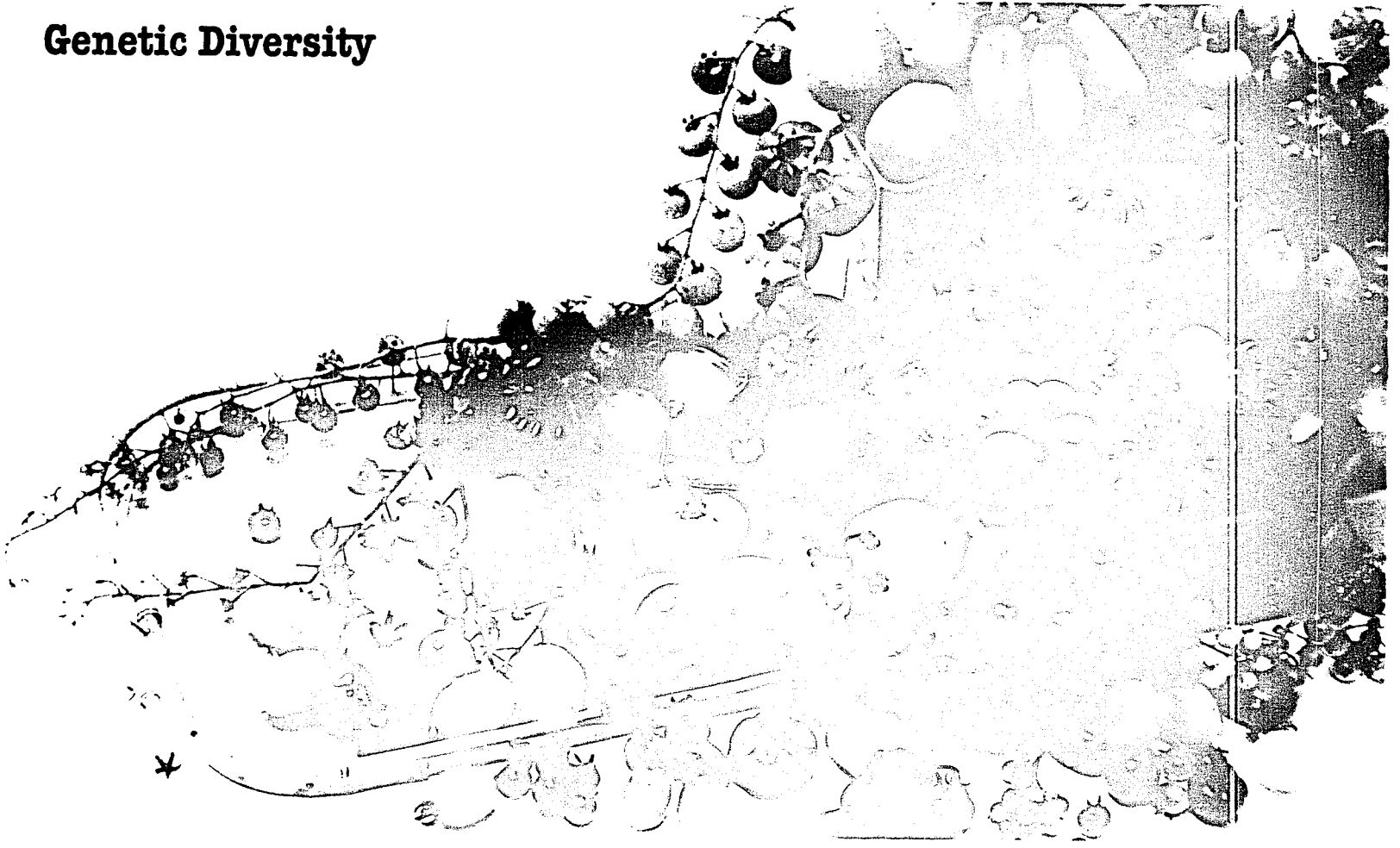
CROP GENETIC RESOURCES OF EAST ASIA

Genetic Diversity



CROP GENETIC RESOURCES OF EAST ASIA

Proceedings of the International Workshop on Crop Genetic Resources of East Asia
10 – 13 November 1987
Tsukuba, Japan

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IBPGR
International Board for Plant Genetic Resources

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INTRODUCTION

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Chairman
K. Hayashi

Inaugural Address

KEN ICHI HAYASHI
Director General
National Institute of Agrobiological Resources

Distinguished Guests and Participants,

On behalf of the Workshop Organizing Committee of the National Institute of Agrobiological Resources and other related institutions, I have the honour and privilege to open the International Workshop on Crop Genetic Resources of East Asia. It is also my great pleasure to extend the most sincere greetings and best wishes to all the distinguished guests and participants in this International Workshop. In particular I would like to extend a cordial welcome to the participants who have come over to Japan from abroad.

The present Workshop has been sponsored by three organizations: the International Board for Plant Genetic Resources, the Ministry of Foreign Affairs and the Ministry of Agriculture, Forestry and Fisheries of the Government of Japan.

I particularly would like to take this opportunity to express my deepest appreciation to the Director of the IBPGR, Dr J. T. Williams, for his thoughtful consideration in sponsoring this International Workshop. I have to express sincere thanks also to some important members of the IBPGR, including Dr F. Kikuchi, professor of University of Tsukuba and a member of the Board of Trustees of the IBPGR, Dr J. L. Creech, Senior IBPGR Advisor and Ir D. H. van Sloten, Head, IBPGR Field Programme, for their constructive advice and collaboration in drafting and completing the detailed program of this Workshop. It is really a very good experience for us and particularly for the Symposium Organizing Committee to have Dr S. Suzuki, Genetic Resources Coordinator of the NIAR, as Organizing Secretary, to put forward all the necessary arrangements and activities from the initial step which was more than one year ago to this final stage. We are now very happy to share with all of you the pleasure of having the opening ceremony of this Workshop this morning.

Despite the presently recorded food surplus, the long-term prospect for food production may not be bright in view of the ever-increasing population growth particularly in the developing countries. Even in the developed countries the application of the conventional agricultural technologies may not guarantee any further upgrading of agricultural production. The agricultural conditions have been changing adversely due to many negative factors including urbanization and environmental deterioration. Under these circumstances it is quite natural that society expects agricultural scientists to develop new technologies that should bring a new breakthrough in agriculture.

The National Institute of Agrobiological Resources (NIAR) was founded in 1983 with objectives to develop pioneering and basic researches on preservation, development and utilization of biological resources in agriculture. Its research areas include genetic resources, applied physiology and cell and molecular biology. In 1986, the Genetic Resources Center, composed of a Genetic Resources Coordinator, two research Departments and one administrative Division was established in NIAR to reorganize and strengthen the activities on genetic resources. The Center aims at the collection, evaluation and preservation of plant, microorganism and animal genetic resources and at promoting related research fields.

The collaboration of NIAR through the activities of this Center with IBPGR involves several aspects. We are part of the international network of the IBPGR base collections and take the responsibility for long-term preservation of the genetic resources of about ten crop plants designated to Japan. Organizing the international group training course on genetic resources is another aspect of this collaboration. This particular training course was proposed originally by IBPGR and has been funded by the JICA (Japan International Cooperation Agency) since 1982, contributing to obtain basic knowledge and technologies on genetic resources for many scientists in Asia, Middle East, Africa and South America.

Today, unlike before, the importance of genetic resources is widely and well understood. It is true that genetic resources are the carrier of genes encoding all the genetic traits that have been accumulated over a period of several billion years. At the same time, genetic resources supply the genetic variation useful for crop improvement through breeding, including biotechnology. In this connection, the present Workshop is very timely, focusing on the use of genetic resources in crop improvement and also on identifying future problems in East Asia and neighbouring regions where the concerted efforts of scientists are really needed.

I sincerely hope that the present Workshop will be sucessful and useful for further promoting activities on genetic resources through our respective countries. I also hope that our friendship and cooperation will be further strengthened through our joint effort in reaching this common target of immense importance. Also, please let me hope that your stay during the Workshop at Tsukuba will be fruitful and enjoyable.

Thank you very much.

Welcome Address 1

TOSHIHIKO NISHIO
Deputy Director-General, Research Council Secretariat, MAFF.

Distinguished Guests and Participants,

On behalf of the Ministry of Agriculture, Forestry and Fisheries, I would like to say a few words on the occasion of the opening ceremony of the IBPGR International Workshop on Crop Genetic Resources of East Asia.

First of all, it is a great pleasure for us that the International Workshop is held here in Tsukuba Science City under the great efforts of the authorities concerned, and I sincerely welcome all participants, including experts from 11 foreign countries and three international organizations, and experts from related research institutes and universities in this country.

This Workshop is designed to strengthen various research activities in the field of the utilization of genetic resources in East Asia, reflecting the results of the IBPGR symposium in 1980 which was also held in Tsukuba. Since it seems to be a most timely Workshop, our ministry decided to cooperate by promoting the participation of the related researchers in the Ministry. I also hope that regional cooperative activities will be further promoted through the success of this Workshop.

On this occasion I would like to explain some research activities in this field. The Ministry is in the process of developing new advanced technologies such as biotechnology in order to make rapid progress in the agriculture, forestry and fisheries, and food industries. In the course of this development, we are placing emphasis on securing genetic resources, and established in 1985 a comprehensive system for genetic resources called the 'Agriculture, Forestry and Fisheries Genebank', which involves a really extensive network for collecting, preserving and evaluating genetic resources such as agricultural plants and animals, micro-organisms, forest trees and marine organisms for more efficient utilization. And from this year, we initiated a project on the breeding of new, epoch-making varieties using that genebank together with the advanced biotechnologies. The Ministry intends to contribute to international cooperation on plant genetic resources through the above research activity.

We fully understand that it is important for many countries and international organizations to work together and exchange information in order to deepen mutual understanding of the subject. We hope that the Workshop will get fruitful results through active discussions by the participants and that further cooperation between countries and international organizations will be developed in the field of training and the development of related technologies on crop genetic resources.

Thank you.

Welcome Address 2

TADASHI FUJITA
Director, Multilateral Cooperation Division,
Economic Cooperation Bureau, MOFA.

Ladies and Gentlemen,

On the occasion of the International Workshop on Crop Genetic Resources of East Asia, I would like to express my hearty welcome to the distinguished representatives of the Asian Region and International Agricultural Research Centers, and extend my sincere respects to Dr J.T. Williams, Director of IBPGR and the members of the Japan Organizing Committee, who have made every effort to realize the holding of this seminar.

First of all, allow me to give briefly an outline of CGIAR and IBPGR. The Government of Japan gives its support to the CGIAR in view of its significant efforts to increase food production and strengthen International Agricultural Research Centers in the developing countries. IBPGR, established by CGIAR in 1974, plays an important role in promoting and coordinating an international network of genetic resources centres to further the collection, conservation, documentation, evaluation and use of plant germplasm and thereby contributes to raising the standard of living and welfare of people throughout the world.

In this context the Government of Japan has been contributing to IBPGR financially and technically.

Let me recall the recommendation by the ASCA Seminar held in November, 1985, which states:

'For ages past, mankind has profound relationships with so many plant species on the earth for the necessities of life, ie. food, clothing, housing, fuel, medicine and so on. The rapid progress in life sciences in recent years has opened new horizons for exploitation and utilization of plant genetic resources in our socio-economic activities, including health and welfare, medicine, agriculture, forestry, fisheries, manufacturing, energy and environmental conservation.'

It is significant in this context that this Workshop is being held with so many distiguished participants from the region and the International Agricultural Research Centers.

I sincerely hope that fruitful results will be produced through the four-day discussions by the distinguished participants, and that the collaborative research network of the IBPGR in the fields of crop genetic resources will be further promoted both regionally and globally.

Thank you.

KEYNOTE ADDRESS

Recent changes in emphasis in international genetic resources work

J. T. WILLIAMS
Director, IBPGR

IBPGR was established by the Consultative Group on International Agricultural Research (CGIAR) in 1974. Its initial task was to promote a global network of genetic resources centres to ensure that the genetic diversity of important food crops and other economic species was adequately collected and satisfactorily conserved, evaluated and documented. This material was to be made available to all *bona fide* scientists.

Since 1985, at the request of our donors, the mandate has been interpreted somewhat differently to include research and this has necessitated a total restructuring of the IBPGR organization. The changes however, have been gradual and many represent changes in emphasis in practical work. In the earlier years IBPGR established a list of priority crops and in the first ten years of its operation, IBPGR made significant progress in establishing major germplasm collections. It had stimulated the establishment of international, regional and national activities and programs, and continues to place high priority on building and strengthening a network of centres in all parts of the world.

One significant change has been a gradual move away from major emphasis on a 'regional' approach. IBPGR has come to emphasize work on crops over the development of regional infrastructures, once a certain level of support has been reached. IBPGR recognizes therefore that it must look primarily to international and national programs as the essential operational units, with the exception of a few regional centres set up with more assured financing by donors.

Regional cooperation is stimulated and becomes especially useful in, for instance, obtaining agreement on arrangements for regional long-term storage of collections of some crops, in organizing regional training courses and in promoting cooperation among the national scientists of a particular region.

IBPGR's task has always been to initiate the urgent action needed to develop a viable global network of activities, but not to act as a technical assistance agency *per se*. By working in this way IBPGR has been able to initiate and catalyze crop genetic resources work in several scores of countries and, in one way or another, has liaison with about 110 countries. Of these, about 50 national programs have emerged in recent years. The emphasis of IBPGR is to help developing countries integrate their activities on a scientific level and readily transfer to them technology on any aspect of collecting, conservation, documentation, and description and use of primitive forms of germplasm. In a number of high priority staple crops, the other international centres of the CGIAR play an important role in the overall network.

Let us recall that, when IBPGR was founded, the major concern about the loss of primitive forms was for the cereals such as rice, wheat, maize, sorghum and millets, and barley because of the recent successes in breeding and the modernization of agriculture which were causing widespread genetic erosion. In less than a decade such erosion was to be found in all other crop groupings, whether food legumes, fruits, vegetables or forages. Hence the priorities for action on crops were expanded rapidly and IBPGR had to initiate work on a very wide range of materials.

The first task was a wide-ranging collecting program to conserve landraces from areas of traditional agriculture and more recently to include related wild species so that the represen-

tation in collection was from the wider genepool of the individual crop. This aspect alone led to the need to chart a new course of action and to initiate much-needed back-up research.

I am delighted to address this workshop and to listen to the progress that has been made since the first IBPGR workshop charted a course for collaborative action 7 years ago for East Asia and the Pacific. We shall also hear of the progress made since five nations of Southeast Asia asked IBPGR for help in initiating a cooperative program. We shall all be involved in looking to the future to see how wider cooperation might be sustained.

This is an opportunity to review progress and identify gaps in ongoing work. It is also an opportunity for scientists to exchange information and I can assure you of the dedication of IBPGR to assist in whatever way possible. In order for you to see the multi-dimensional aspects of the IBPGR network I wish to outline the structure of our global program.

IBPGR program framework

IBPGR's program structure has a number of major functional program elements. These include:

Global Genetic Resources Network
Germplasm Acquisition
Germplasm Characterization and Evaluation
Training
In vitro Culture Research
Genetic Diversity Research
Seed Conservation Research.

This structure has developed because of a series of shifts in activities. For instance there has been a change of emphasis from widespread generalized collecting to selective purposive collecting to fill gaps in existing collections. More attention is now being given to ecogeographical surveys and species mapping and the collection of wild relatives of crops.

IBPGR is actively promoting germplasm characterization of previously collected germplasm and hence the better use of genetic resources. It is also placing greater emphasis on bringing internationally agreed scientific standards into the conservation of germplasm to ensure its safety in the base collections. Another change is that much more attention is being given to active collections linked functionally to base collections and this presents a number of conceptual problems which have not been fully addressed hitherto.

There are also a number of research challenges that IBPGR has identified and it is making efforts to meet these, as well as to lay a basis for accommodating any developments that will occur in the immediate future. For instance, IBPGR is actively supporting research into techniques of *in vitro* conservation and on the maintenance of genetic integrity during storage and regeneration of seed or cultures. Disease indexing to facilitate quarantine and germplasm exchange needs further consideration, and research will include investigations into non-destructive disease indexing methods. In the fast-moving area of molecular biology, IBPGR is closely monitoring developments and incorporating new techniques into its research.

To achieve its strategic research objectives IBPGR provides funds for contract research largely on a competitive basis, organizes workshops of specialists and produces publications so that appropriate research results are effectively transferred to the germplasm community.

The IBPGR program is organized in two sections, the Field Programme and the Research Programme, both serviced by headquarters and a number of staff located in various parts of the world. By far the largest number of staff serve the Field Programme because a substantial part of IBPRG's resources has been and continues to be devoted to strengthening national programs of developing countries which are carrying out, or are willing to carry out, collecting, conservation, documentation, training or other activities which IBPGR considers of a priority character.

IBPGR's strategy with regard to support to national programs of developing countries is to provide a package of appropriate advice and assistance. Moreover, the assistance is tailored to the requirements of each country depending on the strength of the national programs and the physical facilities and expertise available. The priorities of IBPGR play a major role in guiding the Field Programme. With a limited budget IBPGR cannot respond to all individual and localized needs, many must be national or bilateral responsibilities.

Global genetic resources network

The primary aim of IBPGR has been to establish and develop a network. This global network has threads of influence extending to plant breeding organizations, genebanks and university departments. At the moment there is a network of IBPGR-designated base collections of specified material, linked together by the actual or planned duplication of the germplasm as an insurance against loss. Interlocking institutional arrangements and operational strategies function together to conserve and make available germplasm.

Base collections

By the end of 1987, IBPGR-designated base collection of seeds will have been expanded to include tropical and subtropical priority forage legumes and grasses. As many as 37 institutes in 29 countries are now involved, at least half of them in developing countries. All the major cereals, food legumes, root crops and vegetables are covered. For example, designated Asian collections of barley, maize, *japonica* rice and soyabean are held here in Tsukuba, and there are other designated collections, in among other places, Japan, Thailand, the Philippines and China.

Active collections

Active collections hold seed or vegetative materials under medium-term storage conditions for current use. These collections undertake regeneration and evaluate, mutiply and distribute the material. Where the base collection is held separately from any active collection, IBPGR's strategy is for the designated centre to hold the base collection and to enter into collaborative arrangements with one or more centres holding active collections of the crops and which undertake functions of medium-term storage. In the case of many crops these essential links remain to be established or to be fully worked out.

Data management and transfer

IBPGR is providing advice, operating standards, hardware and software to national programs for documentation activities, especially on specific crops. A major effort will be directed towards the development and/or strengthening of the systems of management of collections, in addition to establishing data bases for passport and characterization information and the merging of data from national programs into central crop data bases.

Germplasm acquisition

The collecting program of IBPGR currently concentrates on crops and areas where there are real threats of genetic erosion. For cultivated materials which have already been extensively collected for most major crops, the intention is to concentrate on selective collecting to fill gaps in collections and improve their representative variability.

However, for many wild species we lack information on distribution, times of flowering and seeding and so we have had to initiate lengthy preparatory research using herbaria. Such research is ongoing for okra, *Vigna*, Aurantioid relatives of *Citrus*, wild *Allium* and some

groups of the *Triticeae*. A study on *Mangifera* species was finished in 1985, and is now being translated into field work in Kalimantan, Sumatra and Sabah.

In parallel with such research, IBPGR staff are developing briefing lists for field workers, including practicable and comprehensive keys for identification.

All collected materials are distributed to genebanks for conservation. IBPGR has found it necessary to fund a small Germplasm Distribution Unit at Kew, UK, which cleans and dries material from Africa and adjacent areas, tests viability and sends sealed samples ready for deposition in genebanks of national and international centres. This work will continue until national centres have adequate facilities. IBPGR is also establishing a similar unit in the University of Singapore in 1988 to perform similar tasks for Asian coutries.

Germplasm characterization and evaluation

IBPGR has for many years developed descriptor lists for most of the major crops and a number of minor crops. In parallel, IBPGR provides technical advice and help to genetic resources centres, for the organization of their data on collections. However, this is slow because only a small portion of the germplasm collected so far has been characterized and evaluated. It involves the growing out and data capture of samples, and we have found the need to establish centralized crop data bases as essential to the wide community of users.

IBPGR's support for data bases covers a wide front. Apart from continuing to support the assembling and ordering of passport and descriptive information on existing collections, there is the need to fill gaps in data for older significant collections and cross- referencing to geographical and climatic data bases. Such work will facilitate the value of information to scientists, breeders and other germplasm centres.

Training

IBPGR has always supported training activities. It is essential to ensure the availability of technically skilled personnel to carry out genetic resources work effectively in the global network. The Board's training program has gone a long way in providing manpower for national, regional and international organizations. However, there is still a shortage in most of the developing countries and continued emphasis on training is required.

There are four subprograms: postgraduate courses, specialized short training courses, individual training programs, and intern fellowships.

IBPGR support to training had a major impact on the rate of development of programs in Southeast and East Asia. The more generalized training will eventually be phased out. We shall continue to work with colleagues in the regions to foster the introduction of genetic resource into related degree structures. This has already happened in Indonesia, Thailand and the Philippines. At the same time, training activities of International Centers such as IRRI and in Japan supplement our efforts.

In vitro culture research

IBPGR has taken a lead in initiating *in vitro* research as it is an area full of potential, for the maintenance of clones of interest to breeders and for wider vegetatively propagated materials of crops like sweet potato, cassava, aroids, together with cocoa, coconut, banana and other fruits.

In vitro active genebanks will contain cultures under conditions of slow growth and *in vitro* base genebanks will contain cultures under cryopreservation. Both systems must be linked through multiplication cycles which involve subculturing and monitoring for genetic stability.

In the past 2–3 years, IBPGR has developed the basic principles for the design and operation of *in vitro* genebanks, and a pilot active *in vitro* genebank is currently under test. Materials cultured *in vitro* must represent as wide a range of genotypes as possible and most culture protocols developed for purposes other than conservation need to be researched.

There has been one practical spin-off from the *in vitro* work. Cost-effective methods of germplasm collection in the field of some crops would be greatly enhanced if *in vitro* techniques were used. IBPGR developed such a technique in 1984–85, using simple, rather than sophisticated, technology. So far this has been tested for cacao, wild cotton and cassava and is being developed in other species such as tropical grasses. Another technique has been developed for coconut and will be implemented by national programs in Southeast Asia.

All materials accessed into *in vitro* collections need to be indexed and cleaned to prevent the spread of disease. This is obviously important and IBPGR will be investing time and resources in sponsoring research. One such project has already started. AVRDC is at the moment culturing the meristem tips of sweet potato collected in Asia and the Pacific and indexing them for viruses.

There are virtually no good scientific data on genetic stability of *in vitro* cultures. In 1986, the Board initiated projects to examine the stability of cultures in storage under both slow growth and cryopreservation. It is expected that these will be completed in 1988–89, but further research is expected to be necessary.

Genetic diversity research

Many of the past projects of IBPGR have led to further research on materials collected and to the clarification of species relationships and evolutionary pathways. IBPGR recognizes an urgent need to increase its role in this area because of its emphasis on broadening the genepools in collections with representative samples of related wild species. However, the scientific knowledge base is still slender in many groups of crops of priority to IBPGR and to breeders. For a large number of priority wild species, the limits of distributional areas are uncertain and flowering and seeding times are unknown. In order to promote effective field work, research needs to be carried out.

In relation to wild species and forages in particular, ecological data are needed in the passport data files.

Isozyme analysis

Patterns of genetic diversity can be analyzed by isozyme analytical studies in restriction fragment length polymorphism analysis and correlations with ecogeographic distribution patterns sought with morphological and genetic character combinations.

Seed conservation research

During the past decade the Board's research on seed conservation has focused on basic investigations on seed physiology and practical methods of handling seed in genebanks. The research supported has resulted in both conceptual and technical recommendations for seed conservation. Some of the techniques developed include a new concept of seed drying; the use of extra-low moisture contents and ambient temperatures for long-term storage; the modification of the equation quantifying seed deterioration; and the sequential testing method for monitoring seed viability. The Board's work has resulted in the publication of two benchmark manuals for seed conservation and handling.

Research on seed conservation will be expanded on seed dormancy, regeneration techniques, methods for disease indexing and plant health for seed exchange.

It has now emerged from research that for recalcitrant seeded species embryo storage will

probably be the preferred conservation method but more basic research still needs to be done. Furthermore, in relation to seed storage, changes at the subcellular level which lead to genetic deterioration in stored seed, especially during low-temperature storage, are imperfectly understood and research is needed in this area also. IBPGR will be stimulating such research.

Dormancy

Seed dormancy is a problem in genebanks as it interferes with germination tests. Dormancy is more common in wild materials, in particular amongst the forages. IBPGR will be supporting research on seed dormancy to develop improved techniques for overcoming these problems.

Maintaining genetic integrity is essential during regeneration of germplasm samples, especially in cross-pollination crops. The process involves an understanding of breeding systems and pollination mechanisms of the crop species and their wild relatives. IBPGR intends to carry out investigations which will lead to pragmatic guidelines for isolation and pollination control.

Conclusions

IBPGR operates on a global scale. However, Asia and the Pacific contain some of the world's most important crop genetic diversity. Collecting and conserving these resources remains critical, and IBPGR is committed to accomplishing these tasks. To back these activities, as I have said, IBPGR is urging more advanced areas of strategic research. Much of this research will be directly relevant to the particular problems of Asia and the Pacific, and IBPGR is determined to see solutions that are approriate to local conditions coming out of the projects. I hope that this outline of our international interests and activities has given you some idea of the current direction of IBPGR's programs and updates what we presented 7 years ago. This conference will be reporting much progress and will be presenting an enormous amount of new and valuable information. Let us continue to work together, develop new understandings and devise fresh approaches to much-needed plant genetic resources work.

PART 1

Status Reports

Chairmen
Dr Keun Young Park
Dr R. V. Valmayor

CHAPTER 1

Recent development of research on crop germplasm resources in China

DONG YUSHEN

It is well-known that China is very rich in crop germplasm resources. In the recent years, due to the great importance attached by government to crop germplasm, great progress has been made in the studies of crop germplasm resources in China.

In 1978, the Institute of Crop Germplasm Resources (ICGR) of the Chinese Academy of Agricultural Sciences (CAAS) was established. From 1979 to 1985, several research programs for the exploration and collection of nationwide crop germplasm resources were fulfilled, and a national genebank for long-term storage as well as other provincial genebanks for the medium-term storage were founded at same time. Research works on identification and evaluation of certain crops have been started. And a preliminary nationwide network for cooperative research of crop germplasm resources has been established.

The system of crop germplasm resources studies in China

In China, the research works on collection, conservation and identification of crop germplasm resources are mainly carried out by the National Academy (CAAS) and other provincial academies of agricultural sciences. ICGR is in charge of unified coordination of nationwide crop germplasm research programs. Research departments of germplasm in other institutes of CAAS bea. the responsibility for performing national programs for related crop research. The academies of agricultural sciences in each province, city and autonomous region have their own institutes (or departments) of crop germplasm resources, which are responsible for performing their own provincial research projects. The main tasks of each research unit are listed below.

Institute of Crop Germplasm Resources (CAAS)

This Institute is responsible for

(1) Organization and performance of nationwide key research programs;
(2) Exchange of crop germplasm resources with foreign countries;
(3) Long-term storage of crop (propagated from seed) germplasm for the whole country;
(4) Collection, identification and evaluation of grain crops and grain legume germplasm, medium-term storage of this crop germplasm from some provinces and abroad.

Departments of germplasm resources of other Institutes in CAAS

(1) Organization and supervision of important research programs on related crop germplasm resources;
(2) Medium-term storage of related crop germplasm from some provinces and abroad.
(3) Collection, identification and evaluation of related crop germplasm.

Institutes (or departments) of crop germplasm resources in provincial academy of agricultural sciences (PAAS)

(1) Collection and medium-term storage of local crop germplasm resources;
(2) Fulfilment on research, identification and evaluation assigned by ICGR and other professional institutes in CAAS;

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(3) Fulfilment on provincial programs.

NATIONWIDE RESEARCH NETWORK

ICGR is linked with departments of germplasm resources of other institutes in CAAS by providing guidance and supervising the programs assigned. ICGR also provides guidance to provincial germplasm research units.

A nationwide cooperative research network in respect to crops is organized. There is a unit responsible for each crop as follows.

Rice	ICGR and Chinese National Rice Research Institute, CAAS.
Wheat, Maize	ICGR.
Millet	ICGR.
Grain legumes	ICGR
Sorghum	ICGR and Liaoning AAS.
Barley, Broad bean	Qinghai AAS and Zhejiang AAS.
Oat, Buckwheat	Inner Mongolia AAS.
Broomcorn millet	Shanxi AAS and Ekzhao League Institute of Agricultural Sciences, Inner Mongolia Autonomic Region.
Sweet potato	Xuzhou Prefectural Institute of Agricultural Sciences, Jiangsu Province.
Potato	Keshan Prefectural Institute of Agricultural Sciences, Heilongjiang Province.
Cotton	Institute of Cotton, CAAS.
Oil crops	Institute of Oil Crops, CAAS.
Bast-fibre	Institute of Bast-fibre, CAAS.
Sugar beet	Institute of Sugar Beet, CAAS.
Vegetable crops	Institute of Vegetable Crops, CAAS.
Fruits	Institute of Pomology, CAAS; Zhengzhou Institute of Pomology, CAAS; and Institute of Citrus, CAAS.
Tea	Institute of Tea, CAAS.
Mulberry	Institute of Sericulture, CAAS.
Forage grasses	Institute of Grassland, CAAS and Institute of Animal Sciences, CAAS.

The units responsible accept research projects from ICGR and fulfil them cooperatively with related provincial units.

Collection of crop germplasm resources in China

In the past years, two ways of collecting crop germplasm resources were adopted in China. One was to rely on administrative forces to mobilize the masses to help with the collection. The other was to organize exploration teams from research institutes.

From 1979 to 1984, according to the instructions both from the Ministry of Agriculture, Animal Husbandry and Fishery and State Science and Technology Commission, a popularized and extensive collection of crop germplasm was performed throughout each province of China. It was called supplementary recollection, since there had been a similar collection activity in the 1950s. About 100 000 accessions of germplasm from more than 50 kinds of crops (including some duplications) were collected. And for recording agronomic characters and multiplication, all the materials collected were planted in the fields of provincial academies of agricultural sciences.

From 1979 to 1985, several large-scale activities of exploration and collection were

performed, such as exploration and collection of crop germplasm resources in Yunnan and Tibet, nationwide exploration and collection of wild rice, wild soyabean, Chinese gooseberry (*Actinidia chinensis*) and forage grass, as well as collection of apple and pear germplasm in northwest, southwest and eastern China and of peach germplasm from northwest China. There were also several collection programs at provincial level such as collection of wild kindred plant in Xinjiang Autonomous regions. Totally, about 50 000 accessions of crop germplasm were collected. In the recent years, explorations and collections in Shennongjia Mountains and Hainan Island have been undertaken. Meanwhile, programs financed by IBPGR for the collection of soyabean and *Triticeae* have also been carried out.

I shall now give a brief introduction of exploration in Tibet. This program was performed by ICGR and the Academy of Agricultural Science of Xizang (Tibet) Autonomous Region. In four years the total distance of exploration was 248 000 kilometres (accumulative). About 7 000 accessions of seed samples and 14 000 of specimens of grain crops, oil crops, bast-fibre plants, vegetable crops, fruit trees, forage grass, etc. were collected, many of which are valuable, for example:

Barley

Barley is the main crop in Tibet; it was grown widely and had great diversity. According to the collection of more than 3 000 accessions of cultivated varieties, three types viz. *Hordeum vulgare* L., *H. distichon* L., and *H. intermedium* Carlet, were found. Of which more than 200 varieties were classified according to the traditional taxonomy. Most of the accessions in the collection were naked barley, while the rest were hully barley. Wild barley is distributed in a broad area which was classified into two groups namely, *H. spontaneum* C. Koch. and *H. agriocrithon* Aberg. Wild barley could also be subdivided into two groups according to the length of rachilla. Several types of barley collected have not been seen in other parts of the world.

Wheat

Wheat takes second place in Tibet. About 2 500 accessions of cultivated varieties were collected. Most of them were *Triticum aestivum* L., the rest were *Tr. compactum* Host., *Tr. turgidum* L., *Tr. durum* Desf., *Tr. polonicum* L., and *Tr. turanicum* Jakubz. There was a hexaploid wheat, with spikelets freely separated, which was named *Tr. aestivum*. ssp. *tibeticum* by Professor Qiquan Shao.

Buckwheat

Buckwheat was distributed in a broad area, with great morphological and genetic diversity. More than 200 accessions were collected, including *Fagopyrum esculentum* and *F. tataricum*, as well as annual and perennial habitats of its wild species. It seems that Tibet was one of the centres of origin of buckwheat.

Rape

A large area of wild rape (*Brassica campestris*, *Brassica juncea*) was found to be distributed on mountain slopes of altitudes from 3 200 to 4 000 m above sea level.

Bast-fibre plants

Cultivated species *Cannabis sativa* L., *Linum usitatissimum* L. and *Boehmeria nivea* (L.) Gaudich were collected. Wild hemp could be seen everywhere. Wild perennial flax was distributed at an altitude of 3 500 m above sea level in Changdou.

Fruit trees

Fruit trees in Tibet were characterized by their great diversity, aged trees and wild habitats. Specimens collected included 181 species in 37 genera of 22 families.

The main cultivated fruit trees were walnut, peach, apple, pear, grape and pomegranate.

In Zuogong county, a special smooth shell peach tree was found, which had a circumference of 10 metres and was 1 150 years old. Several grape and pomegranate trees more than 200 years old were also found in the same county.

In a word, there are great varieties of crops in Tibet, and almost all cultivated crops planted in other places of China can be found in Tibet. At present, the materials collected from Tibet are being studied in several institutions.

Conservation of crop germplasm resources in China

Since 1980s, a batch of modern genebanks has been established for the conservation of crop germplasm resources. With support from the Ministry of Agriculture, Animal Husbandry and Fishery, the National Gene Bank at ICGR was put into use in 1985, in which a storage of more than 40 000 accessions has been preserved at present. Another larger new National Gene Bank, which was partly financed by the Rockefeller Foundation, was begun to be built in 1984 and will be used in the near future. More detailed information concerning conservation can be obtained from Professor Jiang Chao-Yu's chapter.

We plan to put 200 000 accessions into our National Gene Bank before 1990. Some 50 000 accessions are required each year. Where does this large number of seeds come from? As mentioned before, we have a nationwide co-research group for each crop which is in charge of compiling a seed catalogue and providing a national accession number after deleting the duplications. Then, according to the seed regeneration schedule, each medium-term storage provides seeds to the National Gene Bank.

Identification and evaluation

In China, most of the agronomic and morphological characters of crop germplasm collected have been observed, and extensive evaluation of characters has being started. In recent years, evaluation of resistance to diseases, insects and stress conditions and quality analysis have also been made. Now, I shall cite some of the related projects sponsored by our Institute.

Resistance to three diseases and two insects for foreign varieties of rice

From 1978 to 1984, evaluation of resistance to diseases viz. bacteria leaf blight, rice blast and yellow dwarf, as well as to two insects namely, rice leaf-roller and brown plant hopper for 3 510 accessions of foreign varieties was carried out cooperatively by 15 research units in 12 provinces, cities and autonomous regions. It was found that there were 186 varieties of rice resistant to blast, 161 varieties resistant to bacteria leaf blight, 39 accessions resistant to yellow dwarf, 142 accessions resistant to brown plant hopper and 6 accessions resistant to rice leaf-roller. About 130 000 records including all the above-mentioned data and 37 items of agronomic character descriptions have been printed, and are available now.

Cold tolerance of rice

From 1979 to 1985, about 2 700 accessions of rice were screened for cold tolerance at six growth stages i.e. seed germination, seedling, tillering stage, meiosis stage, flowering stage and milky stage. Results and methods employed are listed in the following table:

Test stage	Number of cold-tolerant varieties	Temperature (°C)
Germination	79	11
Seedling	168	12 and 8
Tillering	146	17
Meiosis	116	12
Flowering	201	15
Milky[a]	159	

[a] Late sowing at different experiment stations

The following are elite cold tolerant varieties: Zhongxin 134 from Japan was cold tolerant at five growth stages, Heixuan 5 from Yunnan could flower normally below 15 °C and Cer 21 from Zhejiang has high heritability of cold tolerance.

Millet resistance to blast

From 1980 to 1987, with the participation of 13 research units from 7 provinces, a total of 17 962 accessions of millet was evaluated. The result showed that 345 accessions of millet were resistant to blast, which was 1.9% of the total evaluated accessions. The varieties highly resistant to blast and with excellent comprehensive traits were Mingchuan Qinggu (from Henan Province), Zhaonong 1 (from Inner Mongolia Autonomous Region) and Gonggu 6 (from Jilin Province).

Evaluation of wheat resistance to rust, powdery mildew and yellow dwarf

This project is responsible to the Institute of Crop Plant Protection of CAAS. From 1983 to 1987, about 3 529 accessions of domestic wheat varieties and 5 243 accessions of foreign varieties as well as 717 accessions of wild kindred plants have been evaluated. By using Chinese physiological races of pathogen, 5 nurseries with respect to strip rust, leaf rust, stem rust, powdery mildew and yellow dwarf were set up. Several varieties resistant to rust were isolated. Most of the Aegilops accessions were found to be resistant to powdery mildew, while accessions from Agropyron and Leymus were found to be resistance to yellow dwarf.

In the near future, research works on evaluation of resistance to diseases and pests as well as tolerance to adverse conditions will be continued. Cytogenetic and biochemical studies will be also undertaken. We appreciate cooperation with many experts and scientists from all over the world in the field of research and evaluation of crop germplasm.

CHAPTER 2

Recent activities on genetic resources in Japan

S. SUZUKI AND S. WATANABE

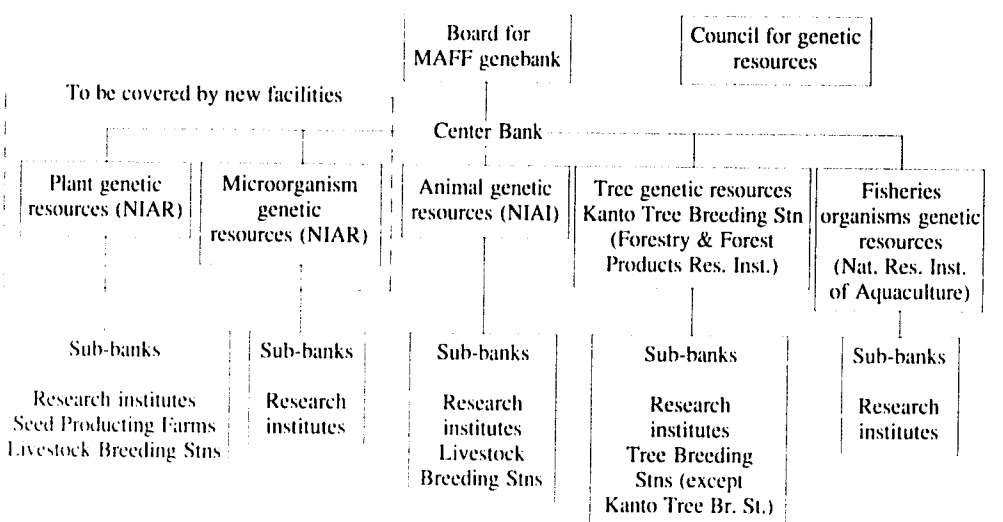
The importance of genetic resources for the increase of agricultural production, and finally for promoting human welfare, has been emphasized in Japan in recent years. Although it is not easy to concentrate the funds and human power needed to raise activities concerning genetic resources in a short time, some progress has been made in several aspects pertaining to this subject in these years. A combined effort has been made to collect, preserve and evaluate genetic resources by universities, prefectural or municipal agencies private sectors as well as by MAFF institutions. An increase in public understanding and interest on the subjects has been helping greatly the recent move to stimulate this trend.

The MAFF system

In December 1986 the Genetic Resources Centre of MAFF, composed of 10 laboratories in 2 departments, was established in NIAR, Tsukuba. The former Department of Genetic Resources was reorganized and strengthened by a significant increase in staffing. Department I takes charge of research problems on introduction, evaluation and information management. Department II is for preservation and its research problems. Subjects covered include not only plant materials but also microorganisms and animal germplasm.

A 10-year plan of the MAFF Gene Bank Project started in 1983 to meet urgent demand for introduction, preservation, evaluation and documentation of genetic resources among MAFF research institutions. It includes plant, microorganism, animal, tree and fisheries organisms as included in Table 1.

TABLE 1 The MAFF Genebank System.



NIAR : National Institute of Agrobiological Resources
NIAI : National Institute of Animal Industry

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Confining the subject only to plants, NIAR's Genetic Resources Center is to serve as a Center Bank, and is expected to play a uniting role in getting cooperation from the related organizations shown in Table 2. As for research institutions, the National Agricultural Research Center, 5 Regional Agricultural Experiment Stations, National Institute of Grassland Research, Fruit Tree Research Station, National Research Institute of Vegetables, Ornamental Plants and Tea, Sericultural Experiment Station and Tropical Agriculture Research Center are cooperating as Sub-Banks covering their specific crop or regional levels in relation to evaluation of new materials, development of breeding materials, seed multiplication and/or preservation and multiplication of vegetative ones. The National Center for Seeds and Seedlings with its 13 Farm Stations throughout Japan and 3 National Livestock Breeding Stations are also participating in the MAFF Gene Bank Project in the evaluation, seed multiplication and /or preservation and multiplication of vegetative plants.

Universities and Colleges and also the private sectors are cooperating with MAFF to efficiently collect, preserve and utilize precious genetic resources for the benefit of national as well as international welfare.

Practical aspects of this project are oriented towards 12 subdivisions of crop species or groups, namely, rice, wheat/barley, potatoes, beans, miscellaneous small grains/industrial crops, forage crops, fruit trees, vegetables, ornamental plants, tea, mulberry and tropical crops.

Twelve curators belonging to various research institutions have been appointed for each of these crop species or group in order to prepare proposals for exploration and collection, long-term integrated evaluation and documentation scheme and for novel budgetal suggestions or to review the status of preservation for the annual report.

Collection

Introduction and collection of genetic resources is a very important issue indeed for island countries especially for those with small and intensively developed land areas like Japan. Until recently introduction of genetic resources in Japan has been under spontaneous movements starting from initiatives of individual scientists. For example, collections of wheat and its relatives at Kyoto University and that of barley at Okayama University are world famous and important ones, but no major systematic multiple crop collections have been reported.

Research has been going on to help collection strategy. Classification and genetic analysis of various characteristics on physiological and ecological levels is opening a new horizon. Examples are given elsewhere in this book.

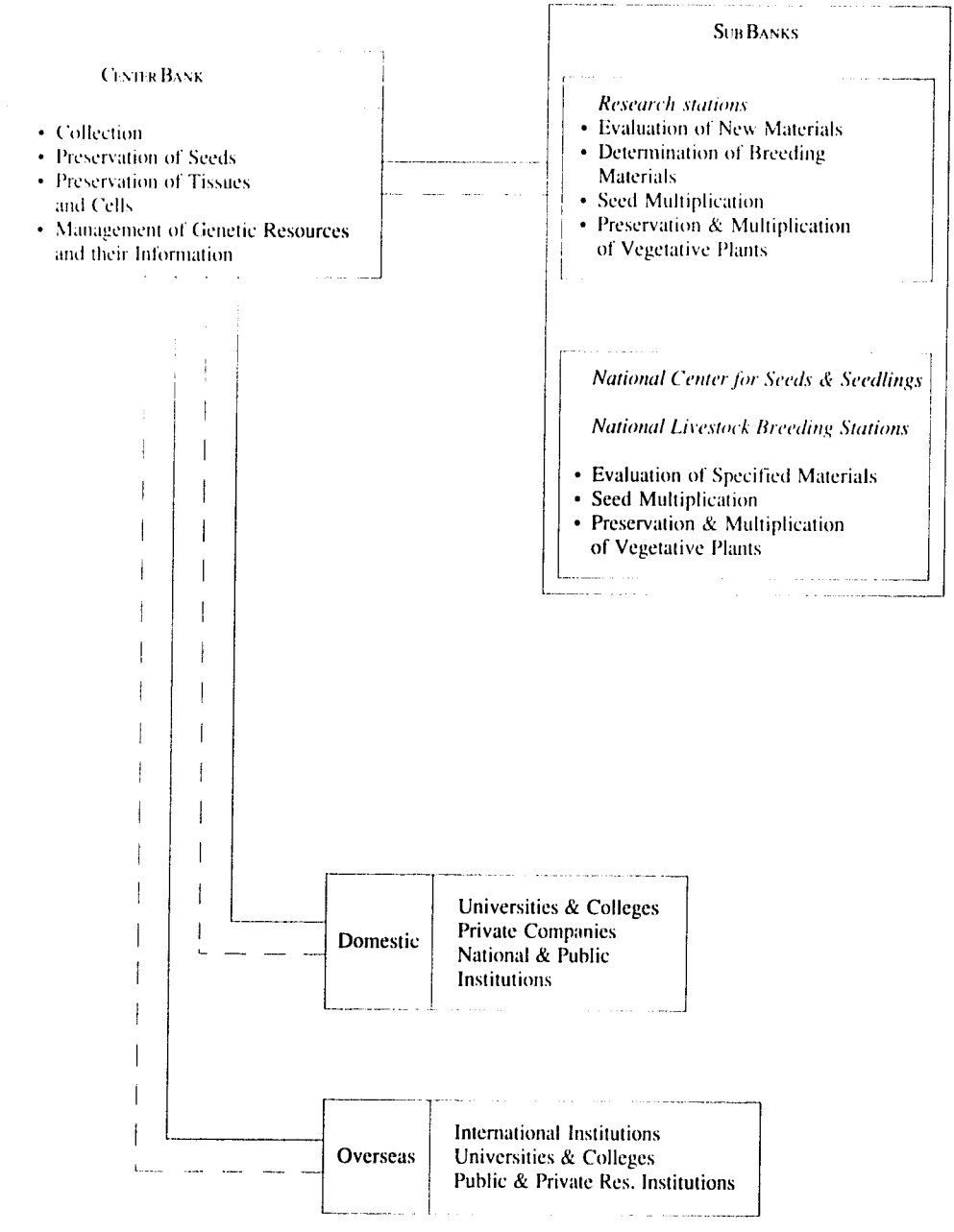
Emphasis has been placed on the importance of collection in recent years, and followed by the initiative of the Resources Surveying Committee of the Science and Technics Agency, Prime Minister's Office, a report on the available means for the acquisition of organisms for gene resources was announced in 1984.

The worldwide trend of genetic erosion, in which biological resources are being lost by social as well as environmental reasons, has been stressed and the growing importance and significant improvement in biological potentiality of new resources have been surveyed. Priorities in collection of resources for the coming years have been discussed (see Table 3).

From the standpoint of research and utilization value, plants and their wild relatives which have potentialities to show characteristics corresponding to various research or industrial purposes should be considered. While, from the standpoint of securing latent genetic resources, plants that have high potential for future utilization are to be examined.

Considering the above two points, the following standards must be kept in mind when collecting: 1) plants which are completely endangered and may be lost forever, 2) plants which are distributed mainly in the areas difficult to reach, 3) indigenous plants or those that

TABLE 2 Plant genetic resources system in MAFF.



indicates flow of genetic resources

indicates flow of information on genetic resources

TABLE 3 Priorities for Crop Introduction of Japan.

Standards I
Research and utilization value
a. Organisms valuable for research and utilized very frequently
b. Organisms suitable for utilization by agricultural, forestry, fisheries, manufacturing ,mining and other industries
c. Organisms suitable for utilization for environment conservation and other public welfare purposes.
Securing latent genetic resources
a. Organisms having high potential for future utilization
Standards II
Organisms liable to become extinct in near future
Organisms maldistributed, difficult to collect
Organisms indigenous to Japan or which have been developed domestically
Organisms not being utilized today but which were widely-used before

are domesticated especially in Japan, and 4) historically important but now almost abandoned plants.

Breeding laboratories have been collecting materials for many years of course. We can cite two examples of systematic internal collection: the local rice strain collection 1962–1965 throughout Japan, ie. from 23 prefectures, and the local corn strain collection 1953–1968 also covering major parts of Japan excluding Hokkaido Island. Over 1 300 and 670 accessions collected by both projects, respectively, have been classified and analyzed from the eco-geographical standpoint and have been used as important breeding materials. Internal collection of local strains and related wild types is again of interest more recently and efforts have been placed by many group of universities as well as by MAFF.

International expeditions are only possible by research cooperation with local scientists. Helped by this cooperation, we have received over 6 000 precious genetic resources from various parts of the world for these 6 years, relating only to MAFF activities, as shown in Table 4. We extend thanks here again for their generous cooperation. Japanese scientists are also taking part in IBPGR international expedition groups recently in various areas, supporting IBPGR activities as stated in its Annual Reports.

The exchange of already collected resources in quantity with established genebanks is very important in assuring a safeguard against unforeseen accidents and also in widening the availability of resources for potential use. Contact has been launched with CIMMYT, AVRDC, INTSOY, IITA and CIAT to extend this program.

Preservation

Preservation of standard (orthodox) seeds seems to have been well standardized for a long time, while, with some compromise, temperature and humidity of many seed storages are often managed at economic, but hardly sufficient levels. Recently a proposal on the use of liquid nitogen has been highlighted for the sake of long-range economy and possible genetic stability during storage. A small experiment has been initiated in NIAR to test its potentiality.

Preservation of vegetative plants is perhaps facing a revolution now. Space and labour-saving dwarfing techniques for field preservation, by special root stocks and/or growth regulating chemicals, has been drawing attention. Still more intensive economization of *in vitro* preservation may need some more time to be standardized in terms of growth regulation, controlled regeneration and also of genetic stability. Several research projects in Japan have been reported to be working.

Universities and institutions in the Ministry of Education hold some important plant collections as seen in Table 5. The wheat and *Aegilops* collection at Kyoto University is

TABLE 4 Plant introduction from abroad in recent years.

Year	Area	Target plants	Number of accessions
1981	Ivory Coast, India, Papua New Guinea Solomon Is., Fiji	Rice (wild), Sorghum, Taro, Yam, etc.	930
1982	Italy, France	Ryegrasses, Alfalfa,	130
1983	Thailand, Laos, Turkey, Egypt, Bangladesh, Indonesia, Mexico, Costa Rica, Peru, Bolivia, Philippines	Citrus, Soyabean, Peanuts, Vigna, Wheat(wild), Rice(wild), Tomato, Pimento, Pumpkin, Devil's tongue	1 240
1984	India, Thailand, Indonesia, North America, Korea, New Zealand	Tea, Grapes, Beans, Forage plants, Rush	1 770
1985	East Asia, South Europe, Argentina	Wheat, Barley, Beans, Root crops	980
1986	Morocco, Portugal, Spain, Thailand, Peru, Tanzania	Rice, Forage plants, Vegetables, Root crops	1 090
Total			6 140

recognized as one of the most important collections in the world for its plant group, and has been listed by IBPGR as an international base collection. The barley collection in Okayama University is also of world-famous value. Only well-known collections are listed in this table; there are still many other minor but specific collections remaining unlisted.

Trees and wild plants mainly in vegetative form are preserved in botanical gardens affiliated with universities or the Ministry of Education. For example, Koishikawa Botanical Garden of Tokyo University is noted for preserving more than 6 000 species besides its 1.4 million herbarium specimens.

The Medicinal Plant Research Station in Tsukuba affiliated with the Ministry of Health and Welfare holds over 2 000 medicinal plants in seed and/or vegetative forms.

TABLE 5 Major crop collections in Universities of Ministry of Education.

Kyoto Univ.	Wheat & its relatives	6 800
	Maize & its relative	800
	Other cereals	1 200
	Vegetables	595
Okayama Univ.	Cultivated barley	4 538
	Wild relatives	271
Tohoku Univ.	*Brassica* spp.	315
	(total)	652
Chiba Univ.	Ornamental plants from Latin America	300
Nat. Inst. Genetics	*Oryza sativa*	3 974
	O.perennis, Asian	203
	O.glaberrima	279
Toyama Univ.	Sesame	389
Kagawa Univ.	Vetch	152
	Vicia spp.	220

In the MAFF Genebank Project scheme, the total collection of genetic resources under all MAFF institutions is reported as 121 600 at March 1987 (see Table 6). Out of 95 700 seed samples, rice, wheat and barley and forage crops all have similar frequencies over 20 000. Vegetables and beans are about half. Among 25 900 vegetative plants preserved, forage crops and fruit trees are more important, followed by root and tuber crops and tea plants.

Because resources have been preserved not only in the Center Bank but also in various Sub Banks around Japan, redundancy in the accession was observed rather frequently at first. But this redundancy has been reduced so we think that this total number of 121 600 is now very close to being related to unique accessions only.

TABLE 6 **Present status of preservation of plant genetic resources in Japan (1986).**

Type of preservation	Crop species	No. of accessions preserved (x 10^2)
Seed	Rice	211
	Wheat & barley	234
	Beans	100
	Vegetables	123
	Forage crops	229
	Others	60
	Sub total	957
Vegetative crop	Fruit	61
	Tea	40
	Root and tuber crops	45
	Forage crops	62
	Others	51
	Sub total	259
	Total	1 216

Seed samples stored at the Center Bank, NIAR now amount to about 50 000 (see Table 7). Duplicate storage of base collection like wheat and *Aegilops* registered at Kyoto University (6 433) is include here. Rice, wheat and barley are most important, followed by beans, forage crops and vegetables. The mechanized seed store built in 1978 with a robotic handling system was designed to house about 50 000 accessions. Faced with nearly fulfilled capacity, a new genebank with a room for about 150 000 accessions is under construction on the NIAR campus. The robotic system is still being improved to save storage room and cost. Advanced research facilities and storage for DNA fragments, microorganism or animal germplasm are also installed in the new genebank. This will be completed in December 1987.

On accepting seed samples from outside, seed germinability is tested by the ISTA standard following the routine of the Germplasm Storage Center of our Center Bank. After a drying process (25°C, 15% relative humidity) for about 10 days, seed moisture is reduced to about 7–8%. Then the sample is divided in two. That for long-term storage, taken out only for regeneration purpose, contains seeds that are vacuum sealed in one or two 180 ml hermetic can(s) and stored under -10°C, 30% RH. The other part is stored in specially prepared vinyl bottles, transparent and square, under 0°C, 30% RH, for distribution purposes. Germinability is checked every 5 years by a stratified sampling technique to assure viability. Decline of germinability to under 50% or decrease of quantity by distribution will initiate the rejuvenation process starting from the original seed lot, selecting appropriate Sub Banks as for crop speciality and environmental adaptability of the accession. Initial germinability is very important for the longevity of seed, so, we are concerned to get good quality seed for

preservation. Attention is paid to the initial seed germinability of accepted seed samples.

Distribution of genetic resources has long been limited to national or public research organizations, but it was made to open to anybody including private companies for research use on January 1986. We now publish the list of available genetic resources, the latest edition of which published on July 1987 includes 42 125 accessions of 230 plants species. Distribution size is fixed for each species at about 50 grains for soybean or maize to 200 for small seeds.

TABLE 7 Present status of preservation of plant genetic resources as seed at NIAR (1986).

Crop species	No of accessions preserved (x 10^2)
Rice	123
Wheat & barley	185
Beans	62
Vegetables	35
Forage crops	55
Others	39
Total	499

The number of distributed samples may indicate the activity of a seed bank in a sense. A 4-year record is given in Table 8. Statistics here follow the calendar year. Cropwise statistics for the 1986 fiscal year (April to March) is shown in Table 9, of which beans are most important. Wheat and barley or rice follows this.

TABLE 8 Number of seed accessions accepted or distributed at NIAR in recent years.

Calendar year	Preserved accessions	Accepted accessions	Distributed accessions
1983	34 025	2 777	4 167
1984	35 793	1 768	6 933
1985	38 091	2 298	6 510
1986	42 125	4 034	7 141

TABLE 9 Accessions distributed as seeds of genetic resources at NIAR (1986).

	No.	%
Rice	1 483	18
Wheat & barley	2 731	33
Beans	3 205	38
Vegetable crops	601	7
Forage crops	212	3
Others	140	2
All accessions	8 372	100

Evaluation and documentation

Evaluation of genetic resources is of prime importance in making a large collection available for wide use. Specialists of some crop might know the potentiality of a certain accession only by the name or by place of origin, but when a result of primary evaluation is recorded, anyone

can select the right material for breeding. Recent advancements in biotechnology have broadened the potential users of genetic resources for various unexpected purposes, so evaluation data can practically help develop hidden availability of resources. In the MAFF Genebank System, the manual for primary evaluation of important crop species has been compiled and distributed to related research institutions and farms for practical use. Here primary evaluation means measurement or rating on mainly morphological or physiological traits easy to evaluate and also stable for places and for years. For more complicated but important characters which need care or expertise to measure, such as stress tolerances, productive or adaptive characters or chemical contents and others, cropwise manuals for standard evaluation methods are under preparation.

However, it is not an easy task to grow a large number of accessions at one time for primary evaluation, so data from separate evaluation trials following some standards are being assembled to construct the integrated database in a long range plan. Table 10 shows an outline of the evaluation and documentation process in the MAFF Genebank Project. There have been several separate databases of various characteristics before 1984, but data following the standard manual have been compiled since 1985. Some of the data taken for 1985 and 1986 still need standardization to be created into the integrated databases. All these databases are now open to related scientists through the MAFF Computer Network for on-line retrieval.

TABLE 10 Evaluation and documentation of collected genetic resources.

Crop name	No. of accessions in databases (until 1984)	Databases created in 1985–86		Total No. of evaluated accessions
		No. of accessions	No. of databases	
Rice	9 178	3 035	1	12 213
Wheat & barley	9 623	3 449	3	13 072
Beans	437	482	1	919
Potato	368	845	1	1 213
Forages	3 876	654	9	4 530
Fruit tree	797	492	11	1 289
Vegetable	1 090	167	3	1 257
Flowers	184	50	1	234
Tea	1 730	0	0	1 730
Mulberry	91	195	1	286
Miscellaneous	429	0	0	429
Total	27 803	9 369	31	37 192

Specific characters like stress tolerance, electrophoretic zymogram patterns, enzymatic activities, or amino-acid contents have been screened on an extensive scale for certain breeding or research purposes. Those data are essential in finding out very rare but useful genes out of a large number of resources collected worldwide. At present these data are mainly in the form of field notes or in mimeographs, so a move is now being taken to compile these and attach them to the integrated databases.

Passport data have been processed to construct the inventory database and those of accessions throughout different Sub Banks of the MAFF Genebank System were reported to the Center Bank. These are now under careful check and will be ready to be merged with those of accessions in the Center Bank in a while to establish the integrated inventory database throughout MAFF institutions. The Science and Technics Agency of Prime Minister's Office has been planning to establish a nationwide database of plant inventory through institutions of MAFF, universities, botanical gardens and even of private companies in order to help

efficient utilization of precious resources already stored in this country. A scheme for necessary descriptors to be filled in has been discussed for two years, and an initial step to start data filing is scheduled in 1987.

Utilization

Some of the collected resources have been utilized very effectively in plant breeding in recent years in this country. Introduction, collection and preservation of large amounts of genetic resources need a great deal of human power, investment and funding. These can only be rationalized by the successful utilization of resources in plant breeding for the welfare of whole mankind.

Traditionally, transfer of genes between organisms was only limited to sexual crossing and chromosome pairing. Useful genes in collected resources could only be transferred by crossing. But remarkable progress of biotechnology in recent years has opened new possibilities of gene introduction through asexual fusion of cells or even direct gene engineering. Utilization of useful genes in genetic resources is now not limited to the range of intercrossing groups but extended to any organisms in life.

Utilization of exotic genetic resources in plant breeding has become still more active recently. Yielding ability is sometimes much improved caused by heterosis effect using wild relatives in several crop species. Specific quality character like starch content or carotene content in exotic germplasm was efficiently used in sweet-potato breeding. Protein and amino-acid content in soyabean were thoroughly screened in domestic as well as exotic collections and isolated variants have been successfully utilized in breeding. Some enzyme-deficient mutant strains screened out of very many accessions of soyabean are expected to yield new cultivars without an unpleasant smell in cooking and to revolutionalize soyabean consumption in the diet.

Tolerance to excess humidity or wilting by some virus disease has been transferred to new cultivars from exotic germplasm located after screening among a large collection.

Another example of the utilization of electrophoretic variant caused by a new isoenzyme banding gene for the analysis of eco-geographical transient picture is presented in part 6 of this book.

Because it is proposed to discuss mainly the successful utilization of precious and in some sense very expensive genetic resources, several presentations on this topic will be thoroughly reviewed and examined in later chapters.

International cooperation

Genetic erosion has been going on rapidly in the developing areas important for genetic diversity. Need for genetic variability in view of research and industrial utilization is also claimed to be urgent. Distribution of these resources does not always overlap with that of availability of advanced technology to develop these resources. International cooperation guided by IBPGR will be a practical solution to this situation.

NIAR has been appointed as a registered genebank for international base collection for some plant species (Table 11). Some Japanese specialists have been participating in the IBPGR missions for expedition in recent years. Japan is continuously cooperating with and supporting IBPGR activities.

NIAR and cooperating institutions have been holding a training course on genetic resources sponsored by JICA since 1982. A general outline of the course is shown in Table 12. Until the 6th class in 1987 we have received 67 junior scientists from 19 countries as indicated in Table 13. JICA has been making significant contributions for establishing storage facilities in some Asian countries and also planning to extend its activity to help related research cooperation among these countries.

TABLE 11 Genetic resources designated by IBPGR for preservation.

Institutions	Plant genetic resources
NIAR	Maize, Wheat, Barley, Sweet potato, Cruciferous crops, *Allium* sp., Sugarcane, *Vigna*, *Glycine*, *Panicum*, *Chloris*
FTRS	Aurantiodeae
University of Kyoto	*Triticum* and *Aegilops*
Tohoku University	Cruciferous crops and wild species

TABLE 12 Current plan of the curriculum.

(1) General Orientation at Tokyo International Center, JICA (one week). Introduction of Japanese culture, history, geography, etc.
(2) Lectures of researchers of NIAR and other institution of Ministry of Agriculture, Forestry and Fisheries and from universities (three weeks)
  (i) Genetic resources in the world and international cooperation
  (ii) Principles of germplasm exploration and collection
  (iii) Test of seed germinability
  (iv) Long-term storage of vegetatively propagated crops
  (v) Management of information on genetic resources
  (vi) Diagnosis and treatment of viral diseases
  (vii) Production of virus free stocks
  (viii) Maintenance of germplasms by tissue,etc.
(3) Technical practices guided by instructors from institutions affiliated to the Ministry of Agriculture, Forestry and Fisheries (two weeks)
  (i) Seed testing
  (ii) Information processing in computers
  (iii) Plant tissue culture
  (iv) Production of virus free scions, etc.
(4) Individual training and report making (two weeks)

Future perspectives

We are trying to enlarge the range of our plant species even to include wild species as far as possible. We invite collecting further diversity of resources at national as well as international levels. Safety and longevity will be assured by more strict maintenance of temperature and humidity; measures to monitor genetic stability will be one of the important research items in the near future. We plan to increase our field activities through cooperation with local scientists to collect endangered resources in Asian countries. Exchange of stored resources for evaluation and utilization is to be promoted more extensively under mutual understanding.

Information of good quality should be aimed at through easier and more convenient forms. The list of available accessions is to be revised to incorporate more information useful to users. Information exchange on the availability of resources as well as evaluated data should become more accessible through floppy disks or data communication lines. In the near future we may start distributing useful genes in the form of DNA fragments in vitro instead of in seed or live plant forms. We need to be well prepared technically as well as on the informational aspects to meet these circumstances. Small but frequent meetings to exchange technical progress in East Asia are earnestly recommended under the regional activities of IBPGR.

TABLE 13 Group training course in plant genetic resources.

Country	1982	1983	1984	1985	1986	1987	Total
Bangladesh	1				1	1	3
China	2	1	1	1	1	2	8
India			1	1	1	1	4
Indonesia	2	1	1	1			5
Korea	1	1	1	1	1		5
Malaysia	2	1	1				4
Pakistan				1	1		2
Philippines	2			1	1	1	5
Sri Lanka		1		1	1		3
Thailand	1	2	1	1	1	1	7
Iran				1			1
Argentina	1	1	1	1	1		5
Brazil		1	1	1	2	2	7
Chile		1					1
Peru		1	1				2
Fiji	1	1					2
Burma						1	1
Nepal						1	1
Panama						1	1
Total	13	12	9	11	11	11	67

CHAPTER 3

Recent development of plant genetic resources in Republic of Korea

WAN SIK AHN

It is well known that Korea has remained a typical agricultural society for several thousands of years up to the present. Accordingly, many valuable crop genetic resources are being maintained in nature.

More than 3 000 temperate plant species out of 90 families are found in Korea. From these more than 1 100 plant species are used for various purposes including 150 species of food crops or fruit trees, 711 species of medicinal palnts, 250 species of vegetables, and other ornamental plants. In Korea, it is advisable to collect and preserve the land race of food crops such as grain legumes, cereals and especially the medicinal and allied plant species.

Due to small land area and the preference of farmers for the newly developed crop varieties, most of the indigenous crop species except for a few minor crops were very rapidly replaced by the new varieties. Though much effort has been given to collecting, preserving, multiplying, and propagating the crop genetic resources from the early days of crop improvement, many indigenous crop species have already disappeared or are near extinction.

Integrated and systematic conservation of crop genetic resources was started in 1976 when the Seed Management Function was established under the Rural Development Administration (RDA, formerly the Office of Rural Development). But the organization and the operation of the Seed Management Division are still in their infancy in respect of modern management of plant genetic resources.

In Korea, more efforts are being given to the collection and preservation of land races from the wide range of crop species. At the same time, the utilization efficiency of plant genetic resources is being included through extensive evaluation for the resources preserved and computerization of detailed information obtained.

Organization

Germplasm Management Division (GMD) is being organized as one of the four divisions of the Research Bureau under Rural Development Administration. There are fourteen affiliated research institutes and experiment stations which are directly controlled by the Research Bureau. Nine Provincial Rural Development Administrations have also indirect connection with GMD through the Research Bureau of RDA.

As shown in Figure 1, GMD plays the central role for any researches related to plant genetic resources. With a consultation from the Crop Advisory Committee, it sets up a basic plan for the related researches or plant genetic resources and undertakes its responsibility for base and active collection.

At the same time, it carries out some basic research on plant genetic resources. GMD is also responsible for computerization of the data obtained at the affiliated research institutes and experiment stations and affords a gateway for international cooperation between RDA and other foreign countries. After collection, multiplication and rejuvenation the plant genetic resources are evaluated at related research institutes and experiment stations such as the Crop Experiment Station, the Horticulture Experiment Station, and the Wheat and Barley Research Institute. The seeds multiplied at the affiliates are sent to GMD for storage.

Colleges and private seed companies have no direct relationship with RDA, but they are advised to store the crop seed at RDA with the exception of some germplasm they keep.

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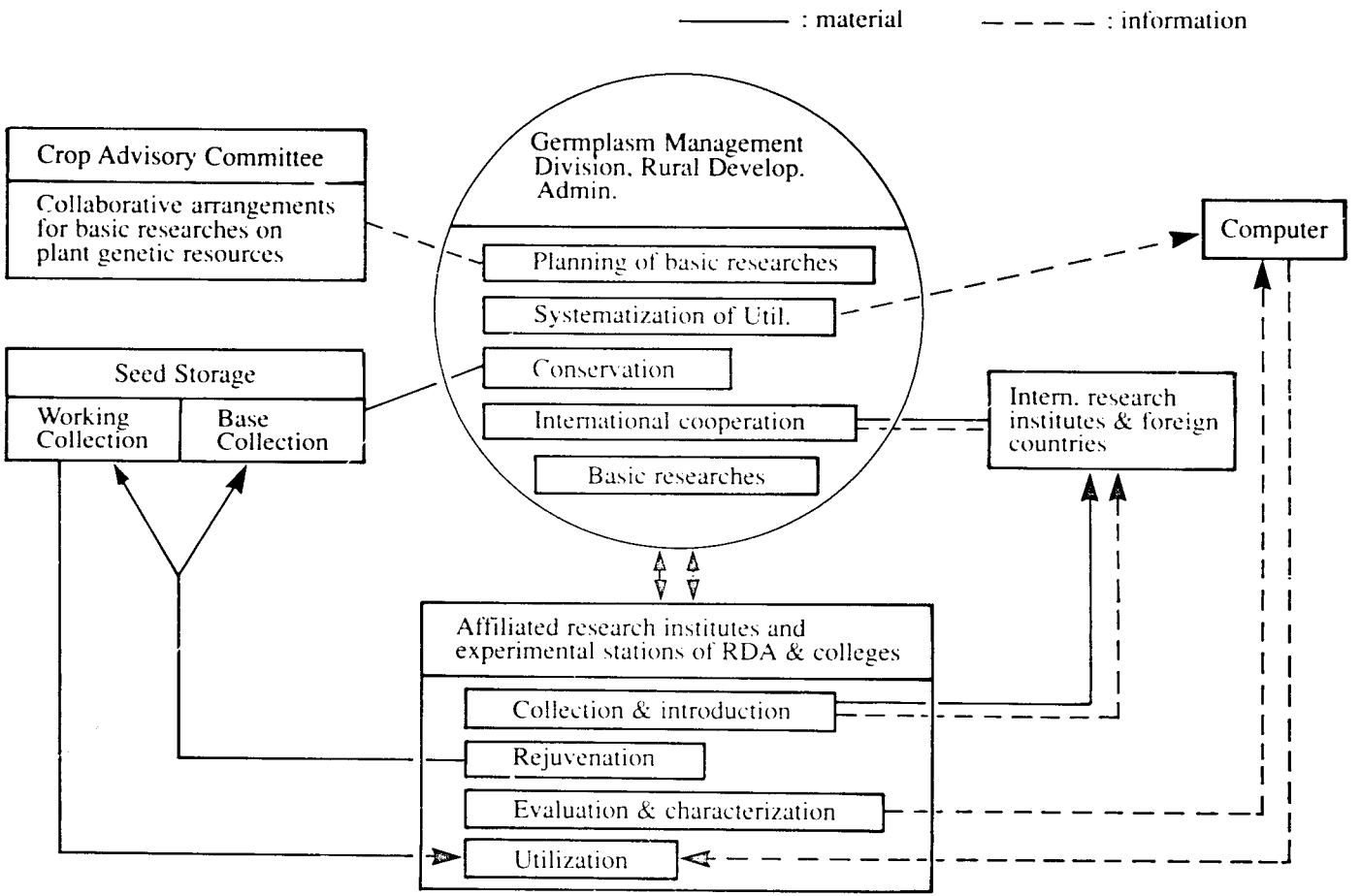

FIGURE 1. Major working components for national crop genetic resources in Korea.

Facilities of seed storage at GMD

Total room area of current seed storage at GMD is 103 m² of which 34 m² is for the base collection (-10°C, 30% RH) and 69 m² for active collection (-1°C, 30% RH).

New facilities will be constructed in Suwon, RDA headquarters by early 1988 for exclusive use. There will be about 3 times the existing floor space to house storage facilities, and laboratories to international standards as shown in Table 1.

Each room is designed to be equipped with the mobile rack systems so that the space can be effectively used.

TABLE 1 The new seed storage facilities of GMD in the Rural Development Administration.

Descriptions	Temperature (°C)	Humidity	Floor area (m²)
Cold rooms 1,2	-18 ± 1	Frost free	43.2 x 2 rooms
Cold rooms 3-6	4 ± 1	40% RH	43.7 x 4
Seed drying room	20 ± 1	10% RH	34.6 x 1
Laboratories and machine room			1 259.2
Total			1 536

Present activities

BASE AND ACTIVE COLLECTION

At the present time, approximately 59 000 seed samples out of 133 crops species are under conservation at the seed storage of GMD. The number of seed samples preserved for major crop species is shown in Table 2. Many of the seed samples are those of staple food crops, such as rice, barley, wheat, soybean and corn.

Seeds preserved for the base collection are either canned or put in aluminium boxes and stored in the room at 30% RH and -10°C. For the active collection, seeds are put in cans or paper envelopes and stored in the room at 30% RH or constant moisture content of 8% and at -1°C.

In the new seed storage under construction, the seeds for base collection will be dried to a 4–5% moisture content and put in three-fold laminated aluminium foil. The foils will be stored in frost-free conditions at -18°C. For active collections, the dried seed will be put in rasine (ie. plastic) bottles and stored at 4°C and 40% RH.

RDA has intensified its genebank management since 1985 in various fields of collection, preservation, evaluation and utilization for the plant genetic resources, especially the collection of land races faced with eradication. As in other countries, many land races of staple food crops such as rice, barley, wheat and legumes were rapidly disappearing along with the dissemination of the newly developed leading varieties. Hence, the collection and preservation of the remaining land races was believed to be one of the most urgent tasks related to plant genetic resources.

About 8 000 agricultural extension workers in every sub-county all through the country were efficiently recruited to collect genetic materials.

Extension workers have two merits: 1) they have good pre-information about crop varieties and regional circumstances; 2) they can collect materials all through the country at one time. To get a better response, they were told the reason why and how to collect materials. And, of course collection envelopes were sent to each extension worker.

In all, 1 731 extension workers responded and 5 171 seed samples covering 57 crops were collected in 1985 (Table 3).

The majority of crop seeds among the collection were those of grain legumes — 2 158 seed

TABLE 2 Number of major crop seeds stored at the seed storage of the Rural Development Administration in 1986.

Crops	No. of samples
Rice (*Oryza sativa*)	16 823
Barley (*Hordeum vulgare*)	11 501
Wheat (*Triticum aestivum*)	5 722
Soyabean (*Glycine max*)	4 871
Wild soybean (*G. ussuriensis*)	266
Adzukibean (*Vigna angularis*)	1 212
Mungbean (*Vigna radiata*)	507
Cowpea (*Vigna unguiculata*)	280
Kidney bean (*Phaseolus vulgaris*)	285
Corn (*Zea mays*)	4 168
Sorghum (*Sorghum bicolor*)	1 122
Red pepper (*Capsicum annuum*)	432
Radish (*Raphanus sativus*)	582
Tomato (*Lycopersicon esculentum*)	373
Chinese cabbage (*Brassica rapa*)	396
Peanut (*Arachis hypogaea*)	758
Perilla (*Perilla frutescens*)	628
Sesame (*Sesamum indicum*)	1 904
Rape (*Brassica campestris*)	256
Adlay (*Coix lacryma*)	361
Others	6 928
Total	59 375

samples or 41.7% of the total collection. Some 993 seed samples of soyabeans were collected, which suggested that Korea is a good storehouse of soyabean germplasm.

It is well known that the Korean peninsula is near or the original site of soyabean including Machuria. Seed samples for adzukibean, mungbean, cowpea, and kidney bean numbered 483, 199, 240 and 215, respectively.

Many seed samples of cereals and other crops were also collected: corn (293), sorghum (293), proso millet (64), Italian millet (177), Job's tears (183), red pepper (211) and pumpkin (108), oil crops seeds, perilla (215), sesame (162), castor bean (112), and rape (7) were also collected.

Landraces, as shown in Figure 2, were evenly distributed all through the country presumably due to small land area of the Republic of Korea. Upland crops such as *Glycine max*, *Vigna angularis*, *Sorghum bicolor*, and *Setaria italica*, etc. were collected in the mountainous area.

Evaluation

Before 1980, no evaluation of the plant genetic resources preserved in GMD was performed, but only parts of the materials used for breeding purposes were characterized at the related experiment station and research institutes. Characterization of 12 865 varieties for 49 characteristics was accomplished during 1980–1984. Along with the extensive germplasm collection from 1985, characterization for the materials preserved at GMD or newly collected was partially started by plant breeders at the related institutes. Since 1985 about 14 000 germplasms were characterized as shown in Table 4. The computerized results will be distributed to the people concerned.

Rejuvenation and multiplication

Every year, GMD tests the germinability for the germplasm preserved in both base and active

TABLE 3 The land races of crop plants collected in Korea in 1985.

Common name	Scientific name	No. collected
Rice	*Oryza sativa*	22
Barley	*Hordeum vulgare*	56
Wheat	*Triticum aestivum*	22
Rye	*Secale cereale*	42
Soyabean	*Glycine max*	993
Adzukibean	*Vigna angularis*	483
Mungbean	*Vigna radita*	199
Cowpea	*Vigna unguiculata*	240
Kidney bean	*Phaseolus vulgaris*	215
Pea	*Pisum sativum*	28
Maize	*Zea mays*	293
Sorghum	*Sorghum bicolor*	293
Italian millet	*Setaria italica*	177
Millet	*Panicum miliaceum*	64
Buck wheat	*Fagopyrum esculentum*	143
Sesame	*Sesamum indicum*	162
Peanut	*Arachis hypogaea*	10
Rape seed	*Brassica napus*	7
Perilla	*Perilla frutescens*	215
Castor bean	*Ricinus communis*	112
Job's tears	*Coix lacryma*	183
Red pepper	*Capsicum annuum*	211
Spinach	*Spinacia oleracea*	57
Cucumber	*Cucumis sativus*	14
Edible burdock	*Arctium lappa*	6
Potato	*Solanum tuberosum*	44
Pumpkin	*Cucurbita moschata*	108
Welsh onion	*Allium fistulosum*	54
Garlic	*Allium sativum*	41
Chinese cabbage	*Brassica campestris*	35
Egg plant	*Solanum melongena*	4
White gourd	*Benincasa hispida*	34
Chinese mustard	*Brassica chinensis*	17
Radish	*Raphanus sativus*	19
Lettuce	*Lactuca sativa*	44
Cotton	*Gossypium nanking*	11
Others		513
Total		5 171

collections. Any materials showing less than 70% germination are sent to the related research institutes for rejuvenation of the seed.

