction and growth and kinetin for promoting callus differentiation. Thus, for efficient production of microspore callus of high morphogenetic potential, both auxin and kinetin are required.

Comparison of the efficiency of 2,4-D and NAA indicates that the two synthetic auxins are about equally effective in inducing callus formation and in supporting callus growth, but callus formed in the presence of 2,4-D is less capable of plant regeneration than that formed on medium with NAA. Thus 2,4-D may be inhibitory to morphogenesis in the callus. The inhibitory effect is probably due to preculture of the callus on the induction medium, since 2,4-D is a rather strong auxin and is not easily decomposed in rice callus (12).

The data in Table 2 show that the effects of kinetin on callus differentiation can be promotive or inhibitory depending on the kind and concentration of auxins in the culture medium. This complex nature of effects of kinetin has also been observed by Inoue and Maeda (13) in rice callus derived from seed. How kinetin interacts with auxins to give these divergent effects is not known.

In this study, histological examination of plant formation was made only on callus initiated on medium with 2 mg/litre NAA and 1 mg/litre kinetin. Our results showed that during plant formation the callus first formed leaf-like structures on the surface, and subsequently embryoids and adventitious shoots arose from the leaf-like structures. According to Vasil (23), the leaf-like structures found in tissue cultures of many cereals are really the enlarged scutella of the embryoids. Thus, the association of a discrete embryoid, which has a coleoptile, shoot-root axis, and a closed vascular system, with a leaf-like structure (scutellum) clearly indicates that typical embryos are formed in rice microspore callus under the

conditions used in this study. Embryogenesis has also been observed in rice microspore callus initiated on medium containing 2 mg/litre NAA (9) but not in callus initiated from various tissue (including microspores) on medium containing 2,4-D (1,18,22). These observations, together with the quantitative data on plant regeneration obtained in this study, suggest that the growth substances in the callus induction medium may play an important role in regulating the pathways to plant formation. A test of this hypothesis by histological examination of callus formed on medium containing various growth substances is urgently needed.

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RICE ANTHOR CULTURE: A TOOL FOR PRODUCTION OF COLD TOLERANT LINES

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Rice anther culture proved to be a very useful tool for obtaining cold tolerant lines by immediately fixing the homozygosity of F_1 hybrids. A shuttle scheme was established in collaboration with the Rural Development Administration of Korea for the production of cold tolerant lines from F_1 hybrids through anther culture techniques. Results show that the genotype is a crucial factor in the culturability of anthers of F_1 sexual crosses. In the cold screening test at the seedling stage, some anther culture (AC)-derived lines have shown increased tolerance relative to both parents, which became more evident at longer durations of cold treatments. In the cold screening nursery, 3 of 22 lines tested were better than the resistant check in percentage fertility and vigor, while in the replicated yield trials 3 of 6 AC-derived lines outyielded both parents. The results show that different recombinants from the same cross are expressed in the homozygous lines regenerated through anther culture. The hybrid vigor and cold tolerance of the AC-derived lines may be due to the synergistic, additive, or complementary effect of the genes from both parents.

Rice is the major source of calories for 40% of the world's population (1). Worldwide, rice ranks second to wheat in area harvested; but it ranks first as a food crop, providing more calories/ha.

In South and Southeast Asia, about 7 million ha cannot be planted to modern rice varieties because of cold water and cold weather. In some areas, two cropping seasons a year would be possible if cold tolerant varieties were developed.

Cold tolerance is an important plant character in areas where low temperature at the seeding and seedling stages is a problem. Incorporat-

ing cold tolerance at the seedling stage is difficult, and the progeny of many crosses of cold tolerant parents show little or no cold tolerance. Backcrossing seems to be effective, although it is a very slow process. Another way of producing cold tolerant lines is by using anther culture techniques in which anthers of F_1 hybrids having one or both parents cold tolerant are cultured. Anther culture offers the advantage of attaining homozygosity immediately.

In 1983, a shuttle scheme for the production of cold tolerant lines from hybrids was established in collaboration with the Office of Rural Development, Suweon, Korea (Fig. 1). This paper presents preliminary results of callus production and plant regeneration from F_1 crosses for cold tolerance, cold tolerance screening, and replicated yield trials of plants regenerated from anther culture.

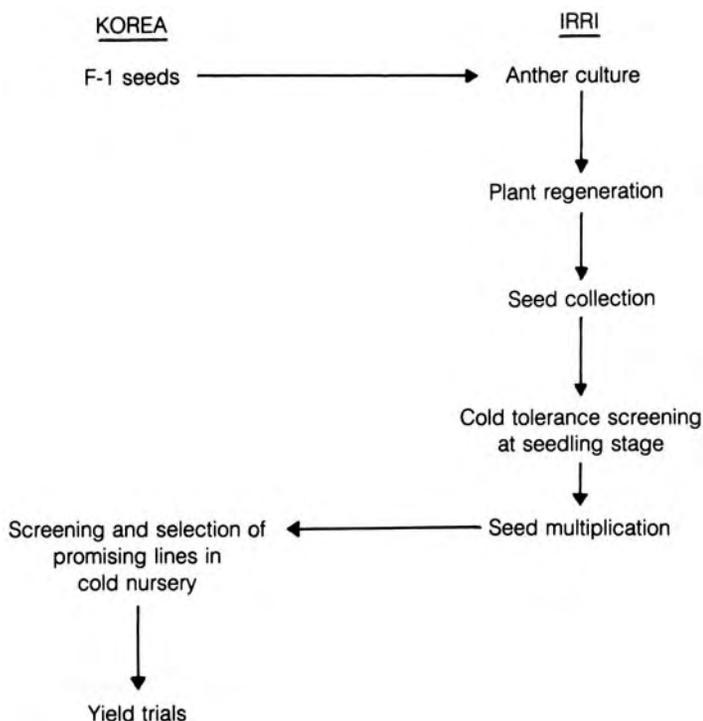


Fig. 1. IRRI-Korea collaborative program for the production of cold tolerant rice through anther culture.

MATERIALS AND METHODS

Anther culture of F₁ crosses

Thirteen SR (Suweon Rice) crosses from Korea were tested for callus induction and plant regeneration. The F₁ seeds were grown in pots in the screenhouse, and panicles were collected at the stage where the auricle distance of the flag leaf to that of the subtending leaf was 5–8 cm. The anthers were plated in various callus-induction media when the pollen grains were at the miduninucleate to early binucleate stage of development. The plated anthers were kept in the cold at 8 °C for 8 days and were then transferred under dim light at 25 °C until calli formed.

Calli of about 1mm diameter were plated on paper bridges in N-19 medium (4) and incubated under 1,000 lux at 25 °C for 4 weeks. The calli were then transferred to N-19 semisolid medium until the plantlets were ready for transfer to normal growth conditions.

The plantlets were grown in nutrient solution (3) for 2 weeks. The plantlets from each callus were individualized, with each one considered as a line. These were then transferred to pots and grown in the phytotron until maturity.

Screening for cold tolerance at the seedling stage

Some of the anther culture-derived lines from this program were subjected to cold tolerance tests at the seedling stage by the IRRI Plant Physiology Department. The seedlings were germinated in enamel trays, grown in the screenhouse for 10 days, and then transferred to a water bath maintained at 10 °C. Scoring was done following the IRRI scoring system (2) when all the plants of the susceptible check (IR8) died, which in this study took 10 days. Some SR crosses were subjected to cold water treatment for 3 more days to compare the tolerance of the anther culture-derived lines to that of the parents at extended periods of exposure to cold water treatment.

Screening of anther culture-derived lines in cold screening nursery

Twenty-two anther culture (AC)-derived lines from IR crosses found to be cold tolerant at the seedling stage were sent to Chuncheon, Korea for further evaluation in the cold screening nursery. The field was laid out such that the inlet temperature was 17 °C and the outlet 25 °C. The

agronomic characteristics of AC-derived lines were checked and compared with susceptible (Setbyeolbyeo) and resistant (Sangpungbyeo) checks.

Replicated yield trials of AC-derived lines

Six anther culture-derived lines and their parents were subjected to yield trials with two replications in Suweon, Korea. Data on agronomic characteristics and yield were gathered and compared with the parents.

RESULTS AND DISCUSSION

Anther culture of F₁ crosses

Variation in culturability in terms of callus induction and plant regeneration among the SR crosses was observed (Table 1). There was no correlation between the ability to produce callus and efficiency in regenerating plants, demonstrating that genotype plays an important role in anther culturability. From a total of 11,500 anthers plated, callus production was 53.56%. Average green plant production per callus plated for regeneration was 1.99, while 50.95% (988 lines) of the total green plants produced set seeds. The remaining plants were either haploids or aneuploids or were not able to survive the stress of being transferred to soil.

Cold tolerance screening at the seedling stage

Variability in response by the various lines from the same cross was observed (Table 2). This shows that different recombinants from the same cross were obtained and expressed in the homozygous lines produced. Among 278 lines tested of the F₁ hybrid RAC 3/Ishiokamochi (SR 11452), 6 exhibited increased tolerance relative to both parents after the seedlings were subjected to a water bath for 10 days at 10 °C. The increase in tolerance could have been due to the synergistic, additive, or complementary effect of the genes from both cold tolerant parents. The increase in tolerance of the anther culture-derived lines became more pronounced when the seedlings were scored after 13 days of cold treatment; 157 lines (56.5%) showed higher tolerance than both parents. The stability of the anther culture-derived lines in their tolerance to cold after a long exposure was evident. Since the plants studied were homozygous diploids, cold tolerance characteristics will be maintained in future regenerations. These lines were tested in the cold screening nursery in

Table 1. Efficiency of callus production and plant regeneration of F₁ hybrids from Korea for cold tolerance studies. Planted in 1983 wet season.

Cross	Anthers plated	Callus production		Calli plated	Calli-producing green plants		Green plant production		Albino plant production		Anther culture plants with seeds	
	(no.)	(no.)	(%) ^a	(no.)	(no.)	(%) ^b	(no.)	(%) ^c	(no.)	(%) ^c	(no.)	(%) ^d
SR11426	476	438	92.00	64	2	3.12	11	17.81	31	48.43	1	9.09
SR11428	1,236	587	47.49	113	1	0.88	1	0.88	61	53.98	0	0
SR11433	678	1,266	186.70	192	35	18.23	276	143.75	144	75.00	108	39.13
SR11436	941	735	78.10	105	3	2.85	22	20.95	46	43.81	8	30.36
SR11451	1,475	710	48.14	90	19	21.11	117	130.00	66	73.33	81	69.23
SR11452	1,066	1,407	131.98	257	68	26.46	979	380.93	225	87.50	439	44.84
SR11453	1,081	440	40.70	72	38	52.77	518	719.44	46	63.80	342	66.02
SR11455	968	88	9.09	50	6	12.00	16	32.00	28	56.00	9	56.25
SR11440	491	33	6.72	21	0	0	0	0	0	0	0	0
SR11238	969	0	0	0	0	0	0	0	0	0	0	0
SR11242	338	0	0	0	0	0	0	0	0	0	0	0
SR11191	857	4	0.46	4	0	0	0	0	0	0	0	0
SR11193	924	14	1.51	9	0	0	0	0	0	0	0	0
Total	11,500	6,160		976	172		1,939		650		988	
Average			53.56			17.62		198.67		66.60		50.95

$$^a \text{Callus production (\%)} = \frac{\text{callus production (no.)}}{\text{anthers plated (no.)}} \times 100$$

$$^c \text{Green/albino plant production (\%)} = \frac{\text{green/albino plant production (no.)}}{\text{calli plated (no.)}} \times 100$$

$$^b \text{Callus producing green plants (\%)} = \frac{\text{calli producing green plants (no.)}}{\text{calli plated (no.)}} \times 100$$

$$^d \text{Anther culture plants with seeds (\%)} = \frac{\text{anther culture plants with seeds (no.)}}{\text{green plant production (no.)}} \times 100$$

Table 2. Cold tolerance score at the seedling stage of anther culture-derived lines from F₁ SR sexual crosses. First generation seeds used.

Designation	Parents	No. of lines tested	No. of lines with a score of				
			1	3	5	7	9
A. After 10 days at 10 °C							
SR 11453	RAC 3/Fujihikari RAC 3 Fujihikari	262	0 x	244 x	18	0	0
SR 11452	RAC 3/Ishiokamochi RAC 3 Ishiokamochi	278	6	239 x x	33	0	0
SR 11451	RAC 3/Hamaasahi RAC 3 Hamaasahi	27	0	11 x x	16	0	0
SR 11426	Fujihikari/Fukei 126 Fujihikari Fukei 126	1	0 x x	1	0	0	0
SR 11433	Chulweon 32/Fujihikari Chulweon 32 Fujihikari	39	16 x x	23	0	0	0
B. After 13 days at 10 °C							
SR 11452	RAC 3/Ishiokamochi RAC 3 Ishiokamochi	278	2	155 x x	102 x	16	3
SR 11433	Chulweon 32/Fujihikari Chulweon 32 Fujihikari	39	0	19 x x	20	0	0

Korea in 1985, promising lines being forwarded for testing in observational yield trials.

Screening of anther culture-derived lines in the cold-screening nursery

Among 22 lines tested, 3 promising ones — AC 608-1 from Taipei 309/Tatsumi mochi and ACs 1166 and 1166-4 from Tatsumi mochi/BG90-2 — were identified to be better than the resistant check in some aspects (Table 3). For example, all three lines had a higher fertility percentage than the resistant check at the inlet. The variation in percen-

tage fertility at the inlet and outlet of AC lines 1166 and 1166-4 was less than the resistant check, suggesting that these two lines are more stable. Another point of importance was that anther culture-derived plants were more vigorous than the resistant check.

Replicated yield trials

Among six anther culture-derived lines tested in replicated yield trials, three gave higher grain yields than both parents (Table 4). AC 599A was 19.5% higher than BG90-2 and 10% higher than Taipei 309. In addition, this plant combined the cold tolerance of Taipei 309 and the plant type of BG90-2. Two lines from Suweon 290/BG90-2 were also superior to both

Table 3. Promising anther culture-derived lines in cold tolerance test, Chuncheon, Korea, 1984.

Observed traits	Anther culture line no.			Check varieties	
	608-1 ^a	1166 ^b	1166-4 ^c	Setbyeolbyeo (susceptible)	Sangpungbyeo (resistant)
Discoloration: 1	2	2	2	9	1
2	4	5	6	6	2
Heading: in (17 °C)	136	136	133	144	137
out (25-27 °C)	109	116	118	127	128
% fertility: in	7	53	51	0	8
out	90	76	78	18	91
Spikelet no.: in	59	78	79	71	102
out	140	130	77	132	101
Culm length: in	55	46	39	—	53
out	87	54	53	—	69
Panicle no.: in	24.6	21.4	22.0	—	22.0
out	23.2	22.2	22.8	—	20.6
Panicle exertion	4	6	6	9	5
Phenotypic acceptability	5	4	5	9	5
Vigor	1	1	1	7	5

^aTaipei 309/Tatsumi mochi; ^bTatsumi mochi/BG90-2.

parents. AC 700 gave 2% higher yield than Suweon 290 and 21% higher than BG90-2, while AC 683 was 1.2% higher than Suweon 290 and 19.6% higher than BG90-2. In field experiments conducted at IRRI, AC 700 also gave a higher yield than the higher yielding parent Suweon 290.

Table 4. Replicated yield trials of AC materials, Suweon, 1984.

AC plant no.	Parents	HD (days)	Culm length (cm)	Panicle length (cm)	Panicle no.	Yield ^a		
						R ₁	R ₂	Mean
Taebaegbyeo (check)		112	77	24	15	835	808	822
BG90-2		141	85	27	13	684	711	698
AC 599A	BG90-2/T. 309	116	77	22	12	818	848	833
Taipei 309		121	112	22	15	762	748	755
AC 683	S.290/BG90-2	117	83	21	12	829	841	835
AC 700	S.290/BG90-2	117	85	22	12	820	870	845
Suweon 290		116	87	21	12	805	851	828

Seeding: April 15

Transplanting: May 25

Fertilization: N-P-K

^a Rough rice (kg/ha)

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CALLUS INDUCTION AND PROTOPLAST CULTURE IN RICE

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Successful cell culture technique is important for rice genetics and breeding. In this paper, the effects of genotype on callus induction and protoplast isolation are discussed. One hundred twenty-seven strains belonging to 12 *Oryza* species and two series of isogenic lines with marker genes—varieties Shiohari (Hokkaido variety) and Taichung 65 (japonica variety) as recurrent parents—were used. In the induction and proliferation of seed calli, a remarkable difference was observed among *Oryza* species. Seed calli from *O. sativa* and *O. glaberrima* showed better proliferation than other species. In the isogenic lines, seed callus proliferation varied depending on the major genes involved. In protoplast isolation from seed calli, the effect of genotype was also prominent, showing some relation to callus growth. Although the strains of high prolific calli were not always superior for the yield of protoplast, a significant correlation was detected between both characters in Shiohari and the dwarf isogenic lines.

Anther culture of rice was pioneered by Niizeki and Oono (6), who obtained haploid and diploid plants from microspores. Since then, many technical problems have been solved, and the method is used for practical breeding in various countries. In Hokkaido, Japan this technique is extensively used for obtaining promising true breeding lines from F_1 plants for development of high quality rice.

As for the cell fusion technique, Niizeki and Kita (7) succeeded in obtaining cell hybrids between rice and soybean protoplasts, but regeneration from the hybrid calli was unsuccessful. In cell fusion a series of techniques extending from protoplast isolation, fusion and culture, and regeneration of the protoplast is important. However, there are many problems to be solved in this culture method such as the stable isolation of protoplast, protoplast culture, selection of fused cells, and regeneration of the whole plant. Many reports related to cell culture are mentioned in a recent review by Yamada and Loh (9). Genotypic effects are noticed in callus induction and proliferation (1, 3, 4). A similar problem is also found in protoplast culture (2, 5, 8, 10).

In this paper, the effects of marker genes and interspecific variation on callus growth and protoplast yield of *Oryza* seeds (provided by Y. Sano) are examined.

MATERIALS AND METHODS

The strains used in this experiment are shown in Tables 1 and 2. Isogenic lines are different from the recurrent parent by only one marker gene, and were produced by successive backcrossing.

Dry seeds were hulled and rinsed in 70% alcohol for 1 min, surface sterilized in 10% commercial bleach solution for 20–30 min, and then rinsed two or three times in sterile distilled water. Then they were planted in 100 ml flasks on a modified N6 medium supplemented with 4.4 mg/liter of 2,4-D, 50 g/liter of sucrose, and 10 g/liter of agar, adjusted to pH 5.7 and autoclaved at 120 °C for 15 min. Calli induced

Table 1. Isogenic lines used.

Isogenic line	Name or gene
ID-S	Shiokari (recurrent parent)
ID-2	<i>d-2</i>
ID-7	<i>d-7</i>
ID-8	<i>d-8</i>
ID-10	<i>d-10</i>
ID-12	<i>d-12</i>
ID-13	<i>d-13</i>
ID-15	<i>d-15</i>
ID-17	<i>d-17</i>
ID-18h	<i>d-18h</i>
ID-18k	<i>d-18k</i>
ID-19	<i>d-19</i>
ID-27	<i>d-27</i>
ID-35	<i>d-35</i>
ID-42	<i>d-42</i>
ID-47	<i>sd-1</i>
ID-51	<i>d-a(t)</i>
ID-52	<i>d-b(t)</i>
T65	Taichung 65 (recurrent parent)
T65-wx	<i>wx</i>
T65-CI	<i>CI</i>
T65-g-1	<i>g-1</i>
T65-gl-1	<i>gl-1</i>
T65-Dn-1	<i>Dn-1</i>
T65-dl	<i>dl</i>
T65-pgl	<i>pgl</i>
T65-fgl	<i>fgl</i>

Table 2. Comparison of callus growth among *Oryza* species.

Species name	No. of strains	Diameter of callus (mm) ^a										Mean	
		0	1	2	3	4	5	6	7	8	9		10
<i>sativa</i>	63	1		3	10	5	14	15	4	6	3	2	5.4
<i>ssp. japonica</i>	30				1	1	7	8	2	6	3	2	6.7
<i>ssp. indica</i>	33	1		3	9	4	7	7	2				4.3
<i>glaberrima</i>	5					1	1	2	1				5.7
<i>perennis</i>	38	1	1	7	19	8		2					3.0
<i>punctata</i>	4			2	2								2.4
<i>minuta</i>	2		1	1									1.6
<i>officinalis</i>	6		2	3	1								1.6
<i>australiensis</i>	2				2								3.2
<i>latifolia</i>	1	1											0.0
<i>grandiglumis</i>	1				1								2.8
<i>brachyantha</i>	2	2											0.1
<i>breviligulata</i>	2				2								3.2
<i>nivara</i>	1				1								3.0

^a50 days after plating.

were subcultured in modified N6 medium. They were kept in the dark at 27°C and transferred to fresh media every 10 days. At each subculture, the diameters of the calli were examined.

For protoplast isolation, about 0.5 g of calli were transferred into 10 ml of enzyme solution (2% Cellulase "Onozuka" RS, 2% Cellulase "Onozuka" R-10, 2% Macerozyme R-10, 0.2% Pectoryase Y-23, 0.7 M sorbitol at pH 5.6) (10) contained in 50 ml flasks and incubated at 28 °C in the dark following 6 h gentle shaking on a reciprocal shaker (50 strokes/min). Protoplasts were filtered through 40 µm nylon mesh and carefully washed free of enzymes by repeated centrifugation (5 min at about 80 g). Then the materials were resuspended in an osmotic solution containing 1 mM CaCl₂ and 0.6 M sorbitol. Protoplasts were cultured in the dark in 35 × 12 mm petri dishes.

RESULTS AND DISCUSSION

Callus induction and proliferation

Seed callus induction and proliferation were examined at 50 days after plating in 127 strains belonging to 12 *Oryza* species. As presented in

Table 2, all species except *O. latifolia* produced seed calli, showing a considerable variation in callus proliferation. The seed calli from cultivated strains of *O. sativa* and *O. glaberrima* exhibited better proliferation than those from the other species. In *O. sativa*, subspecies japonica showed better proliferation than indica. A wide range of variation in callus proliferation was also detected within japonica types. In *O. perennis*, callus growth was equivalent to that of indica types, while the other wild species generally showed poor callus growth, with the diameter of calli ranging from 0 to 3 mm after 50 days.

By using m8marker genes, all lines of the isogenic lines and their recurrent parents produced sound seed calli. As shown in Figure 1, the dwarf isogenic lines and Shiokari exhibited a wide range of variation in callus growth at 50 days, and the callus growth of all lines fell into one of two classes: high or low. The isogenic lines involving the genes *d-7* (cleistogamous dwarf) and *sd-1* (Dee-geo-woo-gen dwarf) showed more superiority than the recurrent parent, Shiokari. On the other hand, as shown in Figure 2, there were no clear differences in callus growth among Taichung 65 and the isogenic lines of marker genes with the exception of the *fg1* line, which was characterized by inferior callus growth.

It was concluded that several genotypes affect callus growth as shown by the pleiotropic effect of the marker genes. Dwarf genes such as *d-7* and *sd-1* showed a superior effect in comparison with normal genotypes.

Protoplast isolation

Protoplast isolation from calli of *Oryza* species is shown in Figure 3. The protoplast yield of Shiokari was used as the standard. There was no correlation between callus proliferation and protoplast yield in *Oryza* species. The species showed high callus growth, resulting in wide variation in protoplast yield. However, there were no strains that exceeded the yield of Shiokari. By contrast, as shown in Figure 4, there was a significant correlation between callus growth and protoplast yield, showing $r = 0.778^{**}$ (significant at the 1% level) in the dwarf isogenic lines and Shiokari. However, no correlation was found in the group of Taichung 65 and its isogenic lines (Fig. 5).

It was proved that the culture method used in this experiment is most adaptable for cultivated varieties of *O. sativa* L., because the other species resulted in inferior callus growth and protoplast yield. However, there was quite a large variation among the genotypes in *O. sativa* L., and some genotypes with marker genes such as *d-7*, *sd-1*, and *g-1* showed better results in comparison with the normal genotype. The significant reduc-

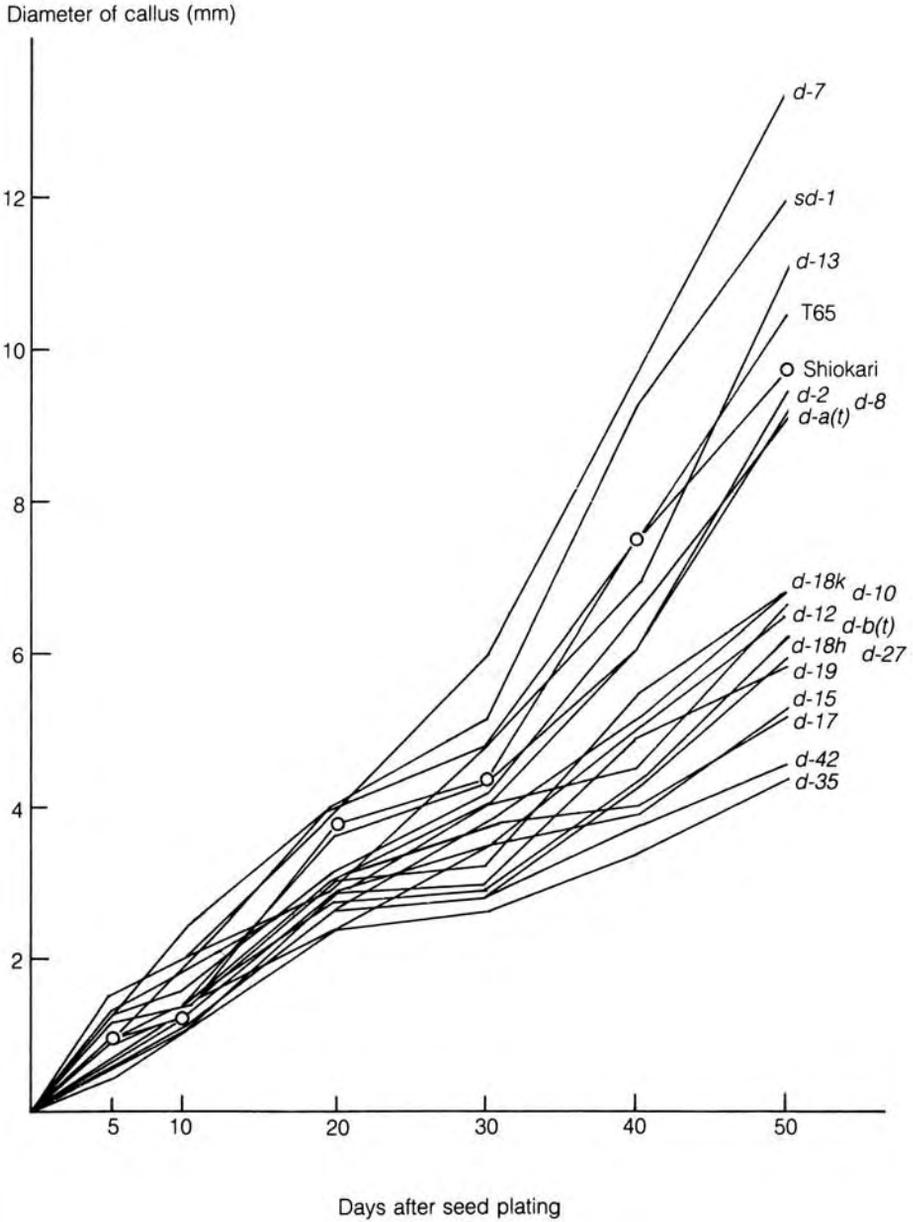


Fig. 1. Growth of seed callus in Shiohari and dwarf isogenic lines.

Diameter of callus (mm)

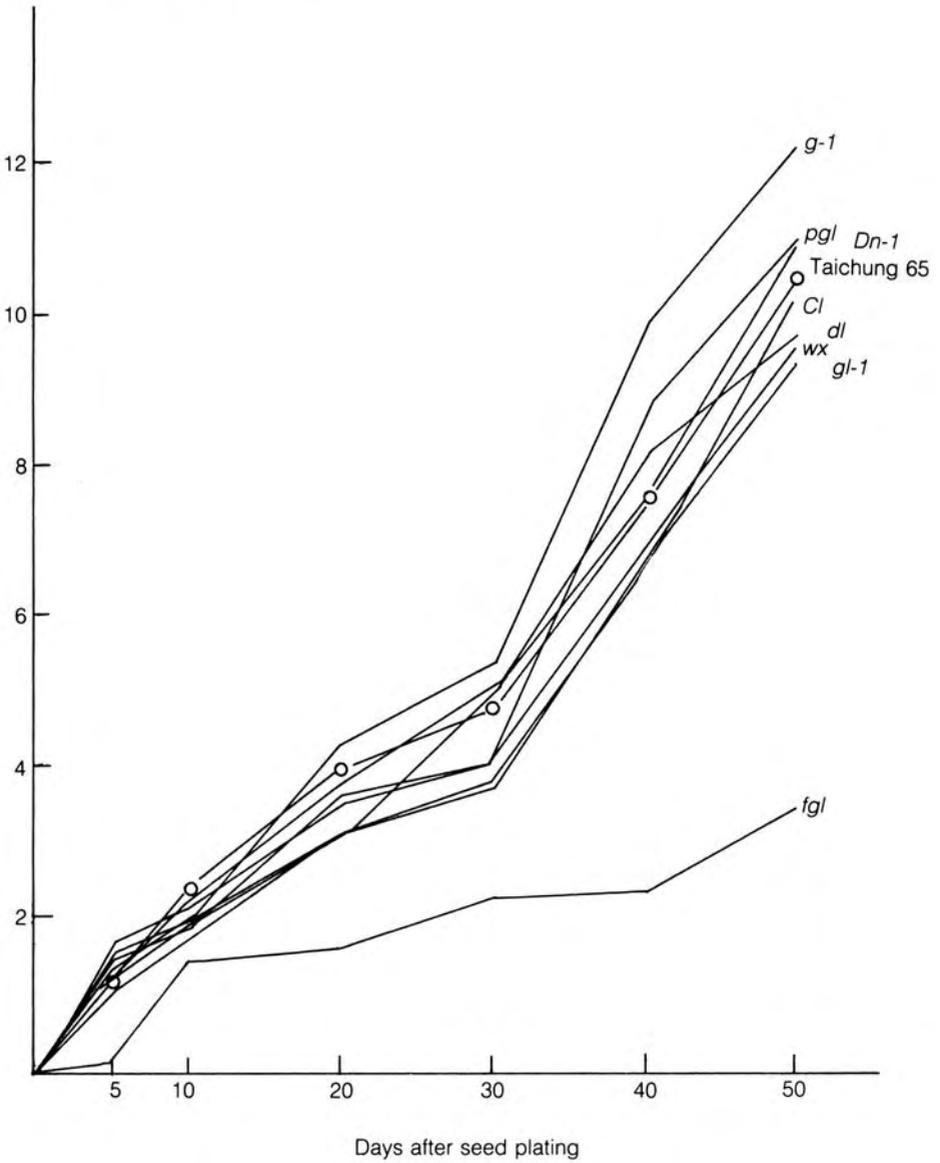


Fig. 2. Growth of seed callus in Taichung 65 and marker isogenic lines.

