Improving Reading Achievement

Left to right:

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Present and Future Strategies for Potato Breeding and Improvement

REPORT OF THE XXVI PLANNING CONFERENCE

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F O R E W O R D

This is the twenty-sixth Planning Conference and the fourth on breeding and genetics research sponsored by the International Potato Center. The purpose of the Conference is to review current research and to develop guidelines recommendations for the Center's research. Previous Conferences on research in Thrust II were held in April, 1974; October, 1977; and November, 1980.

The exploitation of the world germplasm collection maintained by the International Potato Center is aimed at improvement in potato yield with minimum farmer risk due to diseases, pests and environmental stress.

The objectives of the Planning Conference are:

1) To review the progress in the breeding program in the previous three years (November 1980-November 1983).

2) To determine the best strategies for incorporating new germplasm material into CIP's breeding priorities and under CIP's capabilities of multiplication to speed up distribution of advanced material with characteristics acceptable to national programs.

O. T. Page

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C O N T E N T S

	Page
Recommendations	1
Brief Review of Recommendations presented in "Utilization of Genetic Resources of the Potato III", November, 1980	3
Selection of Parental Stocks for Heat Tolerance and Earliness	7
Genetic Studies and Breeding of Resistance to Potato PLRV, PVY, and PVX	17
Breeding Bacterial Wilt (*Pseudomonas solanacearum*) Resistant Germplasm	45
Ploidy Level Manipulation Approach: Development of Diploid Populations with Specific Resistance and FDR 2n Pollen Production	57
Inbreeding in TPS Progenies: Implications for Breeding and Seed Production Strategy	71
Selection of Uniform Progenies to Use TPS in Commercial Potato Production	87
Apomictic Approach to Introduce Uniformity and Vigour into Progenies from True Potato Seed (TPS)	99
Utilization of Wide Crosses in Potato Breeding	115
Use of Meiotic Mutations in Breeding	133
Population Breeding Applied to Improvement of Unadapted Solanum Cultivated Species	143
Use of CIP's Germplasm in National Program: The Brazilian Program	163
Microspores, Protoplasts, and Somatic Hybridization as Tools in Potato Breeding	175
A Perspective View of the Potato Variety Improvement Programme of India	189
Participants	197
Agenda	201

AUTHOR INDEX

	Page
Atlin, G.	71
Brown, C.B.	17
Chujoy, J.E.	133
De la Puente, F.	163
Foroughi-Wehr, B.	175
Hermsen, J.G.Th.	99,115
Hermundstad, S.A.	133
Iwanaga, M.	57
Jongedyk, E.	99
Kohler, F.	175
Leue, E.F.	133
Mendoza, H.A.	7,87
Nayar, N.M.	189
Okwuagwu, C.O.	133
Page, O.T.	3
Peloquin, S.J.	133
Plaisted, R.L.	143
Ramanna, M.S.	99
Schmiediche, P.	45
Schroeder, S.H.	133
Schuchmann, R.	175
Stelly, D.M.	133
Wenzel, G.	175

Recommendations

General

The efficient utilization of the variability available in the *Solanum* genetic resources through long term population breeding with recurrent selection is the basis of the production of cultivars for new as well as traditional potato growing areas.

The continued long-term breeding for agronomic usefulness of the *Solanum* germ plasm should be given constant high attention.

First Priority

1. Continue breeding for improved resistance to PLRV using both cultivated and wild species and for immunity to PVY.

2. Breed for *Pseudomonas solanacearum* resistance and combine it with *Meloidogyne* resistance for hot climates.

3. Continue breeding for earliness, fast bulking and high yield.

4. Continue to breed for resistance to *Globodera* nematodes taking into account sources of tolerance and race non-specific resistances.

5. Continue parental line breeding at the 2x level for disease and pest resistances and agronomic characteristics. Exploit the manipulation of ploidy levels in hybrid production.

6. Breed for resistance to tuber moth using depending screening methods.

7. Expand research in identifying superior parental materials and the most suitable mating systems for production and use of TPS.

8. Develop a computer data management system to record, store and retrieve information from local and regional germ plasm evaluations.

9. Continue breeding for resistance to *Phytophthora infestans* and *Pseudomonas solanacearum* and utilization of new sources of resistance to these pathogens.