EXCHANGE AND DISTRIBUTION

RDA adopts an open-door policy on the basis of mutual fairness for international cooperation in the utilization of crop genetic resources. Up to date, the crop genetic resources were exchanged between the affiliates of RDA and the international research institutes or foreign countries. Accordingly, a small number of seeds only were disseminated to outside the country as shown in Table 5. But international cooperation on plant genetic resources including the introduction and distribution of crop seeds will gradually come under the jurisdiction of the Germplasm Management Divison of RDA.

Ahn : Recent Development in Korea (10)

FIGURE 2. Some Landrace collections of crops in Republic of Korea in 1985.
• Large : 100 items; · Medium : 50 items; · Small : 10 items

TABLE 4 Germplasm characterized at RDA affiliate research institutes in 1985–86.

Crop	Scientific name	Number characterized
Rice[a]	*Oryza sativa*	12 865
Rice	*Oryza sativa*	1 516
Barley	*Hordeum vulgare*	2 614
Wheat	*Triticum aestivum*	980
Oats	*Avena sativa*	244
Rye	*Secale cereale*	97
Corn	*Zea mays*	1 956
Triticale	*Triticale*	269
Soyabean	*Glycine max*	1725
Adzukibean	*Vigna angularis*	785
Sorghum	*Sorghum bicolor*	371
Sesame	*Sesamum indicum*	835
Groundnut	*Arachis hypogaea*	869
Rape seed	*Brassica campestris*	17
Radish	*Raphanus sativus*	203
Red pepper	*Capsicum annuum*	167
Tomato	*Lycopersicon esculentum*	174
Foxtail millet	*Setaria italica*	119
Cucumber	*Cucumis sativus*	55
Melon	*Cucumis melo*	36
Water melon	*Citrullus vulgaris*	10
Others		963
Total		24 469

[a] These evaluations are for the period 1980–84.

TABLE 5 Number of seed samples distributed by the Rural Development Administration.

Country	1983	1984	1985	1986	June 1987
Domestic[a]	3 145	2 246	1 788	1 288	8 336
Foreign	-	-	-	28	313

[a] Distributed to the research institutes, experiment stations and universities.

Future plan

RDA plans to centralize the preservation of crop genetic resources which are now maintained in colleges and private seed companies. This will be done when the blueprinted new seed storage, 1 536 m² in total area or about three times larger than the present seed storage, is built in early 1988.

RDA lays more emphasis on the preservation, evaluation, and characterization of seed-propagated crops and exploration of the wild relatives in Korea.

Better systematization and utilization of the information obtained on plant genetic resources is also one of the most important tasks facing RDA. Hundreds of seed samples and characterized information from the affiliates of RDA are sent to GMD each year; the data are well computerized for better use in the future. Up to date, no complete inventory for the crop genetic resources preserved at GMD has been made ready to use.

The most urgent need for RDA genebanks is to have personnel professionally trained on

plant genetic resources. RDA will give first priority to training of research personnel for the better management and handling of RDA genebanks in future.

Moreover, RDA fully supports the free exchange, cooperative evaluation and utilization of plant genetic resources among the countries and institutes concerned. At the same time, RDA would like to propose a cooperative exploration of plant genetic resources in any countries or regions interested.

CHAPTER 4

Present status, management and utilization of tropical vegetable genetic resources at AVRDC

CHONG-SENG TAY

The Asian Vegetable Research and Development Center (AVRDC) has a mandate for research and development of vegetable crops in the humid and subhumid tropics. Since AVRDC's establishment in 1971, genetic resources activities have been emphasized. Recently, a new facility was constructed with a triple cold-store capacity in accordance with the International Board for Plant Genetic Resources Resources (IBPGR) cold-store standard. The Genetic Resources and Seed Unit (GRSU), therefore, is adopting an even more ambitious plan for the future. Presently, the GRSU goals concerning germplasm are to:

- Promote the utilization of the collections through evaluation and comprehensive documentation;
- Build up base and active collections of vegetable species popular in the tropics;
- Strengthen AVRDC's cooperation within the international network of genebanks;
- Ensure that incoming and outgoing seed samples are free of pests and diseases.

Currently, AVRDC is the repository for the world mungbean collection and the Asian and Pacific sweet potato collection. Negotiations are currently underway between AVRDC and IBPGR concerning AVRDC maintenance of the *Capsicum* base collection. This chapter summarizes the present status, management and utilization of selected tropical vegetable germplasm at AVRDC.

Genebank hardware

The GRSU's functions include germplasm acquisition, conservation, characterization, coordination of evaluation, documentation, utilization, distribution, seed technology research and training. The new GRSU building consists of a genebank with a 75 000-accession capacity, a seed laboratory and a seed conditioning plant built at a total cost of US$353 032 in 1985. The floor plan of the different controlled environment seed stores, drying facilities and supporting laboratories with their controlled conditions are given in Figure 1. A detailed description of the building is given in Tay (1986). The performance of these facilities has been satisfactory during their 2 ½ years of operation. Continuous monitoring of cold-store temperatures and relative humidity is done with a 32-channel Molytek electronic recorder with alarm system. An electrician is on a 24-hr standby basis, and a spare generator is available in case of an electrical mainline power cut. Three levels of safety devices were incorporated in the cold store to prevent the accidental locking of workers inside.

Documentation is done on an HP 3000 minicomputer with a HP 150 microcomputer as terminal.

An aphid and whitefly-proof post-entry quarantine screenhouse was constructed with IBPGR funding to facilitate new introductions into the Asian and Pacific sweet potato collection. This facility had been test-run during the past 1 ½ years. The first batch of 100 accessions from Thailand was successfully introduced, tissue-cultured and met local quarantine regulations.

Present status of the collections

The current status of germplasm maintained at AVRDC, totalling 27 025 accessions is shown in Table 1. Efforts are concentrated on the principal crops, namely tomato, Chinese

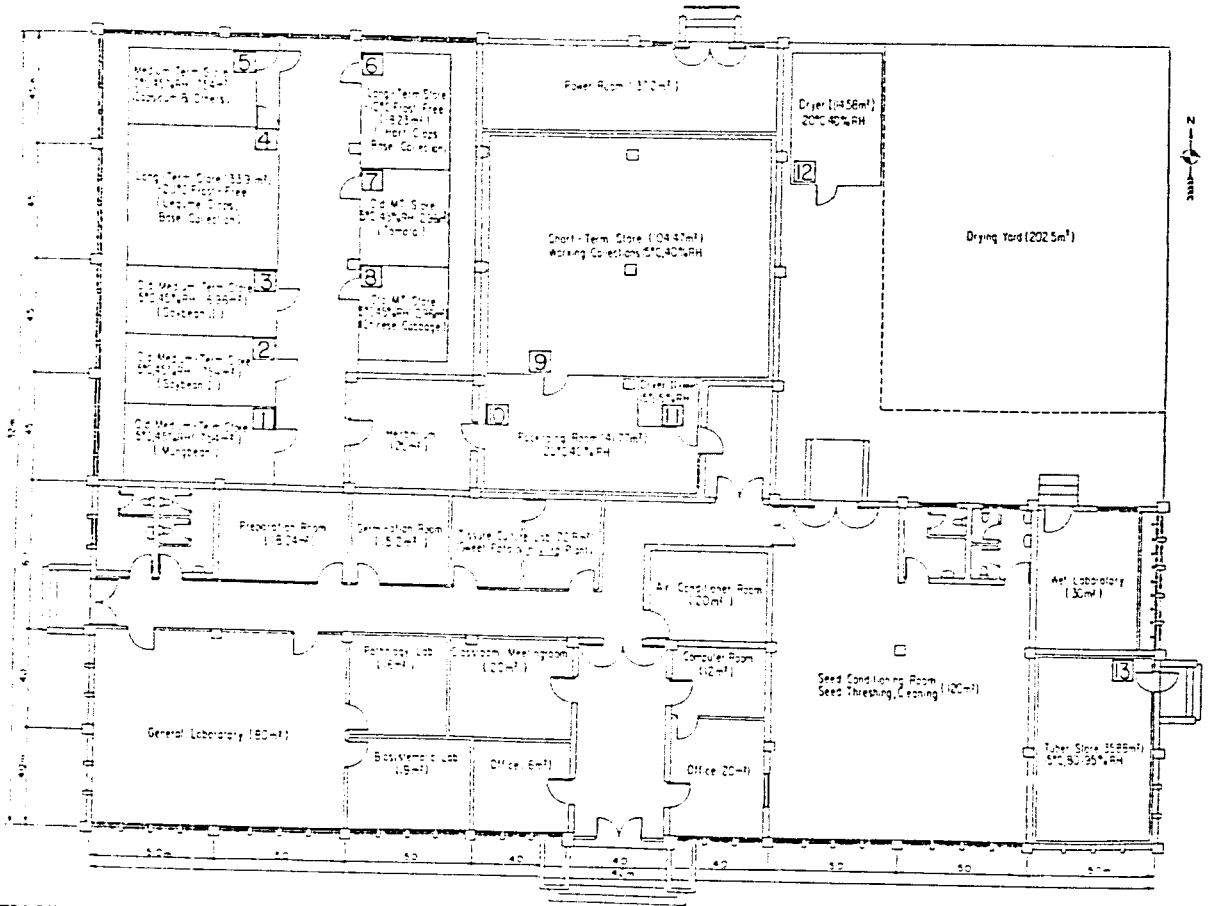

FIGURE 1 Floor plan of the GRSU building.

cabbage, mungbean, soyabean, sweet potato and pepper. Pepper is a recent addition. Until now, 3 475 accessions of pepper have been acquired including accessions from all of the major collections in the USA, Costa Rica, the German Democratic Republic, Thailand, Malaysia, etc. Batches of these accessions are being characterized and evaluated to support the utilization work. Accessions for base and active collections are multiplied in nylon net-houses.

TABLE 1 Germplasm maintained at AVRDC by GRSU in 1987.

Crop	Accessions	Missing	Available	Temporarily numbered accessions	Suspected duplicates[z]
AVRDC Principal Crops					
Soyabean	12 505	835	11 670	99	2 111 (9 034)
Mungbean	5 274	167	5 107	256	985 (5 112)
Tomato	5 484	138	5 346	617	40 (4 734)
Sweet potato	1 219	32	1 187	157	9 (1 200)
Chinesse cabbage	846	42	804	217	-
Pepper	2 500	30	2 470	1 005	-
Subtotal	27 828	1 244	26 584	2 351	3 145 (20 080)
Other Crops					
Adzuki bean	125	-	125	25	-
Amaranth	86	-	86	-	-
Black gram	158	-	158	539[y]	-
Rice bean	72	-	72	50	-
Sub total	441	-	441	614	-
Total	28 269	1 244	27 025	2 965	3 145 (20 080)

[z] Figures in parentheses indicate the total number of accessions from which the suspected duplicates were counted.
[y] Including 356 accessions (lines) of wild black gram purified from four populations.

The germplasm is maintained under five levels of storage, namely base collection (seeds are for permanent storage at -20°C and -10°C in hermetically-sealed packs), active collection (seeds are for distribution and are kept at 12° ± 3°C, 40 ± 5% RH) and working collection (15° ± 3°C, 40 ± 5% RH) for seed propagated vegetables, and a field genebank and *in vitro active* collection (20° ± 2°C) for sweet potato. The current base collections at AVRDC, including the recommended seed moisture content and minimum number of seeds stored per accessions, are listed in Table 2; the active collections at the medium-term storage are presented in Table 3. The working collections consist of seeds which are designated for long or medium-term storage, but are still under processing. The Asian and Pacific sweet potato collection is maintained in a field genebank as described in Tay (1985), and the *in vitro* collection as in Tay et al. (1986). Most of the accessions in the collections of the six crops have been duplicated in other major genebanks in Costa Rica, Japan, the Philippines, Taiwan (China), UK and USA. The viability of seeds in long-term storage is monitored every five years during the first ten years. Depending on the subsequent seed quality the monitoring interval will be adjusted and shortened accordingly.

Characterization and evaluation of germplasm are emphasized at AVRDC to identify the subgroups within each species, specific traits and the duplicates. The IBPGR descriptor lists are closely followed in characterizing mungbean, soyabean, sweet potato, tomato and

TABLE 2 Current status of base collections in long-term storage at AVRDC.

Crop	Accessions stored (no.)	Total accessions (%)	Storage room (units)	Seeds / accession[z] (no.)	Moisture content (%)
Soyabean	2 620[y]	22.45	5	4 000	8.0
Mungbean	1 437[x]	28.14	5	4 000	8.0
Tomato	713	13.34	5	12 000	5.0
Chinese cabbage	341[w]	42.41	5	12 000	5.0
Pepper	75	3.04	5	12 000	5.0

[z] Recommended number of seeds per accession.
[y] Non-USDA accession.
[x] Another 3 000 accessions (58.7%) will be stored in early 1988.
[w] Open-pollinated varieties.

TABLE 3 Current status of active collections in medium-term storage at AVRDC.

Crop	Accessions stored (no.)	Mimimum no. of seeds/Acc.[z]
Soyabean	11 769	500
Mungbean	5 363	800
Tomato	5 963	2 000
Chinese cabbage	1 021	2 000
Pepper	3 475	2 000

[z] When seed quantity reaches this minimum level, seeds are multiplied.

pepper. Some descriptor states were modified. Additional useful characters were formulated for each crop where necessary (Tay 1985; Shanmugasundaram et al. 1985). The GRSU prepared and field-tested a descriptor list for *Brassica campestris* for the IBPGR (Tay and Kuo 1985; IBPGR/AVRDC 1987). The characters evaluated, and the status of characterization are summarized in Tables 4 and 5, respectively.

Management of the genebank

The management of AVRDC's genebank is divided into three main sections. Firstly, the introduction and conservation section with activities which encompass introduction, post-entry quarantine, multiplicatiion, characterization, identification of duplicates, accessioning, seed processing, viability testing, packaging, storage, further characterization and evaluation. Utmost care is taken to prevent mechanical and genetic mixing and loss of accessions. The genetic selection and drift of each accession are minimized by multiplying the collections in the best seed production seasons at AVRDC (Table 6), growing more plants per accession and taking equal representative seed samples from each plant of an accession to obtain a composite sample for storage. The recommended plant numbers for germplasm regeneration of the AVRDC's principal crops are 160 plants (4 m × 2 rows) for mungbean and soyabean, 30 plants (7.5 m × 2 rows) for pepper and tomato, and 150–200 plants (15 m × 6 rows) for Chinese cabbage.

Secondly, the documentation section is responsible for monitoring the flow of an accession into and out of the conservation system, and the status of utilization and distribution.

TABLE 4 Characterization of AVRDC principal crops.

Crop	No. of morphological and physiological characterizations		Chemical characterizations
	IBPGR descriptor list	Additional	
Mungbean	23	13	protein, starch and fibre
Soyabean	26	1	protein, fat, starch and fibre
Chinese cabbage	66[z]	-	-
Pepper	37	2	capsaicin
Sweet potato	27	9	protein, sugar, starch, fibre and dry matter
Tomato	36	2	% brix

[z] AVRDC/IBPGR descriptor list (IBPGR/AVRDC 1987).

TABLE 5 Status of AVRDC germplasm characterization in 1987.

Crop	No. of morphological and physiological characters		Chemical characters	
	Accessions characterized	% of total	Accessions characterized	% of total
Mungbean	4 945	96.8	4 945	96.8
Soyabean	2 672[z]	22.9	2 622	22.5
Chinese cabbage	315[y]	39.2	-	-
Pepper	508[x]	20.6	-	-
Sweet potato	1 168	98.4	734[w]	61.8
Tomato	1 250[x]	22.8	-	-

[z] Non-USDA accessions.
[y] Open-pollinated cultivars.
[x] Ongoing in the fall of 1987.
[w] Accessions flowering at AVRDC.

TABLE 6 Best seed production seasons at AVRDC.

Crop	Season
Mungbean and Soyabean	fall
Tomato and Chinese cabbage	winter
Pepper	summer and winter

Passport, characterization, evaluation, inventory and distribution data are documented using an HP-3000 minicomputer together with an HP-150 microcomputer as terminal. It consists of germplasm, inventory and distribution databases. All the databases except for the inventory database are managed using the MINISIS database software package which was developed by the International Development Research Center (IDRC) of Canada. The passport data of all the crops are integrated in one data base. Similarly, the distribution data are together in another data base. The characterization and evaluation data are kept according

to crop. The inventory data base is constructed with self-designed and self-programmed software.

The current status of documentation of AVRDC's principal crops is given in Table 7. The Chinese cabbage catalogue (open-pollinated accessions) has been distributed and the world mungbean collection catalogue is now in the final stage of preparation.

TABLE 7 Current status of documentation at AVRDC GRSU in 1987.

	Soyabean	Mungbean	Tomato	Chinese cabbage	Sweet potato	Pepper
Passport data entry	100%	100%	100%	100%	100%	100%[z]
Characterization data entry	25%	97%	0%	37%	98%	0%
Germplasm catalogue	Spring 1988	Fall 1987	-	1986 (for OP[x])	Summer 1988	-
Seed inventory data input	0%[y]	0%[y]	0%[y]	100%	0%[y]	0%

[z] New crop in temporary database.
[y] Will be completed in spring 1988.
[x] OP = Open-pollinated.

Finally, the distribution section ensures that the collections are put to maximum use. AVRDC's genebank has one of the world's largest and most diverse collections of mungbean, soyabean, tomato, Chinese cabbage and pepper. Effort is carried out to distribute these materials anywhere in the world. A total of 166 different countries and territories have received AVRDC germplasm. The two prerequisites to satisfy any seed request are, firstly, that the germplasm should be used for research purposes only and secondly, the quarantine requirements of the recipient country can be fulfilled by AVRDC.

Activities of the three sections are interrelated and the interactions and major activities are summarized in Figure 2.

In order to manage the AVRDC genebank efficiently, a new introduction scheme was formulated in order to centralize and streamline germplasm activities from introduction to storage (Figure 3).

The unique features of the new introduction scheme are as follows:

- Centralized registration of all new introductions (temporary number allocation);
- Identification of duplicates in the new introductions;
- Implementation of suitable quarantine standards (in cooperation with AVRDC pathologists);
- Centralized allocation of accession numbers for new introductions;
- Centralized documentation and management of all germplasm databases.

The unique feature of the new scheme is that the accessioning process by the GRSU and the evaluation of new introductions by AVRDC's Crop Improvement Program scientists can proceed simultaneously. Furthermore, all scientists are kept up-to-date on new introductions. This scheme demands a close cooperation among scientists and has, so far, functioned smoothly.

At present, scientists continue to introduce new germplasm as before. However, now, new

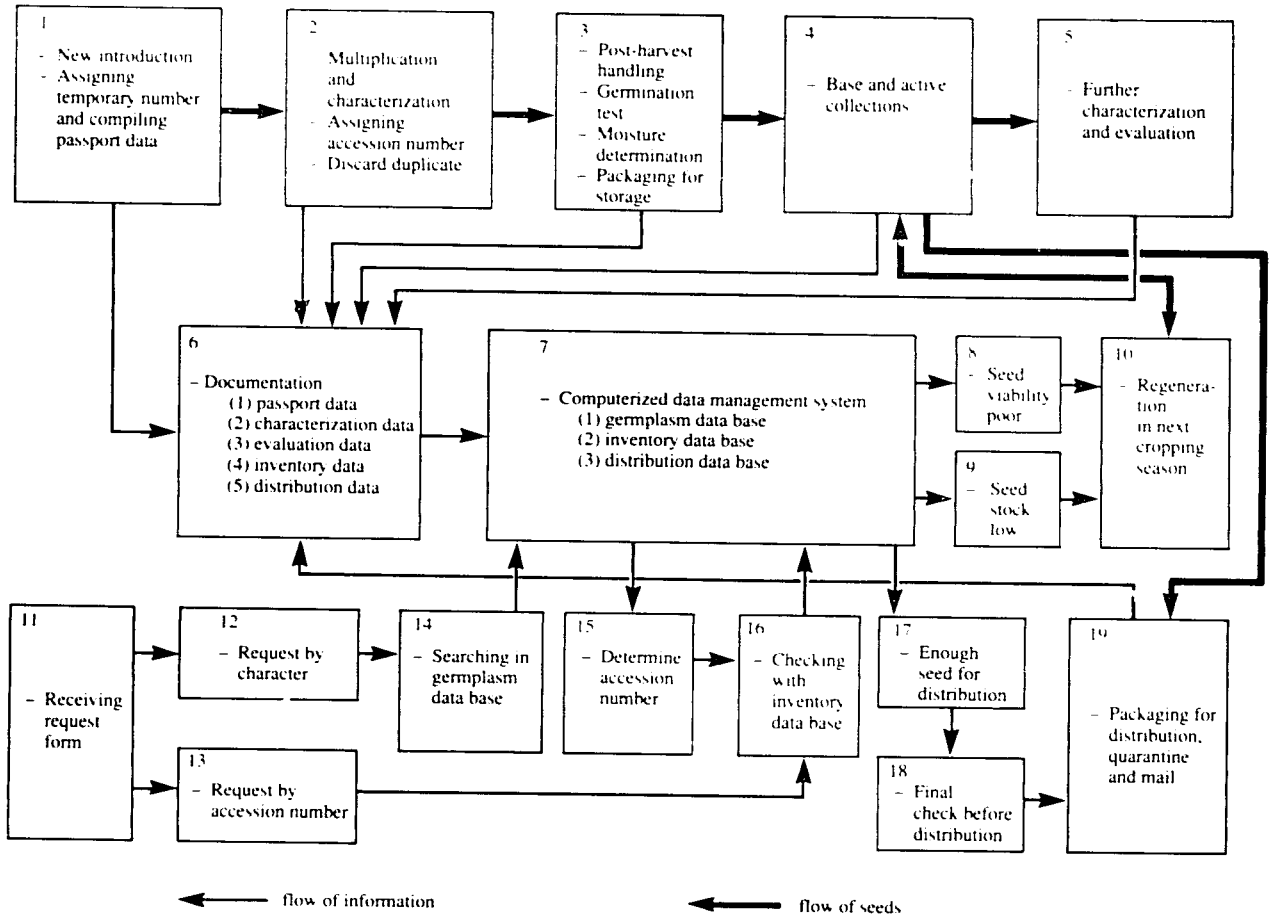

FIGURE 2 Genebank management scheme at AVRDC.

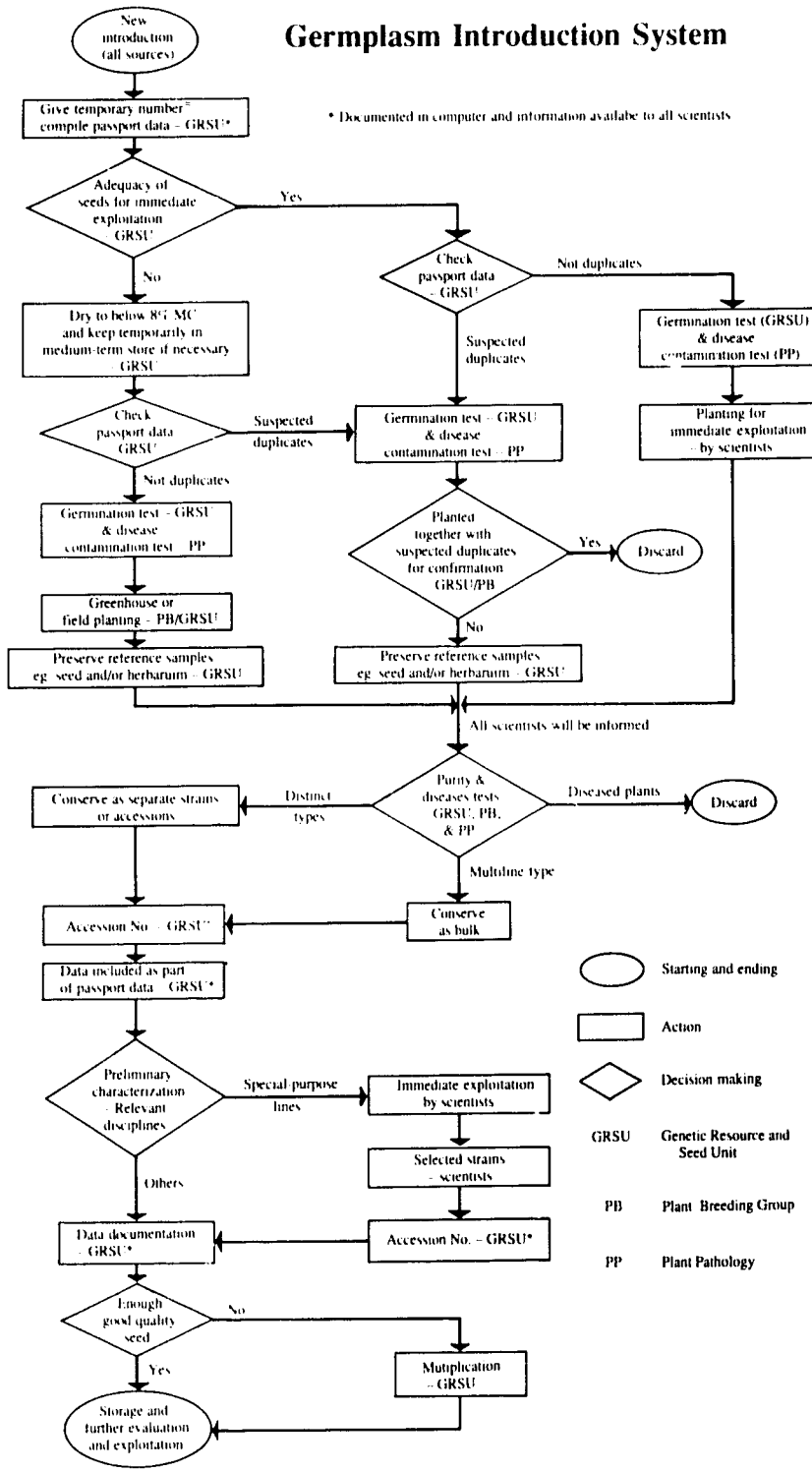

FIGURE 3 Centralized germplasm introduction scheme at AVRDC.

introductions are reported to the GRSU which allocates temporary numbers. The scientists use the temporary number during the preliminary screening and evaluation until a permanent accession number is given by the GRSU after successful multiplication and testing for duplicates. With this improved system, any clerical mistake or incomplete passport data can be requested immediately from the donor before documentation is complete.

During conservation, the Ito two-step seed-storage system is adopted for accession regeneration (Ito 1972). It allows for seeds to be multiplied for active distribution without advancing the regeneration cycle of the accession at every seed multiplication.

Sweet potato is a heterozygous vegetatively-propagated crop. In order to maintain the integrity of each clone a scheme was formulated and implemented at AVRDC for introduction, conservation and distribution (Figure 4).

Utilization of the genetic resources

At AVRDC, the entire collection has been screened and evaluated by AVRDC scientists for specific traits; 137 925 seed packets have been used for varietal improvement since 1975 (Table 8). Cooperation with scientists from national programs in the screening of germplasm for specific characteristics is also encouraged, and during the same period, 225 054 seed packets have been shipped to cooperating scientists in 166 countries (Table 8). The distribution of AVRDC's materials in the last decade has resulted in the release by local authorities of 118 cultivars of the five principal crops in 35 countries (Opeña et al. 1987). The systematic exploitation of the germplasm at AVRDC has resulted in an average of 8.11% of the collections being utilized for crop improvement by AVRDC's breeders (Table 9). Many of the identified traits for biotic and abiotic stress, adaptability and yield have been combined together (Opeña et al. 1987).

TABLE 8 Internal and external seed distribution by crop, 1975–86.

Crop	No. of seed packets		Total
	Internal	External	
Soyabean	48 770	85 856	134 626
Mungbean	49 911	57 156	107 067
Tomato	21 867	45 657	67 524
Chinese cabbage	8 321	16 806	25 127
Sweet potato[1]	5 011	5 732	10 743
White potato	2 347	1 401	3 748
Pepper	62	189	251
Others	1 636	12 257	13 893
Total	137 925	225 054	362 979

[1] Includes fresh roots, cuttings, *in vitro* plantlets and true seeds.

Future work and plans

The GRSU is working to further streamline the genetic resources activities at AVRDC with the newly-built genebank and auxiliary facilities, and a reorganized computerized data management system. Projects in the following areas will be given high priority:

- Further characterization and evaluation will be emphasized both in cooperation with AVRDC disciplinary scientists and researchers from national programs. It is the responsibility of the GRSU to further enhance the utilization of the genetic resources within and outside AVRDC.

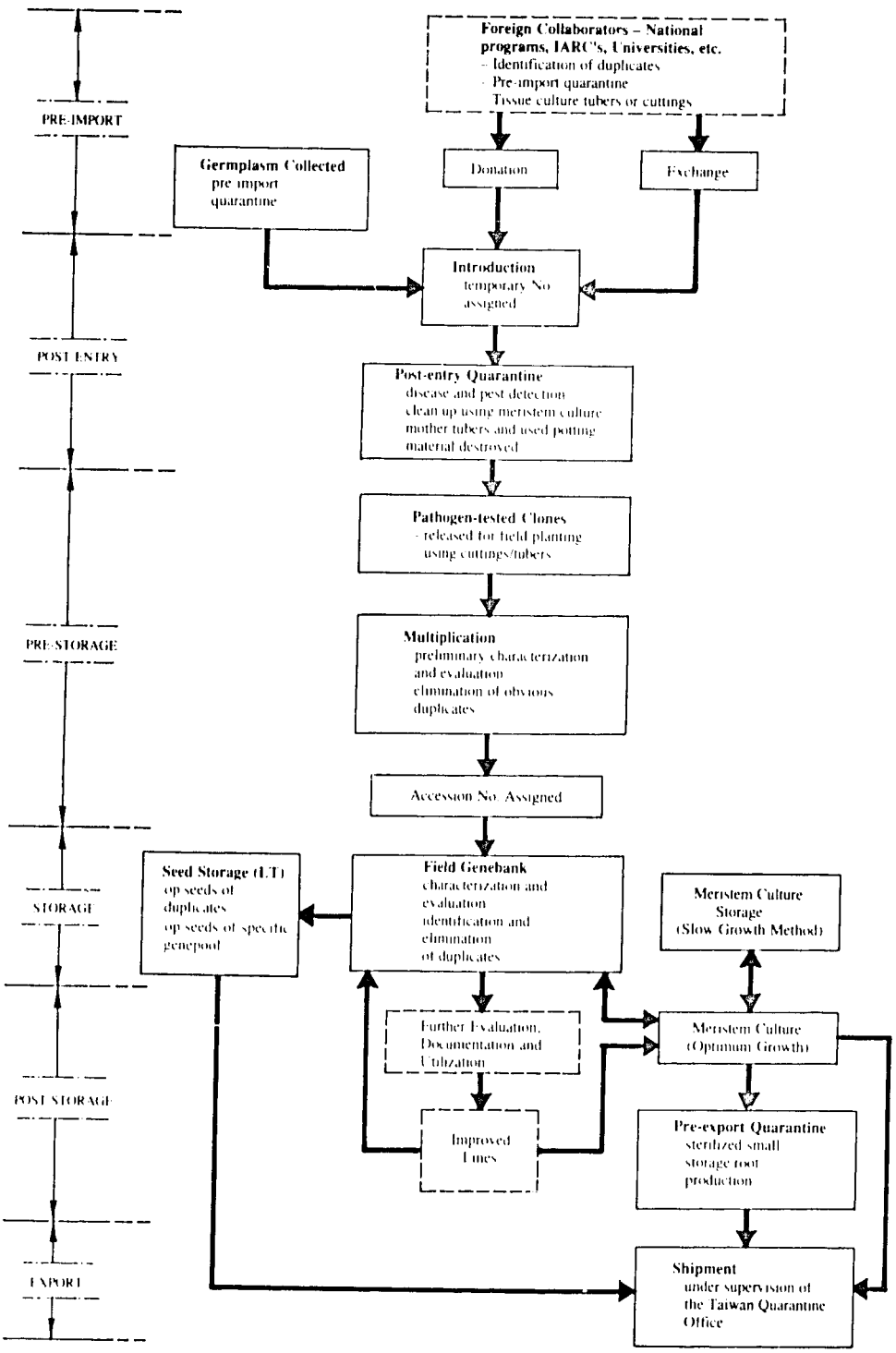

FIGURE 4 Sweet potato germplasm activities at AVRDC.

- Gaps in the collection of AVRDC's principal crops will be identified, exploration recommendations formulated, and collecting expeditions will be organized.
- Since the conservation of 'minor' tropical vegetables is generally neglected, the GRSU recently initiated activities in this area. So far, 113 accessions of 25 'minor' vegetables have been acquired from Southeast Asia, the Middle East and Africa, and stored at the GRSU.
- Training programs on genetic resources will be organized in cooperation with AVRDC's Training Office and other outside agencies.

TABLE 9 Number of accessions and percentage of each collection used in AVRDC breeding programs.

Crop	Period	Total crosses	Number of accessions used			
			As female	As male	As female or males	% of collection
Mungbean	1972–85	4 200	262	274	343	6.72
Tomato	1972–85	6 045	-	-	373	6.98
Soyabean	1973–87	2 487	-	-	501	4.29
Sweet potato	1973–87	1 875	132	92	135	11.37
Chinese cabbage	1973–87	374	-	-	90	11.19

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CHAPTER 5

Recent developments in plant genetic resources conservation work in Southeast Asia

RICARDO M. LANTICAN

Southeast Asia has its share of richness in genetic resources. But owing to population pressure, vast areas of forest cover and natural sanctuaries of wild life have been destroyed. In agriculture technological advances in production could cause a displacement of a significant segment of the natural genetic complex of crops. These realities have raised concern and mobilized people for action to forestall any further threat of genetic erosion.

During the symposium on Southeast Asian plant genetic resources held at Bogor, Indonesia in 1975, a resolution was passed which called for the creation of a working group to assess the problems in the region and prepare a plan of action for genetic resources conservation. The working group was convened in the Philippines the following year, constituted by representatives from Indonesia, Malaysia, Thailand, Philippines and Papua New Guinea. As a final organizational spin-off, the Regional Committee for Southeast Asia (RECSEA) came into being under the aegis of the IBPGR. A full-time regional coordinator was appointed by IBPGR with headquarters located in the FAO Regional Office in Bangkok.

The passage of a decade of work since that momentous year of organization has seen favourable developments in genetic resources conservation in Southeast Asia. For the details of such developments, two reports have been given excellent reviews - The IBPGR/RECSEA Newsletter 9, 1985 issue; and IBPGR's 'A cooperative regional programme in Southeast Asia, a five-year plan of action, 1985-89'.

This chapter highlights the significant developments mentioned in the two reports and provides personal viewpoints on certain issues.

Significant developments in Southeast Asia

1. Through independent in-country or regional initiatives of national programs and with funding from IBPGR, work on germplasm collection and maintenance of important crop species has intensified. Some 40 collection missions were conducted, adding to the existing collections some 20 000 new accessions. Collection work focused on crops representing the varied interest of participating country groups — mungbean, soyabean, groundnut, *Vigna* spp., winged bean, tropical vegetables, taro, sweet potato, yam, cassava, mango, durian, rambutan and banana. Indonesia and Malaysia undertook collection work for rubber, oil palm and cocoa. Collection for spices was undertaken in Indonesia. Philippines and Indonesia did exploration work for sugarcane and coconuts.

Under a collaborative arrangement involving the International Rice Research Institute (IRRI), national centres and IBPGR, some 11 000 seed samples of rice were added to the collection, bringing the total number to more than 75 000.

2. The international research institutes located outside the region collaborated with national programs in undertaking comprehensive plant collection missions for certain mandated crops and therefore, have assisted greatly in the rescue and conservation of germplasm materials native to the region.

3. The regional collaborative endeavours on germplasm conservation have transcended national barriers and protective interests. There has been a sharing of genetic resources and responsibilities for safe-keeping of the materials. For example the banana collections of Malaysia, Thailand, Indonesia and Papua New Guinea have duplicate sets brought to and

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grown in Davao, Philippines. The seed storage facility of the National Plant Genetic Resources Laboratory (NPGRL) of the Institute of Plant Breeding in the Philippines has agreed to keep a duplicate collection of mungbean, tomato and cabbages from AVRDC and the winged bean accessions from Papua New Guinea. Similarly, the storage facility of the National Gene Bank at the Thailand Institute of Scientific and Technological Research serves as the global repository for winged bean and the Asian facility for maize.

4. A number of countries in Southeast Asia have sought to strengthen their activities on germplasm conservation by enlisting governmental support and unifying the different activities on germplasm conservation among in-country research agencies.

5. Countries in Southeast Asia, namely, the Philippines, Thailand, Indonesia and Malaysia have established adequate physical facilities for medium- and long-term storage through national funding or funds provided by IBPGR, bilateral assistance of international bank loan assistance programs. The FAO/UNDP* projects in Laos and Vietnam have funded the establishment of medium-term storage facilities. For *in situ* conservation, a number of national parks, forest reserves and botanic gardens have been established in Indonesia, Malaysia, Thailand and the Philippines. For vegetatively propagated crops and those which produce recalcitrant seed, field genebanks have been established.

6. A program of manpower development for the region has been pursued through short-term technical training in selected institutions within the region or postgraduate training in the University of Birmingham, UK. Some 200 scientists from Southeast Asia have undergone short-term training and 23 have completed their masters or doctoral degree program in genetic resources work, thus providing a corps of professional people.

7. In recent years, plant breeding programs in the Philippines, Indonesia, Malaysia and Thailand have developed their capability for intensified research. The scope of research activities in breeding has grown to the extent that the ecological needs of the different production areas are addressed. The expanded activities will thus ensure that the available gene pools are more thoroughly evaluated, expanded in size and eventually utilized in varietal improvement work. In fact, in planning for the succeeding five-year period of operation (1985–89) for germplasm collection for Southeast Asia, the needs of the different breeding programs received priority consideration through formal consultations within each of the seven inter-Asian working groups established for the region. The seven working committees have met and identified the gaps in the collections that need further attention for plant exploration.

Some issues related to genetic resources work in the region

1. The past collection activities have resulted from a hurried response to a difficult situation and the strategies and the procedures followed lacked the refinements of adequate sampling to represent the array of genotypes across divergent ecologies. Primitive or wild relatives of cultivated species were noticeably lacking and probably not deliberately sought by the collectors.

2. Characterization and evaluation work has not kept pace with the growing size of the collections. The volume of work to be accomplished is overwhelming. The work is also regarded as something routine and may not attract the best of talents. But the real problem is the limited availability of expertise, in relation to the extent of the tasks and detailed requirements in documentation. For example, the descriptor lists that have been developed for each crop group are very elaborate. The job of characterization is important, if the collected materials are to be made valuable to the users. It is also the only means by which redundancies in the collection can be minimized. The development of a computerized data storage and retrieval system is also essential.

* United Nations Development Programs of the Food and Agriculture Organization.

3. The rate of utilization of genetic resources for breeding purposes has been regarded as too low to warrant the high costs of exploration and maintenance. The low rate of usage is due in part to the time lag in detecting the presence of genetic traits in the gene pool and verifying their worth. But it can be argued that once a valuable genetic trait has been successfully utilized in enhancing the value of a cultivated crop, and as experiences have shown, the economic pay-offs are usually large enough to justify the costs of maintaining the entire germplasm collection.

4. The cost of maintenance of germplasm collections is very high and places a burden on the usually thin resources of national programs. The energy requirements to operate cold storage facilities, the high costs of maintaining a field genebank or alternative ways of *in vitro* culture and cryopreservation are problems that national programs will continue to grapple with.

5. The RECSEA has had an auspicious beginning and a productive life. Now it faces uncertainty as to its future operations and role. But even if the regional program ceases to function, the national programs have already developed their capabilities to serve needs.

CHAPTER 6

Recent developments in Nepal

S.B. MALLA

Nepal is located between latitude 26°22' and 30°27' N and longitude between 80°12' and 88°12'E. Its altitude varies from 60 m above sea level in the southern plains to 8 848 m at Mt. Everest in the north. The steep gradient of altitude, temperature and rainfall, and micro-environments has provided a suitable homeland for genetically diverse crops and rich flora.

Food crops

The description of the crops and the genotypes in Nepal result from a Japanese Expedition made in 1952–1953 (Kihara 1956). These results recognized many different landraces of wheat and barley. Witcombe (1975) made a collection of wheat in the eastern hills. Rice germplasm has been fairly well collected and maintained at IRRI in the Philippines.

IBPGR sent the special multicrops collection mission to Nepal twice under the leadership of Professor M. Iizuka, Japan. The first one, sent from 1 November 1984 to 12 December 1984, collected germplasm from Western Nepal (Jumla to Nepalgunj) and Eastern Nepal (Lukla to Dhankuta). The mission collected over 2 000 samples of 80 genera (Iizuka 1986). The second mission in October/November 1985 was sent out to Western Nepal (Humla to Dipayal). This mission collected over 2 800 samples of 86 genera (Iizuka 1986). In addition to this, IBPGR arranged for intern Mr S. Yanagihara, Japan in 1985–1987 to collect germplasm of crops for 15 months in the areas not covered by Professor Iizuka's groups. Mr Yanagihara has collected germplasm from different parts of Nepal except from the restricted areas in the northern region bordering Tibet. The duplicate samples of the collected germplasm have been stored and maintained at the Department of Medicinal Plants and the Department of Agriculture in Nepal.

Economic plants

The Royal Botanic Garden (RBG), Godavari has allocated a separate unit for the germplasm conservation of Medicinal and other economic plants. Over 170 species of medicinal and aromatic plants are being grown for demonstration and genetic conservation. More than 900 species of herbs, shrubs and trees have been growing in the garden premises. Crop germplasm has been conserved in the form of seeds in a deepfreezer.

In vitro culture of some of the important species of medicinal orchids, herbs and aromatic plants, valuable forest species and fodder plants, some agriculture crops, horticultural plants, etc. is being carried out in the RBG. Important medicinal plants e.g. *Rauwolfia, Atropa belladonna, Dioscorea, Pyrethrum* etc. are also being maintained *by in vitro* culture. Nepal keeps plants of great potential value ranging from important agricultural crops, medicinal and economic plants, and other valuable wild plants inclusive of fruits and forest species. If these are properly assessed and maintained through *in vitro* culture technique as well, they will be an asset and of immense benefit leading to effective conservation and development of plant resources.

Four different Herbal Farms of the Department of Medicinal Plants established at different agro-climatic regions are test growing the potent medicinal and aromatic plants. Vrindaban Herbal Farm at Hetauda in Southern Nepal at the central development region has so far

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collected over 559 plants consisting of 339 accessions of medicinal and aromatic plants belonging to 135 genera and 175 species. They are being conserved in field genebanks and in seed store. Of these 45 have been evaluated for their agronomy and chemical constituents (Dept of Med. Plants 1986–87).

In addition to this, forestry genetic resources have been conserved *in situ* in nine National Parks and Wild Life Reserves of the Country. Seeds of some 40–50 indigenous tree species have been conserved at 3–5°C at the Community Forestry Development Office, Hattisar in Katmandu.

Conclusions

In Nepal the plant genetic resources have not been collected from the northen remote and restricted areas (for the foreigners) adjoining the Tibetan Autonomous Region of the Republic of China. It is necessary to recruit and train Nepalese personnel as IBPGR interns for the germplasm collection from these restricted areas to collect medicinal and aromatic plants, the crops and fruit trees.

His Majesty's Government of Nepal is quite aware of the importance of the plant genetic resources. With its limited resources it has started a plant genetic resources program. To make it a fully-fledged program, the existing facilities set up in Nepal need to be extended for fruitful achievement. International agencies such as IBPGR, FAO, UNDP, Unesco, Japan International Cooperation Agency (JICA), etc. in this respect, can play an important role by providing support in accelerating the pace of plant genetic resources activities in Nepal and leading to conservation and utilization undertaking for the benefit of country and mankind as a whole.

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CHAPTER 7

The current status of plant genetic resources activities in Papua New Guinea

R. N. KAMBUOU

The genetic erosion of wild and cultivated germplasm of food crops and other beneficial plants may not be seen as serious in Papua New Guinea (PNG) at this stage. However, there will come a time when all of this rich diversity of germplasm materials will be lost forever if no careful planning is put into conserving these materials now. PNG is very fortunate to have joined the IBPGR South East Asian Regional Program. It is through this program that the country has taken an active role in conserving its diverse and rich heritance of plant genetic resources (PGR). Although the country is limited in technical manpower and financial resources, it has undertaken a number of PGR activities in the fields of exploration and collecting, conservation, characterization, evaluation, documentation, utilization, storage and training.

The two National Institutions that are involved in the PGR programs are the Department of Agriculture and Livestock (DAL) and the University of Technology (Unitech), Department of Agriculture. The country has a national committee for PGR made up of ten personnel from these two Institutions.

The national collections of various crops are maintained on DAL Research Stations throughout the country. They are maintained as active collections or in 'field genebanks'.

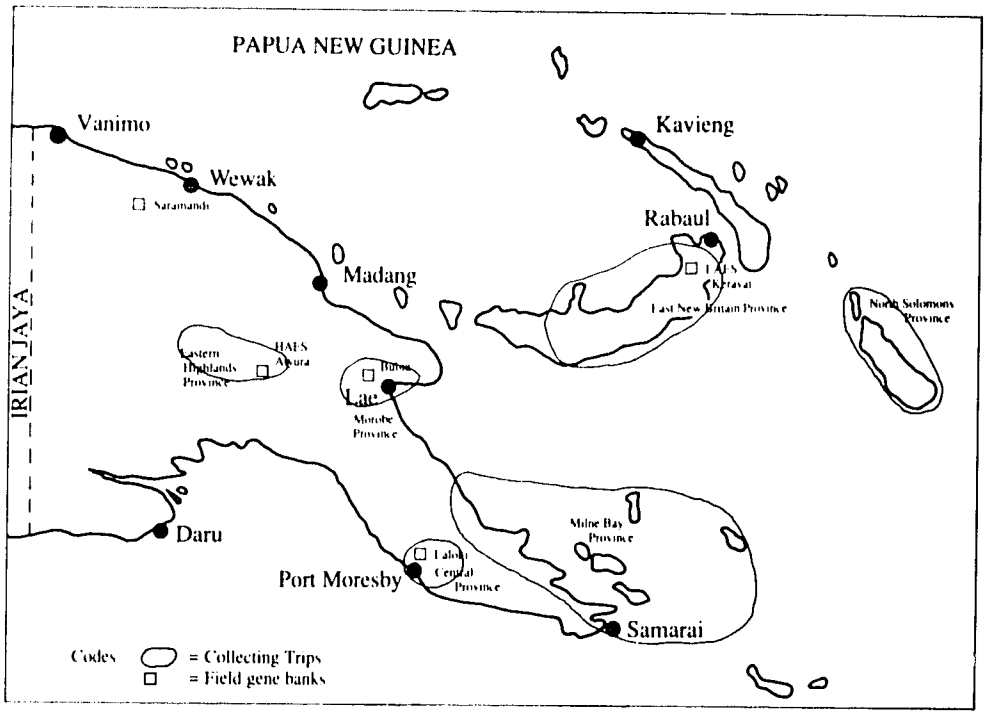
In this Chapter, I will only cover the current germplasm activities in the country. What has happened during the last ten years has been summarized in a paper entitled 'A Decade of Plant Genetic Resources Activities in Papua New Guinea' (IBPGR Regional Committee for Southeast Asia Newsletter, Vol. 9, No. 3, pp 13-15, 1985, R. N. Kambuou).

Exploration and collecting trips

A number of collecting trips was undertaken in past years sponsored either by IBPGR or other International Organizations. The staff of DAL continuously collects germplasm materials when they go on garden surveys.

A major collecting trip was recently carried out in October 1986. This trip was sponsored jointly by IBPGR and the Japanese Government to collect traditional root and tuber crops, green leaf vegetables and bananas. The collecting team visited six Provinces, but collection was done mostly around West New Britain and the North Solomon Island Provinces. The crops collected during the trip were: taro (*Colocasia esculenta*) 94 accessions, sweet potato (*Ipomoea batatas*) 54, banana (*Musa* spp.) 52, aibika (*Abelmoschus manihot*) 2, cassava (*Manihot esculenta*) 2, yam (*Dioscorea* spp.) 11 and taro kongkong (*Xanthosoma* spp.) 8 accessions.

All collected materials except bananas were deposited at Bubia Agriculture Research Centre in Lae. The banana materials were added to the Papua New Guinea Biological Foundation (PNGBF) collection at Laloki Agriculture Research Station in Central Province. In September this year the IBPGR and the Japanese Government sponsored a second collecting trip to PNG covering the southern part of the main island around Popondetta, Milne Bay and Central Provinces. The collecting trip was undertaken during the time this paper was being written. Therefore, it is not possible to discuss the content of the collection. The main crop species likely to be collected are sweet potato, taro, aibika and other minor root/tuber crops.



The Lowland Food Crops Research Team (LFCRT) from Bubia carried out a Rapid Rural Appraisal (RRA) on taro-based subsistence gardens in the Morobe area. They recorded 57 cultivars of taro from five villages visited. Most of these cultivars are duplicated between villages. A total of 19 taro accessions were collected and are now held in the national taro collection at Bubia.

Conservation and collections

The germplasm materials are maintained in three ways. All vegetatively propagated crops are conserved in field genebanks or in active collections. The germplasm in these collections came from all over the country. The main ones are sweet potato, banana, taro, yam, aibika, sugarcane (*Saccharum* spp.), cassava and sago (*Metroxylon sagu*).

All the grain crops are conserved as seeds in coolroom facilities operating at 10°–15°C. These seeds are only stored for short-medium purposes. The seed is tested for viability every 2–3 years. Almost all this germplasm is introduced from overseas genebanks for research purposes. The main seed collections are rice (*Oryza sativa*), peanut (*Arachis hypogaea*), maize (*Zea mays*), sorghum, soyabean (*Glycine max*), mungbean (*Phaseolus aureus*), adzuki bean (*Vigna angularis*) and winged bean (*Psophocarpus tetragonolobus*).

The sweet potato virus-free materials are stored *in vitro* or as tissue culture materials. The *in vitro* technique is new in the country and with no proper facilities and inadequately trained personnel, the current work program is limited to handling only sweet potato virus-free materials.

There are a number of field genebanks in the country (See Appendix). The PNGBF banana collection was assembled in the early 1970s at the University farm in Lae under the leadership of Dr George Argent. Altogether approximately 800 accessions were initially collected,

but three-quarters of the materials were lost, due to a maintenance problem. The remaining collection was then transferred to Laloki Research Station. The collection now has 286 accessions. A total of 178 accessions from the Laloki Collection were sent to the Regional Banana Germplasm Collection at Davao in the Philippines. The National collection is held at Laloki and is maintained with internal funding.

Sweet potato collections are held at each agriculture research station. The national collection is at the Highlands Agriculture Experimental Station (HAES), Aiyura. Some 1079 accessions are maintained. The Aiyura collection is further duplicated at Kuk Agriculture Research Station in Mount Hagen.

The lowland collections are maintained at Laloki (953 accessions), Bubia (68), Keravat (267), Saramandi (81) and Unitech farm (91 accessions). There are duplications in the lowland collections, but it is hoped that once these collections are characterized this problem will be solved. The Saramandi collection consists of 16 accessions from within the country and 65 breeding lines from the International Institute for Tropical Agriculture (IITA) in Nigeria. The Unitech collection is made up of 30 cultivars from the local areas and 61 breeding lines from the University Plant Breeding Program.

The National Taro Collection is held at Bubia with over 430 accessions. Keravat holds ten accessions while Aiyura and Saramandi have 48 and 23 accessions, respectively. Again there are duplications in these collections.

Six yam species are maintained in active collections in the country. These are *Dioscorea esculenta*, *D. alata*, *D. bulbifera*, *D. hispida*, *D. pentaphylla* and *D. nummularia*. The Laloki collection is the largest with 187 accessions. The collection initially had 900 accessions but most were lost through maintenance problems.

The Saramandi collections consist of 20 *D. esculenta* and 16 *D. alata* while Bubia maintains 24 *D. alata* and 39 *D. esculenta*.

The collection at Aiyura holds 16 *D. alata*, 3 *D. bulbifera*, 10 *D. nummularia* and 1 *D. pentaphylla*.

The cassava collections are maintained at four locations. The collection at Keravat is the largest with 34 accessions. The Aiyura collection has 12 accessions, Laloki has six and Bubia only four accessions.

The national collection of Aibika is held at Bubia with 42 accessions. Keravat has 41, Laloki 68 and Aiyura has 5 accessions. Most of the Aibika accessions are duplicated between sites.

The PNG sugarcane germplasm collection is held at Bubia. From the original 206 accessions collected, only 72 are now surviving. Poor maintenance of the collection has contributed to the loss of germplasm materials. From the 72 surviving accessions, 32 are *Saccharum officinarum*, 28 are *S. edule*, and 12 are *S. spontaneum*. The Aiyura collection holds 25 varieties of *S. officinarum* and 4 of *S. edule*, while Keravat maintains 14 accessions of *S. edule*. The only sugar manufacturing company in PNG, Ramu Sugar Ltd, also maintains an active collection of sugarcane germplasm. The collection currently holds 297 cultivars and breeding lines all introduced from overseas genebanks. Fourteen cultivars from the collection are commercially used in the production program.

The traditional green leaf vegetable collections are maintained at Aiyura and Keravat. Aiyura maintains ten species — all have less than ten accessions each. The Keravat collection has four lowland species each with fewer than five accessions. The common species are aupa (*Amaranthus* spp.), kumu mosong (*Ficus* spp.), tulip (*Gnetum gnemon*), valangur (*Polyscias vertiallata*), moku (*Rungia klossii*), nase (*Oenanthe javanica*), karakap (*Solanum nigrum*) and highlands pitpit (*Setaria palmifolia*).

Sago is the main staple food in some parts of the country, yet very little has been done in the country to conserve sago germplasm in *ex situ* set-ups. Most edible varieties are grown wild and are harvested from their natural habitats. The Saramandi Research Station has

collected 13 varieties from the local area and they are maintained in the collection on the station.

Well over 20 species of tropical fruits and nut trees are held at Keravat. Most germplasm is collected within the country. There are varieties in the collections that were introduced from overseas genebanks for research purposes.

All cash crops such as cocoa, coffee, coconut and oil palm are now in the hands of the Research Institutes. They are solely responsible for any research/development programs for these crops. The arabica coffee collection is held at Aiyura while the robusta collection is at Keravat.

The main cocoa collection is held at Keravat with 364 accessions altogether. The oil palm germplasm is maintained by the estates responsible for oil palm production in the country.

Characterization and preliminary evaluation

Some collections have been characterized using the IBPGR descriptor lists, while others are yet to be characterized properly. Most of the accessions in these collections are agronomically characterized after they are screened by preliminary evaluation studies.

Recently no characterization work has been done on the existing collections.

The two national collections properly characterized are the sweet potato collection at Aiyura and the taro collection at Bubia. The sweet potato collection was characterized three years ago by Dr H. Takagi, an IBPGR intern to PNG at that time. The taro collection was partially characterized last year by the members of the Lowlands Food Crops Research Team at Bubia.

Various other collections mentioned in the previous section are agronomically characterized and information is kept in manual form at each Research Station where the collections are located.

A preliminary evaluation study is carried out on all accessions that are introduced into the collections. Observations are made on their overall growth performance, yield potential and pest/disease resistance. Results from these studies enable the agronomists to carry out further agronomic evaluation on the promising accessions.

Some 30 cultivars of sweet potato from the Laloki collection are being screened in preliminary evaluation studies conducted at ten different locations in the Central Province. A group of 27 tissue cultured lines from AVRDC, Fiji and Japan were released from the insect screen house and are being evaluated in the field at Laloki station. About 65 sweet potato breeding lines from IITA are currently undergoing a preliminary evaluation trial at Saramandi. A total of 61 breeding lines from the University are being evaluated at the University farm in Lae.

The Taro Agronomy Program at Bubia includes a preliminary evaluation of 30 accessions of taro from the national collection. Results from this study will enable the Food Crop Research Team to identify suitable varieties of taro for advanced agronomic trials.

The sugarcane accessions are evaluated by the agronomist of the Ramu Sugar Company. Preliminary testing is an essential part of its research/production program.

Further evaluation

Further evaluation in this context refers to agronomic evaluation. After the preliminary evaluation the superior materials are selected and further tested for their agronomic potential.

There are several agronomic trials in the field at the moment on various crops. Ten banana cultivars from the PNGBF collections were selected and are being tested in trials on variety × sucker size × pruning and variety × bunch cover.

A number of promising cultivars from the collections are used in variety testing programs at different locations in the country. Some superior cultivars from the Laloki collection were

selected and further tested in a variety × manure trial on the station. A study on the control of sweet potato scab is being conducted at Laloki using a cultivar from the collection that is very susceptible to the scab disease.

Some good cultivars from the national collection are being put through the variety testing program at Aiyura and Kuk.

Six common cultivars of taro from the collection are further tested for their yield performance under farmers' field at Malahang farm near Lae. Trials are being carried out on taro beetles and taro leaf defoliators using some accessions from the collection at Bubia.

Some promising *D. alata* cultivars are used in a study on the control of anthracnose disease at Saramandi. The common varieties of yam in the Sepik Province are being tested in a variety trial on the station.

The current evaluation study conducted on Aibika is in the field of entomology. Some common cultivars are used in trials on the control of aibika leaf-chewing insects and the insect population dynamic studies are conducted at Bubia. Two popular varieties in Central Province are being evaluated at Laloki in the chicken manure trial.

Some promising sugarcane cultivars and breeding lines are tested in variety trials in the Ramu valley. The recent problem of the Ramu disease has prompted the agronomists and plant pathologists to screen their cultivars thoroughly and conduct trials on the control of Ramu disease.

Agronomic evaluation of cash crops takes place at a number of sites in the country. Varietal studies on cocoa are conducted at Keravat. The arabica coffee evaluation studies are carried out at Aiyura by the agronomist of the Coffee Research Institute.

The DAL hybrid coconut testing program that was commenced five years ago is continuing. The hybrid coconut blocks were set up at a number of sites in each coastal Province in the country. Observations are being made on the overall performances and yield potential of each type of hybrid under those particular environmental conditions.

Utilization and documentation

The genetic materials from the collections are utilized in two ways, in the breeding and the production programs. There is not much research going on in crop breeding at the moment owing to lack of proper facilities and technical know-how. The two on-going activities on utilization of germplasm in breeding are carried out on coconuts and sweet potato. For coconuts, pollen from the selected local tall varieties at Bubia and Keravat are used as a male parent in the breeding program.

The sweet potato germplasm used in the breeding work at Unitech is from the collections at Aiyura and Laloki.

Utilization of germplasm materials through the production programs has been the main emphasis in the country. When accessions are collected or introduced they are put through the preliminary evaluation trials. The elite ones are selected and further tested in agronomic trials under adverse environmental conditions. After some years of testing the best accessions are released as varieties to farmers for production.

Eight sweet potato varieties from Aiyura have been released and are widely grown in the highland areas for subsistant consumption. Some varieties from the collections at Bubia, Keravat and Laloki have been distributed to farmers for production.

Three varieties of dessert bananas from the PNGBF collection are now commercially grown in Lae and Port Moresby for the domestic market. A number of cooking bananas have been distributed to subsistence farmers for cultivation.

The common cultivars of taro, aibika, yam and cassava are grown widely by farmers throughout the country for subsistence consumption.

The germplasm collections in the country are documented in two ways — in computer form and as manual records.

Computer documentation is done for two collections only. The national sweet potato collection at Aiyura and the taro collection at Bubia. The taro collection is partially documented. Those accessions not documented on the computer are kept in manual form. The sweet potato documentation was done by the UNDP/FAO Team in Fiji with local assistance from Dr H. Takagi. The computer printout and the diskette are held at Kuk Research Station. The taro documentation was done by Dr Peter Cox and the Lowlands Food Crops Team on a computer at Bubia.

Information on other collections discussed in the section on conservation and collections above are documented manually and are kept in files at each research station where the collections are held.

Storage

The germplasm of seed crops like grains and vegetables are maintained in short –medium term cool-room stroage facilities based at each research station. the cool-room storage facilities operate at 10°–15°C and are not always reliable especially in areas where power cut-off is frequent. Germplasm of rice, maize, sorghum and pulse crops are conserved under these conditions. Most of this germplasm is introduced from overseas genebanks for experimental purposes.

The winged bean germplasm is local, bred by the University through their plant breeding program. A complete collection of 510 accessions of winged beans is currently held at the Regional Genebank in the Philippines.

Conclusions

The general idea of conserving crop germplasm in collections is being recognized by many agriculturalists in the country. The agricultural administrators and higher authorities are to some extent aware of the importance of preserving the germplasm materials of various crops, particularly the traditional food crops that have a rich diversity of genetic materials.

The country's main concern at the moment is in the maintenance of the existing collections and to ensure that the diversity in food-crops germplasm are safe from genetic erosion. Emphasis is also placed on utilization of selected varieties from the collections for farmers' production.

Not much is being done in the country at the moment in the fields of characterization, documentation and utilization (breeding). A shortage of funds and inadequate technical know-how are the main obstacles to progress in PGR activities in PNG.

The country is grateful to IBPGR through the South East Asian Regional Program for all the financial and technical assistance given during the past years.

We appeal to IBPGR to continue its support and financial assistance in future, for the betterment of conservation of plant germplasm.

APPENDIX

Field genebanks of traditional food crops in PNG

Common name	Scientific name	No. of accessions[a]	Locations
Roots, tubers & starch			
(1) Sweet potato	*Ipomoea batatas*	1079	HAES (Aiyura)
		953	Laloki
		68	Bubia
		267	LAES (Keravat)
		91	United Farm (Lae)
		81	Saramandi
(2) Taro	*Colocasia esculenta*	430	Bubia
		10	LAES (Keravat)
		48	HAES (Aiyura)
		23	Saramandi
(3) Yams	*Dioscorea* spp.	187	Laloki
	D. alata	56	Saramandi, Bubia, Aiyura
	D. esculenta	59	Saramandi, Bubia
	D. bulbifera	3	Aiyura
	D. nummularia	10	Aiyura
	D. pentaphylla	1	Aiyura
(4) Cassava	*Manihot esculenta*	34	LAES (Keravat)
		12	HAES (Aiyura)
		6	Laloki
		4	Bubia
(5) Banana	*Musa* spp.	286	Laloki
(6) Sago	*Metroxylon sagu*	13	Saramandi
Leafy vegetables			
(1) Aibika	*Abelmoschus manihot*	42	Bubia
		41	LAES (Keravat)
		68	Laloki
		5	HAES (Aiyura)
(2) Aupa	*Amaranthus* spp.	3	HAES (Aiyura)
(3) Kumu mosong	*Ficus capiosa*	1	HAES (Aiyura)
		1	LAES (Keravat)
(4) Tulip	*Gnetum gnemon*	1	LAES (Keravat)
(5) Valangur	*Polyscias verticillata*	4	LAES (Keravat)
(6) Moku	*Rungia klossii*	8	HAES (Aiyura)
(7) Nase	*Oenanthe javanica*	5	HAES (Aiyura)
(8) Karakap	*Solanum nigrum*	1	HAES (Aiyura)
(9) Highlands pitpit	*Setaria palmifolia*	24	HAES (Aiyura)
Sugarcane			
	S. Saccharum spp.	297	Ramu Sugar Company
	S. officinarum	57	Bubia, Aiyura
	S. edule	46	Bubia, Aiyura, Keravat
	S. spontaneum	12	Bubia

[a] As at 31 August 1987

PART 2

Utilization of genetic resources for productivity

Chairman
K. Hashimoto

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CHAPTER 8

Utilization of genetic resources for productivity of oil crops in Korea

JUNG IL LEE

The utilization of vegetable oil in Korea is lower than that of any other nation. Starch is a major component of Korean traditional diet. Most oil has been for condimental use in Korean food, particularly from sesame and perilla. Industrialization has enriched the social economy and has led to an increase in consumption of vegetable oil. Not only has the pattern of use changed to salad from flavouring oil, but also has consumption increased.

Sesame cultivation has been thought to be very long established in Korea. The record shows that it spread to Japan in AD 538 through 'Backje' one of the medieval countries, and it was thought to be introduced from India or China with Buddhism.

Records on rapeseed are found in *Economy of Forestry* written in 1643, and much earlier than Japan. But its cultivation, for use as salad oil, has been intiated only since 1957.

To ensure self-sufficiency in sesame, studies on sesame and its cultivation and breeding have been emphasized. By price stabilization and farming loans, production has been promoted, and sesame has become one of the most contracted cash crops. So far no variety has been improved in resistance to diseases. This has become the most important objective in sesame breeding along with the search for high yields. Diverse germplasm collection from the temperate region is needed for this resistance breeding.

Rapeseed grown by double cropping after rice has a great yield potential to support self-sufficiency in salad oil in response to increased demand. The quality and price should be increased to that of soyabean and Canadian canola oil and its production needs support and promotion. Productivity of rapeseed could be increased by development of hybrid varieties. And because the male sterile and maintainer line has already been developed in Korea, germplasm collection has to be focused on restorer line source.

Utilization of genetic resource in sesame and rapeseed

Although oil has been one of the most lacking components in traditional Korean diet, total national demand has greatly increased from 30 000 ton two decades ago to 400 000 ton in 1986. The ratio of self sufficiency has dropped from 74% to 18% during the same period. Moreover, vegetable oil consumption is expected to continuously increase (Table 1).

As much as 47% of the vegetable oil consumed is found to be of low quality and containing

TABLE 1 Current supply and demand of edible oil in kilotons.

Year	'67	'70	'75	'80	'83	'86
Production	21	22	23	41	32	70
Import	8	30	74	158	278	324
Total	29	52	97	199	310	394
Domestic supply (%)	74	42	24	21	12	18

Source: Ministry of Agriculture and Fishery

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much saturated fatty acid. Furthermore, more palm and coconut oils which are cheaper in price are being imported (Table 2).

Two major oil crops in Korea are sesame and rapeseed. Sesame is one of the most important

TABLE 2 Domestic consumption of several kinds of edible oil (1986).

Edible oil supply	Vegetable oil									Animal oil			
	Sesame	Rape	Perilla	Rice bran	Soyabean	Palm	Coconut	Others	Total	Tallow	Marine	Others	Total
394 (Kt)	13.1	2.2	4.8	27.8	141.7	89.0	23.0	20.6	322.2	53.3	10.4	8.2	71.9
100 (%)	3.3	0.6	1.2	7.1	36.0	22.6	5.8	5.2	81.8	13.5	2.6	2.1	18.2

Note: Palm+Coconut+Animal = 183.9 Kt (46.7%)

oil sources. It is mainly used for condimental oil and for giving flavour to side dishes. Some 90 000 hectares are planted for sesame which is the largest among the oil crops in Korea. Domestic production of sesame is 48 000 ton per year, being able to supply 98% of total national demand. National self-sufficiency is expected to be achieved in the near future.

Rapeseed has a high yield potential to support self-sufficiency in vegetable oil and has great value if used in double cropping systems as a winter crop after paddy rice. But the area under cultivation has been reduced gradually, because of its low price. It cannot compete with imported soyabean. But in spite of its low price its use as a winter crop after rice gives extra income to growers during the winter season.

To promote the production of sesame and rapeseed, it is necessary to increase the yield potential urgently to make them more competitive in price compared with imported soyabean. The objectives of sesame improvement involve manipulating the branch type plant with tricapsule, (instead of the non-branch type with a monocapsule) and changing the inflorescence to determinate from indeterminate type to have balance with extended sink capacity and source. And the other target is to introgress the resistant gene of major diseases attacking sesame severely, such as seedling blight, phytophthora stem rot caused by *P. parasitica*, fusarium wilt cased by *F. vasinfectum* and leaf blight caused by *Corynespora cassicola* to the ideal plant type. Such plants would increase yield 50% or more (Table 3).

TABLE 3 Sesame breeding goals.

Character	Goal	
Plant type	Nonbranched: tricapsule, bicarpellate	Branched: tricapsule, tetracarpellate
Germination at low temperature	Below 12°C	
Inflorescence	Determinant	
Disease resistance	Seedling blight, *Phytophthora*, fusarium wilt, *Corynespora*	
Oil content	Above 55%	
Oil quality	High linoleic acid Above 50%	
Seed coat colour	White, Black	

In rapeseed, first of all, oil quality should be improved to enable it to compete with soyabean and sunflower as a salad oil. Oil quality can be improved by changing its fatty acid composition, reducing the erucic acid and eicosenoic acid, which is toxic to humans. Oil cake can be fed to livestock when its quality is improved by eliminating the toxic substance glucosinolate. Early maturity and cold tolerance should be enhanced for double cropping with rice and to allow cultivation further north. The last goal is to develop a hybrid variety by utilizing the cytoplasmic-genetic male sterility to avoid yield reduction caused by in-breeding in pedigree selections. Achievement of these sequential objectives in rapeseed breeding would make it possible to be self sufficient in vegetable oil (Table 4).

TABLE 4 Rapeseed breeding goals.

Character	Goal
Oil content	Above 50%
Oil and gas quality	Zero-erucic acid, low-linolenic acid, low-glucosinolate
Seed coat colour	Yellow
Maturity	Early
Cold tolerance	Survival at lower than -5°C
Disease resistance	*Sclerotinia*
High yield	Above 300 kg/ha in pedigree Above 500 kg/ha in hybrid

The actual breeding program on sesame and rapeseed was begun nearly twenty years ago in Korea. Hybridization started in 1954 for sesame and 1964 for rapeseed.

Germplasm collection has been successful for breeding material compared with other crops and collection areas are restricted to certain countries. The germplasm in continental China is thought to be very plentiful and diverse in variability of traits, and to be easily adapted to our country because of the similarity of climatic conditions in the Korean peninsula, so it is very necessary as breeding material for sesame and rapeseed. But few Chinese accessions are used for breeding material in Korea. Among a total of 797 sesame germplasm collections, most of the accessions are the Korean landrace except for 281 accessions introduced from 13 countries, and 634 collections of rapeseed introduced from 12 countries including mainly Japanese collections (Table 5). We screened the collections for some desirable traits and selected 4 varieties with high oil content, 5 varieties with good quality oil high in linoleic acid, 351 varieties of branching type, 491 varieties of tricapsule and 3 varieties of determinate type (Table 6). Some varieties showed resistance to seedling blight (3), to phytophthora stem rot (2), and to leaf blight (4) (Table 7).

In rapeseed, 35 cultivars or lines of high oil (over 45%), 290 with zero-erucic acid and 283 with low glucosinolate (lower than 3 mg/g) were selected as the breeding materials

TABLE 5 Source of sesame and rapeseed germplasm collected.

Crops	Total	Korea	Japan	Taiwan	Europe	Canada	USA	Africa	India	USSR	Aust.
Sesame	797	544	53	15	71	–	55	26	22	10	1
Rapeseed	634	109	274	20	200	10	4	–	–	4	13

TABLE 6 **Sesame germplasm evaluation for varietal improvement.**

Characters	Oil content (> 55%)	Linoleic acid (> 50%)	Branched	3 capsules	Determinate	Indehiscent
No. of varieties	4	5	351	491	3	7

TABLE 7 **Sesame germplasm evaluation to breed disease-tolerant varieties.**

Diseases	No. of varieties	Resistance					Tolerant varieties
		R	MR	M	MS	S	
Seedling blight	759	3	15	75	452	218	Suwon 20, Nigeria, Nepal
Phytophthora blight	479	2	2	28	320	127	Nigeria, Nepal[a]
Leaf blight	438	4	22	230	166	16	Kwanguye, Renner, Nigeria, Nepal

[a] Nigeria and Nepal collections are wild species

in the quality improving program. Five lines with high quality of oil cake were obtained as cytoplasmic-genetic male sterile plant, and 1105 cultivars were classified as restorers with filament index over 70% for the hybrid breeding to develop high yielding F_1 hybrid varieties (Table 8).

The characters transferred from germplasm were plant type and disease resistance gene in sesame, and oil and oil cake quality and cold tolerance in rapeseed (Table 9).

TABLE 8 **Rapeseed germplasm evaluation for varietal improvement.**

Characters	Oil content (45–50%)	Zero-erucic acid (0–0.99%)	Low glucosinolate (0–2.99 mg/g)	Zero-erucic + low glucosinolate	Restorer fertility index (>70%)	CGMS	CMS
No. of varieties	35	290	283	179	1 105	5	3

TABLE 9 **Number of varieties used for crossing program.**

Crop	Quality	Tolerant to diseases	Early maturity	Cold tolerant	Plant type	Seed coat colour	Yielding
Sesame ('54-'87)	47	236	47	–	357	49	156
Rapesed ('65-'87)	81	27	56	72	19	11	87

The cultivar developed by the pure line selection method in the early breeding period showed 400 kg/ha, but Hanseomggae showed 930 kg/ha, which is the first variety developed for branching type and tricapsule and released recently. The breeding target of sesame would be focused on introgressing the disease resistance and determinate inflorescence gene to this variety (Figure 1).

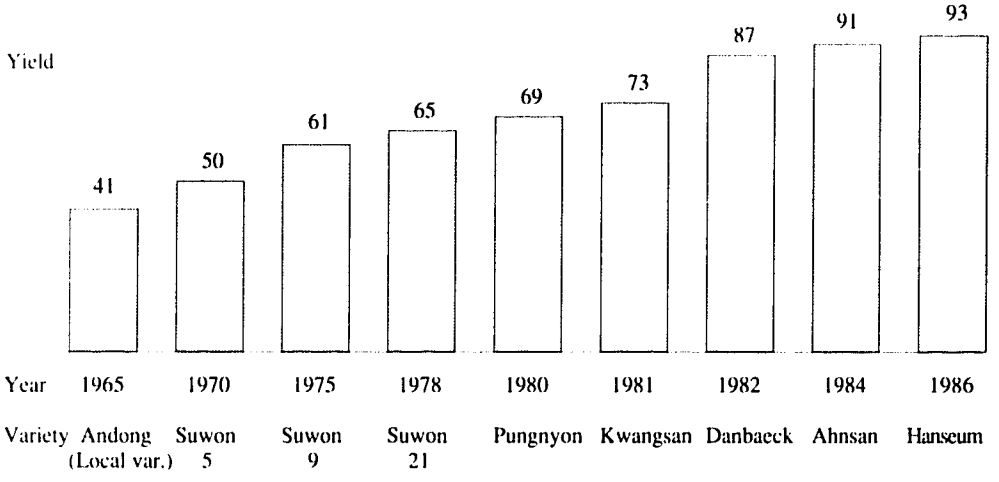

FIGURE 1 Increase of sesame yield by varietal improvement in Korea.

Rapeseed yield was increased to 2.89 t/ha with the developed cultivar by pedigree method, while the landrace showed only 1.94 t/ha. It was thought that to increase this yield level is difficult in rapeseed, because of its inbreeding depression. By hybrid breeding the varieties Cheongpungyuchae and Tankyo 19 were developed. These hybrids showed dramatic yield increases.

Rapeseed varieties Yongdang and Nojeock have zero-erucic acid and Naehanyuchae, Yeongsanyuchae, Hallayuchae and hybrid varieties Cheongpungyuchae and Tankyo 19 have zero-erucic acid and low glucosinolate, that is, they are double zero varieties (Table 10).

TABLE 10 Improvement of rapeseed quality in Korea.

Variety	Released year	Yield (kg/ha)	Oil content (%)	Protein content (%)	Fatty acids (%)			Glucosinolate (mg/g)[a]			
					Oleic	Linoleic	Erucic	BI	PI	OZT	Total
Yudal	1969	2 046	45.0	22.5	10.6	12.4	56.8	1.82	0.61	8.69	11.12
Tower	1975	1 938	42.5	19.7	58.3	22.5	0.0	0.53	0.06	1.13	1.72
Yongdang	1977	2 128	43.0	24.9	68.5	21.2	0.0	2.13	1.62	11.21	14.96
Nozeog	1979	2 640	42.0	24.1	54.4	28.1	0.0	1.25	1.19	8.50	10.94
Youngsanyuchae	1980	2 889	43.9	24.5	66.0	17.1	0.0	0.06	0.03	0.0	0.42
Naehanyuchae	1980	2 752	43.3	23.9	58.7	22.7	0.0	0.28	0.17	0.58	1.03
Hanlayuchae	1985	2 550	44.5	24.1	64.8	18.9	0.0	0.45	0.30	0.76	1.51
Cheongpungyuchae (F_1)	1983	4 120	45.0	24.2	63.9	19.2	0.0	0.20	0.55	0.50	1.25
Tankyo 19(F_1)	1985	4 570	44.8	23.4	61.0	21.6	0.0	0.36	0.47	0.60	1.43

[a] BI: butenylisothiocyanate; PI: 4-pentenylisothiocyanate; OZT: 5-vinyl-2-oxazolidinethione.

To increase sesame yield to 1.5 t/ha, it is necessary to acquire germplasm resistant to diseases and to introgress desirable characteristics, and to extend the sink capacity to balance with source potential of sesame plants. The complex disease resistant gene could be introduced to the cultivated species from the wild species. The barriers to interspecific cross incompatibility between cultivated and wild sesame could be overcome by tissue culture, which is commonly used in other crops. The indeterminate branching type plant with tricapsule can be supplemented by the determinate inflorescence gene by the mutant developed by Ashiri in Israel.