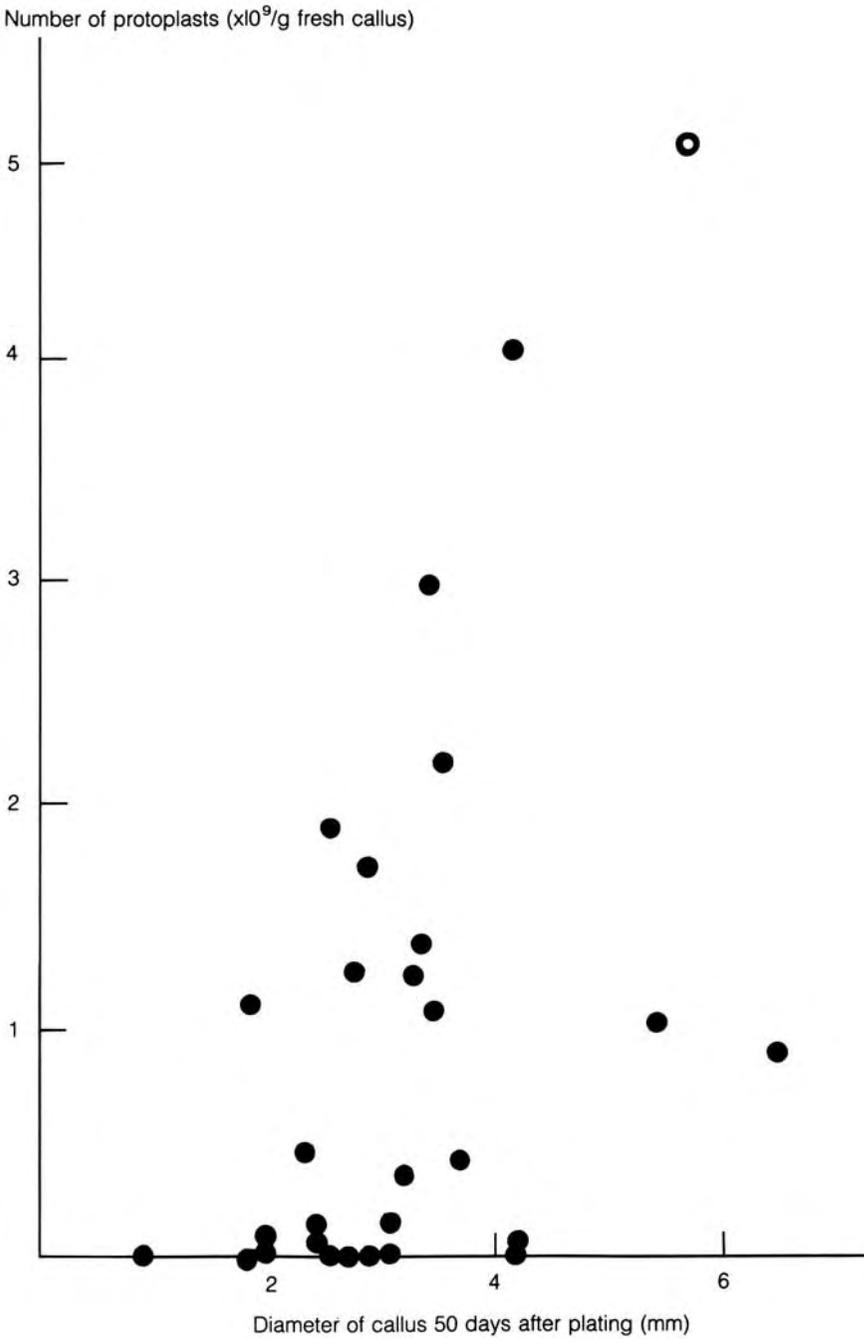


Fig. 3. Correlation between callus growth and protoplast yield in wild species, O = Shiokari, ● = wild species.

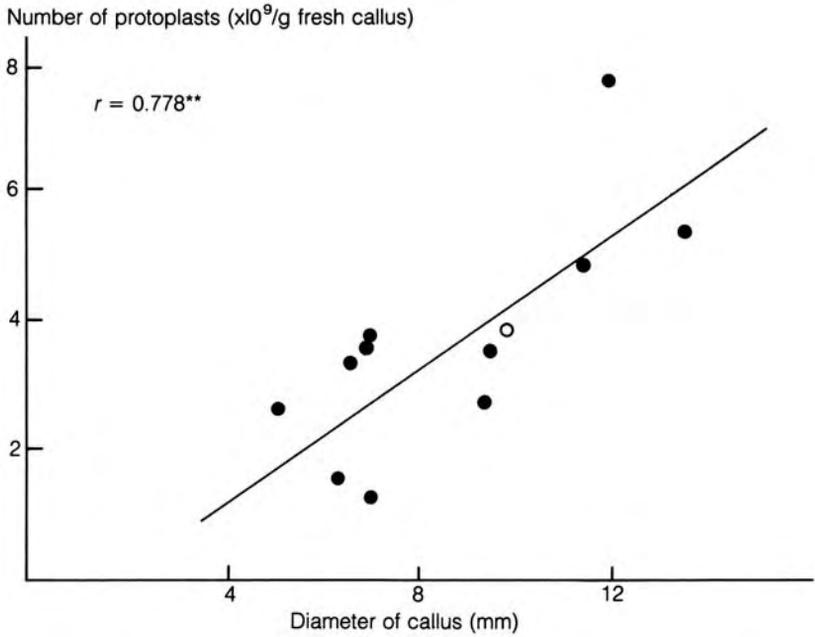


Fig. 4. Correlation between callus growth and protoplast yield in Shiohari and dwarf isogenic lines. ○ = Shiohari, ● = isogenic lines.

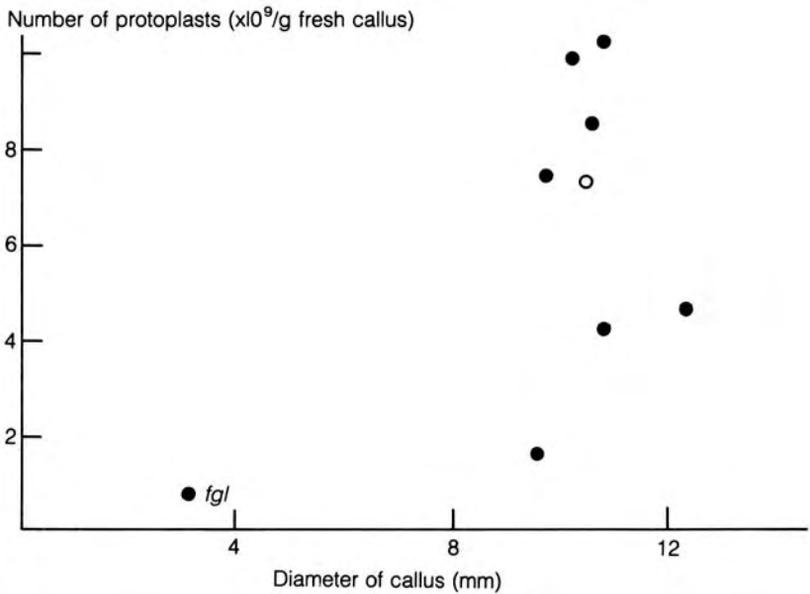


Fig. 5. Correlation between callus growth and protoplast yield in Taichung 65 and marker isogenic lines. ○ = Taichung 65, ● = isogenic lines.

tion detection in the *fg1* line may be caused by the physiological side effects of this gene showing chlorophyll aberration. Thus it is important to consider the specific effects of genotypes and species in the application of culture methods in cell breeding.

Protoplast culture

Protoplasts isolated in the above experiment were used for callus induction on several media. Though the experiments have not been completed, it is possible to continue the cell division until at least the eight-cell stage.

The establishment of regeneration of whole plants is most important for the use of protoplast fusion. In addition, screening of hybrid cells by the use of appropriate markers, preparation of media suitable for a wide range of materials, and chromosomal elimination during the culture must be solved for the improvement of the new breeding techniques.

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RICE PROTOPLAST AND CELL CULTURE*

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Reproducible and efficient protoplast and cell culture systems are an essential requirement for the *in vitro* genetic manipulation of rice. For the application of certain techniques such as somatic hybridization to rice improvement it must be possible to regenerate plants from protoplasts isolated from particular genotypes. Embryogenic cell suspension cultures have been shown to be a source of totipotent protoplasts in other species of the Gramineae. This approach, together with the isolation of protoplasts directly from the plant, has been used to attempt to establish reproducible rice protoplast culture systems. Callus formation from cell suspension culture protoplasts has been achieved for a number of genotypes of rice. Difficulty has been encountered in the maintenance of embryogenic competence of these cell suspensions, and the protoplast-derived calli have been found to be nonmorphogenic. Conditions found important in achieving the division of cell suspension culture protoplasts are being applied to protoplasts isolated directly from the plant. By using conditioned semisolid culture media, reproducibly high frequencies of initial divisions of protoplasts isolated from the immature scutellum have been obtained. Experiments are in progress to obtain sustained division of these protoplasts.

The potential of novel genetic manipulation techniques in rice improvement has been the subject of some interest in recent years (8). However, before these techniques can be applied, reproducible and efficient protoplast and cell culture systems must be established. Progress towards this objective in the cereals has been slow, due largely to the poor response seen when using cultural conditions found favorable for other species. However, the recognition and maintenance of cell cultures with embryogenic competence has given new insights into the response of many species of the Gramineae. Embryogenic callus has been initiated in all the major cereal species, and embryogenic cell suspension cultures have been found to be a source of protoplasts from which plantlet regeneration has been possible. Although this approach has proved successful with five species of the Gramineae (3), to date no mature plants have developed from embryogenic cell suspension protoplasts. Success in the culture of protoplasts isolated directly from cereal plants has been

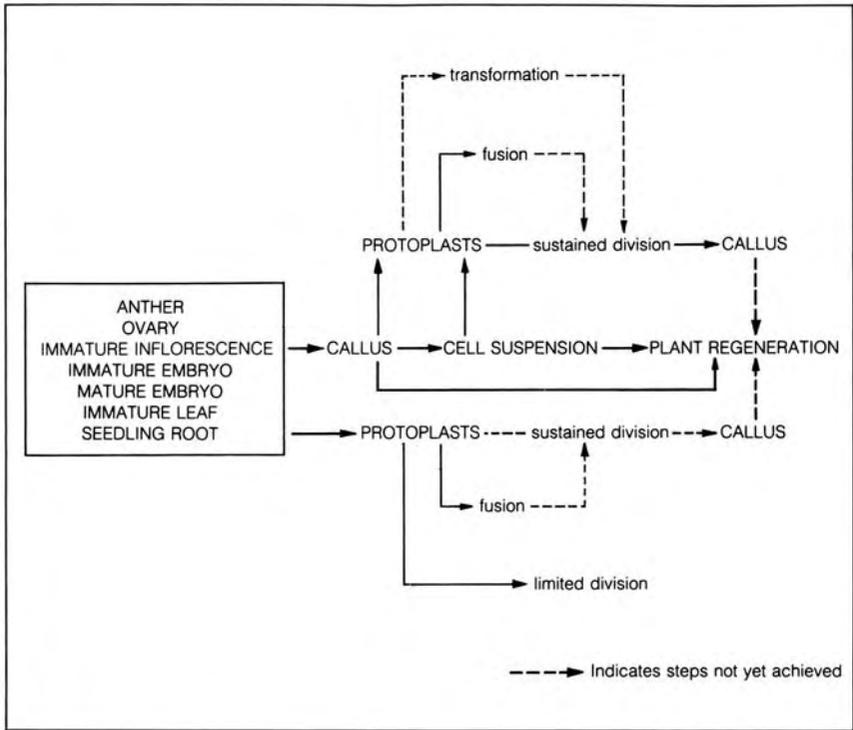


Fig. 1. Current status of rice cell and protoplast culture.

very limited. In only isolated and unreproducible instances has the sustained division of such protoplasts been reported (for review see 9).

The current status of cell and protoplast culture of rice is indicated in Figure 1. A number of juvenile or immature plant parts can yield callus cultures, many of which have been reported to have embryogenic capability, and from which plants can be regenerated (for review see 3). The ease of green plant regeneration is dependent both on genotype and duration in culture (5). To some extent the response of particular rice genotypes can be improved by modifying the culture medium used (7). However, our knowledge of the control of initiation and maintenance of embryogenic competence in rice is still limited. While plant regeneration from cell suspension cultures of rice has been reported (13), the establishment of embryogenic cell suspensions that may be a source of totipotent rice protoplasts is still awaited.

Callus formation from protoplasts isolated from nonmorphogenic rice callus or cell suspension cultures has been reported several times (Table 1). Root regeneration has been reported from callus derived from

Table 1. Reports of rice protoplasts undergoing sustained division.

Protoplast source	Response	Reference
Callus	callus	4
Leaf sheath	callus and roots	4
Callus (obtained from anthers)	callus	1
Callus (obtained from mature seed)	callus	6
Cell suspension (from immature embryos)	callus	E. Göbel, P. Ozias-Akins, and H. Lörz, personal communication
Callus (NR deficient anther-derived cell lines)	callus	12

rice leaf sheath protoplasts (4), but no reproducible protocol has been published for the culture of protoplasts isolated directly from the plant.

Both cell suspension culture protoplasts and protoplasts isolated from the meristematic parts of the rice plant are being used to develop reproducible culture systems of Nottingham. The objective of this work is ultimately to obtain efficient plant regeneration from rice protoplasts and to establish procedures for rice protoplast fusion and genetic transformation.

CULTURED CELL SYSTEMS

Callus was initiated from immature and mature rice embryos using various levels of 2,4-D or 3,6-dichloroaniscic acid (Dicamba), either alone or in combination with kinetin and/or tryptophan (7). Embryogenic cultures were established and maintained using essentially the method described by Vasil (10). Cell suspension cultures were obtained by culturing individual compact calli in approximately 0.5 ml of liquid medium in repli dish squares on a shaker at 120 rpm. Fresh culture medium was added at regular intervals, and the resulting proliferating cell clusters were transferred to 100 ml conical flasks with approximately 20 ml of culture medium.

Embryogenic calli from 26 cultivars of *O. sativa* have been used to attempt to establish cell suspension cultures using this method. Representative observations are shown in Table 2. In responsive genotypes (12 out

Table 2. Establishment of rice cell and protoplast systems.

Cultivar	Callus type	Cell suspension type	Protoplast response
Tetep	embryogenic	limited growth	n.a. ^a
IR36	embryogenic	limited growth	n.a.
Silewah	nonembryogenic	cell line	sustained division-callus
OS 6	embryogenic	cell line	limited division
Taipei 309	embryogenic	plant regeneration-cell line	sustained division-callus
Calrose 76	embryogenic	limited growth	n.a.

^an.a. = not applicable.

of those tested), 3 types of cell suspension culture developed. The majority of cultures consisted of an enriched population of root and embryo meristemoids that were heterogenous in nature and had limited plant regeneration ability. These cultures were not found to be a suitable source of protoplasts and could not be maintained for extended periods. In two cases cell suspensions developed that appeared to consist almost entirely of small embryogenic-type cell clusters. Plant regeneration occurred when these cultures were plated on semisolid medium with reduced auxin levels. However, the frequency of regeneration was found to be low, and a high proportion of albino regenerants occurred. Prolonged culture was found necessary to obtain reproducible yields of protoplasts from these cultures and resulted in these lines appearing indistinguishable from the nonmorphogenic cultures initiated from other genotypes. This third type of culture consisted of fast growing, finely divided cell aggregates that were found to be a suitable source of protoplasts for cultural studies.

High yields of protoplasts ($1-10 \times 10^6/g$ fresh weight) can reproducibly be obtained from such rice cell suspension cultures during the log phase of growth, 4-6 days after subculture. These protoplasts are being used to establish the important parameters for reproducible rice protoplast culture. Improvements in division response have been found by using conditioned agarose solidified culture medium and short-duration isolation procedures. The first division of protoplasts occurs after 3-5 days in culture, and subsequent divisions give rise to cell colonies after 3 weeks. Experiments are in progress to improve the plating efficiency of cell suspension protoplasts above the levels achieved so far (0.1 - 3%). Further attempts are also being made to establish cell suspension cultures from different rice genotypes. Early recognition of embryogenic callus, rapid establishment in liquid medium, and manipulation of the

culture conditions (10) are being used to attempt to establish rice cell suspension cultures that can yield totipotent protoplasts.

PROTOPLAST ISOLATION FROM THE RICE PLANT

Those parts of the rice plant found responsive in initiating morphogenic cell cultures (Fig. 1) are likely to provide the best source of protoplasts with division potential. Protocols have been developed for the isolation of protoplasts from various rice plant parts. Seedling basal leaf tissue and roots were used in early experiments. However, the frequency of cell wall regeneration and the viability of these protoplasts in culture were found to be low.

In recent experiments, immature embryos have been used for protoplast isolation. Cultured excised immature embryos of rice show a high frequency of callus response; up to 100% of plated embryos readily form callus. There is good evidence to show that single cells in the grass scutellum are totipotent (11), protoplasts isolated from the embryo scutellum are therefore particularly likely to be responsive in culture.

Despite the relatively small amount of tissue available, workable numbers of protoplasts can be isolated from the immature scutellum of rice. A number of cultivars of rice including indica and japonica types have been assessed for the release of embryo protoplasts. Protoplasts from all genotypes tested have undergone division. Up to 30% of plated protoplasts have divided reproducibly in conditioned culture medium. Experiments are in progress to achieve sustained division of these embryo protoplasts. Conditions found important in obtaining the sustained division of suspension culture protoplasts are being used. It is envisaged that this approach can be extended to include the culture of protoplasts isolated from the mature embryo scutellum. In many rice genotypes this explant appears to have similar potential to the immature scutellum in terms of the initiation of callus with embryogenic capability.

PROSPECTS FOR THE APPLICATION OF RICE PROTOPLAST AND CEU CULTURE TECHNIQUES

The development of reproducible protoplast and cell culture systems in rice will enable the investigation of several areas of genetic manipulation. Protoplast fusion between sexually incompatible species of rice and the transfer of cytoplasmic male sterility have been proposed (8). Intergenous

and interspecies rice protoplast fusions are planned at Nottingham to investigate the transfer of salt tolerance and disease resistance characteristics. Assessments of the genetic transformation of rice protoplast and cell cultures to introduce specific traits are also envisaged.

For these techniques to make an impact on rice improvement it is necessary to have efficient cell culture systems for particular genotypes. The development of embryogenic rice cell suspension cultures is an important approach to pursue. Such cultures would be of considerable interest for *in vitro* selection studies and may provide a source of totipotent rice protoplasts. However, there are clear difficulties that must be overcome before this approach can be applied to specific rice genotypes. More basic knowledge is required of the causes of genotypic variability seen in culture to enable the response of desired genotypes to be improved. Problems also clearly exist in the regeneration of albino plants from such cultures, which appears to be linked to genotype. Lack of vigor and the occurrence of albino plantlets has so far prevented the regeneration of viable mature plants from cell suspension protoplasts of other cereal species (3). The time required to establish embryogenic cereal cell suspensions that can yield dividing protoplasts can clearly lead to the occurrence of genetic and developmental abnormalities. Dale (3) has suggested, however, that this approach may be the best general method for cereal protoplast culture, provided that genetic stability can be controlled.

The establishment of culture systems using protoplasts isolated directly from the rice plant would largely overcome the problems encountered using cultured cell systems. Meristematic tissue identified as containing totipotent cells is the best choice of material for protoplast isolation.

Immature and mature embryos, immature inflorescences, seedling roots, and juvenile leaves have been found to be sources of embryogenic callus or contain single cells that can give rise directly to somatic embryos (2). Immature rice scutellum protoplasts clearly have division capability. Difficulties exist in obtaining large protoplast yields, and exacting cultural conditions may be necessary to achieve sustained division. However, culture systems for such protoplasts may be more readily reproducible and applicable to a range of rice genotypes.

Nonmorphogenic rice cell suspension cultures can provide much information on factors important in obtaining reproducible, sustained protoplast division. They may also be useful in assessing genetic transformation systems and as fusion partners with rice protoplasts from other sources.

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TISSUE CULTURE IN RICE AND ITS APPLICATION IN SELECTING FOR STRESS TOLERANCE

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The use of tissue culture in selecting for stress tolerant rice is discussed. Special emphasis is placed on developing methods for reliable regeneration of plants from rice callus cultures. An International Plant Biotechnology Network (IPBNET) that is being established to promote research on stress tolerance of major food crops is also described.

According to Dudal (8), only about 10% of the world's total land is nonstressed, good cropland. The vast majority of arable or potentially arable land is limited, in terms of crop productivity, by one environmental stress or another. Among the environmental constraints limiting food production, excess salts, drought, and soil acidity play a major role. Decreases in crop yield associated with these constraints can be limited in two ways. Either the effects of a given stress can be avoided by modifying cropland (irrigation, chemical additives, etc.), or the crop plants themselves can be modified.

In some cases environmental modification is impossible. Climatic variations such as temperature, for instance, are primary factors in determining limits of crop productivity. Problems of water availability cannot be universally addressed by increased irrigation. As it is, 80% of the currently available freshwater supply is already used in agriculture (9).

In other cases, environmental modifications only serve to mask the underlying problems for a finite period. For instance, irrigation water can decrease the concentration of salts in a soil during periods of irrigation; however, the ultimate effect of irrigation water is to add salts. When the amount of irrigation water is insufficient to leach salts completely, the problem is intensified (25). Even where environmental modification may do much to alleviate problems associated with unfavorable soil conditions, costs may be prohibitive and resources scarce.

Nonetheless, as the world's food needs increase, more and more marginal land will have to be brought into full production, and, given the inherent costs and other problems associated with large scale environmental modification, much of the future improvement in crop productivity will necessarily come from genetic improvements in crop plants themselves. The task at hand, then, is to tailor plants capable of producing well in less than favorable environments.

Traditional plant breeding will undoubtedly play the major role in crop improvement in the foreseeable future. Breeding rice for salt tolerance has already shown considerable success (1). It is essential that those breeding efforts be stepped up and that the world's stores of germplasm be continually screened to identify nontraditional crop species and cultivars of traditional crops resistant to various environmental stresses.

Along with plant breeding, tissue culture and other emerging plant biotechnologies will contribute to crop improvement. In many areas plant biotechnology is making considerable headway. Tissue culture selection for herbicide resistance in tobacco has been accomplished, and the resistance has been genetically characterized (6). Salt tolerant plants have been selected in tissue cultures of oats and tobacco (20, 21). At a more molecular level, specific genes have been inserted into dicots using *Agrobacterium tumefaciens* as a vector (4, 13, 19). Recent evidence suggests that *A. tumefaciens* may also prove to be a suitable vector for inserting genes into monocots as well (12), thus making the technique potentially applicable to cereals.

It is also true that there continue to be a number of technical problems associated with some proposed uses of plant biotechnology. It is still not possible to regenerate plants from suspension cultures or protoplasts of most major cereals. At the molecular level (despite a few isolated examples), there is still very little known about specific plant genes in general.

However, progress is being made, and some of the early problems associated with using plant tissue culture as a means for crop improvement have been largely overcome. One of the major problems that faced tissue culturists 5 years ago was the rapid loss of morphogenetic capacity of cereal callus cultures, including rice (7). The lack of long-term regeneration methods severely restricted the usefulness of rice tissue culture in genetic experimentation (5).

Consistent, reliable regeneration techniques are necessary to the production of stress tolerant tissue cultures. Clearly, the production of cell lines tolerant to external stressing agents is not of much practical value unless whole plants can be regenerated. Sporadic, short-term plant

regeneration is of little value, considering that valuable *in vitro* mutations are rare and several months of culturing may be required before mutant cell types become predominant in a given culture.

Over the last few years, our laboratory and others have made considerable progress in overcoming the problems of regeneration in rice and other cereal tissue cultures. Long-term (greater than 31 months), high-frequency (approximately 55 regenerates/g of callus) regeneration is now possible from somatic embryogenic cultures of both *indica* and *japonica* rices. To achieve these levels of regeneration we developed systematic experimental strategies. It will be useful to review these strategies for two reasons:

- We have found the strategies to be widely applicable to many genotypes of many cereal species (22).
- Such a review may serve as an example of how a major problem facing plant tissue culturists as a whole was overcome, given sufficient inputs of time and resources.

PLANT REGENERATION FROM RICE TISSUE CULTURES

The central element to consistent, reliable regeneration from cereal tissue cultures is the identification of embryogenic (E) cell lines (29). Currently, long-term, high-frequency regeneration is possible only when embryogenic cultures can be established and maintained (22).

Embryogenic cultures consist of small isodiametric cells averaging 31 μm in diameter. Nonembryogenic (NE) callus consists of long tubular cells averaging 52 μm in width and 355 μm in length (22). E callus can be distinguished from NE by macroscopic visual examination. Rice E callus is dense and white, producing distinct globular somatic embryos at the callus surface. Some of these somatic embryos differentiate further under appropriate cultural conditions, becoming notched and eventually developing a well defined root-shoot axis, complete with coleorhiza and coleoptile, attached to a scutellum-like structure (15). NE callus in rice is generally light yellow to tan color, loosely packeted, friable, and by definition, does not produce somatic embryos. The ability to distinguish between the two cell types is the first and most critical step in developing regeneration methods for a particular species or cultivar.

Given that embryogenic callus will produce regenerates for long periods of time and a high frequency, the questions become:

- How is embryogenic callus induced in tissue culture?

- Once induced, how is embryogenic callus maintained?
- How can embryogenic callus be routinely manipulated to express its morphogenetic competence?

Unfortunately, no one specific formula can be given to answer these questions for all genotypes of all plant species. However, a systematic approach can be proposed to identify and quantify certain important parameters. Among the factors most important to the production of embryogenic callus and the subsequent regeneration of plants are:

- genotype of the donor plant and environmental conditions under which donor tissue is grown;
- the age and source of explant material used to initiate a tissue culture;
- the composition of induction, maintenance, and regeneration media;
- conditions under which callus cultures are grown; and
- medium conditioning.

Genotype of donor and environmental conditions under which donor tissue is grown

We currently work with two indica rices (Pokkali and IR36), two japonica rices (Calrose 76 and G-159), and one japonica/indica hybrid (Mahsuri). We have achieved good rates of regeneration from callus with all of these by paying particular attention to choosing embryogenically competent explant tissue and systematically defining the best media for production of E callus and regeneration (see below). Although there are some differences in regeneration between these cultivars, it is not immediately apparent whether the differences are primarily genotypic or are due mainly to environmental conditions under which the donor plants were grown.

Genotype has often been indicated as a major factor in the regeneration of cereal tissue cultures (2). While in some cases genotype seems to be a key factor in regenerability, in other reports it is difficult to determine whether “genotype” differences may actually be differences in “preconditioning” of explant material. It has been our experience, for instance, that there are sometimes differences between the responses in tissue cultures of individual seed lots of the same cultivar; i.e., explant material of the same genotype grown at different times or places can behave differently in culture. Environmentally induced differences may be very difficult to distinguish from true genotypic differences, except under very controlled conditions. This should be kept in mind where differences in “genotype” are described below.

Age and source of explant material used to initiate tissue culture

The choice of explant tissue is critical to the successful induction of embryogenic callus. Regarding cereals in general, immature sources of donor tissue produce E callus at a higher frequency than mature sources. The most widely used source of explant material for initiating cereal tissue cultures has been the immature embryo cultured at a specific stage of development (11, 29). Other frequently reported sources of embryogenic callus in cereals are young inflorescences (3, 27) and unexpanded leaves (17, 30).

More recent data have shown that mature embryos of some cereals, including rice and wheat (C. MacKinnon, personal communication), can be used with equal effectiveness in initiating embryogenic callus as immature embryos—given the appropriate conditions. We have successfully induced E callus in rice using immature embryos, whole mature embryos (excised), scutella excised from mature embryos, seeds, and leaves. The degree to which these different explant sources are embryogenically competent, however, may depend on the cultivar. For most cereals, the scutellum of immature embryos (generally less than two mm in length) will give the highest frequency of E callus formation, at least during first passage on tissue culture medium. Although it is somewhat cumbersome to excise embryos at different stages of development, the exercise is useful to visually define exactly what E callus looks like. For any new genotype to be tested for E callus production, the best approach is to test a variety of explants at different stages of maturity.