10. Continue breeding for frost and heat tolerance.

11. Continue evaluation of the world collection for important characters according to priority.

Second Priority

1. Continue to breed for immunity to PVX.

2. Continue to improve the back-up population of the cultivated world potato collection for increased gene frequency of the desired characters.

3. Encourage the use of *in vitro* culture and screening techniques whenever they are an appropriate aid to the breeding program.

4. Maintain a close watch through professional contacts on international developments in gene technology. The major development of such techniques should take place in institutions other than CIP.

Brief Review of Recommendations Presented in
"Utilization of Genetic Resources of the Potato III"
November, 1980

O.T. Page

Since the last planning Conference three years ago several events have occurred which relate to a review of the recommendations proposed in the report of the 1980 conference. The January, 1982, "Profile 1972-2000" outlined eight important areas of research concentration in breeding together with a graphical presentation of research effort to the year 2000. Following the Congress in February, 1982, a "Decennial Anniversary Post-Congress Workshop" was held. Recommendations were developed for five areas of research. While breeding was not a specific topic, nevertheless, there were recommendations which involved breeding input. Finally, in February, 1983, the overall research program of CIP was the subject of CIP's second quinquennial review. In response to the recommendations of the review Panel CIP's research Thrusts were reorganized.

I would like to digress for a moment to orient the non-CIP participants concerning our matrix research organization. For administrative purposes CIP scientists are assigned to departments. Through research projects the special expertise of individual scientists is integrated and directed toward solving specific problems. These interdisciplinary projects, each involving a leader and cooperators, are grouped into Thrusts. A Thrust is a unit of common research activity. Members of the Department of Breeding and Genetics are involved in research projects in eight of the ten Thrusts. In Summary, Departments serve an administrative function while Thrusts provide the organization for integrating interdisciplinary projects into research units to solve broad research objectives.

This Conference is concerned with a spectrum of research in breeding that has a very important impact not only on other source research here in La Molina but also in CIP's Regional Research program. While the recommendations arising out of the quinquennial review would have structured a major portion of CIP's research on agroecological zones this was deflected by the creation of two Thrusts concerned with the cool climate and hot climate potato production. It should be noted that the Post-Congress Workshop included a session on potatoes for the hot-humid climates with recommendations relating to early screening for heat tolerance and increased stability under hot-humid climates with

recommendations relating to early screening for heat tolerance and increased stability under hot-humid conditions. Recommendations were also made in the Profile of the Year 2000 concerning adaptation and utilization of heat tolerant material as well as breeding for cold tolerance. The 1980 Conference Report made four recommendations relating to abiotic stresses: 1) to breed for combined resistances to late blight, bacterial wilt and root-knot nematode for lowland tropics; 2) to improve earliness and resistance to abiotic stress; 3) to increase dry matter content under lowland conditions; and 4) to identify plant materials tolerant to high temperatures.

A dominant theme, now exemplified in two new Thrusts, is to continue with two breeding lines, one for cool, temperate climates and one for hot, humid environments. Because the potato can be grown through 120 degrees of latitude and 4 kilometers of altitude is irrefutable evidence of its wide adaptability. By conceiving environments as either "hot" or "cool" is a common sense approach to characterizing climate for potato production. In practice, field screening for adaptation is carried out initially in Peru at cool highland and hot lowland field stations. Selected clones are further evaluated at other global locations in South America, Africa and Asia. It is hoped that a computerized prediction model, now in its sixth year of testing, will be of assistance in reducing the number of field adaptation trials.

An additional consideration in maintaining a broadly based approach to climatic adaptability is the necessity to incorporate disease and pest resistance. Viruses such as PVX, PVY, PLRV and the viroid PSTV have wide adaptability to climatic conditions as do the late blight fungus, Phytophthora infestans, and the several races of Pseudomonas solanacearum the cause of bacterial wilt. Similarly, biotypes of the potato moth, Phthorimaea operculella, are widely adaptable through a range of 40°N to 30°S if the equator - from Virginia to South Africa. In contrast, Meloidogyne nematodes are generally associated with hot climate conditions while Globodera nematodes are characteristically found under cool highland conditions in the tropics.

So far I have referred to eight of 10 priority recommendations from the 1980 Planning Conference relating to breeding for climatic adaptation combined with resistance to globally important viruses, pests and diseases. Four of eight priority areas for research concentration listed in the Profile to the Year 2000 are concerned with the incorporation of pest and disease resistance appropriate for highland and lowland tropical conditions.