Germplasm used in producing high yielding rapeseed should have proper characters for hybrid breeding. Because rapeseed is outcrossed as much as 25%, inbreeding is depressed and causes yield reduction when the seed produced by self pollination is used to keep seed purity in pedigree selections. Actually yield dropped 14% after self pollination for three years (Table 11).

TABLE 11 Yield depression (kg/10a) by three generation selfing in rape (Mokpo).

Varieties	Inbreeding yield	Selfing yield (3 years)	Depression (%)
Norin 14	279	246	12
Norin 25	264	238	10
Norin 26	276	224	29
Yudal	329	279	16
Mean	287	247	14

To overcome this inbreeding depression, hybrid breeding was attempted by using cytoplasmic-genetic male sterile (CGMS) plant. A CGMS line, Mokpo-MS was developed from the cross between cytoplasmic male sterile Tower and genetic male sterile Isuzu (Figure 2).

The oil and oil cake quality was improved in this male sterile line. The filament and anther does not grow at all, but also the sterility does not restore in high-temperature growing conditions unlike the Shiga and Tompson CGMS (Table 12).

TABLE 12 Comparison of flower characters and quality in each male sterile line.

Line	Flower characters			Quality		
	Length of filament (mm)	Length of anther (mm)	Relative position (0–6)	Oleic (%)	Erucic (%)	Glucosinolate (mg/g)
Shiga	3.1	1.2	2.41	9.6	54.4	13.20
Tompson Bronowski	4.2	1.2	3.12	0.8	9.6	0.81
Yudal	3.2	1.2	2.14	10.4	58.2	11.74
Mokpo	0.2	0.0	0.0	65.1	0.0	0.78

F_1 progenies of 511 combinations crossed with the Mokpo-MS showed heterosis in yield and some agronomic traits. The progenies flowered four days later, grew more vigorously in plant height and number of branches with longer fruiting branch, bore more pods, and

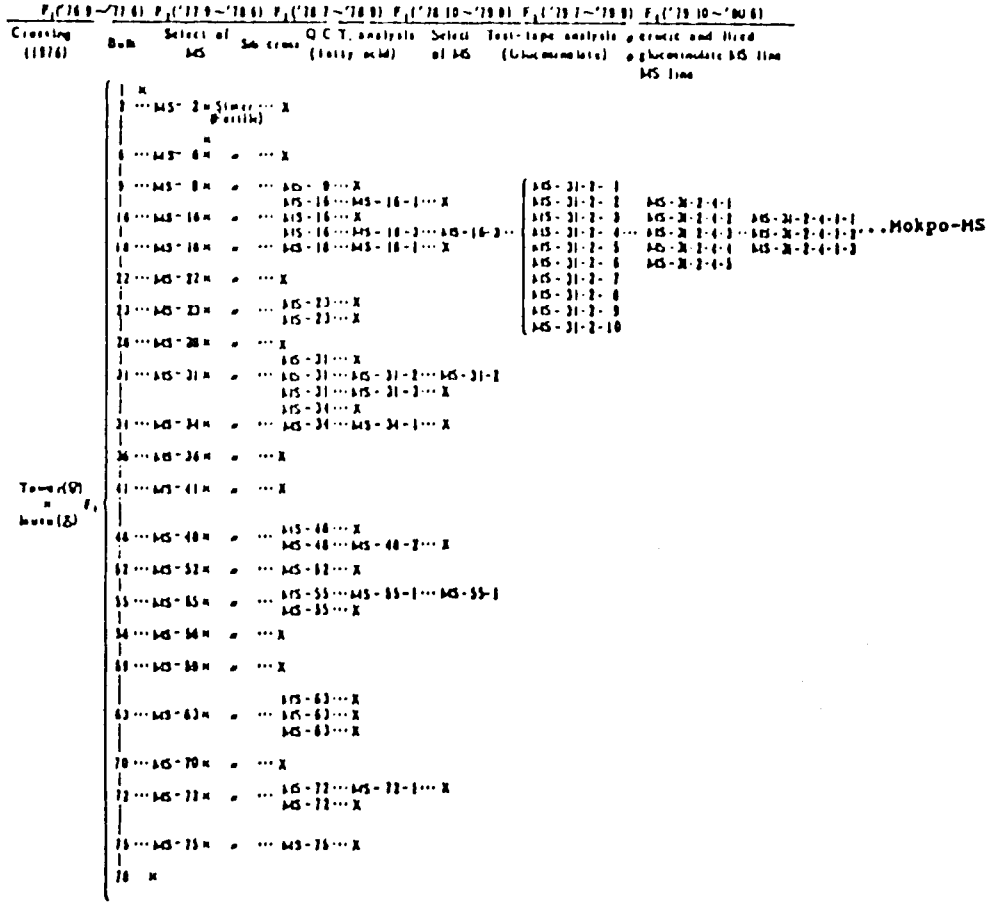

FIGURE 2 Pedigrees of cytoplasmic-genetic male sterille line, Mokpo-MS.
Note: X indicates discarded plant (Lee et al. 1980)

TABLE 13 Comparison of agronomic characteristics between F_1 and its mid-parent in rapeseed.

Generation	Date flower	Plant ht. (cm)	Ear length (cm)	Pods /ear	Total branches	First branches	Density pod setting (cm)	Pod length (cm)	Seeds /pod	1000-seeds (g)	Seed wt./plt. (g)
MP	4.7	119	41	37	36	14	8.0	6.1	26	2.5	172
F_1	4.11	164	49	45	48	18	7.8	5.5	26	2.9	263
F_1-MP	4.0	45	8	8	12	4	-0.2	-0.6	0	0.4	91

produced significantly heavier grains than the mid parent of them. The heterosis was expressed in most of the yield components as increasing yield (Table 13). Some 463 combinations or 91% of total crosses produced higher yield than mid parent (Figure 3).

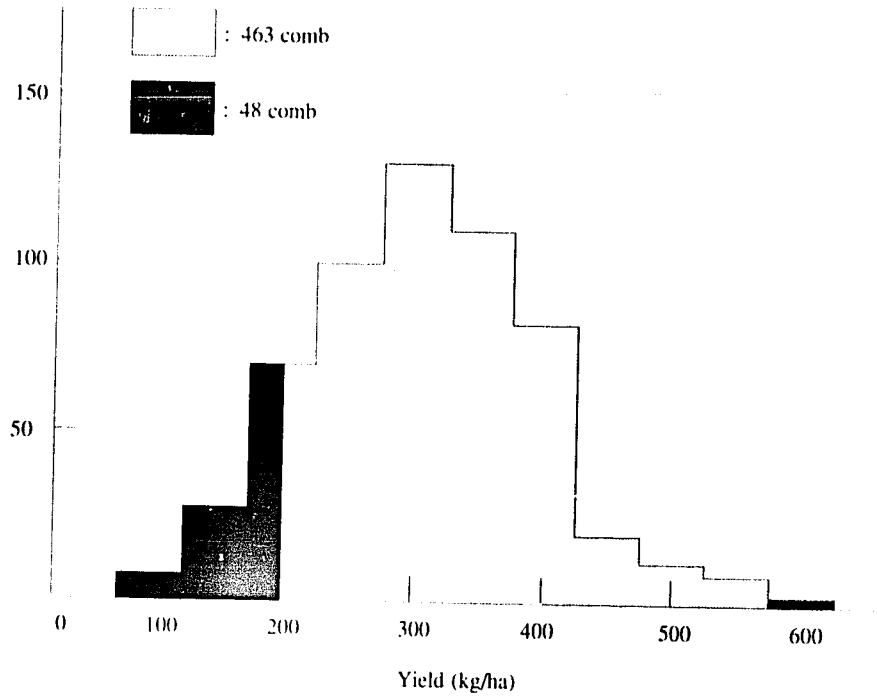

FIGURE 3 Heterosis of F_1 using MS and high quality restorer.

Hybrid varieties Cheongpungyuchae and Tankyo 19 were developed by using the Mokpo-MS in 1983 and 1985 respectively. They were improved in oil and oil cake quality and showed as much as 55% and 67% yield increase compared with the check variety. Cheongpungyuchae produced 4.12 t/ha and Tankyo 19 produced 4.43 t/ha in regional yield trials (Table 14).

Many crosses were made to develop the maintainer for Mokpo-MS. Four B- lines were selected as maintainers by introgressing the male sterile gene from the fertile sister line of Mokpo-MS to Altex bearing normal cytoplasm (Figures 4 and 5).

TABLE 14 Agronomic characters and quality of F_1 varieties developed in Korea.

Hybrids (F_1)	Agronomic characters					Yielding		Quality	
	Plant height (cm)	Branches	Ear length (cm)	No. of pods per ear	No. of seeds per pod	Yield (kg/10a)	Index (%)	Erucic acid (mg/g)	Glucosinolate
Cheongpung	172	31	47	46	30	412	155	0.0	1.25
Tankyo 19	165	34	41	47	31	443	167	0.0	1.49
Naehan (check)	152	25	32	28	27	266	100	0.0	1.03

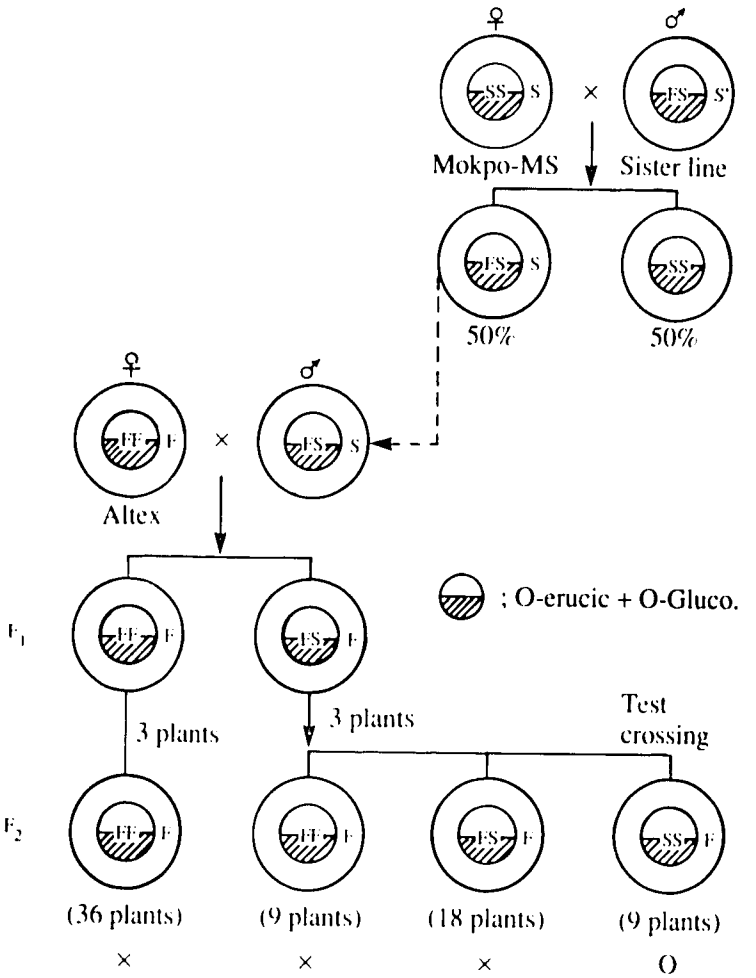

FIGURE 4 Breeding scheme of zero-erucic, zero-glucosinolate maintainer.

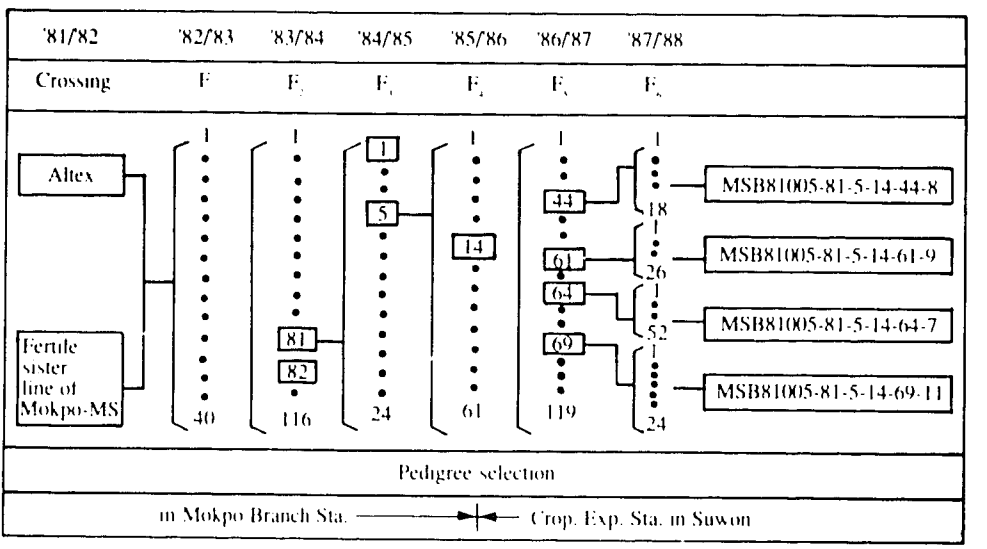

FIGURE 5 Pedigree diagram of Mokpo-MS maintainer lines in rapeseed.

TABLE 15 Classification of F_1 fertility restoration based upon fertility index (F.I.) from relative position of anther to stigma and width of petal.

Source	Restoring F_1 (Above F.I.70)		Partial restoring F_1 (F.I.40-69)		Non-restoring F_1 (Below F. I. 39)		No. of observed plants	Frequency
	No.of F_1	Rate (%)	No.of F_1	Rate (%)	No.of F_1	Rate (%)		
Europe	86	74	18	16	12	10	116	74:16:10
Japan	2	5	14	39	20	56	36	5:39:56
Korea	2	25	2	25	4	50	8	25:25:50
Domestic bred lines with zero-eru. and low-gluco.	412	81	25	5	74	14	511	81: 5:14
Total	502	75	59	9	110	16	671	75: 9:16

Selections were made for restorer line by filament index from introduced cultivars and quality improved pedigree lines. We were very fortunate to obtain 86 cultivars from European collections and 412 lines among the pedigree selections as restorer R-line for Mokpo-MS (Table 15). They showed great variability in agronomic characteristics and could be utilized for successful hybrid breeding.

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CHAPTER 9

Utilization of exotic germplasm in sweet potato breeding

MASASHI KOBAYASHI AND SATOSHI SAKAMOTO

Since the introduction of the sweet potato into Japan about 370 years ago, the cultivated area had spread to a major portion of the country in a span of two centuries. A cultivated area of about 150 000 ha in 1880 increased to 440 000 ha in 1949. Although the acreage began to decrease after 1950, over 300 000 ha of sweet potato plantings were still maintained mainly as raw material for starch production until 1965. Then, a drastic decrease of the acreage occurred due to competition with starch produced from imported raw materials. The total area planted with sweet potato had decreased at the rate of about 10% per year and 65 000 ha of sweet potato cultivation was reported in 1986.

The average yield was about 12 t/ha before World War II and it increased gradually attaining 22 t/ha at present by the adoption of improved varieties and improvement of the cultivation method.

The main use for sweet potato before the World War II was as food for farmers. During the War about 20% of total production was used as raw material for alcohol production. After the food shortage ceased around 1950, the sweet potato was mainly used for starch production and about 50% of the total was consumed for starch production in 1963. At present, the figures for sweet potato consumption for table use, raw material for starch production, animal feed and alcohol production are 40, 30, 13 and 8% of total production, respectively (MAFF 1982–86). The trend of consumption is shown in Figure 1.

Roles of exotic germplasm

Systematic sweet potato breeding started in 1962 in Japan by using indigenous varieties as parents for crossing. Several excellent cultivars such as Norin 1, Norin 2 and Okinawa 100 were released as a result of the initial phase of sweet potato breeding.

However, additional improvement by breeding became increasingly difficult to attain mainly due to common genes amongst parental varieties. To overcome this difficulty, exotic breeding materials from other countries and wild relatives were used as gene sources to introduce new superior genes into offspring plants. They played important roles in the breeding of new cultivars.

More than 130 cultivars have been introduced mainly from South America, Oceania and Southeast Asia. D.E. Yen's collection which includes about 230 cultivars of Southeast Asia, Oceania and South America was also introduced and some of the varieties have been used as genetic resources for the breeding (Takemata and Sakai 1975).

About 300 accessions of wild relatives have been collected and introduced into Japan mainly from tropical America — Mexico, Guatemala, Colombia, Ecuador and Costa Rica. They were classified into 2 groups based on crossability with the sweet potato and about half of them belong to group 1 which could be crossed with the sweet potato. *Ipomoea trifida* is the only species belonging to group 1 and there are diploid, tetraploid and hexaploid forms in it (Kobayashi 1978). *I. trifida* has also been used as crossing material to introduce new genetic diversity into the sweet potato. After the production of interspecific hybrids, different varieties of sweet potato should be used as the recurrent parent in backcrosses to eliminate undesirable characters of wild species and accumulate the useful genes relating to yielding ability and quality (Sakamoto 1970).

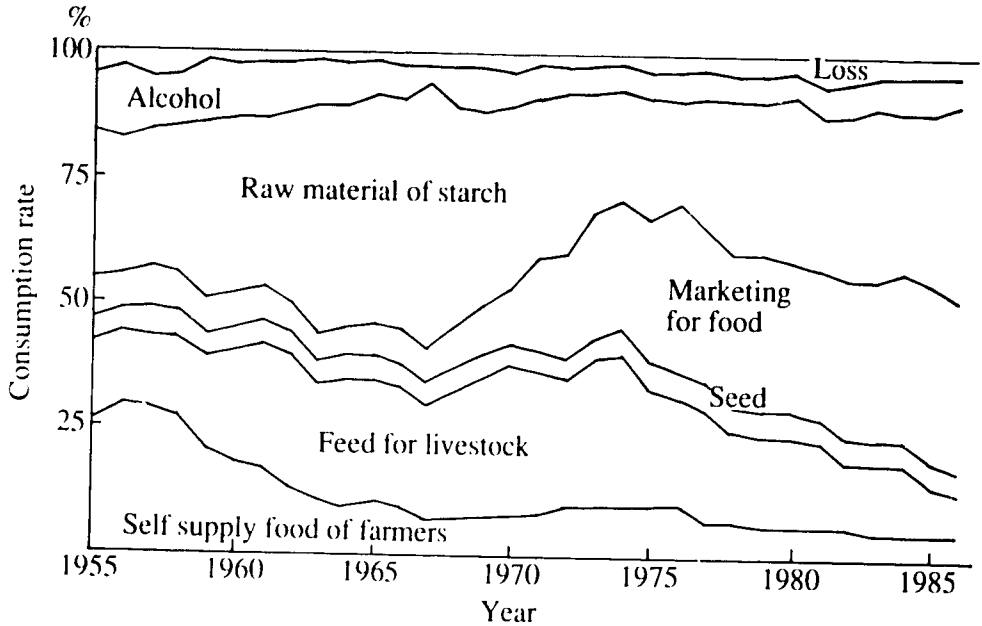

FIGURE 1 Trend of consumption of sweet potato in Japan.

Methodology of using genetic resources for breeding

To use the genetic resources as parental materials, it is necessary to characterize them. Some 50 morphological and 25 biological characteristics are being investigated for all genetic resources at 3 National Breeding Laboratories in Japan. The results of evaluation of wild relatives, *Ipomoea trifida*, for southern root-knot nematode, *Meloidogyne incognita*, and coffee root-lesion nematode, *Pratylenchus coffeae*, are shown in Table 1 and most accessions were recognized to be highly resistant to both nematodes (Sakamoto 1979). Some varieties in Yen's collection were reported resistant to block rot, *Ceratocystis fimbriata*, and scurf, *Monilochaetes infuscans* (Yen 1974).

Some germplasm in Yen's collection shows bush or erect type growth habit which is suitable for mechanical vine harvesting (Takemata and Sakai 1975).

Up to now, single crosses have been mainly employed for sweet potato breeding in Japan and exotic cultivars have been crossed in some cases with the Japanese cultivar so as to introduce new morphological or physiological genes from the foreign cultivar or accumulate the resistant genes. To use wild relatives as parental materials, it is recommended to use a cultivar as the female parent to get higher seed set percentage and cultivar as recurrent parent of back crossing.

Organization of sweet potato breeding

Hybrid seeds were produced in Okinawa and used for breeding at several Prefectural Agricultural Experiment Stations in the early era of sweet potato breeding in Japan. At present, sweet potato breeding is being carried out systematically at 3 National Breeding Laboratories; hybrid seeds produced at Ibusuki (Kyushu National Agricultural Experiment Station) are sent to Kumamoto (Kyushu National Agricultural Experiment Station) and Tsukuba (National Agriculture Research Center, NARC) for selection. All genetic resources have been conserved and evaluated at these laboratories and most of them have been

conserved at several places to minimize the risk of loss due to rotting or drying up during storage. Promising lines selected at the Breeding Laboratories are distributed to the related Prefectural Agricultural Experiment Stations for regional trials. The lines that show excellent performance are finally registered as new varieties and released for extension in the recommended area by the Ministry of Agriculture, Forestry and Fisheries. Up to date, 40 cultivars have been registered by MAFF.

Besides that system, sweet potato breeding is being done at some Prefectural Agricultural Experiment Stations and some private companies on a small scale.

TABLE 1 The degree of resistance of wild relatives *of I. trifida* to nematodes.

Accession No. Polyploidy	Line No.	RKN	RLN	Accession No. Polyploidy	Line No.	RKN	RLN
K123	T1	S	R	K233	Li 1	R	M
6x	2	M–R	R	4x	2	R	R
	4	R	R		3	R	M
	5	S	R		4	R	M–R
	6	M–R	R		5	R	R
	7	R	R		6	R	M–R
	8	R	R		7	R	R
	9	M	M	K300	1	R	S
	10	R	R	4x	2	R	M
	11	R	R		3	R	R
	12	R	R		4	R	R
	13	S	S		5	R	R
	14	R	R		6	R	R
	15	R	R		7	R	R
	16	R	R	K222	1	R	R
	17	R	M–R	3x	2	R	R
	18	R	M		3	M	M–R
	19	R	R		4	R	R
	20	R	R		5	R	R
	102	M	R		7	R	R
	103	R	R	Sweet potato	Norin 1	SS	R
IB	63001	R	M	6x	Norin 2	R	S
	63002	R	R		Koganesengan	M	M
6x	63003	S	M		Minamiyutaka	R	R
	63004	R	R				
	63005	R	R				

RKN: Degree of resistance for southern root-knot nematode, *Meloidogyne incognita*.
RLN: Degree of resistance for coffee root-lesion nematode, *Pratylenchus coffeae*.
R: Resistant; M: Moderate; S: Susceptible; SS: Very susceptible.

Breeding objectives

Different breeding objectives for sweet potato are set up according to the consumption of the varieties. Varieties for table use are required to possess delicious taste, attractive appearance (ie. good skin and flesh colour and good shape) and storability. Varieties for starch production should have high yielding ability and high starch content. Varieties for animal feed require vigorous vine growth and high yield of storage roots with high dry matter content. For alcohol production, varieties for starch production are in use. In addition to those special characters, acceptable high resistance to major pests and diseases are required generally.

Major pests and diseases of sweet potato in Japan are nematodes (southern root-knot nematode, *Meloidogyne incognita*, and Coffee root-lesion nematode, *Pratylenchus coffeae*), black rot, *Ceratocystis fimbriata*, stem rot, *Fusarium oxysporum* f. sp. *batatas*, soil rot, *Streptomyces ipomoea*, and viruses (feathery mottle and russet crack-like symptom). Since sweet potato weevil, *Cylas formicarius*, and scab, *Shaceloma batatas*, have not been observed on the main island of Japan, selection for these insects and disease has not been done.

Results of breeding

Official release of an excellent variety 'Koganesengan' in 1966 highlighted the value of foreign varieties. This variety takes in the good attributes of Pelican Processor (a US variety) and T. No. 3 (an Indonesian variety). In comparison with other standard cultivars Koganesengan is 2–3% higher in starch content, 20–30% higher in yield and shows a short growing period adapted for early harvesting. This cultivar covered 30% of the total sweet potato cultivating area of the country in 1971. It has, however, some defects in resistance against nematodes and black rot, and in storability (Sakai et al. 1967).

'Minamiyutaka' registered in 1975 was bred to improve the above defects of Koganesengan. For the breeding of this variety, a wild relative, *Ipomoea trifida*, was used as parental material to improve the resistance to nematodes and black rot and storability. In this case, sweet potato was used twice as a recurrent parent of backcrossing to improve the economic traits of wild relatives. The pedigree of Minamiyutaka is shown in Figure 2 (Sakamoto 1976).

'Tsurusengan', released in 1981, is a cultivar with high vine yield for green animal feed. This cultivar was developed from the cross between F53–6 and a Japanese selected line. F53–6 is a line selected from progeny of the US cultivars. Tsurusengan shows erect plant type with vigorous top growth and recorded 177 ton in fresh vine weight per hectare by cutting at three times under direct planting cultivation (Shiga et al. 1983).

'Benihayato' is a new cultivar for cooking and processing released in 1985. This variety developed from the cross between Centennial (a US cultivar of high carotene content) and a Japanese breeding line which was derived from the second backcross generation of interspecific hybridization between cultivated sweet potato and wild species, *I. trifida* (Sakamoto et al. 1987).

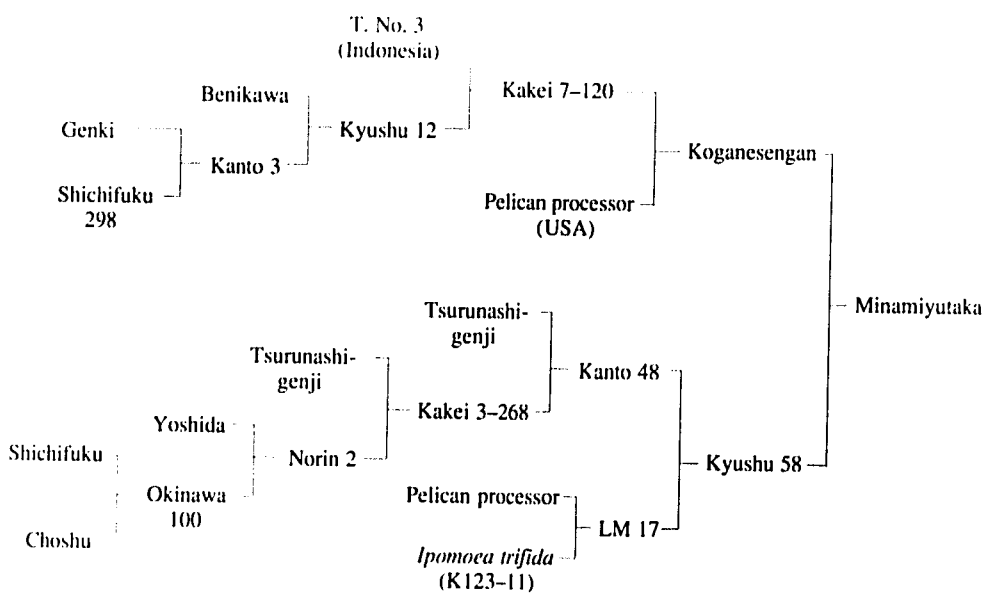

FIGURE 2 Pedigree of Minamiyutaka.

Conclusion

Production of the several successful varieties demonstrates the value of exotic germplasm to broaden genetic background and to introduce specific attributes (i.e. resistance to nematodes). Allelic diversity in sweet potato is well known to result in higher yield (heterosis). Exotic germplasm is an excellent source of such allelic diversity to improve yield potential of sweet potato cultivars in Japan. Successful use of the *I. trifida* accession presents a good example of the value of wild germplasm for allelic diversity and for specific attributes (i.e. nematode resistance).

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CHAPTER 10

The development and utilization of maize germplasm in China

HSU CHI-FENG

Maize is one of the main crops in China. After rice and wheat, it ranks third. In north China it is the second most important crop after wheat. It is distributed in a broad area between 20° and 50°N, from the Northeastern region to the Southwestern provinces, forming a diagonal belt across the country. The total area sown to maize is about 18.15 million hectares and the total annual yield is 64.25 million tonnes (Tong 1987). After America, China has become the second leading maize producing country in the world.

Along with developing and utilizing the maize germplasm, as well as using single cross and double cross hybrids to replace the varieties, the yield and quality of maize have been raised increasingly. The maize improvement work in China, in the last thirty years, has gone from using local varieties, intervarietal hybrids, double cross hybrids and finally single cross hybrids. Up to now, the single cross hybrids occupy about 80% of the total maize acreage. The average yield per unit area increased from 1.43 t/ha in 1952 to 3.54 t/ha in 1985. The higher yield (163% more than 32 years ago) has turned maize from being a staple food for people to a feed for animals. In addition, about two million tonnes have been exported annually in recent years.

Since maize did not originate in China, there is not too much germplasm for breeding work. According to the written record, maize was first introduced into Southeastern China about 450 years ago, and probably within the last 200 years, it had spread in most provinces and gradually formed many local varieties. They number about 11 000 (BAU 1976). With respect to the sources of inbred lines, much attention was paid in the use of these varieties and the inbred lines usually selected from them. At the same time, great emphasis was put on introducing exotic germplasm including varieties, population, synthetics, inbred lines and hybrids. Several hundreds of breeding materials have been introduced from more than 20 countries such as USA, Mexico, Italy, France, Yugoslavia and some Asian countries. Before being released, they should be isolated and inspected by the Institute of Germplasm. The evidence revealed that when the materials came from regions of similar latitude and climate condition, as well as not having serious shortcomings, they could be used immediately. But most of them have to be acclimatized or improved and then used as source materials.

The most efficient work is introducing exotic elite inbred lines especially those having better combining ability, wide adaptability, resistance to prevailing diseases and no photosensitivity. They are generally combined with domestic inbred lines into various hybrids. The breeding of single cross hybrid 'Zhong Dan No 2', combined with exotic line Mo17 and domestic line Zi 330, was the most remarkable example. The planting area is about 1.97 million hectares and the increased production added up to 1.75 million tons within the last eight years (Li 1987).

In order to reduce the damage from diseases in maize production, some mutant genes have been introduced to improve the domestic materials. In the late seventies, single major resistant genes, Ht_1, Ht_2, Ht_3... Ht_N and rhm have been introduced and transferred into different lines so as to raise the resistance to northern leaf blight and southern leaf blight. By using multiple resistant inbred lines and their hybrids, the serious prevailing diseases have been controlled and the damage reduced.

Another success involves developing and utilizing hybrids with high lysine content. Lysine and tryptophane are the limiting amino acids to man and other monogastric animals

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such as pigs. Normal maize contains low levels of lysine and tryptophane, only 0.2% and 0.05% respectively, on the basis of whole kernel weight, while with maize a high lysine content it is 0.4% and 0.103%. The nutritive value is similar to defatted milk powder for children. Hence many countries have been developing the program of quality protein breeding. Since 1974, the source materials of high quality protein such as opaque-2 have been introduced from Yugoslavia, USA and CIMMYT*, and a quality breeding program has started in China.

Opaque-2 gene (op-2) is a single recessive mutant gene with the expression of opaque phenotype on endosperm and raises the percentage of lysine and tryptophane (Table 1). It should be transferred into normal lines and then combined into high lysine content hybrids. For example, to convert normal line Mo17 into op-2 line Mo17, you should cross it with op-2 source material, back cross F_1 with normal line Mo17 and selfcross several times. The following diagram shows the procedure:

Genetic constitution(%)			Back crosses and self crosses
5052	Mo17	P	Mo17^{++} x 5052**
			↓
50	50	F1	(Mo17 x 5052)$^{++}$ x Mo17^{++}
			↓
25	75	BC1	(Mo17^2 x 5052)$^{++}$
			↓⊗
		S1	(Mo17^2 x 5052)$^{++}$ (Mo17^{2}x 5052)**x Mo17^{++}
			↓
12.5	87.5	BC2	(Mo17^3 x 5052)$^{++}$ x Mo17^{++}
			↓
6.25	93.75	BC3	(Mo17^4 x 5052)$^{++}$
			↓⊗
		S2	(Mo17^4 x 5052)$^{++}$ (Mo17^4 x 5052)**x Mo17^{++}
			↓
3.125	96.875	BC4	(Mo17^5 x 5052)$^{++}$
			↓⊗
		S3	(Mo17^5 x 5052)$^{++}$ (Mo17^5 x 5052)**
			↓⊗
			op-2 Mo17**

$^{++}$ normal endosperm ** opaque endosperm

TABLE 1 Comparison of nutritive elements of op-2 and normal maize.

Variety	Protein (%)	Lysine (%)	Tryptophane (%)	Nicotinic acid (%)	Free nicotinic acid (%)
op-2 BAU101	9.24	0.35	0.103	0.029	0.0178
Wu Dan Zao (ck)	8.75	0.19	0.049	0.015	0.006
BAU 101/ck	+5.6	+84.2	+102	+93.3	+196.6

* CIMMYT is the International Maize and Wheat Improvement Centre, Mexico City.

After backcrossing four times with recurrent parent Mo17 and selfing three times, the normal inbred line Mo17 has been converted into opaque-2 Mo17, whose genetic constitution is composed of 96.875% of Mo17 and 3.125% of op-2 line 5052. If compared with normal Mo17 and op-2 Mo17 visually, only the phenotype of endosperm is different. Crossing the op-2 inbred lines with each other, op-2 hybrid may be selected. That is the high nutritive hybrid with high lysine and tryptophane content.

The biochemical test revealed that not only were the lysine and tryptophane contents of kernel higher than that of normal maize but also the nicotinic acid rates doubled and the free nicotinic acid tripled (Zheng and Hsu 1981). So it was very efficient for preventing and curing the human disease 'Pellagra', caused by lack of free nicotinic acid. For example, during the prevailing period from January to April, 400 peasants who consumed the opaque-2 hybrid (BAU101) as staple food, were free from the disease, while those who used common maize as diet, 2.4% of persons fell ill. Results of testing the content of metabolic products (N^1-methylnicotinic acid) from the urea showed that the N^1-MN content of the tested group who dieted on opaque-2 maize was raised from 0.12 mg to 0.22 ± 0.03 mg. It was near the standard level necessary for healthy people (0.24 ± 0.02 mg). But N^1-MN content of the untreated group remained at a level of 0.12 mg (MAC 1980).

When fed to young pigs, opaque-2 maize had a great effect on the daily weight gain. Less feed was consumed and there was a high feed efficiency (Dou 1981). The nutritive value of opaque-2 maize equalled normal maize plus 5% soyabean meal. That means 25% of soybean meal would be saved. At the level of 9.0–14.6% protein, as feeding with opaque-2 maize, the daily weight gain was 29.8–124% higher than that of normal maize and 0.47–2.13 kg of feed was saved for increasing 1 kg of pork. At the same time, the results showed that the opaque-2 maize was a better feed than rice. The daily weight gain was 21.5% higher than from rice and 0.44 kg of feed was saved for increasing 1 kg of pork (Shi 1987).

In 1987, the planting area of opaque-2 hybrid was about 15 000 hectares and the average yield per unit area is similar to or 5% less than that of normal control. But the production of lysine and tryptophane is doubled.

TABLE 2 The effect of feeding young pigs with normal and op-2 maize.

	Normal maize +5% soybean meal (1)	op-2 maize* +5% soybean meal (2)	(2)/(1) (%)	Normal maize +10% sunflower meal (3)	op-2 maize + 10% sunflower meal (4)	(4)/(3) %
Daily weight gain (g)	453	474	+4.6	302	454	+50.3
Daily feed consumed (kg)	1.74	1.65	-5.28	1.66	1.94	+16.9
Ratio of feed consumed to weight gain	3.84	3.49	-9.1	5.50	4.27	-22.4

In order to improve the soft chalky endosperm, the secondary effect of opaque-2 mutant gene, populations and synthetic varieties with hard or semi-hard endosperm have been introduced from CIMMYT and USA. The phenotype of hard or semi-hard endosperm is controlled by modify genes, and the kernel weight can be raised. But the lysine content is somewhat lower than that of pure opaque-2 endosperm, but higher than that of normal kernel.

The breeding approaches are as follows:

1. Inbred lines with hard or semi-hard endosperm are selected directly from these materials by selfing several times.
2. As source materials of modifier, cross them with opaque-2 lines and backcross several times with recurrent parent to integrate modifier genes into opaque-2 endosperm or select lines from the progeny of crossing.
3. Non photo-sensitive plants can be selected from exotic tropic populations and synthesized into a new population from which new inbred lines suitable for the temperate zone, can be selected. Or they may be crossed with early matured opaque-2 lines and inbred lines selected from its progeny.

In recent years, a lot of lines with hard or semi-hard endosperm have been selected. The lysine content ranges from 0.3–0.5% per kernel weight and the hardness scale of endosperm is about 1.5–3.5.

Incorporating the high-lysine maize with high oil content material is an important method for quality breeding. The normal maize contains about 4% of oil of which 85% distributes in the germ. The role is the higher content of oil, the larger size of germ, so that the lysine content is raised. Many such lines have been selected. The average content of lysine is over 0.5% and the oil percentage is about 10. The kernels of new hybrids will have a high lysine content in the endosperm and a high oil content in the germ. Thus it possesses both high nutrition and high energy.

The source materials of sweet corn were introduced from USA, some from Thailand. Besides the common sweet corn ($su_1 su_1$), the supper sweet ($sh_2 sh_2$) and sweet enhancer gene (se se) have been developed in recent years. There are two ways to use them: either harvesting the prematured ears within 18–21 days after pollination and can the kernels immediately; or harvesting prematured ears within the same number of days after pollination, transport them to urban districts for boiling within 24 hours and sell them as breakfast food. The biggest market is in Hong Kong and some cities on the mainland. Canned kernels are exported to Japan and other countries.

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CHAPTER 11

General condition of citrus germplasm research in China

YE YINMIN

Citrus fruit trees mainly include three genera, *Fortunella*, *Poncirus* and *Citrus*. Undoubtedly, China is the original home and centre of *Poncirus*, *Fortunella* and most of the species in the genus *Citrus*. According to historical literature, citriculture in China appeared 4000 years ago, and fruits were sent as tribute to the imperial court. Because of their extremely abundant natural resources and long growing history, citrus fruits consist of many variant types. These rich and varied germplasms are of tremendous worth to the human race.

Long ago, our ancestors knew mandarin (*Citrus reticulata*), citron (*C. junos*) and trifoliate orange (*Poncirus trifoliata*), but they considered these three plants as one class and spoke of them together. In other words, our ancestors had recognized that these three plants were closely related. Zhang Hua stated 'there are many types of mandarins and pummelos. The mandarin, trifoliate orange and orange are distributed widely' in *Bowuzhi* published in the third century. In *Zhouli*, published in the early first century it was mentioned that 'mandarin introduced to north of Huaihe river will become trifoliate orange', which apparently recorded the natural distribution of mandarin and trifoliate orange. Han Yenzhi used two-thirds of the contents of *Julu*, published in 1178, to record 27 citrus types, varieties and strains in Zhejiang Province, China.

With the development of modern agricultural sciences, people have realized the importance of plant germplasm. Investigation of fruit tree resources was carried out in our country in the 1950s. The Chinese Academy of Agricultural Sciences established the Research Institute of Citrus in the 1960s, in which the following works were carried out: wild and cultural citrus resources were widely investigated; a central citrus germplasm orchard in China was set up; and the survey, collection, preservation and utilization of the citrus germplasm in our country were systematically done.

The main cultivated types, varieties and growing area

The citrus producing area in China is distributed at about 33°N latitude, and covers 17 growing provinces. Mandarin production ranks first in commercial cultivation, followed in order by orange, pummelo, kumquat and others.

Mandarins

Mandarins grow widely and represent about 63% of total citrus production in China.

a) Zhu-ju (*C. erythrosa* Hort. ex. Tan.). An old cultivation group, mainly distributed in the provinces of Jiangsu, Jiungxi, Gansu, Shanxi, Hunan and Hubei has strong tolerance to cold.
b) Hong-ju (*C. tangerina* Hort. ex. Tan.). Mainly grown in Sichuan and Fujian provinces.
c) Ponkan (*C. reticulata* Blanc.). Mainly grown in provinces of Guantong, Fujian and Taiwan and also in some regions of Guanxi, Yunnan and Zhejiang provinces.
d) Ru-ju (*C. kinokuni* Hort. ex. Tan.). Principally distributed in provinces of Jianxi and Zhejiang; its dominant variety is Nanfeng mandarin with small, sweet and almost seedless fruits and monoembryonic seeds.
e) Jiaokan (*C. tankan* Hort. ex. Tan.). Widely grown in the provinces of Guanxi, Fujian,

Taiwan and Guantong; a high accumulated temperature is needed for growing. Although it can bear in the northern part of the citrus producing area, fruit quality declines significantly and it has almost no commercial value.

f) Satsuma (*C. unshiu* Marc.). This group is most widely cultivated in every growing area and its production accounts for about 60% of total mandarins. The main varieties, mostly introduced from Japan are cultivated in the provinces of Hunan, Jiangxi, Hubei and Zhejiang.

There are some special local varieties of mandarin, with very concentrated growing area in some regions and having some commercial yield. Bendizao (*C. succosa* Hort. ex Tan.) is mainly grown in the provinces of Huangyan, Zhejiang. Man-ju (*C. tardiferax* Hort. ex Tan.) is grown in Huangyan too with monoembryonic seeds. Ou-ju is a local variety in the provinces of Wenzhou Zhejiang, early in maturity and with monoembryonic seeds. Trifoliate orange (*Poncirus trifoliata*) is used as the main rootstock for mandarins.

Oranges

Orange (*C. sinensis* (L.) Osbeck) is mainly cultivated in provinces of Sichuan, Guangtong, Fujian, Guangxi, Hubei, Jiangxi and Taiwan. Its production accounts for 25% of total citrus production in China and common orange dominates. In China, the traditional varieties of orange can be divided into two types, the acidless orange and the acid sweet orange.

a) The acidless orange type is pricipally distributed in provinces of Guangdong, Fujian, Guangxi and Taiwan. Its main cultivars are Xinhue and Liuchen.

b) The acid sweet orange type is mainly grown in Sichuan province, but it is also distributed in provinces of Guangxi, Guangdong, Fujian and Taiwan. The main varieties are Xiangfeng, Jinchen and Xuegan. Additionally, some orange varieties were introduced from abroad, including Navel, Valencia and Hamlin; they are cultivated mainly in Sichuan Province. The Hamlin orange adapts well to different climates and tends to extend its growth.

Rootstocks for orange generally are Trifoliate orange in northern area, and Limonia in the southern producing area. Sometimes, Tangerina is used as rootstock too, in the provinces of Sichuan and Fujian.

Pummelo (*C. grandis* (L.) Osbeck)

Pummelo types are extremely varied and distributed very widely. Some production takes place in provinces of Sichuan, Guangxi, Fujian, Taiwan, Jiangxi, Hubei and Hunan. The main varieties include Satien, Lianpin, Kweifu, Wendan, Wanbei and Sijipao.

Air-layer propagation is a traditional method for pummelo, and grafting is on domestic seedling free rootstock.

Kumquat (*Fortunella*)

Except Changyejingan (*F. polyandra* (Ridl.) Tan.), other species are sporadically grown in every citrus producing area. They are mainly used for the fresh market, candy and ornament. Their main producing area is in the provinces of Guangxi, Jiangxi, Fujian and Hunan. Kumquats mostly use Trifoliate orange as their rootstock. Additionally, pummelo is also used as rootstock in a few producing areas of Fujian Province. Ornamental kumquats use cuttings of Citron (*C. medica*) as rootstock in Guangdong Province.

Others

a) Citron (*C. medica* L.) grows sporadically in the provinces of Yunnan, Guangdong,

Guangxi and Fujian, in which Buddha's hand (*C. medica* var. *sarcodactylis)* is mainly used for ornament cultivation by cutting propagation.

b) Lemon (*C. limon* (L.) Burm. f.) mainly grows in the eastern part of Sichuan Province. Eureka lemon is the main variety introduced from abroad. In early growing periods, Trifoliate orange was always used as rootstock for lemon in China. Later, we found that exocortis disease was very serious on lemon trees using Trifoliate orange as rootstock. Recently, we have taken Tangerina as rootstock instead of Trifoliate orange.

c) Sour-orange (*C. aurantium* L.) grows sporadically everywhere and is mainly used for medicine. Daidai is used as essential material for flower processing in the provinces of Fujian and Jiangsu. Goutouchen is used as rootstock on account of its strong tolerance to salt and tristeza disease.

Investigation and collection of citrus germplasm resources

Investigation and collection of citrus germplasm resources were carried out in most provinces in the late 1950s with supplementary investigation in Tibet in the 1980s.

Wild citrus germplasm

During investigations of the wild germplasm resources, we found some new species such as Honghechen, a new species in subgenus *Papeda* of genus *Citrus*, found in 1974. Many types of wild mandarins were also found in Hunan Province from the 1960s to the 1970s. Moreover, in the provinces of Guangxi and Jiangxi, different types of wild citrus are found at different times, of which about 6–7 types have the position of botanic species.

Distribution and classification

Distribution and classification for the wild citrus species which have been known to originate from China have been made clearly. Ichangchen (*C. ichangensis*) is not only naturally distributed in Hubei Province, but it also has wild distribution in the provinces of Sichuan, Guizhou, Yunnan, Hunan and Guangxi, and some new types of it have been found. Xiangchen (*C. junos*) also has wild distribution. In the past, there were some different opinions about these species. I believe that Xiangchen should possess the position of botanic species because of wild distribution and stability of its characters. Limonia (*C. limonia*) has wild distribution in provinces of Guangxi and Guizhou and is mainly of yellow-peel type. It is similar to Kusaie lime which grows in India. Therefore, I consider that the limonia possesses the position of botanic species.

Variant types

Abundant variant types have been collected, such as trifoliate orange, which has many natural variant types. Besides big-leaf and small-leaf strains, we have found early-bearing 74-1 dwarf trifoliate orange and 85–90 thorn-few types. A tetraploid (4 ×) trifoliate orange with large leaves, thick branches and thrifty trees was collected in Jiangsu Province. Many types of small citron (*C. medica* var. *ethrog*) were found in Yunnan Province.

Studies on citrus relationship

Since the 1970s we have made some progress with morphological taxonomy, based on the citrus pollen characters by use of a scanning electron microscope, analysis of isoenzyme zymograms, and citrus genome analysis. All of these methods have provided important evidence for citrus taxonomy and determination of citrus relationships.

Tissue culture techniques for citrus germplasm

In 1975, Wang Dayuan studied the speeding up of germplasm propagation with tissue culture. He researched on whether the callus can maintain high differentiation abilities and the inherent stabilities of regenerated plantlets. Zhang Jinren and others continued to observe the callus for eight years and subcultured 38 generations. They proved that the chromosome ploidy of callus and regenerated plantlets have not changed, and that the tissue culture technique is a promising method for preserving citrus germplasm. Moreover, by *in vitro* culturing epicotyls and young shoots in *F. margarita*, a large number of green tufted-shoots and complete regenerated plantlets have been obtained. By using young seeds of Xuegan (*C. sinensis*) some complete regenerative plantlets were formed and blossomed in six months. This could promise to solve the question of the long juvenile stage in citrus. Chen Zhenguang and others obtained plantlets from anthers of Calamondin (*C. madurensis*).

Citrus germplasm orchard

Field preservation is a major method for preserving and evaluating citrus germplasm nowadays. In recent years, the Chinese Academy of Agricultural Sciences has established 16 national fruit germplasm orchards, including citrus, apple, pear, peach, grape and so on. The citrus Germplasm Orchard is located in and managed by our Institute (Beibei, Chongqing). Today, besides traditional collection, preservation and evaluation of citrus germplasm, we are starting to evaluate and propagate the virus-free varieties in main germplasm to prepare for the establishment of a virus-free citrus germplasm orchard.

Studies on utilization of citrus germplasm

At present, citrus germplasm is mainly used for cross-breeding. Since the 1950s, Zhejiang Citrus Research Institute, Hunan Horticultural Research Institute, Huazhong Agricultural University and our Institute have done much work in cross-breeding and induction of mutation, and obtained a lot of useful new varieties. The cross-breed 1-1232 is a long-lasting and good quality tangor variety bred from Valencia × Hongju. A new Hongju variety with fewer seeds and high yield, also has been obtained by ^{60}Co irradiation. The cross-breed 432 is selected from the breeds of Ougan × Gailiangchen (which is a chimera) and their later generations have high yield, good quality and last well. With colchicine, a tetraploid variety, 4× Shiyueju, has been obtained, having high grade and yield.

Our Institute has been exploring and comparing new citrus rootstocks for a long time. It merits attention that pummelo (*C. grandis*) usually grafts on the same varieties, but this year Wan Liangchai has grafted it on trifoliate orange and *Atalantia* Corr. and Jindou respectively. The test results showed that this *Atalantia* is a very good dwarf rootstock, and bears fruits 2–3 years earlier than those in common use. It also can maintain the characteristics of the scion varieties. In addition, Chen Shanchun and others have recently studied the effects of salt on the growth characteristics and biochemical markers of cell clone, from the embryos of orange (*C. sinensis*) in order to select a salt-tolerant cell clone of citrus *in vitro*.

The citrus germplasm in China has made a very important contribution to the citrus industry of the world. We believe that with the development of modern science and technology, the contribution will be greater than ever before.

PART 3

Utilization of genetic resources for pest and disease resistance

Chairmen
C. Kaneda
Shao Qiquan

CHAPTER 12

Breeding for clubroot resistance of crucifer crops in Japan

HIROAKI YOSHIKAWA

Clubroot disease caused by *Plasmodiophora brassicae* Woronin, is considered one of the most serious diseases of crucifer crops. In Japan the occurrence of clubroot was first recorded in 1892 on cabbage and turnip, and in 1978 on Chinese cabbage.

For a long time the infested area was limited and severity of clubroot was not high except in a few fields where traditional turnip cultivars were grown successively. Since about 1965, however, clubroot has been reported in many crucifer crop growing regions, and the severity and percentage of diseased plants have increased considerably. At present, clubroot disease can be found almost all over Japan, especially in the central and northern parts, where it is a serious problem. Intensive crucifer cropping was undoubtedly conducive to a build-up of the organism in the soil.

High concentrations of pathogen in soil were apt to make chemical control ineffective, so that crucifers are forced to be replaced by other crops. Clubroot is a constant threat to crucifer crops, especially to cabbage, Chinese cabbage, turnip, cauliflower and broccoli. Cultivation of resistant cultivars is one of the safest and cheapest methods available to combat clubroot. Breeding work for clubroot resistance was started first on cabbage at the Vegetable and Ornamental Crops Research Station (now the National Research Institute of Vegetables, Ornamental Plants and Tea) in 1974. At present, breeding for clubroot resistance has been practised by one national, and 6 prefectural research stations as well as by 28 seed companies. Through their great efforts, many resistant varieties have been released in increasing numbers, especially in Chinese cabbage. An outline of the breeding works for clubroot resistance in Japan is presented here.

Inoculation methods

For breeding work, a simple and reliable inoculation method is required for stable and uniform disease development under normal environmental conditions. To test accurately and simultaneously the resistance of a large number of plants, it is important to use the minimum effective quantity of pathogen to avoid its unnecessary spread. From such standpoints, Yoshikawa et al. (1981) established a screening method they called the 'insertion method'. According to their comparison tests on spore concentrations and inoculation methods, the insertion method showed the highest percentage of diseased plants and highest disease index under any condition of spore load and soil pH than other dipping, mixing and pouring methods.

Details of the insertion method are as follows:

1) *Insertion method* — The inoculum is prepared by the method of Williams (1966). A mixture of one part clay powder, three parts perlite and one part peat constitutes a good medium for plant growth and clubroot development. The mixture was adjusted to pH 5.5–6.5 and the spore load to 5×10^6 per gram of dry mixture. Cylindrical blocks of the mixture (infested soil), 4 cm in length and 3 cm in diameter, were made mechanically and inserted either into pots or fields. Seeds were sown on these blocks and covered with a thin layer of perlite or noninfested soil (Figure 1).
2) *Favourable conditions for infection* — A high spore load, usually 5×10^6 per gram of dry soil, is optimum for disease development. A soil pH of 5.5 is best, but pH 5.5–6.5 is

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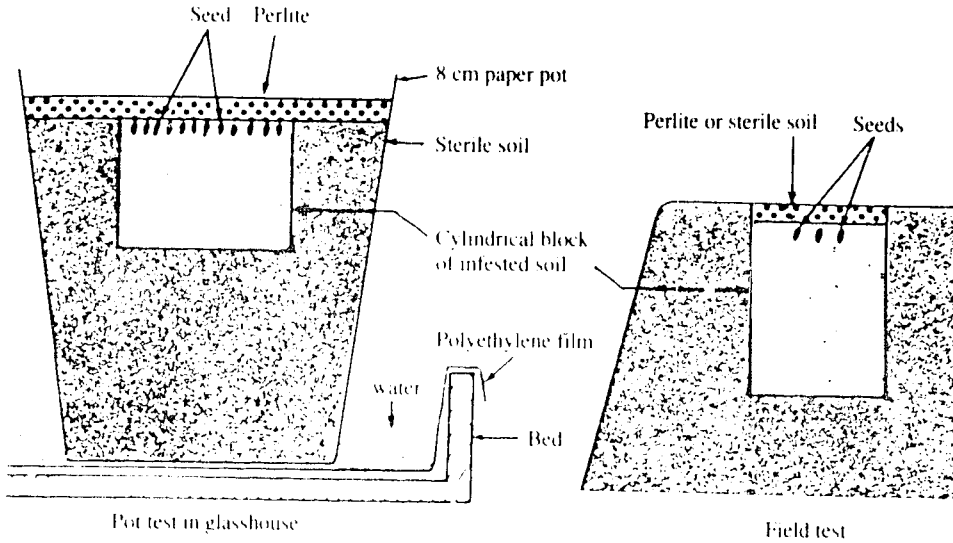

FIGURE 1 A sketch of the insertion method for glasshouse and field tests.

adequate. The soil should be kept damp throughout the experiment. Soil temperature should be kept between 18° and 30°C, preferably between 18° and 24°C. At high temperatures soil drying becomes a problem and frequent watering is necessary.

3) *Observation and evaluation of resistance* — The best time for observation is when the susceptible cultivar is completely and severely affected. Observations on clubbing were usually carried out 6 weeks after sowing when conditions favoured clubroot development. When conditions were less favourable observations were made one or two weeks after clubs became visible with the naked eye on the root base of the susceptible check.

The degree of infection was rated on a 0 to 3 scale, where 0=no infection, 1=few small clubs, 2=considerable clubbings, 3=severe clubbing. The number of plants in category 1 was multiplied by 20, in 2 by 80, in 3 by 100 and the sum of the products was divided by the total number of plants to give the disease index. The trials were usually replicated three with 10 plants per pot.

Cultivar difference

This field of research has received much attention in recent years. Hitherto it has generally been considered that the *B. campestris* group is more susceptible to clubroot than the *B. oleracea* group. In Chinese cabbage marked cultivar differences have been noticed on soil infested at lower inoculum levels, but this has not been observed on soil infested at high inoculum levels. From the breeding point of view, it was thought that Chinese cabbage almost completely lacked resistance to clubroot, although some cultivars, including some from northern China, were less severely clubbed. Japanese turnip and mustard green are highly susceptible and lack a resistance source like Chinese cabbage. European turnip showed a wide range of resistance and suspectibility and some cultivars are highly or completely resistant to race 2. In the *B. oleracea* group, kales are thought to be the most resistant to clubroot. Cabbage showed a wide range of resistance and susceptibility, and none of the commercial cultivars was highly or completely resistant. Cauliflower, broccoli and kohlrabi are highly susceptible and lack a resistant source. Brussels sprouts are more resistant than

cauliflower or broccoli. Rutabaga exhibited a wide range of susceptibility to resistance, while oil rape, Hakuran and Indian mustard were very susceptible. Radish was more resistant than cabbage or rutabaga, and Japanese radish and *Raphanobrassica* showed the highest resistance of all the crucifer crops (Table 1).

Aside from crops, high resistance to clubroot race 2 was found in weeds, such as Sukashitagobo (*Nasturtium palustre* DC.), Inugarashi (*Nasturtium indicum* DC.) and Tanetsukebana (*Cardamine flexuosa* Withering), and in flowers, such as stock (*Matthiola incana* R.Br.) and wallflower (*Cheiranthus cheiri* L.).

TABLE 1 Breeding materials resistant to the race 2 of *P. brassicae* in crucifer crops.

Chromosome No.	Crop	Resistance[a]	Cultivars[b]
n=10	Turnip	RR	Novitas, Novitas 4×, Meedjeslander, Leielander, Ponda, Zelder GT, Halflong Purple Top LB, Mosa, Tigra 4×, Marco, Debra, Siloga, Bicolar, Talonda, Klepa 2×, Vollender Zelder MB, Croppa, VIR 1200, VIR 1201, VIR 1153, ECD 02, ECD 04, Orion, Mommersteeg, Polybra, Waalslander, Roskilde, Civasto R, Gerlia R
		R	Green Top Market, Manchester Market, Golden Ball, Green Top Scotch, Milan White, Red Globe, Green Top Stone, T-3, Milan Purple Top
n=9	Cabbage	R	Bindsachsener 72754, Böhmerwaldkohl 72755, Böhmerwaldkohl 72756, G-6-0, Vologodskaja S, Zimnjaja, Winter White
		M	Ladozhskaja 22, Losinoosarovskaja 8, Zimnjaja Gribovskaja 13, Tajninskaja, Closed Early, Aichibaibansei, Badjer Shipper
	Cauliflower	M	Walcheren Winter Manston
	Brussels sprouts	R	Fillbasket, Winter Harvest, Seven Hills, Roodaerf Seven Hills
	Kale	RR	K 269, K 173, K 271, K 278, K 158, Extra Curled Scotch, Dwarf Green Curled, Sinjaja Kyrgavaja
n=19	Rutabaga	RR	Wilhelmsburger, W. F. Neckless
n=9	Radish	RR	Sakurajima, Shogoin, Uchikigensuke, Miyashigesobuto, Shirokubimiyasigemarujili, Nezumi, Wakayama, Moriguchi, Hooryo, Chubukura, Ookura, Akitsumari, Hakata 40 days, Round Scarlet Giant, Natsuminowase No. 1 (F_1), Evelest, Hayamaru (F_1)

[a] RR: high resistance, R: semi-resistance, M:moderate resistance.
[b] No resistant cultivars in Chinese cabbage, salt greens, broccoli, oil rape and Hakuran.

Physiological races

So far it has been found that seven of the *P. brassicae* physiological races (using the term 'race' as defined by Williams) are present in Japan. Those are named races 1, 2, 3, 4, 5, 8 and 9 (Yoshikawa et al. 1978). This diversity of races has a direct implication on the breeding work and on the types of resistance in the host. Yoshikawa et al. (1978) tested 14 common cabbage, 2 turnip and 13 Chinese cabbage cultivars to observe their reactions to physiological races 1, 2, 3, 4 and to a mixture of all the four. Spore loads were 2×10^6 per gram of dry soil for the individual races and 4×10^6 per gram for the mixture. Cultivar differences in reactions

to the 4 physiological races were relatively small. Highly significant correlation coefficients between percentage of diseased plants or disease indices were observed for each pair of races. Mixing races did not enhance the incidence of disease. This proves that plants which are strongly resistant to one physiological race are also strongly resistant to others, as described by Nieuwhof and Wiering (1963). It also shows that selection of one of the most virulent races is helpful in screening and breeding work.

Inheritance of resistance to race 2

As Chinese cabbage has no resistant source at all, we crossed Chinese cabbage with resistant turnip, to transfer the resistant gene to Chinese cabbage. Resistant turnip 77b; a selection of ECD No. 2, was crossed with susceptible Chinese cabbage; Matsushima Shin No. 2, to yield F_1, F_2, BC_1, BC_2 populations. The F_1 was completely resistant. The F_2 segregated in the ratio of three resistant to one susceptible. The BC_1 generation segregated in the ratio of one resistant to one susceptible (Table 2). This shows that the resistance of 77b is controlled by a single dominant gene. Similar results, produced by the influence of a single dominant gene, were observed in 9 different F_2 generations of crosses between 5 Chinese cabbage cultivars: Kenshin, Hiratsuka No. 1, Matsushima Shin No. 2, Nozaki No. 2 and Choosen, and three cultivars of turnip: Siloga, Gelria R and Debra. The resistant gene is considered to be the same as in 77b and is the same as the resistant gene found in the group of European fodder turnips. Leaf morphology does not correlate with clubroot resistance in any of the 10 F_2 generations described above. Both clubroot resistance and leaf character of Chinese cabbage can be combined in one plant. The dominant resistant gene found in this study should be useful in developing clubroot resistant cultivars of the *B. campestris* group and others, especially Chinese cabbage, and the heading character can be improved easily by back-crossing.

TABLE 2 Frequency distribution of clubroot resistance in crosses between Matsushima Shin No. 2 (Chinese cabbage) and 77b (turnip).

Generation and progeny		Number of plants with disease index:				Mean disease index	Segregation ratio observed[a]
		0	1	2	3		R:S
P_1	Matsushima New No.2 (M)	0	0	0	22	3.00	
P_2	77b (b)	32	0	0	0	0.0	
F_1	M × b	44	0	0	0	0.0	
F_2	M × b	247	5	6	35	0.42	247 (220) : 46 (73)
BC_1	(M × b) × M	101	12	9	50	1.05	101 (86) : 71 (86)
BC_2	(M × b) × b	174	0	0	0	0.0	174 (174) : 0(0)

[a] R:resistant plants (disease index=0); S:susceptible plants (disease index=1,2,3).
Figures in parenthesis indicate theoretical ratios expected from 3:1 segregation in F_2, 1:1 in BC_1, and 1:0 in BC_2.

Similar genetical analyses were done by Yoshikawa et al. (1978), using the race 2 of the pathogen. In a cross between a resistant cabbage; Böhmerwaldkohl 72755, and a moderately susceptible cabbage: Aichidaibansei, the resistance of Böhmerwaldkohl 72755 was inherited by a polygene of four pairs (Table 3). Similar results were shown in the crosses of resistant cabbage; Bindsachsener 72754 or Böhmerwaldkohl 72756, with moderately susceptible cabbage, Aichidaibansei. In the cross of a resistant curled kale, K 269, with a susceptible

TABLE 3 **Frequency distribution of clubroot resistance in crosses of cabbages between Aichidaibansei and Böhmerwaldkohl 72755.**

Generation and progeny		Number of plants with disease index: 0	1	2	3	Mean disease index
P_1	Aichidaibansei (A)	0	10	48	28	2.21
P_2	Böhmerwaldkohl 72755 (B)	58	17	1	0	0.25
F_1	A × B	14	31	34	1	1.28 (1.23)
F_2	A × B	28	81	90	22	1.48 (1.26)
BC_1	(A × B) × A	0	14	133	53	2.20 (1.75)
BC_2	(A × B) × B	106	72	29	0	0.63 (1.23)

$r = (p_1 - p_2)^2/8(V_{F_2} - V_{F_1})$ r = 3.69

TABLE 4 **Frequency distribution of clubroot resistance in crosses of cabbage between Masagosanki (cabbage) and K 269 (kale).**

Generation and progeny		Number of plants with disease index: 0	1	2	3	Mean disease index	Segregation ratio observed[a] S:R
P_1	Masagosanki (M)	0	0	1	96	2.99	
P_2	K 269 (K)	99	0	0	0	0.0	
F_1	M × K	0	12	87	0	1.88	
F_2	M × K	157	95	214	130	1.53	439(447) :157(149)
BC_1	(M × K) × M	3	11	142	192	2.50	345(348) : 3 (0)
BC_2	(M × K) × K	168	87	99	10	0.87	196(182) :168(182)

[a] Same as Table 2.

cabbage, Masagosanki, the resistance of K 269 was inherited by single recessive gene and inheritance of leaf character was independent of that of clubroot resistance (Table 4).

Present status and problems of breeding

CHINESE CABBAGE AND OTHER *B. CAMPESTRIS* CROPS

The breeding work on Chinese cabbage has been carried out by crossing Chinese cabbage with resistant European fodder turnips since 1978 under a practicable plan. Fortunately the resistance is inherited by a single dominant gene, independently of the leaf character. These facts made the breeding work very successful. By 4 or 5 backcrossings of Chinese cabbage to the hybrids between Chinese cabbage and turnip, a good heading quality can be obtained. In the crosses between Chinese cabbage and turnip, lines of various types, such as heading, semi-heading, non-heading Chinese cabbage types, turnip type, *chinensis* type, nabana type (with buds and young clusters for eating) were segregated. Bolting character was also widely segregated in the F_2 generation.

Today, national and prefectural experiment stations as well as many (12 at present) seed companies are devoting their efforts to breeding many types of Chinese cabbage, 'Kanamachi, Nozawana, Sugukina and Oonobeni' types of turnip and 'Nabana, Mizukakena, Meikena and Oosakina' types of salt green. Up to date, 12 F_1 hybrids and 5 parental lines in Chinese

cabbage, 1 F_1 hybrid in turnip, 2 varieties and 1 parental line in salt green, and 1 F_1 hybrid in heading cabbage were released (Table 5), and some other breeding lines have reached the stage of testing their practical use, and for some of them hybrid seed production systems were established by using self-incompatibility. On the other hand there still remain some problems, namely that some lines have undesirable characters such as descreased virus disease resistance, liability to calcium deficiency, poor quality, etc.

TABLE 5 Clubroot resistant cultivars recently bred in Japan.

Crop	Cultivar	Release year	Bred by	Maturity	Head shape	Leaf colour
Chinese cabbage	Strong CR 75 (F_1)	1985	Watanabe Seeds Co.	late	round type	dark green
	Kuhkai 65 (F_1)	1985	Takii Seeds Co.	early	cannon type	green
	Kuhkai 70 (F_1)	1985	Takii Seeds Co.	medium	cannon type	dark green
	CR Kanki (F_1)	1985	Co. Nihon-nourin	medium	cannon type	green
	CR Kanki No. 100 (F_1)	1986	Co. Nihon-nourin	medium	cannon type	green
	CR Ryutoku (F_1)	1986	Watanabe Seeds Co.	semi-late	round type	dark green
	CR Ryoyu (F_1)	1986	Watanabe Seeds Co.	early	cannon type	dark green
	Dentou (F_1)	1986	Matsunaga Seeds Co.	early	cannon type	dark green
	Hukkatsu (F_1)	1986	Matsunaga Seeds Co.	semi-late	cannon type	dark green
	Renpa (F_1)	1986	Matsunaga Seeds Co.	semi-early	cannon type	dark green
	CR Hourai No. 1 (F_1)	1986	Touhoku Seeds Co.	early	cannon type	dark green
	CR Hourai No. 2 (F_1)	1986	Touhoku Seeds Co.	early	cannon typ	dark green
	P. L.[a] :Noh No. 1	1984	NIVOT[b]	early	round type	light green
	P. L. :Noh No. 2	1984	NIVOT	medium	round type	green
	P. L. :Noh No. 3	1984	NIVOT	medium	long type	green
	P. L. :Noh No. 4	1985	NIVOT	early	ball type	dark green
	P. L. :Noh No. 5	1985	NIVOT	medium	round type	green
Cabbage	YCRSE (F_1)	1984	NVOES			
Turnip	Hida-Tobime Kabu	1985	Kosaka Shubyo Co. Ltd.			
Salt greens	Shinano [Nozawana]	1982	NVOES			
	Wakimizuna	1986	Sizuoka Agr. Exp. Sta. Koureichi Branch			
	P. L.: Noh No. 1	1986	NIVOT			

[a] Parental line.
[b] NIVOT: National Research Institute of Vegetable, Ornamental Plants and Tea.

CABBAGE AND OTHER *B. OLERACEA* CROPS

The breeding work on cabbage was started earlier than that of Chinese cabbage, using mainly Böhmerwaldkohl 72755 as a resistant source. But the resistance of Böhmerwaldkohl 72755 is not complete especially when the temperature is very high, and was inherited by polygene. In 1984, Tsukada et al. (1986) bred a clubroot resistant F_1 hybrid, YCRSE (Choya Ko No. 9) using Böhmerwalkohl 72755 as a resistant source. In our case, heading character is much improved but the resistance is not yet enough for commercial use. The breeding work of heading cabbage has faced much more difficulty than that of Chinese cabbage. On the other hand, the resistance of kale 'K 269' is much higher than that of 'Böhmerwaldkohl 72755' and inherited by single recessive gene. The breeding work using 'K 269' is still at an early stage, and needs many years as compared to the above cross, but lines with complete resistance can

be obtained as a genotype of 'aa', and for its effective breeding biotechnology must be introduced. In this cross, plant characters segregated widely and various types of plant, such as heading cabbage type, broccoli type, cauliflower type, Brussels sprout type, etc., were obtained in F_2 generation. So it is possible to breed those various types of line with complete resistance, in a cross of heading cabbage and kale.

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CHAPTER 13

Utilization of genetic resources for soyabean dwarf virus in soyabean breeding

HIROHARU BANBA

Soyabean dwarf virus (SDV) is one of the most serious virus disease of soyabean (*Glycine max* (L.) Merr.) in Hokkaido and the northern part of Honshuu (Tamada 1975; Yanagida et al. 1978). This virus causes remarkable yield loss. For example, a 50% incidence of field infection may result in as much as 40% in yield loss (Tamada 1975). Intensive research to identify the causal agent of the disease has been carried out by the plant pathology division of Hokkaido Central Agricultural Experiment Station since 1966. The disease is found to be aphid-borne, and the natural host plants are ladino, white and red clover (*Trifolium* spp.) (Tamada 1970). The virus is transmitted by the foxglove aphid (*Acyrthosiphon solani*) in a persistent (circulative) manner. Two strains of SDV with a different host range and symptom have been identified. One of them is the dwarfing strain which caused a dwarfing of soyabean plants, with shortened petiole and internodes, and the other is yellowing strain which caused a slight chlorosis of leaflets and interveinal yellowing of old leaves (Tamada 1973). Single infection with yellowing strain or mixed infection with both strains induces highly rugose or wrinkled leaflets causing serious damage to soyabeans under natural conditions (Tamada 1975).

The soyabean breeding program to develop an SDV resistant cultivar has been similarly carried out by the soyabean breeding division of Hokkaido Central Agricultural Experiment Station since 1966. Firstly there were screening tests for resistance to SDV in foreign and domestic germplasm of soyabeans. Secondly crosses between SDV resistant cultivars and the recommended cultivars in Hokkaido were performed. The soyabean breeding program was able to develop the promising lines with moderate SDV resistant high yield and good quality. It was released as a new soyabean cultivar, 'Tsurukogane', in southern Hokkaido in 1984 (Banba et al. 1985).

Germplasm collections for SDV resistance

As many as 2682 cultivars were evaluated for SDV resistance during the past 17 years (Tanimura et al. 1982). The country of origin and the number of soyabean cultivars introduced for SDV screening test are shown in Table 1. These screening materials were offered by several foreign and Japanese soyabean breeding laboratories as follows: (1) U.S. Regional Soyabean Laboratory Urbana, Illinois, USA; (2) Mea Jo Agricultural Experiment Station, Thailand; (3) Plant Breeding and Acclimatization Institute Soyabean Laboratory, Poland; (4) National Institute of Agrobiological Resources, Japan; (5) Tohoku National Agricultural Experiment Station (Kariwano Soyabean Breeding Branch), Japan; (6) Hokkaido Prefectural Tokachi Agricultural Experiment Station, Japan; (7) Nagano Prefectural Agricultural Experiment Station, Japan; (8) Kumamoto Prefectural Agricultural Experiment Station, Japan; and (9) Iwate Prefectural Agricultural Experiment Station, Japan. Screening for SDV resistance was evaluated by both natural infection and artificial inoculation tests.

Screening methods

NATURAL INFECTION

This method was first performed at Naganuma in 1966, and since 1978 it has been performed at Date, where SDV usually occurred very seriously.

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TABLE 1 Countries of origin and the number of soyabean cultivars or lines introduced for SDV screening from 1966 to 1982.

Country	No. of cultivars and lines	Country	No. of cultivars and lines
USA	131	USSR	14
China	120	Taiwan	13
Thailand	64	Canada	12
West Germany	54	Hungary	11
Korea	43	Australia	10
Yugoslavia	42	South Africa	9
Poland	41	Rumania	2
Sweden	30	Unknown	142
France	28	Japan	1 892
Brazil	24	Total	2 682

The seeds were sown in the middle of May. The infection of soyabean with SDV was evaluated in late August by scoring the percentage of infection and the disease index for each soyabean plant (graded from zero to four on the basis of symptom intensity as follows: 0=no symptoms, 4=severe symptoms). The degree of SDV resistance in each soyabean cultivar was evaluated on the basis of the results of continuous screening tests conducted for two or three years, as the occurrence of SDV varied from year to year in the field tests. The percentage of SDV infection in the susceptible and resistant cultivars at Naganuma from 1971 to 1980 is shown in Figure 1.

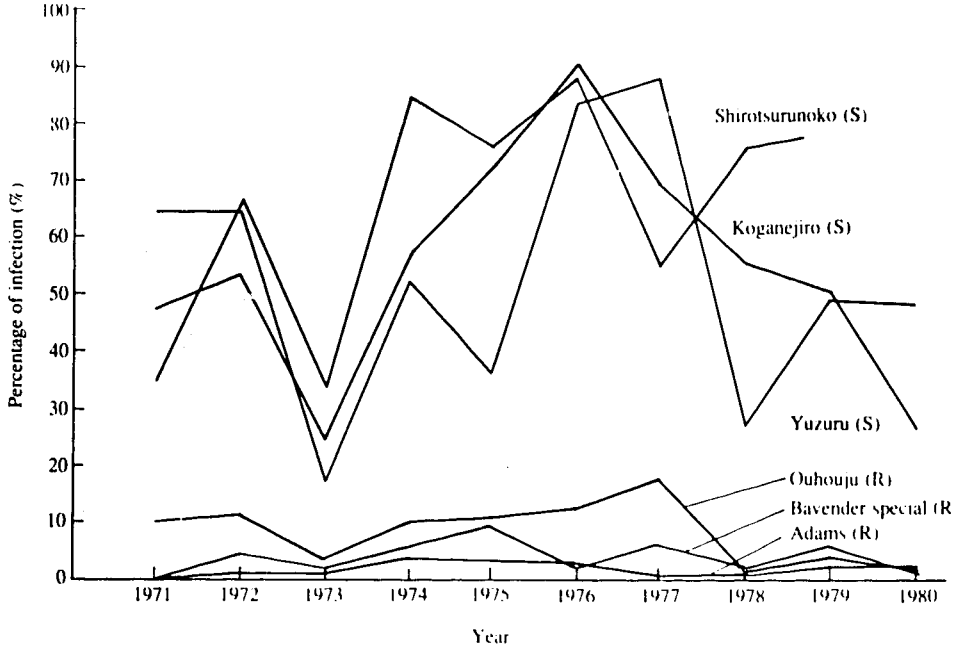

FIGURE 1 Seasonal changes in the percentage of infection on susceptible and resistant cultivars at Naganuma from 1971 to 1980.

ARTIFICIAL INOCULATION

It is difficult to evaluate for SDV resistance under natural conditions because occurrence varies from year to year. Therefore attempts were made to develop an efficient artificial inoculation method. The tested field is surrounded by fine mesh nylon cloth 3 metres high, to prevent infection by viruliferous aphids from nearby places. Dwarfing and yellowing strains of SDV were inoculated by viruliferous aphids onto each plant at the third leaf stage in early July. Seedlings of each cultivar were inoculated by a group of 5 viruliferous aphids and these aphids were killed with insecticide spray 7 days after inoculation. At the same time, non-inoculated plots were prepared for each similar cultivar. Insecticide was applied to the check plots at sowing time. The fine mesh nylon cloth was removed in late July. Symptoms appeared on the plants 3–6 weeks after inoculation. The main agronomic characteristics of the infected plants in each cultivar were measured at harvest time. The relative percentage was calculated as (I/H × 100), where I is the value obtained in the infected plots and H is that in the check plots.

Screening for SDV resistant soyabean cultivars

NATURAL INFECTION

It was found that 20 out of the 2 682 cultivars evaluated were resistant to SDV as in the case of the moderately resistant cultivar 'Ouhouju'. These cultivars are listed in Table 2. Infection ranged from 0.0 to 16.9% in the moderately resistant cultivar 'Ouhouju' during 17 years. The cultivars evaluated for resistance to SDV showed mean values less than approximately 5% of infection. On the contrary, the susceptible cultivars 'Shirotsurunoko' and 'Yuzuru' showed a high percentage of infection. Three cultivars 'Gokuwase Chishima', 'Kimame' and 'PI 90763' showed no disease symptoms during the test periods at Naganuma. However, it was found that 'PI 90763' and 'Kimame' were infected at low percentage in a seriously infested field at Date, whereas 'Gokuwase Chishima' still showed no symptoms.

TABLE 2 Resistance to SDV of soyabean cultivars introduced from 1966 to 1982 at Naganuma.