Induction, regeneration, and maintenance media

While choice of explant material is important for initiating rice tissue cultures with embryogenically competent cell types, the production of E callus can be significantly promoted by manipulation of exogenous growth regulators and nutrients (14, 24). We use Linsmaier and Skoog's basal medium (16). For callus induction, a potent auxin such as 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) must be used. A range of auxin concentrations should be used in initial experiments to determine the maximal amount of E callus induced. (It is important to note that the concentration producing maximal E callus may not be the same as the concentration producing maximal *total* callus.) In general, for rice, we have achieved the best results using between 0.5 and 1.0 ppm 2,4-D; however this may vary from cultivar to cultivar.

In addition to 2,4-D, we also routinely test for interactive effects of kinetin or benzylaminopurine (BAP) in the induction medium, at a number of concentrations. Testing a range of concentrations for each hormonal additive is essential. What is published as an appropriate medium for one genotype is likely to be inappropriate for another genotype.

It has been our observation that *initiation* of embryogenic callus is generally a function of explant source; i.e.; if the explant source is not embryogenically competent, media manipulations are of little use. However, medium additives do have an effect in terms of causing certain cell types to divide or be maintained preferentially (24). In rice, kinetin enhances preferential division and maintenance of E cells for all cultivars that we have worked with — but again, the optimal concentration of kinetin varies with the cultivar.

We have also noted that tryptophan has a positive effect on the production of E callus for some rice genotypes (28). Tryptophan enhances selective production of E cells in Calrose 76, IR36, and Pokkali but has no apparent effect on G-159 or Mahsuri. For tryptophan as well, concentrations at which a tissue culture responds depend largely on the genotype being tested.

Maintenance of embryogenic callus can usually be accomplished by transfer of callus from the appropriate initiation medium to successive passages on the same medium. If, however, the proportion of E callus to NE callus decreases significantly over time, adjustments in the medium composition are probably necessary.

For regeneration, 2,4-D is removed from the medium. We often replace 2,4-D with another auxin such as indole-3-acetic acid or on a naphthalene acetic acid. Also, cytokinins such as BAP or kinetin promote high rates of regeneration. The best approach is to test a number of different cytokinins and auxins interactively at various concentrations to determine the best regeneration medium for each genotype.

It is important to stress that the media manipulations described here are those that we have observed to be the most significant for the production of E callus and for regeneration of rice. Other workers, using different rice genotypes and tissue culture systems, have indicated a variety of other media modifications to be important (31).

The lesson to be learned from these various reports seems to be that no one medium or set of media is appropriate to all rice tissue cultures. The best approach is to define empirically appropriate media for each ge-

notype. To do this, a range of concentrations of various plant growth regulators should be tested in a systematic way.

Medium conditioning

Preconditioning of regeneration media can also be performed in order to maximize rates of regeneration. Preconditioning involves placing a piece of E callus on regeneration medium for about 2 weeks, removing it, and then placing a second piece of E callus on the same medium. The first piece of callus appears to condition the medium by altering its composition. In the case of regenerating callus, medium conditioning by the first piece of callus greatly enhances regeneration from other callus subsequently placed on the same medium (26).

Environmental conditions under which callus cultures are grown

There are a number of environmental factors that may influence the growth of tissue cultures of given crop plants, including temperature, light intensity, light quality, and photoperiod.

Light intensity has been shown to be an important factor in callus production for the cultures we work with. Calrose 76, G-159, and Pokkali produce maximum E callus when cultured in the dark during the first passage on culture medium, while Mahsuri and IR36 produce maximum E callus when first cultured in the light.

We have also observed that handling of tissue during transfers can influence maintenance of E callus. Necrotic tissue must be carefully cut away during transfers or the necrotic tissue can be detrimental to further growth of healthy E callus in a culture. NE callus should also be carefully excised. The degree of precision to which these excisions are carried out relies on the technicians performing the excisions.

Tissue culture is largely an empirical science. A great deal can be learned simply by observing individual researchers/technicians in different laboratories to see how they do things. For instance, one cannot be expected to distinguish easily between E and NE callus for a particular species simply by reading the literature. Hands-on experience is essential. It is particularly important in this area that laboratories exchange information and personnel frequently if maximum progress is to be achieved.

SELECTION OF TISSUE CULTURES FOR STRESS TOLERANCE

Tissue cultured cell lines can be selected *in vitro* for resistance to various stresses. Selections are performed by placing a stress causing agent in tissue cultures containing dividing cells. The stress agent should be incorporated into the tissue culture medium at a high enough level to restrict growth of the culture to 1% to 10% of normal. For selecting cell lines tolerant to salt we use NaCl, for Al, AlCl₃, and for drought, polyethylene glycol as stress agents. To date, the majority of our work has been directed at developing salt tolerant cell lines. In several cases we have shown that plants regenerated from salt tolerant cell lines are also salt tolerant. For oats and tobacco, we have shown that the tolerance exhibited in regenerates is heritable (20, 21).

It is important to note that the appropriate level of stress agent in any given selection medium may vary between genotypes of a given species and at various growth stages of the culture. In general for callus cultures, the level of stress agent used in the induction medium should be increased for successive passages. The amount of increase will depend on the genotype.

The overall effect of stressing callus on a selection medium depends not only on the concentration of stress agent but also on the length of time a tissue culture is exposed to that concentration. A cell line may be able to withstand a very high level of salt for a short time but completely die off over longer periods, allowing insufficient time for selection of tolerant cells in a population of dividing cells.

Ideally, selections for tolerant cell lines are done in suspension cultures. Suspensions insure that all cells of a culture are exposed more or less uniformly to the selective medium. Unfortunately, reliable regeneration methods for major cereal crops have not been reported from suspensions. While lack of techniques restricts the use of suspension cultures in rice genetic experiments, callus cultures have been used successfully in other selection systems. We have successfully used callus cultures to select for salt tolerance in oats (20) and tobacco (unpublished data). Other workers have also used callus cultures to isolate successively mutants as well. Chaleff and Ray used tobacco callus to select for herbicide tolerance (6) and Gengenbach et al (10) used callus cultures to select for pathotoxin resistance in maize.

A number of workers have successfully isolated tissue cultures tolerant to salt (for a recent review see 25). The majority of these tolerant lines should be correctly termed variants because the cause of phenotypic change (epigenetic or genetic) was not determined (18). Our lab has

successfully identified salt tolerant mutants selected from tissue cultures of tobacco and oats (20, 21). More recently, variant cell lines in rice have produced regenerates whose progeny are more tolerant than controls during the seedling stage. Levels of tolerance at the flowering and seed set stages for rice regenerated from variant cell cultures are now under way, and heritability studies are forthcoming.

NETWORKING

Many emerging biotechnologies have the potential to be of immense benefit to scientists in developing countries (DCs.) However, much of the current resource investment in agriculture-related biotechnologies is being made by private corporations in more developed nations that focus their research on large-scale, state-of-the-art farming systems.

Therefore, there is a need to focus more resources and biotechnology-related technical inputs on specific local problems in DCs. In order for new technologies to be implemented in DCs, they must first be made accessible.

Increased accessibility will require a number of things, including: increased educational and training opportunities for scientists and administrators in DCs, financial support through both national and international programs, and material support.

Considering that resources for training and support for this type of development are limited, international cooperative programs are becoming increasingly useful. Given the appropriate framework, international collaborative research can be effectively facilitated by networking (23).

An International Plant Biotechnology Network (IPBNET) is now being organized by the Tissue Culture for Crops Project (TCCP), headquartered at Colorado State University, Fort Collins, Colorado, USA. The primary purpose of IPBNET is to establish working partnerships among researchers worldwide with interest in using plant biotechnology to accelerate the development of stress tolerant crops. IPBNET will be made up of basic and applied research groups in DC laboratories, US laboratories, and plant breeding programs worldwide with interests in stress tolerance. The Network will carry out multinational and multi-institutional collaborative research and serve as a vehicle for information exchange and technology transfer.

Collaborators in the Network will work on specific agriculture-related problems and crops of local interest. They will be scientific counterparts to the TCCP and its other collaborators (including International Agri-

cultural Research Centers, several US universities, and others) and will have priority for Network support services. Support services to these equal-partner collaborators will include: a training course for staff and technicians of DC labs (as needed), exchange of scientists, a technical assistance program (as needed), technical workshops to include crop and stress-specific working groups, yearly conference (at various locations) of Network members to promote information exchange, a biannual Network newsletter to include research results of Network members, a computerized filing system of titles to appropriate technical literature with a reprint service, and a limited number of direct grants from the TCCP to peer-selected Network collaborators. To be effective, it is important that the Network be made up of plant breeders as well as tissue culture scientists.

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APPLICATION OF ANTHOR CULTURE TO HIGH VOLUME RICE BREEDING

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Recent advances resulting in increased callus induction and plant regeneration combined with modifications in methods for handling large numbers of anthers/calluses have reduced the problems that formerly restricted the application of anther culture to a high volume rice improvement program. The difficulties of screening/selecting at the level of callus induction, plantlet regeneration, or R_1 plants are discussed. The problems involved in generating and mass screening 20,000 or more R_2 diploid lines are presented.

Although it has been 17 years since Niizeki and Oono (6) demonstrated that diploid rice plants could be regenerated from haploid anthers, the practical application of this technique for varietal improvement has been largely neglected. The exception has been China, where several commercial varieties have been produced via the anther culture method. The slow adoption of anther culture as a rice breeding tool is a result of the many difficulties involved in generating useful diploid plants. The low rate of callus induction from anthers combined with the small percentage of calli that produce useful plants certainly has not been appealing to the plant breeder. Furthermore, the methods employed in extracting anthers and placing them on media for callus induction, with subsequent transfer to regeneration media, are suitable for conducting small experiments but are not appropriate for a breeding program that requires tens of thousands of regenerated plants. Significant progress has been made in recent years in anther culture technology, but the technique still remains largely in the laboratory in the hands of the tissue culturist and not in the field with the rice breeder.

Rice anther culture research at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia began only a few years ago. Initial efforts were mainly to familiarize ourselves with the existing techniques and to construct the necessary infrastructure. More recently, we have concentrated on developing methodology to produce large numbers of regenerated plants. This reflects our interest within the Rice Program in

incorporating anther culture into the mainstream of our existing breeding program.

CIAT Rice Program responsibilities include providing improved lines to the national programs of Mexico and the countries of Central America, the Caribbean, and South America. Experience has taught us that it is not feasible to breed plant in Colombia that are adequately adapted to all the diverse environments in these regions. A more practical approach may be the development of regional breeding programs that should be more capable of addressing localized production constraints. For example, the Southern Cone of South America, which includes southern Brazil, Uruguay, northern Argentina and central Chile, is a major rice producing area, with a complex of problems most of which cannot be addressed at CIAT. Rice varieties in these areas must possess cold tolerance, be reasonably resistant to the local blast complex, and have tolerance to Fe toxicity (southern Brazil) or resistance to straighthead disease (Argentina). The principal limitation to rice varietal improvement in this region is its slowness, given only one generation/year with the conventional breeding methods, resulting in 10 or more years to produce a commercial variety. Theoretically, the anther culture procedure could reduce the time required to generate a commercial variety to under 4 years. Consequently, our ultimate goal at CIAT is to develop anther culture technology that can be used by national programs in areas where conventional breeding methods are inadequate. This may take one or two forms — provide for local evaluation of regenerated R_2 lines from CIAT crosses between parents offering appropriate traits and/or assisting in the establishment of regional anther culture facilities for local production of regenerated lines.

CONSIDERATIONS FOR ANTHER CULTURE BREEDING

Induction problem

A primary limitation in using anther culture in varietal improvement has been the low percentage of anthers that form calli. This problem remains, although considerable advances have been made during the last 10 years. Anthers having pollen in the mid- to late uninucleate stage are the most suitable for callus induction, and this stage is easily identified in the field. Cold shocking anthers at 8 °C for 4–8 days stimulates callus formation. Improvements in media composition, including liquid nutrient cultures containing 3–4% sucrose and 1–2 ppm 2,4-D, or NAA, have resulted in

further increases in callus induction. Through the use of these improved methods, it is not uncommon to observe 25–50% callus induction in many varieties. This is a remarkable achievement, as 15 years ago reports of less than 1% callus formation were common. However, the low rate of callus induction of indica types is still a major obstacle.

The influence of media composition has been one of the most researched areas in anther culture. However, there is no general agreement on which media are superior, as variety \times medium interactions are always significant. The universal medium, in which anthers from all varieties are capable of high percentage callus induction, has not been developed, nor is there reason to believe that such a medium exists. Varietal differences in response to soil nutrient levels constitute a well known phenomenon, and one would be surprised not to observe a similar response for callus induction in media of varying nutrient and hormonal composition. Another problem is that almost all research conducted on media has utilized japonica types. Consequently, it is not surprising that the media presently available are more suitable for japonica than indica types. Chinese scientists have reported that anthers from F_1 hybrids of japonica/indica crosses cultured on the N_6 medium (originally developed for japonicas) resulted in a biased callus induction and subsequent regeneration of japonica types (2). The generalization that anthers from japonicas are easier to culture may well be due to the extensive research conducted on these rice types. If equal research efforts were devoted to indicas, we might find that indicas respond to anther culture as well as japonicas.

Fortunately, it is not necessary to rely upon only one induction medium for a program where mass production of R_2 lines is the goal. We routinely use two or three media for cultivating anthers of F_1 hybrids — the N_6 medium supplemented with sucrose and auxin, and two versions of the potato extract media also containing auxins. These media are also used extensively by Chinese scientists. By employing several media and by staggering plantings of F_1 hybrids and ratooning, one can significantly increase the probability of generating a sufficient number of calli. The staggered planting/ratooning permit assessment of callus induction in the various media. If callus induction is superior in one of the media, anthers from the later planting are cultured only in the best medium.

Successful *in vitro* selection for desirable plant traits at the callus induction level has not been demonstrated. The relatively low inherent percentage of callus induction combined with the differential responses of F_1 hybrids and varieties to media composition and other unknown factors render selection at this stage extremely difficult.

Plant regeneration

Callus growth and the subsequent regeneration of plants have received much less attention than callus induction. This may be due to the large amount of information already available on regeneration of plants from callus tissue in several crops. The most significant achievement in rice has been the employment of the two-step process in which calli are induced on liquid media containing auxins and are transferred to solid media containing cytokinins (4). The stage of callus transfer appears to be critical, as calli allowed to grow excessively on induction media seldom regenerate plants when transferred to regeneration media. There is without a doubt a strong varietal influence on the efficiency of plant regeneration from callus. However, recent work, again with japonica types, routinely reports 50% regeneration, but regeneration of indica types is significantly less.

The possibility of applying *in vitro* selection pressure at the regeneration level is frequently proposed. Differences in callus growth in media containing Al have been demonstrated in several crops, e.g., carrot, tomato, and sorghum (5,7,10). However, varietal response to Al at the regenerative level may not be related to field reactions. Even at the whole plant level, there does not appear to be a significant correlation between the reaction in nutrient solutions containing Al and responses in the field of acid soils. This is not surprising, since soil acidity is complex, involving toxic levels of Al combined with deficiencies in Ca and/or P as well as other micronutrients. It is doubtful that one can simulate such a complicated factor as the soil acidity complex *in vitro*.

It has also been suggested that amending another culture media at the callus or regeneration stage with pathogen-produced toxins could be an effective means of prescreening regenerated plants for disease resistance (8). While this is an attractive idea, and should be explored, we feel that the evidence is lacking to support a large-scale effort in this area. Very few pathogen-produced toxins are primary determinants of disease, and thus they are inappropriate for such a method. We suspect that screening *in vitro* for toxin tolerance would merely select for easily overcome resistance (akin to a hypersensitive response). Furthermore, it is highly unlikely that diseases with very complex etiology will be overcome by exposing callus *in vitro* to only one or a few of the hundreds or thousands of pathogen products that result in a disease relationship between the rice plant and the pathogen. A final concern is how well the response of undifferentiated tissue will correspond to that of a maturing plant in the field. Can we expect a plant regenerated from callus and tolerant to one

or more toxins to be resistant to neck blast? In a field situation even adult plants resistant to leaf blast may be very susceptible to neck blast.

Parental selection

Evaluation of parents for callus induction and regeneration abilities is often considered important. However, convincing data are lacking to support this conclusion. Generally, pollen from the F_1 exhibits hybrid vigor for regeneration, but callus induction does not appear to follow any consistent pattern. More data are required to demonstrate the benefits of parental selection before one can justify restricting the crossing program based upon callus induction and regeneration capabilities. At CIAT, we prefer to increase either the number of crosses or the number of anthers cultured as opposed to confining the breeding program to a few crosses of known parental capability.

R_2 lines

With the presently available techniques and knowledge of anther culture, the only feasible breeding approach involves evaluation and selection of homozygous R_2 lines. Consequently, the anther culture procedure becomes no more than a means of reaching homozygosity in a reduced period of time. In this light, breeding via anther culture is similar to the single seed descent (SSD) method (1). The SSD method has been shown to be an effective breeding procedure for many important crops, especially in areas where only one generation/year can be advanced. Both methods also have the same limitation in that thousands of fixed lines are produced that have not received prior selection pressure. Consequently, success in using anther culture for varietal improvement depends to a large extent upon the development of screening techniques capable of handling thousands of fixed lines using only small quantities of R_2 seed. Another serious problem confronting breeding via anther culture is the capacity to regenerate sufficient material to have a reasonable chance of encountering large numbers of useful R_2 lines.

Population requirements

Experienced rice breeders using conventional methods normally attempt to obtain an F_2 population of 4,000–5,000 plants/cross. Although it is difficult to make an analogy with anther culture, Chinese scientists have calculated that an equivalent population would be on the order of

100–150 regenerated plants (9). Although we question the equivalence of a large F_2 with a reduced number of unevaluated regenerated plants, we accept 125 R_2 plants/cross as a reasonable target. Thus, a rough estimate can be made of the number of anthers and calluses required to regenerate the desired amount of R_2 material. One panicle taken from the field when the distance between the auricle of the flag leaf and the subtending leaf is 4–7 cm will produce approximately 25 florets containing pollen in the mid- to late uninucleate stage. From each floret approximately 2 or 3 anthers can be separated with ease, resulting in approximately 60 anthers/panicle. Assuming a 25% callus induction rate, each panicle would produce about 15 calli. If a 25% regeneration rate is obtained, four plants would result, and approximately half of these R_1 plants might be diploid. Consequently, from each panicle two diploid plants can be expected. However, only about 50% of the diploid R_1 plants might be agronomically acceptable, resulting in about one useful R_2 plant from each panicle harvested. To obtain the target population of 125 R_2 plants, roughly 100 F_1 panicles will be required from each cross. For a national program, it may be considered that 100 crosses/year is adequate. Consequently, if anther culture is to be used as a breeding tool, one must be capable of handling approximately 10,000 panicles from F_1 hybrids, 600,000 anthers, 150,000 calli, and 75,000 R_1 plants/year. The 75,000 R_1 plants would result in about 15,000 useful diploid R_2 lines. This illustrates the problems of how to regenerate such a large volume of lines and how to expose them to selection pressures to identify elite lines.

METHODS FOR GENERATING R_2 LINES

Induction phase

The techniques normally used for isolating anthers from panicles and placing them on induction media are laborious. The most time consuming step is the cutting of individual florets to extract the anthers. The method we employ consists of the following:

- Approximately 100 panicles are harvested from each cross at the uninucleate stage, wrapped in aluminum foil, and cold shocked for 3–10 days at 8 °C.
- Immature spikelets are surface sterilized, and the top and bottom portions of the panicle are discarded, leaving approximately 25 florets, which are removed and dissected at the base.
- Anthers are extracted from the cut florets by tapping the cut floret

on the induction flask. Another method in which anthers are extracted in mass appears to be promising, but more work is required to reduce the high amount of contamination.

- The induction flask is sealed and placed in the dark at about 20 °C for callus induction.

Each flask contains the anthers from one panicle. All procedures are conducted under a flow hood using sterile conditions. Using this method approximately 150 panicles (9,000 anthers) can be prepared daily.

Regeneration

In each induction flask approximately 15 calli are normally produced. Close observation is required, as callus formation is never uniform. When there are more than 10 small calli/flask they are transferred to the regeneration medium. When only a few calli are present it is necessary to decide to transfer or wait for more callus formation. If the latter is the case and new calli appear, the first formed calli are discarded, since large calli seldom regenerate plants. Calli are transferred in mass by filtering the entire contents of the induction flask in a Buchner funnel. It is important to use a flat vacuum filter to obtain a uniform dispersion of the calli on the filter paper. Uninitiated anthers and small calli are collected on a 9 cm filter, which is the same size as the regeneration container. The filter paper is inverted and placed on top of the induction medium and tapped lightly to embed the calli in the medium. The filter paper is removed, and the calli remain on the medium. The regeneration container is sealed and placed under artificial lighting until regeneration. With this method, one person can prepare approximately 150-200 flasks/day containing a total of 2,000-3,000 calli. This is equivalent to 1.5-2 crosses/day. The advantage of this method is that the unit of work is the panicle, eliminating the need to transfer thousands of calli individually to test tubes.

In summary, two laboratory assistants using the previously indicated methods can handle 100 crosses/year. In developing these techniques a deliberate attempt has been made to employ locally available glassware. This is important, as imported scientific glassware is expensive, and with the large volume of material the total cost would be prohibitive for many developing countries. Baby food jars (125 ml) are ideal for callus induction flasks and are readily available. Regular fruit jars (9 cm diameter) can be cut and made into regeneration containers. In Colombia, we have purchased sufficient glassware to handle 100 crosses/year at a total cost of approximately US\$1,200.

Evaluation of R_2 lines

R_1 plants are inappropriate for screening as they are weak, and diploids cannot be distinguished until flowering. Once R_2 seeds (approximately 20 g/plant) are obtained, the procedure for selecting elite lines is similar to any other breeding method. Due to the large number of lines involved, screening in the field under heavy pressure is required. The material is first screened for tolerance to the easiest character, eliminating many of the lines. Material selected in the first test is further screened for tolerance to the next easiest constraint, and the process continues until the number of lines has been reduced to a sufficiently small number that can be handled in an observation nursery.

We have made approximately 100 triple crosses designed for southern Brazil, which should result in approximately 15,000 R_2 lines. A 3 g sample is evaluated for blast resistance, resulting in about 50% rejection. Since there is a high correlation between leaf blast and panicle blast resistance, this screening is done only at the leaf stage and requires only 50 days. Duplicate 3 g samples of the blast resultant R_2 lines are screened for Fe tolerance. Again, 50% of the entries are discarded; this test requires approximately 60 days. White belly determination is conducted using remnant 3 g samples of the 3,000 R_2 lines possessing blast resistance and Fe toxicity tolerance. The remaining 10 g of remnant R_2 seed of about 1,500 selected lines are planted in 4—row plots in southern Brazil, where the lines are evaluated for phenotypic characters and cold tolerance. Elite selected lines are sent to Uruguay, which has the same yield constraints. This whole process, commencing with panicles of F_1 hybrids, requires approximately 1.5 months for callus induction, 1.5 months for regeneration, 4 months for R_1 growth, 2 months for testing for blast resistance, 2 months for Fe tolerance and 1 month for white belly determination. Consequently, in 1 year, lines are available for observation nurseries. Using conventional breeding methods to arrive at the same stage, at least 4 years would be required at CIAT, where 2 generations/year are produced. If the anther culture process were applied to Brazil, which has only 1 field generation/year, the savings in time would be considerably greater.

A similar scheme is being used for Chile and Argentina. In the latter case, a 3 g sample of R_2 seeds is used to screen for blast and the selected lines are tested for straighthead resistance. Resistance to straighthead disease can be evaluated using arsenic, which has been shown to mimic the disease (3). Lastly, white belly is determined. Again, the remnant R_2 seeds are tested in observation nurseries in Argentina.

PERSPECTIVES FOR BREEDING VIA ANTHER CULTURE

The varied rice ecologies in Latin America offer distinct examples of possible application of anther culture to varietal improvement. These include the Southern Cone, upland rice for acid savanna soils, and tropical Mexico. These examples are characterized by vast production potential addressed by conventional breeding programs restricted to one generation/year.

We consider that anther culture has the potential to reduce considerably the time required to produce fixed lines. Its utility in high volume breeding programs will depend upon resolving remaining limitations and uncertainties, including:

- Continued improvement in the efficiency of callus induction and plant regeneration in indica crosses is essential to reduce the volume of panicles, florets, and anthers processed.
- Field experience with R_2 lines from many crosses is required to estimate the population desired per cross to simulate progeny sizes used in conventional rice breeding.
- Similarly, only extensive field experience will indicate whether doubled haploids give reasonable numbers of normal, agronomically useful lines.

The CIAT Rice Program is concentrating on these and other problems with the goal of moving anther culture from the laboratory to rice breeders' fields. If success is achieved, we believe that serious consideration should be given to establishing anther culture facilities within selected national programs. We would continue to improve methods and train national scientists for local application of anther culture to high volume rice breeding programs.

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DISCUSSION

SESSION 13: TISSUE AND CELL CULTURE

Q – Brar: Could part of the somaclonal variation be due to variation in somatic cells vs. generative cells?

A – Zakri: Not likely.

Q – Virmani: Do we know why japonica rices have better culturability than indica rices?

A – Chen, C. C.: I do not know.

Q – Brar: Did you get embryos directly from the cultured anthers with small intervening callus stage?

A – Chen, C. C.: No. Plant formation in rice anther culture usually is intervened by a stage of callus formation.

Q – Kucherenko: You have reported very high rates of plant regeneration from anther-derived callus. What proportion of these plants are green and is this percentage somehow influenced by growth substances in the medium?

A – Chen, C. C.: The percentage of green plants varies with many factors such as genotype of the donor plants, stage of the microspores, and culture conditions. Usually it varies from 0 to 80–90%.

Q – Zhao: How many generations did these cold tolerant types pass through, and are they stable genetically?

A – Zapata: These materials have passed through four generations and are genetically stable.

Q – Kucherenko: What in your opinion are the reasons for the fact that the same explant, in the same cultural conditions, produces different types of cells?

A – Dykes: It is not always clear why the same explant will produce different cell types under the same cultural conditions. It could be that cells of the explant are not genetically homogenous or that they are exposed differently to the hormones in the medium because of their position.

Q – Brar: What is the frequency of R_1 progenies showing increased salt tolerance? Were there any differences in the cultures stressed with salt and those not stressed?

A – Dykes: Unfortunately, we have not yet been able to screen a high number of R_1 rice plants that were derived from cell lines selected for salt tolerance. To date we have screened less than 100 regenerates of salt-

selected lines. Two of these have shown increased tolerance at the seedling stage. We have not yet evaluated these for tolerance at the flowering stage. Concerning regenerates from cell cultures not selected for tolerance to salt, we have found *no* increased tolerance in the R₁ progeny.

Q – Kucherenko: How do you select for drought tolerance in such tissue cultures?

A – Dykes: We use polyethylene glycol (PEG), which sets to create a kind of chemical “drought.” However, the techniques to do this have proved more difficult than with salt, and we have not yet done very much selection work for cultures tolerant to PEG.

C – Lörz: The mutants and variants found after tissue culture are in principle different from mutants obtained by conventional mutagenesis. The advantage of somaclonal variation is seen mainly in the combination of somaclonal variation and *in vitro* selection to make use of the large number of cells potentially available to be changed in their genomic composition.

GENETIC ENGINEERING

SESSION 14

MOLECULAR CLONING AND SEQUENCING OF RICE GENES*

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We have isolated and determined the DNA sequence of several genes from the nucleus, chloroplast, and mitochondrion of rice (*Oryza sativa*). A histone 3 (H3) gene was analyzed; it codes for a typical H3 protein. An H3-like gene was also isolated. The codon usage of the rice H3 gene is highly unusual in that the third position of the codon contains 98% G and C. We have sequenced two clones that contain rice chloroplast genes. Within these two clones, there is one functional and one truncated gene for the large subunit of ribulose-1,5-bisphosphate carboxylase, two different pseudogenes of the β subunit of ATPase, and two genes which can code for the E subunit of ATPase. Thus, rice chloroplast DNA is highly heterogeneous at the DNA sequence level. Based on the DNA sequences, we have compiled a list of the amino acid sequence of the cytochrome oxidase subunit II (COII) from ten organisms. Cysteine 229 was not found to be one of the 57 conserved amino acids. Thus, this amino acid is not invariant as was assumed by other investigators.

In the last 25 years or so, major improvements in rice have been made by plant breeders and geneticists. However, studies on rice at the gene level using molecular biology techniques have been extremely limited. As a first step, one must isolate genes from rice and study their structures, regulation, and expression. In this paper, we will present data on the cloning and sequencing of several nuclear, chloroplast, and mitochondrial genes.

NUCLEAR GENES

There are five histone proteins (H1, H2A, H2B, H3, and H4) found in all eucaryotic organisms (8). These small, basic proteins specifically interact with DNA and are the fundamental structural proteins of chromosomes. The genes for histone proteins from several species have been cloned using recombinant DNA techniques (8). DNA sequence analysis of a wheat histone gene (H3) showed that the deduced amino acid sequence was identical to that of the pea (12). A rice genomic clone carrying H2A, H2B, and H4 histone genes was identified by Thomas and Padayatty (13).