Summarizing, it has been recommended that breeding for heat tolerance

be combined with bacterial wilt, late blight, virus and root-knot resistances, Guidelines breeding recommendations for cold adaptation include frost tolerance and resistance to late blight, cyst nematodes and viruses. It is apparent that there is an overlapping of environments favorable for several important diseases and pests including viruses, late blight tuber moth and, to a lesser degree, bacterial wilt.

CIP has an interest in the development of technology to grow potatoes from true seed (TPS). A priority recommendation from the 1980 conference states, "Expand research in identifying superior parental materials and the most suitable mating systems for production and use of TPS." The 1982 Workshop recommended the developement of progenies with high yield, uniformity, earliness (about 90 days after transplanting), a low level of glycoalkaloids and seedling vigor. The year 2000 Profile, in referring to breeding for adaptation to propagation by true seed, states, optomistically, that TPS will receive major breeding emphasis to 1990 "When it terminates".

Other priority recommendations proposed in the Planning Conference stress the need to, "Improve promising accessions in the world potato collection through selection for increased gene frequencies and combining of resistances." This recommendation was considered to have a lower priority than the need to, "continue to emphasize breeding at the 2x level for use in producing 4x hybrids." This recommendation also included the desirability of exploiting ploidy level manipulations in hybrid production. This theme is reiterated in CIP's Profile 1972-2000 with reference to genome engineering referring to ploidy level manipulations using haploids and 2n gametes and also in maximizing heterozygosity and yield through 2n gamete breeding approach.

The 1980 conference made a number of general recommendations which, because of their very wide scope offer little guidance for research. However, I would like to draw special attention to the following:

"It is recommended that a continued close watch through professional contacts be maintained on international developments in <u>in vitro</u> techniques must now be seriously considered as a component part of future strategies for potato improvement. Moreover, associated developments in genetic engineering are emerging from the level of promises to practical performance. CIP must be aware of the gradual confluence among biotechnologies including breeding, <u>in vitro</u> methodologies and manipulations at the molecular level. That is the future.

Selection of Parental Stocks for Heat Tolerance and Earliness

H. A. Mendoza

Introduction

The International Potato Center started its research work to adapt the potato to the hot environments in 1974. This was started by screening, in San Ramon, a sample of about 6000 genotypes represented by local and foreign cultivars and breeding lines from a varied origin (Mendoza,1976). From this original sample only 34 clones were selected and later on tested in Yurimaguas, a hot and humid site in the Amazonian basin (Mendoza and Sawyer, 1983). At this second location only four clones (N565.1, DTO-2, DTO-28, and DTO-33) yielded from 9.5 to 17.4 T/Ha in a period of 60 days (Mendoza and Estrada, 1977). This result meant that from the initial set of 6000 clones tested only 0.06% showed adaptability to hot and humid conditions. However, from this small frequency and after five cycles of recurrent selection there has been a significant improvement of 10 to 15% of selected clones (Mendoza, 1980). By that time four more clones had been selected: María Tropical, LT-1, LT-2, and LT-4. From the experience accumulated by testing and observing a wide variety of genetic materials, the main attributes for which selection was applied were: earliness (25); high yield and stability of performance (30); resistance to bacterial wilt, root-knot nematodes, and PVY (30); and acceptable tuber quality, mainly dry matter content (15). The numbers in parenthesis indicate the relative importance of these characteristics in a scale of 0 to 100 points (Mendoza, 1981). In 1982 three more heat tolerant and early maturing clones were selected for regional distribution, LT-5, LT-6, and LT-7. The clone LT-7 has also proven to be an outstanding general combiner for yield and tuber uniformity (1982 CIP Annual Report).

Experimental Results

The experimental results which will be presented were obtained in the period 1981-1983. These are mainly referred to earliness, genetic variability for heat tolerance and selection of parental materials for these attributes.

Earliness: This is a most important attribute for which not enough research has been devoted. Earliness not only gives more flexibility to adapt the potato to a cropping system but also is a manner to avoid certain biotic or abiotic stresses from which an early maturing cultivar can escape. Earliness also may permit a more uniform distribution of potato production by staggering harvesting periods over a longer time.

A study to investigate the inheritance of earliness was completed in 1981. A sample of 11 clones, six early maturing and five medium late,

Table 1. Estimates of general combining ability effects ($\hat{g}i$) for tuber initiation and percent tuberization.