Cultivars	Country of introduction	Test period (year)	SDV infection (%) Mean	Range
Adams	USA	16	1.4	0.0 – 3.8
Akanna	Japan	5	2.7	0.0 – 4.7
Bavender Special	USA	14	2.7	0.0 – 6.9
Gokuwase Chishima	Japan	7	0.0	0.0
Habrinska 231	Yugoslavia	5	1.4	0.0 – 4.8
Kimame	Japan	5	0.0	0.0
M-14	Yugoslavia	6	0.6	0.0 – 2.4
Montreal Manchu	China	5	1.4	0.0 – 6.3
Ouhouju	China	17	5.6	0.0 – 16.9
Pando	Korea	5	3.8	0.0 – 19.1
Peking	China	3	0.9	0.0 – 2.8
Peking Kurodaizu	China	4	2.5	0.0 – 4.4
PI 89772	China	4	3.5	1.8 – 4.9
PI 90763	China	5	0.0	0.0
Shutai 4	China	4	1.4	0.0 – 4.6
Timiriazerevskaja 144	USSR	6	0.6	0.0 – 2.2
Yoshiokachuryu	Japan	7	0.2	0.0 – 1.6
Wabash	USA	3	3.3	0.0 – 5.5
634-13-108-9B	Sweden	6	1.3	0.0 – 5.6
840-2-7	Sweden	6	0.5	0.0 – 3.1
Shirotsurunoko[a]	Japan	14	53.9	11.2 – 87.8
Yuzuru[a]	Japan	12	47.9	2.7 – 89.9

[a] 'Shirotsurunoko' and 'Yuzuru' are susceptible cultivars.

ARTIFICIAL INOCULATION

Artificial inoculation was performed in 20 soybean cultivars which showed a lower percentage of infection in a field at Naganuma. The results are shown in Table 3. The relative percentage for each agronomic characteristic of the cultivars inoculated with yellowing strain was lower than that for the cultivars inoculated with the dwarfing strain. For the dwarfing strain, the relative percentage for each agronomic characteristic in 20 resistant cultivars was much higher than that of the susceptible cultivars 'Shirotsurunoko' and 'Yuzuru', suggesting that these 20 cultivars were much more resistant to dwarfing strain. On the other hand, inoculation tests of the yellowing strain showed that the relative percentage for the seed weight varied with the cultivars. Eighteen cultivars, but not 'M-4' and 'Wabash' showed a high percentage for the seed weight which is one of the important characteristics indicative of resistance. It is suggested that these 18 cultivars are moderately resistant to the yellowing strain. No cultivars immune to SDV were found.

TABLE 3 Relative percentages from artificial inoculation tests using two strains of SDV, yellowing and dwarfing.

Cultivars	Plant height[a]		Number of pods		Weight of seeds		Weight of 100 seeds	
	Dwarf	Yellow	Dwarf	Yellow	Dwarf	Yellow	Dwarf	Yellow
Adams	86	84	60	39	49	34	88	94
Akanita	90	69	90	61	85	35	99	96
Bavender Special	102	75	77	72	65	47	89	74
Gokuwase Chishima	100	83	82	67	90	63	89	78
Harbinaska 231	81	70	77	46	76	31	95	88
Kimame	98	100	65	85	42	35	60	60
M-44	83	77	37	23	39	10	91	67
Montreal Manchu	60	71	50	50	37	23	91	80
Ouhouju	80	83	75	67	66	59	79	75
Pando	95	100	89	55	85	44	81	74
Peking	82	82	63	45	74	54	96	89
Peking Kurodaizu	98	106	94	71	86	63	99	98
PI 89772	116	107	103	73	109	55	92	86
PI90763	97	87	68	57	92	40	98	80
Shutai 4	63	79	49	39	33	25	88	79
Timiazerevskaja 144	84	92	53	46	46	30	94	82
Yoshiokachuryu	105	95	99	63	97	46	94	77
Wabash	80	73	73	24	80	15	90	73
634-13-108-9B	103	100	90	34	91	22	92	53
840-2-7	94	106	68	51	55	35	100	89
Shirotsurunoko[b]	48	97	23	6	9	1	85	73
Yuzuru[b]	36	92	13	38	10	16	86	84

[a] 'Dwarf' is dwarfing strain of SDV and 'Yellow' is yellowing strain of SDV.
[b] Susceptible cultivars.

Breeding for resistance to SDV

It was recognized that the soyabean cultivars 'Adams', 'Bavender Special' and 'Ouhouju' are moderately resistant to SDV from results obtained until 1971 (Tanimura and Tamada 1976). But the agronomic characteristics of the resistant cultivars are inferior to those of the recommended cultivars in Hokkaido. The crosses between these resistant cultivars and the recommended cultivars have been performed since 1971. Hitherto, we have attemped to cross 134 pairs to breed SDV resistant cultivars with high yield and good quality. The moderately

resistant cultivars used for the crosses were 'Adams' (31 pairs), 'Bavender Special' (8 pairs), 'Kimame' (12 pairs), 'Ouhouju' (23 pairs) and hybrid lines originating from the above mentioned moderately resistant cultivars and the recommended cultivars in Hokkaido (68 pairs). In the early generations (F_1 to F_4), selection was based on the absence of symptoms of SDV, good plant type, early maturity and good quality from segregated populations at Naganuma. In the middle generations (F_5 to F_7), the resistance of the breeding lines was evaluated in the field at Date. Performance tests for the selected promising lines and artificial inoculation tests using dwarfing and yellowing strains of SDV were carried out to evaluate the resistance. Breeding lines resistant to SDV have been selected with desirable agronomic characteristics. They originated from the 134 crosses performed at Naganuma. The reaction of 14 breeding lines among them and of check cultivars was evaluated under natural infection at Date and artificial inoculation at Naganuma. The mean value of the percentage in the breeding lines was intermediate between those in the susceptible and moderately resistant cultivars. Also the mean value in two promising lines (Chuiku 14 and Chuiku 17) was especially less than those of other breeding lines tested under natural infection at Date or by artificial inoculation. Moreover, breeding lines were established in a preliminary test at Naganuma. Five lines were selected from many promising ones showing SDV resistance, high yield and good quality (Table 4). They were 'Chuiku 14', 'Chuiku 15', 'Chuiku 16', 'Chuiku 17' and 'Chuiku 18'. In order to determine whether promising lines can be released to farmers they were tested in insecticide-applied and non-applied fields at Date in 1983. The results are shown in Table 5. In the non-applied fields, the percentage of infection and reduction of yield of the three promising lines were lower than those of 'Ouhouju' and 'Adams', whereas the susceptible cultivars 'Yuzuru' and 'Koganejiro' showed low yield.

TABLE 4 Agronomic characteristics of several breeding lines of soyabean in insecticide-applied fields at Naganuma in 1981.

Line or cultivars	Date of maturity	Lodging score	Seed yield (t/ha)	Relative yield (%)	Weight 100 seeds (g)
Chuiku 14	Oct. 7	1.0	3.13	115	36.4
Chuiku 15	Oct. 12	0.5	2.69	99	38.6
Chuiku 16	Oct. 9	0.3	2.79	103	31.5
Chuiku 17	Oct. 11	2.0	2.88	106	38.0
Chuiku 18	Oct. 11	1.0	3.04	112	33.1
Yuzuru (S)	Oct. 16	1.0	2.71	100	40.9
Ouhouju (R)	Oct. 4	0.5	2.01	74	25.3

A new SDV resistant cultivar 'Tsurukogane'

One resistant line 'Chuiku 14' designated as a new cultivar 'Tsurukogane' was originated as an F_{12} line developed from the cross 'Chuiku 1' × 'Ouhouju'. 'Chuiku 1' was selected by pure line selection from the landrace 'Tsurunoko' which had been cultivated at Otobe town in southern Hokkaido. This cultivar has a white hilum, gives high yield and has a large seed size (approximately 45 g per 100 seeds). 'Ouhouju' was similary selected by pure line selection from landrace 'Yonryuki' which had been cultivated in the Jilin districts of mainland China. It has a white hilum, gives high yield and has a medium seed size (approximately 25 g per 100 seeds). It is also moderately resistant to SDV. The cross was performed in 1971 and early generations were evaluated in the field at Naganuma, while the later generations were evaluated in the field at Date. Also, it was evaluated by artificial

TABLE 5 **Results of performance tests for infection with SDV in insecticide-applied and non-applied fields at Date in 1982.**

Line or cultivar	Infection with SDV (%)	Plant height (cm)	Seed yield (t/ha)	Relative yield (%)	Weight of 100 seeds (g)
Non-applied					
Chuiku 14	13.3	80	1.78	81	26.4
Chuiku 15	36.0	84	1.77	82	34.1
Chuiku 17	12.3	82	1.82	88	25.3
Koganejiro (S)	96.6	58	0.31	17	13.8
Yuzuru (S)	92.5	71	0.27	17	32.3
Adams (R)	30.2	103	1.15	53	14.7
Ouhouju (R)	–[a]	85	1.27	64	18.0
Applied					
Chuiku 14	6.3	81	2.20	100	26.4
Chuiku 15	12.6	88	2.15	100	33.1
Chuiku 17	11.8	84	2.06	100	26.7
Koganejiro (S)	14.2	78	1.85	100	18.9
Yuzuru (S)	34.5	78	1.62	100	37.4
Adams (R)	13.0	114	2.18	100	16.3
Ouhouju (R)	10.9	90	1.99	100	20.9

[a] Not measured.

inoculation tests at Naganuma in 1981, 1982 and 1983. The performance tests were conducted in both insecticide-applied and non- applied fields in 1983 at Date and Biratori where SDV was usually serious. The results are shown in Table 6. In the non-applied fields at Biratori, the percentage of infection and yield loss of 'Tsurukogane' were much less than those of the susceptible cultivar 'Kitahomare'. Also, 'Kitahomare' remained immature at harvest time because plants received serious damage. In the non-applied fields at Date similarly the percentage of infection and yield loss of 'Tsurukogane' were much lower than those of 'Yuzuru'. It was shown that 'Tsurukogane' is as resistant to SDV as 'Ouhouju'. The

TABLE 6 **Results of performance tests for infection with SDV insecticide-applied and non-applied fields at Date and Biratori in 1983.**

Insecticide	Cultivars	Infection with SDV (%)	Maturity date	Plant height (cm)	Seed yield (t/ha)	Relative yield (%)
Date						
Non-applied	Tsurukogane	23.0	Oct. 11	66	2.28	80
	Yuzuru	52.9	Oct. 16	54	0.82	39
Applied	Tsurukogane	11.3	Oct. 11	68	2.84	100
	Yuzuru	22.5	Oct. 16	57	2.10	100
Biratori						
Non-applied	Tsurukogane	35.9	Oct. 12	78	2.49	87
	Kitahomare	96.4	Immature	40	0.33	11
Applied	Tsurukogane	15.1	Oct. 11	87	2.86	100
	Kitahomare	35.7	Oct. 11	58	2.94	100

performance tests showed that the mean yield for 3 years was 2.89 t/ha in the insecticide-applied fields at Naganuma and it was approximately 17% higher than that of a check cultivar 'Toyosuzu'.

'Tsurukogane' was registered by the Ministry of Agriculture, Forestry and Fisheries as 'Soyabean Norin No. 79' and it was released as a recommended cultivar in southern Hokkaido in 1984.

Future strategies

As stated previously no cultivars immune to SDV were detected among the germplasm collections. We have already screened 2682 soyabean cultivars for SDV. It would be desirable to evaluate the resistance to SDV on about 10 000 specimens of the world soyabean germplasm which are collected in the USA, Taiwan and other countries.

Almost all the lines selected for resistance to SDV originated from 'Ouhouju', whereas only 4 lines were derived from other materials. The agronomic characteristics of the breeding lines originating from crosses with 'Ouhouju' are superior to those of other SDV resistant cultivars, eg. 'Adams', 'Bavender Special', etc. 'Ouhouju' has excellent agronomic characteristics except lodging and small seed size. However, based on the results of natural infection at Date, the percentage of infection in 'Ouhouju' was found to be higher than in other resistant cultivars. Although attempts were made to cross 'Ouhouju' with other SDV resistant cultivars, almost all breeding lines derived from these crosses were inferior in agronomic characteristics. Therefore, we should breed hybrids with a high combining ability and SDV resistance. A new cultivar having better agronomic characteristics than 'Tsurukogane' should be bred by crosses between these excellent hybrids and the recommended cultivars in Hokkaido.

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CHAPTER 14

Utilization of genetic resources for maize and soyabean disease resistance in Korea

KEUN YONG PARK

Soyabean is one of the oldest native crops and has been grown for about 2500 years on the Korean peninsular (Kwon 1972). The wild soyabean (*Glycine ussuriensis*) which is believed to be the ancestor of today's cultivated soyabean is easily found throughout the country until now.

As a source of protein and fat, soyabean has been grown as one of the major food crops. However, the acreage cultivated and production have been reduced because of its low productivity and low income, and imports for oil extraction/feeding have been increased year after year in recent times.

Maize is assumed to have been derived from China in the mid-sixteenth century (Choe 1986).

The consumption of maize grain has been rapidly increasing particularly for animal feeding. However, most demands are met by imported maize, since the domestic production is limited by the shortage of cultivated acreage. On the other hand, the cultivated acreage of silage maize has increased in recent years and it is predicted to continue to increase as animal production continues to increase.

In this chapter I introduce the general aspects of germplasm collection, evaluation and utilization particularly for the study of virus diseases of soyabean and the maize breeding programs in Korea.

Major diseases of soyabean and maize

SOYABEAN DISEASES

Major soyabean diseases in Korea are listed in Table 1. Among the diseases soyabean mosaic virus (SMV) and its necrotic mutant strains (SMV-N) are the predominant and cause the most destructive damage in soyabean production.

TABLE 1 Major soyabean diseases in Korea.

Common name	Scientific name
Soyabean mosaic virus (SMV)	
Bacterial blight	*Pseudomonas glycinea*
Anthracnose	*Glomerella glycines*
Purple seed stain	*Cercospora kikuchii*
Bacterial pustule	*Xanthomonas phaseoli*
Downy mildew	*Perenospora manshuria*
Pod and stem blight	*Diaporthe phaseolorum*
Black root rot	*Calonectria clotalarise*

SMV is a member of potato virus Y group which includes a large number of important viruses (Edwardson 1974). SMV is primarily transmitted through seeds of soyabean plants producing mosaic symptoms. The rate of seed transmission was reported as high as 68.4% in soyabean cultivar Midwest (Kendrick and Gardner 1924), but generally ranges from 0 to

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35% in most soyabean cultivars (Chung et al. 1973; Iizuka 1973).

Based on virulence to six resistant soyabean cultivars, more than 100 SMV isolates obtained from USDA germplasm collections were classified in seven strains (G1–G7) by Cho (1978) and Cho and Goodman (1979).

Depending on combinations of soyabean mosaic genotypes and SMV strains, SMV causes either mosaic or necrotic symptoms during systematic infection. The necrotic reaction occurs in soybean cultivars resistant to less virulent SMV strains when infected with more virulent SMV strains (Cho and Goodman 1979).

The first symptom of SMV-N was found in 1962 in Suwon at the experimental plots of cultivar Ryukuwoo 3 which was introduced from Japan — a leading cultivar and very resistant to mosaic virus (Lee et al. 1985).

The major leading soyabean cultivars Kwangkyo, Kangrim, Ryukuwoo 3, Pundaedu and Tongbuktae are generally resistant to mosaic but showed necrotic symptoms. The necrotic SMV was tentatively called bud blight in 1974 because the symptoms were very similar to the tobacco ring spot virus (TRSV) (Lee et al. 1985).

Cho and Chung (1986) reported five necrotic strains of SMV; G1, G2, G5, G6 and G7 were identified from 220 leaf samples collected through the country. The strains G5, G6 and G7 occupied 66% of all isolates tested

MAIZE DISEASES

Major maize diseases are listed in Table 2. Black-streaked dwarf virus (BSDV) is the most important disease of maize in southern and central parts of Korea (Kim et al. 1979; Park and Ham 1981). BSDV has also produced epidemics in Japan (Shikata 1974) and China (Tasi et al. 1984). The symptoms produced by BSDV on maize plants are severe stunting of growth, a dark green colouring of leaves with light greyish veins, and numerous swellings along the veins on the lower surface of the leaves resulting from abnormally enlarged phloem cells (Shinkai 1962). Maize plants die prematurely when infected early. In the case of survival, the whole plant is dwarfed as a result of shortening of internodes, the tassels fail to develop and the grain yield is greatly decreased (Yasuo 1969; Youn and Chung 1986). BSDV is transmitted only by insect vectors, (I and Shinkai 1969; Lee et al. 1977).

Similar reports were made for the symptoms and vectors of maize rough dwarf virus (MRDV) (Harpaz 1972). The small brown planthopper (*Laodelphax striatellus*) is the most active vector transmitting BSDV in Japan and Korea (Shinkai, 1962; Lee et al. 1977).

TABLE 2 Major maize diseases in Korea.

Common name	Scientific name
Black streaked dwarf virus (BSDV)	–
Maize dwarf mosaic virus (MDMV-B)	–
Northern leaf blight	*Helmithosporium turcicum*
Southern leaf blight	*H. maydis*
Stalk rot	*Diplodia* spp.
Stalk rot	*Fusarium* spp.
Smut	*Ustilago maydis*
Sheath blight	*Thanatephorus cucumeris*

Genetic resource collections made to date

SOYABEANS

Since the Korean peninsular is close to the origin of soyabean, Manchuria, many countries

have taken an interest in the collections. Scientists from USA, in particular, collected 5496 accessions from Korea during the period 1901 to 1976 (Park 1979).

However genetic resources of several hundred varieties preserved through World War II were lost during the Korean War and only 120 varieties were preserved in 1960. By World War II almost no varietal improvement program had been implemented, although some varietal comparisons had been made and the first hybridization breeding of soyabean was initiated in 1956. The first achievement with hybridization breeding was the 'Kwangkyo' cultivar which is very resistant to SMV and is high yielding.

A great contributor of land race collections of soybean is Dr Shin Han Kwon, a radiation mutation breeder who made collections of more than 2000 accessions throughout the country in 1960s. He also made an elaborate evaluation of all the collections and published *Evaluation of Korean Soyabean Germplasm* in 1978 (Kwon 1978; Kwon et al. 1972, 1974).
The soyabean breeders in the Crop Experiment Station have made continuous efforts in collecting genetic resources of land race or exotic soyabean for their breeding program since the 1960s. Today the total number of accession is 7362 including wild soyabeans (Table 3). The systematic evaluation is not completed yet since the national plant genetic resource program was established only in 1985. But this year, the third year of the program, most of the collections will be evaluated and their valuable characteristics will be computerized, documented and published by 1988.

TABLE 3 **Number of accessions of soyabean genetic resources in Korea.**

	Korea	USA	Japan	China[a]	Others	Total
Cultiv.	4 808	957	387	639	298	7 089
Wild	266	–	2	5	–	273
Total	5 074	957	389	644	298	7 362

[a] China – including Taiwan.

Maize

Before this maize breeding program was initiated in 1959 with several synthetic varieties and double cross hybrids introduced from Nebraska State University in the USA, there were no genetic resources preserved.

The collection of 26 landraces was made through agricultural extension organizations by the Crop Experiment Station in 1962 (Park 1962). Secondly, 242 accessions of local collections were made and major agronomic characteristics were investigated (Park et al. 1971).

Dr Bongho Choe, professor of Chungnam National University, made a valuable contribution in collecting local maize landraces and evaluated various characteristics. He collected 948 ear samples throughout the country (Choe et al. 1978).

Maize breeders in the Crop Experiment Station made an effort to continuously collect genetic resources of local landraces as well as exotic inbred lines and open pollinated populations. The current number of total accessions is shown in Table 4. Evaluation for utilization is going on as for the soyabean collections.

Major achievements in breeding for disease resistance

Soyabean mosaic virus

As already mentioned soyabean mosaic virus is the most destructive virus disease in Korea and I shall introduce some results obtained for the reactions of the differential cultivars to

SMV strains, SMV screening, original genetic resource for the resistance and some considerations.

TABLE 4 Number of accessions of maize genetic resources in Korea.

Grain type	Korea	USA	Japan	China	Others	Total
Dent, flint	2 819	823	5	7	556	5 210
Sweet	-	96	–	1	–	97
Pop	120	74	–	–	–	194
Waxy	325	34	–	1	–	360
Others	-	89	–	–	–	89
Total	4 264	1 116	5	9	556	5 950

Reactions of the differential cultivars to SMV strains

The SMV strains were classified into 7 in 1979 (Cho 1978; Cho and Chung 1986) and two more mutant strains were reported by Cho et al. (1983) (see Lee et al. 1985).

The reaction symptoms of the differential cultivars to the strains are shown in Table 5.

A mutant designated as G5H caused necrosis in Hwanggeum-Kong cultivar and mosaic symptoms in Jangbaeg-Kong cultivar which were immune to other SMV strains. Reactions of soyabean cultivars used as differentials caused by the mutant strain G5H were similar to those caused by G5.

Another mutant designated as G3A caused only mosaic symptoms in Marshall, Ogden and Jangbaeg-Kong cultivars. Reaction in other cultivars caused by G3A were similar to those caused by G3.

SMV strain G5 of G5H is reported as the most prevalent through mid to northern parts of the country (Cho and Chung 1986).

TABLE 5 Reaction of differential cultivars to SMV strains.

	Symptoms to SMV strains								
Cultivars	G1	G2	G3	G3A	G4	G5	G5H	G6	G7
Rampage, Clark, Suwon 120	M	M	M	M	M	M	M	M	M
Davis, York	–	–	–	–	N	M	M	M	M
Marshall	–	N	N	M	–	–	–	N	N
Ogden	–	–	N	M	–	–	–	–	N
Jangbaeg - Kong	–	–	–	M	–	–	M	–	–
Hwanggeum - Kong	–	–	–	–	–	–	N	–	–
Kwangkyo	–	–	–	–	–	N	N	N	N
Buffalo, L78-434	–	–	–	–	–	–	–	–	N

– = symptomless, M = mosaic, N = necrotic

Screening of breeding materials for resistance

Since severe necrotic symptoms occurred in 1974 in Kwangkyo cultivar which had been developed in 1969 with other cultivars resistant to mosaic symptoms, field screening for the resistance was initiated with 373 preserved genetic resources in natural field infection. Nine varieties were symptomless both to mosaic and necrotic SMV, and those were thereafter used as resistant gene source for hybridization breeding. The nine varieties are Patten, Ohdu,

Leekamkwan, 210-2, PI 88300, PI 91646, PI 84751, PI 86737 and KLS 628–1.

Those varieties showing both mosaic and necrotic SMV resistance have poor grain characteristics: black or brown seed coat colour with darkened hilum, small and flat seed shape, etc.

Screenings for SMV resistance have been continued to the present. In 1979 and 1980 a series of experiments to establish a reliable indoor screening for the SMV were conducted at the Crop Experiment Station. Wayne cultivar appeared to be a good inoculum source of necrotic SMV strain propagation since Wayne cultivar produced only mosaic symptom and excellent transmission to other tested cultivars (Kwangkyo) when inoculated with the tissue sap of Wayne plants (Choe et al. 1978).

The other field screening methods were compared to provide better evaluation for the necrotic SMV from 1982 to 1985. Williams cultivar showing mosaic symptoms was used as spreader (propagational host), being inoculated with GH5. In 1982 the inoculated spreader method gave results similar to natural infection in average infection rate of susceptible lines, but in SMV-N susceptible cultivar Kwangkyo infection rate of inoculated spreader method was twice natural. And in 1983 the inoculated spreader method showed a big difference from natural infection.

New genetic resources resistant to SMV strains

The results obtained from the recent screening for resistance to SMV strains indicate that there has been a great advance since the resistance breeding for SMV was initiated in 1974 with the hybridization with the resistant genetic resources which had a poor grain quality. The results of recent screening for the resistance to SMV strains of various breeding lines and other materials are shown in Table 6.

TABLE 6 **Indoor inoculated screenings for SMV resistance of various breeding lines in recent years (Crop Exp. Stn. Suwon: 1984–86).**

Year		Breeding materials					
		OYT[a]	PYT	AYT	RYT	Others	Total
1984	Tested	194	48	15	–	105	362
	Selected[b]	19(10)[c]	1(2)	2(13)	–	2(2)	24(7)
1985	Tested	106	30	12	11	195	354
	Selected	11(11)	3(10)	2(17)	1(9)	12(6)	30(8)
1986	Tested	92	36	12	12	162	314
	Selected	13(14)	11(31)	2(17)	2(17)	14(9)	14(13)

[a] OYT = obervational yield trial; PYT = preliminary yield trial; AYT = advanced yield trial; RYT = Regional yield trial.
[b] Selection was made for the lines resulted resistance both to mosaic and necrotic SMV.
[c] Percentage of lines selected in parenthesis.

BLACK STREAKED DWARF VIRUS IN MAIZE

Screening methods

Since 1979 the screening for BSDV resistance has been carried out in the field at Taegu where the natural infections are the most severe in the country. However about once in three

years very light infections occurred and the screenings were not accurate. Hence, an indoor artificial inoculation method was developed by the virologist collaborating with maize breeders.

The vector insect, small brown planthopper (*Laodelphax striatellus*) can be multiplied in the cage where rice seedlings are grown as host plant. Multiplied planthoppers can absorb BSDV in another cage where BSDV infected rice plants are grown. BSDV infected planthoppers are then released into a net tunnel for inoculation of the maize seedlings.

Utilization of genetic resources

Since BSDV was found in 1977 as a destructive virus disease in maize production in the central and southern plain areas, continuous screenings for the resistance have been carried out with various genetic materials. In 1977, H93, H95 and Ga209 were selected as moderately resistant among the exotic inbred lines and a single cross hybrid 'Kwang-ok' (Suweon 29) which showed moderate resistance to BSDV was released to the farmers from 1979 (Kim et al. 1979). And a three-way cross hybrid 'Jinju-ok' (Kwang-ok × Ga209) which showed a little higher resistance to BSDV compared to Kwang-ok was released to farmers in 1983 (Park et al. 1974).

Nine accessions were selected in the 1979 screening among the 170 local lines collected throughout the country. Those were highly resistant and were derived from the areas of BSDV prevalence, and it was considered that BSDV was epidemic for a long time in the areas and natural selection has been continued.

A comparison of the resistance to BSDV made in 1970 between local and exotic lines is shown in Figure 1.

Nampyong-ok (Ga209 × KL10), a single cross hybrid was released to the farmers, which showed further higher resistance to BSDV compared Jinju-ok hybrid (Park et al. 1987). KL10 is an inbred line developed from a local collection selected in 1979. But KL10 has some defects in agronomic characteristics such as leaf blights, ear height, grain size and lodging tolerance, etc.

Several populations has been under improvement since 1983, being sib-pollinated with resistant sources of local collections, and the exotic sources of moderate resistance and other agronomic characteristics were improved. This indicates that high BSDV resistance can be selected among the local collections, but they have to be improved in other agronomic characteristics.

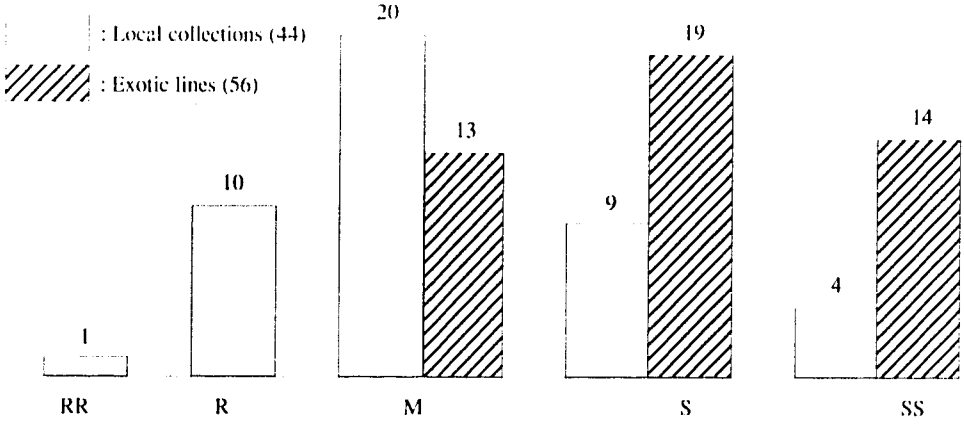

FIGURE 1 A comparison of resistance to BSDV between local collections and exotic lines (1980).

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CHAPTER 15

The resistance of sorghum to head smut and its inheritance

CAO RUHUAI, WANG XIAOLING, REN JIANHUA AND NAN CHENGHU

Head smut [*Sphacelotheca reiliana* (Kühn) Clint] is one of the major diseases of sorghum. The seriousness of the disease is closely correlated with resistance of varieties. As resistant varieties took the place of susceptible ones, head smut decreased rapidly. But, when varieties lost resistance, it became serious again. For this reason, it is considered by researchers everywhere that growing resistant varieties is the main practical solution for the control of this disease (Bai Jinkai 1980; Hu Jicheng 1983; Lu Qingsan 1985). In order to find out new resistant resources and provide a thoretical basis for resistance breeding programs, studies on the resistance to head smut and its inheritance were carried out during 1981–1985.

Materials and methods

Some 1 239 sorghum germplasm resources were identified during the past five years. The test materials were planted in late April or in early May. In each experiment material was planted in two rows, each row was 7.5 m long and they were spaced 33 cm apart. Each row had 25 holes and each hole contained two plants. Seeds in each hole were covered with 100g of soil containing 0.1% spore of head smut fungus. The experimental fungus race was collected from diseased ears of JinZa No. 5 sorghum (Fenyang county, Shanxi). When disease symptoms were fully expressed, the incidence of disease on the main stalks was examined. Resistant types were classified according to the following standards: varieties with 0–0.5% of diseased plants were considered as highly resistant; 5.1–10.0% as resistant; 10.1–30.0% as susceptible and above 30% as highly susceptible.

On the basis of resistance identification, in 1984 nine restoring lines (eg. Shangtingsui) and eight male-sterile lines (eg. Cuo I A) were used in an inheritance experiment with incomplete diallel cross design, from which 72 hybrid combinations were obtained. In 1985, F_1 of 72 hybrid combinations and their parents were planted according to a randomized block arrangement with three replications. The methods of planting and inoculation were the same as that used in resistance identification. Healthy plant percentage was used as the resistance index and aresine transformation of the percentage was carried out in the analysis of variance. On the basis of theoretical composition of each variance, genotypic variance of combining ability and population heritability were estimated according to Model II (Liu Laifu 1984).

Results and analysis

Identification of Resistance in Sorghum Varieties/Lines

Of the 1 239 sorghum germplasm resources used for identification, highly resistant varieties included Shangtingsui, Baibowang, Bairuangaoliang, A_3TX624, A_2TAM428, ATX622, ATX623 and ATX624 (8 varieties, accounting for 0.6% of the total). Resistant varieties amounting to 16 (1.3%) were Duansanchi, Zhuyeqing, Erhuangjiao, Hedong, Langweibagaoliang, 4198, Dadaobo, Wudalang, Xinzhougaoliang, Dahuange, Dadiangxiang, Zhengyansan, Bayeqing, Erfanglan, Mugewo, and Poxiepian. The rest were susceptible or highly susceptible. Head smut of sorghum in China has the phenomenon of physiological differentiation. The resistance of varieties mentioned above is relative in the light of fungus races. When utilizing these resistant varieties, attention must be paid to the composition of the local physiological strains (Wu Xinlan 1982).

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Inheritance of Resistance

Expression of resistance in sorghum hybrid combination

Table 1 shows that there exist two types of resistance inheritance to different varieties — quantitative inheritance and qualitative inheritance. For example, A_3TXA624, A_3TAM428, ATX622 and ATX624 were crossed with nine of their parents in P2 group, F_1s appeared all immune, and their resistance was completely dominant, possessing qualitative inheritance characteristics, whereas the nine parents in P2 group were crossed with Cuo 1A, 3197A and A_3TX3197, the resistance of F_1 was in the middle of their parents approximating the value of midparents. Healthy plant percentage in their hybrid combinations showed consecutive distribution; types of resistance and susceptibility could not be classified distinctively, it possessed quantitative inheritance characteristics.

TABLE 1 Healthy plant percentage of P1 and P2 parents and F_1 hybrid combinations.

P2 \ P1	Cuo 1A 62.32	3197A 63.78	A_3TX3197 50.14	A_3TX624 100	A_3TAM428 100	ATX622 100	ATX623 100	ATX624 100
Shangtingsui 100	87.74	89.40	78.22	100	100	100	100	100
Wudalang 92.8	82.74	80.30	69.84	100	100	100	100	100
Baibewang 100	82.90	78.91	73.44	100	100	100	100	100
Dabayeqing 82.21	79.47	72.20	72.76	100	100	100	100	100
Duansanchi 80.26	77.90	74.01	71.02	100	100	100	100	100
Xinzeugaoliang 87.15	74.68	75.48	65.28	100	100	100	100	100
Huanggaoliang 78.33	77.84	83.83	75.05	100	100	100	100	100
Xinliang 60.45	66.25	61.66	66.92	100	100	100	100	100
Sanchisan 64.54	72.40	68.05	49.49	100	100	100	100	100

Resistance of different parents and hybrid combinations and quantitative inheritance

As shown in Tables 2 and 3, the analysis of variance indicates significant difference in resistance between varieties/lines and hybrid combinations belonging to quantitative inheritance. Therefore, resistant sources for disease resistant breeding can be screened from abundant sorghum germplasm resource through identification of disease resistance, and disease resistance hybrids can be obtained by using these resistant sources.

Resistance of varieties belonging to quantitative inheritance and additive effect genes

Table 4 shows that the general combining ability of parents' resistance was highly significant, while their specific combining ability exhibited no significant difference. In addition, broad sense heritability and narrow sense heritability was 65.53% and 57.58%, respectively; and the variance of resistant general combining ability and that of specific combining ability was 95.14% and 4.87%, respectively. This indicates that resistance of varieties/lines belonging to quantitative inheritance was mainly governed by additive effect genes. This resistance can be fixed and stably inherited. F_2 may produce transgressive

segregation. In addition, the environmental conditions have also some effect on resistance. Therefore, in the process of screening and identifying resistant sources, not only should experimental conditions be strictly controlled, but also repeated identifications should be conducted.

TABLE 2 Variance analysis of twelve parents.

Variance resource	D.f.	Sum of squares	Mean square	F value
Varieties	11	7172.9443	652.086	29.15**
Replications	2	149.4316	74.72	3.34
Error	22	492.1668	22.37	

** Significant.

TABLE 3 Variance analysis of hybrid combinations.

Variance resource	D.f.	Sum of squares	Mean square	F value
Replications	2	44.003	22.00	1.19
Combinations	26	2373.225	91.28	4.94**
Error	52	961.30	18.49	

** Significant.

TABLE 4 Variance analysis of combining ability.

Variance source	D.f.	Sum of squares	Mean square	Model I F value	Model II F value
g_i(P1)	2	514.26	257.13	13.91**	11.37**
g_j(P2)	8	149.70	187.13	10.12**	8.27**
S_{ij}(P12)	16	361.92	22.62	1.22	1.22
Error	52	961.30	18.84		

** Significant.

Results and discussion

1. The resistance of different sorghum varieties/lines to head smut shows significant difference. Some useful resistant sources can be screened through identifying of resistance, and some resistant hybrids can be developed by using appropriate breeding methods.
2. There are different ways of inheriting resistance between varieties/lines. Some varieties express characteristics of qualitative inheritance, while some express characteristics of quantitative inheritance. The resistance of varieties/lines belonging to quantitative inheritance is mainly governed by additive effect genes.
3. The conventional phytopathological methods have long been used for classifying types of resistance. This study adopted genetic methods to classify resistant types of varieties.

Preliminary results show that filial generations can be obtained by means of certain crosses and that experiments on resistance inheritance can be conducted on the basis of certain conditions of artificial inoculation with only one race. When resistance presents quantitative inheritance it may be considered as non-race-specific resistance (horizontal resistance); when resistance presents qualitative inheritance, it may by considered as race-specific resistance (vertical resistance) (Zeng Shimai 1979).

4. In recent years, some resistant hybrids (eg. Jinza 4 and Jinza 5) have lost their resistant ability because of variation of head smut fungus, and have turned into susceptible varieties. Some scholars have pointed out that head smut of sorghum like stripe rust in wheat, powdery mildew of wheat and blast of rice has the problem of losing resistance. They also think that the fundamental way to make resistance last for a long time is to rationally utilize race-specific resistance (vertical resistance), and to exploit and utilize non-race- specific resistance (horizontal resistance) (Zeng Shimai 1985). This study for the first time shows that resistance of sorghum to head smut is controlled by two kinds of gene types, major and minor ones, respectively, while it has provided a theoretical basis for rationally utilizing these two kinds of resistance.
5. Production practice has shown that hybrids made from 3197A were almost immune to head smut. However, with the variation of head smut fungus, they gradually have lost their resistance and turned into susceptible varieties. This indicates that 3197A possessed race-specific resistance to the original race of fungus. However, in this experiment 3197A exhibited characteristics of quantitative inheritance. It was because of the variation of the fungus that the major gene of 3197A had not resisted to infection of the new race, while the effect of minor genes was not strong enough to overcome the toxicity of the new race, so the hybrids developed by 3197A crossing with susceptible varieties all appeared susceptible.

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CHAPTER 16

Genetic resources utilization in barley breeding in Japan

HIROSHI SASAKI

Some examples of new cultivars of wheat and barley that have played an important part will be introduced first. One is the wheat cultivar Chihoku-komugi released in 1981. In Hokkaido, in the north of Japan, the area planted with wheat has increased tenfold in the past 10 years. Until then Horoshiri-komugi and Takune-komugi released in 1984 had been grown there. Although the two cultivars accounted for more than 40% of the wheat planted area, they were not of good quality for making Japanese noodles, owing to the semi-hard properties of the flour, but not suitable for bread. As the planted area of these cultivars has increased, so has the demand for improved quality by processors and farmers. Just in time, a new cultivar, Chihoku-komugi, of good quality was released and it became an effective replacement.

The second is the new 2-rowed malting barley cultivar, Misato Golden. Since 1980, barley yellow mosaic virus (BYMV) spread to Kanto and Kyusyu, main cultivation districts of 2-rowed barley. Cultivation of 2-rowed barley with non-resistance became impossible in contaminated fields. The first resistant cultivar to BYMV as malting barley, Misato Golden, was released by Tochigi Prefectural Agricultural Experiment Station. Cultivation is now possible in the contaminated area. After release of the two cultivars the seeds were distributed to the farmers as rapidly as possible.

Barley cultivation in Japan

In Japan 'mugi' is a general term that refers to wheat and barley; wheat is called 'komugi' while barley is 'omugi'. Wheat and barley have been major crops after rice. Until about 1920, barley was grown more than wheat because it was an important staple food supplementing rice.

The planted area of barley which amounted to about 1.3 million hectares in the 1910s decreased to 0.75 million in the 1930s. During the 1950s and the 1960s the area decreased considerably due to increased rice production, lack of profitability to the farmers, and the large quantity of barley imported at low prices. In the 1980s the policy to reduce rice production together with the subsidization of wheat and barley cultivation stimulated barley production; consequently the planted area of 2-rowed malting barley and 6-rowed covered barley has increased slightly.

Barley is classified in Japan into 3 categories; 6-rowed covered, 6-rowed naked and 2-rowed covered barley. Six-rowed covered and naked barleys have been used for human or animal feed. Two-rowed barley is mostly used for malting. The five years average yield per hectare is, at present, about 3.0–3.4 ton. Although the national average yield has been increasing gradually, it is still about 1.5 ton lower than in European countries. The relatively low level of yield may be ascribed to various factors such as the double cropping system which restricts the growing period available for barley, in the middle and western part of the country, where about 80% of barley is grown in drained paddy fields, cultivation under heavy snow in the region facing the Japan Sea and high precipitation and temperature at harvest time in many areas.

For example, the climatic conditions of the London area in the United Kingdom are relatively mild throughout the year compared with those prevailing in the Mito, Takada and Takamatsu areas. Monthly mean temperatures, from April to November in the London area

are lower than in the above three areas, and the precipitation ranges from a quarter to a half of those areas. In addition, snowfall at Takada in winter is very heavy and barley lies under the snow for more than four months. Such conditions are not optimal for growing barley. The main districts for the cultivation of 6-rowed covered barley are Tohoku, Hokuriku and Kanto, for 6-rowed naked barley, Shikoku, and for 2-rowed malting barley, Kanto and Kyusyu. Only in Hokkaido is spring barley grown for malt. Barley cultivation in Japan currently accounts for 22 000, 10 400 and 79 600 ha in the case of 6-rowed covered, 6-rowed naked and 2-rowed covered barley, respectively. Covered and naked barleys display a distinctly separate distribution with covered barley being grown in the eastern part, and naked barley in the western part of Japan. The difference in winter hardiness between the types is considered to be the main reason for this. Domestic production of barley has reached 396 000 ton whilst the amount of imported barley is 2 284 000 ton, a self-sufficiency ratio of 16%. Domestic barley is used for food (pearled, mixed and cooked with rice), tea, malt, feed and seed.

Development of barley breeding

In Japan barley breeding started in 1904, but was discontinued in 1932. In 1941 systematic breeding of 6-rowed barley was resumed at 6 Prefectural Agricultural Experiment Stations. Until 1954 the breeding of 2-rowed malting barley had been conducted only by private brewing companies. Since then several designated Prefectural Agricultural Experiment Stations fully subsidized by the National Government have initiated breeding work on malting barley.

At present hulled barley breeding is carried out at three National Agricultural Experiment Stations, and the Nagano Prefectural Agricultural Experiment Station. Naked barley breeding is conducted at the Shikoku National Agricultural Experiment Station. Two-rowed malting barley breeding is carried out at the two Prefectural Agricultural Experiment Stations of Tochigi and Fukuoka as a national project, and at the Kitami Prefectural Agricultural Experiment Station, and by four brewing companies namely Kirin, Sapporo, Asahi and Suntory (Figure 1). There are also several supporting laboratories responsible for wheat and barley germplasm, breeding methods, and malting quality improvement and testing in the national breeding program. In addition, 14 testing stations for specific characters have been established to determine selection indices for resistance to cold weather and snow, or to several diseases including barley yellow mosaic virus, powdery mildew, snow mould, and scab. Eleven Prefectural Agricultural Experiment Stations have been designated to conduct local adaptability tests.

Breeding for early maturity and shortening of the culm

In Japan barley is sown in autumn, except in Hokkaido, as the second crop in rotation with rice or summer upland crop. Consequently, the interval between harvesting barley and the transplanting or sowing of the succeeding crops is very short, which makes the double cropping system impossible in the northern part of the country. In addition, the harvest period for barley tends to fall in the rainy season when the crop matures late. For these reasons it is essential to breed cultivars that mature as early as possible. In the case of 6-rowed covered barley in the Kanto district, tall and late maturing, normal types of barley had been grown. However, due to the development and increased application of chemical fertilizers, they have been replaced by the semi-dwarf uzu type of cultivar which is early maturing and shorter, without any reduction in yielding potential (Table 1). As the breeding object had been directed toward earliness and shortening of the culm, successful results were also obtained for malting barley. The heading date has been advanced by 14 days and the culm length was shortened by 35 cm.

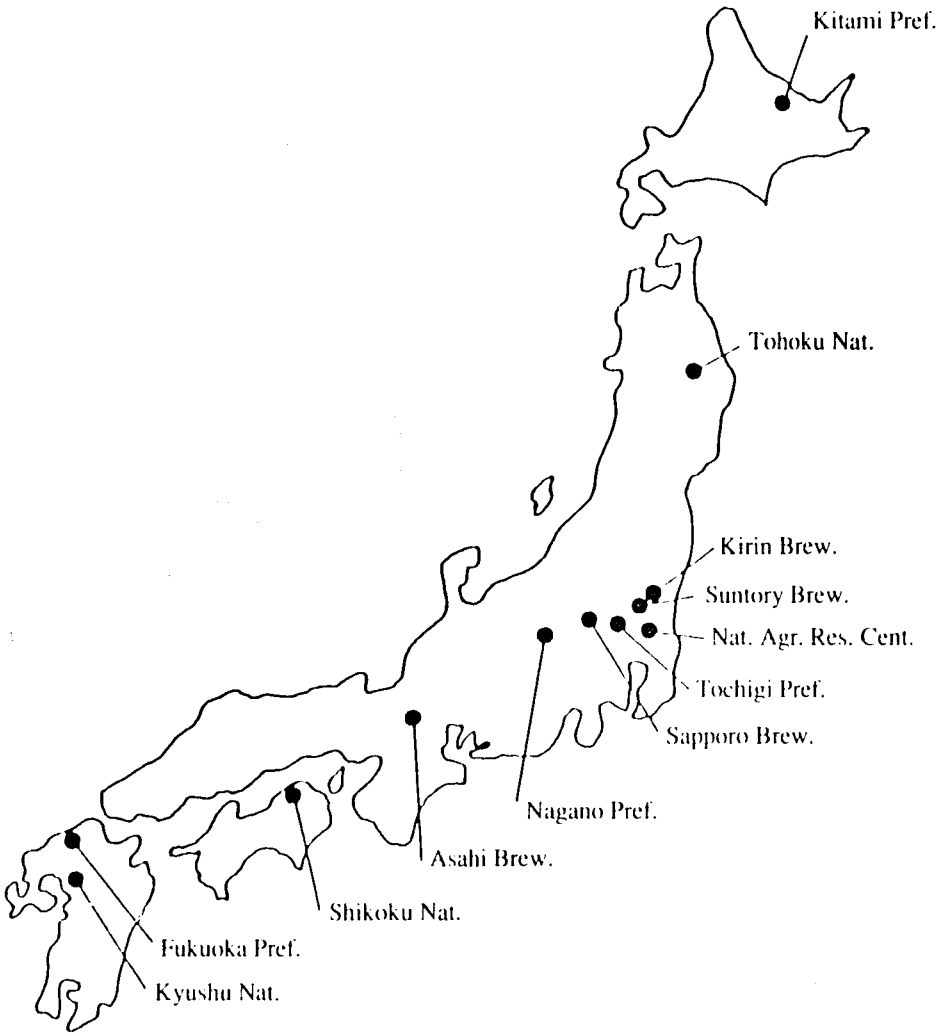

FIGURE 1 Breeding stations for barley in Japan.

TABLE 1 Progress made in the breeding of 6-rowed covered barley.

Year of release	Name of cultivar	Culm length (cm)	Maturity date	Grain yield (t/ha) Standard	Drill
Local var.	Tora-no-o	101	June 4	–	–
1914	Sekitori-sai 1	80	May 30	4.4	6.0
1963	Doriru-mugi	70	May 29	5.0	7.0
1960	Kashima-mugi	75	May 25	4.7	7.0
1976	Katori-mugi	70	May 28	5.0	7.5
1976	Hayami-omugi	72	May 25	4.8	7.3

(Nonaka 1979)

Breeding for straw stiffness

In order to breed cultivars resistant to lodging, two approaches can be considered: shortening of culm length and increase of culm stiffness. Since the culm length of 6-rowed cultivars had been reduced to some extent, further efforts were made to strengthen the culm which is more fragile at maturity than that of wheat or 2-rowed barley. As breeding material for straw stiffness Hagane-mugi has been extensively used. Several cultivars, both in Japan and Korea have been bred from Hagane- mugi crosses. Hagane-mugi displays a heavier culm, a thicker culm wall, a larger culm diameter and stronger resistance to culm breaking compared with the representative uzu type Sekitori-sai 1 (Table 2).

TABLE 2 Comparison of characters relating to lodging.

	Dry wt per unit culm length (mg/mm)	Thickness of culm wall (mm)	Culm diameter (mm)	Bending moment of culm at breaking (g.cm)
Hagane-mugi	18	1.10	5.60	1 110
Sekitori-sai 1	13	0.86	4.54	431

(Ota, Suzuki and Udagawa 1966)

Breeding for resistance to BYMV

To control barley yellow mosaic virus, which had caused serious damage to 2-rowed barley, the first resistant selections bred from Mokusekko 3 at the Tochigi Prefectural Agricultural Experiment Station were crossed with lines with a high malting quality. As a result, many cultivars and parental lines have been bred from there (Figure 2). Another resistant cultivar was released from Fukuoka Prefectural Agricultural Experiment Station in 1986. Because malting barley breeding groups have made extensive use of the Mokusekko 3 gene, most of the currently promising lines are resistant to BYMV. On the other hand, Hagane-mugi, mentioned above as a source of straw stiffness, was found to have another resistance gene to BYMV. Kyushu National Agricultural Experiment Station released the first BYMV resistant 2- rowed non-malting barley cultivar Ishukushirazu in 1981. In the 6-rowed barley breeding program the mutation line Ea 52 has been used and promising lines have been bred from it. As a result of cooperation with the virus research section, new information is being obtained on race differentiation, and efforts will have to be made to introduce further resistance genes in future breeding programs.

Breeding for quality

In 6-rowed barley breeding programs quality selection has been undertaken for yield of pearled grain, pearling time and whiteness of pearled grains using a test pearler. On the other hand, the nutritional quality of naked barley has been improved and several promising lines bred at the Shikoku National Agricultural Experiment Station. However it remains difficult to combine the high whiteness of pearled grain with high nutritional quality. In malting barley breeding programs priority has been given to quality selection. Recently high quality cultivars such as Amagi Nijo, and Haruna Nijo have been released as a result of extensive work on quality selection. As shown in Table 3, considerable progress has been made in malting quality improvement.

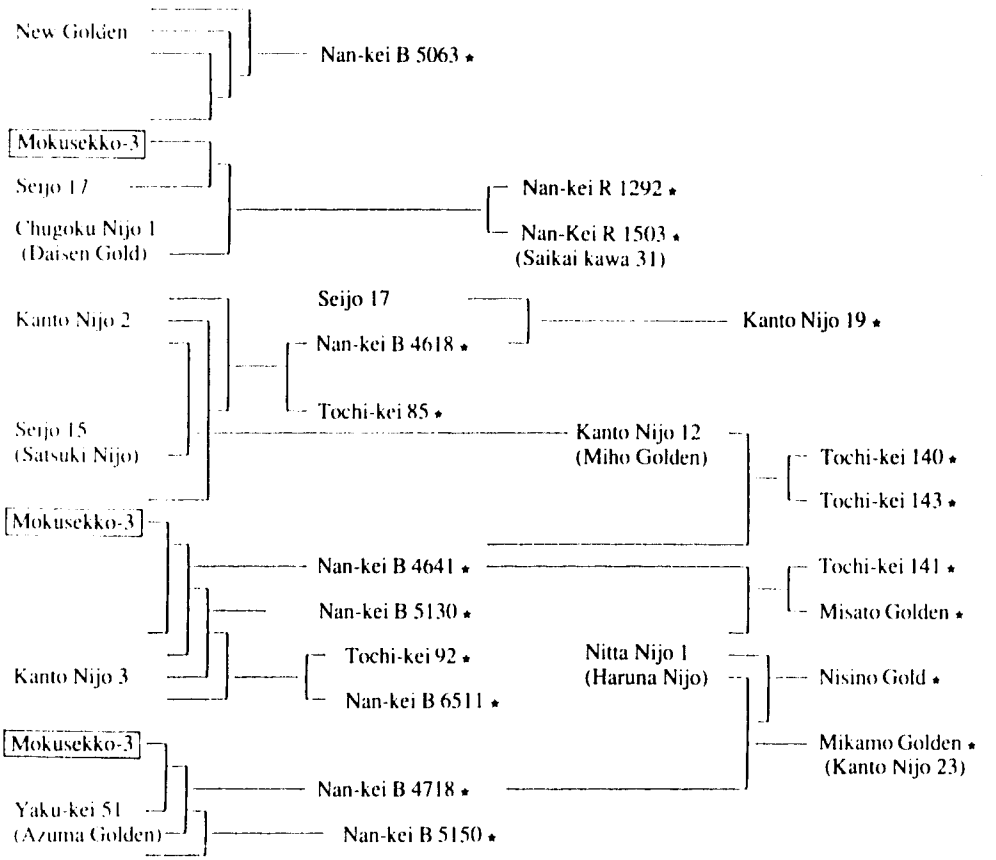

FIGURE 2 BYMV resistant cultivars and lines using Mokusekko 3 as the gene source.

TABLE 3 Improvement of malting quality.

Cultivar	Year of release	Malt extract (%)
Tochigi Golden Melon	1915	78.2
Hakata 2	1935	76.0
Shunsei	1953	77.4
Seijo 17	1964	78.3
Satsuki Nijo	1964	80.6
New Golden	1965	78.0
Azuma Golden	1971	78.3
Miho Golden	1975	79.2
Fuji Nijo II	1975	80.7
Amagi Nijo	1978	80.4
Haruna Nijo	1979	82.4
Tsuyusirazu	1984	81.4
Misato Golden	1985	80.4
Nirasaki Nijo	1985	83.0
Satsukibare	1985	83.4
Nisino Gold	1986	–
Mikamo Golden	1987	–

(Tochigi 1984)

Utilization of genetic resources

Until the 1950s local cultivars or pure line selections accounted for most of the barleys grown. For example, the uzu cultivars of the Sekitori group for 6-rowed covered barley, the uzu cultivar Akashinriki for 6-rowed naked barley, and the normal type Golden Melon introduced from abroad about 100 years ago as well as a group of pure line selections for 2-rowed malting barley. The first 6-rowed covered Norin cultivar Satsuki-mugi developed by cross breeding was released in 1951, the first 6-rowed naked cultivar Norin 1 was released in 1949, and over the subsequent 40 years a further 28 cultivars have been released. For 2-rowed barley, the first Norin cultivar New Golden was released in 1965 and has been followed by 11 cultivars over the subsequent 33 years. At private brewing companies cross breeding started early in the 1920s and Hakata 2 was released in 1935. Since then many cultivars have been developed by cross breeding and mutation breeding.

As Tochigi Prefectural Agricultural Experiment Station had conducted the evaluation with more than 1000 cultivars at the start of 2-rowed malting barley breeding work (from 1954), many genetic resources have been used to search the excellent gene sources for a new breeding objective. But the genetic resources which played an important part in crosses of Norin cultivars were few and almost all were Japanese local cultivars or those introduced in former days. This may be ascribed to the fact that main local or old cultivars and lines bred from them have played a part in adaptation to various conditions in Japan, and many other cultivars have done for improvement of specific characters as such a parental line. Until 1983, the germplasm laboratory of wheat and barley had collected 6 000 wheat and 4 500 barley cultivars. These collections included the breeding lines and Norin cultivars donated by each breeding section, and old cultivars, relative or wild species of wheat and barley by Okayama University and Kyoto University. A catalogue of nearly 10 000 cultivars is to be published. The data will compare cultivars of Japanese and overseas origin. Cultivars of Japanese origin tend towards early maturity and short culm. This result seems to relate to the high ratio of uzu types and selection (Figure 3).

In 1983, the MAFF Gene Bank Project started. It has continued to collect, evaluate and distribute the genetic resources. At present, total collections of wheat and barley at the Gene Bank Center amount to 26 000 and 13 000, respectively.

Over four years about 3 500, 900, 500, and 340 cultivars were investigated for primary evaluation, powdery mildew, scab and BYMV, respectively (Table 4).

TABLE 4 Evaluation of barley varieties, cultivars and breeding lines by Gene Bank Project (1983–86).

	Primary evaluation (12 characters)	Powdery Mildew	Scab	BYMV
6-rowed covered	1 500	881	104	–
6-rowed naked	500	40	150	–
2-rowed barley	1 500	–	260	340

There are as yet many uninvestigated cultivars and more practical characters to be evaluated (secondary evaluation, Table 5), and the catalogue is to be published.

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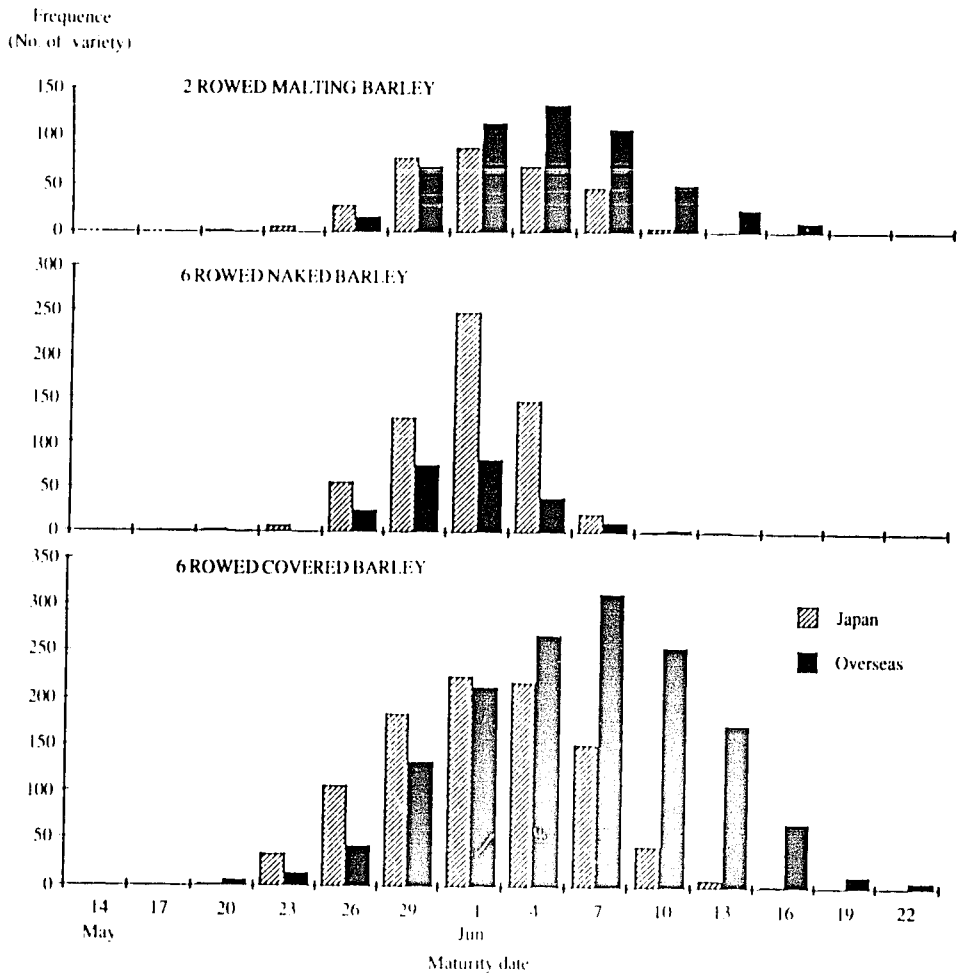

FIGURE 3 Comparison of maturity for barleys of Japanese and overseas origin.

TABLE 5 Characters investigated by Gene Bank Project.

Primary evaluation	Secondary evaluation[a]
1. Growth habit	1. Rust resistance
2. Uzu or normal type	2. Mildew resistance
3. Culm length	3. Scab resistance
4. Ear length	4. Resistance to snow injury
5. Number of rows of spike	5. Cold tolerance
6. Awnness	6. Yellow mosaic resistance
7. Glume colour	7. Green mosaic resistance
8. Grain size	[b]8. Lodging resistance
9. Grain colour (only naked)	[b]9. Resistance to pre-harvest sprouting
10. Distinction of naked or covered	[b]10 Protein content
11. Heading stage	[b]11. Yield and related characteristics
12. Maturity stage	[b]12. Quality and related characteristics

[a] The manual is being prepared.
[b] Not tested.

CHAPTER 17

Rice breeding for cold and blast resistance in Yunnan Province, China

N. HORISUE, WANG YONG-HOUA, JIANG ZHINONG, T. HIGASHI, WANG HUAIYI, K. MORIYA AND T. KUNIHIRO

The Tropical Agriculture Research Center of Japan and Yunnan Academy of Agriculture Science Of China have restudied fundamental research programs of the joint research project on rice breeding once every three years. The first and the second period programs conducted from 1983 to 1987 covered three major subjects.

First, the purpose was to breed new varieties having cold and blast resistance, and good capacity even in high altitude areas in Yunnan. Second, to test cold resistance and the utilization in breeding of genetic resources provided by Japan and China. Third, to test blast resistance and genetical analysis of the genetic resources. The following gives an outline of the results obtained so far.

Breeding new varieties having cold and blast resistance, and high yield

1. The problem was taken charge of by a breeding work group. The research, concerned (1) breeding new varieties for cold and blast resistance, (2) raising yield and quality for the altitude zone in Yunnan, (3) selecting intermediate parents on important charateristics, (4) accumulating genes on cold and blast resistance and high yield, and (5) studying an ecotype of rice.
2. The object of a Japan and China joint breeding project is to raise new varieties of an intermediate type between the Japanese and Chinese varieties. Plant type features, e.g. culm length, the number of panicles and grains of a panicle, should have several varieties according to each area. The main object is to find new varieties having cold and blast resistance, large sink size, good ripening ability, and a high yield and good quality. Recently, cultivation of intermediate-type varieties such as Sinan-175, Yunyi-1-hao, Dianyu-1-hao, and Chungang-3-hao is becoming prevalent in Yunnan. These varieties show better qualities than those of Yunnan-type varieties. However, cold and blast resistance, and lodging resistance are not good enough.
3. Crossing of Japanese and Chinese varieties in this project began in 1983. The most advanced generation of the material reaches F_6 as of this year. Table 1 shows the yield ratio results of a performance test for recommendable varieties conducted at thirteen places in Yunnan province in 1986. Results of the test showed that yield ability of growing lines in a joint study was very great at heights of less than 1900 metres, and also comparatively good in areas at over 1900 metres when the lines had rather cold resistance. These lines featured a little earlier maturing, culm length ranging from short to middle, good ripening, high yield, and good quality on the whole. Cold resistance ranged from middle to middle-strong. Blast resistance was also good on the whole. However, testing was not perfectly executed.
4. It is difficult to tell whether elite lines can be raised or not by crossing Japanese and Yunnan varieties. However, after studying characteristics obtained in F_1 and F_2 generations of Japanese and Chinese crossings, we found that there were many characteristics featuring either hybrid vigours or weakness in heading date, culm length, grain size, etc. in F_1 generations. Some individuals exceeded their parents possibly resulting in higher yield capacity in F_2 generation. We feel that Yunnan and Japanese varieties possibly belong to the same Japonica type, so cross compatibility is high. But genetic backgrounds

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TABLE 1 Yield ratios of growing lines compared with standard variety Yungeng 136.

Name of lines and variety	Place and height (m) above sea level												
	A	B	C	D	E	F	G	H	I	J	K	L	Average
	1580	1653	1745	1774	1840	1860	1906	1990	1920	1900	1916	2040	
Hexi 1 hao	103	112	126	108	98	126	96	86	69	113	117	81	104
Hexi 2 hao	117	113	133	111	115	119	95	97	54	110	110	95	116
Hexi 3 hao	123	111	112	115	119	102	89	97	60	96	107	78	104
Hexi 4 hao	107	91	129	120	95	116	56	48	109	126	109	85	99
Hexi 5 hao	114	119	142	116	122	90	87	87	100	112	85	75	106
Hexi 6 hao	90	114	128	122	90	105	87	104	50	94	94	75	95
Hexi 7 hao	137	112	109	126	116	120	90	115	69	78	94	70	105
Hexi 8 hao	120	111	135	129	103	107	83	81	103	90	88	79	104
Hexi 9 hao	123	104	142	128	110	87	99	102	141	92	86	103	113
(S) Yungeng 136	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
(C) Yungeng 9 hao	91	107	66	88	73	144	93	96	109	109	97	110	94
(S) Yungeng 136	63.8	67.3	62.4	64.0	56.8	62.9	72.6	76.9	18.9	59.2	69.2	43.2	

Cold weather damage was severe in Zhaotong and Wuding. Hexi 4 hao is an early maturing variety. There was bird or rat damage in some places. The bottom line shows the purified grain yield (kg/ha) of standard variety Yungeng 136, using 80% husked rate.

A: Houajie B: Baoshang C: Jiengjiang D: Kuangning E: Luliang F: Anning
G: Quqing H: Dali I: Zhaotong J: Xishang K: Kunming L: Wuding

are different. So there may be hybrid vigour in the F_1 generation. On the other hand, in the F_2 generation alternative charateristics are combined in various cases and yield capacity may increase. (Figure 1).

5. Amongst the Japanese varieties used so far, Todorokiwase, Toyonishiki, Himenomochi have shown good parental features. Among the Yunnan varieties, Yungeng-135, Jinhon-1-hao, 79-219, and Dianyu-1-hao can be named. These Japanese varieties have a good reputation even in Japan. Yunnan varieties, except Dianyu-1-hao, are classified as Yunnan- type having ultra heavy panicles, long culms, and big ears. It is believed that these Yunnan varieties have become good parents, after various types of individuals and lines were segregated and created. Wide variation has been introduced by crossings with Japanese varieties.
6. When Yunnan-upland rice was used for the purpose of producing varieties having high blast resistance, many lines having good plant posture with high blast resistance were found in the crossing lines of upland rice with Dianyu-yu-1-hao. Race specificity of fungus strain must be borne in mind because blast resistance of Yunnan-upland rice may be influenced by a major gene.

Testing cold resistance, and use of genetical resources for breeding rice having cold resistance

1. In this research by a cold resistance-work group, items included (1) researching cold resistance of genetical resources provided by Japan and China, and of materials raised by the breeding work group, (2) selecting and breeding intermediate parents for cold resistance, (3) studying genetical analysis and establishing breeding methods, and (4) selecting standard varieties and establishing testing methods of cold resistance.
2. About 500 samples provided by both China and Japan were tested to study cold resistance in several environmental conditions such as natural cool weather conditions, temperature controlled conditions, and cool-water irrigated conditions from 1983 to 1986.
   As a result, many Yunnan-native varieties showed more resistance than Japanese ones

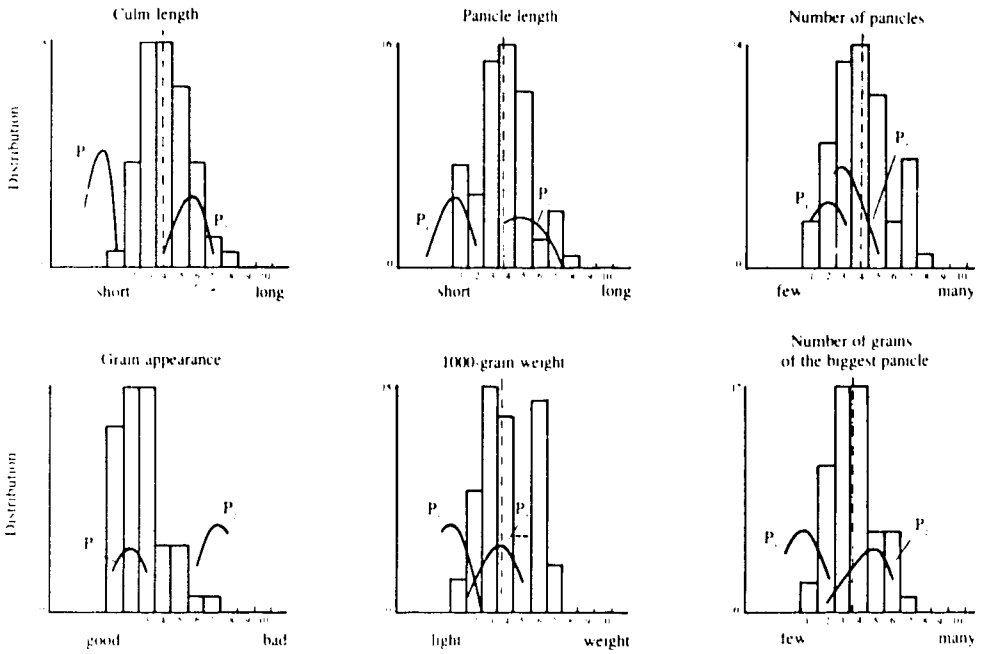

FIGURE 1 Examples of genetic features of individuals in Japanese and Chinese varieties crossing in F_2 generation (Toyonishiki / Gendiao 3 hao). P_1: Toyonishiki P_2: Gendiao 3 hao

(Table 2). Especially Lijian-xintuan-heigu, Kunming-xiao-baigu, Banjie-man, and Gandiao-3-hao showed the high resistance at booting and flowering stage in natural cool conditions. Generally these varieties featured long culms, big ears, and ultra heavy panicles. Plant posture and grain quality were inferior to Japanese ones. Among Japanese varieties, (Fuji) Chubo 42, Somewake, Hayayuki, and Otori showed medium-strong resistance. Delizaoxian and Chuxian, indica varieties, showed medium cold resistance.

3. It is possible that Japanese varieties are regarded as dwarf and unsuitable ones in Yunnan because of differences between China and Japan in daylength and other meteorological conditions. It is also possible that Japanese ones show lower resistance to cold there than in Japan. They may have been evaluated unfairly. Considering this situation, it is possible that Yunnan varieties show the same trends when they are tested in Japan. It is interesting to use both varieties to study various characteristics including resistance to cold when the conditions, daylength, temperature and soil, are changed, and also interesting to study what kind of lines can be produced when both varieties are crossed.
4. In Yunnan province there are more than ten stations breeding Japonica-type paddy rice. Recently, many improvements not only in general characteristics but also in cold resistance among the breeding varieties have been recognized. But among the intermediate-type varieties between China and Japan, there are only a few beyond the class of medium-strong in resistance to cold. Intermediate types including Kunming, showing characteristics of medium-strong level cold resistance and a constant yield even in colder zones at heights of between 1800 and 2200 metres, are expected to be developed in the near future. At the same time, intermediate type having characteristics of medium level cold resistance and high-yield capacity at heights of between 1500 and 1800 metres are expected to be developed.

TABLE 2 Varieties highly resistant to cold under natural cool conditions.

Level of cold resistance	First provided materials		Second provided materials[a]	
	Yunnan varieties	Japanese varieties	Yunnan varieties	Japanese varieties
High	Lijian xintuan heigu Gangdiao 3 hao Banjiemang Kunming xiao beigu			
Resistant	Pannong 1 hao Zhaotong maxiangu Kunming 830 Liezihouan	Fuji Chubo 42	04-108	
Medium resistant to resistant	Yun 83-139 Yungeng 9 hao	Somewake Ootori	80-2 04-2865 Dianhoua 3 hao 82-148 Yunertian 022 151 nuo Weihonggu[b] Zapu[b]	

[a] Among the second provided materials, some varieties were tested for only one year. Levels of resistance will change from now.
[b] Upland rice.

5. Meteorological conditions during the rice breeding season in Yunnan, located at high altitudes, leads to continuing cold weather damage. In Kunming, average 10-day temperatures, from the middle of May to the end of August, change slowly from 18.5° to 20.2°C showing the highest temperature at the beginning of August. For this reason, cold weather damage only takes place in some specific early and late maturing varieties unlike Japanese ones. There are various types of cold-weather damage each year. Thus it does not take so many years to find varieties presenting cold resistance.
6. In testing breeding lines, the cold resistance tests must be completed in only one year. It is thought to be possible to use subterranean water at the Yunnan Academy of Agricultual Science in summer. It is roughly 17°C and comparatively stable. We thought there was a high possibility of finding ways of testing cold resistance by means of proper irrigation using this water. In addition, we have prepared cool-water supplying facilities for testing cold resistance at various growing stages, and are going to begin studying testing methods using irrigated fields where temperature is controlled.
7. The cold resistance of the progeny after crossing Japanese and Yunnan-varieties generally features a middle point between parents in the F_1 generation, and is mostly distributed in the middle of parents after the F_2 generation. Therefore we must use varieties having high resistance for both parents in cold resistance breeding. There is a possibility of obtaining progeny having greater cold resistance than both parents because the resistance gene is accumulated when the parents having cold resistance are crossed (Figure 2).
8. Recently, Suzuki has stated that there is a close relation between cold resistance and the length of anther and stigma. We found that there was a close relation between cold resistance and the length of anther, but not between cold resistance and the length of stigma. We also found that the length of anther among the varieties was not much

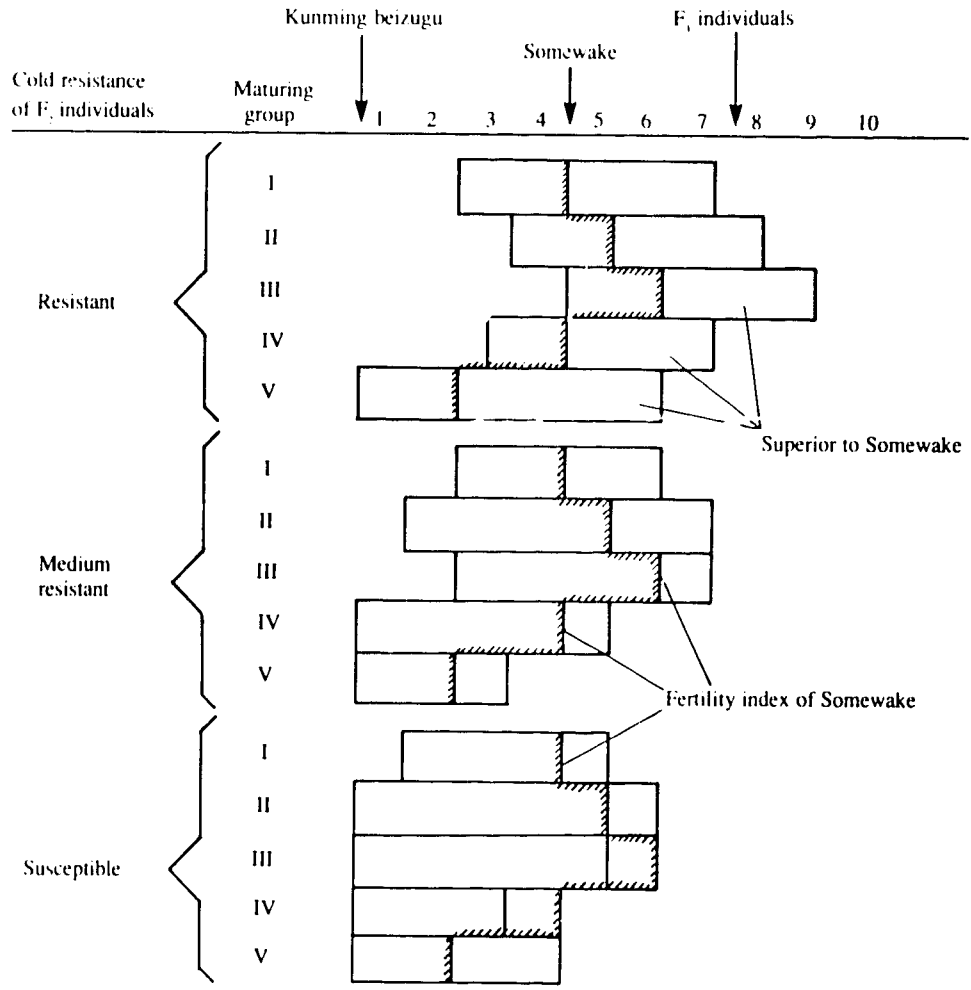

FIGURE 2 Distribution of fertility in F_1 individuals derived from crossing between Somewake and Kunming beizugu. Fertility index 1–10.

influenced by environmental conditions while a sterile rate, an index of cold resistance, was greatly influenced by them (Figure 3). That means anther length can be used for the index of cold resistance whenever or wherever cold weather damage takes place.

9. We attempted to use anther length for cold resistance breeding by using the crossing populations and lines of varieties from both Japan and Yunnan. As a result, anther length generally indicated overdominance in the F_1 generation. Correlation between anther length and sterility rate of the next generation became closer and closer as generations advanced. So did correlation between parent and sibling anther length (Table 3). It was thought the selection of varieties on the basis of anther length was more effective in an advanced generation than in an early generation.
10. We have determined standard varieties having cold resistance in accordance with the different ripening times after several experiments in natural conditions (Table 4).

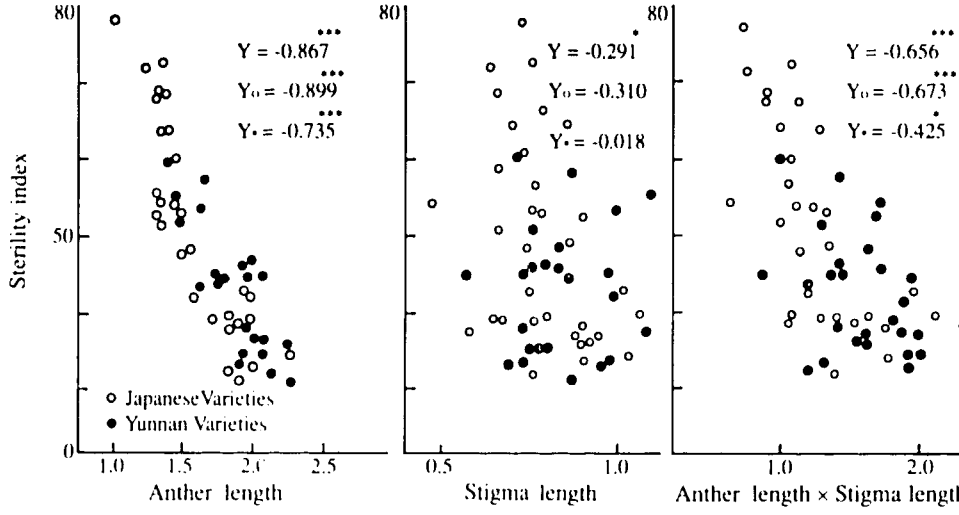

FIGURE 3 Relationship of anther length and stigma length to grain sterility.

TABLE 3 Correlation of anther length with grain sterile parameter and anther length of next generation.

Combination	1985 Number of materials & generation	Place	F_4 generation Grain sterile	Anther length	1986 Number of materials & generation	Place	F_5 generation Grain sterile	Anther length
Chubo 42/ Kunming xiaobeigu	74 F3	Kunming	*** -0.411	** 0.343	36 F4	Iliang	*** -0.761	*** 0.839
	74 F3	Shuwangshio	*** -0.400	* 0.273	36 F4	Shuwangshio	*** -0.769	*** 0.801
Nishihomare/ Yungeng 135	40 F3	Kunming	*** -0.446	* 0.435	35 F4	Iliang	** -0.524	*** 0.757
	40 F3	Shuwangshio	* -0.309	-0.185	35 F4	Shuwangshio		*** 0.678

Statistical significance — *** 0.1%, ** 1%, * 5%

TABLE 4 Standard varieties with cold tolerance in Kunming.