Cloning and analysis of rice histone 3 gene and pseudogene

Total rice DNA from the variety IR26 was partially digested by *Sau*3A restriction enzyme. Fragments of approximately 15–20 kb were isolated and ligated to *Bam*HI-cut λ EMBL4 DNA to construct a genomic library (carried out by Y. Xie in our laboratory) of over one million independent isolates. Using a sea urchin H3 gene fragment as a probe, we obtained eight hybridizing plaques, and one of them, 1RH3-17, was analyzed in detail. Restriction mapping and hybridization analysis showed that two regions of this 13.8 kb fragment contain H3 or H3-like genes. These two regions were separately cloned into the pBR322 plasmid and resulted in plasmids of pRH3-1 and pRH3-2.

The 1.3 kb rice DNA insert in pRH3-2 was sequenced and the following features were revealed.

- The insert includes a 405 bp coding sequence that starts with ATG and ends with a stop codon TGA (Fig. 1). The DNA sequence shows 80% homology with the H3 gene from sea urchin sp17, and 92% homology with the H3 gene of wheat.
- It contains a 233 bp 5' noncoding sequence that includes a TATA box (at position -120) and a GCCTCC sequence about 10 bp upstream from the TATA box (5). A sequence resembling the cap box, of the form 5'-pyCATTcPu-3', is found at position -89. The nucleotide sequence 63–83 bases (#470–490 in Fig. 1) downstream from the termination codon shows hyphenated dyad symmetry (11), 5'-^gGTAATGGCTT^gGTTGCTGTTGC-3'. This sequence can form a hairpin loop and may serve as a putative terminator for the transcription of histone genes (5).

Figure 1 also gives the amino acid sequence of rice H3 as deduced from the DNA sequence. In comparing the amino acid sequences of rice H3 with wheat H3, three differences are found (data not shown): Amino acids in locations 86, 90, and 98 are thr, arg, and arg in rice H3, and they are ser, ser, and ala in wheat H3. It is interesting to note that both rice and wheat are monocots, and their evolutionary distance is closer than that between wheat and pea (a dicot). Yet, the H3 protein in wheat is identical to that of the pea (12). By comparing the amino acid sequence of rice H3 to that of sea urchin sp17, seven differences are found.

A 0.97 kb region in pRH3-1 was sequenced. There is 94% nucleotide sequence homology between this H3-like gene and that of the H3 gene between nucleotides 1 and 405 (Fig. 1). We found no initiation codon in the H3 gene from clone pRH3-1. Moreover, there are alterations of six amino acids and five 3 bp deletions when compared with the H3 gene

from clone pRH3-2. Thus we assume that the H3-like gene from pRH3-1 is a "pseudogene," because it is unlikely that it can give a functional H3 protein (usually highly conserved) with such large sequence deviations.

Codon usage

The codon usage of the rice H3 gene is highly unusual in one respect. We noticed that the third position of the codon is almost exclusively G or C. As shown in Table 1, out of 135 codons, G + C occurred 132 times (or 98%) and A + T only 3 times. In contrast, the third position of the codon for the sea urchin (sp17) H3 gene contained only 59% G + C (11). Interestingly, the rice H3 "pseudogene" also contained 93% G + C at the third position of the codon. We noticed that wheat H3 gene and H4 gene show the same high bias for using G or C for the third position of the codon (12, 14). The bias is much less pronounced for the H4 genes of sea urchin (pSP2), *Xenopus Laevis* (*peX1H4W2*), and mouse (14). We speculate that the very high bias for selecting G or C for the third positions of the codons of H3 and H4 genes may provide survival advantages for rice, wheat, and perhaps other higher plants. We believe that the effect of high occurrence of G or C is manifested at the mRNA level. It is likely that selecting more G and C for the third position of the codons will allow more stable interaction between the mRNAs and the anticodons of the tRNAs at higher temperature. Thus, synthesis of H3 and H4 can occur at relatively high temperature. It is likely that these proteins play a more vital role in the survival of the cells than other proteins. It is also possible that mRNA, with a higher content of G and C, is more likely to form stable hairpin structures. This may allow more efficient interaction with protein factors. Plants are especially vulnerable to high temperature because, unlike animals, they are unable to move away from an unfavorable environment.

CHLOROPLAST GENES

The chloroplast DNA (ctDNA) in each plant consists of circular molecules. Size variations among many species of angiosperms range from 120 to 210 kb (10). The ctDNA in most plants includes a pair of inverted repeats (10 kb to 65 kb in size). Essentially all the site-specific recombination activity in the chloroplast is postulated to occur between two strong recombination sites located in the inverted repeats (10). Other than these recombinations, the chloroplast genome is generally considered to be stable within a species.

Table 1. Codon usage for the third position of H3 and H4 genes. For the first four lines, the frequencies of third codon usage of the H3 gene or pseudogene are given (total number of codons is 135 for H3). Data for wheat H3 gene are taken from 12 and those for sea urchin H3 from 11. Data for the H4 gene given in the last four lines are taken from 14.

Gene	C	G	A	T	% G + C
Rice H3	75	57	0	3	98%
Rice H3 pseudogene	71	49	5	4	93%
Wheat H3	83	49	1	2	98%
Sea urchin H3 (sp17)	48	30	30	24	59%
Wheat H4					98%
Xenopus H4(pcXIH4W2)					76%
Sea urchin H4 (pSp2)					54%
Human H4 (pHu4A)					76%

Isolation of rice chloroplast genes

Using the spinach *rbcL* as a probe, we have isolated two clones (Ct-1 and Ct-2) from a HindIII library of the rice chloroplast genome. Restriction maps for Ct-1 and Ct-2 were constructed, and the location of the *rbcL* was determined by Southern blot analysis. Further Southern blot analysis using the wheat genes coding for the **b** and **e** subunits of ATPase (*atpB* and *atpE*) as probes and DNA sequencing delineated the locations of *atpB*, *atpE*, and *tRNA* genes (Fig. 2). In both Ct-1 and Ct-2 clones, these genes are organized in an order similar to that found in chloroplast genomes of other plants (10).

DNA sequences of chloroplast genes

Comparison of DNA sequences in these two clones reveals several differences. The clone Ct-2 contains a complete *rbcL* that can code for a functional large subunit of ribulose-1,5-bisphosphate carboxylase (RuBP-Case) consisting of 468 amino acids. However, the clone Ct-1 contains a truncated *rbcL* that codes for only 278 amino acids (about 60% of the complete RuBPCase). Comparison of the sequences for the first 278 amino acids of the *rbcL* reveals 21 bp substitutions, 12 of which are silent, thus resulting in only 9 amino acid changes. In 5 of these 9 cases the Ct-1 amino acid is the same as that of maize, and in 3 cases Ct-2 and maize share the same amino acids. The 5' noncoding sequences of *rbcL*

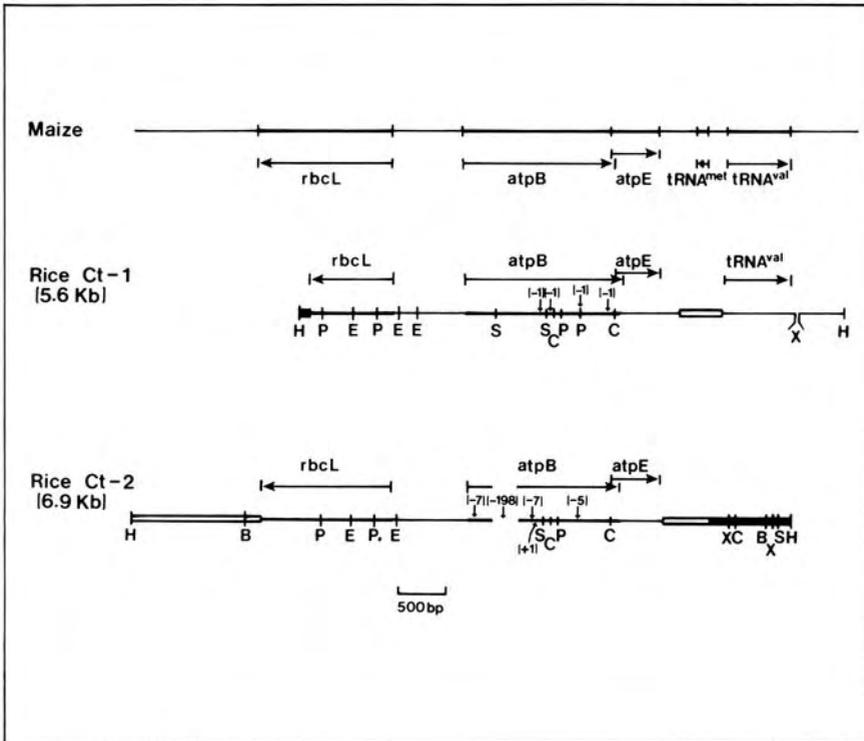


Fig. 2. Physical maps of portions of rice and maize chloroplast *rbcL*, *atpB*, and *atpE*. The ctDNA coding sequences of clones Ct-1 and Ct-2 are shown by heavy dark lines, and the two regions marked with a filled-in box are sequenced, but the coding information has yet to be assigned. The three regions marked with open bars are not sequenced. A gap or region marked with a number in parentheses indicates deletions. B = Bg1II, C = ClaI, E = EcoRI, H = HindIII, P = PstI, S = SstI, X = XbaI, rbcL = RuBPCase large subunit gene, atpB = CF₁β subunit gene, atpE + CF₁ε subunit gene.

in Ct-1 and Ct-2 are highly homologous. However, a significant difference was observed: The *rbcL* of Ct-1 has a typical prokaryotic ribosome binding site (Shine-Dalgarno sequence), 5'-GGAGG-3', that is found in all other *rbcL*, whereas that of Ct-2 has an altered sequence, 5'-GGGGG-3'.

DNA sequence analysis revealed that the rice *atpB* of neither Ct-1 nor Ct-2 is functional. Although the *atpB* of Ct-1 is nearly perfect, it has four single-base deletions that disrupt the normal reading frame as compared to the corresponding maize gene. The *atpB* of Ct-2 is grossly defective due to one 198 bp deletion, two 7 bp deletions, one 5 bp deletion, and one 1 bp insertion. Apart from these deletions and the insertion, there are 19 bp substitutions between the *atpB* of Ct-1 and Ct-2, which result in 7

amino acid changes. If a functional *atpB* exists in the rice chloroplast, it may be included in a 10 kb fragment that we have recently identified and that appears to contain the functional *atpB*. If it is found, it would mean that there are at least three different versions of the *atpB* gene in rice. In contrast, the *atpE* of both Ct-1 and Ct-2 appear to be functional based on comparison of their nucleotide sequences with other *atpE*. The two *atpE* of Ct-1 and Ct-2 differ in only two base pairs, which result in two amino acid changes.

The *tRNA^{val}* gene was identified in Ct-1 but not in Ct-2. The *tRNA^{met}* gene has not yet been identified in these clones. DNA sequences of the *tRNA* regions of these two clones are found to be different.

There are several hundred ctDNA molecules in each plant cell. It has generally been assumed that the DNA molecules in the chloroplasts of a given plant cell are identical (10). Our preliminary results indicate that there are different chloroplast DNA molecules in rice. So far, we have sequenced only two clones that hybridized with the *rbcL*, and we found two versions of the *rbcL*. Furthermore, if there is a functional *atpB* in the chloroplasts, as in most plants, it would indicate that there are at least three versions of the *atpB* in rice chloroplasts. The *atpE* in Ct-1 and Ct-2 are similar but not identical. The *tRNA^{val}* gene is present in Ct-1 but not in Ct-2. Thus, within a 6.9 kb region of the rice chloroplast DNA, several striking differences are found by analyzing only two clones. Thus, the rice ctDNA appears to be heterogeneous at the DNA sequence level. When more sequence information is available, and when other clones similar to Ct-1 and Ct-2 are isolated and sequenced, it will be possible to determine just how many different versions of the chloroplast DNA molecules are present in rice. We realize that our chloroplast DNA contains some mitochondrial DNA; thus we have yet to prove conclusively that Ct-1 and Ct-2 clones are chloroplast in origin.

MITOCHONDRIAL GENES

Mitochondrial cytochrome oxidase consists of three polypeptides synthesized in the mitochondrion, and at least four others synthesized in the cytoplasm. Cytochrome oxidase contains two heme groups and two protein-bound Cu atoms per monomer. Cytochrome oxidase subunit II (COII) is especially interesting because it binds one heme and one Cu and it is in close contact with cytochrome *c*. The binding sites on COII that interact with cytochrome *c* are believed to involve aspartate (D)-136, (D)-187, and glutamate (E)-227 (numbering refers to that

given in Fig. 3). The binding sites on COII that interact with the Cu are believed to involve cysteine (C)-225, (C)-229, and histidine (H)-232. It is likely that (H)-41 and possibly (H)-190 bind heme. Thus these eight amino acids are most important for the biological activity of COII, and they must be conserved. The information summarized above is consistent with that derived from an alignment of the amino acid sequences of COII from seven organisms (15).

Cytochrome oxidase subunit II gene

The COII genes from ten organisms have been sequenced. In this paper, we present an alignment of the amino acid sequences of COII, which are deduced from the DNA sequences. The amino acid sequences in COII that are common to all ten species of eucaryotes are given in line 14 of Figure 3. Fifty-seven amino acids (around 23% of the 227–260 COII amino acids in these organisms) are common to these ten eucaryotes. Most of these amino acids have been conserved (between yeast and human) over 1.1 billion years of evolution, presumably because they are important for the biological function of COII. Information derived from Figure 3 shows that amino acid 229 is a cysteine (C) in all organisms except for wheat (3). Thus, one must conclude that C-229 is not important for binding to Cu in COII, assuming that the result of sequence analysis is correct. This important new information derived from DNA sequence analysis can help in understanding the mechanism of action of COII.

The amino acid sequences of COII from five plant species share 80% homology (Fig. 3, line 6). The three monocots—rice, wheat and maize—share 99% amino acid sequence homology. The plant and yeast COII molecules are longer than those of animal COII by about 30 amino acids. Seventeen of these amino acids are present at the amino terminus. This fact, together with a higher percentage amino acid homology between plants and fungi, indicates that yeast and *Neurospora* are more similar to plants than to animals. Sequence comparison also shows that the amino acid sequence of COII of the five plants is closer to yeast than to *Neurospora*.

The amino acid sequences of COII from three animal species share only 68% homology (Fig. 3, line 12). Thus, there is greater COII sequence divergence among animals than among plants so far studied. Yet the evolutionary distance between human and mouse (around 80 million years) is probably considerably shorter than that between rice (or maize) and pea. The reason for the considerably slower rate of evolution

	1	1	1	1	1	1	2	2
	4	5	6	7	8	9	0	1
	123456789012345678901234567890123456789012345678901234567890							
1 Rice	DEQSI.TFDSYTI	PEDDPEL	QSRLL	EVNDR	VVVP	AKTHLR	MIVTP	ADVP
2 Wheat	DEQSLTFDSYTI	PEDDPEL	QSRLL	EVNDR	VVVP	AKTHLR	MIVTP	ADVP
3 Maize	DFQSLTFDSYTI	PEDDPEL	QSRLL	EVNDR	VVVP	AKTHLR	MIVTP	ADVP
4 Pea	DEQSLTFDSYTI	PEDDPEL	QSGLL	EVNDR	VVVP	AKTHLR	IIVTP	ADVP
5 <u>Oenothera</u>	DEQSI.TFDSYTI	PEDDLEL	QSGLI	.FVNDR	VVVP	VKTHRL	IIVTP	ADVP
6 Common to plants	DEQSLTFDSYTI	PEDD	ELGQS	L	EVNDR	VVVP	KTH	I
7 <u>N. crassa</u>	NDEFIEFDSYI	VSPESD	LEEGAL	RML	EVNDR	VILPEI	THVRF	IITAG
8 Yeast	SCHTVEFESYV	IPDELLEE	QRLRL	LDT	DTSJ	VVP	VDTHIR	FVVTA
9 Human	---	LIFNSY	MLPPL	FL	PGDL	RLL	DVDNR	VVLP
10 Bovine	---	LSFDSY	MIP	TSEL	KP	GELR	LLEVDNR	VVLP
11 Mouse	---	LCFDSY	MIP	TNDL	KP	GELR	LLEVDNR	VVLP
12 Common to animals		L F SYM P	L PG	RL	LL	VNDR	VVLP	E
13 Common to RMPYHMo		F SY P	G LL	D V P			DV H A P	G K DA
14 Common to all		F SY P	G L D P				DV H A P	G K DA P R N
	2	2	2	2	2	2	2	
	1	2	3	4	5	6		
	1234567890123456789012345678901234567890123456789012							
1 Rice	TSISVQREGV	YVYGC	SEIC	GTN	HAF	TP	IVVE	AVTL
2 Wheat	TSISVQREGV	YVYGC	SEIR	GTN	HAF	TP	IVVE	AVTL
3 Maize	TSISVQREGV	YVYGC	SEIC	GTN	HAF	TP	IVVE	AVTL
4 Pea	TSISVQREGV	YVYGC	SEIC	GTN	HAF	-P	IVVE	AVP
5 <u>Oenothera</u>	ISMSVQREGV	YVYGC	SEIC	GTN	HAF	MP	IVIE	AVS
6 Common to plants	S	SVQREGV	YVYGC	SEI	GTN	HAF	PIV	FAV
7 <u>N. crassa</u>	VSVFIN	REGV	FYGC	SEIC	GI	LHSS	MP	IVIES
8 Yeast	VSAI	IQREGV	FYGC	SEIC	GT	G	HAN	MP
9 Human	TTFTATR	PGV	YVYGC	SEIC	G	AN	H	S
10 Bovine	TTL	MSSR	PGL	YVYGC	SEIC	G	SN	H
11 Mouse	ATV	T	SN	K	P	L	F	Y
12 Common to animals	T	RPG	YGQC	SEIC	G	NH	S	F
13 Common to RMPYHMo		R G	Y G C	S E	C G	H	P I	F
14 Common to all		R G	Y G C	S E	G H	P I	E	

sequences that are common to rice, maize, pea, yeast, human, bovine, and mouse. These seven species are grouped together because their COI sequences have also been compared (data not shown). The amino acid letter code is as follows: A = Ala, R = Arg, N = Asn, D = Asp, C = Cys, E = Glu, G = Gly, H = His, I = Ile, L = Leu, K = Lys, M = Met, F = Phe, P = Pro, S = Ser, T = Thr, W = Trp, Y = Tyr, V = Val.

of the plant COII DNA sequences and amino acid sequences relative to animals remains to be determined.

Cytochrome oxidase subunit I

We also aligned the amino acid sequences of cytochrome oxidase subunit I (COI) from seven organisms corresponding to line 13 (see legend to Fig. 3). We found that the general conclusions made for the evolution of COII are applicable to COI as well. One notable difference is that the sequence in COI is more conserved than in COII. By comparing the same seven organisms, COI showed 47% homology (data not shown), whereas COII (Fig. 3, line 13) showed only 26% homology. Furthermore, there are many more long stretches of homology in COI (up to 10 in a cluster) than in COII (up to 3 in a cluster). A more detailed comparative analysis of the sequence data of COI and COII both at the amino acid level and at the nucleotide level is in progress.

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THE Ti PLASMIDS OF *AGROBACTERIUM* AS VECTORS FOR GENE TRANSFER TO PLANTS

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Agrobacterium tumefaciens can only transfer T-DNA after induction of the Ti plasmid-encoded *vir* genes. The identification of plant-synthesized metabolites that mediate this virulence may possibly help in the attempt to transfer T-DNA into rice cells. The availability of chimeric marker genes already allows the selection of T-DNA-transformed cells in several monocot species of the Liliaceae. New chimeric genes may be needed for the Gramineae.

It is now well documented that Ti plasmids are efficient and reliable gene vectors for genetic engineering of dicotyledonous plants. Already many plants, transformed with a variety of new genes, have been constructed. This has resulted in the opening of a new field in plant genetics: the study of the structure and functions of the plant genome.

The demonstration that several monocotyledonous plant families from order Liliales (7, 12) could be engineered via the Ti plasmids is encouraging for rice genetic engineering projects.

An urgent objective is now the construction of selectable marker genes that, upon expression in rice cells, will allow the identification of these transformed rice cells. Meanwhile, it becomes essential to isolate genes that could confer advantageous traits to rice plants, as well as to identify and construct DNA cassettes that allow tissue and developmentally controlled expression of the engineered genes.

Ti PLASMIDS

All crown gall-inducing *Agrobacterium* strains have been shown to harbor plasmids (6). These plasmids, 200 kb long on the average, contain two characteristic sets of genes, both essential for tumor induction. One set, called the onc genes, is localized in the T-DNA region. This DNA segment, 10–20 kb long, is confined by two 25-base pair sequences in direct-repeat as the right and the left border sequences. One or more

copies of the T-DNA segment can be transferred and integrated into plant DNA. The orientation of the right border 25-bp repeat determines the sequential order of this DNA transfer (18).

To “activate” the T-DNA for transfer, another series of genes, localized in what is called the virulence region (*vir* genes) is needed. These *vir* genes are in the repressed state (15, 16). The induction of these *vir* genes occurs when *Agrobacterium* enters into contact with a class of compounds produced by plant cells. These compounds are released by wounded plants, by plant cell or organ cultures, as well as by plant protoplasts (16), and they have been purified and their structure determined (S. Stachel, E. Messens, M. Van Montagu, and P. Zambryski, unpublished data).

The role of the different *vir* genes has not yet been identified at the molecular level. However, it is clear that they are essential for the early events in T-DNA transfer, in particular for the “activation” of the right border 25-bp sequence (K. Wang, and P. Zambryski, unpublished data).

These two sets of genes, the T-DNA genes and the *vir* genes, are actually very different in design. The genes encoded by the *vir* region are typically prokaryotic (S. Stachel, unpublished data). The T-DNA genes have evolved to be functional in the plant cell and hence have the feature of eukaryotic genes.

T-DNA

Among the T-DNA-encoded genes (Fig. 1), three are important in tumorous growth. One gene codes for an enzyme for cytokinin biosynthesis (AMP-isopentenyl transferase), and two genes code for enzymes involved in auxin biosynthesis (tryptophane-2 mono oxygenase and acetamide hydrolase). The expression of these genes allows the auxin and cytokinin independent growth of these plant cells, a property that can often be used to select transformed cells among a majority of untransformed cells.

None of the T-DNA sequences is essential for the T-DNA transfer. This means that all the DNA localized between the 25-bp repeats can be removed and replaced by genes that one wants to transfer to plant cells. So DNA of any origin, once flanked by these 25-bp sequences, will behave as a T-DNA element. These findings were essential for the design of efficient plant gene vectors.

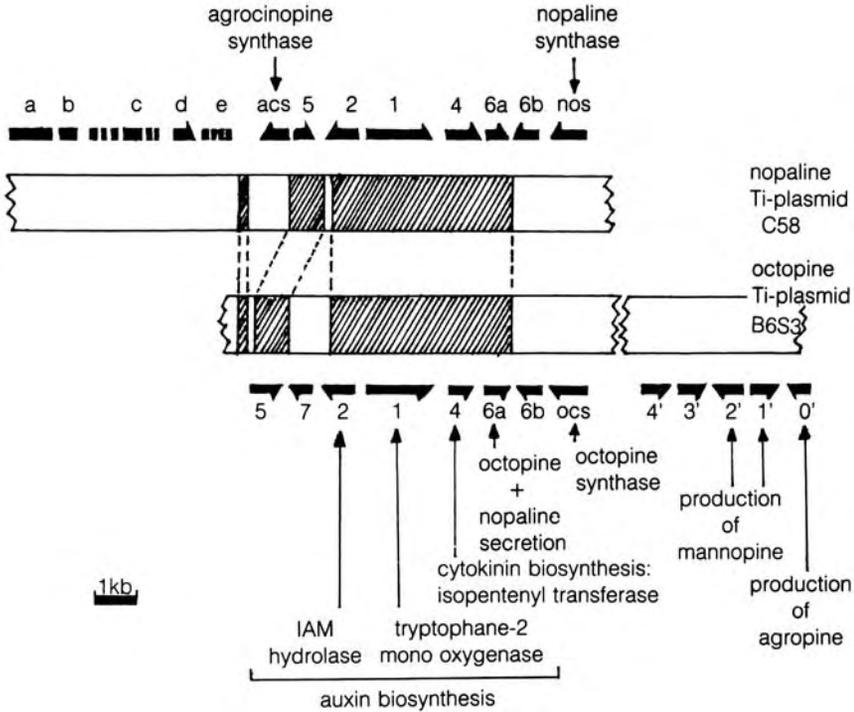


Fig. 1. Functional organization of the T-DNA of nopaline (upper) and octopine (lower) Ti plasmids. The arrows indicate the different genes and their direction of transcription in the transformed plant cell. The gene nomenclature follows the original designations according to transcript size. The dashed areas represent regions of homology between the two types of plasmids.

PLANT GENE VECTORS

As explained above, a first class of efficient Ti plasmid-derived gene vectors was obtained by replacing most of the T-DNA, including genes 1, 2, and 4, with pBR322. A typical example is the nopaline pTi C58-derived plasmid pGV3850 (20). Foreign genes cloned into pBR322 can be cointegrated with this Ti plasmid via homologous recombination.

A variation on this principle is a construction wherein the whole of the T-DNA, including the 25-bp repeats, has been replaced by pBR322 (e.g., the octopine pTi B6S3 derivative pGV2230) (2). Here, the foreign genes have to be cloned in a pBR322 derivative containing the 25-bp border sequences.

A typical representative of such a cloning vector, called an intermediate vector, is pGV929 (R. Deblaere, unpublished data). Here, a marker gene, expressible in plant cells, and a polylinker sequence are flanked by chemically synthesized right and left border sequences (Fig. 2). The foreign genes, and if necessary additional marker genes, can be inserted in the polylinker sequence.

A second class of Ti plasmid-derived gene vectors is a binary vector system. This system consists of a broad host range plasmid with the cloned T-DNA borders and a complementary plasmid containing the Ti *vir* functions (4, 11).

GENE EXPRESSION IN PLANT CELLS

The first attempts to express directly prokaryotic, yeast, or mammalian genes in plant cells failed. It became clear that the regulatory sequences from plant genes are different. It was therefore decided to construct "chimeric genes" consisting of a plant (or a T-DNA gene) promoter, the coding sequence of a bacterial gene that could function or a selectable marker in plant cells, and the termination signals of a plant gene (or T-DNA gene). Such genes were introduced into plants via a Ti plasmid system and shown to be expressed (1, 5, 8, 9). The chimeric genes have been used as dominant selectable markers in the isolation of transformed plant cells from these cells. Phenotypically normal and fertile plants have been regenerated. The new trait (e.g., kanamycin resistance) is inherited by the progeny in correct Mendelian fashion (3).

EFFICIENCY OF GENE TRANSFER

Cocultivation of plant protoplasts with agrobacteria containing the vector Ti plasmids can result in highly efficient gene transfer, sometimes up to 20%. This efficiency is, however, highly species- and even cultivar-dependent. Since regeneration of plants from protoplasts is still restricted to a limited number of species, alternative approaches were needed.

A coinfection method was developed whereby a whole plant could be used as the starting material. Here the engineered Ti vector carrying *Agrobacterium* is mixed with a shoot-inducing oncogenic *Agrobacterium* strain. The bacterial mix is used to infect the plant to be transformed. Among the proliferating shoots, some will contain only DNA coming from the engineered strain and not the oncogenic T-DNA of the helper

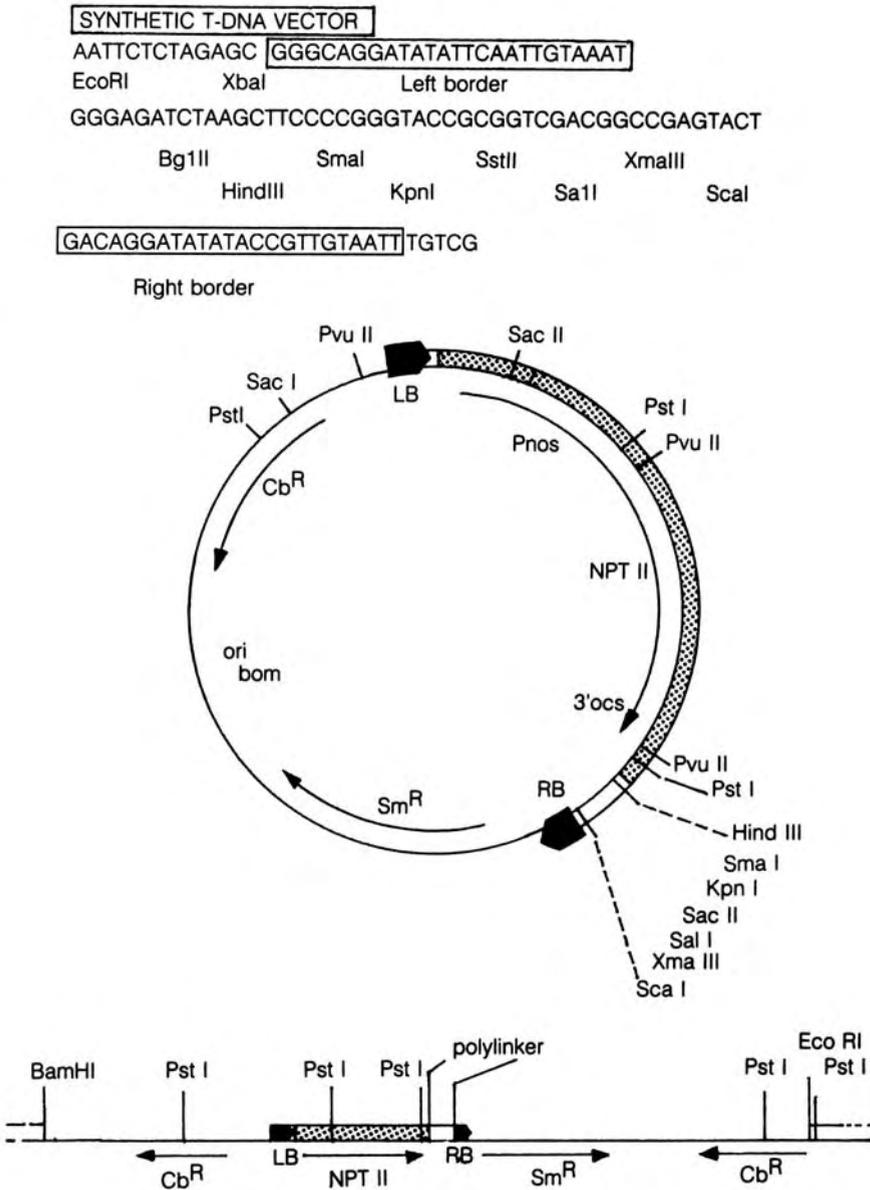


Fig. 2. Physical map of the 7.5-kb intermediate vector pGV922. The DNA that will become transferred and inserted into the plant DNA is located between the left border sequence (LB) and the right border sequence (RB). It consists of a chimeric gene *PnosNPT (II)3'ocs* and a polylinker sequence. This gene was constructed by joining the nopaline promoter (*Pnos*) to the coding sequence of the neomycin phosphotransferase II of the bacterial transposon Tn5 (*NPT-II*) and adding to this the 3' termination sequence of octopine synthase (*3'ocs*).

strain. These shoots can be selected for if the foreign gene fragment contained the necessary marker genes (19).