Clones	Tuber initiation	Percent tuberization	
		Seedlings	Clones
DTO-33	-3.877	4.949	4.588
LT-1	-4.492	10.686	5.834
R268.1	-3.668	8.679	5.368
DTO-28	-3.526	7.738	-2.669
65-ZA-5	-1.235	- 3.960	1.036
M. Tropical	-2.611	10.459	1.517
R100.1	2.964	- 2.834	-3.428
R209.2	2.442	- 7.311	-2.232
R111.2	6.013	-13.290	-6.623
R13.7	3.986	- 7.564	-1.648
R120.20	4.004	- 7.552	-1.743
s.e. ($\hat{g}i$)	0.776	2.178	2.377
s.e. ($\hat{g}i-\hat{g}j$)	1.151	3.231	3.526

was taken from the breeding stocks. These clones were mated following a diallel mating design without reciprocals. As a result a population of 55 progenies was obtained. This population was evaluated for tuber initiation in the greenhouse and the results were presented by Mendoza, 1980.

Trials were also conducted at the field where seedlings were transplanted. Three harvestings were made 30, 60, and 90 days after transplanting. At the last harvest tubers were taken from each plant to repeat the trial but in a clonal stage. Also harvestings were made 30, 60, and 90 days after emergence. Data in Table 1 indicates the estimates of General Combining Ability (GCA) effects for each of the clones for days to tuber initiation in the greenhouse experiments, as well as percent tuberization in seedlings (30 days after transplanting) and in plants from tubers (30 days after emergence).

Data in Table 1 show that the first six clones (early maturing) have negative effects for their GCA estimates. This means that their tuber initiation start at an earlier time that the medium late clones. It is also noticeable that within each group of clones there is considerable variability for their estimates. The estimates of the GCA effects for percent tuberization in seedlings coincides very well with those for tuber initiation. For those clones having an early tuber initiation, the percent tuber initiation 30 days after transplanting is high. This agreement was expected since both characteristics were measured in seedling populations. The estimates for GCA effects for percent tuber initiation in the clonal generation presents certain disagreement with that of the seedlings. This deviations are important in the clones DTO-28, María Tropical, R-209.2, R-13.7 and R-120.20. This could be due to environmental effects and also to the fact that seedlings, and plants produced from tubers have two different physiological conditions, i.e. plants from tubers have two sources for tuberization induction, their foliage and mother tuber, while the seedlings only have their foliage. This may also indicate that in the work of selection for tuber initiation one should use plants from tubers rather than seedlings.

Table 2 shows estimates of GCA effects for foliage senescence, average weight per tuber, and average tuber weight per plant. The group of early maturing clones, on the average, transmit to their progenies the characteristic of an early foliage senescence at a greater extent than the group of medium late clones. However, for this attribute, there are no differences in GCA effects within each group of clones. For average weight per tuber there is a great deal of variation in the GCA estimates among clones. For this characteristic, the clones DTO-28 and María Tropical transmit to their progenies an average at both seedling and clonal generations. The early clone LT-1 and the medium late R-100.1 do, but only at the clonal generation. For the attribute average tuber

Table 2. Estimates of GCA ($\hat{g}i$) effects for various traits at the seedling and clonal generations

Clones	Foliage senescence		Average wt. per tuber		Ave. tuber wt/plant	
	Seedlings	Clones	Seedlings	Clones	Seedlings	Clones
DTO-33	-.207	.145	-.035	.007	-.019	.032
LT-1	.181	.189	.003	.052	-.046	.040
R268.1	.181	.150	.004	-.011	.036	.036
DTO-28	.172	.206	.068	.059	.064	.041
65-ZA-5	.127	.151	.013	-.014	.048	.023
M. Tropical	.078	.207	.048	.072	-.037	.013
R100.1	-.210	-.082	.026	.060	-.032	-.045
R209.2	-.176	-.175	-.024	.001	-.024	-.044
R111.3	-.157	-.295	-.058	-.087	-.025	-.039
R13.7	-.192	-.271	-.013	-.101	.031	-.038
R120.20	-.213	-.225	-.031	-.037	.004	-.019
s.e. ($\hat{g}i$)	.048	.055	.018	.023	.027	.030
s.e. ($\hat{g}i-\hat{g}j$)	.071	.081	.026	.034	.075	.044

weight per plant only the clones R-268.1 and DTO-28 had GCA estimates larger than their s.e. at both seedling and clonal generations. The clones 65-ZA-5 and R 13.7 did but only at the seedling generation while DTO-33, and LT-1 did only at the clonal generation.