Resistance	Maturing group: Early maturing	Middle maturing	Late maturing
Resistant	Pannong 1 hao	–	Kunming 830
Medium resistant	Hamaasahi	Kunming 217	Yungeng 20
Medium	Fujiminori	Jingning 78-102	Xinan 175
Weak	Towada	Hidekomochi	Nipponbare

Testing blast resistance and gene analysis of genetic resources

1. The blast resistance group was concerned with (1) testing blast resistance on genetic resources supplied by Japan and China and on breeding materials, (2) selecting and breeding on intermediate parents, (3) breeding isogenic lines having different types of blast resistance, (4) testing pathogenicity of Yunnan blast fungi, (5) analyzing genetics of blast resistance and establishing its breeding methods, (6) selecting standard varieties, and establishing testing methods for blast resistance.
2. We studied how responsive the varieties in Yunnan were to typical fungi of blast seen in Japan and China and compared the result with the standard Japanese varieties. As a result, the varieties in Yunnan were assumed to have *Pi-k*^{s}, *Pi-a*, *Pi-i*, *Pi-k*^{m} as blast-resistance gene. They are thought to be comparatively simple (Table 5). Japanese varieties, to which true resistance from foreign varieties had been applied, are thought to have wider varieties of blast genes.
3. After examining the pathogenicity of blast fungi collected from various places in Yunnan, using the standard Japanese varieties, we found that its race composition in Yunnan was more complicated than in Japan. For example, there were many fungi invading *Pi-i*, *Pi-ta*. There were some fungi showing unstable pathogenicity to the varieties Kanto-51, Tsuyuake and Pi No 4. Studying the differences in distribution of blast fungi races in places where Japonica, Japonica–Indica, and Indica rice were cultured, we found that the distribution was simpler in the Indica–Japonica cultured zone where rice was mainly grown, than in the zones where Japonica or Indica were cultured separately.
4. For the purpose of studying utilization effects of true and field resistance, we conducted experiments in response to blast at about 20 places in Yunnan, using several kinds of varieties such as (1) specific varieties for judging blast, (2) true-resistance accumulated varieties, (3) isogenic lines of a kind of Nipponbare having different genes for blast, (4) possible standard varieties for the study of field resistance, and (5) Japanese and Chinese upland rice.

TABLE 5 Estimated true resistance genes to rice blast in Yunnan paddy rice varieties.

+	Lijian xintuan heigu Zhaotong maxiangu (Yungeng 129) Xihong 131 Xinan 175 Jinning 65-36 (Yun 83-140) (Yun 83-144) Yungeng 9 hao
Pi-k^{s}	(Banjie man) Lianshuigu (Gandiao 3 hao) Yixi 8126 Xijin 1 hao xuan (Jinnuo 1 hao) (Dali 75-64) Keqing 3 hao (Jingguo 9-2) Jingguo 9-3 Jianjiang 65-113 Yungeng 5 hao
Pi-a	Yungeng 79-635
Pi-k^{s} Pi-a	(Kunming xiaobeigu) Ximinghuanpigu Yungeng 21 Jinning 277 Dali 59-701 Dali 635-781 Yungeng 20 Yun 83-147
Pi-i	(Lizihuang) (Zapu) (Pannong 1 hao) (Yungeng 136) Yungeng 133 (Yungeng 79-219) Jinghong 1 hao (Jinning 768) Yun 83-128 (Yun 83-147)
Pi-k	(Jiniandao)
Pi-k^{m}	Kunming 830 (Yun 83-138)
Pi-z	Qingnong 1033

TABLE 6 **Frequency of pathogenic races which have virulence to each differential variety in Yunnan.**

Differential varieties	Resistant gene	Race distribution (%)	
		A	B
Shin 2	Pi-k^s	96	95
Aichiasahi	Pi-a	86	85
Ishikarishiroge	Pi-i	81	35
Kanto 51	Pi-k	59	52
Tsuyuake	Pi-k^m	21	17
Fukunishiki	Pi-z	8	6
Yashiromochi	Pi-ta	59	49
Pi No4	Pi-ta^2	7	5
Toride 1	Pi-z^t	0	6
BL 1	Pi-b	3	14
K59	Pi-t	53	35
Lijian xintuan heigu	+	100	100

A: Collected around the YAAS.
B: Collected from various parts of Yunnan.

TABLE 7 **Blast response of varieties in different cultivated zones.**

Variety	Resistant gene	Indica			Indica-Japonica						Japonica			
		A	B	C	D	E	F	G	H	I	J	K	L	M
Lijian xintuan heigu	+	S	S	S	S	S	S	MS	S	S	S	S	S	S
Shin 2	k^s	S	R^h	S	S	S	S	S	S	MS	S	M	S	S
Aichiasahi	a	S	S	S	S	S	S	S	S	S	S	M	S	S
Ishikarishiroge	i	S	R	S	S	R	S	M	S	M	S	M	S	S
Kanto 51	k	MR	S	MR	S	M	MS	S	S	MS	M	M	S	M
Tsuyuke	k^m	MR	MS	S	S	MS	R	S	S	M	M	S	R	MS
Fukunishiki	z	R	M	R^h	R^h	R	R^h	R^h	M	R^h	R^h	M	R^h	R^h
Yashiromochi	ta	M	R^h	M	R^h	MR	R	R^h	R	M	M	S	MS	M
Pi No4	ta^2	R	R^h	R	R^h	R	R^h	R^h	R	R^h	R^h	M	R^h	R^h
Toride 1	z^t	R	R^h	R^h	R^h	R^h	R^h	R^h	R	R^h	R^h	M	R^h	R^h
BL 1	b	S	R	R	R^h	R^h	R^h	R^h	R	M	R^h	M	R^h	R^h

A: Sumao B: Ruili C: Menzu D: Chuxing E: Iliang F: Shiping G: Baoshang
H: Yuanjiang I: Jianshui J: Kunming K: Zhanyu L: Zhaotong M: Wuding

The experiments were performed in a nursery test. As a result, there were some features in responses of true resistance genes. Varieties having +, *Pi-k*^{s} and *Pi-a* did not show any resistance in almost all the places.

Those having *Pi-i*, *Pi-k*, *Pi-k*^{m}, *Pi-t*, *Pi-ta* indicated resistance in some places. On the other hand, those having *Pi-z*, *Pi-ta*^{2}, *Pi-z*^{t}, *Pi-b* presented high resistance in almost all the places.

5. As to field resistance, interaction between varieties of the same gene and places in disease severity was recognized to be significant. However, resistance level did not vary between places. We concluded that field resistance took effect. Japanese and Chinese upland rice showed high resistance in all of the places.
6. All of the Japanese upland rice was susceptible to the injection of blast fungus, race 037. But some of the Yunnan upland rice was resistant. Judging from this, those varieties are thought to have some true resistance.

CHAPTER 18

Vegetable germplasm at AVRDC and its utilization in crop improvement with special reference to disease resistance breeding

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The primary goal of AVRDC is to develop improved technology in the form of tropically adapted vegetable cultivars and appropriate management practices. Since its inception, AVRDC has vigorously underscored the importance of diverse germplasm assembly to support the crop improvement programs for its principal crops, namely, tomato, Chinese cabbage, sweet potato, soyabean, mungbean, white potato and pepper, which was added only recently. Over the past decade, the elite genetic materials of these crops have been utilized by AVRDC scientists to develop new cultivars that are more resistant to disease and tolerant of environmental stresses in the tropics. AVRDC has been active in disseminating improved breeding lines and elite accessions for use by national programs, either directly as commercial cultivars or as parent stocks in breeding.

In this chapter we present the the genetic resource and crop improvement activities on AVDRC's principal vegetables (except white potato and pepper) with special emphasis on disease resistance breeding.

Disease constraints of vegetables in the tropics

A review of the diseases capable of attacking AVRDC's principal vegetables in the tropics is given below:

Tomato

Tomatoes grow fairly well in the cool, dry regions of the tropics but are often attacked by many diseases during the hot, humid season. Some 9 or so of 51 tomato diseases are important in the hot, humid tropics (Villareal 1979; Yang 1979). These are bacterial wilt (*Pseudomonas solanacearum*), rootknot nematode (*Meloidogyne incognita*), tomato mosaic virus, leafmold (*Cladosporium fulvum*), grey leafspot (*Stemphyllium solani*), Septoria leafspot (*Septoria lycopersici*), southern blight (*Sclerotium rolfsii*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), and powdery mildew (*Erysiphe polygoni; Leveillula taurica*). Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) has also become a severe problem in the humid tropics (Opeña et al. 1986a)

Chinese cabbage

Three diseases among several afflicting Chinese cabbage, namely, softrot, downy mildew, and turnip mosaic virus, are considered important in the hot, humid tropics (AVRDC 1974). Softrot (*Erwinia carotovora*) is most damaging during the hot season. Downy mildew (*Peronospora parasitica*) is a serious problem only in the early or late summer planting in the fringes of the tropics or in the cool, moist highland regions. Turnip mosaic virus, while relatively unimportant during the hot, rainy periods, could be damaging during the hot, dry season.

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Sweet potato

Although relatively less ravaged by diseases than many other vegetables, sweet potato is attacked by several pathogens such as black rot (*Ceratocystis fimbriata*), Fusarium wilt (*Fusarium oxysporum* f. sp. *batatas*), Java black rot (*Diplodia tubericola, D. gossypina*), scab (*Elsinoe batatas*), scurf (*Manilochates infuscans*), soil rot (*Streptomyces ipomoea*), witches' broom (a mycoplasma-like organism) and viruses.

Diseases did not receive a particularly strong emphasis in the first decade of the AVRDC' sweet potato program. AVRDC's production survey of Asia and the Pacific in 1982 indicated, however, that diseases are also important production constraints (Lin et al. 1983). Of the diseases cited, scab was the most prevalent, followed by Fusarium wilt and witches' broom.

Mungbean

Several diseases, especially Cercospora leaf spot (*C. canescens, C. cruenta*), powdery mildew (*Erysiphe polygoni*), mungbean yellow mosaic virus, the root disease complex (*Pythium* sp., *Rhizoctonia solani, Fusarium* sp.), reniform nematode (*Rotylenchulus reniformis*), and root-knot nematode (*Meloidogyne* sp.), can cause large yield losses in mungbean (Morton et al. 1982). Cercospora leaf spot is severe during the warm rainy season; whereas powdery mildew thrives well under cool, dry weather. The root disease complex is favoured by cool temperatures. More than 20 viruses have been reported to infect mungbean. The more common ones are mungbean yellow mosaic virus (MYMV), blackgram leaf crinkle, and mungbean mottle virus (Anon. 1984), of which MYMV, transmitted by whitefly (*Bemisia tabaci*), is the most severe, especially in the Indian subcontinent.

Soyabean

Thirty-five economically important diseases have been reported for soyabean worldwide (Sinclair 1986). Among them, the following have been frequently cited and researched in the tropics (Shanmugasundaram and Tschanz 1985; 1986): fungal diseases—soyabean rust (*Phakopsora pachyrhizi*); anthracnose (*Colletotrichum truncatum; Glomerella glycines*); target spot (*Corynespora casiicola*); frogeye leaf spot (*Cercospora sojina*); purple seed stain (*Cercospora kikuchii*); downy mildew (*Peronospora manshurica*); pod and stem blight (*Phomopsis sojae*), and root and stem rot (*Rhizoctonia solani; Fusarium oxyporum* f. sp. *traccheiphilum, Pythium* sp., *Sclerotium rolfsii, Sclerotinia sclerotiorum*); and bacterial diseases—bacterial pustule (*Xanthomonas campestris* pv. *phaseoli*); bacterial blight (*Pseudomonas syringae* pv. *glycinea*), and wildfire (*Pseudomonas syringae* pv. *tabaci*); virus diseases—soyabean mosaic virus, soyabean yellow mosaic virus, soyabean dwarf virus, and blackeye cowpea mosaic virus.

The root gall-causing *Meloidogyne javanica, M. arenaria* and *M. incognita* are the most important nematodes attacking soyabean in the tropics.

Of the fungal diseases, soyabean rust, downy mildew, anthracnose, purple seed stain, and pod and stem blight are the most serious in the tropics. Soyabean rust occurs both in the rainy and dry seasons but is more serious in the dry-season crop with irrigation. Downy mildew is also a dry-season disease. Bacterial diseases, purple seed stain, and pod and stem blight are common in the rainy season. Among the viruses, the whitefly-transmitted yellow mosaic virus is the most serious; but it is endemic only in the Indian subcontinent.

Genetic bases for the crop improvement program at AVRDC

Germplasm assembly: The need for genetic diversity

The diversity of diseases that attack AVRDC's principal vegetables presents a formidable

challenge to their survival and productivity. Crop improvement programs aimed to meet this challenge require broad genetic pools from which to derive the resistance genes that the new breeding lines must have. Over the years, AVRDC has built up its genetic resources to sizeable numbers (Table 1). These genetic resources are being maintained, characterized, evaluated, catalogued and distributed worldwide by the AVRDC's Genetic Resources and Seed Unit (GRSU) in collaboration with the institute's crop improvement scientists. The genetic bases supporting AVRDC's crop improvement activity for each principal crop are further elaborated below.

TABLE 1 AVRDC germplasm collection as of September 1987.

Commodity	Accessions
Chinese cabbage	846
Mungbean	5 274
Soyabean	12 505
Sweet potato	1 219
Tomato	5 484
Pepper	2 500
Total	27 828

Tomato

AVRDC maintains one of the world's largest collections of tomato germplasm, totalling some 6 099 accessions. Of this total, more than 90% belong to *Lycopersicon esculentum*. Other species included are *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium*, *L. hirsutum*, *L. pervianum*, *L. cheesmanii*, and a few interspecific hybrids (*esculentum* × *pimpinellifolium* and *esculentum* × *hirsutum*). In addition, 528 cultivars and other genetic stocks are still in the breeders' active collection.

Chinese cabbage

The AVRDC Chinese cabbage collection comprises 846 accessions. An additional 200 accessions have not been permanently numbered. The emphasis on collection has been on the subspecies of interest, *Brassica campestris* ssp. *pekinensis*, which constitutes more than 90% of the total collection. Other materials collected were *B. campestris* (ssp. *chinensis*, ssp. *parachinensis*, ssp. *narinosa*, ssp. *japonica*, and ssp. *rapa*), *B. juncea* and *B. napus* (Tay and Kuo 1985).

Sweet potato

A total of 1 219 accessions from 32 countries is in the AVRDC sweet potato collection. Germplasm from Papua New Guinea, Taiwan and the USA account for about 70% of the total. AVRDC has been officially designated by the IBPGR as the repository for sweet potato germplasm in Asia and the Pacific. Efforts are now underway to introduce the sweet potato germplasm from countries in the region for maintenance, disease elimination and eventual distribution.

Mungbean

AVRDC has been designated by IBPGR as the genebank for the world mungbean base

collection. A total of 5 274 mungbean accessions from all over the world are kept as both base and active collection (Tay and Kuo 1985).

Soyabean

AVRDC has assembled 12 330 soyabean accessions originating from 53 countries. Among them, 11926 are *Glycine max*; 344 are *G. soja*; and 60 belong to 11 other wild *Glycine* species (Shanmugasundaram et al. 1985).

Identification of disease-resistant stocks

The development of disease-resistant tropical breeding lines at AVRDC has been well-supported by plant pathological research on the nature of diseases, screening techniques, and disease scoring methods. Results from these studies have been applied in screening the AVRDC germplasm collections and breeding populations for disease resistance. Through the years, the AVRDC plant pathologists have amassed valuable information on disease-resistant germplasm for use by AVRDC plant breeders and other researchers worldwide. A summary of the important tropical diseases of AVRDC's crops and the availability of resistant germplasm is given in Table 2 and Table 3. The identification of resistant stocks is further elaborated below:

Tomato

The multiplicity of diseases infecting tomatoes in the humid tropics necessitated the prioritization of goals in disease resistance breeding (Opeña 1985; Opeña et al. 1986b). Bacterial wilt (BW) received the highest priority at the outset. Leaf-clipping of young seedlings with a pair of scissors dipped in a mixture of highly infective BW inoculum was the standard BW screening technique for many years (Opeña et al. 1986b). However, this method has inherently high variability for amount of inoculum delivered per plant and has thus been replaced recently by a field screening technique (AVRDC 1985; Opeña and Tschanz 1987). Some of the most important BW-resistant stocks that have been identified and used in the breeding program are as follows: the VC lines series from the Philippines; cultivars Venus and Saturn from North Carolina, USA; L 336 (unknown); and PI 406 994.

Four genetic differentials have been used at AVRDC to identify the major strains of tomato mosaic virus (ToMV) (Green et al. 1986). ToMV resistance is conditioned by two dominant genes. Gene Tm-1 imparts resistance to Strains 0, 2, and 2^2; whereas, gene Tm-2 conditions resistance to all strains except Strain 2. Tm-2 has an allele, Tm-2^2, which confers resistance to all strains except Strain 2^2. As the most common strains in Taiwan and Southern Asian countries are Strain 0 and 1, the incorporation of Gene Tm-2^2 into AVRDC's tropical lines was adequate (AVRDC 1985; Green et al. 1986). Using artificial inoculation techniques, AVRDC virologists have identified a number of genetic stocks carrying the different resistance genes, singly or in combination. The ToMV-resistant stocks that have been used in the AVRDC tomato program are as follows: L 127 (ah Tm-2^2), L 5340 (Ohio MR-12), L 5341 (Ohio MR-13), L 5337 (Moperou), L 5338 (Mocimor), and a number of recently released cultivars.

The AVRDC tomato collection has also been explored for resistance to other viruses, such as tomato yellow leaf curl virus (TYLCV), potato virus Y (PVY), and cucumber mosaic virus (CMV). Good resistance to PVY has been found in *L. hirsutum* (VL-111 series). Promising resistance to TYLCV has been observed among accessions of *L. hirsutum* (LA 38683 and LA 129583) and *L. peruvianum* (VL-115).

Rootknot nematode resistance is controlled by a single dominant gene, *Mi* derived from *L. peruvianum*. *Mi* is tightly linked to *Aps-1* (an isozymic locus for acid phosphatase) which

TABLE 2 **Important tropical diseases of AVRDC's horticultural crops and availability of resistant genetic materials.**

	Causal organism				Resistance sources [a]	
Crop/disease	bacteria	fungi	virus	nematode	accession	breeding line
Tomato						
Pseudomonas solanacearum	•				+	+
Xanthomonas vesicatoria	•				+,p	p
Tomato mosaic virus			•		+	+
Potato virus Y			•		+	p
Meloidogyne incognita				•	+	+
Cladosporium fulvum		•			+	-
Stemphylium solani		•			+	-
Septoria lycopersici		•			+	-
Sclerotium rolfsii		•			n	-
Alternaria solani		•			+	-
Chinese cabbage						
Erwinia carotovora	•				q	e
Peronospora parasitica		•			+	+
Turnip mosaic virus			•		+	+
Sweet potato						
Elsinoe batatas		•			+,p	p

[a] Please refer to the text for examples of resistant germplasm.
e Early crop maturity under adverse tropical conditions sought as alternative solution.
n Genetic resources have not been evaluated yet.
p Evaluation/breeding activities in progress.
q Varietal differences observed but questionable value in breeding.
Disease resistance breeding not yet activated.
+ Resistant genetic materials available.

gives a unique isozyme banding pattern that can be used as a marker in resistance screening (Rick and Fobes 1974). At AVRDC, the technique of electrophoresis is used to screen segregating materials and germplasm originating from California's nematode-resistant stocks. As the linkage between *Mi* and *Aps-1* has already been broken in other stocks, notably those from Hawaii, we also use the nematode egg inoculation method as an alternative screen (Opeña et al. 1986b). AVRDC pathologists and chemists have screened a number of germplasm stocks, especially those reportedly carrying the *Mi* gene. Some of the stocks that were confirmed as nematode-resistant and used initially in the AVRDC tomato program are as follows: L 97 (Healani), L 274 (Kewalo), L 275 (Hawaii 7795), L 345 (Cal-Mart VFN), and L 3890 (72 T6). Recently, we have began to use a number of nematode-resistant cultivars released by commercial seed companies as parent stocks because of their improved horticultural characteristics such as large fruit size and better fruit firmness.

Chinese cabbage

Varietal differences in reaction to artificial softrot infection have been observed; however, no reliable source of resistance has been sucessfully exploited, thus far, in the breeding program.

TABLE 3 Important tropical diseases of AVRDC's legume crops and availability of resistant genetic materials.

Crop/disease	Causal organism				Resistance sources[a]	
	bacteria	fungi	virus	nematode	accession	breeding line
Mungbean						
Cercospora canescens, *Cercospora cruenta*		•			+	+
Erysiphe polygoni		•			+	+
Poty virus			•		+	-
Unknown virus			•		+	+
Soyabean						
Phakopsora pachyrhizi		•			+,t	t
Xanthomonas campestris pv. *phaseoli*	•				+	+
Peronospora manshurica		•			+	+
Soyabean mosaic			•		+	+
Poty virus isolate 220			•		+	+
Pseudomonas syringae pv. *phaseoli*	•				+	-

[a] Please refer to the text for examples of resistant germplasm.
t Accessions and breeding lines tolerant to soyabean rust are availabe.
- Disease resistance breeding not yet activated.
+ Resistant genetic materials available.

The development of turnip mosaic virus (TUMV) resistant genotypes had depended previously on complex infection until a specific strain concept was established (Provvidenti 1980; Green and Deng 1985). Five distinct strains of TuMV have been recognized so far. AVRDC virologists have successfully identified resistance to TuMV strains C1, C2, and C3 among Chinese cabbage germplasm (AVRDC 1985). Resistance to the above strains appears to be common in Chinese cabbage. On the other hand, resistance to strains C4 and C5 is rare, having been observed only in Accession B 730 (AVRDC 1985, 1987a). Another useful accession, B 708 (PI 418957), carries immunity to strains C1 and C3 and resistance to C2 and C4 (AVRDC 1987a).

Accessions, breeding lines and segregating populations are ordinarily exposed to downy mildew (DM) epiphytotics, either natural, artificial, or both. An *in vitro* technique using sporangia production on detached cotyledons, inoculated and incubated for 12 hours at 14°C, promises to be a useful tool to screen a large number of seedlings under controlled environment (AVRDC 1987a). The most promising sources of DM resistance used by the AVRDC breeders were Korean introductions, particularly accession B 742 (AVRDC 1985). B 639 (Hakuran), a synthetic amphidiploid between *B. campestris* ssp. *pekinensis* and *B. oleracea*, has also shown an excellent DM resistance and has been the important donor parent in a backcross program to transfer the resistance to *B. campestris* ssp. *pekinensis* (AVRDC 1987a).

Sweet potato

Lack of methodology for rapid artificial multiplication and effective screening for sweet potato scab resistance had blocked progress in the past (Opeña et al. 1987). Scab is not

endemic under AVRDC conditions, occurring rather infrequently during the hot, humid season, thus mitigating the use of natural field screening technique, the AVRDC pathologists have now developed a suitable *in vitro* propagation techique of the causal organism, the rapid screening method via artificial inoculation, and the disease scoring method (AVRDC 1987c). These techniques have been employed in identifying scab-resistant germplasm. In several screening tests, accessions I 1175 (V2-30), I 1206 (GI 6), and I 1213 (GI 13) have been confirmed as resistant (AVRDC 1987a, b).

Mungbean

The methodology for rapid production of Cercospora leafspot inoculum and the inoculation technique were developed and used in resistance screening at the early stages of the AVRDC mungbean program (Mew et al. 1975).

Accessions, segregating populations and advanced breeding lines are evaluated normally under natural and/or artificial epiphytotics of Cercospora leafspot (CLS) and powdery mildew (PW) during these seasons: spring (March-May), summer (July-September), and fall (September- November). Resistances to CLS and PM were identified from thousands of mungbean accessions screened at AVRDC. Examples of accessions with high levels of CLS resistance are V 2773, V 4718, and V 5000, all from India, and V 1160, an amphidiploid from the Philippines. PM-resistant stocks include V 4717 and all of the above CLS-resistant accessions except V 5000.

Several viruses causing mottling, mosaic and leaf crinkle symptoms on mungbean have been isolated at AVRDC and at Pingtung, Taiwan (Anon 1984). One of the viruses, with a host range confined largely to the legume family, is seed-transmitted and is serologically related to cucumber mosaic virus. A new, aphid-transmitted poty virus has also been recently isolated in Taiwan. The virus is serologically related to blackeye cowpea mosaic virus, adzuki bean mosaic virus and the NY-15 strain of bean common mosaic virus (Anon. 1984). Resistance to the seed-transmitted, unknown virus has been found among accessions V 3686, V 2010, and V 298, and among elite breeding lines and parental stocks (AVRDC 1987b). Resistance to the new poty virus was not detected among elite breeding lines but immunity is present in accessions V 1682, V 1153, and V 1745 (AVRDC 1987b).

Soyabean

Much of the known disease-resistant soybean germplasm (Palmer and Kilen 1987) has been re-evaluated at AVRDC. Additional sources of resistance to other diseases have also been identified from the AVRDC collection.

Four independent, major dominant genes conferring resistance to four different races of soyabean rust have been identified (Hartwig 1986). The resistant stocks and the specific genes they carry are PI 200492 (*Rps1 Rps1*), PI 230970 (*Rps2 Rps2*), PI 462312 (*Rps3 Rps3*) and PI 459025 (*Rps4 Rps4*). More than 10 000 accessions have been evaluated for rust resistance at AVRDC since 1973. However, all of the reported genes for resistance were found compatible with the virulent rust races present on Taiwan. It appears that the pathogen can readily evolve new races to overcome the specific resistance genes. Tolerance, defined as 'the relative yielding ability of a genotype under severe disease epidemic', was therefore explored as an alternative avenue to combat soyabean rust.

Two screening methods have been used to evaluate rust tolerance. The first method, applied specifically to variable populations, involves the growing of test materials during a severe rust epidemic and concurrently selecting for yield and yield loss potentials. Soyabean rust reduces the seed size but not the pod size. Therefore, yield loss potential can be evaluated by comparing seed size to seed cavity size. A standard tolerant cultivar is used for comparison (Shanmugasundaram and Tschanz 1985, 1986). The second method, applied to stable lines

and accessions, uses a split-plot design where the *with* and *without* fungicide treatments serve as main plots and lines as sub-plots. Both high yielding and tolerant check cultivars are included for comparison. Selection for tolerance is based on both low yield loss and high yield. Using these techniques, tolerant materials have been identified in the AVRDC germplasm collection and breeding lines (Tschanz and Tsai 1983).

Based on reaction to downy mildew under natural field infection, Shih Shih was identified as an excellent source of downy mildew resistance.

The procedure used in screening for pustule (BP) resistance has been described earlier (Shanmugasundaram and Tschanz 1985, 1986). Eighteen accessions were noted to be highly resistant to BP at AVRDC. The resistance levels in these accessions were better than those observed in CNS (AVRDC 1987a). A few examples of these BP-resistant accessions and their original identities are as follows: G 2025 (Hutton), G 2035 (Bragg), G 2296 (Bossier), G 4493 (PI 171445), G 5087 (Cobb), G 5090 (Tracy), and G 2424 (SRF 400).

Accessions G 38, G 2042, and G 4919 were found to be immune to several soyabean mosaic virus strains.

Utilization of AVRDC's disease-resistant vegetable germplasm

Breeding for disease resistance among AVRDC's principal vegetables

The identification of disease-resistant germplasm is only a necessary first step in crop improvement. In many cases, the disease-resistant stocks lack the desired array of characters that commercial cultivars require and must, therefore, be genetically manipulated so as to bring their useful genes into commercially acceptable genetic backgrounds. This is the major task of crop improvement scientists.

AVRDC scientists work as a multidisciplinary team to achieve the desired goals of the crop improvement program (Opeña et al. 1986b). For disease resistance breeding, the combined efforts of plant breeders and plant pathologists are very crucial prerequisites.

Among self-fertilized species such as tomato, mungbean, and soyabean, hybridization schemes such as biparental crosses, three-way and double crosses, or other complex parental matings have been used to combine disease resistance with other desirable characters.

Segregating populations are then handled by the pedigree, bulk or modified bulk methods, with selection for disease resistance, as appropriate, either through artificial inoculation, natural field infection, or both. Other special methods like single-seed descent (SSD) have also been employed for some crops, either independently or in combination with other methods such as the backcross method (Villareal 1979; Opeña et al. 1986a,b). In recent years, the backcross method has been used more frequently to pyramid the resistance genes for different disease (Opeña et al. 1986a,b; Opeña and Tschanz 1987). This method is also a well-known standard for 'gene domestication' from the wild species. A suitable example of its use in the AVRDC program is the transfer of PVY resistance from *L. hirsutum* to cultivated tomato (AVRDC 1987c).

The breeding strategy for cross-pollinated species like Chinese cabbage has focused on the development of homozygous inbred lines carrying desirable traits such as tolerance and disease resistance. These stable lines are then utilized to develop F_1 hybrids and open-pollinated populations (Opeña and Lo 1980). The segregating populations from intervarietal crosses are invariably handled by the pedigree method, with selection for important characters such as heat tolerance, disease resistance and self-incompatibility in the segregating stages.

The disease resistance breeding among AVRDC's principal crops is further discussed below.

Tomato

Heat tolerance and BW resistance received the highest priority in the first decade of the tomato program (Villareal 1979; Opeña 1985; Opeña et al. 1986b). The first series of tropical tomato lines such as CL 8d, CL 11d, CL 32d, CL 143, and others carry these major traits. Breeding ToMV resistance received impetus afterwards, resulting in the development of new tropical lines which simultaneously carry BW and ToMV resistance. Good examples of such lines are those in the CL 1131 series, CL 5915 series, and others. In the last three years, AVRDC tomato scientists have emphasized the incorporation of rootknot nematode resistance into the tropical tomato. Some of the new lines with multiple resistance to three diseases (BW, ToMV and rootknot nematode) are now evolving from the tomato program (AVRDC 1987c). Good examples of these advanced lines are those belonging to the CLN 229, CLN 236, and CLN 337 series.

Chinese cabbage

Breeding for softrot resistance has so far not been highly effective; therefore, an acceptable alternative was achieved by developing early-maturing cultivars which are able to escape the disease. Hybrids 58, 62, 82–156, 82–157, and others are early maturing and heat tolerant, enabling them to perform well during the hot, humid season. Furthermore, some of these hybrids, eg., 58, 59 and 62, as well as open-pollinated populations like 77 M(3)–27 and 77 M(3)–35 carry varying levels of resistance to downy mildew (AVRDC 1985) and turnip mosaic virus.

Sweet potato

Breeding for scab resistance has been initiated recently. The screening of the population derived from polycrosses, using accessions GI 6 and GI 13, and cultivar V2–30 as scab-resistant parents, is now underway.

Mungbean

Resistances to CLS and PM from selected germplasm have been successfully incorporated into advanced breeding lines with good yield potential. Examples of breeding lines with good resistance to CLS and PM are as follows: CLS resistance — VC 1137A, VC 1560D, VC 2720A, VC 3689A, VC 3543A, and VC 3748A; PM resistance — VC 1560A, VC 1560C, VC 1482A, VC 3528A, and VC 3543A.

Soyabean

Promising rust-tolerant lines have been derived from crosses using the following parents: Shih-shih, PI 230971, PI 248407, Shinshei, Sato–3, Akiyoshi, and PI 64728. Some of the best lines, when evaluated across at least five environments with fungicide protection and five without, showed yield increases of 5 to 10% over the best check cultivar, Asian *Glycine* selection (AGS) 129, and a higher level of yield stability (AVRDC 1987c, data unpublished). Thirteen breeding lines, resulting from crosses with earlier SRF 400 or PI 297550, carry high levels of bacterial pustule resistance. Also, a majority of the AVRDC soyabean breeding lines possess downy mildew resistance.

Several high-yielding and early-maturing selections from various crosses were found to be immune to either SMV or a combination of SMV, peanut mottle virus and a new poty virus. The AGS 9, 19, 103, 112, 115, 129, 147, 174, 209, 214, 216, 218, 231, and 239 are immune to SMV isolates G1 and G4, and the new poty virus isolate 220.

USE OF ELITE DISEASE-RESISTANT STOCKS BY NATIONAL PROGRAMS

The major concerns of the national vegetable programs in the tropics are adaptability and disease resistance. AVRDC's research and development activities dovetail well with these constraints. Thousands of improved breeding lines and elite accessions are distributed yearly by AVRDC to national programs and private concerns worldwide for further tests and utilization. Over the past decade, a number of AVRDC genetic materials have been used by national programs and private firms in further breeding. More importantly, a number of these improved germplasms have found their way to the farmers' fields through official releases by several national programs. To date, 118 AVRDC breeding lines and/or genotypes derived therefrom, have been officially released as commercial cultivars in 35 countries (Table 4).

TABLE 4 Official cultivar releases by national programs utilizing germplasm from the AVRDC crop improvement program (September 1987).

Year	Mungbean	Soyabean	Tomato	Chinese cabbage	Sweet potato	Total
1978	1	2	3	–	-	6
1979	–	–	6	–	–	6
1980	1	2	4	–	–	7
1981	2	1	6	2	–	11
1982	5	3	5	3	–	16
1983	3	2	21	3	1	30
1984	3	1	1	2	9	16
1985	4	3	2	–	1	10
1986	5	2	3	–	–	10
1987		2	2	2		6
Total	24	18	53	12	11	118
Countries[a]	15	9	22	6	3	35

[a] Countries/territories and number of releases (in parentheses if more than one cultivar released).

Mungbean Australia (2), China, Costa Rica, Ecuador, Fiji (3), India, Indonesia (4), Korea (2), Malaysia, Philippines (2), Sri Lanka, Taiwan, Tanzania, Thailand (2), Vietnam (2).

Soyabean Honduras, India, Indonesia (4), Malaysia, Taiwan (4), USA, Philippines, Korea (5), Thailand, Zimbabwe.

Tomato Barbados (3), Brazil, Congo, Cook Islands (4), Fiji (2), Gabon (2), Guam (2), Indonesia (3), Malaysia, Mauritius (5), Panama, Papua New Guinea, Philippines, Reunion (2), Senegal (2), Seychelles (11), Singapore (2), Sri Lanka (2), Taiwan (4), Tanzania (Zanzibar), Thailand, Trinidad/Tobago (2), USA (Virgin Islands), Zaire.

C. cabbage China (2), Indonesia (2), Japan (2), Korea (2), Philippines (3), Taiwan.

S. potato Bangladesh (3), Philippines (2), Tahiti (6).

Future prospects

The AVRDC crops improvement program on five principal vegetables has sucessfully combined adaptability to tropical conditions with resistance to one or more major diseases. The improved genetic materials are now serving the needs of the national programs either as commercially released cultivars or as elite parental stocks for further breeding. Emphasis in future AVRDC re. earch, with special reference to diseases, will be in the following areas: extended evaluation of germplasm for resistance to other important diseases; continuing development of advanced cultivars with multiple disease resistance; and, further enhancement of the stability of resistance, especially when its long-term value is dubious.

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PART 4

Potential of wild relatives

Chairman
J. L. Creech

CHAPTER 19

Potential of wild relatives of *Citrus*

I. UENO AND T. AKIHAMA

The genera *Citrus, Fortunella, Poncirus* belong to subfamily Aurantioideae, family Rutaceae. Aurantioideae have 35 genera according to T. Tanaka. The many species of the genus *Citrus* originate from Assam. Some species reached South China, through north Burma, other species spread to Indonesia through Burma, Thailand and Malaysia. In addition, several species (citron, lemon and sour orange) were introduced into Europe via Persia.

Surrounding these, there are some regions where various citrus species, both wild and cultivated, are growing, representing a secondary origin. These areas are considered to be an important pool of citrus gene resources.

'Exploration, collection, characterization and documentation of *Citrus* genetic reources in East Asia' has been a joint Japan/IBPGR project in the period 1983–86. Its purposes have been to include within it the collection of germplasm possessing variability in characteristics such as disease resistance, high-yield, fragrance, root-stock capability, etc. It also included the collection of materials from as wide a range of aurantioid genera as possible. We have completed explorations in Thailand, Malaysia, Brunei, Sumatra and Java, and have collected about 300 kinds of citrus species and their relatives. These collections are to be used for the benefit of citrus breeders in the future as breeding materials.

Number of *Citrus* and related genera collected

About 300 citrus samples were obtained and investigated during the exploration. As shown in Tables 1 and 2, about 10 citrus species and hybrids were collected as scions and seeds. Among the collected materials, there are 18 citrus related genera. These are *Aegle, Atalantia, Clausena, Feronia, Hesperethusa, Murraya*, etc. The plants are being used as rootstocks or drug. The citrus germplasms collected in Thailand were most numerous, because the country has many pummelo types. Consequently, primitive types of this species were found. In Indonesia, Java is most advanced in its citrus cultivation. However some expansion of orchards is now taking place on Sumatra, Madura and Bali. Concentrated cultivation is mostly done with *C. reticulata* and some with *C. grandis*. Primitive types of mandarin were found in the present exploration. Many citrus related genera have grown in Kalimantan Island, East Malaysia, but citrus and wild citrus are scarce.

Potential of germplasm collected

Variation in *C. grandis* continued to be of special interest to us. It has been suggested that pummelo has its greatest diversity centred in southern Thailand. It is more diverse in this region when compared to the variation in specimens examined from Malaysia. Hence southern Thailand near the Malaysian border may be considered as the centre of origin. Pummelo types from this area have varied traits; red or white rind, red, pink and white pulp, elliptical, oblate and pyriform fruit shape, and some with peel hair. Pummelos collected at Srmpang Tiga in Sumatra must have a parthenocarpy gene since seedless fruits are found among a few fruits on the tree. We could not collect *C. ichangensis* hybrids of great interest, but we collected hybrids of something else with *ichangensis*.

A *C. reticulata* hybrid collected at Bali Berguret in Sumatra has polyembryonic large

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TABLE 1 Citrus germplasm collection in Thailand, Malaysia, Brunei and Indonesia.

Countries	1983			1984			1986		
	Scions	Seeds	Total[a]	Scions	Seeds	Total[a]	Scions	Seeds	Total[a]
Thailand	86	130	152	16	20	27	6	9	13
Malaysia	–	–	–	37	15	48	–	–	–
Brunei	–	–	–	–	1	1	–	–	–
Indonesia	–	-	–	–	–	–	17	39	50
Total	86	130	152	53	36	76	23	48	63

[a] The total number of scions plus seeds is different. This is because the samples contained scion, seed, or both from any tree.

TABLE 2 Citrus germplasm collection by genera and species.

Genera or species	1983			1984			1986		
	Scions	Seeds	Total[a]	Scions	Seeds	Total[a]	Scions	Seeds	Total[a]
Papeda spp.	3	3	3	1	–	1	–	–	–
Citrus aurantifolia	6	7	7	2	–	2	–	3	3
C. medica	1	1	1	3	–	3	–	2	2
C. limon	2	1	2	–	–	–	–	–	–
C. grandis	28	73	83	9	9	17	7	13	18
C. sinensis	5	7	7	–	1	1	1	2	2
C. reticulata	15	19	20	2	2	4	3	11	14
C. halimi	–	–	–	2	1	2	–	–	–
C. spp.	–	–	–	–	–	–	2	2	3
C. spp./Hybrids	18	14	20	14	13	21	10	11	17
Fortunella spp	–	–	–	1	2	2	–	–	–
Citrus related genera	8	5	10	19	8	23	–	4	4
Total	86	130	152	53	36	76	23	48	63

[a] The total number of scions plus seeds is different. This is because the samples contained scion, seed, or both from any tree.

seeds, and is of a most primitive type. A *C. reticulata* collected at Larargan in Madura is a mandarin colorate even under tropical conditions. This gene is important for citrus production in tropical areas. The interesting mandarin type has been produced in an orchard near Chiang Mai in northern Thailand. There is no calyx on the fruits of this type, and harvesting is by simple pulling-off. This gene has good character and we can use it for satsuma mandarin, dancy tangerine and sweet orange, etc.

Attempts were made to collect samples of particular *C. halimi*, a wild species reported from southern Thailand and the Malay Peninsula. This represents an important addition to the citrus germplasm resource collection in view of the limited number of reports of this species in the wild and its virtual non-existence in cultivation. It is not certain whether this species is desirable or not for citrus breeding, so we must examine its resistance to disease and its insect and cold tolerance.

The large number of citrus relatives collected is valuable, for among the materials available

now are undoubtedly plants possessing good traits such as humidity tolerance, disease and insect resistance, which are of great potential value in crop breeding programs.

Just how many of these related genera and species will prove to have value in the future is not easy to determine now, but the possibility is there.

Feronia limonia, from citrus related genera, has exceptional water tolerance, and has been used as rootstock of citrus in lowland areas of Thailand. *Murraya paniculata* is a lovely ornamental or hedge plant, but it has been recently suspected of being an intermediate host of mycoplasma (MLO) infection and inducing greening disease of citrus. Therefore this plant will be used as test material for citrus greening disease.

Viral diseases

Citrus in Southeast Asia are largely damaged by viruses, especially with citrus stubbon and greening disease, Citrus psorosis and other viruses are severe. This means that not only may cultivation of citrus become difficult, but also valuable gene resources will be lost. In the areas where farmers have planted commercial crops, trees seemingly valuable as gene resources of citrus were cut down a number of years ago. Therefore, genetic erosion is rapidly advancing.

CHAPTER 20

Potential of wild relatives of solanaceous vegetables with reference to breeding resistant eggplant

TOMOAKI NARIKAWA

Breeding tomato for disease resistance is typical of using some kinds of wild relatives, which are *Lycopersicon esculentum* v. *ceraciforme*, *L. pimpinellifolium*, *L. peruvianum*, and others. In the National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT) tomatoes have been bred for resistance to leaf mould (*Cladosporium fulvum*), Fusarium wilt (*Fusarium oxysporium* f. *lycopersici*, J_1 and J_2), tobacco mosaic virus (TMV), rootknot nematode (*Meloidogyne incognita*), and others.

Eggplant or Brinjal (*Solanum melongena*) originated in tropical Asia (Khan 1979) and has been used as a vegetable for a long time. However breeding for disease resistance has not been advanced, because there are fewer close relatives of eggplant compared with those of tomatoes. In Japan some wild relatives have been utilized as the resistant stocks, for example, *Solanum integrifolium* (a kind of scarlet eggplant) (Niakan 1980) is resistant to Fusarium wilt and comparatively tolerant to bacterial wilt (*Pseudomonas solanacearum*). In addition, *Solanum gilo*, *S. mammosum*, *S. toxicarium* and hybrids between *S. melongena* and wild relatives were tested for resistant stocks to some diseases (Yamakawa and Mochizuki 1978a,b).

Today bacterial wilt and Verticillium wilt (*Verticillium dahliae*) are spread severely in the field of solanaceous vegetables. It is said that such diseases are caused by continuous cropping of the same vegetable. These diseases are soil-borne and it is very difficult to control them by chemicals or cropping management and, accordingly, there have been many programs of breeding for resistance. In this chapter the NIVOT breeding programs are described with reference to genetic resources of eggplant, and some problems in breeding procedures are discussed.

As mentioned above, to control Fusarium wilt and bacterial wilt, *S. integrifolium* was found to be effective as a resistant rootstock in the 1950s. Grafting with *S. integrifolium* was widespread in the region cultivating eggplant (Yamakawa 1978; Mizu, personal communication). This stock has, however, a low tolerance to bacterial wilt and no resistance to Verticillium wilt, and more tolerant stocks have been expected for a long time.

Breeding of 'Okitsu 1,2'

Native varieties of eggplant were screened for resistance to bacterial wilt by Kageyama (1950) and it was clear that 'Taiwan-Naga' was the most resistant of 24 varieties (Table 1). It was said that 'Taiwan-Naga' was introduced from Taiwan and that it was a variety cultivated in southern China. Kotani et al. (1964) made hybrids between 'Taiwan-Naga' and 'Nakate-Shinkuro' for selecting resistant lines. Through the pedigree tests the genetic behaviour of resistance was suggested to be controlled by polygenes. 'Okitsu 1 and 2' have been as resistant lines to bacterial wilt (Table 2) and are suitable as the rootstocks (Suzuki et al. 1964; Kotani et al. 1964). These lines are susceptible to Verticillium wilt, Fusarium wilt and rootknot nematode.

Usefulness of *S. torvum* as a resistant stock

S. integrifolium and *S. gilo* have been familiar rootstocks for farmers, but they are not resistant

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TABLE 1 Resistance to bacterial wilt of eggplant varieties. (Kageyama 1950)

Variety	R D[a]	Variety	R D[a]
1. Kanazawa-hetamurasaki	91%	13. Fusanari-Chunaga	91%
2. Okitsu	73	14. Indo-Fusanari	45
3. Okitsu 6	64	15. Ootoshi	100
4. Okitsu 20	100	16. Harima	82
5. Nakate-Shinkuro	100	17. Ena 5	91
6. Shin-Yamashiro	100	18. Honnaga	55
7. Hookou	100	19. Kurume-Naga	73
8. Sakata Oka 11	91	20. Hakata-Naga	55
9. Oosaka-Chunaga	64	21. Taiwan-Naga	0
10. Kawano-Nakate	91	22. Daisen-Naga	91
11. Kitta	100	23. TsuruHoso-Sennari	73
12. Tashiro	100	24. Nashino-Naga	64

[a] R D(=Rate of damaged plants) = $\frac{\text{No. of plants damaged}}{\text{No. of plants tested}} \times 100\ (\%)$

TABLE 2 Resistance of Okitsu 1 and 2 to bacterial wilt. (Kotani et al. 1964)

Variety	End of August		Middle of Sept.	
	P_0[a]	DI[b]	P_0	DI
1. Okitsu 1	85	13	65	26
2. Okitsu 2	95	5	65	19
3. Taiwan-Naga	–	–	87	5
4. Nakate-Shinkuro	–	–	0	100

[a] $P_0 = \frac{\text{No.of healthy plants}}{\text{No.of plants tested}} \times 100(\%)$ [b] $DI = \frac{\Sigma\ (\text{Disease index} \times \text{No.of plants with the index})}{4 \times \text{No. of plants tested}} \times 100(\%)$

Disease index; 0: healthy ~ 4: damaged and died

to Verticillium wilt or to rootknot nematode and have low tolerance to bacterial wilt. In NIVOT the screening tests of rootstock varieties to some diseases were started by 1977 and a *S. torvum* line from Puerto Rico was highly resistant to Fusarium wilt and more tolerant to bacterial wilt, Verticillium wilt, and rootknot nematode than *S. integrifolium* (Table 3) (Yamakawa and Mochizuki 1978a,b). This line was registered as a new rootstock variety 'Torvum vigor' and widely distributed to eggplant growers.

Breeding of 'Asist', an F_1 cultivar cross of *S. integrifolium* and eggplant

It is reasonable that native cultivars of tropical countries have resistance to bacterial wilt. Because it is hot and highly humid and suitable for the bacteria, solanaceous vegetables resistant to bacterial wilt might be selected artificially and/or naturally. Nath (1975) in India reported that 'Dingaras Multiple Purple' (DMP) introduced from the Philippines, was highly resistant to bacterial wilt. In NIVOT Nath's variety was introduced in 1976. Yamakawa and

TABLE 3 Damage of *Solanum* spp. by bacterial wilt every ten days. (Yamakawa & Mochizuki 1978)

Species	Rate of damaged plants											
	V31	VI10	VI20	VI30	VII10	VII20	VII30	VIII9	VIII19	VIII29	IX8	IX18
S. melongena	6	13	25	31	38	63	88	94	94	100	100	100
S. sisymbrifolium	0	0	0	0	30	60	60	60	100	100	100	100
S. torvum	0	0	0	0	0	0	0	0	0	0	0	0
S. toxicarium	0	0	0	0	0	0	0	0	0	0	0	0
S. integrifolium	0	0	0	0	0	0	50	100	100	100	100	100

Mochizuki (1977) tested the resistance to bacterial wilt with some introduced eggplants and relatives and verified that DMP was the most resistant (Table 4). The fruit quality of DMP is not acceptable to the Japanese and it seemed that DMP was a good material for breeding resistant stocks. F_1 between *S. integrifolium* and DMP was resistant to bacterial wilt and Fusarium wilt and registered as 'Asist' in 1983. NIVOT now uses DMP for parental line to breed eggplants resistant to bacterial wilt.

TABLE 4 Screening of introduced eggplants and relatives for bacterial wilt. (Yamakawa & Mochizuki 1977)

Collection No.	Species or Variety	P_n				DI			
		1[a]	5	9	13	1	5	9	13
1. LS 207	*S. gilo*	78	56	34	0	13	32	52	83
2. 216	D M P	100	100	100	97	0	0	0	1
3. 217	*S. incanum*	59	41	22	0	23	50	71	95
4. 233	Big size	72	66	63	34	14	28	34	51
5. 234	Jumka-1	84	63	59	34	7	32	40	49
6. 235	Jumka-2	81	69	59	31	10	26	35	52
7. 236	*S.* spp	81	66	53	19	7	23	39	59
8. Check-1	Nakate-Shinkuro	25	9	3	0	54	88	92	98
9. Check-2	Taiwan-Naga	91	78	78	69	6	15	16	21

[a] Figures show the date in June.

TABLE 5 Relationship between hosts and pathogen. (According to Ozaki & Kimura 1986)

Group	No. of samples	*S. melongena* (cv. Senryo 2)	*S. mammosum*	*S. integrifolium*	*S. torvum* (cv.Torvum vigor)
I	19	S	R	R	R
II	1	S	S	R	R
III	3	S	S	S	R
IV	3	S	S	S	S
V	3	S	R	S	R

S:Susceptible and R:Resistant

Use of *S. incanum* to resist Fusarium wilt

S. incanum is the closest relative to *S. melongena* (Rao 1979) and can be easily crossed with *S. melongena*. It is said that *S. incanum* may be the wild type of *S. melongena* or that it may be a variant of eggplant. One line of *S. incanum* introduced from India was resistant to Fusarium wilt. From the backcrossed population of (*S. melongena*^{3} × *S. incanum*), several lines were selected as resistant to Fusarium wilt (Komochi and Nishio 1984). Further investigation is underway.

Pathogenic groups of *Pseudomonas solanacearum*

Ozaki and Kimura (1986) have studied groupings of bacterial wilt. They collected 29 plants damaged by bacterial wilt, 12 of which were eggplants, 12 tomatoes, 4 peppers, and 1 potato. Bacteria were isolated and then inoculated into the four species *S. melongena*, *S. mammosum*, *S. integrifolium* and *S. torvum*. According to the difference of pathogenicity, five pathogenic groups were classified as shown in Table 5. From the results, it seems resonable that *S. integrifolium* was used for resistant rootstock to bacterial wilt and that in some regions it was susceptible. Now *S. torvum* is the most resistant stock but it is possible that it will be damaged by new group such as group IV.

Joint research on biotechnological development of tropical microorganisms and plants

Joint research, sponsored by the Science and Technology Agency of Japan, was advanced for three years and in NIVOT the Joint Research on Collection and Utilization of Solanaceous Plants was started with the Malaysian Agricultural Research and Development Institute (MARDI) (Komochi 1985; Sakata 1986). Three collection trips were made to the areas shown in Figure 1. There were 17 collection locations. The list of collected species is shown in Table 6. The collected seeds were shared between MARDI and NIVOT.

TABLE 6 Number of lines of solanaceous plants collected in Malaysia.

Species	1984	1985	1986	Total
S. melongena	20	21	10	51
Capsicum spp.	44	15	14	73
L. esculentum	30	9	21	60
Solanum spp.	81	40	18	139
Others	1	2	0	3
Total	176	87	63	326

In 1986, Sakata et al. screened for resistance to Verticillium and bacterial wilts with *S. torvum* lines and some rootstock cultivars (Table 7). It was clear that there were varietal differences of resistance to *Verticillium dahliae* and *Pseudomonas solanacearum* groups among the tested lines. These lines will be useful for breeding new rootstock cultivars.

In 1986 screening tests of Verticillium wilt were done with *S. melongena*, *S. incanum* and *S. ferox* (Table 8). There were no resistant cultivars of *S. melongena* and *S. incanum*, but there were resistant lines of *S. ferox*. In this experiment *S. melongena* and *S. incanum* could not be differentiated, because the two species were very similar. Tests for resistance to bacterial wilt are being conducted at NIVOT.

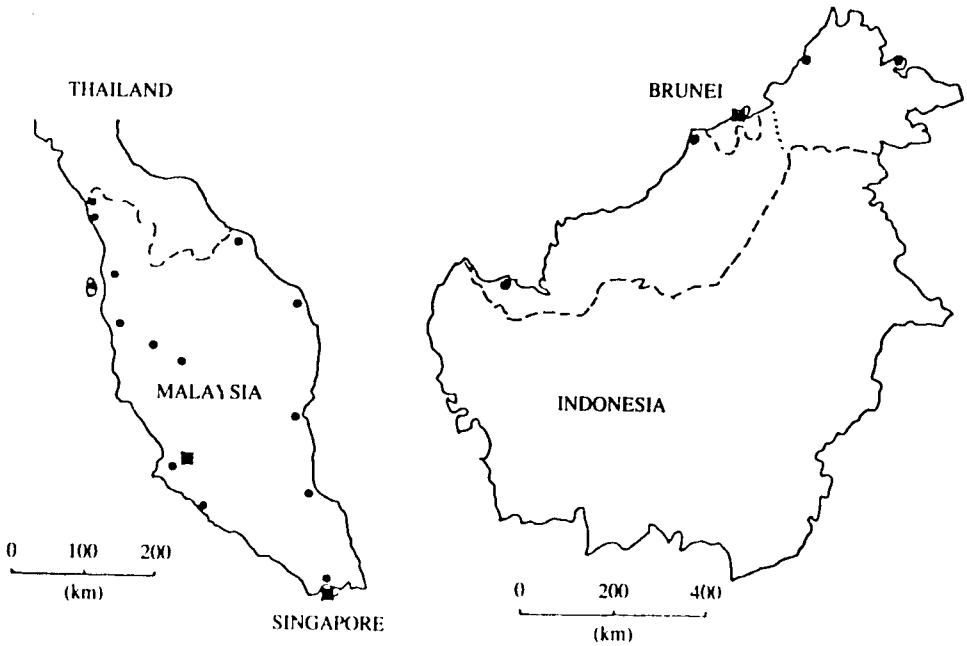

FIGURE 1 Points for collection in Malaysia. (Komochi 1985, and others)

Discussion

Eggplant is a very important solanaceous vegetable in tropical and temperate regions of Asia and other Continents. So too are tomato and sweet and hot peppers. The latter were introduced from the New World, but eggplant originated in tropical Asia. So it is likely that there are many resistant cultivars or relatives that have been naturally and/or artificially selected for resistance to bacterial wilt or Verticillium wilt under hot and humid conditions.

In tropical countries new cultivars are being imported from Japan, Taiwan and other countries, and this is causing the erosion of genetic resources. Hence the surveying, collection, evaluation and preservation of the native cultivars and the wild relatives are urgently needed. In Japan grafting is a good method for avoiding soil-borne diseases in cultivating eggplant. The method should be recommended to other countries. 'Torvum vigor' is a resistant rootstock, but as shown in Table 5 it is susceptible to pathogenic group IV. It is interesting that there are some differences of resistance to bacterial wilt between native *S. torvum* lines. Although resistance to bacterial wilt is controlled by polygenes, it is useful to breed a new line of *S. torvum* by crossing between lines with differing resistance. Moreover, resistance to Verticillium wilt is necessary for rootstock cultivars. In these breeding programs the genetic resources from tropical Asia are most useful.

In this paper the resistance of pepper and tomato has not been discussed. Their diseases, however, are as severe as those of eggplant. In the IBPGR movement many genetic resources are being gathered up now by many scientists, but further surveys and germplasm exchanges by researchers may be more important.

TABLE 7 Screening of *S. torvum* lines for two diseases. (Narikawa et al. 1986)

Collection No.	Verticillium wilt				Bacterial wilt	
	No.5[a]		No.41[a]		III[b]	IV[b]
	DI[c]	GI[d]	DI	GI	DI	DI
(*S. torvum*)						
1. LS 1843	0.84	0.94	0.25	1.21	–	–
2. 1878	0.45	0.79	0.35	1.17	–	–
3. 1880	0.70	0.70	0.10	0.73	0.14	0.75
4. 1881	0.58	0.71	0.25	1.01	0.25	0.86
5. 1898	0.44	0.75	0.55	1.14	0.25	1.50
6. 1915	1.16	0.51	0.15	0.52	0.45	0.75
7. 1921	0.65	1.28	0.25	1.41	0.27	0.82
8. 1922	1.00	0.45	0.25	0.73	0	1.00
9. 1928	0.90	0.47	0.25	0.97	–	–
10. 1947	1.26	1.14	0.30	1.51	0.24	0.28
11. 1948	1.15	0.68	0.35	0.69	0	0.46
12. 1955	0.17	0.72	0	0.60	–	–
13. 1957	0.37	0.77	0.30	1.03	0	1.64
14. 1964	0.50	0.49	0.15	0.62	0.29	0.54
15. 1196[e]	0.70	0.69	0.35	1.19	0.14	1.42
16. 1783[e]	0.80	0.72	0.30	0.85	0.27	0.83
17. MT-7[e]	0.85	1.00	0.25	1.10	0	1.25
(Checks)						
18. Torvum Vigor	0.90	0.70	0.30	0.66	0.27	0.88
19. Meet	1.25	0.73	0.89	0.49	0.08	0
20. Taibyo-VF	3.16	0.38	1.85	0.59	–	–
21. Asist	1.80	0.58	1.84	0.39	0	0
22. D M P	3.94	0.08	2.55	0.29	0.23	0
23. Nakate- Shinkuro	3.85	0.10	1.80	0.59	2.62	2.18

[a] Collection No. in NIVOT.
[b] Pathogenic group by Ozaki & Kimura (1986).
[c] Disease Index.
[d] Growth Index = $\frac{\text{Fresh weight of inoculated plant}}{\text{Fresh weight of non-inoculated plant}}$
[e] *S. torvum* introduced from other countries.

TABLE 8 Screening of *S. melongena*, *S. incanum* and *S. ferox* for Verticillium wilt. (Narikawa et al. 1986)

Test 1			Test 2			Test 3		
Coll.No.	No.5	No.41	Coll.No.	No.5	No.41	Coll.No.	No.5	No.41
LS 1859	3.8	3.9	LS 1884	2.9	3.5	LS 2206	4.0	3.5
1860	3.2	3.9	1896	3.5	2.7	2209	4.0	3.5
1883	3.5	3.9	1942	0.3	0	2219	3.3	3.3
1891	3.8	4.0	2014	2.6	1.8	2223	3.5	2.3
1902	3.7	3.5				2240	3.2	3.0
1903	3.3	3.3	1953[a]	1.0	1.7	2242	3.5	3.6
1907	3.3	2.2	1954[a]	1.6	0.9	2254	3.6	2.3
1910	2.3	2.4	1956[a]	2.3	0.4	2262	3.3	3.7
1911	3.4	3.9	1958[a]	2.6	0.4	2263	2.6	3.2
1913	3.5	3.8	1959[a]	2.3	0.4	2271	4.0	3.5
1931	3.4	2.9	1960[a]	2.5	0.5	2280	4.0	3.0
1934	3.7	3.3	1961[a]	2.3	2.1	2281	4.0	3.7
1949	3.3	3.3	1981[a]	2.5	2.8	2282	4.0	3.8
1951	3.3	3.1				2284	3.4	3.2
1998	4.0	4.0				2287	3.5	3.7
1999	3.4	4.0				2288	4.0	4.0

[a] *S. ferox*.
S. melongena and *S. incanum* could not be distinguished.

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CHAPTER 21

Potential use of Korean filbert (*Corylus* spp.) landraces

Inheritance of several characters in cross combinations between the Korean native filbert strains and introduced cultivars

HYEON-MO CHO

Most world-wide cultivars of the filbert have been bred by selection from the European species *C. avellana*, *C. maxima* and *C. americana* and the crosses among these species.

The most valuable of these cultivars originated mainly from *C. avellana*, which has a large kernel and high quality.

However, these cultivars are not cold-resistant because they have been domesticated and adapted in regions having mild winters and uniform rainfall throughout the year.

Domestic cultivation of *C. avellana*-originated cultivars in Korea since 1974 has led to cold damage in the trees during winter and consequent abnormal flowering and fruiting due to it.

Although filberts are now cultivated in 10 countries or more, research on the genetics and breeding has not been conspicuous.

The main cultivars are still confined to the selection of native cultivars or chance seedlings. Genetic studies are also limited because of self and cross-incompatibility in the filbert genus. According to Thompson (*Euphytica* 1977, 26, 465-474), the characters of nut weight, kernel ratio, pellicle fibre, shrinkage of kernel and number of nuts per cluster and so on, except for husk length, had high correlation between progeny mean and mid-parent value in genetic study of the cultivars originated from *C. avellana*.

Thompson also assumed that their genetic mechanism was due to the additional action of polygenes.

This study was carried out to clarify the inheritance mode of morphological characters of nut, disease resistance and cold hardiness in cross progenies, of which maternal strains were *C. heterophylla* F., a native of Korea, and paternal *C. avellana* introduced from abroad.

Materials and methods

EXPERIMENTAL MATERIALS

Experimental materials used in this study were obtained from crosses between Korean native filbert and European ones from 1976 to 1978 (See Table 1).

All these materials were planted and managed in the field for filbert breeding in the Horticultural Experiment Station in Suwon, Korea.

INVESTIGATION METHOD

Nut characters

a) *Nut weight* — This signifies mean weight of one hundred nuts sufficiently dried after harvest and was measured by 0.01 g unit.

b) *Nut length and diameter* — The meaning is the same as the case of nut weight. Maximum value of nut length and diameter was measured by 0.01 cm unit.

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TABLE 1 Number of progeny in 13 *Corylus* crosses.

No.	Cross	Number of progeny
1	HF-2 × Butler	31
2	HF-2 × Barcelona	4
3	HF-13 × Barcelona	39
4	HF-1 × Ennis	18
5	HF-2 × Bollwiller	31
6	HF-1 × Bollwiller	12
7	HF-1 × Royal	11
8	HF-322 × HF-605	18
9	HF-501 × HF-605	17
10	HF-237 × HF-803	13
11	HF-16 × HF-803	16
12	HF-1 × HF-803	16
13	HF-1 × HF-13	17
	Total	243

Kernel characters

a) *Kernel weight* — The same as for nut weight. This was measured by 0.01 g unit.
b) *Kernel ratio (%)* — (Kernel weight/nut weight) × 100. This was indicated by 0.01% unit.
c) *Fibre amount attached to pellicle* — This was classified into 4 grades:
1 : none
2 : a little (small amount)
3 : medium (moderate amount)
4 : abundant (very much).
d) *Shrinkage of kernel* — This was classifield into 4 grades:
1 : never shrunk
2 : slight (or a little) (small amount)
3 : medium (moderate amount)
4 : extremely shrunk (very much).

Husk length

This was classified into 4 grades:
1 : shorter than three-fourths of nut length
2 : either longer or shorter than nut length by a fourth
3 : longer than nut length by a fourth to three fourths
4 : longer than twice nut length.

Disease resistance

The degree of susceptibility was classified into 4 grades:
1 : The rate of infected leaves is 25% or less
2 : The rate of infected leaves is 26% to 50%
3 : The rate of infected leaves is 51% to 75%
4 : The rate of infected leaves is 76% or more.

Cold hardiness

The degree of chilling injury in experimental trees which encountered very low temperature of -24.8°C on 5 January 1981, was classified into 4 grades:

1 : no damage
2 : catkins were killed
3 : catkins and twigs were killed
4 : the whole tree was killed.

Precocity

This was classified into 5 grades:
1 : fruited in three-year old tree
2 : fruited in four-year old tree
3 : fruited in five-year old tree
4 : fruited in six-to seven-year old tree
5 : fruited in above eight-year old tree.

Results and discussion

Characteristics of parental strains or cultivars

The main characteristics of parental strains or cultivars are shown in Table 2.

The introduced cultivars of Barcelona, Bollwiller, Butler, Ennis and Royal were not only all fruit types with heavy nut and kernel and long nut length and diameter but also comparatively abundant in fibre amount attached to pellicle and excessive in kernel shrinkage. And they appeared to be resistant to anthracnose but not resistant to powdery mildew. They were also weak in cold hardiness and late in precocity.

On the other hand the native strains of HF-1, HF-2, HF-16, HF-237, HF-322, HF-501, HF-605 and HF-803 were mostly small fruit ones and with little fibre attached to pellicle and low kernel shrinkage. It appeared that they were resistant to powdery mildew but not to anthracnose. As to cold hardiness and precocity they were strong and early, respectively. Table 3 indicates the means and the standard deviations in the investigation values for all characters according to cross combinations.

Genetic variation of important characters in cross progeny

Nut characters

a) *Nut weight* — As shown in Figure 1 the distribution of nut weight of cross progenies, in 3 combinations such as HF-2 × Butler, HF-1 × Ennis and HF-1 × Bollwiller of 7 combinations between native strains of small nut and introduced cultivars of large one, the frequency of individuals above mid-parent value was high, but in the other 4 combinations the tendency was opposite. Therefore no regular tendency in the inheritance of nut weight appeared.
However, in 5 of 6 cross combinations among native strains the frequency of individuals below mid-parent value was high. Consequently the appearance of individual lighter values had a tendency to be more frequent.

b) *Nut length* — The distribution of nut length in cross progenies is described in Figure 2. In 4 of 7 combinations between native strains and introduced cultivars the number of individuals above mid-parent value in nut length was overwhelming while in the other 3 combinations the individuals below mid-parent value was prevalent. All the 6 combinations among native strains showed the prevalence of the individuals above mid-parent value. On the whole, the frequency of the individuals above the mid-parent value in nut length tended to be a little high.

c) *Nut diameter* — As shown in Figure 3, the distribution of nut diameter in cross progenies did not show a regular trend. In 3 of 7 combinations between native strains and introduced cultivars the individuals above mid-parent value appeared more frequently, while in the

TABLE 2 The characteristics of 14 *Corylus* or strains used as parents.

Cultivar or strain	Weight		Kernel		Kernel ratio	Nut		Number of nuts per cluster	Husk length	Resistance		Cold hardiness	Precocity
	Nut	Kernel	Fibre	Shrinkage		Length	Diameter			Anthracnose	Powdery mildew		
Barcelona	2.52±0.11	1.00±0.09	3	2	40±2.1	2.30±0.03	2.50±0.12	2	2	2	4	4	5
Bollwiller	2.69±0.14	1.07±0.13	4	2	40±2.6	2.20±0.04	2.10±0.14	2	3	2	4	5	5
Butler	2.52±0.16	1.12±0.15	3	2	45±2.5	2.10±0.03	2.20±0.22	2	2	2	4	4	5
Ennis	3.36±0.13	1.48±0.12	4	4	44±2.9	2.40±0.06	2.30±0.19	2	3	2	4	4	5
Royal	3.28±0.20	1.34±0.17	4	3	40±2.1	2.30±0.02	2.30±0.22	2	3	2	4	4	5
HF-1	1.70±0.15	0.63±0.09	2	1	37±1.7	1.20±0.02	0.08±0.11	2	4	5	2	1	1
HF-2	1.64±0.21	0.58±0.02	2	1	35±1.8	1.20±0.02	0.80±0.13	2	4	3	2	1	1
HF-13	1.58±0.23	0.54±0.05	2	1	34±1.9	1.20±0.03	0.80±0.15	2	4	2	2	1	1
HF-16	1.31±0.09	0.54±0.06	1	1	41±2.1	1.00±0.02	1.60±0.16	3	4	2	2	1	1
HF-237	1.66±0.10	0.75±0.06	3	1	45±2.3	1.00±0.03	1.10±0.14	3	4	2	2	1	1
HF-322	1.03±0.15	0.49±0.09	1	1	40±2.2	1.20±0.04	1.10±0.12	3	4	4	3	1	1
HF-501	1.00±0.13	0.48±0.06	2	1	47±2.0	1.20±0.03	1.40±0.13	2	4	2	3	1	1
HF-605	1.58±0.19	0.59±0.07	1	1	37±1.3	1.20±0.04	1.30±0.15	4	2	4	2	1	1
HF-803	1.74±0.20	0.69±0.05	2	1	40±1.2	1.80±0.05	1.90±0.17	3	3	3	2	1	1

TABLE 3 Overall characteristic analysis of 13 crosses of *Corylus* cultivars or strains.

Cross	Weight		Kernel		Kernel ratio	Nut		Nuts per Cluster	Husk length	Resistance		Cold hardiness	Precocity
	Nut	Kernel	Fibre	Shrinkage		Length	Diameter			Anthracnose	Powdery mildew		
HF-2 × Butler	2.47±0.62	1.02±0.26	2.41±0.70	2.73±3.14	41.28± 4.63	1.79±0.30	1.63±0.23	1.75±0.47	3.41±0.61	1.16±0.36	1.29±0.45	1.09±0.29	1.25±0.50
HF-2 × Barcelona	1.97±0.53	1.15±0.38	2.25±0.43	2.50±0.50	44.91± 2.83	1.47±0.28	1.24±0.35	2.36±0.35	3.25±0.43	1.00	1.50±0.50	1.00	1.25±0.43
HF-13 × Barcelona	1.74±0.59	0.71±0.28	3.10±1.03	2.71±0.98	40.40± 9.88	1.61±0.28	1.51±0.28	2.33±0.55	3.66±0.52	1.23±0.52	1.56±0.67	1.07±0.26	1.28±0.55
HF-1 × Ennis	2.55±1.14	0.88±0.31	2.72±0.93	2.83±0.83	36.76± 7.90	1.90±0.48	1.82±0.48	1.96±0.65	3.55±0.60	2.87±0.94	2.33±0.57	1.05±0.22	1.16±0.37
HF-2 × Bollwiller	1.48±0.54	0.61±0.37	2.38±0.82	2.32±0.69	37.39± 5.17	1.51±0.21	1.40±0.26	2.08±0.69	3.48±0.66	1.22±0.41	1.47±0.54	1.03±0.17	1.19±0.39
HF-1 × Bollwiller	2.60±0.46	1.05±0.43	3.66±0.62	2.58±0.75	39.39±10.85	1.90±0.19	1.47±0.21	1.97±0.48	3.33±0.62	3.50±0.50	1.50±0.50	1.08±0.27	1.25±0.43
HF-1 × Royal	2.28±0.73	1.03±0.33	3.27±1.21	2.36±0.64	44.32± 6.92	1.79±0.42	1.49±0.32	2.44±0.50	3.72±0.44	2.00±0.42	1.81±0.71	1.09±0.28	1.36±0.64
HF-322 × HF-605	1.03±0.25	0.42±0.10	3.11±0.73	2.22±0.41	42.00± 7.42	1.38±0.11	1.24±0.17	2.66±0.71	2.77±0.62	2.05±0.62	1.72±0.44	1.16±0.37	1.05±0.22
HF-501 × HF-605	1.46±0.35	0.72±0.17	3.52±0.77	2.29±0.74	49.93± 5.67	1.54±0.29	1.38±0.19	2.35±0.53	2.41±0.77	1.17±0.38	1.88±0.32	1.05±0.23	1.05±0.23
HF-237 × HF-803	1.30±0.21	0.62±0.11	2.00±0.67	1.61±0.48	47.81± 5.28	1.53±0.18	1.27±0.20	2.00±0.48	3.30±0.72	1.15±0.36	1.23±0.42	1.07±0.26	1.15±0.36
HF-16 × HF-803	1.16±0.26	0.47±0.13	2.62±0.78	2.00	41.44± 7.57	1.36±0.19	1.17±0.19	2.65±0.76	3.37±0.85	1.18±0.39	1.18±0.39	1.18±0.39	1.12±0.33
HF-1 × HF-803	1.35±0.42	0.54±0.19	2.93±0.82	2.18±0.52	40.51± 4.62	1.56±0.25	1.23±0.20	2.29±0.61	3.31±1.04	3.31±0.58	1.31±0.36	1.06±0.24	1.06±0.24
HF-1 × HF-13	0.95±0.19	0.41±0.12	3.23±0.80	2.70±0.74	43.06± 5.85	1.00±0.17	1.25±0.17	3.27±0.88	3.82±0.38	1.35±0.58	1.05±0.23	1.11±0.32	1.11±0.32

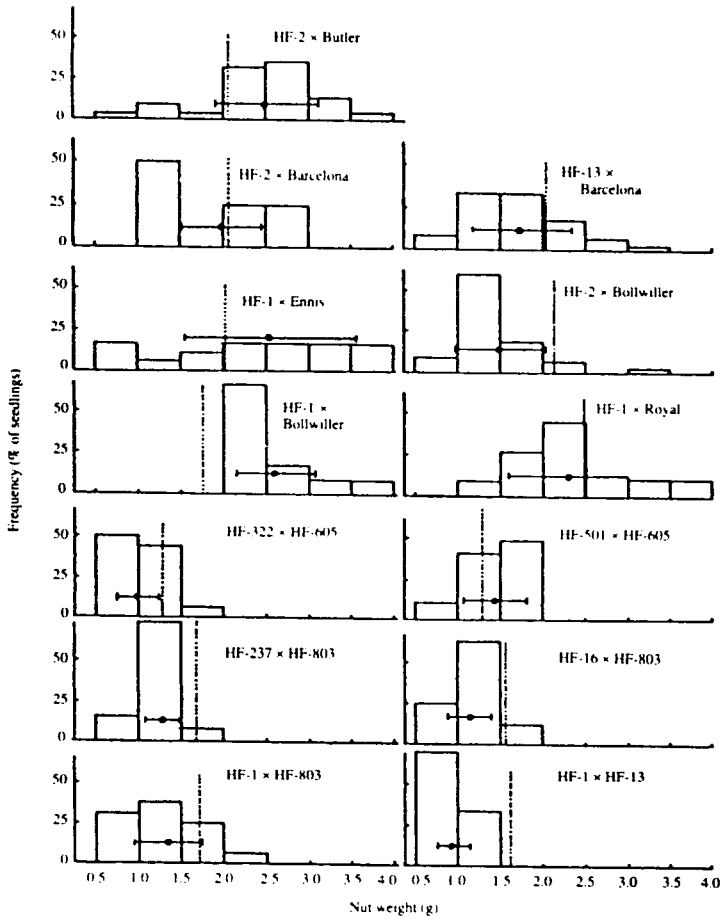

FIGURE 1 Distribution of nut weight in 13 filbert crosses.
–●– : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

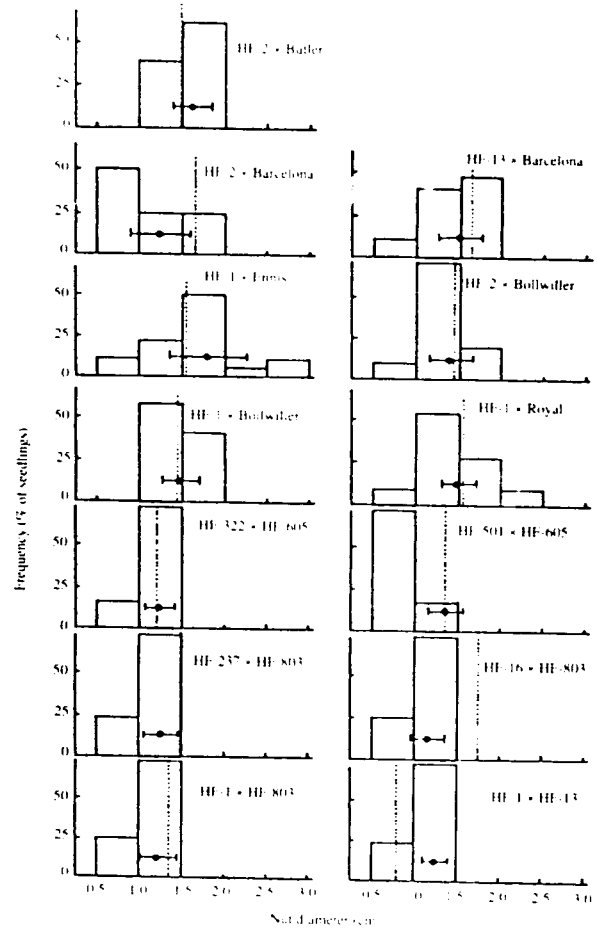

FIGURE 2 Distribution of nut length in 13 filbert crosses.
–●– : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

other 4 combinations the individuals below mid-parent value appeared more frequently. In 6 combinations among native strains there were 2 combinations of which progeny mean was slightly greater than mid-parent value and 1 combination of which progeny mean was much greater than mid-parent value and 3 combinations of which progeny mean was smaller than mid-parent value. On the whole it appeared that individuals below mid-parent value in nut diameter were more frequent.