Recently, methods were introduced wherein tissue pieces (leaf discs, stem sections, root sections) are incubated with the *Agrobacterium* vector. Again, the results are species-dependent. For each use the optimal conditions have to be worked out.

In some cases it has also been possible to use cell suspension cultures. A further analysis of the optimal conditions and the availability of the “*vir* region-inducing compounds” may hopefully generalize the use of suspension cultures.

TISSUE-SPECIFIC GENE EXPRESSION

In plants, as in other eukaryotes, one class of genes is constitutively expressed; they are the “household” genes. Many genes, however, are regulated in a developmental or tissue-specific manner. In several cases, it was possible to show that sequences 5' upstream of the coding region control this gene expression. Hence the construction of chimeric genes where these upstream sequences were fused to the coding sequences of a marker gene could result in the controlled expression of the marker gene. That this is feasible was demonstrated by obtaining a photoregulated expression of the bacterial chloramphenicol acetyltransferase (*cat*) gene when the 5' noncoding region of one of the pea small subunit-ribulose-1,5-bisphosphate carboxylase (ss-rubisco) genes was fused to the *cat* gene (10).

Furthermore, it can be shown that the enzyme encoded by the marker gene becomes transferred to the chloroplasts if the constructs keep the sequences encoding the transit peptide (14, 17).

Both these results are of great importance to plant genetic engineering. It can indeed be expected that a continuous synthesis of a foreign protein at a high level, encoded by an engineered gene, will interfere with the normal development of the plant. Hence it will be of importance to express the newly introduced gene only in specific tissues (leaves, roots, fruits) of a mature plant. Furthermore, it is likely that the accumulation of a new protein in an organelle (vacuole, storage body) will allow a higher amount of this protein to be synthesized.

In many laboratories, efforts are now concentrated on the identification and isolation of DNA cassettes, derived from developmentally controlled plant genes.

THE TRANSFORMATION OF MONOCOTS

Recently, tumor induction by *A. tumefaciens* on the monocotyledonous plant *Asparagus officinalis* has been described (7). The failures previously reported were perhaps in part due to the fact that cell proliferation, a simple criterion for judging transformation, is much more difficult to obtain with monocots.

That at least transient transfer of T-DNA is sometimes possible can be observed by the temporary presence of T-DNA marker products such as nopaline or octopine (12).

The crown gall induction has now been obtained with representatives of several plant families, all from order Liliales (J. P. Hernalsteens, unpublished data). In the case of *Asparagus*, it can be shown by Southern blot that the T-DNA segment that becomes integrated is identical to the T-DNA fragment found in dicots.

It has also been possible to obtain kanamycin-resistant *Asparagus* tissue after a Ti plasmid-mediated transfer of a chimeric aminoglycoside phosphotransferase gene.

However, as was observed with several dicots (e.g., soybean) crown gall development in Liliales was not only cultivar-dependent but also very dependent on the type of tissue inoculated, on the growth conditions of the plant, and on unidentified factors, since often only some percentage of the inoculations proliferated.

Considering that the different steps involved in T-DNA activation, transfer, and integration are still unknown at the molecular level, it is not immediately clear how to design experiments that can overcome the low efficiency of transformation. Difficulties in the identification of the transformed cells form additional problems in appreciating the transformation efficiency. So, one has to cross the level of expression of dicot genes when introduced in different monocots. This can be done by introducing such genes as naked DNA into plant cells and monitoring their transient expression. By this method, it was shown that some T-DNA promoters such as the nopaline synthase (*nos*) promoter are expressed in maize (L. Herrera-Estrella, personal communication; J. Messing, unpublished data; V. Walbot, personal communication; and M. Lörz, personal communication).

Interestingly, naked DNA uptake can result in a stable transformation in the case of wheat cells (13). If this could be obtained with sufficient efficiency, it would be an important alternative to the Ti plasmid methods.

CAN *AGROBACTERIUM* TRANSFORM RICE?

Until now it is not possible to isolate rice cells that stably express a foreign gene, be it a chimeric antibiotic resistance gene or a gene conferring "hormone-independent" growth.

It is possible that transformation of rice is a rare event. So one has to consider the different steps involved and to control which events occur less efficiently with rice. We have already observed that the first event, the induction of the *vir* genes, occurs only at a very low level in the presence of rice cell exudates. External addition of a "*vir* region-inducing" compound resulted in full induction (R. Dekeyser and S. Stachel, personal communication). The transfer event could be followed by studying the transient expression of marker genes under the condition that the chimeric gene constructs presently available allow a sufficiently high level of expression. The latter can be tested by naked DNA transformation.

For the time being, it is very difficult to select for transformed rice cells. The background level of resistance shown by the untransformed rice cells makes it unlikely that any of the marker genes used until now will allow a distinction. Hence, the construction of new selectable marker genes becomes an urgent objective.

RICE GENE CLONING

The rapid progress in the development of Ti plasmid-derived gene vectors has resulted in an uncomfortable situation. It is possible efficiently to engineer new genes in a variety of dicots. However, the genes that could confer new traits have not been isolated.

To avoid such a situation with rice it is imperative that a concerted effort is made to identify and isolate such genes.

Concurrent with this, the cloning of tissue and developmentally controlled genes should be pursued. The isolation of the regulatory DNA segments that precede or flank the coding sequences will allow the construction of DNA cassettes. Such cassettes are needed for the controlled expression of the new genes. They should be available once the rice transformation and the consequent regeneration into fertile rice plants are worked out.

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IN VITRO CULTURE OF RICE AND DIRECT GENE TRANSFER TO CEREAL CELLS

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Prerequisites for the application of the new methods of genetic engineering and plant biotechnology for crop improvement are successful in *invitro* culture and plant regeneration. We studied the potential of immature embryo and other explant-derived cultures to form embryogenic callus and embryogenic suspensions from which plants can be regenerated. Single cells and protoplasts are considered the most promising tool for the genetic manipulation of plants. *Oryza sativa* L. cv. Yamabico was used to establish a fast growing cell suspension. Numerous parameters were studied to develop a procedure for the isolation and culture of protoplasts from this cell line. Protoplasts can be regenerated to the callus stage. Despite the limitation due to the lack of morphogenetic capacity, an established cell line provides suitable material to study DNA-mediated gene transfer into rice cells. Evidence for direct gene transfer into cultured cereal cells is presented. Transformation of *Triticum monococcum* protoplasts was achieved by using plasmids containing a selectable gene coding for kanamycin resistance. These experiments showed for the first time that plasmid constructions carrying a selectable marker gene, which have been used so far only for the transformation of dicotyledonous plant species, can also be used successfully for transformation of Gramineae species.

Plants can be regenerated from *in vitro* cultures via shoot and subsequent root morphogenesis or via somatic embryogenesis. Both regeneration systems lead to complete plants; however, somatic embryogenesis is the most direct way for regeneration from tissue culture. Embryogenic cultures of cereals have been obtained from immature embryos, young inflorescences, and young leaves (21). Embryogenic cultures are used to initiate cell suspensions, and consequently these cultures represent good material for the isolation of protoplasts. The long-term stability of the embryogenic character of the cell line, however, is not always guaranteed and is influenced by factors that are not easily manageable. The best and most reproducible results of somatic embryogenesis from cereals are obtained when immature embryos are used as the explant.

Plants of *Oryza sativa* cv. Yamabiko and cv. Taipei were grown as experimental materials in the greenhouse. Immature embryos were excised from milk stage kernels when the embryos had reached 0.8 to 1.2 mm.

The immature embryos were cultured, embryo axis downwards, on agar medium. The media used in our experiments were either based on MS, CC, or N6 medium and supplemented with different amounts of auxin - mostly 2,4-D — coconut water, and sucrose. Most suitable media were CC, MS-EE-I, MS-23, and N6-12 (Table 1). During cultivation at 26°C in the dark or dim light the scutellum proliferated and formed callus within 3–5 weeks. The developing callus was classified either nonembryogenic (translucent watery type, yellowish or brownish) or embryogenic (compact, white or yellow, smooth surfaced callus; compact globular structures). Only embryogenic type cultures were used for further subculturing and regeneration experiments.

Embryogenic callus was subcultured on CC or MS medium supplemented with 1 or 2 mg/litre 2,4-D and cultured in dim light at 26°C. Under these conditions the proliferation of somatic embryos continued over several subcultures, and the embryogenic capacity of the tissue could be maintained for many months. With each subculture, visual selection and physical separation of embryogenic tissue was made and nonembryogenic tissue discarded. Plant regeneration from embryogenic tissue was achieved by transferring morphogenic callus to MS-23K medium to allow proliferation of the somatic embryos and shoot and root formation. A further transfer to hormone-free MS medium allowed the development of complete plants, which were transferred to soil and grown to maturity. Rice plants can therefore be regenerated from mul-

Table 1. Media used for immature embryo, callus, suspension, and protoplast culture of rice.

Medium	Reference	Modifications or supplements
CC	17	none
DI	13	1 mg/litre Dicamba, 0.4 mg/litre thiamine HCl, 30 g/litre sucrose
MS-EE-1	13	1 mg/litre 2,4-D, 0.5 mg/litre thiamine HCl, 30 g/litre sucrose, 50 mg/litre coconut water
MS-23	13	1 mg/litre 2,4-D, 0.1 mg/litre thiamine HCl, 100 g/litre sucrose, 100 ml/litre coconut water
MS-23K	13	0.5 mg/litre Kinetin, 0.1 mg/litre thiamine HCl, 100 g/litre sucrose, 100 ml/litre coconut water
N6-1	3	2 mg/litre 2,4-D, 2 mg/litre glycine, 60 g/litre sucrose
N6-12	3	1 mg/litre 2,4-D, 2 mg/litre glycine, 20 g/litre sucrose, 0.5 g/litre caseinhydrolysate, 690 mg/litre proline
TC-2	13	2 mg/litre 2,4-D, 0.4 mg/litre thiamine HCl, 30 g/litre sucrose
TC-K1	13	1 mg/litre Kinetin, 30 g/litre sucrose

ticellular explants such as immature embryos following a defined protocol.

The potential of root meristems for somatic embryogenesis and plant regeneration was investigated. Root tip cultures were started in liquid TC-2 medium, and developing callus was subcultured and propagated thereafter on solid medium of the same composition. The first evidence for somatic embryogenesis and shoot formation was observed after transfer of white, compact callus material to D1 medium supplemented with Dicamba. Plantlets from root tips of *O. sativa* cv. Taipei were formed on TC-K1 medium about 8–12 weeks after culture initiation (J. Zimny, unpublished data).

EMBRYOGENIC AND NON-MORPHOGENIC CELL SUSPENSIONS

Immature embryo-derived callus exhibiting somatic embryogenesis and with obvious potential for plant regeneration was used to initiate cell suspension cultures. The material was subcultured into liquid media of various compositions for initiation of an embryogenic cell suspension. After several subcultures the initial callus material changed its morphological characteristics in liquid culture and obviously lost its embryogenic character. The resulting cell suspension was a fast growing and non-morphogenic cell line. The media finally used for culturing the rice suspension cells were CC and N6-1. The suspension cultures were kept on a gyratory shaker at 140 rpm at 27°C in the dark and were subcultured twice a week. Cell clumps consisting of 10 to about 100 cells showed rapid proliferation.

In more recent experiments, newly immature embryo-derived cultures were used again to initiate embryogenic rice suspension cultures that retain the potential for morphogenesis over a longer period of time. Two months after culture initiation, callus tissue exhibiting somatic embryogenesis was used to establish an embryogenic suspension. When suspension-derived cells were plated (1 month after suspension culture initiation) onto solid medium, small cell clumps proliferated and formed somatic embryos. Thus, it also appears possible to retain the embryogenic capacity of a rice cell line during subculture as a cell suspension. Further investigations will show how long these suspension cultures retain their embryogenic potential and whether mature plants can be regenerated after prolonged subculture. Additionally, these suspensions may be used as a source of material for the isolation of potentially totipotent protoplasts.

ISOLATION AND CULTURE OF PROTOPLASTS

Single cells and protoplasts are considered the most promising tools for genetic manipulation of plants (12). A necessity for the use of protoplasts in experimental approaches towards genetic modifications of plants is their successful regeneration into mature plants. In cereals, progress in somatic cell genetics is still limited by the lack of regenerative ability of protoplasts in culture. With the exception of a few reports describing plantlet regeneration, the culture of protoplasts has led in most cases only to callus formation, and until now no mature plants have been obtained from protoplasts (14). Thus, for most cereals nonmorphogenic cell lines remain the only accessible material to study protoplast isolation and culture procedures. Despite obvious limitations they offer a suitable system for initial experiments towards introducing foreign genetic material into cereal cells. Experiments on the isolation and culture of rice protoplasts have been published previously using either leaf base-derived or cell culture-derived protoplasts (1, 4, 5, 20, 22).

O. sativa cv. Yamabiko was used in our studies to optimize protoplast isolation and culture procedures (Fig. 1). For protoplast isolation, cells were harvested from a nonmorphogenic suspension 3–4 days after the last subculture and incubated in an enzyme solution containing 1% Cellulase Onozuka RS, 0.5% Macerozyme R 10, 0.05% Pectolyase Y-23, and 0.6 M mannitol (700 mOsm/kg H₂O). The cells were incubated at 27°C in the dark for 2–4 hours. At the end of the enzyme incubation the protoplasts were separated from the remaining cell clumps and undigested cells by filtering through stainless steel sieves (pore sizes 100–50–25–20 μm). The protoplasts were pelleted twice in seawater (adjusted to 700 mOsm/kg H₂O) by centrifugation for 3 min at 50 g to remove the enzyme solution and cell debris. Thereafter the cells were resuspended in regeneration medium, plated at a density of $1-5 \times 10^5$ cells/ml, and incubated at 27°C in the dark. The regeneration medium was CC supplemented with mannitol to obtain an osmotic value of 700 mOsm/kg H₂O. After 4–7 days in culture, up to 40% of the protoplasts reformed a new cell wall. The first dividing cells could be observed 7–10 days after culture initiation. A maximum of 1% of the initially plated protoplasts underwent sustained divisions and gave rise to colonies. No shoot formation was observed in the protoplast-derived callus material.

From the protoplast-derived calli new suspension cultures were initiated in CC and N6-1 media. Protoplast isolation and regeneration from cells of these suspensions were performed under the same conditions but with improved results (up to 5% plating efficiency).

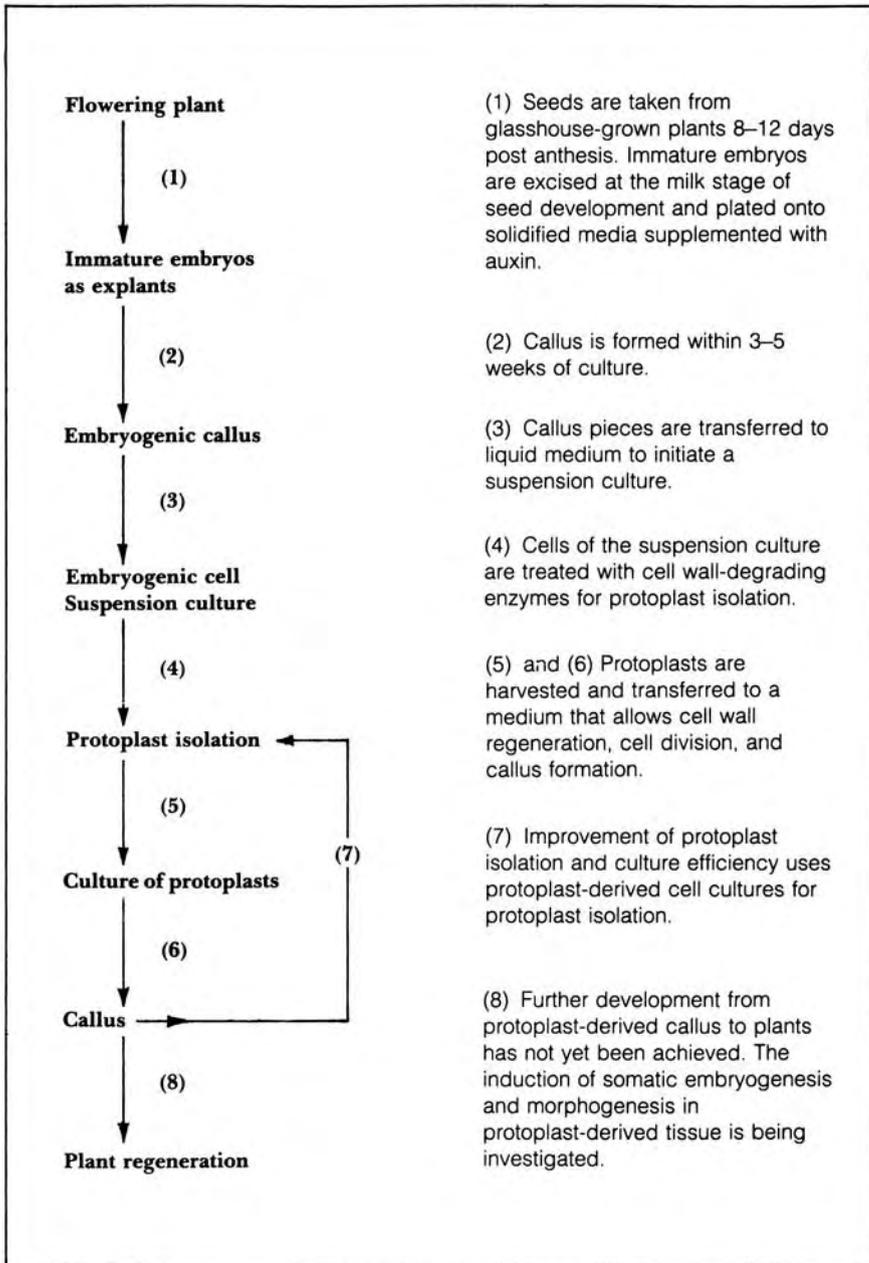


Fig. 1. Scheme of the major steps for the development of a rice protoplast culture system. At present special emphasis is given to the use of embryogenic tissue for the isolation of protoplasts that are expected to be regenerable to plants.

These conditions for tissue and protoplast culture of Yamabiko and Taipei are considered optimal at our present stage of experimentation, but we are aiming for still further improvements.

GENETIC MANIPULATION OF CEREAL CELLS

Different techniques for manipulating cells by protoplast fusion, indirect gene transfer, or direct gene transfer have been described, as follows:

- Somatic hybridization by protoplast fusion leading to
 - new combination of two genomes
 - new combination of nuclear genome and plastidal genome
- Transfer of fractions of the genome by
 - fusion of irradiated protoplasts
 - fusion with subprotoplasts (miniprotoplasts, cytoplasts)
 - transfer of isolated nuclei, chromosomes or plastids
- Transfer of isolated and defined genes by
 - *Agrobacterium* as vector
 - cauliflower mosaic virus as vector
 - DNA uptake into protoplasts or pollen
 - capillary microinjection of DNA into somatic cells, pollen, zygotes or ovaries

However, only a few results have been published concerning gramineous species. The major limitations are the low division response, the generally low plating efficiency of cereal protoplast systems, and the lack of efficient selection systems.

Ti-plasmids from *Agrobacterium tumefaciens* have been developed as useful gene vectors for dicotyledonous plants (8, 19). Transfer of recombinant T-DNA constructions into plant cells is accomplished by “natural methods” making use of *Agrobacterium* infection in planta or by cocultivation in vitro of plant cells with agrobacteria. More sophisticated methods are the fusion of bacterial spheroplasts with plant protoplasts, liposome-mediated delivery of DNA, direct uptake of DNA into protoplasts, or microinjection of DNA molecules into protoplasts. So far gramineous species have not yet been shown to be transformable by agrobacteria. New efforts, however, are being made to test a wide range of plant species for their susceptibility to *Agrobacterium*. It has been found that certain monocotyledonous species can be transformed with *A. tumefaciens* (7, 9). Of special interest for cereals are gene transfer techniques independent of agrobacteria, Ti-plasmids, and T-DNA sequences. New constructions are being developed with selectable marker genes and promoters derived from a plant viral genome (2) or with promoters

isolated from different plant genes (16). Such constructions can now be used in DNA-mediated transformation studies. DNA-mediated transformation of tobacco protoplasts was described first by Krens et al (10) with T-DNA, but more recently chimeric genes containing selectable markers have been used (6, 15) for tobacco transformation.

The first evidence for DNA-mediated transformation of cereal cells was achieved by transforming *Triticum monococcum* protoplasts with the plasmid pBL 1103-4 (11). The pBR322-derived plasmid contains a selectable chimeric gene comprising the protein region of the Tn5 aminoglycoside phosphotransferase type II gene (*NPT II*), the nopaline synthase promoter (*pNOS*), and the maize controlling element *Ac*. To induce DNA uptake, protoplasts isolated from a cell suspension of *T. monococcum* were incubated with plasmid DNA (10 µg plasmid DNA with or without 50 µg carrier DNA/1–2 × 10⁶ protoplasts). After an initial culture in liquid medium without selection, regenerating and dividing cells 4–7 days after culture initiation were plated into agar medium containing 100 µg/ml kanamycinsulfate. This level was found to be inhibitory for nontransformed *T. monococcum* cells. Selected colonies were tested for expression of the *NPT II* gene, and the enzyme activity was assayed. No activity was found in untreated callus tissue of *T. monococcum*. However, NPT II enzyme was found in protoplast-derived tissue treated with pBL 1103-4 DNA.

These results and the successful transformation of *Lolium multiflorum* protoplasts (18) indicate that vectors containing a functional promoter and a selectable gene allow the transfer of foreign genes into cereal cells.

CONCLUSIONS

The development of procedures to induce somatic embryogenesis in callus and suspension cultures opens up the possibility of regenerating very large numbers of rice plants from in vitro cultures. The occurrence of somaclonal variation offers the opportunity to search for new variants and mutants developing under in vitro conditions.

At present, many limitations remain for practical genetic manipulation of cereals in general and rice in particular. Reproducible and efficient protoplast culture and regeneration are possible to the callus stage only. Suitable vectors for gene transfer have been constructed, and successful DNA-mediated transformation of protoplasts from Gramineae species will stimulate further efforts to improve protoplast culture and regeneration conditions for all of the cereal crops.

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GENOMIC LIBRARY AND GENE TRANSFER SYSTEM IN *ORYZA* *SATIVA*

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This article outlines the experimental approach used in developing a genetic transformation system in *Oryza sativa*. It includes the construction of a genomic library in a phage lambda (EMBO-4) vector. Technology has been perfected for obtaining protoplasts and uptake of added donor DNA. Attempts to isolate certain selectable markers and to identify centromeric and telomeric DNA sequences that would enable the construction of artificial chromosomes in the future are presented.

Plant tissue culture has reached a state of sophistication that makes it possible to regenerate whole plants from calluses derived from protoplasts, haploid cells of anthers, or diploid somatic cells. In rice, success in this direction has been reported (12). However, the frequency of plant regeneration is low. Thus there is a need to carry out experiments involving gradients of hormones in tissue culture medium as in the case of soybean suspension cultures (4). Introduction of biotechnological methods at the tissue culture level and regeneration of modified plants would enable obtaining plants with resistance to certain diseases, to salinity, and to Al (18). Introduction of transposons like Tn-5 or the AC element of maize should provide selectable markers in a transformation system, identify certain development genes, and provide mutations. Some of the approaches I have taken during the last 6 months are presented below.

CELL CULTURE

Hulled rice seeds are sterilized and placed on R-2 medium supplemented with 0.8% agar. Calluses appear after 8–10 days and are transferred to liquid R-2 medium in spinner flasks or roller bottles. The R-2 medium formulated by Ohira et al (13) is very simple and suitable for growth of large amounts of suspension cultures. The suspension cultures are mixtures of different cells. By gradient fractionation and isolation of different types of cells by micromanipulator, it was clear that only small, compact,

dense cells are capable of good growth and callus formation. Calluses generated from single cell types are presently being checked for their ability to regenerate into plantlets. We preserve these cells in a medium of R-2 containing 5% dimethyl sulfoxide. When thawed, the growth of the rice cells resumes after a very short lag period of a few hours. Then the growth rate is identical to untreated cells, and their viability is about 60–65% of unfrozen cells, as observed by Sala et al (15). Organ redifferentiation and full plant formation from rice calluses have been reported (12) using the basal medium of Linsmayer and Skoog (8). In this case neither the optional constituents nor the cytokinins were added. Only 2–4 dichlorophenoxyacetic acid at 10 mM concentration was included. We have used this medium successfully, although the frequency of regeneration to plantlets was only 3% of the callus inoculated. Currently, haploid culture studies have been initiated by using anther culture techniques perfected at IRRI (21) using E-24, N-19, and G-4 media.

CONSTRUCTION OF GENOMIC LIBRARY OF RICE

The DNA content of rice diploid cells varies between 3 and 4 pg (11). A haploid genome contains 2 pg, which is 2×10^9 base pairs. In making a genomic library the probability of having any DNA sequence represented in the library (99% probability) can be calculated if one knows the average length of the DNA fragments obtained from the restriction enzyme digest of the genomic DNA (5). For an average fragment length of rice DNA of 2×10^4 base pairs one needs 4.6×10^5 recombinant phages to complete the library. We isolated high molecular weight DNA using the regimen of Taylor and Powell (19), which employs CTAB (cetyltrimethylammonium bromide). Using this protocol one can obtain about 50 μ g of DNA from 4 g of callus cells. Genomic DNA was then cleaved partially with Bam H1 (although other enzymes could also be used) and fractionated on a sucrose gradient. Each fraction was then checked for the DNA molecular weight by agarose gel electrophoresis. Fractions containing the desired length of DNA were pooled and cloned into the Bam H1 site of the lambda EMBL 4 vector (6). Thus far we have made the library for a japonica variety of rice only, but we plan to extend this for important varieties like IR-36 in collaboration with IRRI scientists.

ISOLATION OF PROTOPLASTS AND UPTAKE OF HETEROLOGOUS DNA

Actively dividing 1-2 week-old suspension cultures of rice in R-2 medium are pelleted and then incubated overnight with a sterile mixture containing 4% cellulysin, 0.6% pectinase, 0.2% hemicellulase, 0.2% potassium dextran sulphate, 1 mM CaCl_2 , and 0.8 M mannitol at pH 5.4 at room temperature. Usually the efficiency of protoplast formation depends on the age and type of the cells. The protoplasts are then washed with 0.6 M mannitol solution containing 1 mM CaCl_2 (9) and are resuspended in R-2 medium with 0.6 M mannitol along with naphthylene acetic acid (1 mg/liter) and kinetin (0.2 mg/liter) at a concentration of 1×10^6 protoplasts/ml. Transformation is performed according to Cassells and Cocker (3) using polyethylene glycol and CaCl_2 . In all our experiments we have used three linked marker genes from *Bacillus subtilis* that have been cloned into the plasmid pBR322 or phage lambda (20). These three genes control the biosynthesis of leucine. The identification of these genes is easy and very sensitive. After transformation the protoplast DNA is isolated and assayed on competent cells of a *B. subtilis* leucine auxotroph. These experiments can tell whether the DNA is taken up by the rice cell protoplasts and whether the donor DNA suffers nucleolytic cleavage inside the cell as observed by loss of biological activity and loss of linkage. By doing kinetic experiments we can also get information on how long the DNA stays in the cytoplasm and when it reaches the nucleus. Carrier DNA (rice DNA) at 100 $\mu\text{g/ml}$ and marker DNA at 200 $\mu\text{g/ml}$ are mixed and added to the protoplasts. A DNA $\text{Ca}_3(\text{PO}_4)_2$ precipitate forms in the tubes in about 30 m at 25°C. The tubes are shaken gently; clumping and precipitation of protoplasts occur. The next step is gradually to dilute the polyethylene glycol and simultaneously increase the Ca concentration by adding appropriately supplemented R-2 medium. Finally, the protoplasts are washed with R-2 medium containing 0.5 M sucrose and treated with DNase 1 to remove any free donor DNA. Results of this type of experiment show that at least 4-7 μg of DNA is taken up by 1×10^6 protoplasts. Thirty percent of the 4.5 kb DNA sequences that enter the cell do not suffer any loss of genes, at least up to 6 h after uptake. Most of the detectable biological activity is associated with the cytoplasmic fraction only in the initial 4 h. Nuclear fractions show transforming activity only after 9 h. The frequency is too low to quantify, and further experiments are in progress to increase the sensitivity of the system. Other markers

that are presently being tried in the rice transformation system are the transposon, TN 5, as has been done successfully in *Nicotiana* cells (7), and the cloned Activator (Ac) element from maize (6). In all the transformation experiments lack of selectable markers is a key problem. One of the markers we are presently selecting is for resistance to the drug aphidicolin, a tetracyclic diterpinoid obtained from *Cephalosporium aphidicola* and an inhibitor of replicative alpha DNA polymerase in animal cells. It has been shown by Sala et al (16) that rice cells contain a replicative DNA polymerase that is inhibited by the drug. Aphidicolin inhibits the incorporation of thymidine into the DNA of cultured rice cells. The inhibition is reversible upon removal of the drug. Experiments in vivo show that the drug at 15 mM concentration inhibits cellular DNA synthesis without any effect on RNA or protein synthesis. Selection of cell lines resistant to higher concentrations of the drug would enable the identification and isolation of the polymerase gene by the usual recombinant DNA technology.