Considering data presented in Tables 1 and 2 it is interesting to contrast the breeding behavior of some of the clones from the sample included in these experiments. The clone DTO-33 transmitted to its progenies a fast tuber initiation as well as a high percent tuberization at 30 days after transplanting (Table 1). However, the GCA estimates for average weight per tuber as well as the average tuber weight/plant were practically no different from 0 (Table 2), indicating that this clone does not transmit these attributes, on the average, to its offspring. Contrastingly, the clone DTO-28 has GCA effects for all the traits considered in both tables greater than the s.e. and in most cases highly significant. This indicates that this clone transmits these attributes to the average of its progenies and, therefore, it is a valuable progenitor, the clone R 100.1 transmits late tuber initiation but the GCA estimates for average weight per tuber are high indicating that its progenies have a fast bulking rate. However, the GCA estimates for yield per plant are not different from 0. This suggests that it transmits on the average the capacity of producing large tubers but in small number. A very similar behavior is observed in the clone M. Tropical.

Estimates of narrow sense heritability (h^2) for various traits at different harvest times are presented in Table 3. Percent tuberization at 30 days had a heritability of .41 which indicates that adequate response to selection can be expected. Since this trait is an expression of earliness of tuberization it is an important component of the total length of the growing period and its heritability indicates that it can be improved. Foliage senescence at both 60 and 90 days is very highly heritable and also indicate that in breeding for earliness this trait will be very responsive to selection. Since another important component of the length of the growing period is the speed of bulking, the average weight per tuber and the average tuber yield per plant at 30, 60 and 90 days are very important. The heritability estimates for average weight per tuber are higher than for average yield/plant. However, since these two attributes have a phenotypic correlation of about .7 (Thompson and Mendoza, 1984), a large gain in average weight per tuber will also be accompanied by a gain in average yield/plant.

These results would indicate that there is a great deal of genetic variability in the various components of earliness as time to tuber initiation, percent tuberization, speed of bulking as reflected by tuber weight per plant at the various harvest dates and vine senescence. If some of the parental stocks do not posses the ability to transmit all these attributes a careful study and choice of progenitors could permit to complement

Table 3. Estimates of heritability for various traits at 3 different harvest times.

Trait	h^2
Percent tuberization (30 days)	.41
Foliage senescence (60 days)	.80
Foliage senescence (90 days)	.89
Ave. weight per tuber (30 days)	.58
Ave. weight per tuber (60 days)	.70
Ave. weight per tuber (90 days)	.62
Ave.tuber wt/plant (30 days)	.49
Ave. tuber wt/plant (60 days)	.27
Ave. tuber wt/plant (90 days)	.31

in a progeny the necessary components of earliness and achieve a significant progress in breeding for this attribute.

Heat tolerance: In the program of selection for adaptation to the hot environments, heat tolerance and earliness are two major attributes. In 1981, a population of 127,000 seedlings was evaluated at both San Ramon, and La Molina. Also, a sample of 1700 clones from selections made at San Ramon and La Molina in the winter of 1980, from seedling populations were evaluated.

A sample of 1172 of these clones originating from hybrids of 8 parental clones from a broad genetic background were evaluated at Yurimaguas during the summer of 1981. Results of these evaluations are shown in Table 4. It is noticeable that one of the clones derived from 377904.10 yielded 860 gr/plant in a growing period of 70 days. The average yield of the selected clones was superior to that of the clones utilized as checks. This was showing that there was sufficient variability for heat tolerance and earliness in that population. Also the clones DTO-28, 377904.10 and 14XY.4, were showing a good ability to transmit these attributes to their progenies.

A group of 120 female clones selected in previous years were topcrossed to a pollen bulk and their progenies were evaluated in replicated trials at both San Ramon and La Molina. From the results of these progeny tests several potential parental clones were selected transmitting earliness and heat tolerance. Among the most important clones we can count 7XY.1, 377250.7, 377892.7, 378015.13, 378015.16 and 378017.2. There were other selected clones which were eliminated later for transmitting some undesirable attribute. Also at that time a PVY immune early maturing clone 379706.34 (LT-1 x XY Bulk) was selected at San Ramon.