Kernel characters

a) *Kernel weight* — As to the distribution of kernel weight in 7 combinations between native strains and introduced cultivars, in 3 combinations the frequency of individuals below mid-parent value was high. However, there was 1 combination in which the progeny mean was greater than mid-parent value and 5 combinations in which progeny mean was smaller than mid-parent value in 6 combinations among native strains. Therefore, in the case of kernel weight the trait of small kernel appeared to be dominant gene action to that of large kernel (Figure 4).

b) *Kernel ratio* — The distribution of kernel ratio in cross progenies is described in Figure 5. In 5 of 7 combinations between native strains and introduced cultivars, the frequency of individuals above mid-parent value in kernel ratio was high while in the other 2 combinations the frequency of individuals below mid-parent value was high.
And 5 of 6 combinations among native strains showed the prevalence of the individuals above mid-parent value. On the whole, it appeared that the individuals above mid-parent value in kernel ratio were more frequent.

c) *Amount of fibre attached to pellicle* — The distibution of fibre attached to pellicle in cross progenies is described in Figure 6. In 9 of 13 combinations containing 4 of 7 combinations between native strains with a little fibre and introduced cultivars with much fibre, and 5 of 7 combinations among native strains the frequency of individuals above mid-parent value in the amount of fibre attached to pellicle was high. Therefore, in this case, the dominant gene leads to much fibre.

d) *Shrinkage of kernel* — As shown in Figure 7, in all combinaitons the frequency of individuals above mid-parent value (more shrink) in shrinkage of kernel appeared more frequently. Therefore, in the case of shrinkage of kernel the trait of more shrinkage appeared to be the dominant gene action over that of little shrinkage.

Number of nuts per cluster

As shown in Figure 8, the distribution of number of nuts per cluster in cross progenies did not show a regular trend.

Husk length

As shown in Figure 9, the distribution of husk length in cross progenies did not show a regular trend. For example, in 5 of 7 combinations between native strains with longer husks and introduced cultivars with shorter ones the frequency of individuals with husk longer than mid-parent value was prevalent, but contrarily in all 6 combinations among native strains with longer husk the distribution of individuals with husk shorter than mid-parent value was overwhelming.

Disease resistance

As shown in Figures 10 and 11, the frequency of individuals more resistant to anthracnose and powdery mildew than mid-parent value was overwhelming in all 13 combinations. Therefore, in the case of these two diseases the trait of resistance appeared to be dominant gene action over that of susceptibility.

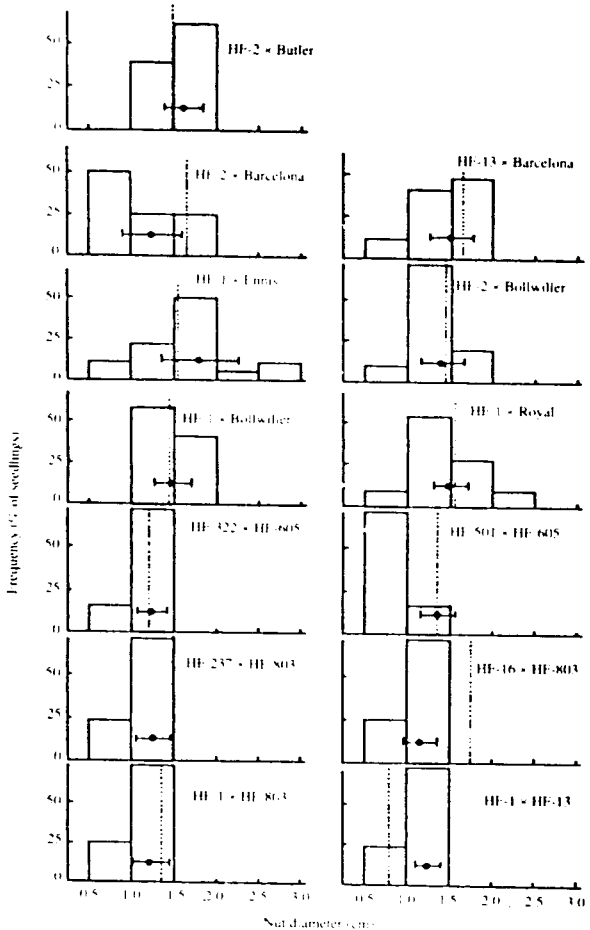

FIGURE 3 Distribution of nut diameter in 13 filbert crosses. –●– : progeny mean and standard deviation. Vertical dotted line indicates mid-parent value.

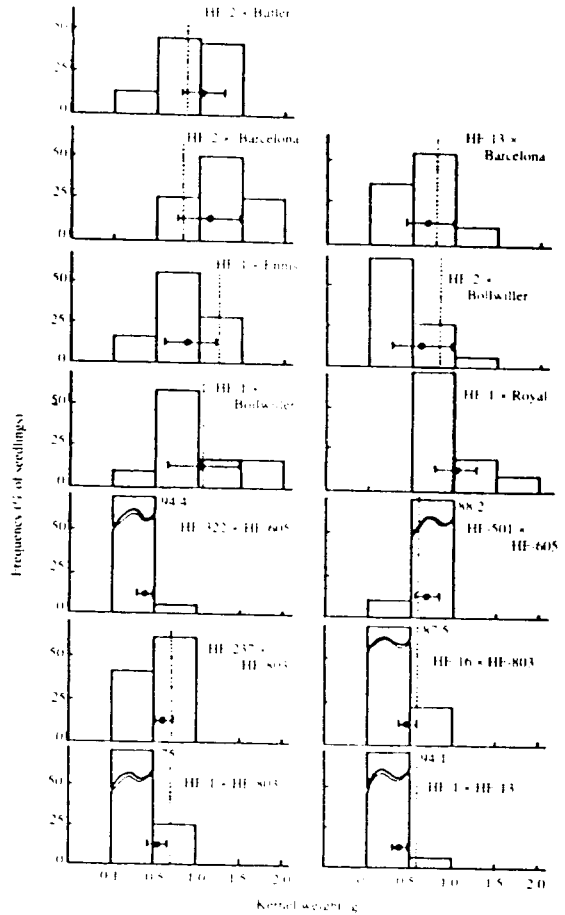

FIGURE 4 Distribution of kernel weight in 13 filbert crosses. –●– : progeny mean and standard deviation. Vertical dotted line indicates mid-parent value.

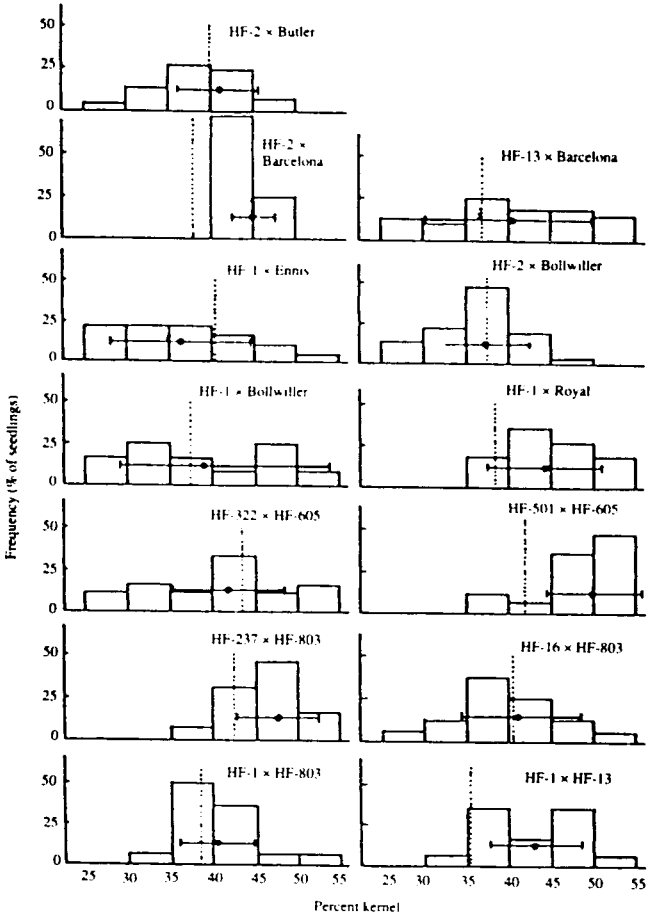

FIGURE 5 Distribution of kernel ratio (%) in 13 filbert crosses.
-●- : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

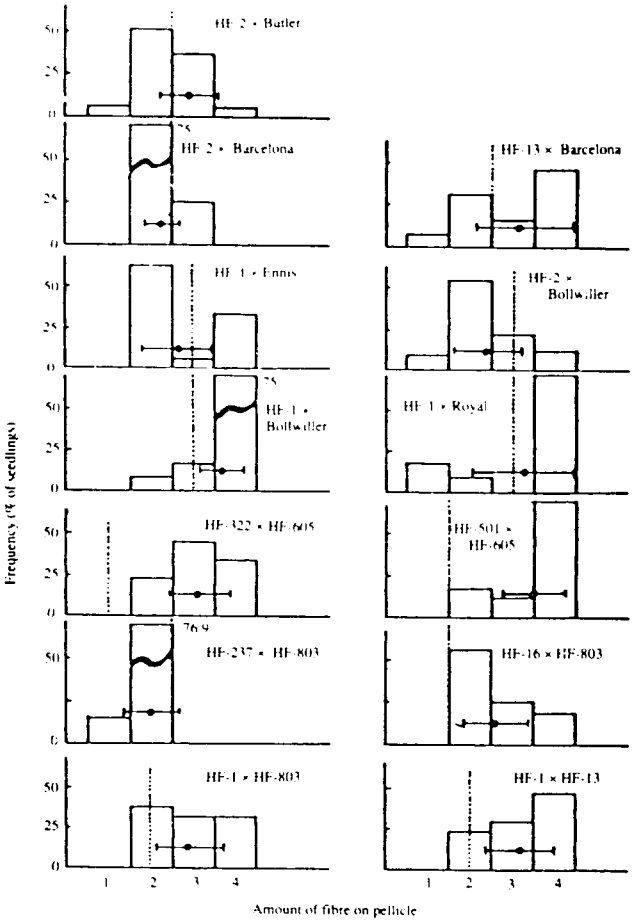

FIGURE 6 Distribution of amount of fibre on pellicle in 13 filbert crosses. Ratings for amount of fibre on pellicle: 1=none, 2=small amount, 3=moderate amount, 4=very much.
-●- : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

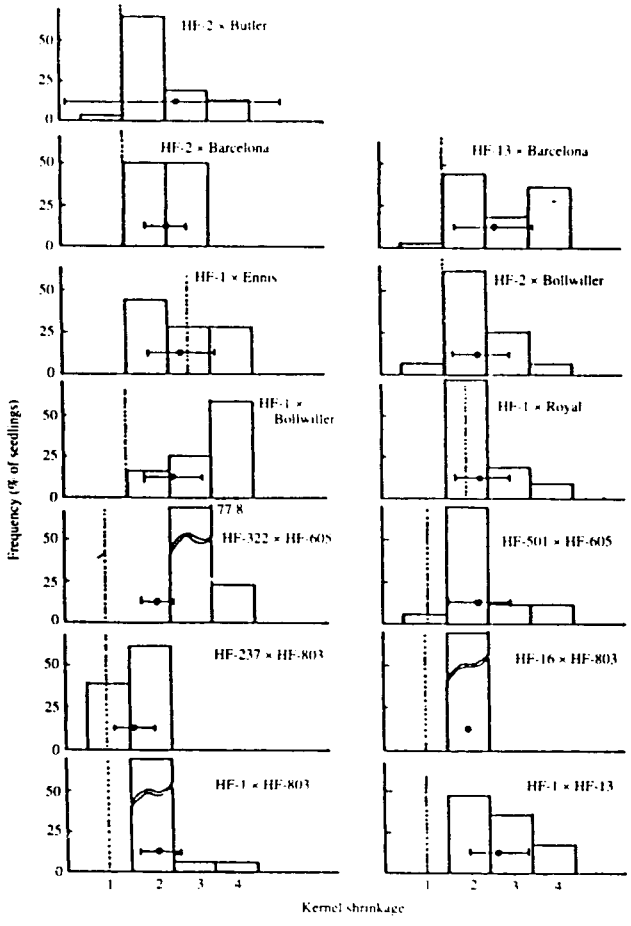

FIGURE 7 Distribution of kernel shrinkage in 13 filbert crosses. Ratings for kernel shrinkage: 1=no shrivel, 2=small amount, 3=moderate amount, 4=very much.
—●— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

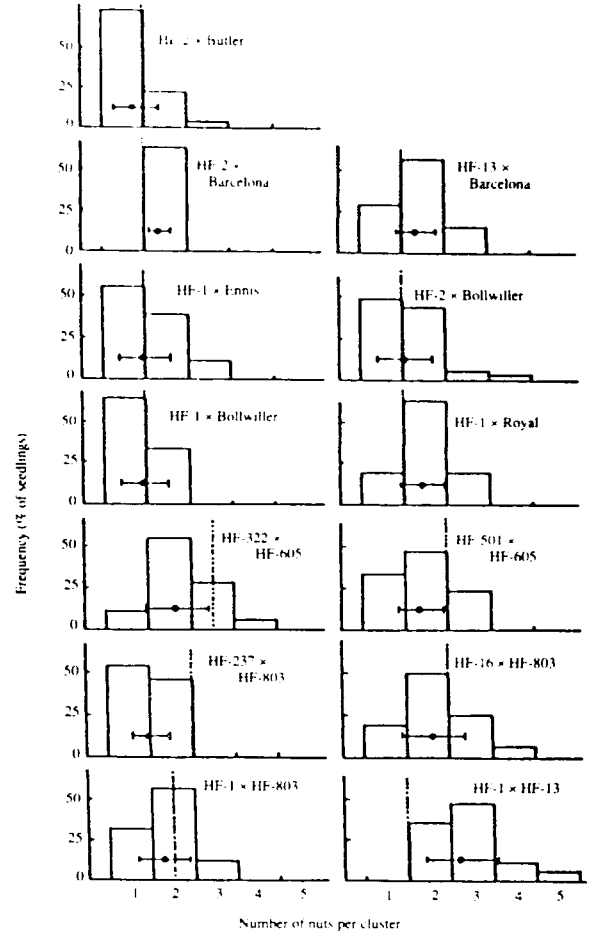

FIGURE 8 Distribution of number of nuts per cluster in 13 filbert crosses. Ratings for number of nuts per cluster: 1=mostly single, 2=mostly 1s, 3=mostly 2s, 4=mostly 3s and 4s, 5=mostly 4s and 5s, 6=many with 5, or more.
—●— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

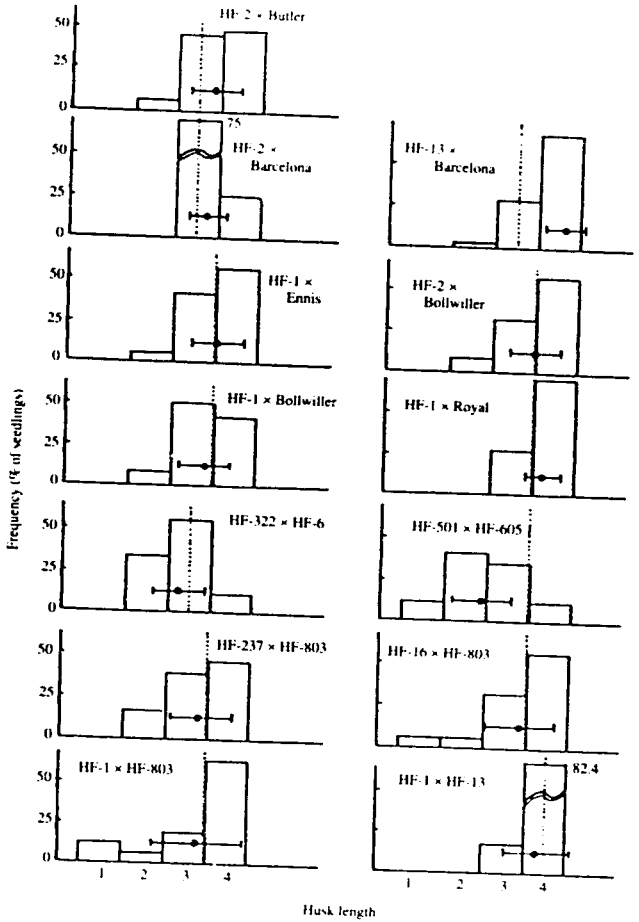

FIGURE 9 Distribution of husk length in 13 filbert crosses. Ratings for husk length: 1=less than ¾ nut length, 2=±¼ nut length, 3=¼ to ¾ longer than nut length, 4=twice length of nut, or larger.

—⊙— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

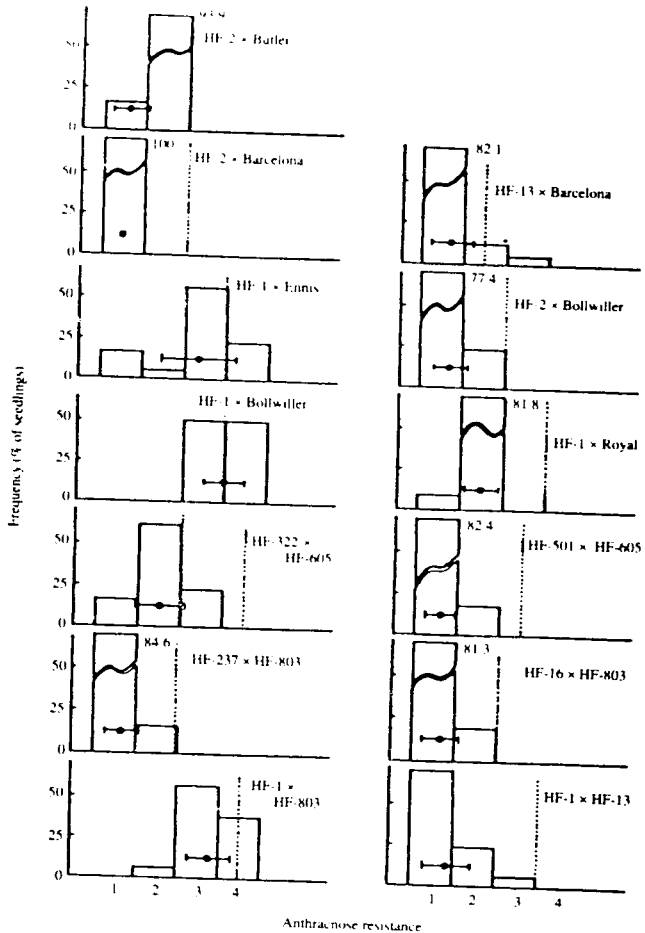

FIGURE 10 Distribution of anthracnose resistance in 13 filbert crosses. Ratings for anthracnose resistance: 1=less than 25% of leaves infected, 2=26–50% of leaves infected, 3=51–75% of leaves infected, 4=more than 76% of leave infected.

—⊙— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

Cold hardiness

The distribution of cold hardiness in cross progenies is described in Figure 12.

In the case of 7 combinations between native strains with cold hardiness and introduced cultivars with relatively lower cold hardiness, the frequency of individuals hardier than mid-parent value was from 81% to 100%. Therefore, in this case the trait of hardiness appeared to be the dominant gene action over that of susceptibility.

Precocity

The distribution of precocity in cross progenies is shown in Figure 13. In all 13 combinations the frequency of individuals with more precocity than mid-parent value was overwhelming. All the native strains have high precocity bearing nuts in 3–4 year old trees even in case of seedling. To the contrary, most of the introduced cultivars bear nuts very late in 8-year old seedlings.

In all combinations between native strains and introduced cultivars in this experiment most of the cross progenies bear nuts in 5-year old trees. In addition, there are about 73–94% of progenies bearing in 3-year old seedling. Therefore, in the case of precocity the trait of early bearing appeared to be dominant gene action over that of late bearing.

Summary

The main introduced cultivars have been derived from *C. avellana* because of advantages of large nut and good quality.

In Korea, however, normal fruition and reasonable yield in introduced cultivars cannot be expected because of damage to the floral organ by low temperatures during the winter season. On the other hand *C. heterophylla* F. and *C. heteropylla* F. var. *thunbergii* B., natives of Korea have some advantages of cold-resistance and precocity in that three-year-old seedlings can bear nuts, even though the nuts are very small.

Genetic relationships were investigated by comparing the distribution states of each characteristic measured in cross progeny with mid-parent value of corresponding characteristic in 7 combinations in which *C. heterophylla* F. and *C. heterophylla* F. var. *thunbergii* B. were used as female parent and *C. avellana* as pollen parent and in 6 combinations among strains. Judging from the above results the conclusions about nut weight and diameter of nut characters were not clarified, while in nut length, long nuts appeared to be more or less dominant over short nuts. As there were some cross combinations in which individuals above mid- parent value in nut size always appeared more frequently, the importance of parent selection was recognized. Light kernels appeared to be slightly dominant, while in kernel ratio, high ratios were more or less dominant. In respect of fibre attached to the pellicle and shrinkage of kernel, important factors affecting nut quality, abundant fibre amount and extremely shrunk kernel were dominant, respectively. In the genetic study with nut weight, kernel ratio, fibre amount and shrinkage of kernel in cross combinations between the cultivars derived from *C. maxima*, Thompson had reported that cross progenies in most combinations were concentrically distributed with mid-parent value in the centre but there were some differences in the aspect of distribution. The present study also showed a similar tendency to Thompson's result.

For anthracnose and powdery mildew, a serious disease in filbert, resistance appeared to be dominant over susceptibility. In cold resistance, the most important character, the strong was dominant over the weak.

It took 5 to 6 years for most introduced cultivars to bear nuts; in the case of seedlings it took more than 6 years from grafting to bear. However, *C. heterophylla* F. and *C. heterophylla* F. var. *thunbergii* B., native strains in Korea, have such precocity that even 3- to 5-year old

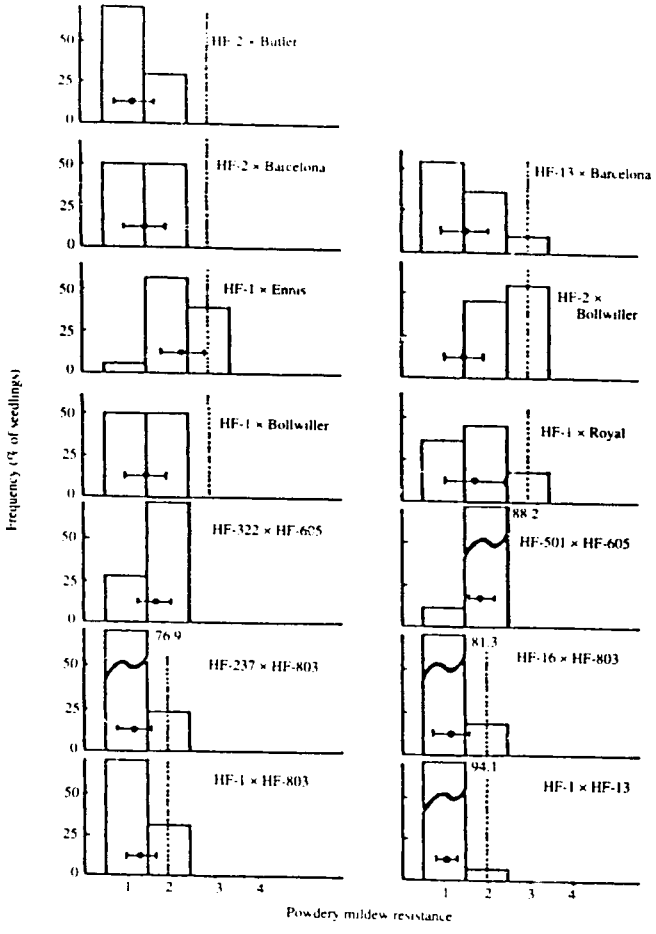

FIGURE 11 Distribution of powdery mildew resistance in 13 filbert crosses. Ratings for powdery mildew resistance: 1=less than 25% of leaves infected, 2=26–50% of leaves infected, 3=51–75% of leaves infected, 4=more than 76% of leaves infected.
—o— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

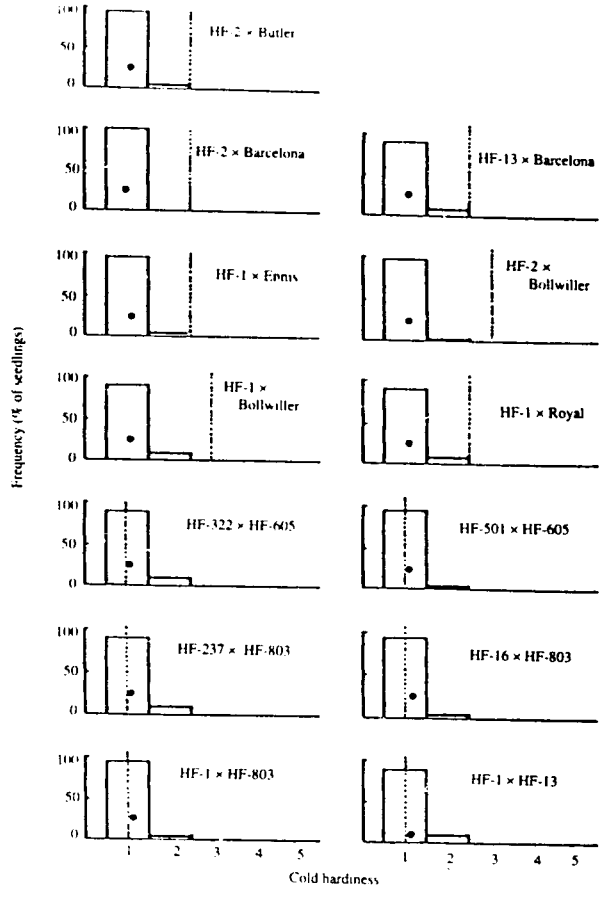

FIGURE 12 Distribution of cold-hardiness in 13 filbert crosses. Ratings for cold-hardiness (freeze damage ratings): 1=no damage, 2= catkins were killed, 3=catkins and twigs were killed , 4=catkins, twigs and branches were killed, 5=the whole tree was killed.
—o— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

seedlings can bear nuts. And when they were crossed with *C. avellana* of non-precocity, the precocity appeared to be dominant. On the basis of above results the author concludes that *C. heterophylla* F. and *heterophylla* F. var. *thunbergii* B. could be used as a source for cold resistance, precocity and powdery mildew resistance and *C. avellana* as a source for large nut and anthracnose resistance.

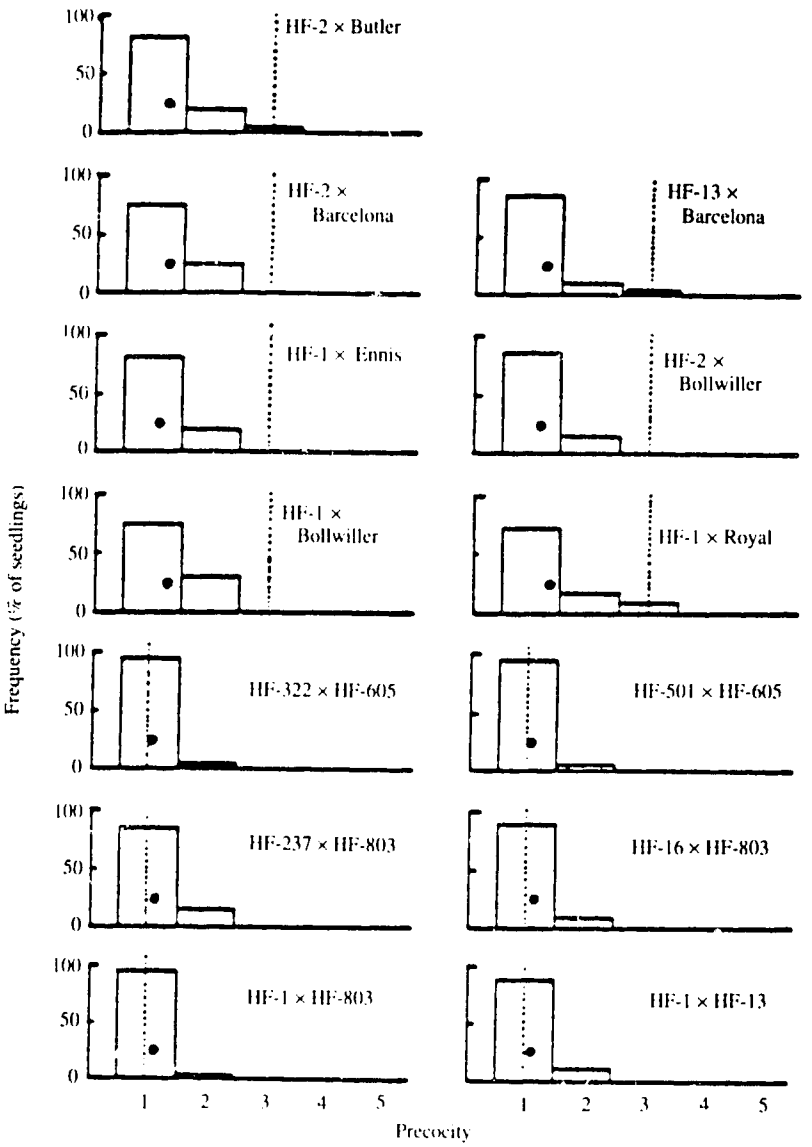

FIGURE 13 Distribution of precocity in 13 filbert crosses. Ratings for precocity: 1=fruited in three-year old tree, 2=fruited in four-year old tree, 3=fruited in five-year old tree, 4=fruited in six to seven-year old tree, 5=fruited in tree above eight years old.
—●— : progeny mean and standard deviation.
Vertical dotted line indicates mid-parent value.

CHAPTER 22

Crop improvement through utilization of wild relatives in China

SHAO QIQUAN

The collection, study and utilization of native varieties for every crop allows us to make use of wide genetic diversity for crop improvement and gain large benefits in breeding and agriculture generally. Breeders sometimes face great difficulties because of a lack of appropriate genes in the species themselves. It is then especially important thing to use wild species in the search for disease resistant, male sterility, stress resistant gene resources for crop improvement. In recent years, wild relatives of rice, wheat, barley and cotton have been used for crop improvement with great successes.

Importance of wild species for rice improvement

Success in the development of hybrid rice is a great breakthrough in rice breeding and a technological innovation in rice production. It has markedly increased rice yield on a large scale. It has been necessary to develop suitable cytoplasm-genetic male sterile, maintainer and restorer lines in order to make hybrid rice a commercial success. In the course of collecting wild rice sources for distant crosses, a male-sterile wild rice (*Oryza sativa* f. *spontanea* L.) was found by Yuan Longping's assistant Li Bihu in Hainan Island in 1970. It was named 'wild abortive', or more shortly 'WA' type. This type of wild rice was used as the female parent and cultivated rice was used as the male parent: from F_1 used cultivated rice for back crossing and from this combination were created more than 200 male sterile lines derived from 'WA' cytoplasm. Some of them such as Zhe-Shan 97A and V20A, widely used in production, have been backcrossed 30–35 times and still have high stability in male sterility under various environmental conditions.

Many excellent hybrids with early maturity, multiple resistance to diseases and insects and good grain quality have been developed (Yuan Longping 1986).

The hybrid rice plays a great role in increasing yield. For example, among the japonica hybrids, Li-you 57 gave highest (13.7 t/ha) in Liaoning Province. Japonica hybrid variety Xiu You 57 yielded an average 28% more than improved japonica variety Jing Yin 39 in Ninxia Province. The average yield from hybrid rice growing in China in 1983–1986 was 6.5 t/ha. In 1985, the hybrid rice-growing area was 26.4% of the total rice area. It contributed 32.7% of the total rice production. The cumulative production increase due to cultivation of hybrid rice in China in 1976–85, has been estimated as 94 million tons (Figure 1 and Table 1) (Yuan Longping 1987).

TABLE 1 Total growing area, yield and average yield of hybrid rice in China 1983–1986.

Year	Total area (10^6 ha)	Total yield (10^6 t)	Average yield (t/ha)
1983	6.74	42.97	6.4
1984	8.84	56.62	6.4
1985	8.27	53.53	6.5
1986	9.13	60.24	6.6

(From Yuan Longping et al. 1987)

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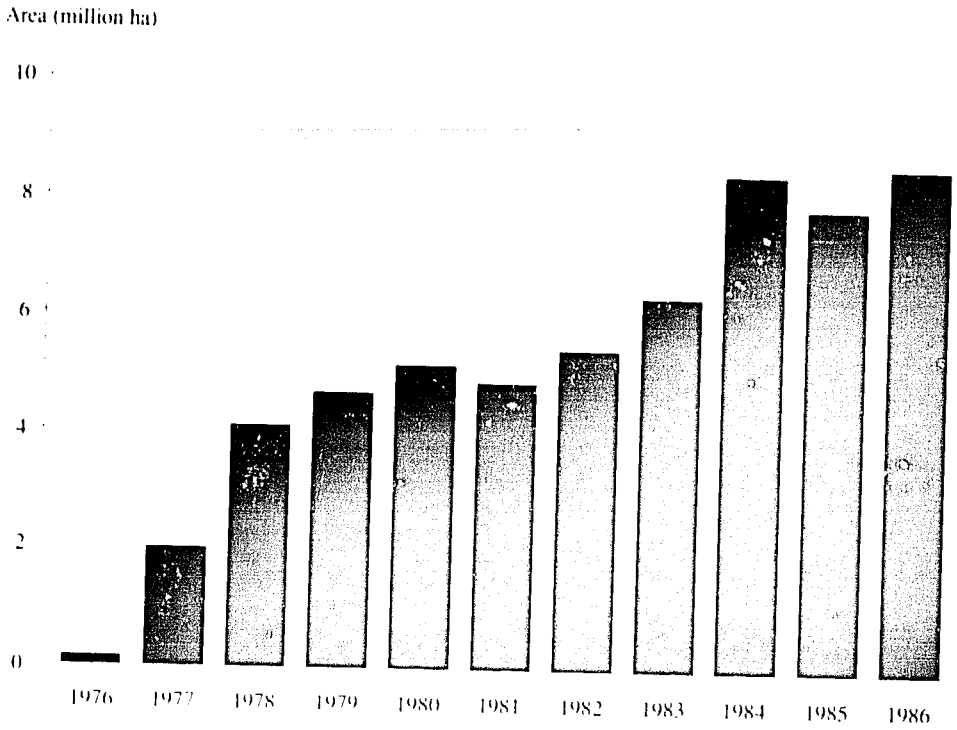

FIGURE 1 Area grown to hybrid rice in China, 1976–86. (From Yuan Longping et al. 1987)

Barley improvement using wild relatives

Interspecific and intervarietal crosses of wild and cultivated barley collected from Qingzang Plateau have been made in the experimental station of the Institute since 1979. Hybrid vigour was observed in F_1 hybrids of some combinations. Male-sterile plants were found in F_2 and F_3 of several crosses. Complete and partially male sterile (90% above) forms were obtained in 1982. Male sterile plants were derived from a cross between two-rowed wild barley (*Hordeum spontaneum*) and the six-rowed cultivated form (*H. vulgare* var. *nudum*); the male sterility was controlled by a recessive gene. There were two male-sterile types identified. One of them was two-rowed with naked grains and tough rachis, while the other had covered grains and tough rachis. Both types had small degenerated anthers with dried and shrivelled pollen grains. Pollen abortion occurred between tetrad and microspore stages, indicating that the two types were sporophytic sterile.

For example, field observation in 1983 showed that the 5 backcross combinations maintained sterility and 16 testcross combinations restored fertility. In order to obtain stable maintainer and restorer traits, both test and backcross are in progress.

Besides these an attempt is being made to solve two practical problems in hybrid barley production. One is to look for mutants of glume-opened pollination as a pollen source of male-sterile barley and the other is to incorporate the tough rachis character into some wild species having good combining ability and glume-opened pollination (Shao Qiquan et al. 1986).

Male sterility sources of wheat from wild relatives

Semi-wild wheat and *Aegilops squarosa* could serve as sources of male sterility for wheat

Semi-wild wheat was discovered during a scientific expedition to Qingzang Plateau in 1974 and a large number of seed and plant samples were collected and then analyzed cytologically and genetically. Semi-wild wheat usually grew as weed in barley and wheat fields. The matured spikelets freely separated and fell on the ground, being thus naturally sown. Identification of genetically stable lines of semi-wild wheat showed that the majority had the stable trait of brittle rachis. Semi-wild wheat could be grouped into various types of varieties and polymorphism in the species was apparently demonstrated. Because of great differences from other species of wild wheat it was separated by us into a new subspecies as *Triticum aestivum* subsp. *tibetanum* Shao and was used for crossing with common wheat (Table 2).

TABLE 2 Comparison of primitive wheat with semi-wild wheat.

Material	No. of chromosomes (2n)	Growing habit	Character of rachis	Disarticulation of rachis
T. spelta	42	spring habit	easy broken	above pedicel
ssp. *yunnanensis*	42	spring habit	easy broken	below pedicel
ssp. *tibetanum*	42	spring habit	natural broken	below pedicel

The F_1 hybrid between common wheat and semi-wild wheat was studied. In the spring of 1979, a cross was made between Zon-kan-ai, a variety of common wheat, with many recessive traits, and semi-wild wheat. Reciprocal crosses were made also.

The data verified that mature plants of F_1 hybrid had a rachis which can be broken easily, a red glume with hair and red grain. These traits come from the male parent, *tibetanum*. That means, the trait of broken rachis of semi-wild wheat is dominant, but the stable rachis of common wheat is recessive. The reciprocal cross gives the same results.

At the same time, the data show us that we have something like a primitive interspecific barrier. It is one of the reasons why semi-wild wheat from Tibet was separated as a new subspecies, *tibetanum*.

Common wheat and semi-wild wheat can usually be crossed. The F_1 hybrid for such a combination is usually fertile, but occasionally it is possible to obtain sterile lines from such hybrid combinations. Semi-wild wheat can serve as original material for breeding male sterile lines of common wheat (Table 3) (Shao Qiquan et al. 1983).

TABLE 3 Behaviour of F_1 hybrid between common wheat and semi-wild wheat.

Hybrid combination	Female traits	Male traits	Hybrid no. mature plants	No. immature F_1 plants	Traits of hybrids
Zon-kan-ai × semi-wild wheat	white glume white grain hairless glume rachis stable	red glume red grain hairless glume rachis broken	11	6	The spikes of all mature plants with rachis can be broken easily, red glume, red grain and glume with hair. Immature spikes have unbroken rachis.
semi-wild wheat × Zon-kan-ai	red glume with hair, red grain, rachis broken	white glume hairless glume white grain rachis stable	6	0	The spike of all mature plants with rachis can be easily broken, red grain, red glume with hair. Some of them are male sterile.

That *aegilops squarosa* cytoplasm can be used as a source for inducing male sterility in common wheat was shown by Zhang Yan and his group (1984). They have succeeded in producing hybrids between *Ae. squarosa* line No. 11, as female parent, and common wheat cultivar 'Kenya' as male parent. This cross combination, gave alloplasmic substitution line. Examination of root tips revealed that chromosomes in BC2 plants were 2n=42. Their morphological appearances were similar to those of the nuclear donor, suggesting that the nuclear genotype of the nucleo-cytoplasmic hybrid was homologous to those of the pure line Kenya. In most cases the substitution line had complete pollen and seed fertility. However, in BC4 self-pollinated progenies, male-sterile plants were obtained .

Ten of a total of 12 000 plants grown had complete or high male sterility. Anthers of all the completely sterile plants were smaller and more slender than normal ones. These materials have been used for breeding male-sterile lines of wheat.

Improvement of cotton through interspecific hybridization

Professor Liang Zhenglan and his group successfully obtained interspecific hybrids between cultivated cotton with several species of wild cotton. Many useful characters were introduced into cultivated cotton from wild relatives. Quite interesting results were obtained from a cross with wild cotton *Gossypium bickii*.

G. bickii is an Australian wild species which belongs to G1 genome. It has many desirable characteristics, such as resistance to insects (due to its intense hairiness), high gossypol stalk and gossypol-free seed, and is very useful for cotton breeding. However, due to difficulty of interspecific hybridization, transfer of these genes into cultivated cotton did not succeed for a long time. Only in recent years,using new techniques in embryo rescue and F_1 grafting and back cross, have hybrids been successfully developed by Liang et al. (1985).

The hybrids of *G. hirsutum* × *G. bickii* and *G. herbaceum* × *G. bickii* could be obtained by the method of spraying phytohormones and embryo culture. When the F_1 of the former is grafted onto the upland cotton and then backrossed with upland cotton under cool-night and short-day conditions, BC1 seeds can be harvested; BC1 and BC2 plants can be grown up later. Therefore, incompatibility of interspecific hybridization may be rejected to a certain degree by this technique. Most recently offspring with red flowers (F_2) and lines with gossypol glanded plants, but with glandless seeded plants, were established and a program for breeding a new cultivated cotton with insect-resistant characteristics is in progress. (personal communication from Professor Liang).

There are still great potentialities for crop improvement in using wild relatives for many crops. Many desirable positive traits from wild species could be transferred into crops (Table 4). The technique for transferring these genes has been developed also.

TABLE 4 Useful traits of wild species for crop improvement.

Name of wild species	Useful traits
Oryza sativa f. *spontanea* L.	Cytoplasmic male sterility, disease resistant, stress tolerant
Glycine soja	High seed protein compounds, disease resistant
Hordeum spontaneum L.	Cytoplasmic male sterility, mildew and rust resistant
Triticum aestivum ssp. *tibetanum*	Cytoplasmic male sterility, rust and mildew resistant
Aegilops squarosa L.	Cytoplasmic male sterility
Gossypium bickii	Gossypol glandless seeded

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PART 5

Collection and evaluation of indigenous germplasm

Chairman
K. Takayanagi

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CHAPTER 23

Collection and utilization of legume vegetable crops in China

WANG SU

A considerable part of China's land lies in the temperate and subtropical zone. Because the vast territory and varied topography give the country a wide viariation in climate, China is extremely rich in vegetable germplasm. There are many legumes used as vegetables. The important ones and their distribution are shown in Table 1.

TABLE 1 The distribution and use of legume vegetables in China.

Name	Scientific name	Distribution	Use[a]
Snapbean	*Phaseolus vulgaris* L.	All over the country	Canning,freezing
Yardlong bean	*Vigna unguiculata* ssp.*sesquipedalis* (L.) Verdc.	All, mainly in the south	Pickling
Vegetable soyabean	*Glycine max* Mern.	Yangtze river valley	Freezing
Garden pea	*Pisum sativum* L.	All, mainly in southwest	Canning,freezing
Hyacinth bean	*Dolichos lablab* L.	All, on small scale	Dry vegetable
Broad bean	*Vicia faba* L.	Yangtze river valley, mainly in the southwest	Dry seed processing
Lima bean	*Phaseolus lunatus* L.	On the south of Yangtze river	
Scarlet runner bean	*Phaseolus multiflorus* Willd.	Southwest and Shaanxi	Pickling
Sword bean	*Canavalia gladiata* DC.	South and southwest	
Winged bean	*Psophocarpus tetragonolobus* DC.	Southwest and south part of the centre	
Velvet bean	*Mucuna capiata* Wight.	Southwest	

[a] All can be used for the fresh market.

Of the vegetable legumes, snapbean, yardlong bean, vegetable soyabean and garden pea are extensively distributed, have more varieties and are relatively more important. In northern China, snapbean is the most important legume vegetable. Snapbean grown in Northeast China accounts for 90% of the total legume vegetables. The distribution of yardlong bean is different to that of snapbean. In Guangdong and Guangxi, the acreage of yardlong bean is 60–70% of total legume area. Vegetable soyabean is produced mainly in Jiangsu province, and Shanghai city, about 30–40% of the total legume production. The area for pea production is about 10–20% in Jiangsu Province and southwest China. Figure 1 shows the distribution of 4 important legume vegetables all over the country.

Collection of legume vegetable germplasm

In the late 1950s, an extensive collection of vegetable germplasm (including legume vegetables) was conducted. Since 1980, we have carried out another extensive collection. Up to the present, 3267 accessions of legume vegetables have been collected and preserved. The quantity and source of various legume vegetables are shown in Table 2.

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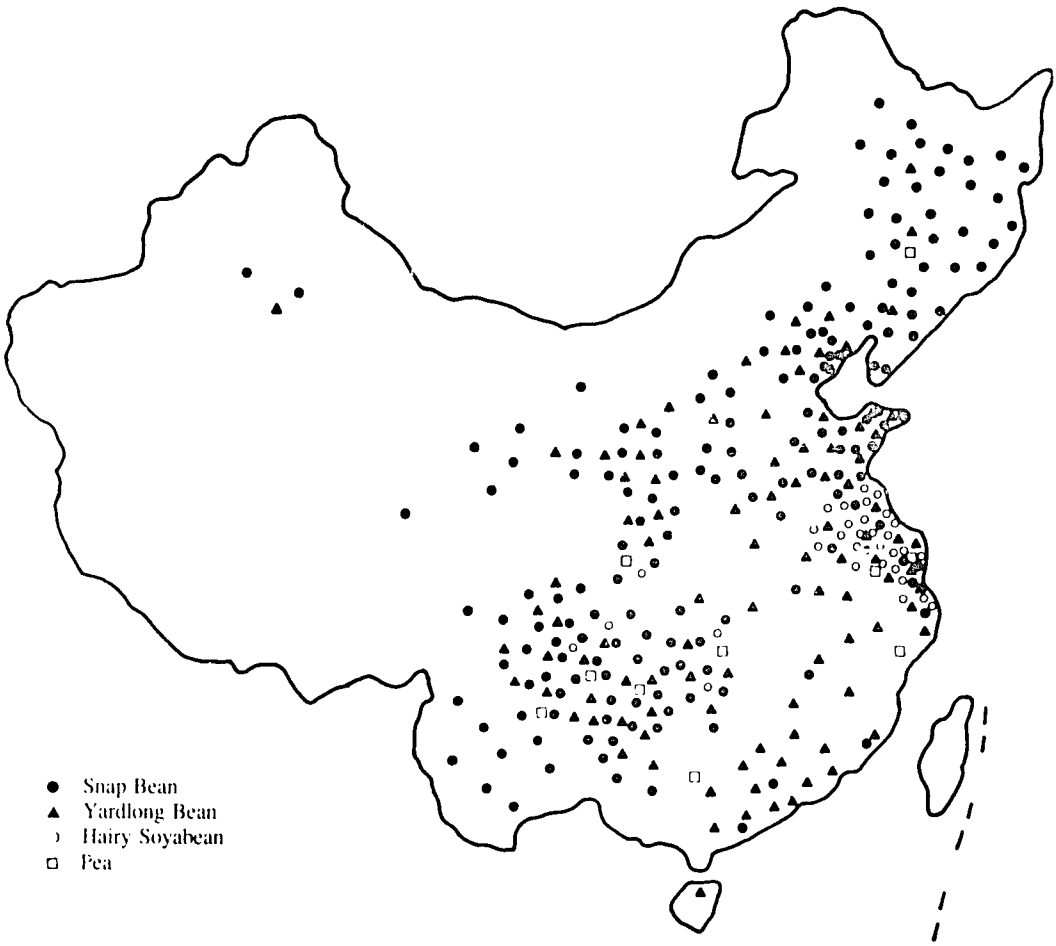

FIGURE 1 The distribution of the four vegetable legumes.

TABLE 2 The number of collections and locations of legumes in China.

Name	Scientific name	No. of varieties	Locations
Snapbean	*Phaseolus vulgaris* L.	1 629	Sichuan, Shaanxi, Shandong, Hebei, Jilin, Hilongjiang,
Yardlong bean	*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc.	837	Sichuan, Guangdong, Shandong Jiangsu, Anhui
Hairy soyabean	*Glycine max* Merr.	326	Shanghai, Jiangsu, Anhui, Hunan
Garden pea	*Pisum sativum*	106	Sichuan, Yunan, Guizhou, Jiangsu
Hyacinth bean	*Dolichos lablab* L.	252	Sichuan, Hubei, Shandong, Jiangsu
Broad bean	*Vicia faba* L.	55	Anhui, Jiangsu, Sichuan, Guizhou
Lima bean	*Phaseolus lunatus* L.	26	Shanghai, Hunan
Sword bean	*Canavalia gladiata* DC.	36	Sichuan, Hunan
Total		3 267	

CLASSIFICATION AND VARIETAL GROUPS OF SNAPBEAN BASED ON BOTANICAL CHARACTERS

Snapbean is one of the most important legume vegetables in China, both in quantity of varieties and growing area it dominates. Snapbean can be classified on botanical characters and horticultural characters of pod.

Classification based on botanical characters

Snapbeans can be divided into several types according to the plant habit, the shape, colour, weight and length of pod, seed colour and seed shape. The percentage of each type is shown in Table 3.

TABLE 3 Snapbean classification.

Character	Type	Percentage(%)
Growth habit	Determinate	20
	Semi-determinate	5
	Indeterminate	75
Flower colour	White	49
	Violet or dark violet	51
Pod shape	Flat	50
	Round	20
	Sickle shaped	25
	Sword shaped	5
Pod colour	Light green	80
	Green	10
	Violet and yellow	3
	Other	4
Pod weight	<8 g	32.4
	8–16 g	55.1
	>16 g	11.8
Pod Length	<12 cm	18
	12–21 cm	75.2
	>21 cm	6.3
Seed shape	Kidney shaped	60
	Round or nearly round	30
	Tubular	10
Seed colour	Single (black,white,brown, yellow,grey,violet, blue)	–
	Mixed (More than 1 colour)	–
Growing period	Early, <60 days	26
	Medium, 60–70 days	40
	Late, >70 days	34

Classification based on horticultural characters

On the basis of shape and colour of fresh pod, snapbean in China can be divided into the following varietal groups:

a) *Coloured pod group* — Most of the pods are wide and flat. The ground colour of the pod is light green (or yellowish green) with red or violet stripes. Tender pod and seed develop simultaneously with raised seed. The quality is excellent. At maturity, the pod becomes yellow. There are no strings along the lines of the dorsal and ventral suture. The pod remains soft throughout the growing period. The fresh pod and seed are both used for vegetable and food. Consumers like it very much. The yield of this group is not high. This group is grown mainly in the northeast.

b) *Round pod group* — Seed development is slow. Pod flesh in thick. Tender pods are for eating mainly. Fibre content is low in tender pods, but becomes higher as the pod matures. Pods are short, round, light green (or green), 10–14 cm long, about 1 cm in diameter. Flowers are white or violet. Plants with white flowers produce white seeds, and plants with violet flowers produce black seeds. The varieties of this group are used for the fresh market, as well as for canning and freezing. White-seeded varieties are ideal for processing. They are grown mainly in part of east and central China.

c) *Meaty pod group* — Pods are 16–20 cm long, nearly round in cross section, slightly curved, light green or whitish green, rough, raised in seed section. They fill well, and have thick flesh and high yield. This group produces an excellent quality fresh vegetable with a very good flavour.

d) *Flat pod group* — Flat varieties can be further divided into long, short, broad, narrow types. They are different in pod length, thickness, colour and quality. Among them, pods of some varieties are flat, slightly raised in seed section, light green or whitish green, low fibre, high quality. Some pods become white or yellow, as if mature, but in fact, they still keep a high quality. The varieties of this group are grown mainly in Shandong Province.

e) *Yellow pod group* — The principal characteristics of this group are yellow pods and white flowers. They are subdivided into round and flat types. Flat pod type has a greater water content, low fibre, good quality. Varietal numbers in this group are small. Distribution is wide.

f) *Purple pod group* — Pods contain anthocyanidin, so they are purplish red or dark reddish purple. After cooking, the pigment is dissolved, and the purple colour disappears. Pod, stem and leaf vein are violet. Flowers are purplish red or dark purple. Most pods are narrow and flat. There are strings in the line of dorsal and ventral suture. Pods have low fibre and good quality.

Classification based on botanical characters and varietal groups of yardlong bean

The growing area and the number of varieties of yardlong bean are second to snapbean in China. We collected 837 varieties of yardlong bean. Their classification follows.

Classification based on botanical characters

The proportion of each type of classification based on botanical characters is shown in Table 4.

Horticultural classification

a) *Dark green pod group* — Pods are green or dark green, round. Pods have firm flesh, good quality and are storable.

b) *Light green pod group* — Pods are light green, 50–60 cm long or more, round. They have loose fleshy texture, good quality and high yield.

c) *Greenish white (or white) pod group* — Pods are greenish white or white, about 30 cm

TABLE 4 **Yardlong bean classification.**

Character	Type	Percentage (%)
Growth habit	Determinate and semi-determinate	5
	Indeterminate	95
Flower colour	Milky white, light yellow	11
	Violet or bluish violet	89
Pod shape	Round	70
	Flat	13
	Coiled	<1
Pod colour	Dark green	5
	Green, light green	70
	Violet	9
	Other	7
Pod weight	<10 g	21
	10–25 g	65.7
	>25 g	12.5
Pod length	<30 cm	22
	30–60 cm	63.3
	>60 cm	14.2
Seed shape	Kidney shape	–
	Near round	–
Seed colour	Black	–
	Purplish brown	–
	With stripes	–

long, earlier in maturity. The plants produce pods in their central and low part. They have a high yield in the early stage, but total yield is not high.

d) *Purple pod group* — Pods are purplish red, round, 40–50 cm long. Seeds are mostly purplish red with dark stripes.

e) *Coloured pod group* — Pods are light green with violet stripes, 30–40 cm long, round or flat. Most seeds are purple with purplish brown stripes.

f) *Coiled pod group* — Pods are dark green with a small number of purplish red spots. Pods coil horizontally for 1.5–2.5 circles. Length is about 30–35 cm. Weight is about 15–20 grams. They are of very good quality and usually late to mature.

THE OTHER LEGUMES

Vegetable soyabean

Vegetable soyabean is grown for its tender seeds. Because there are fine hairs on the pod, it is called Hairy soyabean. It is a special local vegetable in China. There are many vegetable soyabean cultivars in Jiangsu and Shanghai. The varieties collected in China are classified according to their growth period: early, medium and late; according to seed colour: yellow, light yellow, yellowish green, green, dark green, brown, dark brown, black, alternate between the black and green; according to seed shapes: large, small, round, long round, oblate and flat. Flavours are glutinous or nonglutinous.

Garden pea

The cultivation of peas is very common in China, especially in the southwest. Those collected in China are classified as follows. According to growth habit, they are divided into the determinate, semi-determinate and indeterminate; they have white and violet flowers, soft and firm pods. Some varieties are grown for their fresh edible green seeds. The others are raised for the whole edible pods with seeds. Among the varieties grown for fresh green seeds, some are used for fresh market and some for canning or freezing. In addition, there is a special kind of pea called 'no-tendril pea'. They are grown for their tender shoots.

Hyacinth bean

Hyancinth bean is popular but it is not grown on a large scale. It is commonly planted in the front of houses or along the fences. The bean can enrich the table in the off season. Because its cultivation has a long history, the genetic resources are very rich. Most of the accessions collected in our country are of the climbing type, and very few of the bush type. Flowers are white and violet. Pods are dark green, green, light green, whitish green, greenish white, violet and green with violet dorsal and ventral lines. Pod shape is broad flat, long flat, crescent moon-like or semicircle-like. Some varieties have thin pod flesh. Some have thick pod flesh (more than 1 cm), low fibre and are of very good quality.

Utilization of legume vegetable germplasm

The utilization of legume vegetable resources in China is as follows.

Growth of local cultivars for the fresh market

Coloured, broad, flat varieties in which pod and seed develop simultaneously are grown mainly in northeast China. In the east and central parts, round pod varieties are grown for the fresh market or processing. Yardlong bean is tolerant to heat. It is an important vegetable in summer and fall in south China. Its growing area is larger than that of snapbean.

The east and central part of China is also an important region for vegetable soyabean production. There are many varieties in the region, including early, medium and late maturing varieties. They all are very good cultivars and are grown on a large scale.

Extending processing varieties

The development of canning and freezing in legume vegetable promotes the extension of processing varieties. Not only are original processing cultivars grown extensively, but also many new processing and dehydrating varieties will be grown. Freezing types of vegetable will flourish in Hilongjiang and Jilin provinces. The white seeded, round podded, local varieties suitable for processing will be grown more extensively. Snapbean, yardlong bean and vegetable soyabean varieties used for processing are being developed and utilized.

Full use of introduced germplasm from other countries

Although native bush beans in China usually mature early, the quality of some cultivars is not good. Therefore, some high yield and early varieties from foreign countries have an important part to play in snap bean production. Varieties include 'Contender', 'Fullcrop', 'Provider', etc.

Many pea varieties were also introduced from other countries, for example, 'Alaska', 'Lu Zhu', '1341'. They all are grown not only for the fresh market, but also for canning. Their growing area is expanding.

INDIRECT USE OF NATIVE AND INTRODUCED GERMPLASM

In recent years, breeders have developed many new varieties by use of local and introduced varieties. The new variety 'Yun Feng' was bred by crossing a line with a local variety. It is early in maturity and high in yield. New varieties 'Qiu Kang No. 19', 'Chun Feng No. 2', 'Chun Feng No. 4' and 'Qiu Kang No. 6' have been released by Tianjin Vegetable Research Institute.

'Zi Jiang 28-2' is new yardlong bean variety which was developed by crossing 'Red Beak Swallow' with a local variety in Zhejiang Horticultural Institute. It has strong adaptation and high yield. Pods are thick and long. It is an important variety in Zhejiang province and is grown commonly all over the country. 'Yan Dai Jiang' developed in Shanghai is suitable for summer and autumn growth. 'Yue Xia' series are new varieties selected from local varieties in Guangdong Academy of Agricultural Sciences. Afterwards, new varieties 'Fei Cui', 'Jin Sui' and 'Lu Hua bai' were released. 'Bai Jiang No. 2' was bred by crossing a line with a local variety in Nanjing Vegetable Research Institute. It is planted mainly in late spring and summer.

Many organizations, such as Sichuan Academy of Agricultural Sciences, Shanxi Agricultural University, and the Chinese Academy of Agricultural Sciences, have developed a great number of new varieties of peas that are used for vegetable or for both vegetable and dry seed. These new varieties, on the one hand, enrich the germplasm of legumes. On the other hand, they are adopted in production as new varieties to replace the old ones — so it is important for us to collect and preserve local legume genetic resources as soon as possible.

CHAPTER 24

Collection and evaluation of the germplasm resources in the genus *Oryza* in Guangxi, China

LIANG YAO MAO

Guangxi lies at the southern border of China and is located between 20°54'N and 26°20'N and 104°29' and 112°02'E. The tropic of Cancer traverses its central section. The topography, with few plains, and more rivers and streams, makes a complex terrain sloping from northwest to southeast facing the sea. The weather is mainly hot and humid, but in the mountain area and in the northern part some temperate features occur. High temperatures and humidity and plentiful rainfall combined with complex topography make Guangxi a main region of genetic diversity for many crops in China.

Rice is a staple food of Guangxi, and has been cultivated for a long time. The history of rice growing had led to a great number of local varieties spreading throughout this area. But since the improved rice was popularized in the 1950s many old varieties have been quickly eliminated. To protect the rice germplasm of the old varieties from damage, action has been taken three times by the Agricultural, Animal Husbandry and Fishery Department and the Guangxi Academy of Agricultural Sciences from the beginning of the 1950s. These three variety collecting activities were in 1950, 1978 to 1980, and 1983 to 1985, respectively. By these actions, the genetic resources of the genus *Oryza* of each county were surveyed and collected. Thousands of accessions of local cultivars and some stems and seeds of wild rice were collected and reserved, in addition to some improved varieties. They showed that there are two wild species in Guangxi, *O. rufipogon* and *O. officinalis*, and described their geographical distribution and ecological environment. In recent years, some research work on the samples collected was done on morphology, analysis of isoenzymes, resistance, and classification; at the same time the utilization of this rice germplasm by mean of biotechnology was investigated.

An attempt is made below to show that Guangxi is one of the centres of rice germplasm diversity in China according to type of rice germplasm, characteristics and distribution.

Distribution of the genus *Oryza* in Guangxi

CULTIVATED RICE (*ORYZA SATIVA*)

Sorting and classification of thousands of collected accessions showed that rice cultivar types are very complete and plentiful in Guangxi.

First of all, samples could be classified as subspecies Hsien (*O. sativa* L. ssp. Hsien Ting) and subspecies Keng (*O. sativa*. ssp. Keng Ting). The cultivars are very different from each other in characteristics, physiological nature, geographical distribution, and relationship. Rice Hsien has a light leaf colour, more leaf thorn, no awn or short awn, less thorny glume and small and slender grain, it drops easily and is distributed mostly on plains and valleys. Rice Keng is dark green, has little or no leaf thorn, a slightly bent awn, more thorny glume, roundish grain, drops with difficulty, and is found in the northern part or cold mountain area. The rice Keng is very complex, especially in shape of grain and colour. Its leaf blade is wider and grain size is larger, somewhat different from those of Keng in the northern part of China. The zymograms of esterase isoenzymes of both have an exclusive enzymatic banding. The rice Hsien has 7A but no 8A, the Keng the reverse.

Below the subspecies of Hsien and Keng, several ecological groups could be classified, mainly as follows.

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Samples can be classified into two groups according to the cropping seasons—the early and middle-late rice. The main difference is that the early one is sown in spring and heading and ripening occur under a long daylight period, and that the middle-late one is sown in May to June, ripening under a short daylight period. Now, apart from the mountain area where the temperatures are rather low and the altitudes higher and one crop of middle rice is still grown, most of the sites had changed to two-crop rice. But as a result of being accustomed to single cropping system in most areas, more varieties of middle-late rice were collected, accounting for 80%. There are many types like Hsien, Keng, Nuo (glutinous) wetland and upland rice and so on. There is a winter rice that is sown in autumn–winter and harvested in May the following year. This variety is not limited by the short daylight period when it is heading, and could be classified as an early rice. The two groups have little difference in morphology and in the zymogram band of esterase. But in the late rice Hsien, the zymogram has more 10A band, accounting for 52%, but the early rice Hsien has no such band. This could be used as a reference for classification.

Samples can be classified in two varieties, the Zhan (nonglutinous) and the Nuo (glutinous) rice accordinng to the nature of the milling grain. Zhan has a rather transparent body of grain and more straight-chain starches; it is difficult to be pasted and shows a blue colour when it reacts with iodine. Nuo has a milk-white body of grain and more branch-chain starches; it is easy to be pasted and shows a red colour when it reacts with iodine. There is no markedly distinguishable zymogram in the analysis of isoenzymes of the esterase. Amongst the resources of rice varieties of Guangxi, all the subspecies of rice Hsien and Keng are of the two types — Zhan and Nuo, ie. the Hsien and Keng Nuo rices. The grain of Hsien Nuo rice is long and narrow, and the Keng Nuo, round and short. The analysis of isoenzyme of esterase showed that the Hsien Nuo rice has the exclasive 7A band, while the Keng Nuo subspecies have few 7A and the exclusive 8A band. This is a situation worth studying. Of the rice resources of Guangxi the Nuo (glutinous) rice makes up to 20%. Of this percentage there are more Keng Nuo types and middle-late rice varieties with many mutations. The cultivated area of Nuo rice is not large at present and most of it in the mountain area, where the Keng Nuo rice is the staple food for the people living there.

Samples can be classified according to their ecological environment as upland, wetland, and deep water rice. The upland rice that is mainly grown on the slopes of mountains is susceptible to root rot, and plant establishment is poorer when it is planted in wetland. It is different from wetland rice in morphology, having a rather roundish base of the leaf blade. The cultivated area of upland rice in Guangxi is now about 13 000 ha, and makes up 5% of the total amount collected. Upland varieties comprise Hsien, Keng and Nuo types, but they are late rice mainly. The analysis of esterase isoenzymes showed many types of zymogram in Guangxi upland rice, especially for Keng upland rice. The data of 41 accessions analyzed showed 17 types of zymogram.

Deep water rice is now planted at individual sites and the cultivated area may be only about 30 ha in Guangxi. According to the ecological environment and morphological characters, they can be classified in two types. One of them is planted on flooded lowland and the other on beachy lowland where sea water has intruded. Both of them are tolerant to deep water and belong to the late rice group. The former has a thick culm, large leaf blade, and a bigger and roundish body of grain; while the latter a slender culm and leaf, and a long and small grain. Judging from the morphology, both deep water rices belong to the subspecies Hsien but the analysis of esterase isoenzyme showed that the former has the 7A zymogram band possessed exclusively by the Hsien rice and the latter has the 8A one possessed exclusively by the Keng rice. This situation is worthy of further study.

In addition to those mentioned above, there are many special varieties within the resources of rice germplasm of Guangxi. For example, some varieties have sweet smelling leaves and grains, but such varieties mainly belong to the Keng Nuo type, not the Hsien one; some have

a dark purple glume; some have no thorn on the glume (so called 'smooth grain') and others much more thorn on both sides of the leaf blade; and some are adaptive to intercropping. The diversity of the local cultivars in Guangxi could be clarifed by the analysis of esterase isoenzyme. As many as 12 zymogram bands and 52 zymogram patterns were obtained from 775 accessions. Keng Nuo rice possessed the richest zymogram pattern — 145 accessions showed 23 zymogram patterns. Then, 117 accessions of Keng showed 21 zymogram patterns. Finally, 278 accessions of late rice Hsien showed 17 zymogram patterns. The zymogram patterns of early rice Hsien were fewer. Results of the isoenzyme analysis and the morphological observation were identical.

COMMON WILD RICE (*O. RUFIPOGON*)

According to the results of morphological observation and biochemical analysis, Guangxi common wild rice can be divided into the typical type and various variant types.

The morphological character of the typical type are: strong fibrous roots; prostrate habit; extravaginal branching at higher nodes; culm length 100–250 cm; perennial; basal leafsheath and internode colour, purple or light red; auricle with long hairs; membranous ligule with purple lines and without hairs; open panicle type; without 2nd order panicle branching; generally 20-60 grains per panicle; long awns; light red seedcoat; strong shattering.

The morphological characters of variant types were between those of wild species and cultivars, for instance growth habit – prostrate, erect and semierect; culmtype – long-stalked and short stature; leaf shape – broad and narrow; panicle type – compact and open; awn length – long and short; seedcoat – light red and red. Variant types may be produced from the hybridization between wild species and cultivar or belong to the evolution products from wild species to cultivar. Questions about the formation of variant types remain to be studied in future.

Analysis of esterase isoenzyme gave 9 zymogram bands and 18 zymogram patterns from 124 accessions of various types of *O. rufipogon* originating from Guangxi, in which zymogram pattern 1359A had the highest frequency (42.7%), and secondarily the 134579A (9.7%). The typical type showed 9 zymogram patterns from 56 accessions tested, the frequency of 1359A as 62.5%. The variant types showed 16 zymogram patterns from 68 accessions, 1359A represented 26.4%. Results indicated that 1A, 5A, 9A belong to common zymogram band of *O. rufipogon* and cultivars, 3A is an exclusive zymogram band of *O. rufipogon*, 4A, 7A, 8A, 11A, 12A are exclusive zymogram bands of cultivars. In the zymogram patterns of the variant types of *O. rufipogon*, there existed 4A and 7A of Hsien type of cultivars. (4A, 57.4% ; 7A, 63.7%). The result indicated introgression of genes of cultivars into wild species.

O. rufipogon was one of the most widely distributed in Guangxi and was found in 42 counties. The distribution area extends from 106°22'–111°50' E and 21°28'–25°11' N. Most were found in the central and south section of Guangxi, where the rivers and swamps were concentrated and the environment was suitable for the growth of *O. rufipogon*. There exist some habitats of large area. In more than 10 habitats, the area covered by plants of *O. rufipogon* reach more than 6 hectares, the largest one was about 28 hectares.

O. OFFICINALIS WALL.

The morphological characters of *O. officinalis* originating from various ecological environments were basically no different. The morphological characters of Guangxi *O. officinalis* are: strong fibrous root system; decumbent habit; rhizomes; perennial; large and strong culm; culm length 200–300 cm (the longest was 467 cm); long and broad leaf (width 4.6 cm, length 123 cm); ligule tip round or truncate; long panicle neck (the longest was 123 cm); panicle type — open; generally without 2nd and 3rd order branching; panicle length 30–40 cm; generally

200–300 grains per panicle; gra n width 0.2–0.26 cm; grain length 0.4–0.5 cm; seedcoat red; strong shattering.

According to esterase isoenzyme analysis, 3 zymogram bands (1A, 5A, 7A) and 2 zymogram patterns (17A, 157A) were obtained in which the frequency of 157A was 74.4%. This result indicated the genetic homogeneity of *O. officinalis*.

The distribution range of *O. officinalis* was limited. It was found in 16 counties of the southeast section of Guangxi. The distribution area extends from 108°46'–111°50' E, 22°35' –23°59' N. It was located in the ditches between hills where the sunlight was less. *O. officinalis* generally grows in the shade and in rich and acid soil.

Utilization perspectives

Utilization of Guangxi germplasm resources in the genus *Oryza* for rice breeding

Guangxi possesses abundant local cultivar resources. According to evaluation results in recent years many materials had superior agronomic characters, such as good grain quality; high protein content; large panicle; large grain. Some varieties were resistant to major diseases and insects, tolerant to low temperature and drought and to problem soil. Those materials could be used for rice breeding and will contribute to breeding of high quality grain, high yields, resistance breeding and to biological engineering. But the local cultivar also possesses some shortcomings such as high-stalk, leaf-droop, poor tillering, and is susceptible to fertilizers and lodging, etc. These shortcoming should be noted and overcome in utilization.

According to the preliminary evaluation, *O. rufipogon* and *O. officinalis* originating from Guangxi possessed some special superior agronomic characters and resistant abilities which could not be found in cultivars. The germplasm resources of *O. rufipogon* showed great genetic diversity. *O. rufipogon* and cultivars belong to genome A and possess higher combination ability. Therefore the resources of *O. rufipogon* possess high utilization value. Although some superior materials were found in *O. officinalis*, there was a great distance in relative relationship between the *O. officinalis* and cultivars. Overcoming the low combination ability of hybridization and the unfavourable character of wild species were two difficult matters. We are conducting studies of biological techniques to overcome these problems.

The roles of Guangxi resources in the studies of taxonomy, origination and evolution

Kato first comprehensively studied the classification of *O. sativa* in 1928. According to the combination ability of hybridization, *O. sativa* was divided into *Indica* and *Japonica* types. Based on the grain shape and size, Matsuo in 1952 classified *O. sativa* into A, B and C types. Ting Ying divided *O. sativa* into two subspecies (*O. sativa* L. ssp. Hsien Ting and *O. sativa* L. ssp. Keng Ting). Although there existed differences of classification method and nomenclature, the views of the researchers on the classification of Hsien and Keng were identical. There still exist some questions about the classification of *O. sativa*. For instance whether *Javanica* could be divided into the third subspecies, the classification of medial types. Guangxi rice germplasm is of various types, including subspecies Hsien and Keng and medial types. Keng and Keng Nuo rice have many types. The classification of Guangxi local cultivars have not been systematically studied.

Many theories and discussions about the origination, evolution and propagation of *O. sativa* have not yet been satisfactorily agreed upon. Guangxi has abundant local cultivars and wild species, and undoubtedly, has a high diversity of the genus *Oryza* in our country. The geneticist N.I. Vavilov considered that the area of greatest crop diversity would be its origination site. Studies of Guangxi resources of *Oryza* are contributing to studies of the origination and evolution of *O. sativa*.

CHAPTER 25

Collection, evaluation and utilization of genetic resources for barley breeding in Korea

FUN SUP LEE

Barley is used in Korea as food, malt, feed and alcoholic beverages. As a winter crop barley plays an important role in increasing cultivated land by double cropping alongside the summer crop. Barley breeding programs were begun in 1907. But up to the 1970s, barley breeding had mainly been carried out through the conventional pedigree method, specifically for high yielding capacity.

From the early 1970s, the major target for the barley breeding program has been early maturity to aid barley–rice double cropping under paddy fields. Also, Korean breeders have been interested in the resistance to lodging and diseases. A regional peculiarity for barley production in Korea is the need for winter barley with early maturity and cold tolerance. A severe problem was cold injury because earliness is strongly linked to spring types of barley with weak cold tolerance. Utilization of germplasms with earliness, winter habit and cold tolerance resulted in the development in 1977 of a new barley variety Dongbori 1 which is characterized by cold tolerance, early maturity and intermediate winter habit. From this, several varieties that have similar characters to Dongbori 1 were developed. In addition, lodging was largely improved by the development of a new variety 'Kangbori' in 1976, by use of cross material, 'Haganemugi' with high resistance to lodging.

It is only since the late 1970s that the study of grain quality has become important due to demands for high quality foodstuffs following rapid economic growth. Thus, 'Chalbori' (covered barley) and 'Challsalbori' (naked barley) which are characterized by waxiness were developed with the waxy gene.

Since 1980, new barley breeding programs have started on food, malt, feed, starch and the milling process. Studies on barley are being vigorously carried out for these purposes with the collecting, evaluating and utilizing of domestic and foreign germplasms under the balanced budget.

Collection and evaluation of barley germplasm

Strong interest on barley germplasms started in the early 1970s for early maturity because new developed paddy rice required earlier transplanting. Barley germplasm in Korea has been preserved for the long term under low temperature since 1975. The first report on landrace barley was published in 1979. An explanatory pamphlet on evaluation of 1714 barleys collected from domestic and foreign countries was issued in 1983.

Since 1984, a special program on germplasm in Korea was prominently established as shown in Table 1. Studies of barley germplasm were not started until 1983. Since active participation started the germplasm collection has reached 8611. Most of this germplasm has been screened under natural and laboratory conditions.

From 1985, all germplasms screened under field and laboratory conditions (7 019) have been computerized and classified for their breeding potential. The remaining 1 592 accessions which have not been screened will be evaluated for future use. These germplasms are stored at 15°C and 45% relative humidity for the short term. Table 2 indicates that covered barley occupied 87% and 6-row barley, 71%, thus showing that most barley is of the 6-row covered type. However, naked barley occupies 13%. Of these, there are a few 2-row barleys which were recently collected and have been improved for good food quality. For cultivation

TABLE 1 Collection and evaluation of barley germplasm.

Year	Until 1983	1984	1985	1986	1987	Total
Collection	2 243	1 673	937	2 166	1 592	8 611
Evaluation	2 243	–	1 673	937	2 166	7 019
Computerization	–	–	1 673	937	-	2 610
Long-term storage	2 243	–	1 673	937	2 166	7 019

TABLE 2 Types of barley germplasm.

6-rowed barley		2-rowed barley		Total
Covered	Naked	Covered	Naked	
3 062 (59)	631 (12)	1 459 (28)	49 (1)	5 201 (100%)

in Korea, barley is now planted in 6-row naked, 6-row covered and 2-row covered barley in order. In the past it was planted in 6-row covered barley, 6-row naked barley, 2-row covered barley in order.

However, it is considered necessary to change acreage according to changing needs as follows: 6-row naked for manufacturing, 2-row covered for brewing, 2-row naked for high quality food, and 6-row covered for feed.

In 1987, 2510 germplasm samples were collected and screened for the major agricultural characters shown in Table 3.

It is considered that effective barley breeding should be concerned with longer spike, more grains per spike, and higher grain weight. Early maturity is sought in barley production in Korea. However, heading before 20 April leads to damage by chilling. There are many barley materials for earliness, but chilling injury after heading is another problem in the search for improved barley varieties in Korea.

TABLE 3 Range of some characters of Korean 6-row barley (1987).

Trait	Range
Spike length	3–13 cm
No. of grains per spike	22–108
1 000 grain weight	12–49 g
Stem length	43–140 cm
Heading date	15.iv–15.v

Utilization of barley germplasm

Winter hardiness

Winter hardiness varies according to the different cultivation areas. Improvement in cold tolerance will increase the area of stable cultivation. Evaluation of germplasm and breeding line to cold tolerance were carried out at 3 locations in Korea at -5°C, -7°C, and -9°C average minimum temperatures during January under 3 different cultural practices with high, common and low ridges, respectively. Cold damage was measured by survival rate of tillers and individual plants. Korea needs barley varieties with winter hardiness, early maturity and

winter habit. This makes breeding difficult because of cold tolerance going with late heading.

Relations between cold injury and heading date for 528 Korean land varieties are listed in Table 4. Lines with early heading date and winter hardiness were not observed in this experiment. It appeared that lines showing early heading time were the most cold damaged populations. 'Dongbori 1' was selected for winter hardiness under average minimum temperature of -9°C during January and used for crossing materials. Crosses between 'Dongbori 1' and other cultivars resulted in several cold-tolerance barley varieties. One of the limiting factors for expansion of cultivated areas for naked and 2-row barley was extreme susceptibility to coldness during winter months above the middle part of Korea. These varieties should be cultivated only in the southern part of Korea. However, naked barley 'Suwon 236' and 2-row barley 'Suwon 212, were released for cold tolerance by use of covered barley as cross materials. (Table 5).

These new cultivars are being recommended for the middle part of Korea.

TABLE 4 Associations between cold damage and heading date of 528 Korean native varieties.

Association	Observed (O)	Expected (E)	O - E	χ^2
Least cold-damaged				
Early heading	0	7.0	-7.0	7.00**
Mid-early heading	64	48.7	15.3	4.81*
Late heading	21	28.4	-7.4	1.93
Extremely late heading	1	2.0	-1.0	0.50
Mid cold-damaged				
Early heading	14	28.1	-14.1	7.08**
Mid-early heading	190	196.3	-6.3	0.20
Late heading	135	114.5	20.5	3.67
Extremely late heading	8	8.0	0.0	0.90
Most cold-damaged				
Early heading	29	7.7	21.3	58.92**
Mid-early heading	45	53.8	-8.8	1.44
Late heading	18	31.4	-13.4	5.72*
Extremely late heading	3	2.2	0.8	0.29
				χ^2 91.56**

* Significant at 5% level ** Significant at 1% level

TABLE 5 Winter hardiness of major barley varieties in Korea.

Variety	Dongbori	Olbori	Suwon 236	Suwon 212
Row	6-row	6-row	6 row	2 row
Covered or naked	covered	covered	naked	covered
Cold damage(%)	30.1	52.2	52.7	53.4

EARLINESS

Under the double cropping system, it is necessary to harvest barley before transplanting rice. To increase barley production, breeding for earliness is necessary because development of

early transplanting rice cultivars (a cross between Indica and Japonica types) preceded the development of earlier barley varieties.