ARTIFICIAL CONSTRUCTION OF RICE CHROMOSOMES

While the transformation approach is important, in the long run the coupling of artificial chromosomes with transformation of haploid culture either by microinjection or other means would have a far reaching impact on the improvement of cereal crops. Accordingly, our major effort will be focussed on this aspect and is outlined here.

This technology involves the artificial synthesis of minichromosomes and their addition to haploid cells either by microinjection or by packaging in a liposome or by the techniques outlined earlier for transformation. Most of our knowledge is derived from research done on yeast cells or simple protozoans like *Tetrahymena* (10). It is known from classical genetics that centromeres and telomeres are crucial for the meiotic and mitotic stability of chromosomes. A third element that has been identified is a sequence of DNA that gives the chromosome the ability to replicate autonomously (17). These sequences, called autonomously replicating sequences (ARSs), have been identified in the yeast genome. They permit the rapid replication of a plasmid and a high frequency of transformation. Using this assay of high frequency of transformation, several different ARSs have been identified in the genomic library of yeast. Yeast plasmids with ARSs are unstable unless they include a DNA sequence that contains the centromere. When the centromere sequence (CEN) is pre-

sent the number of copies of the plasmid goes down to one or two, but they are capable of proper segregation. The presence of a CEN in a plasmid can be assayed by the meiotic stability of the plasmid, checked by tetrad analysis. CENs in chromatin can be identified by nuclease digestion of the chromatin followed by fractionation of the digested product by electrophoresis on an agarose gel. It is known that the sequences of DNA contained in the chromosome corresponding to the centromeric regions are protected by proteins bound to these regions. For example, centromere DNA shows a very discrete pattern on the gel after micrococcal nuclease digestion and purification (2). Several centromeres have been isolated and analyzed for their structural composition. The centromeric region can be divided into three parts. Regions 1 and 3 are short stretches of 14 and 11 base pairs separated by a region of 82–89 base pairs of extremely AT rich DNA (83%). Thus the 120 base pair segments of DNA are the kinetochores of the yeast chromosomes. Plasmids that contain the ARSs, a selectable marker gene, and the CEN are able to function as minichromosomes.

Another important element that has been identified as a crucial part of chromosome stability and replication is the sequence of the telomeres. Telomeres were identified in classical genetics as the unique chromosomal terminal region. Biochemically these have been identified in the protozoan *Tetrahymena* and in yeast (1). The chromosomal location of telomeric sequences indicates that they are highly conserved and are homologous to each other. They are palindromic sequences present in multiple copies. Each end of a chromosome has tandem repeats of the hexanucleotide CCCCAA. Several single-stranded one-nucleotide gaps were found on the CCCCAA strand at specific positions within the repeats. More than 50 tandem repeats are present at the end of each chromosome, and the gaps are confined to the terminal 100 base pair region. Because of the repeats at the termini, these ends of the chromosome are able to form loops that have a specific role in the replication process (1). From several studies it is clear that most organisms have these conserved sequences in their telomeres and centromeres. We are using specific probes to identify similar sequences in the rice genome. If they can be identified we will then use them in a biological assay of plasmid stability in yeast cells. Recently, autonomous replication of plant genomic fragments has been reported in yeast cells (14). Therefore it is worthwhile to construct rice minichromosomes and then see whether the yeast cells will promote their replication.

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DISTANTLY RELATED HYBRIDIZATION AND GENETIC ENGINEERING OF CROPS

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Hybrids between distantly related plants are cytologically (number and size of chromosomes) and morphologically like the maternal plant, but certain inherited changes are noted in the phenotype. The hypothesis of DNA segment hybridization has been proposed to explain this phenomenon. In China, rice has frequently been used as the maternal plant. In the author's laboratory it was found that differences exist between rice-sorghum hybrids and the maternal rice plant both in esterase isograms and in repetitive sequences. A technique was developed for the introduction of exogenous DNA into distantly related species. The hybrids obtained frequently segregated and after a few generations became stabilized, very similar to what one usually observes in conventional crossing. Such variations have in fact occurred in nature among different varieties of rice as well as other plants. It seems that the mobile elements like repetitive sequences or transposable segments are responsible for such phenotypic variation. This information has been exploited for gene transformation and DNA recombination studies in crops.

Some 10 years ago, when I learned that the revolutionary techniques of genetic engineering had begun to occupy a dominant position in biological research, both pure and applied, I set about doing some work of this kind on plants. Areas of extreme utility like that of transferring N fixation properties to cereals are hard nuts that I did not aspire to crack, but I did hope to be able to do something to improve Chinese agriculture, especially in rice and cotton, to help her basic economy. It is difficult to conceive of a biochemist in that period attempting genetic engineering research on crops. However, I dived into the sea of agriculture hoping to find a starting point.

A PHENOMENON IN NEED OF EXPLANATION

Plant breeders and farmers throughout China had noted that offspring from conventional crosses between distantly related crop species (5, 12), e.g., rice with sorghum (9), maize, wheat, reed (8), grass, or even

bamboo (7), often exhibit phenotype variations that are inherited and stable through several generations. Some of the altered characteristics were very interesting, including resistance to disease, tolerance to drought, and higher yield than the maternal crop. However, these "hybrids" retained the morphological characteristics of the mother plant. Cytological examination with the light microscope of those stable hybridized offspring from rice and sorghum or maize showed that they were similar to the mother plant. Such observations were not explainable by the classical theory of breeding. One could ask: Are these real hybrids? Classical geneticists said "No." They were inclined to dismiss the point as a case of maternal mutation, maternal impurities, "dirty experimentation," or simply a lie. How could one believe in a so-called "hybrid" without the genetic characteristics carried by the pollen from a distantly related species? The people who did the crossing said "Yes," because the crossings were performed with their own hands. The facts were there, though the explanation had to wait. The debate continued from the 1950s through the 1970s with rising vigor and even to the present day. Meanwhile, the experimentalists continued with their experiments, producing more "hybrids" without being able to offer any explanation, while classical geneticists insisted on their viewpoint without carrying out experiments.

THE HYPOTHESIS OF DNA SEGMENT HYBRIDIZATION

Around 1974, it occurred to me that the dispute could be settled if looked upon from a molecular biology point of view (14). It was then already well known that fusion of human and mice cells in the absence of selection pressure results in the complete exclusion of human chromosomes from the progeny of the fused cell. There are only mice chromosomes; yet these are what remain of human genes. I put to myself the following question: Why is it not possible for a mother plant cell to harbor foreign DNA segments in "hybridization" between distantly related species? It seemed to me quite rational, in view of the human-mice fusion, to suppose that foreign DNA segments could be integrated after the pollen chromosomes have degraded. The DNA segments could be structural genes, regulators, repetitive sequences, transposable elements, or any other type. With the exception of structural genes, these segments might in some way affect gene expression and cause phenotypic alteration. Such integrations are below the chromosome level and are thus not easily detectable by simple conventional genetic methods.

Incompatibility grows as the evolutionary distance between organisms grows. However, it may not be farfetched to suppose that incompatible chromosomes should, after degradation (because of their incompatibility), give rise to segments that exhibit greater or less homology with the host cell genome, to such an extent as to be able to find sites for hybridization.

To test this hypothesis of DNA segment hybridization, we started to study the genetic relationships among rice, sorghum, and their hybrids (20). Sorghum and rice belong to different genera of the same order; therefore, genetically they are distantly related. Using rice as the maternal plant to cross with sorghum was a popular hybridization often done in China. I myself have seen six of these in different cities and provinces. They were performed with different lines of rice and sorghum, but the hybrids selected are fundamentally alike. Most of them have bigger spikes, more grain, and higher yield than the maternal rice. Some of them are drought tolerant.

It was shown that the sorghum pollen tubes do grow into the rice ovaries. In early generations, some erratic chromosomes were found in hybrid cells (2). They could have come from sorghum, or they could have grown under the influences of sorghum. But in later generations, especially in those stabilized "hybrids," the G bands of the chromosomes might change, and it was shown that the nucleolus organizer changed from one pair to two pairs of chromosomes (6, 19). It was also found that rice could have two pairs of chromosomes as the nucleolus organizer. Thus, although there are differences in morphology and cytology between rice and rice-sorghum hybrids, there remained a puzzle to be solved.

ESTERASE ISOGRAMS

We started to study the isozymes among hybrids and their parents, rice and sorghum. The material was supplied by S. Chen of the Institute of Crop Breeding and Cultivation, Chinese Academy of Agricultural Sciences, Beijing. We found in hybrids of F_{13-14} a band of esterase isozymes that we called No. II band of the isogram (15). It was present in one third of the hybrid embryos analyzed. This band was also present in the sorghum but not in the rice. This No. II band of the hybrids and the sorghum had the same reaction with different esterase inhibitors. Both were stained red with a mixture of alpha and beta naphthyl.

From thousands of different lines analyzed, it was found that the No. II band was also present in embryos of certain indicas, but not in japonicas.

We analyzed (unpublished data) further the different parts (e.g., each leaf, node, internode, root, spike, anther, ovary) and different developing stages (seed, seedlings, before three leaf stage, three leaf stage, tillering stage, earing stage, late milky stage) of typical japonicas and indicas individually. The isograms were typical of differences between two subspecies but also of those between different lines of japonicas or indicas. They were all genetically stable. By accounting additively for the esterase isozymes on all the isograms of a single line of japonica or indica, we found qualitatively that the number of the esterase could be detected on a one-dimensional PAGE. Esterase isozymes from both japonica and indica are the same; the No. II band was strongly expressed everywhere at any developing period of certain indicas. Some other indicas expressed it only in certain parts or at certain time. In a few indicas and all japonicas that we studied the No. II band was not easily found. The band was stained so weakly on some isograms of japonicas that it sometimes escaped our detection. Anyhow, we found the No. II band in the isograms of the maternal japonica rice several times. The weak No. II band was stained in red, as was that of the hybrids and the sorghum. There was a black band appearing at the same position of some isograms that also did not show in the embryonic isogram of japonica but was obviously present in other tissues both in japonicas and indicas. Therefore, the No. II strong red band of esterase isozyme in the rice-sorghum hybrid does not mean that a new gene has been transferred from sorghum. It may be that the sorghum DNA has influenced the gene expression of the rice. Duan and Chen (unpublished data) made two other rice-sorghum hybridizations in 1978 and 1982 on the basis of a stabilized hybrid from an old (1960) rice-sorghum hybridization. They followed the varied phenotypes of the esterase isograms in the first five generations. It was shown that the No. II band appeared in some offspring of the first generation, then segregated, but appeared most frequently in F_{2-3} , decreased in F_4 , and became steady in later generations. The No. II band could be lost in the offspring of one generation but could reappear in succeeding generations.

We may conclude that the appearance of the No. II band of the esterase isozyme in the rice-sorghum hybrid was due to alterations in gene expression. The same phenomenon has been shown in upland cotton after receiving exogenous DNA from sea island cotton. Some of the offspring changed their phenotypes continuously and dramatically. Some dominant traits that disappeared in one generation reappeared in succeeding generations. It is well known to experimental breeders in Russia and China that "mad segregation" occurs in some offspring of interspecies or intergenus hybridization of plants. Those hybrids segregate

continuously and will stabilize only after many generations. Their phenotypes change dramatically. It seems that "mad segregation" very much resembles the phenotypic variations observable after introducing the transposable element—P factors—into *Drosophila* (1,11).

It might be that in distantly related hybridization certain foreign DNA segments cause disturbances of gene expression of the maternal genome.

REPETITIVE SEQUENCES

From the study of genomic organization of rice (10), we found that 10% are foldback sequences, 15.6% highly repetitive, 27.8% middle repetitive, and 46% single copy sequences. The data we have obtained are similar to those of Deshpande and Ranjekar (3), only they did not separate the foldback part from fractions with Cot smaller than 1.0.

From kinetic reassociation studies (18) of the repetitive sequences, we found 16% homology between sorghum and rice in rapidly reassociated sequences. These 16% homologous sequences may provide a molecular basis for possible hybridization between these two species. With homologous sequences, heterogenous sequences of sorghum might be attracted to hybridize to the rice genome. We used an immobilized rice DNA column to eliminate the major part of the homologous sequences from the sorghum. The latter was used as a probe to hybridize the hybrid, rice, and sorghum genomic DNA. The ratio of the time required for half of the ¹²⁵I-labelled probe DNA to reanneal in the presence of a large excess of rice DNA ($t_{\frac{1}{2}}$ p) and sorghum-rice DNA ($t_{\frac{1}{2}}$ p) was studied. It was found that $t_{\frac{1}{2}}$ p/ $t_{\frac{1}{2}}$ p is greater than unity. Therefore, the DNA sequence homologous to the sorghum probe DNA present in the hybrid was more than that present in the rice genome. Membrane DNA molecular hybridization data (unpublished) indicated the same results for rapidly reassociated sequences. The difference in this repetitive sequence between rice and the hybrids was located in the middle repetitive sequence region, which was shown by comparing the Cot curves. It was also found that different varieties of rice, japonicas or indicas, are different in their repetitive sequences. They are different but genetically stable. That is also true for the different stabilized rice-sorghum hybrids.

It seems that the repetitive sequences including the transposable elements might in some way affect and create changes in phenotype through gene expression.

We have not yet found differences in structural genes transformed. If there are any, it will require a long time for us to find them. The

repetitive sequences, from the point of view of a great number of copies, would not only have more chance to be hybridized but would also be more easily detected.

Thus, differences have been found in plant phenotype, G bands, number of chromosome pairs in the nucleolus organizer, esterase isograms, as well as repetitive sequences between the maternal rice and its hybrids with sorghum. But similar differences could be found between different varieties of rice. There are some 10,000 different varieties of rice with different phenotypes or traits. How were they formed? The answer is: through mutation, hybridization, and selection. The mutation of the primary structure of DNA must be the basic event for changing phenotype. Phenotypes are governed by genes and gene expression. Natural mutation is low; therefore the gene evolution of gene structure is low. But the phenotype varied rapidly. There must be another event playing its part in phenotypic variation. Genetic rearrangement by mobile elements (repetitive sequences and transposable elements) that influence gene expression directly or indirectly should be considered. The tremendous variations of the rice phenotype as well as those of rice-sorghum hybrids might be the result of genomic rearrangement after mutation or hybridization, especially after introducing distantly related noncoding DNA sequences such as mobile elements.

HYBRIDIZATION OF EXOGENOUS DNA SEGMENTS FROM DISTANTLY RELATED PLANTS

Another way to prove the hypothesis of DNA segment hybridization is to put exogenous DNA segments from a distantly related plant into a receptor plant and produce hybridized offspring. This work was begun in 1978. The first problem was how to introduce exogenous DNA into a plant. It was then that the fertilizing process among distantly related plants came to mind. Could one imitate this process? With DNA segments, how might seeds be obtained without intact pollen chromosomes? It was the "dirty" experiments in the field that aroused my great interest; when there is contamination by pollen other than that under consideration, the experiment is considered a "dirty" one. Male sterilization done in the field, as is well known, is hardly complete. Selfing might take place during pollination with foreign pollen, of which the genome after degradation might yield DNA segments and might integrate into the maternal genome. Thus, a simple technique was formulated for the introduction of

exogenous DNA into rice, wheat, and cotton. The principle of this technique is: After selfing, the exogenous DNA is injected into the ovary or put onto the stigma under conditions such that the DNA may pass through the pollen tube to reach the embryonic sac and transform the egg or zygote or early embryonic cells. We succeeded in the attempt. When we worked with cereals, the transformation frequency was 10^{-3} to 10^{-4} judging from the phenotypic variations in offspring after treatment with exogenous DNA. With cotton we got 10^{-1} to 10^{-2} . Recently, Duan and Chen reported they got 10^{-1} to 10^{-2} for rice by treating the exogenous DNA at a precise time in the fertilization process (unpublished). Thus the technique is practical, both for big flower plants like cotton and small flower plants like rice.

The size of the segment of exogenous DNA is usually 10^6 to 10^7 daltons. The DNA of sorghum and *Spartine angelica* co.o. Hubbard was introduced into rice. The phenotypic variations were similar to those of conventional crossing. Some of the offspring were stable even in their first generation; some of them varied greatly. Obviously some of the exogenous DNA segments of the distantly related sorghum were taken up by the rice genome. The segments of DNA that could enter the cell may do so by a random process, but the segments that have integrated into the receptor genome and have been maintained in the stabilized hybrids may not be random. This should be determined by the structure and function of those DNA segments. So, it is not surprising to have similar results in exogenous total DNA introduction to that of hybridization in conventional crossing of distantly related species.

The hypothesis of DNA segment hybridization of distantly related plants is only preliminary, but it gives rise to some new ideas to investigate.

GENETIC ENGINEERING

DNA segment hybridization between distantly related species may be considered as a case of "natural genetic engineering." This idea was a spur to me to try to imitate the process and bridge the gap between natural and experimental genetic engineering. The success of such an attempt would enable us to overcome the experimental difficulties that one encounters in plant genetic engineering.

The techniques for introducing exogenous DNA into plants (17) mentioned above are of definite advantages:

- The introduction of exogenous DNA in or on the ovary soon after selfing may cause the DNA to leak through the pollen tubes, enter the embryonic sac, and transform the fertilized cell.
- There is no or less cell wall problem for transformation.
- Seeds are usually obtained directly; these would give rise to the next generation without applying cell culture and cell screening processes.
- The cultivated plants are manipulated directly and the phenotype with the most desirable properties selected.
- The technique is simple and easily disseminated among growers.

In plant genetic engineering, the proper strategy for the construction of integratable DNA recombinants is critical. From studies on hybridization among remotely related plant species, repetitive sequences vary with genomic structure. These sequences, including transposable elements, may play an essential role in genome rearrangement that may well affect gene expression.

From our work on introduction of exogenous DNA into cotton, the following were observed in their offspring (16):

- With introduction of self-DNA, no phenotypic alterations have been observed in their offspring.
- Few phenotypic changes occur with interspecies DNA. Wilt resistance is, however, easily transferred.
- With interspecies DNA, great variations may occur in some of the offspring.
- With intergeneric DNA, few changes were observed similar to those that occur in hybridizations between remotely related species.

The offspring is like the mother plant.

It is quite probable that exogenous DNA does not integrate with the maternal genome in a random way. Homologous sequences may recognize and find their way to binding sites without affecting the phenotype, while heterologous sequences may not find a proper site to sit on. With interspecies DNA, homologous sequences might undergo mutation but still retain original binding properties to make integration possible, while expressing phenotypic alterations due to heterogeneity in genomic structure.

For genetic engineering to work with higher organisms, the question that has always been raised is how one should transfer a gene so as to enable it to integrate at the correct site of the receptor genome, have it expressed at the correct time, and maintain it stably. It may be possible for us to transfer a segment with an overall different sequence but containing a definite sequence from the receptor genome that may serve

as a vector to bring the segment to the correct size. Attempts are being made to realize some such scheme (12). Recently, using M_p7 clones of repetitive sequences from cotton, it was demonstrated that these sequences appeared in the genome of the cotton seeds.

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STRUCTURE OF RICE CHLOROPLAST DNA*

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Homogenizing rice leaves in liquid N made possible isolation of intact chloroplasts, and chloroplast DNA was isolated from purified chloroplasts. The DNA was digested by several restriction enzymes and fractionated by agarose gel electrophoresis. The results showed that the DNA was pure chloroplast DNA, the size of which was estimated to be 130 kb by the total length of restriction fragments. A physical map of fragments generated by Sal I, Pst I, and Pvu II was constructed. The loci of genes for the large subunit of Rubisco, the 32 KD protein of the photosystem II reaction center, and cytochrome f were determined by using labeled tobacco DNA fragments containing these genes. Rice chloroplast DNA fragments generated by Bam HI and Pst I were cloned in plasmids for studying the precise structure of the DNA.

Like other higher plant cells, a rice cell contains its genetic information separately in its nucleus, chloroplasts, and mitochondria. However, most rice geneticists have concentrated their interest on nuclear genomes, with relatively little attention being paid to chloroplast genomes. Chloroplasts are important energy-transducing organelles. They contain the entire components necessary for photosynthesis, as well as the expression of their genetic information. Moreover, recent progress in molecular biology had established that many genes important for photosynthesis and gene expression in chloroplasts are encoded in chloroplast DNA (ctDNA) itself.

Attempts to modify chloroplast genomes for increasing photosynthetic activity as a part of plant breeding are in progress using cell fusion techniques (1) and searching vectors for ctDNA (9). However, studies of the structure and gene expression of chloroplast genomes are equally important for this purpose.

Since the physical map of restriction enzyme fragments in maize ctDNA was first constructed (2), physical maps of spinach (6), tobacco (5), wheat (4), and soybean (12) have been reported. Thus, physical maps of ctDNA are available for all of the major crops except rice. Because of the accumulation of hard, amorphous silica gel in epidermal cells, rice leaves have been difficult to homogenize in a condition for

maintaining chloroplasts and nuclei intact. We have now overcome this difficulty by homogenizing rice leaves in liquid N and have isolated rice ctDNA from intact chloroplasts (7).

We will present the isolation method, the restriction patterns, the physical map of rice ctDNA, the location of three genes, and the cloning of the ctDNA fragments.

ISOLATION OF RICE CHLOROPLAST DNA

Rice plants (*Oryza sativa* L.) were grown in a growth chamber at 28°C under fluorescent lamp illumination. Leaves were briefly blended in liquid N. The method of Saltz and Beckman (10) for *Petunia* and *Nicotiana* ctDNA preparation was modified and used. After the liquid N was evaporated, the leaf powder was suspended in Kool's buffer A (3) containing 0.1% bovine serum albumin. The suspension was filtered through two layers of gauze and two layers of Miracloth. The filtrate was centrifuged for 10 min at 1,000 g. The pellet was resuspended in Kool's buffer A; loaded on top of a stepwise 20-45-60% sucrose gradient in 50 mM tris-HCl (pH 8.0), 0.3 M sorbitol, and 7 mM EDTA; and centrifuged for 30 min at 2,000 g. The band at the interphase between 20% and 45% sucrose was collected, diluted 1:3 with Kool's buffer B (3), and centrifuged for 10 min at 3,000 g. The chloroplast pellet was resuspended in Kool's buffer B, and the chloroplasts were lysed by adding sodium dodecylsarcosinate at a final concentration of 3%. One twentieth volume of 10 mg/ml pronase E was added to the solution, and the mixture was incubated overnight at 37°C. DNA was extracted twice from the lysate with phenol and once with phenol-chloroform (1:1). The DNA was precipitated from the aqueous phase to which 0.1 volume of 3 M sodium acetate had been added with 2.5 volumes of ethanol. The precipitate was washed twice with 70% ethanol and dissolved in water.

Although the yield of the DNA was low, about 10 µg/200 g fresh weight of leaves, the ctDNA extracted by this method was completely digested by restriction enzymes without any further purification such as CsCl-EtBr density gradient centrifugation. The DNA fragments were also inserted into plasmids as described later. Therefore, rice ctDNA prepared here is suitable for molecular biology experiments.

RESTRICTION ENZYME PATTERNS OF RICE ctDNA

Restriction endonuclease analysis was used to examine the purity and to determine the size of the DNA. Rice ctDNA was first analyzed by Eco RI, Bam HI, and Hind III. Although the pattern shown in Figure 1 indicates that the ctDNA was pure, these enzymes generated a large number of fragments ranging from about 20 to 0.5 kb or less. For example, Bam HI generated no less than 30 fragments. They were not suitable for determining the total size of fragments, i.e., the molecular size of rice ctDNA.

Sal I, Pst I, and Pvu II were then used, and they generated 8, 14, and 9 fragments, respectively, as shown in Figure 1. Since the number and size of the fragments digested by the second group of three enzymes were suitable for determining the size of the DNA and for constructing a physical map of the DNA, we also analyzed the sizes of fragments produced by double digestion among the three enzymes. The sizes of each of the fragments are given in Table 1. The total size of fragments generated by each of the three enzymes was 130 kb; therefore the size of rice ctDNA was determined to be 130 kb.

The size of rice ctDNA is similar to those of other monocot ctDNAs from maize (8) and wheat (4). However, monocot ctDNA is consistently smaller than dicot ctDNA from tobacco (5), spinach (6), and soybean (12). It would be interesting to determine what parts of dicot ctDNA are missing in monocot ctDNA.

CONSTRUCTION OF A PHYSICAL MAP

It was not possible to construct a physical map by the double digestion data alone because there were too many fragments for it, even with digestion by a second group of restriction enzymes. We therefore hybridized nick-translated Bam-2 fragments from tobacco ctDNA with rice ctDNA fragments. In tobacco ctDNA, the Bam-2 fragments are known to occupy most of the short single copy region between two inverted repeats (14). The rice fragments that hybridized to tobacco Bam-2 fragments were tentatively assumed to be located in a short single copy region.

In order to get more information, we cloned the rice Pst-8, Pst-11, Pst-13, and Pst-14 fragments to pUC 8, and the recombinant plasmids were digested by Pst I or Pst I plus other enzymes. The restriction sites of the plasmid inserted Pst I fragments are shown in Figure 2. Finally, we labeled the cloned DNA and hybridized it to restriction fragments from

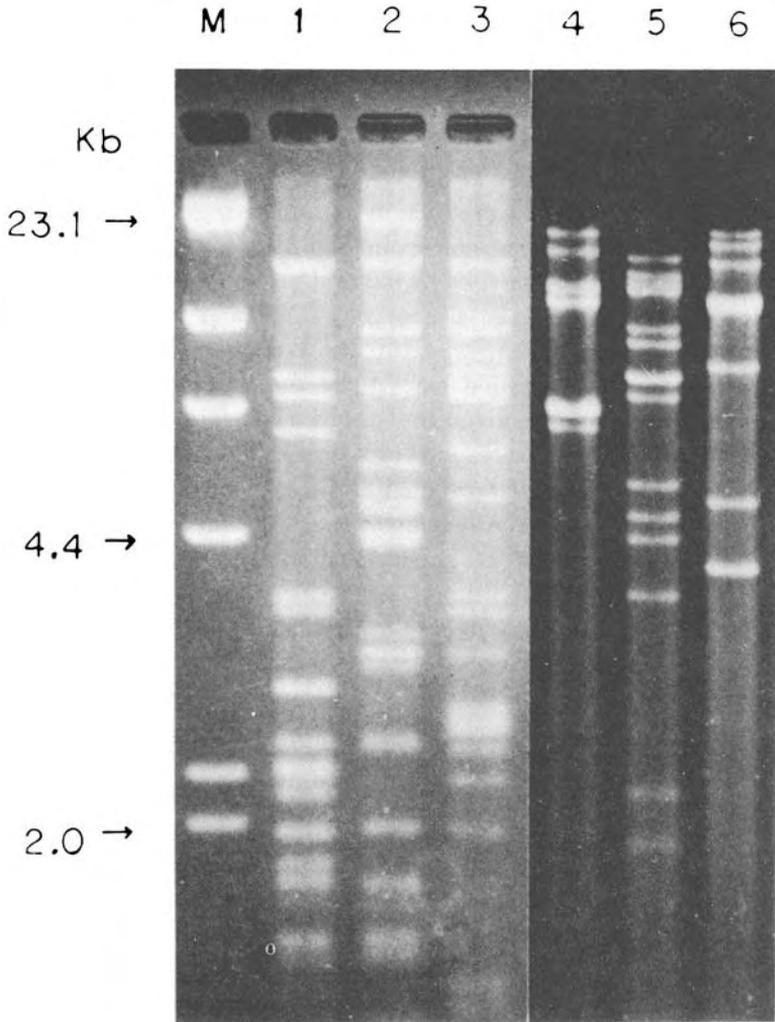


Fig. 1. Restriction fragment patterns of rice ctDNA generated by digestion with Eco RI (1), Bam HI (2), Hind III (3), Sal I (4), Pst I (5), and Pvu II (6). M = lambda DNA digested by Hind III.

Table 1. Size in kb of restriction fragments from rice chloroplast DNA.

No.	Sal I	Pst I	Pvu II	Sal-Pst	Pst-Pvu	Pvu-Sal
1	27.9	19.1	28.0	19.1	19.1	22.6
2	20.8	15.7	22.6	14.6	15.7	12.5
3	14.3	14.6	18.1	11.8	10.0	11.6
4	13.8	13.8	13.0×2	7.8	9.1	9.4
5	12.6×2	10.6	12.7	7.2×2	7.4×2	8.3
6	7.4	10.0	8.8	7.1×2	7.0	8.2×2
7	7.2×2	8.3×2	5.2	6.8	6.2	6.8
8	6.8	7.6	4.2×2	5.5	5.6×2	5.6
9		5.5	1.0	5.0	5.2	4.8×2
10		5.0		4.8	5.0	4.6
11		4.6		4.6	4.0	4.4×2
12		3.8		3.8	3.9	4.3
13		2.1		3.5	2.7×2	2.4×2
14		1.7		2.8	2.3×2	1.8×2
15				2.1×2	1.7×2	1.0
16				2.0	1.5×2	0.8
17				1.7×2	1.0×2	
18				0.8×2	0.6	
19				0.3×2	0.4	
Total	130.6	130.7	130.8	130.5	130.6	130.7

rice ctDNA. These data were analyzed together with double digestion data, and the physical map shown in Figure 3 was constructed.

The length of inverted repeats in rice ctDNA was estimated to be not less than 17.4 kb. However, it could be extended 1 kb in the large single copy region and 4 kb in the short single copy region so that the inverted repeat might be a maximum of 22 kb. This size would correspond to the 21.0 kb inverted repeat of wheat ctDNA (4) and the 22.5 kb inverted repeat of maize ctDNA (8).

THE LOCATION OF GENES IN RICE ctDNA

The cloned fragments containing the tobacco large subunit (*LS*) gene of Rubisco, the 32 KD of photosystem II reaction center gene (*P32*), and *cytochrome f* gene were labeled and hybridized to rice ctDNA fragments (data not shown). The tobacco DNA containing the *LS* gene (11) hybridized mainly with the Pst-14 fragment of 1.7 kb, showing that most of the *LS* gene of Rubisco is contained in the Pst-14 fragments. The tobacco DNA fragments containing the *P32* gene (13) hybridized to

Pst-11, Pst-6, Pvu-5, and Pvu-1 fragments. Rice Pst-1 and Pvu-2 fragments hybridized with the tobacco DNA containing the *cytochrome f* gene. Therefore the rice *cytochrome f* gene locates somewhere in the Pst-1 fragment.

CLOWING OF ctDNA FRAGMENTS

At first we tried to clone all of the fragments of rice ctDNA generated by digestion with Pst I to plasmid pUC 8. This plasmid vector contains the multiple cloning site of M13mp7 in a segment of *Escherichia coli* lac operon that is a selectable marker (15). Four fragments shown in Figure 2 were cloned to pUC 8 repeatedly. However, other fragments could not be

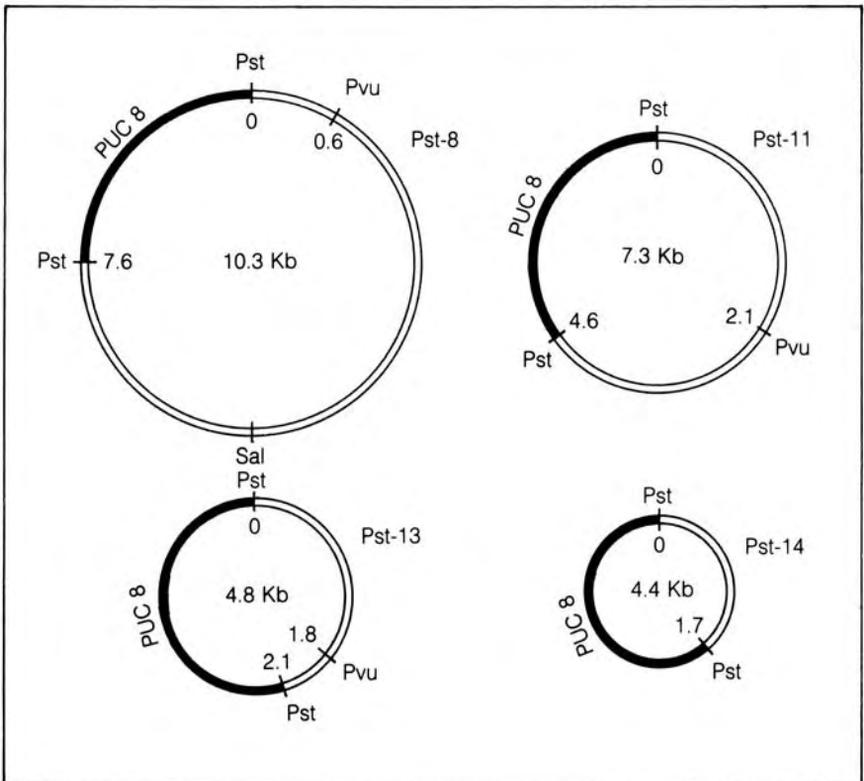


Fig. 2. Physical maps of recombinant DNAs that inserted Pst-8, Pst-11, Pst-13, and Pst-14 fragments (open bar) of rice ctDNA into plasmid pUC 8 (solid bar).

cloned to the plasmid in our laboratory. Therefore we changed the cloning vector and cloned Bam HI and Pst I fragments to pBR 322. Some of the cloned fragments are shown in Figure 4. These fragments are identified as a part of ctDNA and not contaminated nuclear DNA fragments by their sizes after restriction enzyme treatment and by the fact that they hybridize with labeled rice ctDNA.

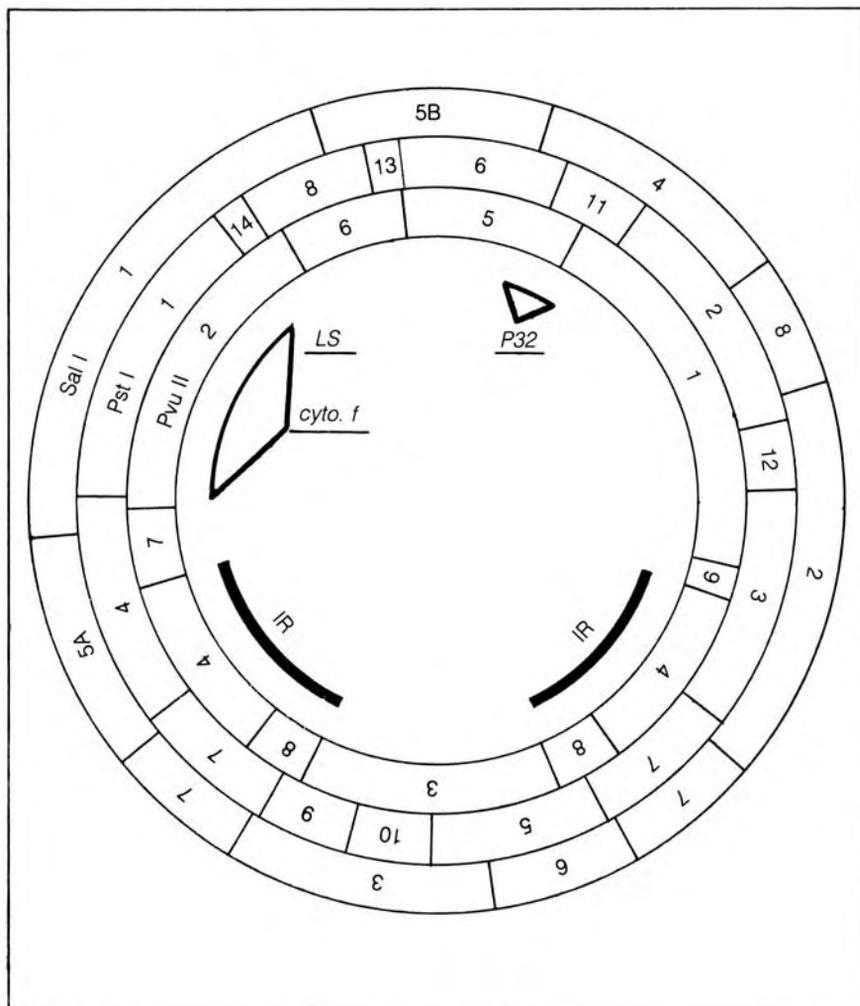


Fig. 3. Circular Sal I + Pst I + Pvu II physical map of rice ctDNA showing the inverted repeats (IR) and the location of the Rubisco large subunit (LS), the 32 KD protein gene (P32), and *cytochrome f* gene (*cyto. f*).

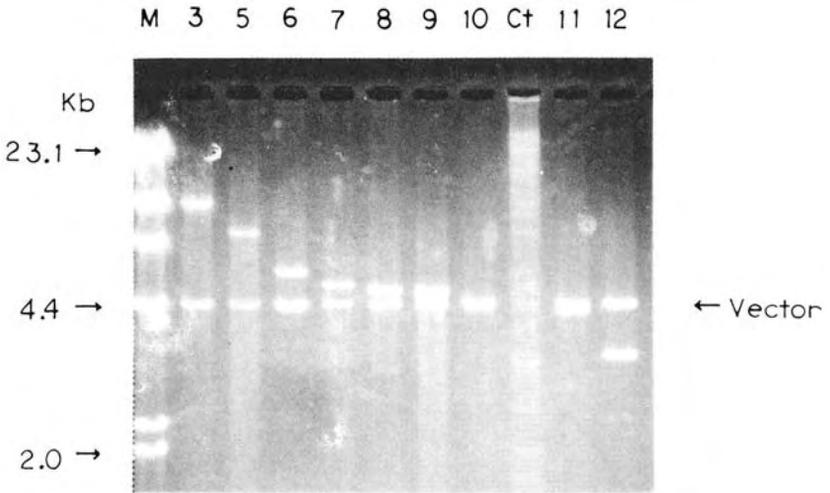


Fig. 4. Restriction fragment patterns of recombinant DNA inserted Bam HI fragments of rice ctDNA (3,5,,,12) and total rice ctDNA (Ct) generated by digestion with Bam HI. 3,5,,,12 = Recombinant DNA inserted Bam-3, Bam-5, . . . Bam-12 fragments, respectively.

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GENETIC ANALYSIS OF RICE STORAGE PROTEINS

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The biosynthesis mechanisms for the storage proteins in rice endosperm were studied using electron microscopy, an in vivo protein synthesizing system, and an in vitro protein synthesizing system. The results indicate that prolamin and glutelin are synthesized by membrane-bound polysomes and that both proteins are formed via pro- or prepolypeptides. Prolamin comes from preprolamins and has a signal peptide 2,000 d larger than mature prolamin; glutelin comes from a preproglutelin, the entire size of which is about 60 kd. After the signal peptide is removed from the preproglutelin, the proglutelin cleaves to form mature 22–23 and 37–39 kd glutelin subunits. mRNA for preproglutelin was purified and then reverse transcribed into double-stranded cDNA. The cDNA was cloned in *Escherichia coli* using pBR322 and Okayama-Berg vector primer. The cloned cDNA thus obtained arrested the formation of preproglutelin in the in vitro protein synthesizing system. Isolation of genomic sequences for glutelin was also attempted after a genomic library was constructed using the EMBL-3-system.

The final goal of our work is to find a means to improve the quality and quantity of the storage proteins in rice endosperm by genetic manipulation. To reach this goal, we began by isolating glutelin and prolamin genes and by analyzing their structure in order ultimately to clarify the control mechanisms involved in the expression of these genes. We are currently engaged in the following experiments.

- To clarify the biosynthesis mechanisms for the storage proteins in rice endosperm. For this purpose we are using the wheat germ and rabbit reticulocyte lysate system and the microinjection technique using frog oocytes.
- To construct a cDNA library for the mRNAs appearing in the ripening rice endosperms.
- To construct a genomic library for rice.

BIOSYNTHESIS AND DEPOSITION OF RICE STORAGE PROTEINS

The average protein content of milled rice is about 10% of the total kernel weight, though this differs among rice varieties (5). The most abundant rice storage protein is glutelin, which accounts for about 80% of the total protein in the starchy endosperm (4). This protein has two groups of polypeptides with Mws of 22–23 and 37–39 kD (9). Prolamin (alcohol soluble protein), and globulin (salt soluble protein) are rather minor components. The former consists of three major polypeptides with molecular weights of 10,000, 13,000, and 16,000. They are found in the protein bodies in the starchy endosperm.

Tanaka et al (10) have made a detailed study of the localization of rice storage proteins in the starchy endosperm. They isolated two types of protein bodies from developing starchy endosperm (Fig. 1a) and analyzed the polypeptide composition. It was concluded that the two kinds of protein bodies, PB types I (PB-I) and II (PB-II), are different in shape, density, and protein composition. PB-I is a small, concentric ring-structured protein body (Fig. 1b), containing mainly prolamin. PB-II is a large, uniformly stained protein body (Fig. 1c), mainly storing glutelin with a small amount of globulin. The former is described by Bechtel and Juliano (1) as a large, spherical PB and the latter as crystalline PB. To date this is the only report of protein body polymorphism in the starchy endosperm of a cereal protein body.

PB-I and PB-II are apparently membrane bound. In the former the ribosomes remain attached to the single membrane in developing starchy endosperm cells (Fig. 1b). In the case of PB-II, however, no ribosomes were observed in the periphery throughout rice development (Fig. 1c). Thus, it is assumed that PB-I originates from the ER membrane, where the polypeptides of prolamin are synthesized and cotranslationally discharged inside of the membrane (Fig. 2a), and that PB-II originates from the large vacuole, polypeptides of glutelin being synthesized by ER-bound polysomes and then transported and deposited into the vacuoles (Fig. 2b).

Yamagata et al (13) demonstrated that two polypeptides of molecular weight 57 kD and 13 kD are strongly labelled when developing starchy endosperm is treated with ^{14}C -amino acid and then analyzed by SDS-PAGE fractionation. In pulse-chase experiments, the 57 kD polypeptide gradually disappeared, and 22–23 kD and 37–39 kD polypeptides concomitantly formed. The ^{14}C label in the 13 kD polypeptide (prolamin) remained unchanged. The 57 kD polypeptide was reported to be a salt soluble protein and the 13 kD one was an alcohol soluble protein (12). It

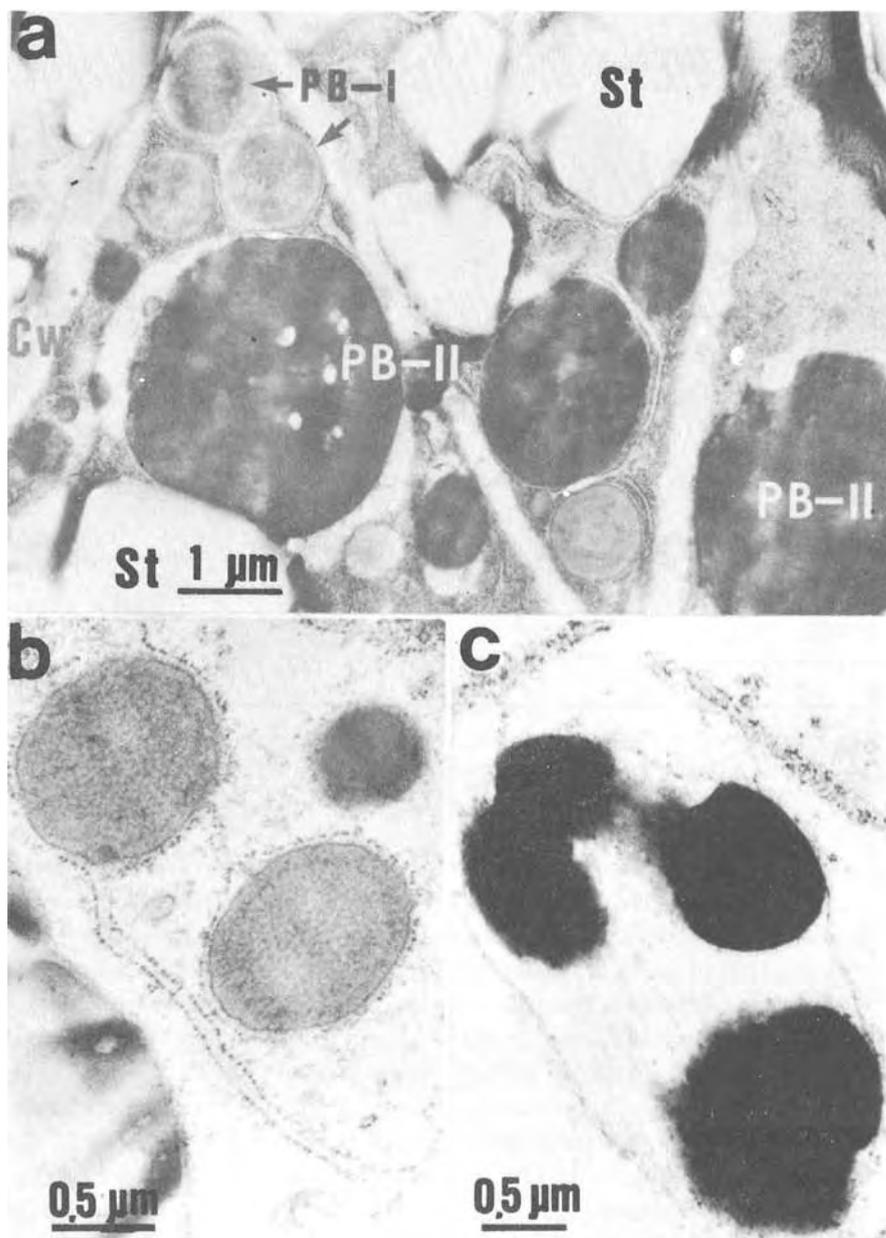


Fig. 1. Electromicrographs of protein bodies in the starchy endosperm. (a) The starchy endosperm cells in a developing rice grain 16 days after flowering. PB-I = protein body Type I, PB-II = protein body Type II, St = starchy grain. (b) Protein body Type I in the starchy endosperm 7 days after flowering, (c) Protein body Type II in the starchy endosperm 7 days after flowering.

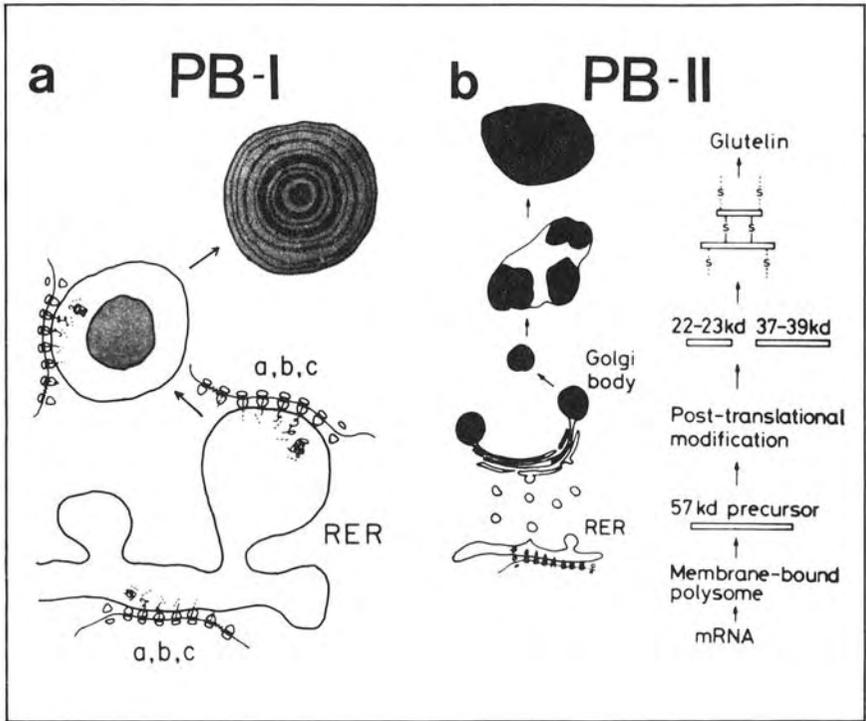


Fig. 2. Schematics of protein body formation systems. (a) Formation system of protein body Type I (PB-I). a, b, c = different prolamin species. (b) Formation system of protein body Type II (PB-II).

was concluded that two subunit groups of glutelin are formed through the post-translational cleavage of the 57 kD polypeptide and also that the prolamin polypeptide did not undergo post-translational cleavage.

A fraction of poly-(A) containing RNA isolated from developing rice grains was added to a cell free translation system prepared from wheat germ (11) and reticulocyte. In the wheat germ system, the major products synthesized *in vitro* were polypeptides of 10, 13, and 16 kD, which correspond to the prolamin protein (Fig. 3a). These polypeptides have relatively higher molecular weights than the polypeptides of the prolamin protein in PB-I. The subunits of glutelin were not detected in any translation system. In the reticulocyte lysate system the major product synthesized was a polypeptide with a larger molecular size than the 57 kD polypeptide that had been observed in *in vivo* labeling experiments (Fig. 3b).

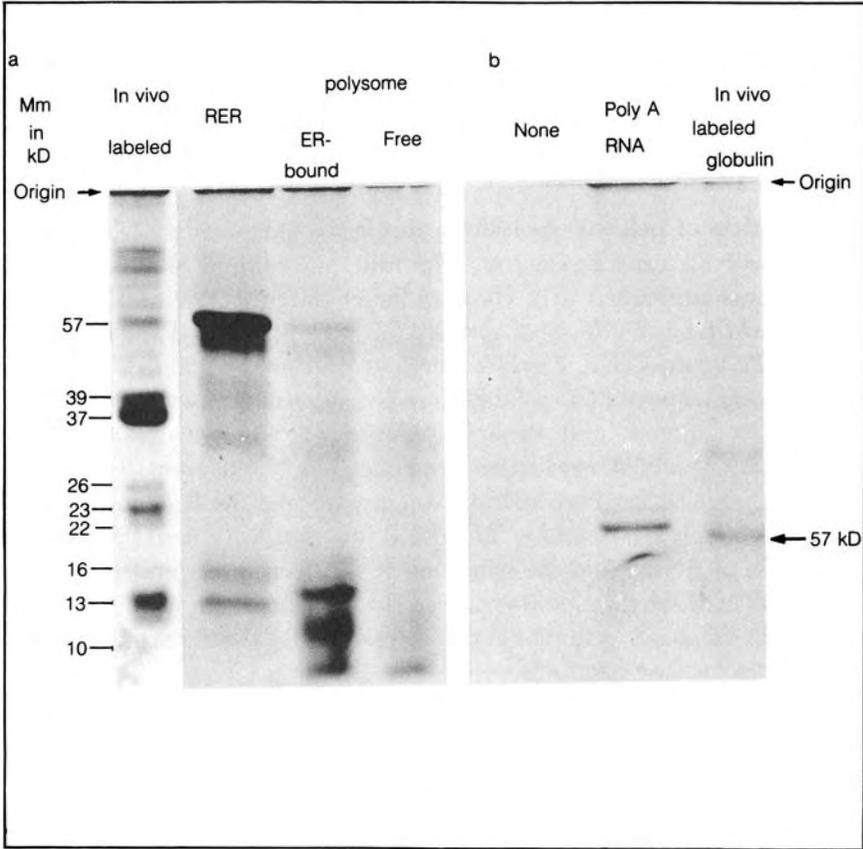


Fig 3. SDS-PAGE analysis of polypeptides synthesized by in vivo and cell free system. A fluorograph. (a) In vitro synthesized polypeptides by wheat germ translation system. In vivo labeled = total proteins extracted by urea-SDS containing buffer from ¹⁴C-leucine-labeled developing seeds, RER = translation products by isolated rough endoplasmic reticulum, ER-bound polysome = translation products of isolated mRNA from endoplasmic reticulum-bound polysomes, free polysome = translation products of isolated mRNA from free polysomes. (b) In vitro synthesized polypeptides by reticulocyte lysate system. None = no addition of RNA, poly A⁺ RNA = translation products by isolated poly A⁺ RNA obtained from ripening rice endosperms, in vivo labeled globulin = protein extracted by 0.5 M NaCl containing buffer from ¹⁴C-leucine labeled developing seeds.

In vitro protein synthesis studies have shown that mRNAs directly coding the 22–23 kD and 37–39 kD subunits of glutelin are absent from developing rice grains. In contrast, polypeptides of prolamin were found to be synthesized as prolamin with a signal sequence.

CONSTRUCTION OF cDNA LIBRARIES FOR mRNAs APPEARING IN THE RIPENING RICE ENDOSPERM

cDNA library constructed by Hind III linker method

Preparation of mRNA. Starchy endosperms of ripening rice seed (5 g) were powdered using an electric coffee mill under liquid N coldness. The powder was suspended with 10 ml of buffer (50 mM-Tricine-KOH, pH 8.0, 10 mM KCl, 5 mM MgCl₂, 0.001% (W/V) polyvinyl sulfate, 2 mM 2',3'AMP, 1 mM DTT, 7 mM diethylpyrocarbonate, 250 mM sucrose) and then centrifuged at 75 g for 3 min. The supernatant was layered on 3 ml of 60% sucrose and then centrifuged at 500 g for 5 min. The supernatant obtained was further centrifuged at 37,000 g for 15 min. The resulting precipitate corresponds roughly to the RER (rough endoplasmic reticulum) fraction. The RER fraction was used for phenol extraction of RNAs, and the extracted RNAs were precipitated by 70% ethanol. The RNA fraction was passed through a column with 0.5 ml bed volume of Oligo dT cellulose and the retained RER-poly(A)RNAs were then eluted by water.

Preparation of double strand cDNA for RER-RNA. Single strand cDNA (sscDNA) was synthesized from 10 days after flowering (DAF) starchy endosperm primed with Oligo dT using an avian myeloblastosis viral (AMV) reverse transcriptase (7). sscDNA was recovered by phenol extraction and ethanol precipitation. Double strand cDNA (dscDNA) was then prepared using DNA polymerase I (large fragment) after boiling the above sscDNA precipitate. Hind III linkers were induced at both ends of the dscDNA after treatment with S1 nuclease. Finally, Hind III linkers-induced dscDNA was treated with Hind III to remove extra Hind III linkers.

On the other hand, *Escherichia coli* cloning vector pBR322 was first digested with Hind III (Hind III-pBR322) and then treated with alkaline phosphatase to remove 5'-phosphate to prevent self-ligation (Hind III-APase-pBR322). This treatment resulted in almost complete prevention of self-ligation of the Hind III treated pBR322. The frequency of the self-ligation of Hind III-APase-pBR322 was less than 1.3×10^{-4} compared to that of Hind III-pBR322. When dscDNA with Hind III linkers prepared as above was ligated with Hind III-APase-pBR322, the rate of

ligation was raised more than 160 times over that of the self-ligation of Hind III-APase-pBR322. The frequency of appearance of Am(r)Tc(s) colony appearance was about 3.0×10^5 colonies/ μg dscDNA.

Identification of colonies carrying cDNA sequences for rice glutelin. According to the usual selection method, Am(r)Tc(s) colonies were obtained on agar plates. The Am(r)Tc(s) colonies were then further screened by ^{32}P -labeled sscDNA produced by reverse transcription from RER-poly(A)RNA, which was then used to produce cDNA libraries for mRNAs of ripening rice endosperm. All Am(r)Tc(s) colonies obtained here hybridized strongly to ^{32}P -labeled sscDNA probe from RER-RNA, suggesting that these colonies have some cDNA sequences for the mRNAs that appear in the ripening starchy rice endosperm. As shown in Fig. 3b, the majority of the poly(A)RNA in the RER-poly(A)RNA obtained above is composed of 57 Kd glutelin precursor mRNA. Thus, it may be expected that many of the Am(r)Tc(s) colonies are carrying the cDNA sequence for rice glutelin.

From one of Am(r)Tc(s) colonies, plasmid DNA was recovered by the cleared lysate method (2), and the size of the plasmid was determined as shown in Figure 4. This plasmid (pRcD644) was larger in size than pBR322, although it was only a few hundred base pairs longer than that of pBR322. The reason why the inserts were so short might be attributed to the decomposition of cDNA during dscDNA preparation or the fact that Hind III sites were included in the glutelin cDNA sequence.

As shown in Figure 5, when purified pRcD644 DNA (1 μg) was added to a RER-poly(A)RNA-directed in vitro protein biosynthesis system (50 μl), the formation of the 57 Kd polypeptide was stopped completely, although pBR322 did not affect its formation at the same concentration. This shows that pRcD644 is carrying a partial cDNA sequence to the mRNA for rice glutelin precursor.

cDNA library constructed by Okayama-Berg method

Although some colonies such as pRcD644 have a cDNA sequence to the mRNA for glutelin precursor, the size of the cDNA is far shorter than expected for a full-length cDNA for 57 Kd glutelin precursor mRNA, which is supposedly longer than 1700 bp. We then tried cloning a full-length cDNA for 57 Kd glutelin precursor polypeptide according to the method of Okayama and Berg (8).

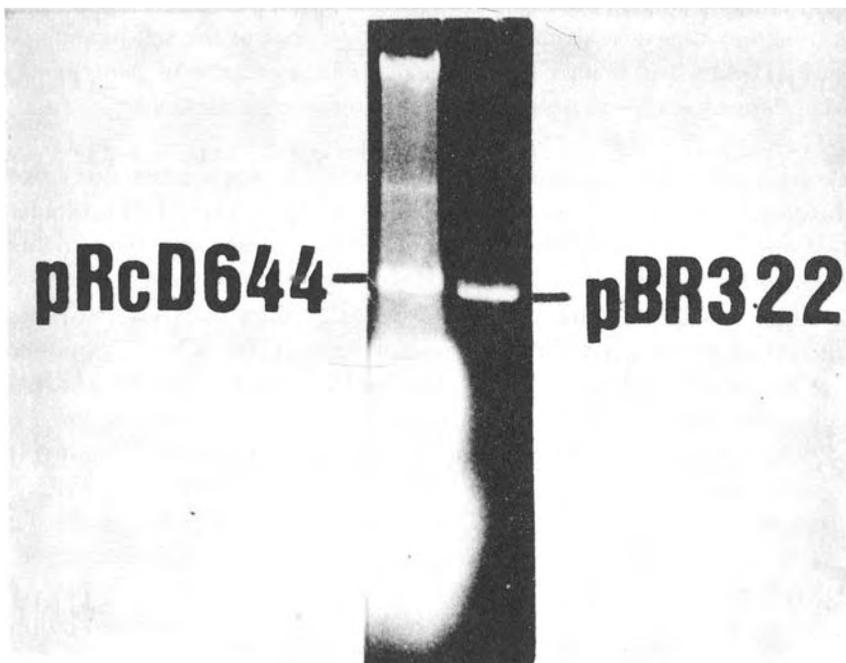


Fig. 4. Agarose gel electrophoresis of isolated plasmids. pRcD644 = a plasmid carrying glutelin cDNA.