Heading in barley is closely related to the physiological factors such as earliness in the narrow sense, photo-response, spring–winter habit and cold tolerance. Cold tolerance has a great influence on heading. In general, winter-hardy varieties resulted in delayed heading. But spring barleys in early heading were damaged by the cold. Table 6 shows the spring–winter habit of barley varieties recently developed.

TABLE 6 Classification of growth habit for Korean land races and bred cultivars (1979–85).

	Degree of growth habit						
	I	II	III	IV	V	VI	Total
Land races	55	3	22	282	37	0	399
Bred cultivars	108	37	95	54	0	0	294

There are many spring types, followed by fewer intermediate and winter types, indicating the selection of earliness as a priority. Many germplasm types have been utilized as crossing materials for development of early varieties since 1960. This effort has led to improvement of many barley cultivars. These bred varieties and breeding lines were evaluated for photo-response. Thus, Table 7, shows photo-responses of 264 released barley varieties and lines since 1980 as 21% extremely insensitive, 51% insensitive, 17% medium and 11% sensitive. This result shows that much effort was given to selecting the insensitive lines to photo-response at late generations for development of extremely early barley varieties with crossing lines insensitive to photo-response.

TABLE 7 Classification of photoperiodism for Korean barley bred cultivars and lines depending on the differnce between short photoperiod (A) and earliness in narrow sense (B).

Classification	Difference (A-B) (days)	Tested year					All years	
		1982	1983	1984	1985	1986	No.	(%)
Extremely insensitive	10	2	2	17	21	14	56	21
Insensitive	11-20	37	19	20	33	26	135	51
Moderate	21-30	16	5	7	7	9	44	17
Sensitive	31-40	6	12	3	2	1	24	9
Extremely sensitive	41	–	1	4			5	2

Insensitive barley lines respond to short photoperiodism before the winter or regrowth stage, and usually head earlier than sensitive lines. Thus breeders have more opportunity to select the insensitive lines, and many insensitive lines selected were utilized as breeding materials. Table 8 indicates the relationships among physiological factors related to field heading time and earliness.

TABLE 8 Correlation coefficient among physiological factors related to field heading time and earliness in 1984.

Character	S	G	H	M
Earliness in narrow sense (L)	0.721**	0.332*	0.683**	0.563**
Short photoperiod (S)	–	0.167	0.697**	0.523**
Growth habit (G)		–	0.269	0.129
Heading time (H)			–	0.730**
Maturing time (M)				–

* Significant at 5% level ** Significant at 1% level

Field heading time showed highly significant correlations with earliness in the narrow sense and short photoperiod. Earliness in the narrow sense was highly correlated with short photoperiod. However, there was no correlation between spring–winter habit and field heading time, indicating that many spring types showed delayed heading time due to the cold damage. Figure 1 shows changes of maturity in Korean bred barley cultivars according to the year of release. Maturity of 'Jogangbori' and 'Tabgolbori' showed 10 days earlier heading than that of 'Buheung' and 'Suwon 18' which were widely cultivated in the early 1970s. The earliest barley variety in Korea matures in late May; it is impossible to shorten the maturity in the southern area. Barley should be headed about 20 April in order to mature by late May. If it is headed before that date, barley plants will suffer sterility, longer maturing period, etc. from chilling.

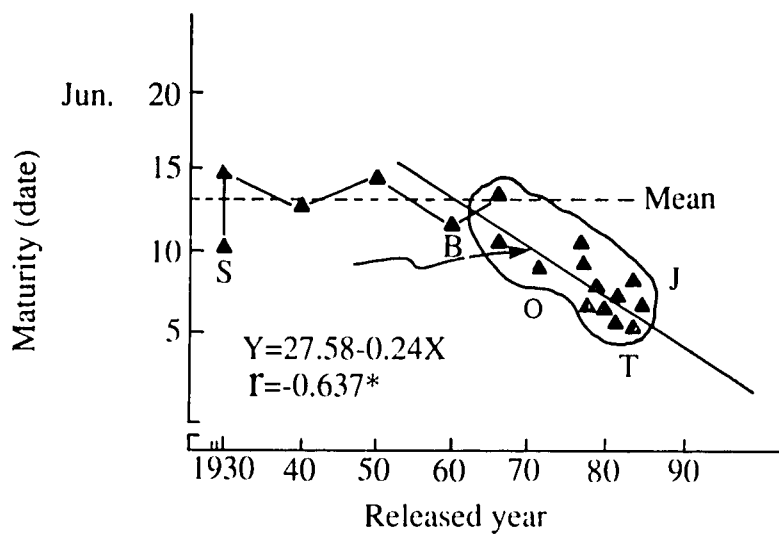

FIGURE 1 Changes in maturity of Korean barley cultivars released from 1930 to 1984. S: Suwon 18 B: Buheung O: Olbori J: Jogangbori T: Tabgolbori

LODGING

To improve lodging resistance, 'Suwon 18' was developed in 1932, and Hangmi and Yeugi with semi-dwarfness of the UZ gene in 1960. In the 1970s Olbori which has a strong culm and high stem flexibility was used for the improvement of lodging resistant varieties, such as 'Albori', 'Buhobori' and 'Paldalbori' as shown in Figure 2.

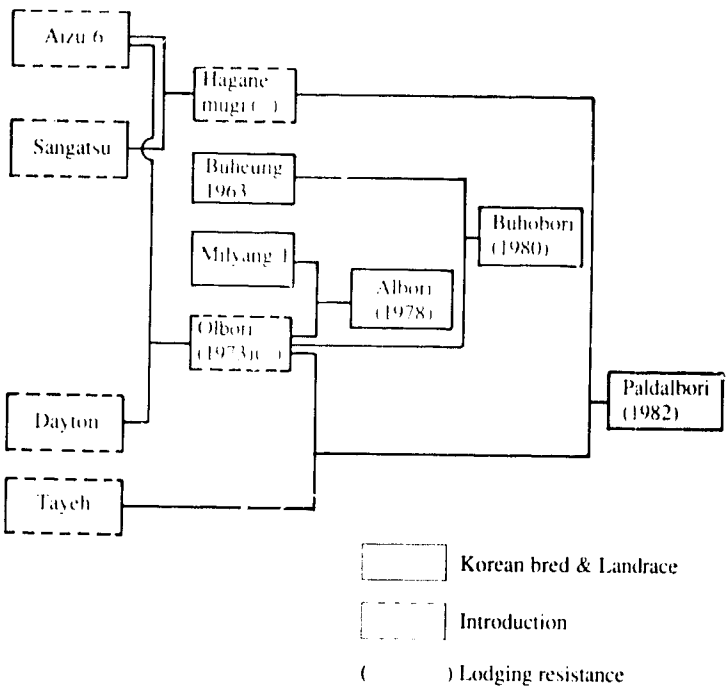

FIGURE 2 Lineage for lodging resistance originated from Olbori of covered barley cultivars bred in Korea.

'Kangbori', selected in 1975 from combination of 'Bisenwase' × 'Haganemugi', is highly resistant to lodging with stiffness of culm and tall plant height (90 cm). Several lodging resistant varieties such as 'Dongbori 1', 'Dongbori 2', 'Durubori', 'Oweolbori', 'Namhaebori', 'Jopungbori', 'Saeolbori' and 'Chalbori' had been developed by use of 'Kangbori' as cross materials as shown in Figure 3. Naked barley such as 'Songhakbori', 'Mokpo 51', 'Muanbori' and 'Saessalbori', were also improved for resistance to lodging by use of the semi-dwarf gene 'UZ'.

SPIKE SPROUT

In the field, barley spikes occasionally germinate at maturing stage during rainy weather. In particular, food barleys have more problems due to spike sprout, because they are waxy and naked, causes of easier sprouting of spike in rainy weather. Thus, one important breeding target is to develop varieties resistant to spike sprout. Experimental procedures and methods

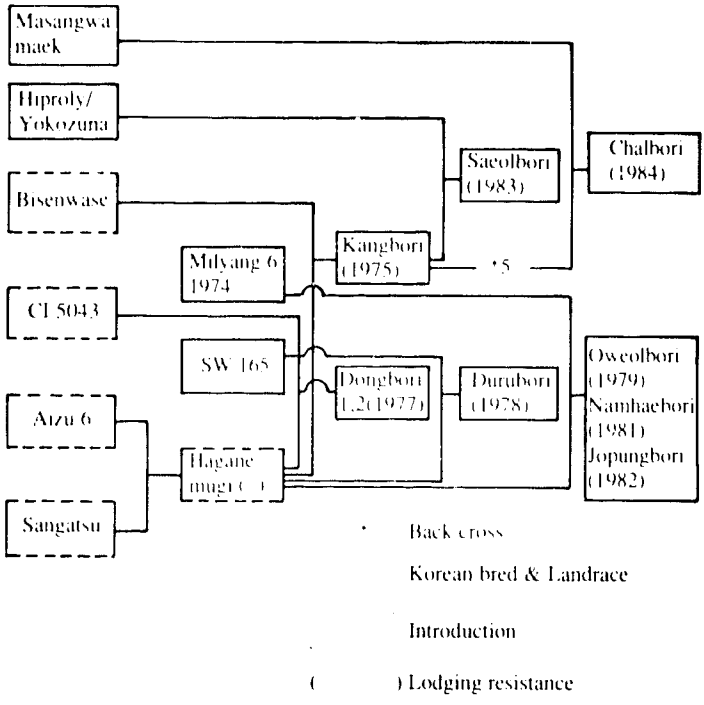

FIGURE 3 **Lineage for lodging resistance originated from Kangbori of covered barley cultivars bred in Korea.**

TABLE 9 **Germination rates 40 days after heading under different treatments.**

	H_2O_2	Moisture	Sand bed
Mean	8.5	5.7	11.2
SD	8.35	8.14	9.36
Range	0.7–30.0	0–38.7	1–44.0

TABLE 10 **Germination rates under the sand-bed method.**

	Days after heading		
	20	30	40
Mean	0.48	0.38	11.3
SD	1.34	7.82	9.36
Range	0–5.0	0–31.0	1–44.0

were developed to find better screening methods for spike sprouting. In these barley seeds were treated with H_2O_2, moisture, and a moistured sand bed. Many germplasm types including breeding lines and recommended varieties were screened, and those selected used as cross materials for development of resistance varieties (Table 9).

The sand bed method is best, showing higher mean germination. Studies on 3 different stages for spike sprout indicate that treatment at 40 days after heading was the best (Table 10).

TABLE 11 Sprouting rates according to wx and naked genotypes (1987).

Classification	No. of varieties	Mean	SD	Range
NN-WxWx	74	6.83	7.27	0–30.6
NN-wxwx	6	18.05	9.02	7.3–31.3
Difference		11.22**		
nn-WxWx	35	8.09	9.62	0–38.5
nn-wxwx	22	18.50	9.94	1.9–40.7
Difference		10.41**		

** Significant at 1% level

TABLE 12 Sprouting degree of major varieties in Korea ('83–'86).

Classification	Range	6-row, covered	6-row, naked	2-row, covered	2-row, waxy
Low	Below 5%	Dongbori 2	Iri 9	Dusan 8	
Medium	5–20%	Saeolbori Paldalbori Bunong	Baekdong Songhakbori	Hyangmaek	Chalbori
Heavy	Above 20%	Durubori Namhaebori Kangbori	Neulssalbori Iri 15	Sacheon 6 Dusan22	Suwon 235 Suwan 236

Spike sprouting on dwarf 2-row barleys such as 'Dusan 8' and 'Dusan 12' for malt also had improved lodging resistance. The development of dwarf varieties resistant to lodging has been continued intensively with them. Table 11 indicates spike sprout on germination using naked and waxy varieties. Waxy barleys showed rapid absorption of water, but there was no difference between hulled and hull-less barley.

Table 12 shows the differences of spike sprout among 18 different cultivars. 'Dongbori 2', 'Iri 9' and 'Dusan 8' showed < 5% of spike sprouting and spike sprouting on 'Suwon 235', 'Sacheon 6' and 'Neulssalbori' was > 20%. Recent studies have concentrated intensively on the development of resistant varieties by use of germplasm with covered varieties having resistance to spike sprout as cross materials.

Disease

Barley diseases are not a serious problem in Korea. However, there are two potentially important diseases, 'powdery mildew' and 'barley stripe disease' which frequently attack plants. Studies were undertaken to select resistant cultivars to control powdery mildew. As

TABLE 13 Distribution of breeding lines and recommended varieties on powdery mildew.

Trait	R	MR	MS	S
No. of varieties	208	20	77	560

TABLE 14 Pearling and cooking characteristics of SW 235 with w × w × nn compared Saessalbori W × W × NN.

Cultivar	Pearled grain							
	Amylose content (%)	Whiteness (%)	MVT (°C)	MV (BU)	Consistency (BU)	Water absorption (%)	Expandibility (%)	Pearling rate (%)
SW 235	5.8	49.3	70.0	1 770	107	261	452	77.4
Saessalbori	22.7	46.6	92.5	950	540	214	400	74.8

a result, many varieties resistant to powdery mildew were selected as shown in Table 13. But breeding for resistance to powdery mildew is not vigorously carried out because the disease causes only sporadic damage to barley plants.

Barley stripe disease occurs under conditions of high temperature and high humidity after winter months. Even though the disease is systemic, it can be prevented by chemical treatment. Recently selections for barley stripe resistance were made. Of those tested, 56% are resistant varieties.

QUALITY

Barley is used as food in Korea. Thus, pearling and cooking quality is very important. Barley is mixed with rice for cooking. Non-waxy barley is characterized by the poor absorption of water. The barley has to be soaked in water for one day before cooking, or boiled before being mixed with rice for cooking. But waxy genotypes easily absorb water. Thus, the waxy variety 'Chalbori' was developed in 1984 by the back-cross method. By use of this variety as cross material 'Suwon 236' and 'Suwon 235' which have waxiness and nakedness were developed in 1987. Table 14 indicates the differences of 'Suwon 235' (waxy and naked) and 'Saessalbori' (non-waxy and naked) on pearling and cooking qualities. Waxy variety 'Suwon 235' showed lower amylose content (5.8%), lower gelatination temperature (70°C), and low consistency compared to non-waxy variety 'Saessalbori'. However, 'Suwon 235' showed better whiteness, maximum viscosity, water absorption, expandibility and pearling rate.

CHAPTER 26

Utilization of ecotypes in forage grass breeding in Japan

Y. TERADA

Forage grasses are not indigenous to Japan, but were introduced from abroad about one hundred years ago, and some grass species, such as orchardgrass, timothy and Italian ryegrass, were adaptable to the Japanese environment. These species have resulted in valuable ecotypes by natural selection for many years. The populations are heterogeneous and have large genetic variability and genes adaptable to the Japanese climate. They have been used as breeding materials along with newly introduced foreign genetic resources.

At present, we have 59 Japanese varieties of 15 species: 18 (32%) are developed from ecotype and 14 (25%) from both ecotypes and foreign germplasms. They are cultivated widely over a large area on grassland in Japan. This indicates that ecotypes have provided very important gene sources for grass breeding in Japan.

A new research project for the utilization of ecotypes started from 1984 in MAFF, and 1 500 ecotype germplasms have been collected and are now being evaluated.

Short history of Japanese forage grass ecotypes

More than ten species of cool-season grasses and legumes and several species of warm-season grasses are cultivated for forage production throughout Japan, because of the diversity of climatic conditions. These species are not native but were introduced from abroad less than three hundred years ago (Table 1).

TABLE 1 Introduction and ecotype formation of forage grasses in Japan.

Species	First introduction	Adaptation area	Acreage under cultivation	Ecotype formation
Italian ryegrass	1870s	C–S	Very large	Very high
Perennial ryegrass	1870s	C–N	Small	Low
Orchardgrass	1870s	N–S	Very large	Very high
Timothy	1870s	N	Very large	High
Tall fescue	1870s	N–S	Large	Medium
Meadow fescue	1870s	N	Large	Low
Smooth bromegrass	1870s	N	Small	Very low
Bahia grass	1952	S	Very small	Very low
Dallis grass	1906	S	Very small	Medium
Rhodes grass	1960s	S–C	Small	Low
Coloured guineagrass	1960s	S–C	Very small	Low
Guineagrass	1960s	S–C	Very small	Very low
Red clover	1600s	N–S	Large	High
White clover	1600s	N–S	Very large	High
Alfalfa	1870s	N–C	Medium	Low

N: Northern Japan, C: Central Japan, S: Southern Japan.

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Red clover, *Trifolium pratense*, and white clover, *T. repens*, were introduced from th Netherlands in the 1600s with other imported goods.

About one hundred years ago, many species of cool-season grasses and legumes wer introduced by the Japanese Government from the USA in order to find out which wer adaptable to Japanese climate, especially to that of Hokkaido. In the experimental field tes in Hokkaido, some of them failed to adapt, but several species survived and became adapte to the climate and soil of Hokkaido (Table 2). Orchardgrass, *Dactylis glomerata*, an Timothy, *Phleum pratense*, were most promising and widespread in Hokkaido and Tohok regions. They were cultivated in pastures and meadows, but some plants escaped and becam naturalized on the road and river side among other native weeds, and changed into naturalize populations.

TABLE 2 **First introduction of grasses and legumes in Hokkaido, Japan, from USA, in 1877, by William P. Bruck.**

Species	Amount of introduced seeds (lb)	Present cultivation and distribution
Orchardgrass	300	Popular in all Hokkaido. Escaped plants are naturalized on roads and riverside.
Timothy	300	The same as above.
Tall fescue	50	Few but promising for beef-cattle.
Perennial ryegrass	40	Small area, less winter-hardiness.
Italian ryegrass	40	Only summer annual cultivation.
French ryegrass	40	No.
Kentucky blue grass	150	Popular in pastures. Escaped plants are aggressive in old pastures.
Red top	150	Extremely few in pastures.
Sweet scented vernal grass	30	No.
Hungarian grass	100	No.
Red clover	150	Popular in mixed grassland. Escaped plants are naturalized in the fields.
White clover	75	The same as above.
Mangold wurtzl	6	No.
Turnip rutabaga	6	Extremely small area.
Turnip swede	4	The same as above.
Meadow fescue	—	Semi-popular in the mixed grassland with orchardgrass, timothy and clovers.
Alfalfa	—	Promising species, recently rapidly enlarged for its excellent quality.

Seventy to eighty years ago, several national livestock farms and a few private livestock farms were formed in all parts of Japan, and some of the cool-season grasses, such as Italian ryegrass, *Lolium multiflorum*, orchardgrass, red clover, white clover, etc., were introduced and cultivated. Harvested seeds were stored for the next sowing.

Through many years of natural selection and regeneration, their progeny has acclimatized to become ecotypes or local domestic strains of Japan.

In 1906, dallisgrass, *Paspalum dilatatum*, a species of warm-season grass, might have come from Australia to the national livestock farm in the southern Kyushu, together with hay, when horses were introduced. It escaped from the field to river sides, spread and grew as a weed everywhere in Kyushu by natural propagation.

After World War II, many species of warm-season grasses were introduced from subtropi-

cal and tropical regions to southwestern Japan, but their ecotypes have not been found so far in these areas.

Characteristics of forage grass ecotypes

All the ecotypes have the characteristics of early maturing, erect growth, stemy plant type, good winter or summer tolerance, medium disease resistance, high seed yield and moderate forage yield. Early maturing plants are commonly found in many ecotype populations in orchardgrass, timothy and red clover. Hokkaido ecotypes of orchardgrass have good winter hardiness and good regrowth in spring after winter injury: Miyazaki and Okayama ecotypes (wild population) have excellent summer tolerance.

Plants with these characteristics are thought to have gained selective advantage over competing populations over a long period.

Orchardgrass and Italian ryegrass ecotypes were not resistant to brown stip disease and crown rust disease, respectively. Natural selection may be less effective for these leaf diseases, because they do not cause serious damage to seed formation.

Since all of the cool-season grasses are cross-pollinated and heterogeneous, these ecotypes have large genetic variability and adaptive genes to Japanese climate and soils. In addition, an important aspect of ecotypes seems to be the potential variation hidden under apparent uniformity of phenotype, and selective effects of the ecotypes are higher than foreign or improved varieties.

For these reasons, Japanese ecotypes have been utilized as valuable breeding materials, along with newly introduced foreign genetic resources, since the 1950s when the Japanese breeding project started.

Utilization of ecotypes as breeding materials of forage grasses

In 1964, a forage-grass breeding organization was established with 13 laboratories of 11 stations located from eastern Hokkaido to southern Kyushu (Figure 1). Several grass species, which have proved to be promising in cultivation trials, were employed to breed new varieties adapted to Japanese environments. Species for breeding were determined in each breeding station according to their environmental condition.

The first step was to collect and evaluate the ecotypes from the old farms and pastures. Comparative breeding methods were used for selection from the ecotype population, and a new variety was first released in 1965.

From 1965 to 1987, 56 varieties of 15 species have been released including public and non-public varieties (Table 3). These varieties are classified into three groups according to breeding materials : 1) varieties selected from ecotypes or domestic strains originated from ecotypes : 2) varieties developed from both ecotypes and foreign varieties ; 3) varieties from foreign varieties or strains.

The first group has 18 varieties (32% of total) including 7 varieties of Italian ryegrass and 5 varieties of orchardgrass.

The second group has 14 varieties (25%) including 4 orchardgrass varieties and 3 varieties of Italian ryegrass and red clover. So, ecotype genes are efficient in 32 varieties of 8 species, which are 57% of all Japanese varieties.

In the early stage of breeding work between 1965 and 1971, the breeding materials were mostly ecotypes of Italian ryegrass, orchardgrass, timothy and red clover. In the period between 1972 and 1979, varieties from ecotypes decreased, and those developed from both introduced and mixed materials increased. In the period between 1980 and 1987, direct selection from ecotypes decreased, and the varieties and strains from ecotypes in the early period of breeding were used as breeding materials. The efficient combination between ecotypes and foreign germplasms is more important for the next stage of breeding.

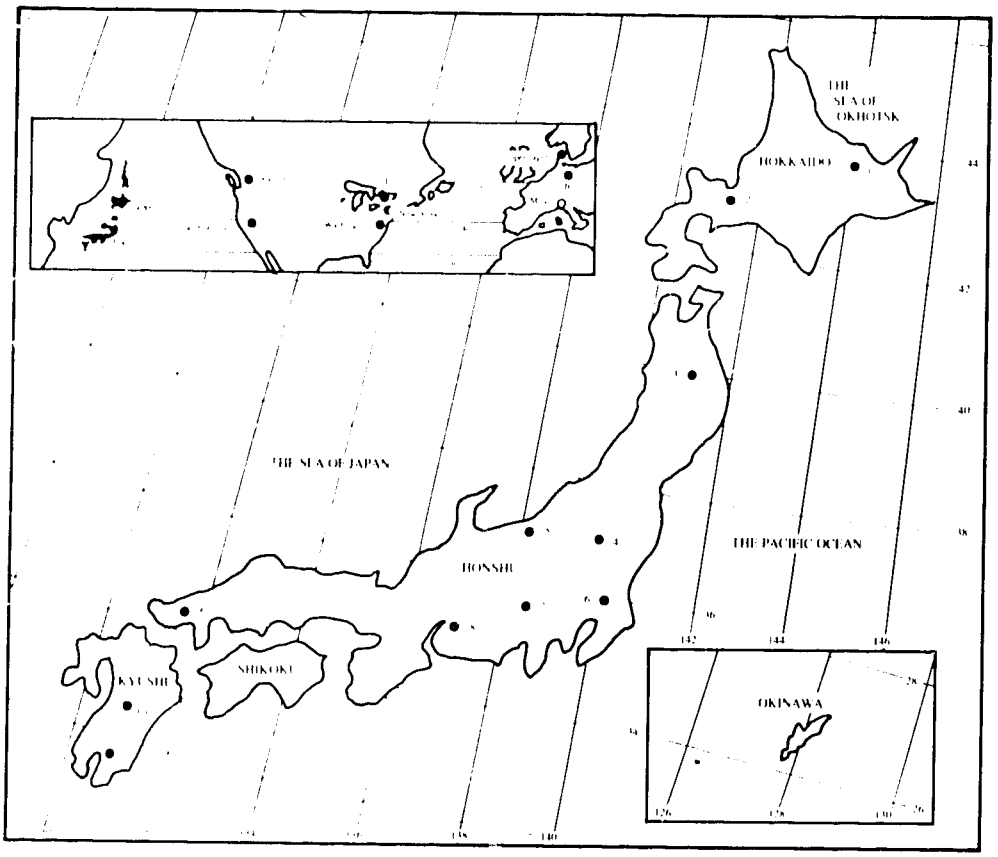

FIGURE 1 Location of Breeding Stations.

Key
1. Kitami Agric. Exp. Sta. : Timothy, Smooth bromegrass
2. Hokkaido Agric. Exp. Sta. : Orchardgrass, Fescue, Red clover, Alfalfa
3. Tohoku Agric. Exp. Sta. : White clover
4. National Grassland Research Institute : Orchardgrass
   Hokurihu Agric. Exp. Sta. : Italian ryegrass
6. Ibaraki Livestock Exp. Sta. : Italian ryegrass, white clover
7. Yamanashi Dairy Exp. Sta. : Perennial ryegrass
8. Aichi Agric. Exp. Sta. : Alfalfa, Panicum
9. Yamaguchi Agric. Exp. Sta. : Italian ryegrass
10. Kyushu Agri. Exp. Sta. : Tall Fescue, Tropical grasses
11. Kagoshima Agric. Exp. Sta. : Bahia grass, Rhodesgrass

Table 4 shows all the varieties originated from ecotypes released by MAFF. In all ecotypes, the Tottori ecotype of Italian ryegrass is most important; it was used to develop four varieties, i.e. Waseaoba, Waseyutaka, Minamiwase and Haruaoba. The Tottori ecotype was registered on the OECD variety list in 1967, named St. Tottori.

TABLE 3 Classification of breeding materials in Japanese varieties.

Species	1965–71			1972–79			1980–87			Total			No. of varieties
	E	M	F	E	M	F	E	M	F	E	M	F	
Italian ryegrass	3	0	0	2	2	3	2	1	0	7	3	3	13
Perennial ryegress	0	0	0	0	0	2	0	0	2	0	0	3	3
Orchardgrass	4	0	1	0	2	1	1	2	0	5	4	2	11
Timothy	1	0	1	0	1	1	0	1	0	1	2	2	5
Tall fescue	0	0	0	1	0	1	1	1	0	2	1	1	4
Meadow fescue	0	0	0	0	0	0	0	0	1	0	0	1	1
Smooth bromegrass	0	0	0	0	0	0	0	0	1	0	0	1	1
Bahia grass	0	0	1	0	0	1	0	0	1	0	0	3	3
Dallis grass	0	0	0	0	0	0	1	0	0	1	0	0	1
Rhodes grass	0	0	0	0	0	0	0	0	1	0	0	1	1
Coloured guineagrass	0	0	0	0	0	0	0	0	1	0	0	1	1
Guineagrass	0	0	0	0	0	0	0	0	1	0	0	1	1
Red clover	1	1	0	0	2	0	1	0	0	2	3	0	5
White clover	0	1	0	0	0	0	0	0	2	0	1	2	3
Alfalfa	0	0	0	0	0	1	0	1	1	0	1	2	3
Total	9	2	3	3	7	10	6	6	11	18	15	23	56

E: Ecotype and domestic strain bred from ecotype.
M: Mixed utilization both of ecotype (E) and foreign germplasm (F).
F: Varieties bred from foreign germplasm.

Hokkaido ecotypes of orchardgrass and timothy are also valuable genetic resources, because the leading varieties in Hokkaido, Kitamidori orchardgrass and Senpoku timothy were selected from the many populations of Hokkaido ecotypes.

In the early stage of grass breeding, mass selection and maternal line selection were dominant, but later the synthetic variety method combining domestic and foreign germplasms became popular in cross pollinated grasses, in order to promote heterosis.

New project for the future utilization of ecotypes

Ecotypes are valuable as breeding materials with their tolerance to environmental stresses, such as their tolerance to cold, snow, heat, wet and drought conditions.

Recently, foreign varieties and newly released Japanese varieties have been grown in all grasslands of Japan; therefore original ecotypes were eliminated and become spontaneously crossed with these varieties. Genetic erosion of the original ecotypes is becoming serious in Japan.

A new research project of ecotypes started in 1984 in 14 laboratories of 5 national and 2 prefectural experiment stations. Collection of ecotype germplasms and development of practical methods of evaluating stress tolerance are our present main concerns.

From 1984 to 1986, we collected 1500 germplasms of seeds and plants from all over the country (Table 5), and they are now being evaluated in the breeding stations. We hope to find materials and methods for developing varieties with broad adaptation and high tolerance to unfavourable environments.

TABLE 4 List of grass and legume varieties developed from ecotypes released by MAFF.

Variety	Year	Breeding Station[a]	Breeding Material[b]	No. of strain E	No. of strain F	Ecotypes
Italian ryegrass						
Wasehikari	1965	4	E	1	0	Chiba Livestock Exp. Sta.
Oobahikari	1965	4	E	1	0	Hillside of Mt.Yatsugadake
Waseaoba	1970	5	E	1	0	Tottori Livestock Farm
Waseyutaka	1972	9	E	3	0	Tottori, Kuroishi, Kochi
Nasuhikari	1974	4	M	–	–	Total 17 strains
Minamiwase	1977	9	M	4	7	Kuroishi, Tottori, Kochi, Ibaragi
Miyukiaoba	1983	5	M	14	4	Nigata
Haruaoba	1987	6	E	3	0	Tottori,Miyazkil,Kuroishi
Orchardgrass						
Aomani	1967	4	E	1	0	Kariwano in Akita prf.
Kitamidori	1968	2	E	6	0	Hokkaido 6 places
Okamidori	1976	2	M	1	7	Eastern Hokkaido
Akimidori	1976	4	M	6	2	Okayama, Fukishima,Tottori
Makibamidori	1981	4	M	4	4	Fufushima
Wasemidori	1987	2	M	5	3	Eastern Hokkaido
Timothy						
Senpoku	1967	1	E	17	0	Hokkaido ecotypes 17 strains
Nosappu	1977	1	M	2	2	Aomori, Kushiro
Kunpu	1980	1	M	2	1	Hidaka and Kitami (Hokkaido)
Tall fescue						
Hokuryo	1972	2	E	1	0	Tukisamu (Hokkaido)
Dallis grass						
Natsugumo	1980	10	E	1	0	Shikine in Kagoshima prf.
Red clover						
Sapporo	1966	2	M	–	35	Hokkaido zairai
Nishiaka	1974	4	M	3	36	2 from Hokkaido, 1 from Chiba
White clover						
Kitaooha	1971	3	M	1	36	Morioka
Alfalfa						
Kitawakaba	1973	2	M	9	5	7 from Hokkaido, 2 fromHonshu

[a] Refer to Figure 1 for member of breeding station
[b] Refer to Table 3 for classification of breeding materials

TABLE 5 Number of ecotypes of forage grasses collected in Japan in 1984–86.

Specoes	North[a]	Central[b]	South[c]	Total
Timothy	50	27	4	81
Orchardgrass	73	76	137	286
Tall fescue	39	38	197	274
Perennial ryegrass	9	4	8	21
Italian ryegrass	5	52	112	169
Rhodes grass	0	0	152	152
Coloured guineagrass	0	36	0	36
Red clover	75	32	52	159
White clover	56	35	126	217
Alfalfa	19	95	2	116

[a] Hokkaido and Tohhoku.
[b] Kanto, Tookai, Kinki and Hokuriku.
[c] Chugoku, Shikoku and Kysushu.

PART 6

Evaluation of genetic diversity through advanced methodology

Chairman
M. Yamada

CHAPTER 27

Ecotype differentiation and geographical distribution of indige nous rice varieties in Yunnan province of China based on esteras isozyme loci

XIONG JIAN HUA, WANG HUSI YI, INOUE MASAKATSU AND NAKAGAHRA MASAHIRO

To improve rice varieties in Yunnan province in China, a collaborative project betwe Yunnan Academy of Agricultural Science, China and the Tropical Agriculture Resear Center, Japan was initiated in 1981. Since then, we have focused on the utilization indigenous Yunnan rices bearing resistance genes for pests and diseases, cold toleran during growth and flowering stages, and the other helpful agronomic traits.

As one of the aims of this collaborative study on rice breeding, we are trying to evalua indigenous varieties of rice in Yunnan province as mother materials for future breeding.

In order to clarify the genetic diversity of cultivated rice varieties in the province and evaluate them on the level of varietal groups, we attempted to find the extent of isozym polymorphism. As the first step, we adopted the esterases, because it is well known that the have many loci and wide variations within the species of *Oryza sativa* L.

Materials and method

MATERIALS ANALYZED

The materials used totalled 426 varieties as shown in Table 1. Of them, 392 varieties wer obtained from Yunnan Province and were mostly indigenous except for 49 improve varieties. In addition, 13 varieties were from other provinces, 15 from Japan and 6 from othe southern countries of Asia.

To make geographical distribution clear, the Provinces were divided into seven regions i accordance with the climatic and agroecological conditions as shown in Figure 1 and Tabl 1. Ten plants seeded were heavily fertilized at 4–5th leaf stage so as to be given a clear enzym activity.

TABLE 1 **Materials used and their seed sources.**

No.	Regional division	Number of varieties		
		Indigenous	Improved	Total
1	Northwest region of Yunnan Province	11	0	11
2	Mid-north region of Yunnan Province	30	38	68
3	Mid-west region of Yunnan Province	6	0	6
4	Southeast region of Yunnan Province	18	11	29
5	South region 1 of Yunnan Province	129	0	129
6	South region 2 of Yunnan Province	65	0	65
7	Southwest region of Yunnan Province	84	0	84
	Total in Yunnan Province	343	49	392
8	Other Provinces of China	6	7	13
9	Japan	1	14	15
10	Other Asian countries	6	0	6
	Total	356	70	426

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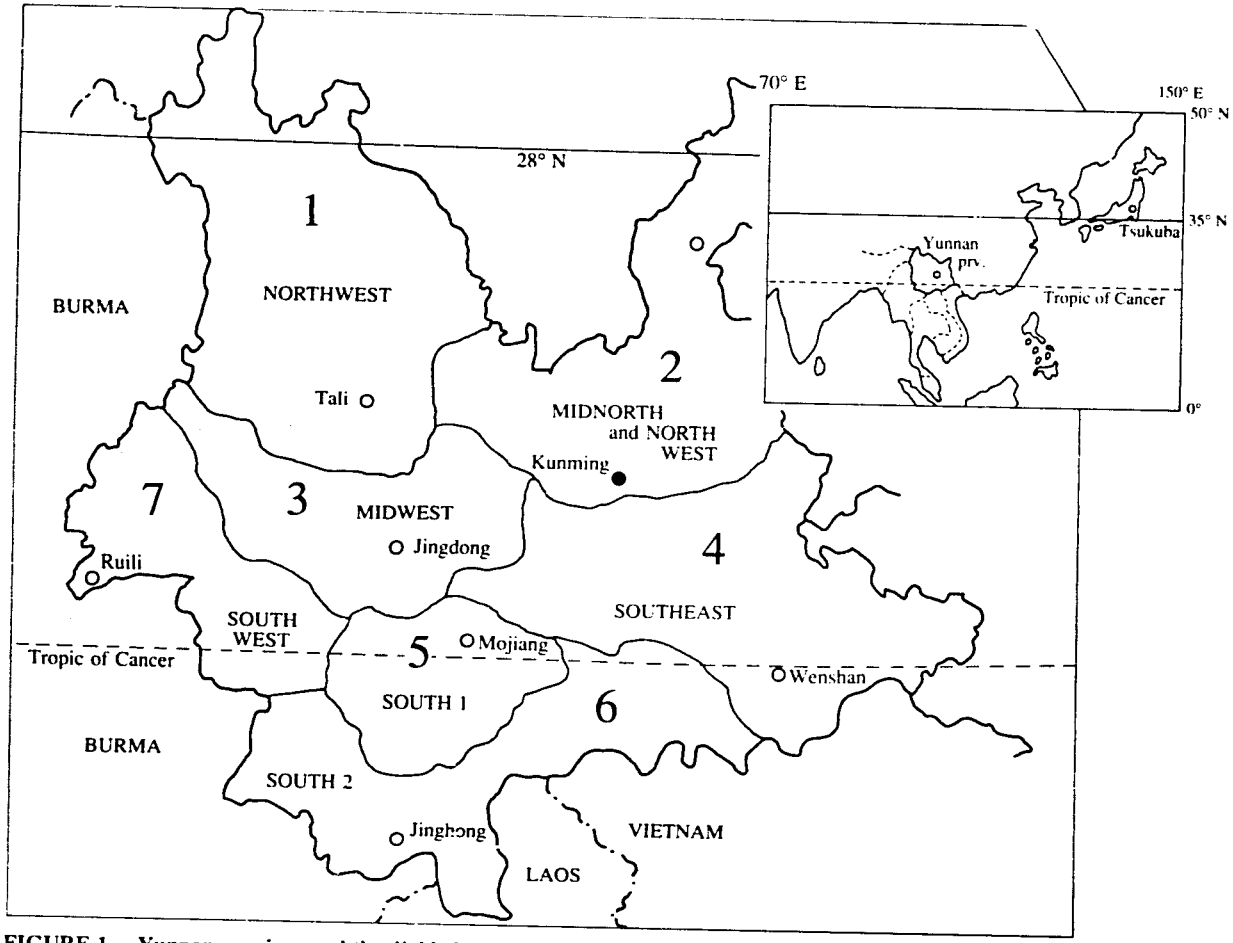

FIGURE 1 Yunnan province and the divided regions.

Extraction of the materials

After four days of fertilizer application, 2–3 plants (whole parts of top) were sampled and immediately stored at -15°C until use. The frozen materials were mashed by a specially developed homogenizer attached to an electric drill to obtain well crushed tissues (Nakagahra et al. 1976). The crude extracts were prepared without the addition of any buffer solutions or distilled water to optimize the intensity of enzyme activity. They were then safely stored under the original conditions for several months.

Electrophoresis

Electrophoresis for esterase isozymes were performed by the horizontal agar-gel thin-layer method (Nakagahra et al. 1975). The gel medium contained 2% of agar and 5% of polyvinylpyrrolidone in a beronal buffer solution (pH 6.8). After electrophoresis, the activities of esterases on the plate were stained by the spray method using α,β-naphthylacetate as the substrates and Fast Blue B salt for the staining.

Results and discussion

Genetic variations on esterases in Yunnan Province

Activities of esterase isozymes were observed in all kinds of bands. Nine alleles out of four loci which were already known from their genetic control and a new allele, Band 2A, were traced as the markers. They were Est-1, Est-2, Est-3, and Est-4 has 2 (1A and null), 3(6A, 7A and null), 2(12A and 13A), and 2(10A and null) alleles, respectively. Band 2A migrated close to 1A but faster than 1A, so that it has a possibility of an allele of Est-1.

Indigenous varieties of Yunnan Province had all the kinds of allelic variations which were theoretically expected, but also had a new allele representing Band 2A (Table 2). Figure 2 demonstrates a zymogram variation concerning Est-1, Est-2 and Est-3. Figure 3 shows the presence of Band 10A (Est-4). Figure 4 is an example of a new zymogram concerning Band 2A. Then, it was generally recognized that the province has wide allelic variations like the neighbouring areas of Burma, Thailand and Laos, where rich diversities were displayed as reported earlier (Nakagahra 1986a).

TABLE 2 Percentage occurrence of each allele in the materials used.

Source of varieties	Alleles observed									No. of varieties
	Est-1	Est-1°	Est-2^{S}	Est-2^{F}	Est-2°	Est-3^{S}	Est-3^{F}	Est-4	Est-4°	
Yunnan Province	90.8	9.2	10.5	14.5	75.0	57.7	42.3	32.9	67.1	392
Other provinces in China	100.0	0.0	0.0	7.7	92.3	61.5	38.5	15.4	84.6	13
Japan	100.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	15
Other Asian countries	16.7	83.3	33.3	0.0	66.7	66.7	33.3	0.0	100.0	6

Distribution of gene frequencies

The gene frequency of materials used was 90.8% in Est-1, 9.2 in Est-1°, 10.5 in Est-2^{S}, 14.5 in Est-2^{F}, 75.0 in Est-2°, 57.7 in Est-3^{S}, 42.3 in Est-3^{F}, 32.9 in Est-4 and 67.1 in Est-4°, respectively (Table 2).

After dividing the province into seven regions based on the climatic and ecological conditions, the relationships between the regions for the gene frequencies of the loci were compared. The number of alleles in the southern regions, 5, 6, and 7, was larger than that in the northern regions, 1 and 2 (Table 3). Average gene diversity (H) was lowest in region 1

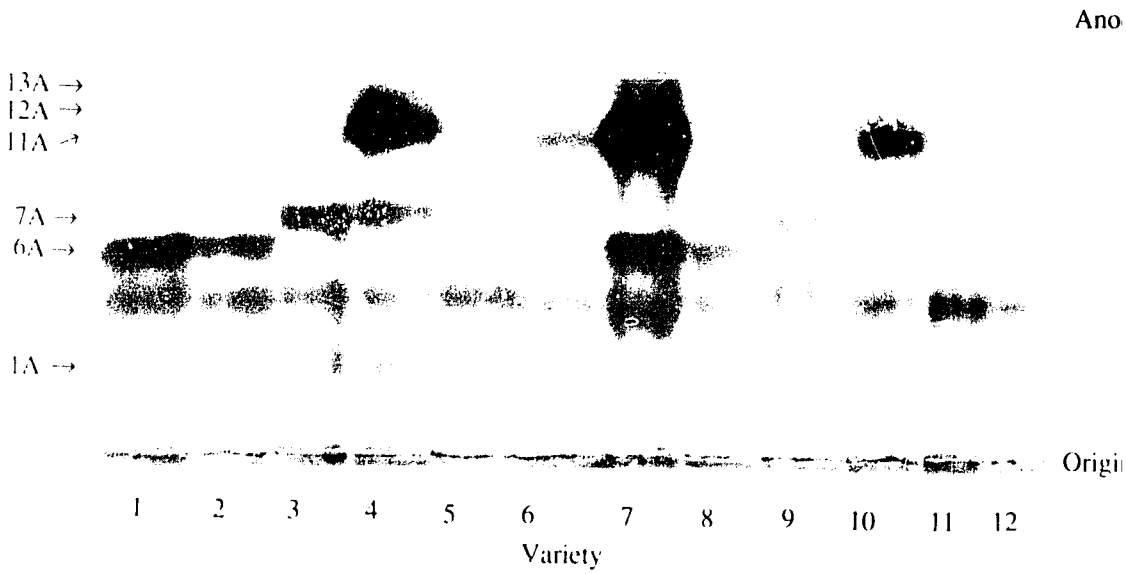

FIGURE 2 All possible combinations of esterase isozyme genotypes expected by 7 alleles of 3 loci found in Yunnan rice.

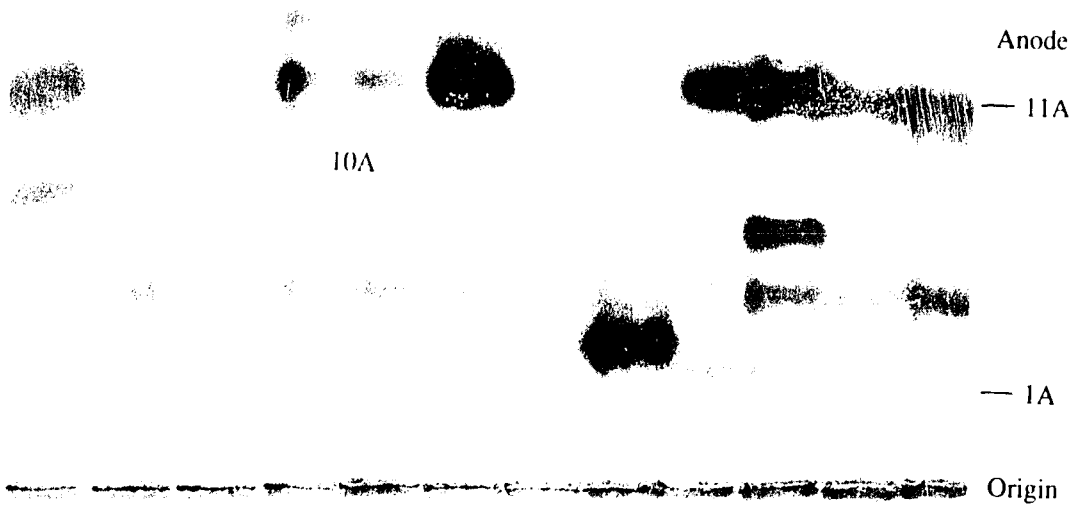

FIGURE 3 Examples of zymogram variations and a new isozyme of esterases, Band 10A, found in Yunnan rice.

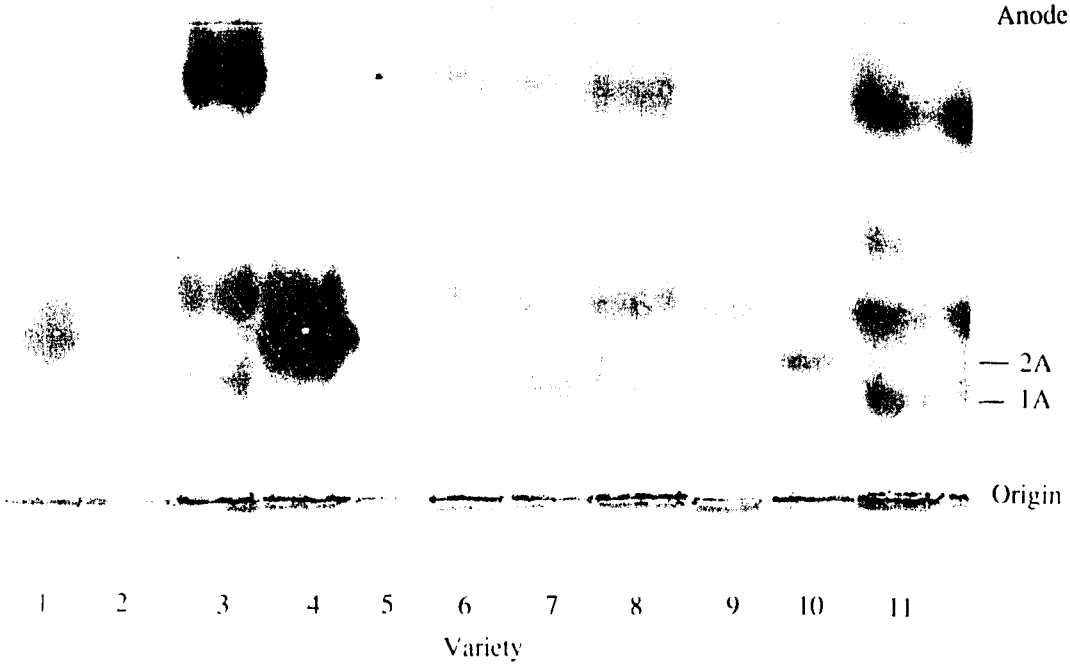

FIGURE 4 **Zymogram patterns showing a newly found isozyme allele, Band 2A, in southern parts of Yunnan province.**

Variety No. 1, 4 and 10 has band 2A, respectively.

TABLE 3 **Distribution of allelic frequency of each region of Yunnan Province and its gene diversity.**

		Frequency of allele in												
No.	Region[a]	Est-1	(2A)	Est-1[c]	Est-2[b]	Est-2[f]	Est-2[e]	Est-3[b]	Est-3[f]	Est-4	Est-4[e]	No. of alleles	H[b]	No. of varieties
1	Northwest	1.00	0.00	0.00	0.00	0.09	0.91	1.00	0.00	0.00	1.00	5	0.04	11
2	Mid-north	1.00	0.00	0.00	0.00	0.00	1.00	0.83	0.17	0.07	0.93	6	0.10	30
3	Mid-west	1.00	0.00	0.00	0.17	0.00	0.83	0.50	0.50	0.50	0.50	7	0.32	6
4	Southeast	0.94	0.06	0.00	0.11	0.11	0.78	0.67	0.33	0.33	0.67	9	0.34	18
5	South 1	0.97	0.03	0.01	0.10	0.09	0.81	0.48	0.52	0.40	0.61	10	0.34	129
6	South 2	0.74	0.26	0.03	0.17	0.28	0.55	0.59	0.52	0.28	0.72	10	0.46	65
7	Southwest	0.83	0.17	0.07	0.14	0.14	0.71	0.45	0.55	0.34	0.66	10	0.42	84
	Total	0.91	0.02	0.07	0.11	0.14	0.75	0.58	0.42	0.33	0.67	10	0.39	392[c]

[a] Regions are divided into 7 as shown in Table 1.
[b] H: Average gene diversity (NEI 1975)
[c] Includes 49 improved varieties.

(Northwest), and increased towards the south. The frequency of each allele has a constant geographic tendency in the direction of south to north. These results indicate that varieties in the southern part of Yunnan Province comprise a wider range of genetic variations than the North in the isozyme alleles.

TABLE 4 Geographical cline in the frequencies of esterase genotypes in Yunnan Province.

Genotype[a]	Regional division						
	Northwest	Mid-north	Mid-west	Southeast	South 1	South 2	Southwest
1				1 (5.6)	3 (2.3)	1 (1.5)	3 (3.6)
2			1 (16.7)		4 (3.1)	1 (1.5)	
3							
4						1 (1.5)	
5					2 (1.6)	4 (6.2)	4 (4.8)
6					6 (4.7)	6 (9.2)	2 (2.4)
7						1 (1.5)	
8							1 (1.2)
9		2 (6.7)	2 (33.3)	1 (5.6)	36 (27.9)	8 (12.3)	14 (16.7)
10		4 (13.3)			14 (10.9)	3 (4.6)	15 (17.9)
11						1 (1.5)	
12							1 (1.2)
13							2 (2.4)
14					4 (3.1)	2 (3.1)	4 (4.8)
15					1 (0.8)		
16						5 (7.7)	3 (3.6)
17						1 (1.5)	3 (3.6)
18					4 (3.1)	2 (3.1)	
19						1 (1.5)	
20						3 (4.6)	1 (1.2)
21		1 (3.3)	1 (16.7)	5 (27.8)	7 (5.7)	1 (1.5)	2 (2.4)
22	11 (100.0)	23 (76.7)	2 (33.3)	11 (61.1)	45 (34.9)	19 (29.2)	21 (25.0)
23							
24					2 (1.6)	3 (4.6)	1 (1.2)
25					1 (0.8)	2 (3.1)	6 (7.1)
No of genotypes	1	4	4	4	13	19	16
Total No of varieties	11	30	6	18	129	65	84

[a] Genotypes in a gamete phase are 1: E-1E-2^{S}E-2^{F}E-4, 2: E-1E-2^{S}E-3^{F}E-4°, 3: E-1°E-2^{S}E-3^{F}E-4, 4: E-1°E-2^{S}E-3^{F}E-4°, 5: E-1E-2^{F}E-3^{F}E-4, 6: E-1E-2^{F}E-3^{F}E-4°, 7: E-1°E-2^{F}E-3^{F}E-4, 8: E-1°E-2^{F}E-3^{F}E-4°, 9: E-1E-2°E-3^{F}E-4, 10: E-1E-2°E-3^{F}E-4°, 11: E-1°E-2°E-3^{F}E-4, 12: E-1°E-2°E-3^{F}E-4°, 13: E-1E-2^{S}E-3^{S}E- 4, 14: E-1E-2^{S}E-3^{S}E-4°, 15: E-1°E-2^{S}E-3^{S}E-4, 16: E-1°E-2^{S}E- 3^{S}E-4°, 17: E-1E-2^{F}E-3^{S}E-4, 18: E-1E-2^{F}E-3^{S}E-4°, 19: E-1°E- 2^{F}E-3^{S}E-4, 20: E-1°E-2^{F}E-3^{S}E-4°, 21: E-1E-2°E-3^{S}E-4, 22: E-1E-2°E-3^{S}E-4°, 23: E-1°E-2°E-3^{S}E-4, 24 E-1°E-2°E-3^{S}E- 4°, and genotype 25 is the others concerning Band 2A. The gene symbol E is the abbreviation of *Est* of the esterase locus.

Geographical cline on the genotypes in Yunnan Province

Clear geographical clines of genetic markers are frequently found by their genotypic combinations in some crop species. Rice plants are a good example as reported earlier (Nakagahra et al. 1975). Genotype frequencies combined by the four isozyme loci, which amount theoretically to 24 combinations as shown in Figure 5, were calculated in each region. The largest diversity of the genotypes was observed in the southernmost regions in the

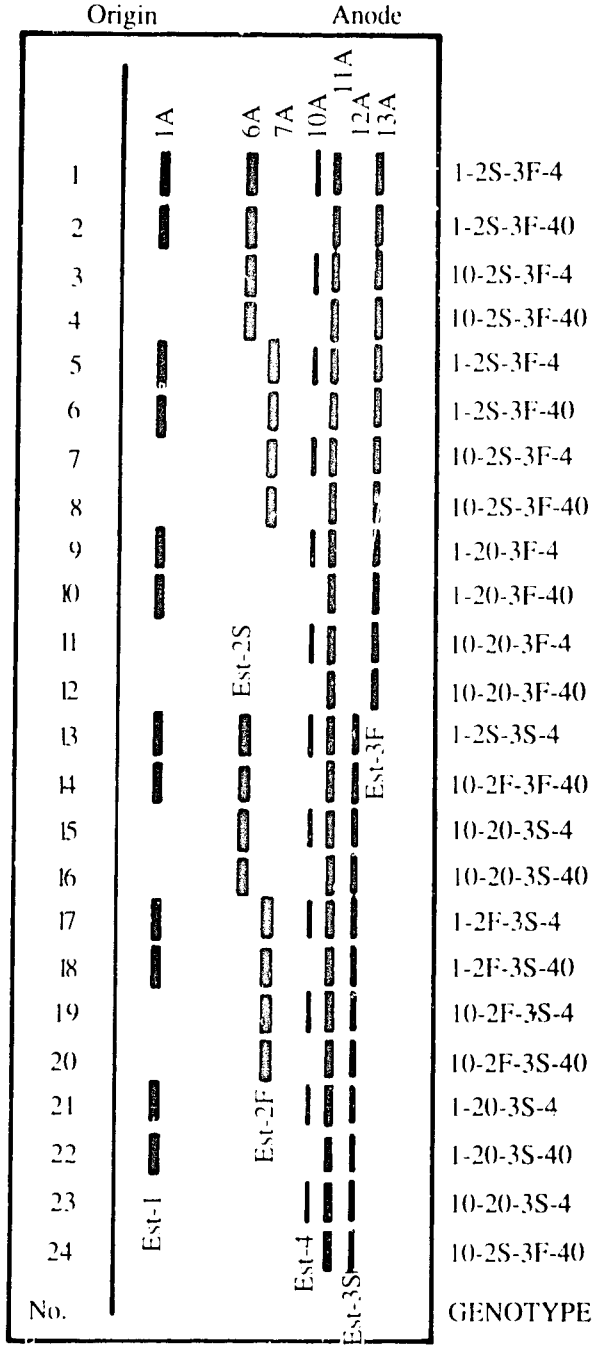

FIGURE 5 Zymogram patterns expected by the combinations of 4 esterase loci. Yunnan rices have all kinds of genotypes except No. 23.

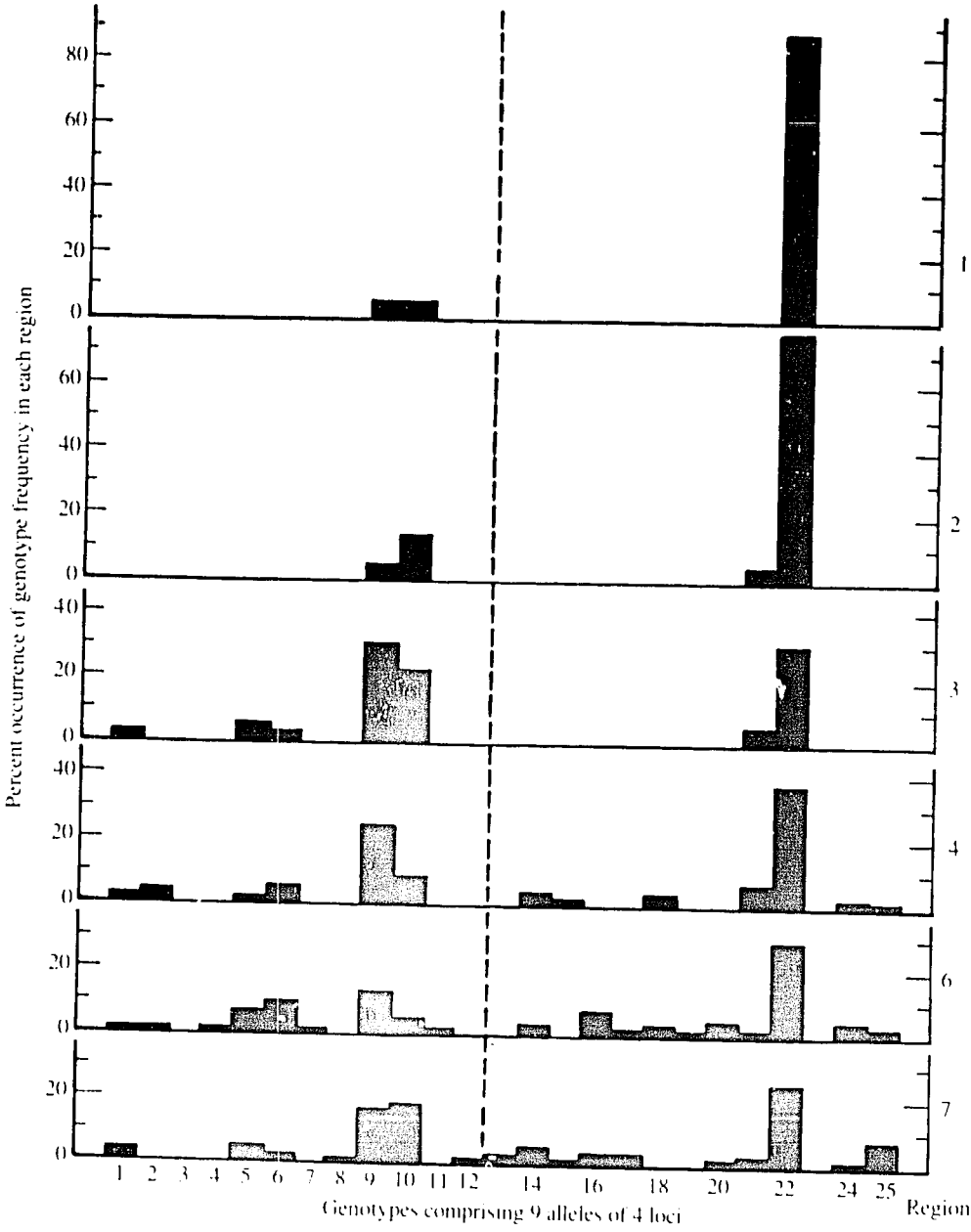

FIGURE 6 Geographic cline of esterase genotypes of native in Yunnan.
More than 90% of varieties of rices can be separated into Indica and Japonica by the vertical dotted line. Varieties on the left side are mostly Indica, and those on the right mostly Japonica.

vicinity of Laos and Burma. No dominant varietal groups were noted in these regions. The distribution of some of the genotype frequencies was clearly dependent on geographical locations. Namely, the degree of diversity exhibited was greatest in the southernmost regions; it decreased toward the north and only a few genotypes were observed in the northernmost regions (Figure 6).

Genetic variations of Yunnan rices and their classification

Yunnan province is located in the subtropical area, but has large differences in climate because of its topography. As an example, the altitude of rice cultivating areas, ranges from 70 m to 2 600 m above sea level in the south–north direction. The northern parts are mostly highland, and the southern parts are hilly areas.

The present experiments indicate that the southern part of Yunnan province is included in the centre of genetic diversity of Asian rice varieties as reported in previous studies.

On the other hand, the materials in the present study include some kinds of varietal groups such as Indica (Hsien rice), Japonica (Keng rice), Javanica (Nuda or Guangqiao rice, or Hill rice). The Japonica group (Keng rice) was cultivated in the northern regions in most cases, whereas all kinds of varietal groups were cultivated in the south.

Furthermore, there are some varieties from the southern regions that are not definitely classified yet, when we use the other qualitative and quantitative characters or agronomic and biological traits such as the resistance of pests and diseases, the water requirement, the termperature stress and some of the reproductive barriers. In fact, Yunnan rices have valuable agronomic characters such as blast resistance, cold tolerance, various degrees of endosperm contents, and so on.

It is noted that indigenous varieties of Yunnan province hold an important position not only to classify and distingush Asian rices but also to evaluate as useful genetic materials for the future. They are still expected to be collected comprehensively and to be analyzed genetically.

Yunnan rices, then, require further study in relation to evaluation of their genetic constitution for various other characters in plant genetic resources work.

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CHAPTER 28

Aspects of the evaluation and utilization of wild germplasm in *Brassica* in Japan

K. HINATA

Seed storage of *Brassica* and its wild allies is carried out in the Laboratory of Plant Breeding, Tohoku University under financial support from the Japanese Ministry of Education. This laboratory is also designated by IBPGR for storage of one of the base collections of wild relatives of cruciferous crops. The wild germplasm is used for studies on the taxonomical and evolutionary relationships in this taxon and on the utilization of wild germplasm for biosciences. Investigations concerning germplasm of wild brassicas recently carried out in our laboratory are reported here.

Consideration of the species relationships in subtribe *Brassicinae* in view of cluster analysis of morphological characters

The taxonomic relationships of 53 species (122 strains) in subtribe *Brassicinae* were studied by using Euclidean distances calculated from 53 metric characters. Through cluster analysis applied to the species and subspecies in the genus *Brassica*, six large clusters were defined (Figure 1). By the same method, *Diplotaxis* was grouped into four clusters, *Erucastrum* into two, and *Sinapis* into two. These clusters coincided in general with the taxonomic sections of Schulz (1936), but some exceptions were noted. Most of the exceptional species were those placed in critical positions by cytological observations. It was found that cluster grouping reflected the cytogenetical relations of species fairly well.

Relationships among clusters and genera are represented by a Euclidean distance matrix, and the evolutionary trends of those taxa are discussed. Cluster B-3 composed of *B. fruticulosa* and *B. barrelieri* cytodems, cluster D-1 composed of *Diplotaxis siifolia*, *D. assurgens*, *D. catholica*, *D. tenuisiliqua* and cluster E-1 composed of *Erucastrum cardaminoides*, *E. varium*, *E. leucanthum*, *E. nasturtiifolium*, were closely related to each other forming a complex. This complex is closely related to *Sinapidendron* species which was considered to retain some characters in common with their putative ancestor by Gomez-Campo and Tortosa (1974). Species in the other clusters are considered to be derived from the hypothetical ancestor in the course of evolutionary divergence (Figure 2).

Chloroplast DNA variation among diploid species in *Brassica* and allied genera

The restriction enzyme cleavage patterns of chloroplast DNA of eleven diploid species of *Brassica* and its allied genera were compared by using five restriction endonucleases. A dendrogram of species relationships as shown by the estimated numbers of recognition site mutations was drawn. The species were grouped into two clusters at the 5 recognition site mutations (Figure 3). The clusters did not always correspond to genera recognized in current taxonomy, but good correspondence was observed with the grouping by fraction I protein large subunits. The species relationship as seen with the present method were significantly correlated with those found in cytogenetical studies, but not with those based on numerical morphological taxonomy. It was suggested that *Brassica* species have strong potential for morphological variation and they have diversified according to their habitats (Yanagino et al. 1987).

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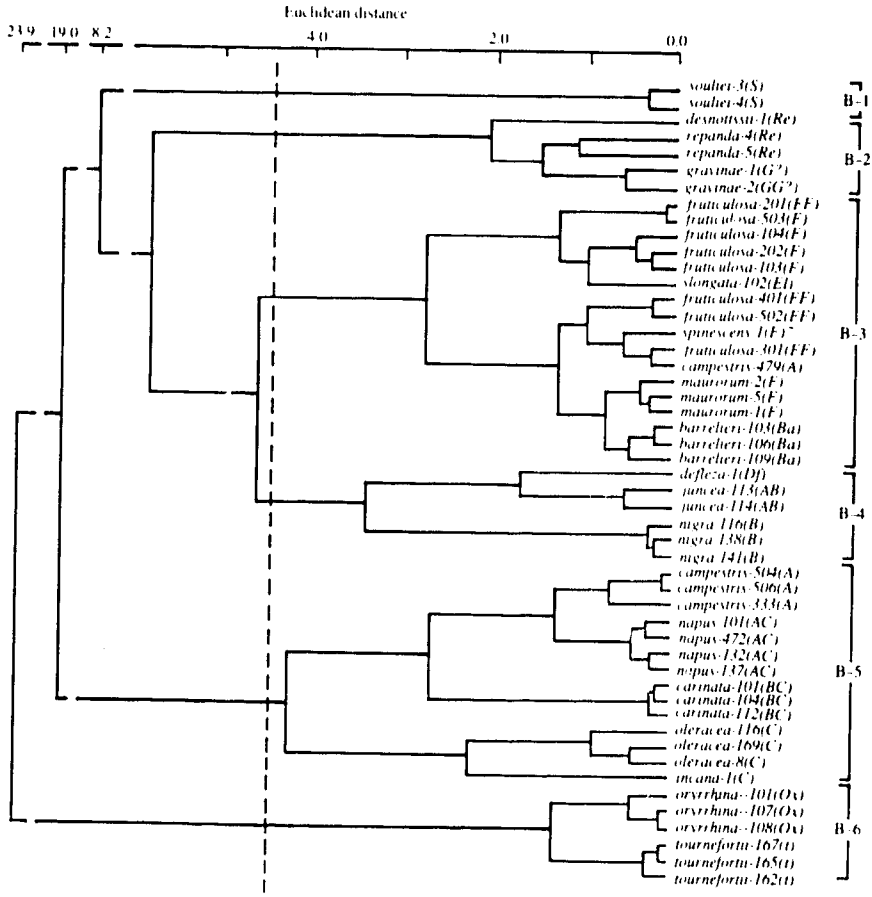

FIGURE 1 A dendrogram constructed by cluster analysis for 50 strains of 19 species in *Brassica*. Parentheses denote genomic symbols. (Adopted from Takahata and Hinata 1986)

Callus growth and shoot regeneration in the wild species of Cruciferae

The ability for callus proliferation and plant regeneration was surveyed for 79 strains of about 50 wild species in the taxon. Leaf segments were cultured on MS medium with 1 mg/l 2, 4-D to produce calli, and the growth of calli was evaluated. Response to a liquid culture was also examined in some species. Shoot regeneration ability from leaf segments and primary callus was evaluated on MS medium supplemented with 1 mg/l NAA, 1 mg/l 6-BA and 100 mg/l casamino acids. Many of the species produced brown coloured calli and their subcultured calli were not vigorous in growth. However, some species produced a whitish-yellow and friable callus, and the calli of *Diplotaxis tenuisiliqua*, *Sinapis arvensis*, *S. turgida*, *Moricandia arvensis* and *Lepidium sativum* were subcultured for more than four years without browning. The calli of *Brassica fruticulosa*, *M. arvensis* and *Armoracia rusticana* produced shoots when transferred to NB medium. Because culture conditions were limited in the present experiment, it is not always true that the browned calli have low potential for growth. However, it is clear that the vigorously-growing calli could be taken as experimental materials for tissue culture. From these data, *Sinapis turgida* and *Moricandia arvensis* were taken for the experiment of protoplast fusion. (Toriyama et al., in press).

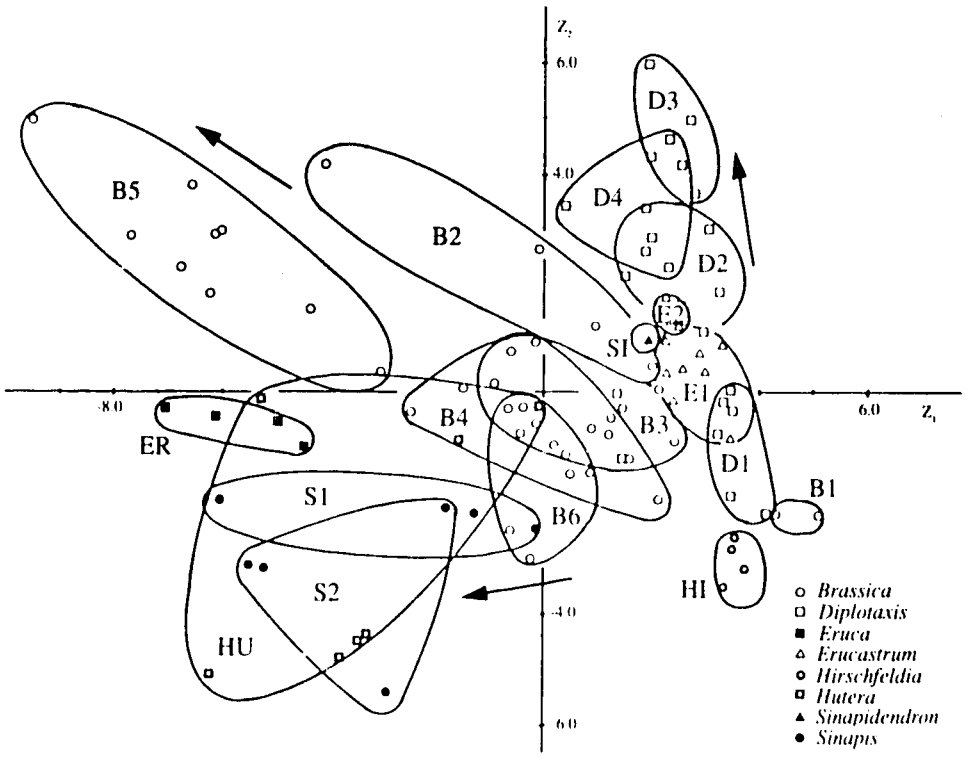

FIGURE 2 Clusters distributed on a plane defined by the first and second principal component axes obtained by Takahata and Hinata (1980). (Adopted from Takahata and Hinata 1986)

Selection of a universal hybridizer in *Sinapis turgida* and regeneration of plantlets from somatic hybrids with *Brassica* species

A double mutant cell line, which was unable to grow in a medium with NO_3^- as the sole nitrogen source and was resistant to 5- methyltryptophan (5MT), was selected from cell suspensions of *Sinapis turgida* by culturing the cell in AA medium supplemented with chlorate and 5MT. Protoplasts of this cell line were fused with mesophyll protoplasts of *Brassica oleracea* with dextran, and six somatic hybrids were selected initially by culture in the NO_3 medium supplemented with 5MT. The somatic hybrids produced embryoids, leaves and plantlets on a regeneration medium. The hybrid characters were confirmed by investigations of acid phosphatase and peroxidase isoenzymes, chromosome number, growth on NO_3 medium, 5MT resistance and capacity to regenerate plants. Somatic hybrids between *S. turgida* and *B. nigra* were also obtained using this method. These results indicate that the double-mutant cell line established here will be able to serve as a universal hybridizer to select somatic hybrids after protoplast fusion with any other wild-type partner (Toriyama et al. 1987a).

Production of somatic hybrid plants, 'Brassico-moricandia', through protoplast fusion between *Moricandia arvensis* and *Brassica oleracea*

Intergenetic plants were obtained through protoplast fusion between *Brassica oleracea* and *Moricandia arvensis*. Protoplast fusion was carried out by the dextran method using

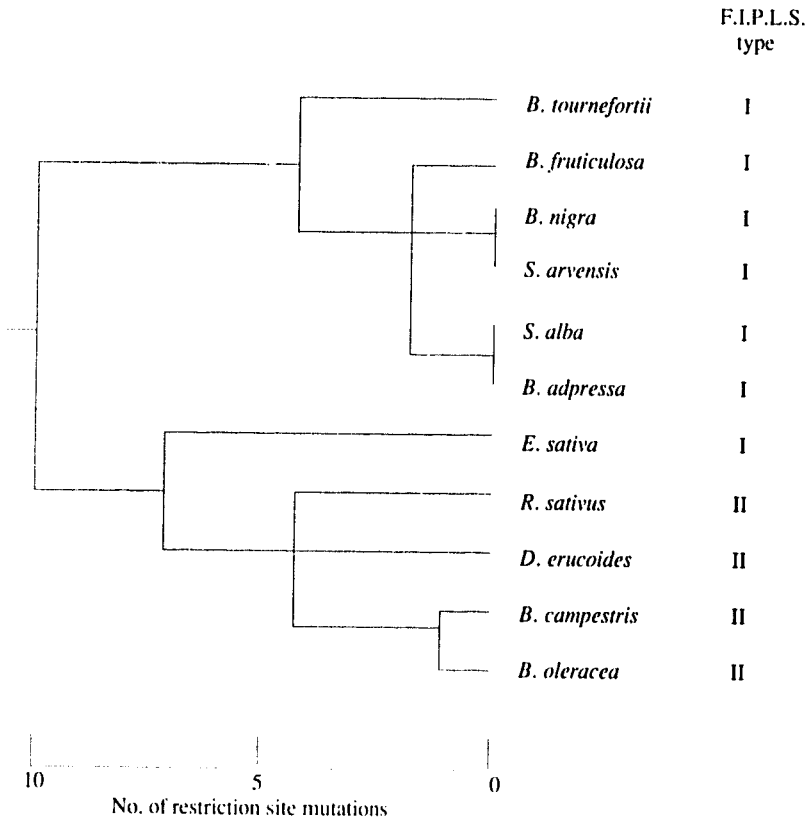

FIGURE 3 **A dendrogram showing the chloroplast DNA divergence and fraction I protein large subunit types. (F.I.P.L.S.: Uchimiya and Wildman 1987) of eleven diploid species. (Adopted from Yanagino et al. 1987)**

protoplasts isolated from hypocotyls of red cabbage and from suspended cells of *M. arvensis*. The fusion-treated protoplasts cultured in B5 medium with high concentrations of 2,4-D and 6-BA produced abundant calli, and from them, 8 calli produced a number of hybrid plants. The hybrid plants have come into flower. All the regenerated plants exhibited combined morphological characteristics of both parental species. Analysis of leaf isozymes for acid phosphatase and aspartate amino-transferase also indicated their hybridity. Even though no selection was performed for hybrid cells, all of the regenerated plants were hybrids. It is thought that the somatic hybrids exhibited hybrid vigour and readily produced shoots. Two somatic hybrid plants were also obtained between cauliflower and *M. arvensis* (Toriyama et al. 1987b).

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CHAPTER 29

Genetic differentiation and geographical distribution of barley

T. KONISHI

It is generally believed that cultivated barley originated from its wild progenitor, *Hordeum spontaneum* C. Koch, in the 'Fertile Crescent' of the Near East about 9000 years ago. By securing a wide range of genetic variation by spontaneous mutation and hybridization, barley has spread and adapted since then to different regions of the world through natural and artificial selections. Therefore, it is true that geographical regularity of gene distribution can be observed in barley varieties grown under different climatic and ecological conditions.

Takahashi (1955) first demonstrated the following geographical regularity of gene distribution mainly for morphological and physiological characters, using a large number of cultivars collected from different regions throughout the world. The 'normal' characters which are generally common to the wild progenitors and genetically dominant, are very prevalent everywhere in the world, while the numerous characters that have probably been generated under cultivation are found in more or less limited regions. From these results, it is apparent that the genetic changes which took place in the barleys of the Oriental region are quite different from those found in the barleys of the Occidental region.

The geographical distribution of several kinds of complementary genes for hybrid abnormality are presented here.

When different dominant complementary genes are combined into a hybrid by a test cross, the abnormal character appears in the hybrid, indicating that the test-crossed parent has the counterpart of the complementary gene in the tester stock. Although such an abnormal hybrid must be eliminated, either of the parents having one of the complementary genes is normal. Therefore, each of the complementary genes is thought to be 'neutral' to or indifferent to natural and artifical selections. The genic changes of various isozymes can also be regarded as neutral ones based on the definition. The territory of the neutral gene which has been included into a plant after mutation or introgression becomes wider by spreading its progeny, giving some useful information about geographical differentiation of barley.

Geographical distribution of complementary genes

Non-brittle genes

As is well-known, cultivated barley and its wild progenitor, *H. spontaneum*, have the same genome (n = 7), and are true diploids with no barrier in hybridization between them. In the process of evolution from wild to cultivated barley, the genic change from brittle to non-brittle rachis after ripening is of the greatest importance. The rachis brittleness is governed by two dominant complementary genes (Bt and Bt_2). And, if either of the genes mutated to be recessive, the plant then has non-brittle rachis. Takahashi (1955, 1963, 1987) has indicated that all the cultivated barleys have either of $bt\,Bt_2$ or $Bt\,bt_2$ genotype with only one exception ($bt\,bt_2$), since both loci are tightly linked with each other as the pseudo-alleles, and a doubly recessive recombinant seldom occurs.

The geographical distribution of these genotypes is not random in the world. The $Bt\,bt_2$ genotype (Type E) is mainly distributed in southern and central parts of Japan, Korea and China, and highlands of Nepal (mostly occupied by naked barley), while the other genotype, $bt\,Bt_2$ (Type W) is found in the remaining regions of the Old World, especially in lowlands

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of Nepal (mainly grown covered barley), Turkey, Europe and Ethiopia, as shown in Table 1. From the geographical distribution of both genotypes for non-brittle rachis, it is clear that the Oriental barley is different from the Occidental one in the evolutionary process of cultivated form derived from the wild progenitor.

TABLE 1 Geographical distribution of two non-brittle rachis genotypes, *BtBt* bt_2bt_2 (Type E) and *btbt* Bt_2Bt_2 (Type W), in cultivated barley.[a]

Region	Type E ($Bt\,bt_2$)	Type W ($bt\,Bt_2$)	No. of varieties tested
Japan (South)	88.9%	11.1%	99
Korea (South)	89.7	10.3	77
China (Proper)	98.5	1.5	69
Nepal (Highland, Naked)	100.0	0.0	74
Nepal (Lowland, Covered)	19.5	80.5	77
India & Pakistan	42.6	57.4	115
Southwest Asia	74.2	25.8	62
Turkey	14.9	85.1	222
Europe	25.5	74.5	267
Japan (North)	50.0	50.0	18
Korea (North)	62.5	37.5	32
China (North)	27.3	72.7	22

[a] Cited from Takahashi (1987).

Hybrid chlorosis genes

Among a large number of test crosses for non-brittle rachis genotypes, a conspicuous phenomenon was found in F_1 hybrids between Hayakiso 2 (a tester stock for non-brittle rachis, $Bt\,bt_2$) and some Ethiopian strains including Addis Ababa 12, which was characterized by chlorosis and stunted growth. Takahashi and Hayashi (1987) showed that the hybrid chlorosis is controlled by two dominant complementary genes, *Ch-a* and *Ch-e*, which are detected in Hayakiso 2 and Addis Ababa 12 respectively, and that their geographical distribution is regular and clear-cut. As is apparent in Table 2, the *Ch-a* gene is found abundantly in Asian regions, especially in Japan, Korea, China, Nepal, Pakistan and Afghanistan. On the other hand, the frequency of varieties having the *Ch-a* gene suddenly dropped in India, Turkey, Europe (including USSR), North Africa and Ethiopia, and the varieties with the recessive *ch-a* gene are abundant there.

As for the hybrid chlorosis, it is useful to examine the genotype in *H. spontaneum*, independent of brittle rachis. More than 60% of the strains of *H. spontaneum* have the *Ch-a* gene, while the other strains possess the recessive allele, *ch-a*, suggesting that the genetic change from *Ch-a* to *ch-a* had already occurred in the wild progenitor by a spontaneously recessive mutation.

Hybrid weakness

One more growth repression in F_1 hybrid was first found in the cross between Turkey 193 and a linkage tester, Col-orange, and named 'hybrid weakness'. The weak hybrid is characterized by only a few spikes on the shorter stems with remarkable shrived, yellowish leaves after the boot stage, and easily distinguished from normal plants. This is shown to be controlled by two different complementary genes, *Wek-a* and *Wek-t* (Konishi 1985).

A total of 1176 cultivars originating from different parts of the Old World, together with

TABLE 2 **Geographical distribution of hybrid chlorosis genes and hybrid weakness genes in cultivated and wild barleys.**

Region	Hybrid chlorosis[a]			Hybrid weakness		
	Ch-a	*ch-a*	No. of vars. tested	*Wek-a*	*wek-a*	No. of vars. tested
Cultivars						
Japan	64%	36%	(149)	98%	2%	(93)
Korea	77	23	(145)	100	0	(111)
China	54	46	(129)	100	0	(82)
Nepal	77	23	(148)	86	14	(126)
India	35	65	(62)	76	24	(90)
Pakistan & Afghanistan	62	38	(47)	79	21	(127)
Southwest Asia	30	70	(27)	77	23	(87)
Turkey	3	97	(137)	16	84	(155)
Europe	4	96	(141)	34	66	(135)
Ethiopia	0	100	(85)	4	96	(170)
Wild progenitor (*H. spontaneum*)	62	38	(100)	72	28	(123)

[a] Cited from Takahashi (1987).

123 strains of *H. spontaneum*, were crossed with each of the two tester stocks, Col-orange and Turkey 193 with the gene *Wek-a* and *Wek-t*, respectively, and the occurrence of weakness in the F_1 hybrids was examined (Konishi 1987). Turkey 193 is the unique variety having the *Wek-t* gene. As shown on the right side of Table 2, the frequency of varieties having the *Wek-a* gene is considerably different in geographical regions. That is, the varieties with the *Wek-a* gene are abundant in Asian regions, especially in Japan, Korea and China. On the other hand, in the regions west of Turkey inclusive, the frequency of the *Wek-a* gene suddenly drops, becoming almost null in Ethiopia. Thus, geographical distribution of the *Wek-a* gene is much similar to that of the *Ch-a* gene for hybrid chlorosis mentioned above. In *H. spontaneum*, 72% of the strains have the *Wek-a* gene and the remaining 28% have the recessive allele, *wek-a*. This result suggests that allelic differentiation at the *Wek-a* locus for the hybrid weakness had already occurred before appearing in the cultivated barley.

Geographical distribution of esterase isozyme genotypes

Kahler and Allard (1981) demonstrated that patterns of geographical distribution of alleles at four loci for esterase isozymes are not at random: European and North American collections are similar to each other, but differ from Asian collections in allelic frequencies. Stimulated by this finding, I have done similar work, using more than 3000 cultivars collected from different regions of the world, together with 157 strains of *H. spontaneum*. However, in the present study, geographical variation has been indicated as the frequency of genotype which consisted of allelic combination at the three esterase isozyme loci, Est_1, Est_2 and Est_4. Beause, these loci are tightly linked with each other on the long arm of chromosome 3 (Nielsen and Hejgaard 1985), and the data of genotypes is thought to offer more detailed information about geographical differentiation of barley.

A total of 30 genotypes for esterase isozymes are observed in the materials, and geographical distribution of 10 major genotypes (A to J) is shown in Table 3. A large genetic diversity for esterase isozyme genotypes is found in Southwest Asia, where cultivated barley

TABLE 3 Geographical distribution of barley varieties with different esterase isozyme in the Old World.

Region	Type	A	B	C	D	E	F	G	H	I	J	Others	
	Est_1	*Af*	*Ca*	*Ca*	*Ca*	*Pr*	*Al*	*Pr*	*Ca*	*Ca*	*Ca*		No. of
	Est_2	—	—	*Un*	*Un*	*Fr*	*Fr*	*Fr*	*Fr*	*Fr*	*Dr*		vars.
	Est_4	*Su*	*Nz*	*Su*	*Nz*	*At*	*At*	*Su*	*At*	*Su*	*Nz*		tested
Japan		++	++	++	++	±	±		±	++		±	732
Korea		++	+	++	+	±	±		±	+		±	462
China		++	++	++	++	+	±		±	+	±	±	178
Nepal				±	+	++	±	+	±	±		±	364
India					++	++	±	++	+	±	±	±	209
Pakistan		±			++	++	+	+	±	±			134
Afghanistan		++	+	+	+	++	++	+		+		+	100
Southwest Asia			+	+	±	+	++	++	+	++		±	100
Turkey				+	+	+	++	++	++	++	+		300
Europe			±	±	+	+	++	++	++	++	+	±	416
Ethiopia						+	++	++	+			+	228
Wild progenitor (*H. Spontaneum*)		+	±	±	±		±	±	+	+	±	++	157

++: >10%. +: 1–9%. ±: trace.

is generally believed to have originated, and 9 of 10 major genotypes (A to I) are detected in this region, together with other minor genotypes. On the other hand, the variation of genotypes becomes smaller in the regions apart from there. In South Asian regions including Nepal, India and Pakistan, only three major genotypes (D, E and G) can be detected. Compared with the regions mentioned above, East Asia (Japan, Korea and China) is occupied by somewhat different genotypes (A to D). While, cultivars of type I in these regions are thought to have been introduced from Europe, since they are distributed in northern parts of East Asia and have the same genotype or characters (eg. *bt* Bt_1 for non-brittle rachis, short-haired rachilla, resistance to Japanese mildew race I) often found in Europe. Further to the west four other genotypes (F to I) are frequently observed in Turkey and Europe that differ from those in Asian regions. It is noteworthy that Ethiopian cultivars have a large genetic variation in visible characters, but the kinds of esterase isozyme genotypes are limited in only two major types (F and G).

In *H. spontaneum* mainly collected from Southwest Asia, there are 24 esterase isozyme genotypes which are more than those of cultivated form there, suggesting that a large genetic variation in the wild species may have resulted from repeated hybridizations between the species and between wild and cultivated barleys, since the species is a partial open-pollinated plant. Further, a new allele, *Sp*, at Est_3 locus is found which cannot be observed in cultivated barley as yet.

Consideration of geographical differentiation of barley

The results presented above revealed that East Asian varieties were much different from those of Western regions of the Old World in their genetic constitution for several neutral genes, as well as for morphological and physiological characters, mentioned by Takahashi

(1955). It is generally believed that cultivated barley had originated in Southwest Asia on the boundary between the two regions, and that cultivated barley has been established through the differentiation–hybridization cycles (Harlan 1970). Thus, it is interesting to know the genic combination or genotype of cultivars for these neutral genes in different regions.

Table 4 indicates the frequencies of genotypes for hybrid chlorosis and hybrid weakness in each varietal group of *Bt Bt*, and *bt Bt*, genotypes for non-brittle rachis of cultivated barley in different regions of the Old World. As to the frequencies of the genotypes for hybrid chlorosis, it is clear that most of the East Asian varieties have the *Bt bt*, and *Ch-a ch-a* genotype (simply called *bt*, and *Ch-a* genotype), while the varieties in Western regions including Turkey, Europe and Ethiopia are mainly of the *bt* and *ch-a* genotype. In South and Southwest Asian varieties, the genetic constitution is much different from those of other regions. Namely, the varieties of *bt*, and *Ch-a* genotype are abundant in Pakistan and Afghanistan similar to East Asia, and those of both genotypes, *bt*, and *Ch-a*, and *bt* and *Ch-a*, are frequent in Nepal. On the other hand, the *bt* and *Ch-a* genotype are often found in India, and the *bt*, and *ch-a* genotypes are in S.W. Asia. A similar situation is observed in the relationship between genotypes for non-brittle rachis and hybrid weakness. East Asian varieties are principally of the *bt*, and *Wek-a* genotype, and those of Western regions are of the other genotype constructed of *bt* and *wek-a* genes. The complex genetic constitution is also found in South and S.W. Asian varieties, as shown in Table 4. The results indicate that East Asian varieties are characterized by the genic combination of *bt*, *Ch-a* and *Wek-a*, while Western varieties have the other genotypes of *bt*, *ch-a* and *wek-a*, suggesting that the three alleles on different loci had been spread as a set to East Asia and Western regions through the dispersal of cultivated barley.

TABLE 4 Frequencies of varieties having for hybrid chlorosis (*Ch-a* and *ch-a*) and hybrid weakness (*Wek-a* and *wek-a*) in relation to *Bt bt*, and *bt Bt*, genotypes for non-brittle rachis in different regions.

Region	*Bt bt*,		*bt Bt*,		No. of vars. tested	*Bt bt*,		*bt Bt*,		No. of vars. tested
	Ch-a	*ch-a*	*Ch-a*	*ch-a*		*Wek-a*	*wek-a*	*Wek-a*	*wek-a*	
Japan	**65%**	24%	3%	8%	(74)	**86%**	0%	12%	2%	(93)
Korea	**68**	19	13	0	(84)	**85**	0	15	0	(111)
China	**62**	20	7	11	(73)	**85**	0	15	0	(111)
Nepal	**44**	13	**35**	9	(126)	**51**	2	**35**	12	(126)
India	15	7	**52**	26	(27)	18	2	**58**	21	(89)
Pakistan & Afghanistan	**56**	19	6	19	(16)	**55**	12	21	12	(131)
Southwest Asia	**32**	**50**	0	18	(22)	**31**	13	**45**	11	(86)
Turkey	2	18	2	**78**	(85)	1	22	15	**62**	(138)
Europe	5	2	1	**72**	(127)	9	15	26	**50**	(139)
Ethiopia	0	2	0	**98**	(41)	1	8	3	**87**	(87)

Bold figures indicate the major genotype(s) in each of the regions.

This becomes more clear from the relationship between the genotypes for non-brittle rachis and esterase isozyme genotypes in cultivated barley, as shown in Table 5. Among esterase isozyme genotypes, the five genotypes (A-E) are abundant in East and South Asian regions, and most of the varieties with either of the genotypes have in common the *Bt bt*, genotype for non-brittle rachis. Meanwhile, the other four esterase isozyme genotypes (F-I) are found frequently in Turkey, Europe and Ethiopia, and their varieties have mainly the *bt*

Bt_2 genotype for non-brittle rachis. It has been made clear that the three esterase isozyme loci (Est_1, Est_2 and Est_4) are tightly linked with each others at the terminal of the long arm of chromosome 3 (Nielsen and Hejgaard 1985; Konishi and Matsuura 1987), while the two loci (*bt* and bt_2) for non-brittle rachis are closely linked as the pseudo-alleles on the short arm of the same chromosome (Takahashi and Hayashi 1964), indicating that the two allelic blocks for esterase isozymes and non-brittle rachis are inherited independently. Nevertheless, combination of allelic blocks is not at random, as if these allelic blocks are linked with each other.

TABLE 5 **Frequencies of varieties having *Bt* bt_2 and *bt* Bt_2 genotypes in different esterase isozyme genotypes.**

Esterase isozyme genotype		A	B	C	D	E	F	G	H	I	J	Others	
	Est_1	*Af*	*Ca*	*Ca*	*Ca*	*Pr*	*Al*	*Pr*	*Ca*	*Ca*	*Ca*		
	Est_2	—	—	*Un*	*Un*	*Fr*	*Fr*	*Fr*	*Fr*	*Fr*	*Dr*		
	Est_4	*Su*	*Nz*	*Su*	*Nz*	*At*	*At*	*Su*	*At*	*Su*	*Nz*		Total vars. tested
Non-brittle rachis genotype	*Bt* bt_2	98%	97%	92%	69%	76%	22%	28%	31%	19%	7%	60%	812
	bt Bt_2	2	3	8	31	24	78	72	69	81	93	40	769
No. of vars.		140	86	196	94	144	338	261	68	165	27	62	1581

As barley is an obligated self-fertilizing crop, a neutral gene once combined into a plant through mutation or introgression has been moved by the spread of its progeny, without any selection. Therefore, the geographical regularity of neutral gene distribution may suggest the regional differentiation and the direction of transmission of cultivated barley originated from its wild progenitor, *H. spontaneum*, in Southwest Asia, and offers valuable information about collecting the genetic resources in barley. It is noticeable that these results are able to be obtained only when the landraces, not improved varieties, are used as the materials. From the viewpoints of phylogeny, genetics and breeding, landraces adapted to different regions should be collected and preserved before genetic erosion occurs.

Acknowledgement

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PART 7

Problems of genetic resource management

Chairman
Dong Yushen

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CHAPTER 30

Management of genetic resources information in Japan

S. IYAMA

It is quite clear that a precise and well-prepared database is indispensable for the efficient utilization of genetic resources. Here I will consider two aspects of management of genetic information. One concerns the catalogues of germplasm conserved in the university that have been published recently in Japan. The other is related to the management systems for genetic resources information.

I am engaged in documentation of genetic stocks and experimental organisms preserved in the universities and research institutes under the Ministry of Education, another administrative category distinct from the Ministry of Agriculture, Forestry and Fisheries. As Dr Suzuki states elsewhere in this book, institutes belonging to MAFF preserve the plant germplasm very extensively. There is also an important collection of plant germplasm in the universities and research institutes mentioned above. They have been accumulated during the course of research work by collection, findings in the materials used, or artificially created mutants, etc. They are to be utilized as test materials for genetic and other biological research work as well as for breeding.

Catalogues of germplasm

Recently two catalogues of important plant germplasm collections in the university have been published.

In 1983, a catalogue of barley germplasm preserved in the Institute of Agricultural and Biological Sciences, the University of Okayama was published. It was edited by Professor R. Takahashi (1983), the world famous barley geneticist in that institute. Barley strains of known origin were collected from various regions of the world for his studies on the geographic distribution of barley varieties and their genetic differentiation. The catalogue contains about 4 000 cultivars with descriptions of some agro-morphological, physiological, pathological and genetic traits, accumulated during the course of research. In all 28 items were listed. They include some interesting characters for consideration by research workers, as well as plant breeders.

In 1984, a catalogue of *Aegilops* and *Triticum* germplasm preserved in the Plant Germplasm Institute, the University of Kyoto was published. It was edited by Professor M. Tanaka (1983). The catalogue listed about 2 400 strains over 24 *Aegilops* species, 4 400 strains over 23 *Triticum* species and 83 strains of artificially produced autopolyploids and amphidiploids. It mainly consists of the collections made by Professor H. Kihara and his students during botanical expeditions to Nepal, Pakistan, Afghanistan, Iran, Iraq, Turkey, Transcaucasus and the eastern Mediterranean countries, which were organized by Kyoto University between 1955 and 1978. Description include geographical and topographical information, such as the place of collection and the altitude of the collection site.

There is also a group of rice strains of marker genes which are mostly preserved in Hokkaido University and Kyushu University. These strains will be used for linkage analysis and other purposes. When the documentation and construction of a database for these materials were attempted by a group of geneticists, they faced the fact that standardization of gene symbols was urgently necessary. Thus geneticists concerned with this matter, formed a committee for rice-genetic resources information and discussed the standardization of gene

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symbols of rice and prepared rules of nomenclature. Later this activity was extended to the international scale and at the end it evolved into the Rice Genetics Cooperative, established by cooperation between the Japanese group and IRRI scientists in 1984.

The Rice Genetics Cooperative publishes the *Rice Genetics Newsletter* annually (Oka and Khush 1986). It contains up-to-date information on rice genetics. For instance, in the third and latest issue is the report from the Committee of Gene Symbols, Nomenclature and Linkage Groups. Dr Kinoshita, the convenor, proposed the rules of nomenclature for rice genes. Information on marker gene strains appear also in the Newsletter. The information on marker gene strains, which contained mutant characteristics, locations and their reference is being put into a computer database.

Information on barley germplasm at the University of Okayama has already been computerized, although it is not yet available to research workers. Information on *Aegilops* and *Triticum* germplasm of Kyoto University is also being processed into a database.

Management systems

I now move to the subject of management systems. Databases compiled so far have been designed for comparatively large-scale computers as part of a system that can respond to various requirements, such as access from any place by on-line inquiry or through an off-line service. However, a personal-type of handy computer became very popular recently and is widely used. Under these circumstances, it is desirable and of benefit to make a database fitted to and capable of being handled on a personal computer. Such a database packed on a floppy disk can be distributed on demand.

Databases may be stored on one or two floppy disks separated according to specific subject matter and served with an adequate management system. For instance, the catalogues mentioned earlier may be contained on such disks.

For the process of compilation, one may conveniently use commercially sold efficient software. After compilation, the database may be converted into more compact form so as to save memory space and to attain faster operation when used.

Another useful type of database contains graphic information. It can be effectively used to illustrate genetic resources. Video and laser disk recording systems connected to a computer will be conveniently used. However, at present, there is a difficulty in multiplication of such graphic information for distribution. I hope this will be solved in the near future.

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CHAPTER 31

Recalcitrant seeds: techniques for conservation

H.F. CHIN

Most seeds in general remain viable for longer periods when they are dried and stored at low temperatures down to -196°C. Harrington (1972) expounded on two rules of thumb which related the effects of seed moisture content and storage temperature. Reduction in moisture content and temperature increases life span of seeds. Roberts (1973) clarified the situation by introducing the terms 'orthodox' and 'recalcitrant' to describe the storage behaviour of seeds. He referred to seeds obeying Harrington's rule that is; seeds which can tolerate desiccation and freezing temperatures as orthodox while many other seeds that are readily killed by desiccation if the moisture content falls below a critical value (12–31%) and cannot tolerate freezing temperatures, these he classified as recalcitrant. Recently Hanson (1984) suggested a more accurate and descriptive term to call orthodox seeds desiccation-tolerant and recalcitrant seeds as desiccation-sensitive.

Recalcitrant or desiccation-sensitive seeds are very important to developing countries in the tropics. They form the backbone of the primary industries, covering crops such as rubber, cocoa, fruits and many important timber species. Yearly, millions of hectares of forests that are extremely rich and diverse in genetic resources have been lost. Forests have been unscrupulously exploited by logging, deforestation for agricultural and urban development. There is urgent need for genetic conservation, and various ways have been implemented to overcome this constant threat. Today it is a well-known fact that little attention has been given to the problems of storage of seeds of tropical fruit tree species (Hanson 1984). There is an abundance of storage problems yet to be solved in tropical plantation crops (Chin 1978). Williams (1984) in the book marking the tenth anniversary of IBPGR outlining the latest strategy, stressed that one of the strategies is that the work on clonal crops should be accelerated for major crops. At present, the conservation methods include both *in situ* and *ex situ* conservation by having forest reserves, botanical gardens and field genebanks.

Although conservation by seed germplasm bank is most economical, today even with the advancement in seed science and technology, there is no way recalcitrant seeds can be stored for long-term conservation. This paper therefore summarizes the present existing techniques for the short-term storage of recalcitrant seeds and also discusses the possibility of long-term preservation techniques. The storage of recalcitrant seeds in genebanks is physically and economically impractical. Hence other techniques such as cryogenic storage of excised embryos will be utilized and emphasized here as a potential technique for long-term conservation.

Characteristics and properties of recalcitrant seeds

Recalcitrant seeds are mainly large seeded, coming from trees under a moist ecological forest-type environment. They include many important tropical crops such as rubber, cocoa, tropical fruits e.g. mango, durian and jackfruit and timber species belonging to Dipterocarpaceae and Araucariaceae. The average weight and size far exceeds that of orthodox seeds. In general, they are heavier, with 1 000 seed weight often exceeding 500 g (Chin et al. 1984). This is both due to their high moisture content (ranging from 30% to 70%) and their large seeds.

Morphologically and anatomically recalcitrant seeds also show a wide range of variation.

These huge seeds are generally spherical or oval in shape. Many of the tropical fruit species have seeds covered with a fleshy or juicy arilloid structure or the fibrous drupes have thick endocarp and mesocarp which surround the true seed, some of them are indeed fruits in structure. They are also variable in their basic structure. For example, rambutan (*Nephelium lappaceum*) have the embryo axis attached at different locations between the unevenly-sized cotyledons (Chin 1975). A number of species display polyembryony e.g. mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*) and langsat (*Lansium domesticum*).

The critical properties inherent in recalcitrant seeds are their physiological storage behaviour which forms the basis of classification. They are very sensitive to desiccation. For example, cocoa seeds perish below 26% moisture content (Hor et al. 1984) and they cannot tolerate low temperature (15°C). This temperature is considered high for orthodox seed storage. The longevity of recalcitrant seeds is very short; it varies from a few days to a few months or a year under proper storage conditions. The other important property of recalcitrant seeds is the high moisture content even after they have been shed from the parent plant. They do not undergo maturation drying unlike orthodox seeds. These large seeds are generally high in moisture content, ranging from 30% to 70%. There is a great deal of variation in moisture content between individual seeds with a high coefficient of variation of about 13 as compared to 2 or 3% in orthodox seeds. In view of their large size, variability and high moisture content, they are indeed hard to handle and are thus labelled as recalcitrant.

Identification of recalcitrant seeds

Recalcitrant seeds are generally large with short life span. The chief characteristics and properties for the classification of recalcitrant seeds are based mainly on their sensitivity to desiccation and freezing temperatures. All these characteristics are typical but are not diagnostic. Roberts et al. (1984) warned that the diagnosis of the recalcitrant condition is not always straightforward. In the last decade there has been a number of cases in which seeds have been wrongly classified as recalcitrant when in actual fact they are orthodox seeds. Good examples are citrus (Mumford and Grout 1979) and cassava (Ellis et al. 1981) which have been formerly classified as recalcitrant but proved to be orthodox. Correct diagnosis and identification are of prime importance. The success or failure of seed storage depends on correct identification as conservation methods for each type are very different.

In view of the uncertainty and difficulty in their identification, IBPGR on the advice of the Advisory Committee on Seed Storage in 1981 ordered a thorough study to be made. The Universiti Pertanian Malaysia undertakes this study using a screening technique involving low drying temperature and humidity. This technique uses drying temperature around 20°C and low relative humidity with the help of desiccants (Chin et al. 1984). The main objective is to develop a method of desiccation ensuring that death is not caused by faulty drying at high temperature when seeds are moist or high in moisture content. Farrant et al. (1986) suggested freshly harvested seeds should be screened and recommended rapid drying because seeds become increasingly sensitive to dessication on storage.

At present a list of over 75 species from 29 families are provisionally classified as recalcitrant (King and Roberts 1979). But this again is subject to change and modification as more recalcitrant species are found and identified while others may be reclassified as orthodox. The list will undoubtedly grow longer as many species are yet to be confirmed for which evidence is still lacking.

Storage techniques for recalcitrant seeds

Reports from the past half century revealed that recalcitrant seeds can only be stored for short periods of a few months to a year. Although long-term storage is presently not possible, any improvement of short-term storage will be appreciated as improvements in these con-

ventional methods are valuable in easing the problems of field collection and transportation to genebanks. Besides, short-term storage is also important for the storage of carry-over seeds for the next season's sale or planting. A good example is the expedition to Brazil in 1876 to collect *Hevea* seeds in which 4% of the seeds were viable on arrival in Kew Gardens. From the latest expedition in 1981, Ong et al. (1983) reported greater success with over 65% germination of rubber seeds on arrival in Malaysia.

At present the successful short-term storage methods are limited to moist or imbibed storage, partial drying method and controlled atmosphere technique. The use of the conventional dry-storage method as for the orthodox seeds is not suitable for application to recalcitrant seeds. Roberts et al. (1984) is of the opinion that it is unlikely that the conventional methods of short-term storage of recalcitrant seeds be used as a method of germplasm conservation. The most promising method according to Roberts et al. (1984) is storage in liquid nitrogen, but so far there has been no success with truly recalcitrant seeds and the technical problems are formidable. Research work on cryogenic storage of recalcitrant seeds with a number of species proved to be a failure basically due to their exceedingly large size. Presently work is focused on using the excised embryonic axes which are very small and practical to store cryogenically and subsequently cultured aseptically in agar medium. This technique has great potential and has had to date some degree of success. Hopefully in the not too distant future, cyrogenic storage of excised embryos will become a practical method of germplasm conservation.

Moist or imbibed storage

As recalcitrant seeds are desiccation-sensitive, it is necessary to store them in moist media or even submerged in water. Moist storage has been in practice for many years, for a number of crops: rubber (Ang 1977), rambutan (Chin 1975) and cocoa (Evans 1953). The storage media are damp charcoal or sawdust or a mixture of both. Moist sand has also been used. The storage temperature ranges from 4°C to 20°C depending on species. With the moist storage technique viability can be maintained for about 3 months. The most extreme moist storage is by storage of seeds under water. Wild rice (*Zizania aquatica*) stored in water gave 86% germination after 14 months (Brown and Scofield 1903). Jones (1958) reported oak (*Quercus robur*) remained viable when stored in water. Ong and Lauw (1963) kept *Hevea* seeds under water; after one month storage, the percentage germination was above 60%. Similarly King and Roberts (1982) stored cocoa seeds using the imbibed storage technique.

Imbibed or moist storage which is short-term in nature has not been very successful mainly because of the various problems encountered in using this technique. The major problems are micro-organisms and germination during storage. Attempts have been made to overcome these problems i.e. prevent germination and control fungal growth during storage. The natural germination inhibitors in fruit juice have been generally unsuccessful for cocoa (Pyke et al. 1934) and rambutan (Chin 1975). The osmotic inhibitory influence of these natural inhibitors on seed storage has been unsuccessful (King and Roberts 1979). Artificial chemical inhibitors have been used such as sucrose, polyethylene glycol (King and Roberts 1979), sodium chloride (Mayer and Poljakoff-Mayber 1975) and abscissic acid (Ohkuma et al. 1963).

Partial dehydration technique

Moist or imbibed storage techniques have their limitations. Hence other methods such as partial dehydration of seeds has been tried experimentally. Based on the fact that recalcitrant seeds can be dried to a certain critical value of moisture content, but still relatively high compared to orthodox seeds, a new method has thus been improvised in our laboratory; this is the partial dehydration method. The process involves partial drying by air at low

temperature (20°C). Rubber seeds are processed, cleaned and treated with fungicide and subsequently surface dried to a moisture content of just above 20% before storage.

Hor (1984) in his method of cocoa seed storage, processed the seeds and reduced the moisture content to 33% to 35% by drying them in an air-conditioned room (22°C and 55% RH). After partial or surface drying, the seeds are then dusted with 0.2% w/w of an equal benlate–thiram mixture and packed in groups of 500 seeds in a thin (0.15 mm) perforated polythene bag (20 × 30 cm). The bags are stored in a loosely closed box in an air-conditioned room. Under these conditions they maintain a germination percentage of over 50% up to 24 weeks. This is an improvement over the imbibed methods where seeds can only be stored for 10–12 weeks.

For rubber this method has been fairly successful in prolonging the storage life of rubber seeds from the normal 3 months in moist storage to a year. Freshly collected rubber seeds are cleaned and soaked in 0.3% benlate and then they are drained and surface air dried. Seeds are stored in perforated polythene bags at ambient temperature (25° + 3°C). Viability of over 50% was achieved after a year of storage.

Controlled atmosphere storage

This method of storage involving the storage of seeds in various gases or in sealed containers has had little success. Seeds of cocoa while still in the pods are stored by waxing the whole pod (Pyke 1934). Durian seeds which are surface sterilized and then stored in sealed containers remained viable only for about 32 days (Soepadmo and Eow 1976). A timber species *Shorea talura* sealed in inflated polythene bag plus fungicide lasted for 6 months (Sasaki 1976). Sealed carbon dioxide atmosphere has been used also for cocoa seeds with slight improvement of up to 45 days (Villa 1962), similarly with rubber seeds when stored in controlled atmosphere of carbon dioxide (Ong and Lauw 1963). From the above examples, it is unlikely that this method has any practical application in the storage of recalcitrant seeds.

Cryogenic storage

For seeds to survive in storage at very low temperatures, they should be dried prior to freezing (Becquerel 1925; Lipman and Lewis 1934; Fedosenko 1974; Sakai and Nishiro 1975). At low-moisture contents there will be no injury even at -196°C (Stanwood and Bass 1978). According to Roberts et al. (1984) the most promising method of germplasm conservation for recalcitrant species is storage in liquid nitrogen. To date there has been no success in storing truly recalcitrant seeds as the technical problems are formidable. Attempts made in our laboratory for storing these very large seeded species have failed; their sheer physical size does not permit the use of this technique. An alternative method is required for vegetatively propagated plants and species with recalcitrant seeds. Withers (1980) and Bajaj (1985) suggested that in such cases where seeds are short- lived, germplasm could possibly be conserved through cryopreservation of excised embryos or their segments. It has been reported that materials dehydrated by drying in an oven (Sun 1958) or under vacuum (Withers 1979) exhibit remarkable resistance to cryogenic damage. Grout et al. (1983) have shown that embryos of oil palm (*Elaeis guineensis*) seeds which were formerly classified as recalcitrant can be successful cyropreserved after desiccation.

Even some orthodox seeds exhibit a higher threshold of moisture content that can withstand freezing temperature i.e. higher, high moisture freezing limit (HMFL). HMFL is the seed moisture threshold that will result in a decrease in viability of a seed sample during cooling in liquid nitrogen. Stanwood (1983) listed a range from the low 9.6% for sesame to a high 28.5% for bean. This shows the possibility that some embryos of recalcitrant seeds can be dried to below their HMFL and subsequently cooled and stored in liquid nitrogen. Grout (1979) has used low-temperature storage of imbibed tomato seeds as a model for recalcitrant seed storage.

In 1986 IBPGR funded a project on dehydration and preservation techniques of recalcitrant seeds at Universiti Pertanian Malaysia. Preliminary studies are concerned with the screening of recalcitrant seeds and embryos for their sensitivity to liquid nitrogen. Initially seeds and embryos were dried to various levels of moisture content, then they were cooled at different rates or plunged directly into liquid nitrogen. With this technique of partial desiccation of *Hevea* embryos to around the critical value of 15–20% (Chin et al. 1981), Normah et al. (1986) in our laboratory found that a moisture content between 14–20% (desiccation for 2–5 hours), 20–69% of the embryos survived cryopreservation for 24 hours and formed seedlings with normal roots and shoots when cultured *in vitro*. This is the first time a truly recalcitrant species has survived liquid nitrogen.

Those species which did not survive without cryoprotection were subsequently tested with treatment of single or combination of cryprotectants such as glycerol, DMSO and L-proline and various enriched media. To date embryos of a few species *Artocarpus heterophyllus*, *Nephelium lappaceum*, *Cocos nucifera* and *Dryobalanops aromatica*, have survived from cryopreservation and on culturing in enriched media showed signs of growth i.e. callus, shoots and roots (Chin et al. 1988). However these techniques have to be refined and they may differ from species to species.

A number of problems are encountered in our work such as browning of tissues in excised embryos with the release of phenolic compunds. In spite of all the problems, *in vitro* culture of embryos after storage in liquid nitrogen will be a potential technique for long-term genetic conservation. The IBPGR Advisory Committee on *in vitro* storage has discussed the advantages and disadvantages of using this technique in germplasm conservation. Today with the rapid advances in tissue culture, biotechnology and cryobiology, I am confident and optimistic that in the near future, this technique will be used in the conservation of germplasm for our future generation.

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CHAPTER 32

Low temperature conservation system for crop germplasm resources in China

HANG CHAO-YU

After the establishment of the People's Republic of China the Ministry of Agriculture and related leading departments organized several large-scale nationwide collections and supplementary collections of crop germplasm resources. In 1959, about 50 species of field crops with nearly 200 000 accessions (including some duplicates), were collected. However, some of these collections were lost during the 'Cultural Revolution'. In 1979 a nationwide supplementary collection was again made, from which around 60 species of crops with nearly 100 000 accessions (including some duplicates) were collected. In recent years, investigations of crop germplasm in Yunnan Province and Tibet Autonomous region have been made successfully. The investigations for certain single crops such as wild soyabeans, wild Chinese gooseberry and fodder vegetables have also been organized, from which a collection of 50 000 accessions of seed specimen has been achieved. If a deduction for duplicates and losses is made, the total number of accessions still reaches 300 000 which ranks China as one of the richest countries of germplasm resources in the world.

The tasks the government entrusts to the Institute of Crop Germplasm Resources (ICGR), Chinese Academy of Agricultural Sciences (CAAS) are responsibilities for: i) long-term preservation of crop germplasm resources; ii) unified management of crop germplasm i.e. introduction from abroad and international seed exchange; and iii) coordination of nationwide collection, preservation and evaluation of germplasm resources. Under the leadership of the state Science and Technology Commission and the Ministry of Agriculture, Animal Husbandry and Fishery (MAAHF) through planning, coordination and organization works, the conservation system for crop germplasm with duplicated accessions at two levels suitable to China has been established, i.e. a combination of national long-term preservation and regional (or professional) medium-term storage has been put into effect. In this system two copies of an accession are preserved in two different places to prevent unexpected losses. Meanwhile reduction in the number of duplicates among preservation units takes place according to a scheme of economic and proper conservation.

Storage at ICGR

Long-term storage of various crop germplasm of the whole country is performed by ICGR, CAAS in Beijing. The germplasm is preserved here as a base collection not for distribution, while short-term and medium-term storage of duplicates takes place for distribution, international exchange, evaluation and identification.

1. There are two national genebanks in ICGR, CAAS.

   Gene Bank No. 1 — This genebank, built in 1985, has a 111 m^2 cold room at a temperature of -10°C. The moisture content of standardized seed samples is reduced to 6–7% in a heating cabinet at not more than 40°C in a dehumidified room. Airtight cans are used for the storage of dried seed. Fixed metal shelves with drawer-like containers allow 50 000 cans to be accommodated. This genebank has also built-up seed processing facilities for seed receiving, examining, listing, cleaning, germination testing, drying and packing. Up to 15 August 1987, 39 499 accessions have been preserved in this genebak since the first acceptance of seed in 1985. Accessions are as follows:

Wheat	4 064	Broomcorn millet	1 500
Rice	7 584	Edible bean	2 767
Corn	4 088	Rapeseed	222
Barley	1 723	Peanut	719
Sorghum	5 263	Wild soyabean	2 597
Millet	3 642	Cultivated soyabean	5 282

Generally, each accession is put into three cans, which contain a sum of 10 000 grains for those below 5 grams per 1 000 such as millet, sesame and rapeseed etc; 6 000 for those with 20-100 g/1 000, such as wheat, rice and sorghum, etc; 2 500 for those with [illegible]-400 g/1 000, such as soyabean, pea and small red bean, etc; and 1 000 grains for those [illegible]ing 1 g each, such as broad bean and kidney bean, etc.

Gene Bank No. 2 — This genebank, partially financed by the Rockefeller Foundation for the construction, has two big cold rooms of 150 m² each, in which the temperature is -18°C and relative humidity 50%, with mobile metal seed shelves. The two rooms can accommodate a total of 400 000 accessions, with 200 000 accessions in each room. Besides, there are four smaller rooms (68 m² in total) in which the temperature and relative humidity parameters can be adjusted for the research work. The built-in seed processing facilities have been available since August 1987. For saving on energy the processed seed at present still goes to Gene Bank No. 1 until its capacity is full. Then one of the big rooms in Gene Bank No. 2 will be turned on and the whole storage in Gene Bank No. 1 will be transferred to it. Gene Bank No. 2 bears the task of long-term storage of 200 000 accessions in the period of the seventh '5-year plan'. It will also hold some international crop germplasm. On 25 March, 1987, The MAAHF consented to the request of the International Board for Plant Genetic resources (IBPGR) for basic collections of wheats from East Asia and worldwide collections of radishes and brassicas to be preserved in Gene Bank No. 2.

2. ICGR has a medium-term storage of 214 m² at 0°C. Fixed metal seed shelves are used to accommodate 100 000 accessions. Its main purpose is for distributing and exchanging germplasm with domestic institutions and foreign countries.
3. ICGR has a short-term storage rooms without an air-conditioner, with an average annual temperature ranging from 13.6°C to 15.3°C. In summer the highest daily temperature can rise to 25.1–30°C. Under such conditions, wheat seed can be preserved safely for 6 years or so. The seed stored here is to be used for identification.
4. ICGR has its own computerized database for the management of data from preservation, identification and evaluation.

The seed that is to be put into the long-term storage must be listed in the national catalogue, characterized in several aspects such as quality, tolerance to stress, etc. The seeds rejuvenated in various regions must be sent together with their documentation to the National Gene Bank. The data are then stored in computer for retrieval.

5. A field genebank and specific storages have been built for perennials and vegetatively propagated crops, respectively. Under the leadership of the MAAHF and with the coordination among regions, about 10 national stock plots for fruit trees and perennials have been built up. Preservation of potato and sweet potato seedlings in test tubes has been conducted in ICGR with an active *in vitro* genebank completed, in which seedlings of 300 accessions of sweet potato and 80 accessions of potato have been preserved up to now.
6. Wild relatives can be preserved either through field genebanks or natural protection zones. ICGR in collaboration with other related units has established germplasm stock plots at two sites for the preservation of wild rices and wild peanuts. In places where rich wild germplasm occurs, *in situ* reserves will be established.
7. The study of germplasm under super-low temperature storage in liquid nitrogen has been

started in ICGR, which may serve as a means of preserving certain specific germplasm resources.

Other storage facilities

Many of the professional institutes, such as the Institute of Cotton, CAAS, Institute of Oil Crop and Institute of Vegetable Crops, have established medium-term storages responsible for preservation of certain domestic and foreign germplasm. The Academies of Agricultural Sciences in provinces, cities and autonomous regions have established medium-term storages for preservation of germplasm material owned by themselves. But, it is not always so. In order to save energy and avoid excess duplications, some regional academies of agricultural sciences, instead of setting up their own medium-term storages, entrust the professional institutes in CAAS to preserve germplasm material for the medium-term range. Based on this condition, with proper adjustment and replenishment, a nationwide network for combination of medium-term storages with experimental bases of crop germplasm research work, is taking shape in the main ecological environments, which, besides medium-term storage of germplasm, carries out the test planting, characterization and evaluation of introduced foreign germplasm and works of propagation and rejuvenation for National Gene Bank. Up to now, medium-term storages that have been built everywhere are as follows:

1. Medium-term storage of 71 m^2 at 0°C located in Heilongjiang Academy of Agricultural Sciences, Harbin.
2. Medium-term storage with two rooms, of which one is of 57 m^2 at temperature 0°C and other 25 m^2 at -10°C located in Institute of Vegetable Crops, Beijing Academy of Agricultural Sciences.
3. Medium-term storage with two cold rooms of 45 m^2 each at -5°C, located in Hebei Academy of Agricultural Sciences.
4. Medium-term storage with two rooms, of which one is of 80 m^2 at 0°C and the other 20 m^2 at -10°C located in Shanxi Academy of Agricultural Sciences, Taiyuan.
5. Medium-term storage with two cold rooms of 66 m^2 each at 5–10°C, located in the Institute of Cotton, CAAS, Anyang, Henan province.
6. Medium-term storage of 30 m^2 at 0°C, in which fixed seed shelves are adopted with a capacity of 1 500 accessions, located in Hubei Academy of Agricultural Sciences, Wuhan.
7. Medium-term storage with two rooms, of which one is of 98 m^2 at 0°C and relative humidity 30% and the other 28 m^2 at -10°C and 30% humidity, located in Guangxi Academy Agricultural Sciences, Nanning.
8. Medium-term storage of 6 rooms with a total of 694 m^2, using natural arid and cold conditions (without air conditioner) located in Qinghai Academy of Agricultural Sciences, Xining. The storage can accommodate 280 000 accessions at an annual average temperature of 8.8°C and relative humidity 68.6% in the room. The germination rate of rice seed reduced from 100% to 95% after 4 years of preservation in paper bags and the germination rate of barley seed dropped to 86.0%.

Besides these, the Institute of Application of Atomic Energy in Agriculture, CAAS, and the Institute of Tobacco, CAAS have their own storages.Guangdong Province and Xinjiang Autonomous region are planning to build their own genebanks.

To sum up, low-temperature conservation systems for crop germplasm in China has just begun to take shape and certainly needs to be improved. ICGR is now facing much more work on coordination, both in scope and depth, with domestic and international institutions. We are ready to cooperate with any related departments and institutions in the world, and we firmly believe that through various ways of global cooperation, the work of conservation, characterization and evaluation of crop germplasm will be improved and advanced.

PART 8

Results and recommendations for action

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CHAPTER 33

The first IBPGR symposium on the Far East and the Pacific

D.H. VAN SLOTEN

Following the IBPGR proposal in 1977, and several planning meetings in Japan in 1978 and 1979, it was agreed that IBPGR would sponsor a 'symposium on the Genetic Resouces of the Far East and the Pacific', 21–24 October 1980, with support from the Government of Japan. The symposium was convened in Tsukuba, and participants included scientists from Japan, China and Korea, the Pacific island countries and countries bordering on the Pacific such as Australia, Canada and USA.

A summary report of the symposium was published by the IBPGR in April 1981 (IBPGR, 1981), while the full papers presented were assembled in a book which was also published in 1981 (Williams and Creech 1981).

Major recommendations of 1980 symposium

One of the major recommendations related to the recognition of two regions, the East Asian and the Pacific. The East Asian region comprised China, Japan, Korea and other countries of the region that wished to join. The Pacific region comprised the countries represented at the 1980 Symposium (Fiji, Solomon Islands, Western Samoa, Vanuatu, Tuvalu, Guam) and other countries encompassed by the South Pacific Commission (SPC) that wished to join.

Although not wishing to see regions of the world as isolated units, IBPGR recognized the usefulness of this subdivision for purpose of collaborative programs. It recognizes that the above two regions are closely linked with each other as well as with South and Southeast Asia.

Conservation

In reviewing the storage facilities available in 1980 in East Asia, the delegates of the symposium recommended the development of a plan for upgrading and expansion. This is described by Dr Tao in Chapter 35.

Exploration

The 1980 Symposium prepared a long listing of crops (and forages) with priority for exploration both within (indigenous resources) the East Asia Region and outside this Region (plant introduction requirements). A new list has been prepared and is reported in the recommendations on page 275.

Working groups and expert crop committees

Recommendations were made in 1980 for the formation of small working groups and/or expert committees to:

(i) facilitate the exchange of genetic material between the participating countries;
(ii) develop specific proposals for each priority crop (exploration, conservation, training);
(iii) finalize a timetable for specific plans for action.

These recommendations were not implemented, in part because of lack of resources, but largely because global advisory committees and working groups of IBPGR were addressing similar questions.

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TRAINING

The need for training has been recognized from the start by IBPGR and a large number of scientists from East Asia have attended IBPGR courses, including one course in Beijing which was entirely in the Chinese language. In addition, the yearly course on genetic resources offered by JICA (Japan International Cooperation Agency) has been and still is an excellent course for scientists from East Asia and neighbouring regions.

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CHAPTER 34

Perspectives of collaborative programs for germplasm collecting

MUNEO IIZUKA

Collection and conservation programs for plant genetic resources by IBPGR should be supported fully by goodwill and the cooperation of countries, organizations, individuals and others which adhere to the IBPGR philosophy. On the way to achieving this purpose, however, many problems surface during the process of determining the specific crops and their close wild relatives, and of specifying collecting sites for investigation. Problems also exist in conservation of materials even if collection was successful, and enormous efforts and close communication are necessary between the conservation centre in the region and IBPGR, to solve these problems.

Recently, with the help of the special project fund presented by the Japanese government to IBPGR, surveying missions of plant genetic resources were conducted twice to each region of Nepal (1984 and 1985) and Papua New Guinea (1986 and 1987). The purposes were a multicrops collection mission in Nepal, and for banana, taro, yam, sweet potato and indigenous vegetables in Papua New Guinea. I participated as a mission leader in all four missions, and here, with the experiences obtained in these missions, I would like to present some of my opinions on the collecting and conservation programs of IBPGR which are supported by international cooperation, and to suggest some future perspectives.

All responsibilities for the contents of this report belong to myself, and I would like to apologize for any erroneous matter due to my misunderstanding and ignorance.

Survey and collecting missions for plant genetic resources should be conducted as IBPGR recommends, and success of the missions will largely depend upon the following factors.

Cooperation with the host country

Cooperation should be sought in the country in which collections are to be made. This includes determination of surveying region and permission for the mission; assistance to and participation in the mission; and special permission to collect in areas designated as 'no foreigners': a) collecting by native personnel, b) special permits, c) others.

Funds

The budget should be finalized as early as possible; it should be determined a year before the mission with more than a 90% probability. This is especially important when the mission is planned in remote regions, because knowledge of the budget in the early stage may allow us to make better use of limited money in reservations for transportation, accommodation and other matters that are very critical in these regions.

When we wanted to use an UNDP aeroplane in Nepal, it was necessary to apply for it a year in advance. It also required 3 to 4 months before we could use aeroplanes and accommodation facilities of the mission in Papua New Guinea.

Survey missions to remote regions with an exceedingly reduced budget might not only face dangers during the mission, but also create a situation in which the plan itself might need to be reduced due to inflexibility.

Mission structure

Missions are best composed of personnel of different nationalities. Participation of members native to the collection region is essential, and it may determine the success of the mission.

The academic expertise of members should include genetics–plant breeding, plant pathology–applied entomology, cropping systems, plant classification, and other related fields. During the mission, members must trust each other and cooperate on every occasion.

Missions with different purposes should sometimes move separately. Joint surveys might reduce efficiency on both sides. For example, a mission surveying specific wild species may require investigations in areas different from those for primitive or indigenous cultivars.

Materials and foods

Some of the materials and foods taken on the survey may sometimes be wasted, but it is still desirable to obtain all necessary goods beforehand. This is particularly important when the mission is conducted in remote regions, as they can be used in exchange for precious seeds (as food or as true seeds and potatoes) as well as for our own food.

Insurance

IBPGR should insure the security of members in the mission. The exact period of the mission and the names of some members who will join in the collection site may sometimes be unknown beforehand. It is thus a common practice at present for members themselves to insure their own security. IBPGR must consider this problem and guarantee their security in the preliminary project report.

Regeneration

When collecting true seeds in remote regions, it is almost impossible to obtain theoretically enough seeds to cover and keep all genetic variabilities which the cultivar has possessed. Therefore, propagation is an important part of the project for long-term conservation, and the cost of this should be included in the budget.

Conservation

Taro and yam are important food crops throughout the countries of East Asia, Southern Asia and Pacific Islands. Construction of a long-term conservation centre for these crops or alternatively appointment of a certain organization for this purpose is strongly recommended. In these countries, main food sources are gradually moving to grain crops and genetic erosion of taro and yam is progressing.

Manpower

Many countries in East and Southern Asia and the Pacific Islands have a shortage of people who are able to handle problems of plant genetic resources, and thus, insufficient effort has been made to improve the situation. IBPGR should provide expanded long-term training sessions in addition to short-to-medium sessions, and East Asian countries should cooperate and support these activities.

Future perspectives

It is good to know that many East Asian countries have contributed as donors to activities of IBPGR. Japan started support to IBPGR in 1979 as a donor country, and since then the main

activity has concentrated on continuous support of collection and conservation of plant genetic resources in East and Southern Asia and the Pacific Islands. Fortunately, surveys in Southest Asia are progressing well, although many problems still exist. On the other hand, those in the Pacific Islands are far behind and need stronger future support.

Conclusions

It is suggested that future investigation and collecting of plant genetic resources should be advanced in East Asia and neighbouring regions of the Pacific Islands and Southeast Asia, and that enormous efforts and friendship of many countries including those in these regions are needed to achieve this purpose. In regard to preservation of these resources, especially taro and yam which are the main food resources in the regions, the establishment of a long-term conservation centre and education of supporting manpower are immediate needs. For that, the active support of East Asian countries is indispensable.

CHAPTER 35

Assessment of physical facilities for genetic resources in East Asia

KAR-LING TAO

During the last decade, IBPGR has developed a global network of base collections to safeguard the genetic resources of various crops. East Asia is obviously a very important link in the global network.

In order to facilitate long-term seed storage for germplasm conservation, a genebank must be well managed and must have proper physical facilities for seed drying, storage and testing. In this chapter we examine current long-term storage facilities in the region and provide some suggestions for future genebank construction.

Current situation of long-term storage facilities in the region

The East Asia region includes five countries, namely China, Japan, Peoples' Democratic Republic of Korea, Republic of Korea and Mongolia. According to the authors' knowledge, there is no long-term seed storage facility for germplasm conservation in either the Peoples' Democratic Republic of Korea or Mongolia at this moment, although a joint collecting mission between the German Democratic Republic and the People's Democratic Republic of Korea was carried out last year. The long-term seed storage facilities in the other three countries are discussed below.

China

In the early eighties, most provincial Academies of Agricultural Sciences (AAS) in China planned to build their own seed storage facilities for long-term conservation. However, it quickly became apparent that it was not economically feasible to have one or more long-term storage facility in each province. Scientists from the headquarters of the Chinese Academy of Agricultural Sciences (CAAS) and IBPGR successfully convinced most of the provincial AAS to establish medium-term storage facilities rather than long-term storage ones. At present, at least two long-term storage facilities at the provincial AAS have been completed, namely the genebank at Guangxi AAS, Nanning, Guangxi and the genebank at the Beijing Vegetable Research Center, Beijing. In these two genebanks, seeds were initially stored in paper bags at -10°C and 30% relative humidity (RH). However, due to the difficulty in maintaining RH at 30%, IBPGR provided aluminized bags and a bag sealer to these two genebanks in order that the seeds could be stored in sealed laminated aluminium bags.

The genebank at the Crop Germplasm Research Institute, CAAS, Beijing will serve as a primary National long-term store for the whole country. The first long-term storage room, 111m^2 in area, was built by the Chinese Government. The walls of the storage room are about 2 feet thick with 5 layers of cork insulation sealed with aluminium. The storage conditions are -10°C and 30% RH. Recently, the construction of the new Chinese National Germplasm Center (CNGC) was completed at CAAS, Beijing. There are 4 small regulated (temperature adjustable) rooms and two large long-term storage rooms. These long-term storage rooms have a storage capacity of 400 000 accessions. This is presently the world's largest genebank in terms of storage capacity. Seed will be stored in sealed cans at -10°C. The RH in the store room could, if necessary, be reduced to 30% RH, however the RH will not be controlled in the storage room since the seeds will be hermetically sealed. This genebank was expected to be in full operation by August 1987.

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Prior to the construction of the new National Germplasm Center, many provincial genebanks sent their collections to Qinghai AAS for storage. Here, germplasm is stored in natural environments without refrigeration and RH control. The storage conditions here are presented in Table 1. Although there has been no obvious decrease in faba bean and pea collections after 15 and 11 year storage respectively, the establishment of the new CNGC will greatly strengthen the storage facility for base collections in China. Furthermore, the BVRC has obtained funding from Japan to reconstruct its storage facility and seed testing laboratory.

In Taiwan, China, there are presently no governmental storage facilities for germplasm conservation. However, the government recently has decided to establish a genetic resource centre for germplasm conservation and utilization. Several million US dollars have been allocated for this purpose.

TABLE 1 **Natural storage of germplasm at Qinghai Academy of Agricultural Sciences.**

Location:	Xining, Qinghai, China (37°N, 102°E)
Natural environment:	Elevation 2295 m; rain 43 cm/yr
Average temperature:	Inside 9.7°C; Outside 12.5°C; Range 0–21°C
Container:	Sealed tin trunk
Result:	Faba bean, no obvious decrease after 15 years
	Pea,no obvious decrease after 11 years

Republic of Korea

The main genebank in the Republic of Korea is located at the Rural Development Administration (RDA), Suwon, which is not far from Seoul. This genebank has been described in detail in a recent article by Ahn (1987). The genebank is supervised by the Seed Management Division (SMD) — one of four research divisions of the RDA. SMD plays a leading role in the management of crop germplasm in South Korea. Facilities for seed storage at SMD include one long-term storage room (34 m^2, - 10°±1°C, 30–35% RH), one seed laboratory (143 m^2) and one machinery room (100 m^2). Seeds are stored in vacuumized aluminium cans. In order to reduce volume per sample, laminated aluminium bags have been recently used for long-term storage.

RDA plans to centralize the preservation of crop genetic resources which are now maintained in college and private seed companies. A new seed storage facility (140 m^2), with about five times the present seed store capacity, is presently under construction in SMD, RDA at Suwon. In addition to the SMD genebank, seed collections of various crops are stored at different experiment stations of RDA (Table 2). It appears that the storage conditions in the experimental stations are not as good as those at the RDA genebank. Upon completion of the new seed store, collections currently held at different experiment stations will be centralized and stored under better conditions.

Japan

In Japan, accessions of seed-propagated crops are maintained by four kinds of agencies: Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of Education, local governments and some private companies (Kawakami and Fujii 1981). MAFF formally coordinates crop-genetic resources activities. The Germplasm Storage Center is located in the National Institute of Agrobiological Resources (NIAR), Tsukuba, Ibaraki. NIAR was formerly called NIAS (National Institute of Agricultural Sciences). The NIAR genebank is well equipped and very modern. Seeds are stored at -10°±1°C, 30% RH in sealed cans for

TABLE 2 **Major seed collectons in different research institutes and experiment stations of the Rural Development Administration, Republic of Korea.**

Research institutes & experiment stations	Address	Major crops
Wheat and Barley Research Institutes	Tap-dong, Suwon 170	Wheat, Barley, Rye, Oats, Triticale
Crop Experiment Station	Seodun-dong, Suwon Suwon 170	Rice, Soyabean, Adzukibean, Mungbean, Cowpea, Kidneybean, Pea, Millet, Sorghum, Peanut, Sesame, Perilla, Rape
Mokpo Sub-Station of Crop Exp. Stn.	Cheongcheonri Cheongkemyun, Muahngun, Chunnam-do	Rape, Soyabean, Sesame, Cotton Sweet potato
Horticulture Exp. Stn.	Yimok-dong Suwon 170	Vegetable crops: Onion, Welsh onion, Burdock, Mustard, Cabbage, Chinese cabbage, Turnip, Red pepper, Garden chrysanthemum, Melon, Cucumber, Squash, Carrot, Lettuce, Tomato, Radish, Spinach
Livestock Exp. Stn.	Omok-dong Suwon 170	Grass and pasture crops
Honam Crop Exp. Stn.	Songhak-dong, Iri 510, Chunnam-do	Rice, Naked barley, Rape
Youngnam Crop Exp. Stn.	Naei-dong, Milyang-up Milyang-gun 605 Kyungnam-do	Rice, Malting barley

long-term storage. The area of the storage facility is 41.7 m^2. However, the NIAR genebank faces a shortage of storage space. A new building and new storage facilites are under construction and they should be completed by the end of 1987.

Evaluation of IBPGR designated base collections in the region

Five genebanks in East Asia have been designated by IBPGR to hold base collections of various crops (Table 3). In 1985, the IBPGR Advisory Committee developed standards for base collections. These standards have been described in detail in the Report of the Third Meeting of the Committee (IBPGR 1985). IBPGR has begun to evaluate all the designated base collections against a check list developed from these standards (Table 4).

The NIAR genebank at Tsukuba has recently been evaluated by IBPGR. This genebank is very modern and well managed. All data are computerized. The accession retrieval system is unique, having a robotic mechanism. Seed samples are obtained at the press of a button, with no need for any person to enter the cold room. As indicated in Table 5, the designated base collections at NAIR, Tsukuba meet all the IBPGR standards.

The AVRDC genebank has also been evaluated against the IBPGR standards. This genebank is well run and well equipped. It should be pointed out that the total cost of the Genetic Resource Unit in AVRDC, including construction and equipment is only about US$ 550 000. However, this genebank meets 7 preferred standards and all the other acceptable standards (Table 5). The floor plan of the AVRDC genebank is presented in Figure 1.

Basides the two genebanks discussed above, the genebanks at the Tohoku University and Kyoto University will be visited and evaluated in November 1987. Since the CNGC at CAAS will not be in operation until late 1987, this genebank will be evaluated in 1988.

TABLE 3 IBPGR designated base collections in East Asia.

Genebank	Designated crops	Geographic responsibility
NIAR, Tsukuba, Japan	Barley	Asia
	Wild wheat	Duplicate of Kyoto Univ.
	Maize	Asian
	Rice (japonica)	Global
	Soyabean	Global
	Vigna angularis	Global
	Sweet potato	Global
	Allium	Asia
	Crucifers	East Asia
	Sugarcane	Global
	Panicum	Global
	Chloris	Global
Tohoku University, Japan	Wild relatives of crucifers	Global
Kyoto University, Japan	Wild species *Triticum* and *Aegilops*	Global
CAAS, Beijing, China	*Brassica oleracea*	Global
	Raphanus spp.	Global
	Wheat	Regional
AVRDC, Taiwan, China	*Vigna radiata*	Global
	Sweet potato	Asia

Aspects needing special attention

The author has visited many genebanks in this region and all over the world. Regarding physical facilities the following considerations merit special attention. In the interest of fairness, the names of specific genebanks will not be mentioned.

Seed drying facilities

Some genebanks use air forced ovens to dry their germplasm collections. Seeds are dried at a temperature between 35°–45°C. When seeds have a high moisture content, they deteriorate rapidly at high temperatures. It should be noted that 40°–45°C has been used in the accelerated aging vigour test (Tao and McDonald 1983). For example, in the accelerated aging test for soyabean, seeds are kept at 40°C and about 100% RH for 64 hours. Low-vigour soybean seed will be killed and the seed moisture content increased from 10–25% during seed aging. Therefore, for germplasm conservation, seed aging should obviously be avoided. In addition, drying polyethylene primed vegetable seeds (such as onion and carrot) at 35° reduced the percentage of germination, increased mean germination time and percentage of abnormal seedlings compared with drying at lower termperatures (Gray et al. 1987). In the case of leeks, drying at 30°C was harmful to the seed. IBPGR strongly recommends drying seeds at 15°C and 15% RH.

Packing and drying rooms

Construction of a combined seed packing and drying room (15°C and 15% RH) was proposed in one genebank in this region, and was actually completed in another region. However, this is very impractical and is not recommended. Personnel working in the drying room will be dehydrated and feel uncomfortable. Furthermore, the average person releases 200 g of water per hour through breathing and evaporation, which could decrease the efficiency of seed drying. Therefore, the seed packing room should be separated from the seed drying room.

TABLE 4 A check list for genebank standards.

1. Free availability of material from core collection		A ___	N ___
2. Back-up generator	P ___	A ___	N ___
3. Fire precautions (Fire fighting equipment ___ , alarm system ___ , high temp. cut off ___ , lightening rod ___ ,		A ___	N ___
4. Security		A ___	N ___
5. Refrigeration standards and equipment	P ___	A ___	N ___
6. Safety of personnel (instruction ___ , alarm ___ , door open from inside ___)		A ___	N ___
7. Construction and insulation standards		A ___	N ___
8. Storage containers (Sealed can ___ , Laminated bag ___ , sealed bottle ___ , other ___)		A ___	N ___
9. Storage temperature (___°C, ___ RH)	P ___	A ___	N ___
10. Temperature monitoring	P ___	A ___	N ___
11. Seed moisture content (___ %)		A ___	N ___
12. Method and equipment for moisture content determination		A ___	N ___
13. Accession sizes	P ___	A ___	N ___
14. Regeneration standards (___ %)		A ___	N ___
15. Methods of regeneration		A ___	N ___
16. Initial viability monitoring		A ___	N ___
17. Viability monitoring period (___ yrs)		A ___	N ___
18. Viability monitoring test method	P ___	A ___	N ___
19. Equipment for germination test detection of empty seeds and removing dormancy		A ___	N ___
20. Handling procedures before storage		A ___	N ___
21. Seed drying equipment and conditions (___ °C, ___ %RH)	P ___	A ___	N ___
22. Seed cleaning		A ___	N ___
23. Information about samples (___ % passport data, ___ % partial passport data, ___ % characterization data)	P ___	A ___	N ___
24. Seed testing laboratory		A ___	N ___
25. Genebank personnel (No. of staff ___ , No. of accessions ___ , Seed physiologist in staff ___)		A ___	N ___
26. Agreement for duplicating samples	P ___	A ___	N ___

27. Site of duplication (If space is not enough, use the back of this paper)

Crop	Site	Base or active

28. Number of accessions of designated crops (If space is not enough, use back of this paper)

Crop	Number

29. Storage volume ___ m^3

30. Storage space availabel? Yes ___ No ___

Date _____ Name of evaluator _____

Genebank _____

(A = Acceptable, P = Preferred, N = Not within standards)

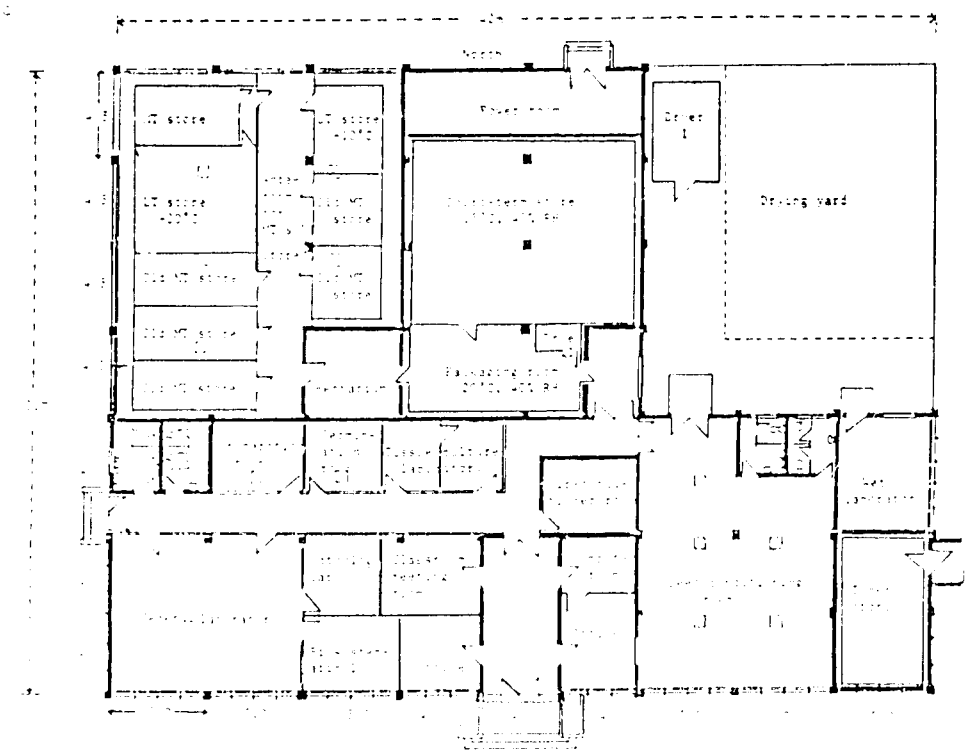

FIGURE 1 Floor plan of the GSRU Building at AVRDC, Taiwan, China.

TABLE 5 Summary of evaluation on designated base collections at NIAR and AVRDC against IBPGR standards.

Genebank	Standards achieved[a]		
	Preferred	Acceptable	Not acceptable
NIAR Tsukuba, Japan	10, 23	1–9, 11–22, 24, 25	None
AVRDC, Taiwan	5, 9, 10, 13, 21, 23, 26	1–4, 6–8, 11 12, 14–20, 22 24, 25	None

[a] Numbers refer to item number on check list in Table 4.

RH CONTROL IN STORAGE ROOM

Several genebanks in this region have problems in maintaining RH at 30%, due to mechanical or design problems. In addition, operating cost is a burden for genebanks, particularly in developing countries. However, as the seeds are stored in hermetically sealed containers, seed moisture contents will not be affected by the RH in the storage room. Therefore, there is no need to maintain RH, at least not as low as 30% RH, in the storage room, if seeds are stored in hermetically sealed containers that are properly designed to avoid water condensation damage and rusting.

TABLE 6 Example of calculation of stroage space for 5 000 accessions of soyabean and onion.

Crop	1000 seeds (cm^3)	4000 seeds (cm^3)	Volume required 5000 acc. (m^3)	Estimated required storage space (m^3)
Soyabean	175–200	800	4	12
Onion	2–9	36	1.8	5.4

STORAGE SPACE

There is a tendency to build large storage rooms regardless of the number of accessions and crops to be stored, particularly if funding is from a foreign county. Yet an oversize cold storage room is not encouraged, since the electricity costs will have to be paid for by the genebank itself. Prior to the construction of any storage facility, the numbers of accessions and crops should be estimated. Table 6 shows two examples of estimations of storage space required for soyabean (relatively large seed) and onion (small seed) collections of 5 000 accessions each.

Conclusions

Many storage facilities for genetic resources in East Asia are newly constructed. The genebanks at NIAR, Tsukuba and AVRDC, Taiwan both meet IBPGR standards. The completion of the NGC, CAAS genebank in Beijing greatly strengthens the germplasm conservation program in China and in the region. Although there are no long-term storage facilities in North Korea and Mongolia, new genebanks in RDA, Suwon, BVRC, Beijing and Taiwan are either under construction or in the planning stage. The outlook for long-term storage is very bright. Some of the experience in genebank construction in this region as discussed here could provide useful guidance for other regions.

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RECOMMENDATIONS

International workshop on crop genetic resources of East Asia

10–13 NOVEMBER 1987

The first 'Symposium on the Genetic Resources of the Far East and the Pacific' was held from 21–24 October 1980 in Tsukuba, Japan. The current Workshop, which can be considered as a follow-up to the 1980 Symposium, comprised 91 participants from ten countries and 8 participants from 3 international organizations. Seven status reports were presented as well as 25 research papers and 3 discussion papers. The Workshop reviewed organizational developments in countries of East Asia and adjacent areas, the utilization of genetic resources for produtivity, pest and disease resistance and stress tolerance, the potential of wild relatives, the collection and evaluation of indigenous germplasm in East Asia and advanced methods of evaluation and management of genetic resources. Towards the end of the Workshop reveiews were presented on progress since the 1980 Symposium, in terms of the implementation of earlier recommendations, collaborative programmes for germplasm collecting and physical conservation facilities in East Asia.

Participants noted the enormous strides forward in plant genetic resources activities in East Asia, especially with regard to germplasm collecting and exchange, the great expansion of physical conservation facilities and the increased utilization of germplasm for crop improvement.

The Workshop, in all its deliberations, reiterated the basic principle of free exhange of germplasm as the basis for current and future collaboration, both within and outside the East Asia region.

Germplasm collecting

1. The Workshop reviewed progress made on germplasm collecting and subsequently revised the exploration priorities. It recommended the use of the revised priorities as presented in Table 1 in planning future collecting missions. Special attention was drawn to the need to collect and conserve medicinal plants, under-utilised crops, and forest tree species.
2. The participants stressed the importance of continuing well-prepared collecting missions, both in East Asia and neighbouring regions. Wherever possible joint missions involving participants from the host country, the country of origin and other countries and/or agencies should be encouraged.
3. The Workshop, recognizing the genetic erosion among wild relatives of crops, their often unknown potential and their frequent use in breeding for disease resistance and other purposes, recommended that increased emphasis be placed on the collecting of wild relatives and also requested IBPGR to accumulate information on those cases where wild species have contributed to plant improvement.
4. Noting the lack of information on collecting missions by various institutions, the Workshop recommended that each country and/or agency nominates a coordinator on genetic resources to act as focal point for information on this and related subjects. The dissemination of such information could be stimulated through the issue of a regional newsletter (see recommendation 14).

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TABLE 1. Priorities for Exploration Activities[a].

Crop	Japan	China	Korea
Wheat	1C	1B	1C
Rice	1C	1A	1C
Barley	1C	2A	1B
Millet (common)	2C	–	3C
Millet (Italian)	3C	1A	3B
Sorghum	1B	2A	3B
Maize	1B	2C	1B
Brassica (oil)	2B	2A	2C
Sesame (oil)	2B	–	1B
Saccharum	3B	2C	–
Aroids	3B	2B	3C
Sweet potato	2B	3C	2C
Soyabean	1B	1A	1A
Mungbean	3C	2B	2B
Red bean	2C	2B	2B
Chinese cabbage	1C	1A	1C
Cotton	3C	2B	3C
Buckwheat	2C	2A	3B
Coconut		3-	–
Cassava	3-	3C	–
Yam	2B		
Eggplant	2C	2B	3C
Tomato	1-	1C	2C
Leek	2A	2B	2C
Cucumber	1C	2B	1C
Deciduous fruits	1B	2A	1B
Vitis	1B	1B	2B
Citrus	1B	1A	2C
Tropical fruits	2B	1A	2C
Forage legumes	1B	2C	1B
Forage grasses	1B	2C	1B
Bananas	3-	2C	3-
Tea	1B	1A	3C
Mulberry	1B	2A	2C
Groundnuts	3C	2-	2-
Bamboo			

[a] Priorities in terms of national needs are rated as 1 (high), 2 (medium) or 3 (low). Indigenous plant genetic resources for these crops (and forages) are rated as high (A), medium (B) or low (C); (-) no indigenous diversity.

Conservation

5. Noting that a large number of institutions in East Asia are involved in genetic resources conservation, the workshop recognized the need for more complete information, and recommended that national plant genetic resources inventories be prepared and relevant catalogues be regularly exchanged.
6. The workshop was informed about the status of those base collections in the East Asia region, which have accepted the IBPGR request for long-term storage of specific crops. It reiterated the principle that each of these should be duplicated in at least one other base collection. The Workshop recognized the need for additional designations for those crops which are not yet covered, such as *Setaria* and buckwheat, and recommended that IBPGR further investigate this in East Asia in consultation with the respective national programs.

7. The workshop recommended that more attention be paid to the conservation of vegetatively propagated crops of importance to East Asia and neigbouring regions, including taro, yam and coconut. Most of this germplasm is currently being conserved in field genebanks, and the meeting recommended that collaborative research be initiated on alternative conservation methods.

Training

8. In order to be able to assess future training needs, the Workshop recommended that IBPGR carries out a survey of past training programs and assesses future needs for trained manpower in East Asia and related regions.
9. The Workshop appreciated the role of the short-term technical training courses as provided by IBPGR, IRRI and JICA, and hoped that these organizations will continue this effort.
10. The Workshop recommended an expansion of the advanced training program to meet increasing needs for trained manpower in East Asia and related regions through MSc and PhD training. The latter might require special measures such as seeking additional funding.

Regional collaboration

11. In view of the rapid progress being made by national programs in East Asia, the Workshop agreed to strengthen collaboration, especially in regard to the exchange, evaluation and documentation/rationalization of existing collections. For this purpose, the Workshop recommended the establishment of Working Groups for crops of high regional importance (eg. wheat, barley, soybean). Such working groups would meet annually or biennially, to develop a plan-of-action and to exchange information and discuss progress being made.
12. The Workshop noted with interest the intention of the CAAS and IBPGR, to establish an IBPGR Field Office in Beijing starting from 1988. The Workshop expressed its desire that this Field Office should also fulfill a regional function in the East Asia programme.
13. Although the Workshop emphasized closer collaboration among countries in East Asia, it recommended continued links with other national and regional programs, especially those in the Pacific and Southeast Asia.

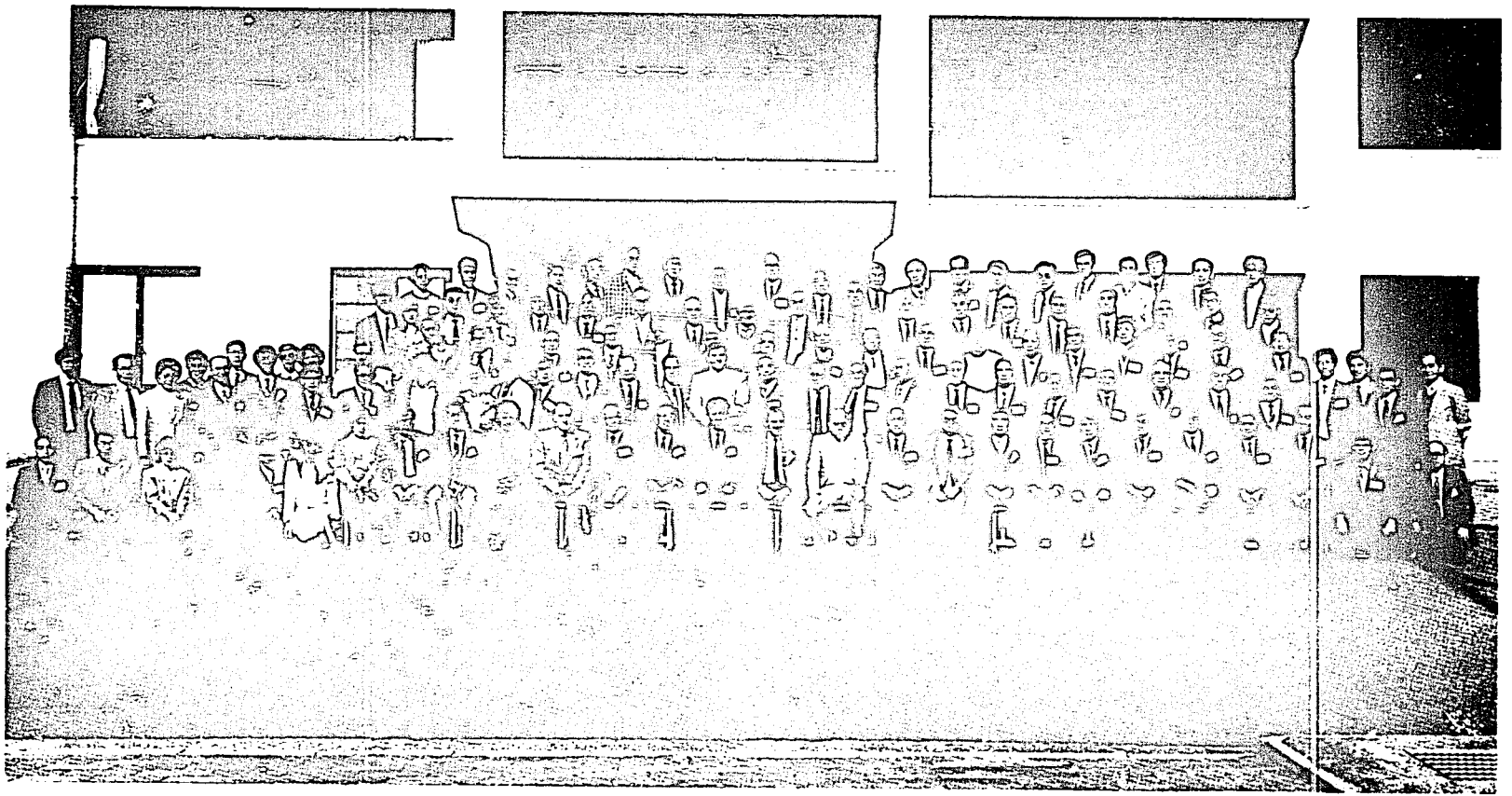
Newsletter

14. Recognizing the need for better communication, the Workshop recommended that IBPGR establishes a regional newsletter. This does not need to be restricted to East Asia alone, but could cover Asia and the Pacific in general.

Next workshop

15. The participants considered the current workshop of enormous benefit to the genetic resources programs in East Asia and recommended that a similar Workshop be organized in not more than five years from now and to involve every country in the region.

The participants of the Workshop wished to record their appreciation to the Government of Japan (Ministry of Foreign Affairs and Ministry of Agriculture, Forestry and Fisheries) for hosting this Workshop and for their generous financial support, also to the local organizing committee for their dedicated and untiring efforts and to the IBPGR for its action in the region and for sponsoring this Workshop.



INTERNATIONAL WORKSHOP ON CROP GENETIC RESOURCES OF EAST ASIA
10.13 NOVEMBER, 1987 TSUKUBA, JAPAN

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