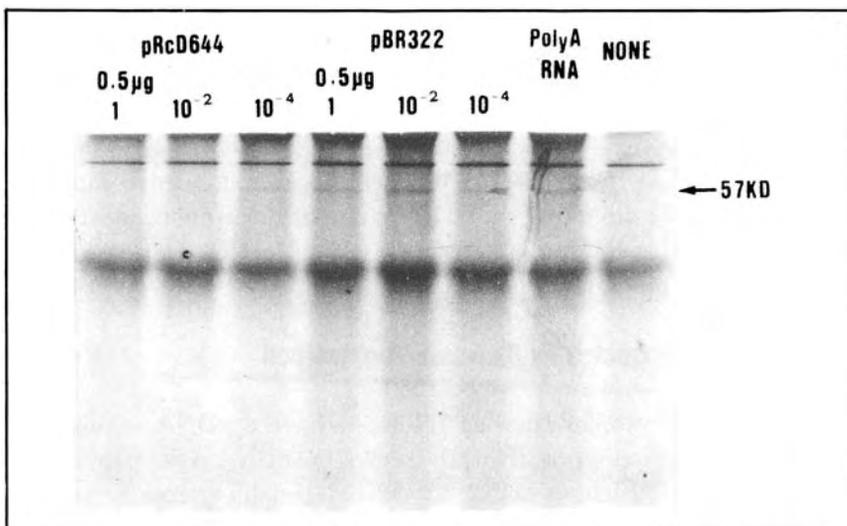


Fig. 5. Polyacrylamide gel electrophoresis of synthesized proteins after hybrid arrest translation. A fluorograph. pRcD644 = addition of isolated pRcD644 plasmid, pBR322 = addition of isolated pBR322 plasmid, Poly A⁺ RNA (1 μg), None = no addition of poly A⁺ RNA.

From the cDNA library constructed by the Okayama-Berg method, we obtained many colonies that were supposed to carry glutelin cDNA sequence using pRcD644 as a probe. Some of them had large inserts that were long enough to carry a full-length cDNA for the 57 Kd polypeptide. A detailed analysis on this insert is now being carried out.

CONSTRUCTION OF A GENOMIC LIBRARY

To insert nuclear DNA into bacteriophage lambda DNA, rice nuclear DNA larger than 50 kbp was prepared by a freeze-thaw method as follows.

Embryos of japonica rice grains were powdered by a bio-mixer (Nihon Seiki Co. Ltd.) under liquid N coldness. The nuclear DNA was extracted by phenol after the powdered embryos were treated with protenase K in 0.5M EDTA, 0.5% sarcosyl for 3 hours at 50°C. The DNA was precipitated by two volumes of ethanol. Finally, the DNA fraction was centrifuged to equilibrium in a CsCl density gradient.

EMBL 3 DNA was harvested from host *E. coli* (Ymel) and then double digested by restriction enzymes Bam HI and Eco RI to produce vector DNA.

The nuclear DNA already prepared was partially digested by Sau 3AI to produce a fragment of about 20 kbp. The DNA fragments were then treated with calf intestinal alkaline phosphatase to prevent self-ligation.

Ligation reaction DNAs (3:1 vector DNA to insert DNA) were mixed and T4 DNA ligase was added to the mixture. The ligated DNAs were used for in vitro packaging reaction.

In vitro packaged bacteriophage carrying recombinant rice DNA were inoculated into indicator bacteria (LE392 or NM539). After plaque formation efficiency was determined. *E. coli* on the surface of the agar plates was rinsed with dilution buffer and amplified to produce the genomic library.

PERSPECTIVES

The nature of rice storage proteins has become clearer by recent molecular biological investigations. One important characteristic of rice storage proteins is that prolamin accumulates in PB-I and glutelin in PB-II by different processes. Moreover, the biosynthesis mechanism of the former

is similar to that of zein, the storage protein of maize; however, the formation mechanism of glutelin is similar to that of legumin, the storage protein of legumes.

Nutritionally, glutelin is a better protein than prolamin. For this reason, it is desirable to breed a glutelin-rich rice, i.e., a low prolamin rice. In molecular biological terms, we must try to breed rice seeds with an enhanced expression of glutelin gene(s), i.e., with a depressed expression of prolamin gene(s).

We have already bred some mutants (6), which are rich in glutelin (such as CM 21) or are low in prolamin (13 Kd) (such as CM 1787). The reason for the appearance of these mutants must be dependent on the changes in glutelin and/or prolamin genes, especially in the promoter regions. We are now in the process of constructing genomic libraries for normal, CM 21, and CM 1787 to analyze the changes in the prolamin and glutelin genes.

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DISCUSSION

SESSION 14: GENETIC ENGINEERING

Q – Okita: The C-value (DNA content of haploid genome) is estimated to be about 0.6 pg according to Bennet and Smith. Where did you get your value of the DNA content of rice?

A – Wu, R.: The value of 1.5 pg DNA/haploid genome of rice came from (1) K. Oono in *Biology of Rice* 1984 (Tsunoda S., and Takahashi, N. eds.), Elsevier, pp. 339–358.

(2) Nagato, Y., Yamamoto K., and Yamashita H. 1981 *Japanese J. Genetics* 56:483–493.

However, a lower value of DNA content, around 0.6 pg DNA/haploid genome of rice, has also been reported in the following reference:

(3) Iyengar, G.A.S., Gaddipati, J.P., and Sen, S.K. 1979 *Theor. Appl. Genet.* 54:219–224.

Thus, there is 2.5–3 fold discrepancy among different reports.

Q – Brar: For many genes governing economic characters, it is difficult to isolate mRNA. How would you clone such genes?

A – Wu, R.: If one can find a probe, a heterologous probe or a synthetic oligodeoxynucleotide probe, then one can probe a genomic library. mRNA is not really difficult to isolate from rice, so one can make a cDNA library.

Q – Hooykaas: Rice, as was mentioned in the presentation, produces only little *vir*-inducing compound. From work in Leiden we know that other cereal species produce as much as dicots. How was the amount measured and with which species or variety of rice?

A – Van Montagu: The HoMo-1 transposon was used for making gene fusions with *vir*-genes. We measured **b**-gal activity (*virB-lacZ*) after induction with a fixed amount of plant cells. At least two varieties of japonica rices (Taipei 309, Taichung 65) gave the same result.

Q – Brar: You have nicely described several methods for genetic transformation using naked DNA, microinjection, and pollen incubation. However no genetic transformants could be recovered. What is your opinion about the nonoccurrence or non-recovery of genetic transformants? Is it due to lack of DNA uptake, integration, or degradation of DNA itself?

A – Lörz: Convincing evidence at the biochemical as well as at the molecular level has been obtained for genetically stable transformation using the method of direct DNA uptake into protoplasts and the micro-

injection technique. In respect to pollen transformation there are several reports, including our own observations, where the plants of the desired phenotype have been selected or have been found, but in all these cases no data are available demonstrating the actual presence and integration of the foreign gene.

Q – Brar: Do we have sufficient evidence to demonstrate the mutagenic effect of alien DNA?

A – Lörz: I am not aware of any molecular evidence for the mutagenic effect of exogenous DNA in transformation experiments. However, observations along these lines have also been made with fusion experiments when irradiated protoplasts have been used as one of the fusion partners. Restoration of, e.g., nitrate reductase activity has been found, but the molecular evidence for the transfer of the foreign DNA into the last cell genome has not been found.

Q – Wullems: Do you have any guaranty that the DNA you measure is inside the cell, and not stuck to the outside?

A – Ganesan: Yes. The protoplasts were sedimented, washed, treated with DNA-1, and washed again before acid precipitation and re-extraction of donor DNA. What I see are the counts from DNA (and the biological activity) that have entered the cell and are not accessible to added DNA-1.

Q – Brar: When you isolated chloroplast DNA, you did not use the CsCl method for purification. I feel there would be contamination of chloroplast DNA by nuclear as well as mitochondrial DNA. Do you think it is not necessary to use CsCl for chloroplast DNA isolation and purification?

A – Hirai: I think we have a small amount of contaminated nuclear and mt.DNA in our chloroplast DNA preparation. However, the stained bands we observed were all from chloroplast DNA, because in all experiments the total size of our bands was about 130 Kb long. Therefore, I think CsCl purification is not necessary for our purpose.

Q – Juliano: Since PBI is the principal PB (spherical PB) of rice endosperm, and PBII (crystalline PB) is mainly in the subaleurone layer, what are the percentages of prolamin and glutelin in PBI? As you know, milled rice has only 3% prolamin but about 80% glutelin. Overmilled rice with only PBI has similar glutelin/prolamin rates.

A – Tanaka: PBI does not contain any prolamin. According to our research work concerning isolation of protein bodies, we found that two types of protein bodies are present in the starchy endosperm. One of them is referred to as spherical PB (PBI) and the other as crystalline PB (PBII). Analysis of protein compositions in the protein bodies has shown that 10, 13, and 16 Kd polypeptides, which are alcohol-soluble proteins, are found only in PBI. On the other hand, PBII contains 22–23 Kd and 37–39 Kd polypeptides, which are 1% lactic acid soluble protein

(glutelin), but not prolamin. Thus, most of the glutelin in the starchy endosperm is localized in BPII, not in BPI.

Q – Van Montagu: You showed that the prolamin polysomes separated with the RER fraction. Can you be sure from your EM work that it is the RER? It could be another class of vesicles to which polysomes once attached as RER. If so, it would be important to know, for it would indicate the existence of a special class of signal peptide for storage vesicles.

A – Tanaka: We are very sure that PBI vesicles are continuous with the widely distributed rough endoplasmic reticulum.

C – Wu, R.: PBI is not necessarily synthesized within the lumen of ER. Our EM evidence shows that small vesicles with attached ribosomes are often associated with the “growth” of PBI.

CONCLUDING REMARKS

OVERVIEW OF THE SYMPOSIUM

BIRTH OF THE RICE GENETICS

COOPERATIVE

PARTICIPANTS

CONCLUDING REMARKS

It has been a great pleasure and honor for me to be able to attend this conference, the International Rice Genetics Symposium, and to be given the opportunity to address you at its conclusion.

On behalf of my colleagues from Japan, I wish to offer our gratitude to Dr. M. S. Swaminathan for hosting the conference and to his IRRI colleagues, especially Dr. G. S. Khush, for their kind efforts in organizing it. Without their hard work we would not have been able to accomplish so much during this meeting, and we are indeed grateful.

We are also grateful to the participants of the symposium, who have shared with us their knowledge of rice genetics and have identified areas for further research and cooperation. The primary objectives of this gathering have been attained. I thank every one of you for your fine contributions.

At this time my memories go back to the International Symposium on Rice Genetics and Cytogenetics, which was held at IRRI in February 1963. The proposal for the 1963 symposium was made by Dr. H. Kihara and his Japanese associates to Dr. R. F. Chandler, the director of IRRI at that time. Dr. Chandler and his colleagues organized that symposium, and Dr. T. T. Chang served as convenor and technical editor of the proceedings. The proceedings, entitled *Rice Genetics and Cytogenetics*, was a useful scientific publication on rice.

I am happy to note that considerable progress has been made in rice genetics since 1963, as well as in the development of new fields such as genetic engineering.

I am glad to note that Dr. H. I. Oka, who helped organize the 1963 symposium, also played a key role in organizing this one. Among those who have attended both symposia are Drs. H. I. Oka, T. T. Chang, C. C. Chen, C. H. Hu, R. Seetharaman, S. C. Hsieh, H. K. Wu, and P. R. Jennings.

I am delighted to see the birth of the Rice Genetics Cooperative (RGC). I am sure the RGC will play a vital role in promoting the cause of rice genetics, and I urge you all to extend your full cooperation to it. The success of this organization will depend upon the concerted efforts of all of

you, the rice geneticists of the world. I also wish to laud the commendable efforts of Drs. H. I. Oka and G. S. Khush in starting the Rice Genetics Newsletter. This newsletter should facilitate the work of the RGC.

M. E. Takahashi

OVERVIEW OF THE SYMPOSIUM

Rice would not be the organism of choice for basic research, so all who undertake its study do so with the objective of helping those who grow and eat rice. In this symposium all our discussions have been devoted to the practical purposes of rice improvement.

I have been surprised at the extent to which work has been described involving the use of Mendelian genetics. It is rewarding to know that formal segregational genetics is still so useful and that it demonstrates the ways in which genes — for example those for insect resistance — can be brought together to maximize the range of resistance.

Questions were raised during discussion on the limited extent to which yield has been raised in new varieties beyond that introduced with the semidwarfing gene. This seems to me to be readily understandable, since breeders have regarded it as their prime responsibility to stabilize the yielding capacity of the new plant type against the limitations imposed by the impact of diseases, insects, and adverse soil and environmental conditions. Such stabilization would enable as many farmers as possible to benefit from semidwarfs. Although considerable success has been achieved in the use of genetic protection against diseases and insects, much still remains to be done, and protection against drought, soil toxicity, and cold still remains to be exploited.

Nevertheless, yield can be advanced by practical plant breeding processes. The appropriate genetic variation is available, as has been shown in studies of quantitative inheritance. If yield can be advanced in this conventional way, questions must be raised about the value of hybrid varieties, but we should not neglect the possibility that similar yields may be produced more easily and at less cost in inbred lines.

While considering hybrid varieties and the use of cytoplasmic male sterility, I note the time and effort that goes into the genetic testing with restorer genotypes of each newly discovered cytoplasmic source of sterility. In maize, molecular probes can be used to test for the lesion in the mitochondrial genome that is the cause of male sterility; it would be useful if such probes could be developed for rice.

In this way and in others we may expect that molecular biology will soon start to contribute to rice improvement. Initially, molecular and cell

biology will provide tools to assist plant breeders and to increase the precision of their work; subsequently, entirely novel genotypes will be created. During this symposium we have heard about the start of the application of molecular biology to rice. Clearly, the work has started vigorously and shows great promise. However, it should not be thought that the molecular approach can replace classical plant breeding; it will not, but it will provide a very valuable complement to it.

At the beginning of the symposium, Dr. Swaminathan spoke of hybrid vigor being generated by the combination of conventional genetics and breeding on the one hand and molecular and cell biology on the other. I have not detected hybrid vigor, but I have observed that there has been a perfectly compatible mixing of disciplines. The symposium participants are an international community of scientists competent in a wide range of disciplines and devoted to common objectives. This sense of community has been further developed by the creation of the Rice Genetics Cooperative.

Consequently, the International Rice Genetics Symposium has reinforced our sense of community and has enabled many international linkages to be made or renewed. The creation of these linkages, the exchange of information about problems and results, and the sense of common purpose that has been generated out of a progressive and dynamic scientific activity have all gone to make the International Rice Genetics Symposium outstandingly successful.

For this and for all their hard work may I, on behalf of the participants, thank Dr. Swaminathan, Dr. Khush, and all their colleagues at IRRI.

Ralph Riley

BIRTH OF THE RICE GENETICS COOPERATIVE (RGC)

The need for an international organization for promoting the cause of rice genetics has been felt for many years. Considerable correspondence took place between Japanese (H. I. Oka, Y. Futsuhara, and T. Kinoshita) and IRRI (M. S. Swaminathan and G. S. Khush) scientists during 1982 and 1983 in this regard. On the basis of this correspondence a discussion paper entitled "Proposal for Organizing a Rice Genetics Cooperative (RGC)" was prepared and distributed to the participants in the opening session of the symposium on 27 May 1985. The text of the discussion paper is given in Appendix I.

Informal consultations were held with many participants during the first two days of the symposium regarding the need, aims, and by-laws of the RGC. A group discussion was held on the evening of 28 May at the residence of Dr. M. S. Swaminathan that was attended by:

A. O. Abifarin	T. Matsuo
A. A. App	Min Shao Kai
A. Bianchi	M. Van Montagu
T. T. Chang	H. I. Oka
Y. Futsuhara	T. Omura
A. T. Ganesan	Qi Quan Shao
M. H. Heu	C. M. Rick
H. Ito	R. Riley
N. Iwata	J. N. Rutger
M. Jacquot	R. Seetharaman
P. Jennings	B. H. Siwi
G. S. Khush	M. S. Swaminathan
T. Kinoshita	M. E. Takahashi
A. Majeed	G. Toenniesen
M. Maluszynski	R. Wu

Based on these discussions, a draft covenant and by-laws for the Rice Genetics Cooperative was prepared and distributed to the participants. It is given in Appendix II. An ad hoc committee under the Chairmanship of Dr. C. M. Rick was set up to suggest a uniform system of numbering

rice chromosomes; it met in the evening of 29 May, and its report is given in Appendix III.

A Nominating Committee under the Chairmanship of Dr. T. Matsuo was set up to recommend the names of scientists who could serve on different standing committees of the Rice Genetics Cooperative. The Nominating Committee met on 30 May 1985 at noon and proposed the names of 14 scientists as members of the Coordinating Committee of the Rice Genetics Cooperative (Appendix IV).

The reports of these committees were presented to the participants during the closing session of the symposium on the morning of 31 May 1985. These reports and the Covenant and By-Laws of the RGC were accepted by the participants and led to the birth of the Rice Genetics Cooperative.

The first meeting of the Coordinating Committee of the RGC was held at IRRI in the afternoon of 31 May 1985. Office-holders of the RGC and Convenors and Members of the Standing Committees were elected (Appendix V).

Appendix I

PROPOSAL FOR ORGANIZING A RICE GENETICS COOPERATIVE (RGC)

The standardization of gene symbols has been an important concern of rice geneticists since the early days. The rapid generation of new information on rice genetics in recent years has resulted in the use of different symbols for the same genes and the same symbols for different genes. It has also brought about different systems of numbering of the chromosomes.

To promote cooperation among rice workers in the adoption of uniform gene symbols and in other areas of rice genetics, an interim committee of Japanese scientists was organized in 1979 and has continued working until today. However, the need for the establishment of an international organization has been felt increasingly by the members. Through discussions among Drs. M. S. Swaminathan, G. S. Khush, H. I. Oka, T. Kinoshita, Y. Futsuhara, and other colleagues, it was agreed to publish the first issue of the Rice Genetics Newsletter (RGN) in 1984 and to hold the International Rice Genetics Symposium (IRGS) in May 1985, as are mentioned in retrospect by Dr. T. Matsuo in the Foreword of RGN Vol. 1.

RGN Vol. 1 was published in December 1984 under the sponsorship of the Japanese Rice Genetics Information Committee with the generous financial support of the Tropical Agriculture Research Center of the Ministry of Agriculture, Forestry, and Fisheries, Japan. In his Foreword, Dr. T. Matsuo stressed the need for an international organization for the long-term management and publication of the RGN. It is hoped that during the IRGS, world rice geneticists will establish a Rice Genetics Cooperative (RGC) to assume responsibility for publishing the RGN.

The RGC, herewith proposed, is a group of scientists who have a common interest in rice genetics and who are organized for the purpose of exchanging information and genetic stocks. The main function of the RGC will be to compile and to distribute to members annual issues of the RGN, to monitor rice gene symbolization and chromosome mapping, to foster cooperation in genetic engineering research, and to organize a network of scientists to facilitate the maintenance and exchange of information and genetic stocks among the scientists.

Apparently, the success of this work depends upon the concerted efforts of the rice geneticists of the world. We now ask for your cooperation in

this activity. A tentative draft of the Covenant and By-Laws is presented for your consideration.

Signed by:

M. S. Swaminathan

T. Matsuo

M. Takahashi

G. S. Khush

H. I. Oka

Y. Futsuhara

T. Kinoshita

Appendix II

RICE GENETICS COOPERATIVE

COVENANT AND BY-LAWS

I. *Organization and purpose*

The Rice Genetics Cooperative (hereinafter designated as RGC) is an informal, incorporated scientific society intended for the advancement of research in the field of rice genetics and its applications to rice improvement.

The major purposes of the RGC will be the following:

- a) To foster cooperative research on all aspects of rice genetics with a view to promoting rice improvement
- b) To develop standard rules for gene symbolization and nomenclature, and to prepare and continuously update linkage maps and develop a standard system of numbering of chromosomes
- c) To ensure the proper maintenance of genetic and chromosome markers including trisomics and translocation stocks and to facilitate their availability to interested researchers through the dissemination of information
- d) To monitor and promote the application of genetic engineering and tissue culture techniques
- e) To organize an International Rice Genetics Symposium (IRGS) at periodic intervals
- f) To assume responsibility for publishing the Rice Genetics Newsletter (RGN)

II. *Membership and dues*

The membership of the RGC shall consist of the following two categories:

- a) *Individual members:* All scientists interested in rice genetics are eligible to join.
- b) *Corporate members:* Institutes and organizations interested in rice genetics.

Membership admissions will be made by the Secretaries of the Coordinating committee. The annual dues will be fixed by the Coordinating Committee and will be submitted for approval by the general meeting of

the RGC or by correspondence among the members. The initial subscription will be US\$4 per year for individual members and US\$100 per year for corporate members.

III. Committees

The Coordinating Committee will be in overall charge of the prices and programs of the RGC. It shall consist of fifteen members including the Chairman, Co-Chairman, Secretaries, Treasurer, and Editors. Five members will represent the major rice-growing regions and will be drawn from Africa, Latin America, North America, Europe, and Oceania. No separate representation is indicated for Asia, since it is expected that many of the office-holders will be from Asia. The Coordinating Committee will be responsible for organizing the International Rice Genetics Symposium.

The Coordinating Committee will be supported by the following four Standing Committees, each consisting of five members with a Convenor:

1. *Committee on Gene Symbolization, Nomenclature, and Linkage Groups*: This Committee will help promote the use of standard gene symbols and chromosome numbering.
2. *Committee on Genetic Stocks*: This Committee will help keep records on genetic stocks with marker genes, chromosomal alterations, and other hereditary modifications maintained in different laboratories. It shall promote the exchange of genetic stocks among rice geneticists of the world.
3. *Committee on Genetic Engineering*: This Committee will promote the exchange of information on DNA vectors, cloned genes, and gene libraries and will monitor progress in this fast-moving field.
4. *Committee on Publication of the Rice Genetics Newsletter*: This Committee will serve as the International Editorial Board for the RGN.

In addition, Ad Hoc Committees may be set up by the Coordinating Committee from time to time for special purposes.

IV. Election and appointment of committees

The Chairman and Members of the Coordinating Committee shall be elected for five-year terms by voting at the general conference of the RGC or by correspondence among the members. The Chairman and Members of other Committees shall be appointed by the Coordinating Committee. The initial Coordinating Committee will be established during the International Rice Genetics Symposium at IRRI in May 1985.

V. Publications

One of the main functions of the RGC shall be to issue the RGN between October and December of each year for distribution among members. The RGN shall contain (a) special notices and announcements, (b) current linkage maps, (c) lists of genes and genetic stocks newly reported, (d) a list of recent publications on rice genetics, (e) research notes, and (f) a membership list. Convenors of the four Standing Committees will send reports for publication in each issue of the RGC. The RGN will be distributed free among Individual and Corporate Members and can be subscribed to by institutional libraries at a price to be determined by the Coordinating Committee.

VI. Meetings

Meetings of committee members shall be held at such times and places as determined by the Coordinating Committee. The meetings shall be announced, and main conclusions reached will be reported in the RGN.

A general conference of the members in the form of an International Rice Genetics Symposium shall be held at suitable intervals as determined by the Coordinating Committee. Initially, it is planned to organize an IRGS once in 5 years.

VII. Amendment

These By-Laws may be amended by simple majority of voting members by mail ballot, provided a copy of the proposed amendment has been mailed to all the active members at least 60 days prior to the balloting deadline set by the Secretaries.

VIII. General prohibitions

The RGC shall be organized and operated exclusively for scientific and educational purposes. It shall not operate for profit or for any political or religious reasons or purposes other than the pursuit of science.

IX. Secretariat

The Coordinating Committee will decide on the location of the RGC and RGN Secretariats from time to time. Initially, the RGC Secretariat will

be located at the International Rice Research Institute, Los Baños, Philippines, and the RGN Secretariat in Misima, Japan.

X. *Foundation members of RGC*

The participants of the International Rice Genetics Symposium being held at IRRI from 27 to 31 May 1985 will be invited to join as Foundation Members of the RGC. If this proposal is approved by the participants at the Closing Session of the symposium on 31 May 1985, they will be given forms for applying for Foundation Membership. Other rice geneticists not attending the symposium will also be given the opportunity to join as Foundation Members throughout 1985.

Appendix III

REPORT OF AD HOC COMMITTEE ON CHROMOSOME NUMBERING SYSTEM

In order to clarify understanding in rice genetics, a unified system of numbering rice chromosomes needs to be adopted. The chromosomes shall be assigned arabic numbers in order according to their pachytene length (or centromere position in case of ambiguity of length) based on studies by Dr. S. V. S. Shastri et al (1960) and confirmed by Dr. N. Kurata et al (1981). The somatic chromosomes shall be numbered accordingly. Complete primary trisomic series have been established and the extra chromosomes identified in three laboratories, but differences exist in the identification of the extra chromosomes of a few trisomics. These differences should be resolved as soon as possible. In this fashion all linkage groups will be identified with their respective chromosomes.

Committee members

C. M. Rick, Chairman
N. Iwata
G. S. Khush
T. Kinoshita
N. Kurata
R. N. Misra
H. I. Oka
R. Riley
R. Seetharaman
H. K. Wu

Appendix IV

REPORT OF THE NOMINATING COMMITTEE

The Committee identified the following scientists to serve on the Coordinating Committee of the RGC.

- Dr. M. S. Swaminathan (IRRI)
- Dr. T. Matsuo (Japan)
- Dr. M. Takahashi (Japan)
- Dr. G. S. Khush (IRRI)
- Dr. H. I. Oka (Japan)
- Dr. Y. Futsuhara
- Dr. T. Kinoshita (Japan)
- Mr. Min Shao Kai (China)
- Dr. J. Neil Rutger (USA)
- Dr. A. Bianchi (Italy)
- Dr. K. Miezán (Ivory Coast)
- Dr. R. Seetharaman (India)
- Dr. B. H. Siwi (Indonesia)
- Dr. M. H. Heu (Korea)

Committee members

- T. Matsuo, Chairman
- A. Bianchi
- M. H. Heu
- T. Kinoshita
- K. Miezán
- Min Shao Kai
- H. I. Oka
- J. N. Rutger
- R. Seetharaman
- M. Takahashi

Appendix V

RICE GENETICS COOPERATIVE

Minutes of the First Meeting of the Coordinating Committee of the Rice Genetics Cooperative (RGC) held at IRRI on 31 May 1985.

Present were A. Bianchi, Y. Futsuhara, M. H. Heu, T. Kinoshita, G. S. Khush, T. Matsuo, K. Miezán, Min Shao Kai, H. I. Oka, J. Neil Rutger, R. Seetharaman, B. H. Siwi, M. S. Swaminathan, and M. Takahashi.

In accordance with the Covenant and By-Laws of the Rice Genetics Cooperative established at IRRI in Los Baños on 31 May 1985 by the participants of the International Rice Genetics Symposium, the Members of the Coordinating Committee met at IRRI at the end of the symposium on 31 May.

Dr. Swaminathan extended a warm welcome to all the members of the Coordinating Committee. The items discussed and the decisions arrived at are given below:

I. Appointment of office-holders

The following were requested to serve in the positions indicated:

M. S. Swaminathan – Chairman
T. Matsuo – Co-Chairman
G. S. Khush – Secretary and Editor
H. I. Oka – Editor
Y. Futsuhara – Secretary

Dr. D. Senadhira, IRRI plant breeder, was requested to serve as Treasurer of the RGC. The following will serve as councilors representing different regions:

A. Bianchi	J. Neil Rutger
M. H. Heu	R. Seetharaman
T. Kinoshita	B. H. Siwi
K. Miezán	M. Takahashi
Min Shao Kai	

It was decided to invite Dr. Mike Cox of Australia to represent Oceania on the Coordinating Committee.

II. *Appointment of convenors and members of standing committees*

The following were chosen to serve as Convenors and Members of the four Standing Committees:

1. *Committee on Gene Symbolization, Nomenclature, and Linkage Groups*

Convenor: T. Kinoshita
Members : G. S. Khush
 J. Neil Rutger
 R. Seetharaman
 H. K. Wu

2. *Committee on Genetic Stocks*

Convenor: T. Omura
Members : T. T. Chang
 N. Iwata
 K. Miezan
 S. D. Sharma

3. *Committee on Genetic Engineering*

Convenor: M. Van Montagu
Members : B. Siwi
 Dayuan Wang
 R. Wu
 H. Yamaguchi

4. *Committee on Publication of the Rice Genetics Newsletter*

Convenor: G. S. Khush
Members : A. Bianchi
 S. Iyama
 H. I. Oka
 J. Neil Rutger

III. *Financial matters*

Dr. Oka mentioned that the Japanese Government would continue to provide support for the Rice Genetics Newsletter. Dr. Swaminathan requested all members to identify suitable institutions to become Corporate Members of RGC. It was decided to approach the Rockefeller Foundation to become a Corporate Member.

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