In 1982 and after several seasons of evaluation, three heat tolerant and early maturing clones were introduced to the seed program to be freed from diseases and distributed to the regions. These clones are:

LT-5	Snowflake x N551.12
LT-6	P_6C_1.16 x María Tropical
LT-7	ASC 77-056

The last clone, LT-7 (378017.2) as indicated previously passes a high general combining ability for yield and earliness.

In the summer of 1982, a sample of 1680 clones selected in the previous year were evaluated in 10-hill plots. Table 5 shows the yields of the 12 highest yielding clones compared to the check Desiree. Notice in the pedigree of the highest yielding clones, the constant presence of the progenitors selected for general combining ability which have been pre-

Table 4. Average yield (kg/plant) of selected clones grouped by the male parent. Yurimaguas, 1981.

Male Parent		No.total clones	No.selected clones	Range of Yield	Average Yield
DTO-28	800169	102	13	.800-.267	.425
7XY.1	N558.43 x OP	128	3	.517-.309	.417
15XY.4	701535 x Neo tbr bulk	30	5	.467-.348	.411
14XY.4	701225 x Neo tbr bulk	120	9	.618-.294	.404
M. Tropical	377260 - (N500.2 OP)	400	12	.687-.270	.390
PS - CR	Bulk PS - CR	40	3	.600-.275	.386
Tbr bulk Yuri	Bulk tbr Yuri	152	7	.537-.263	.368
377904.10	R245.4 x BR-63.65	200	17	.860-.285	.362
Total average		1172	69	.635-.289	.395

Yield of checks: Spunta =.130
LT-2 =.275
377904.10 =.275

Table 5. Evaluation of first generation clones at San Ramon, Summer 1982 (10-hill plots).

Pedigree	Yield (kg/plant)
(377887.25 x 377904.10).15	1.95
(377950.15 x 378017.2).12	1.82
(377877.9 x 378017.2).11	1.66
(65-ZA-5 x 378017.2).1	1.60
(377250.7 x 377904.10).1	1.49
(377882.11 x 378015.16).12	1.45
(Atzimba x 378017.2).3	1.43
(377964.5 x 378017.2).10	1.40
(LT-1 x 378017.2).1	1.36
(377935.7 x 377964.5).13	1.32
(377935.27 x bulk PSR).24	1.31
(377250.7 x 377904.10).24	1.30
Desiree	.70

viously mentioned, i.e. 377904.10, 378017.2 (LT-7), 378015.16, 377250.7, and 377964.5.

In summary, the results presented are indicating that selection of parental material for heat tolerance and earliness has permitted the identification of a number of clones which posses the capability to transmit these attributes to their progenies. For CIP's breeding program this is very important, since the use of these progenitors permits not only to improve the quality of the populations sent to the national programs but also to provide parental clones for breeding purposes.

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Genetic Studies and Breeding of Resistance to PLRV, PVY, and PVX

by C. R. Brown

Introduction

Seed of potato cultivars are multiplied in seed production schemes. In the nineteenth and early twentieth centuries many commentators recorded that potato varieties declined in performance over time (Salaman, 1949). New varieties were tested and introduced primarily to replace older varieties whose yield had fallen to unacceptably low levels. Degeneration of varieties was attributed largely to the effects of viruses by Salaman (1921). Since that time prevention of infection through phytosanitary practices and the breeding of virus resistant varieties have been studied and put into practice as a means of controlling virus-incited degeneration. In developed countries, seed programs have been relatively successful in providing seedstocks with low virus content. Where phytosanitary practices are successful there is relatively less demand for resistance. In most developing countries, however, seed programs have yet to reach an effective level of organization. Virus resistance is highly desireable in new varieties in order to permit efficient multiplication of seedstocks where virus inoculum cannot be excluded from seed production areas. The principles of seed production require that the crop be isolated as much as possible from sources of infection and protected from modes of inoculation, such as mechanical or insect vector transmission. In practice the organization and capital investment necessary to enforce these principles are lacking or scarce in most developing countries. Often seed production cannot be isolated from ware production areas and the control of virus transmission is lacking or erratic.

The objective of the virus resistance breeding program is to develop parental lines that are resistant to the most important viruses and that transmit this resistance to progenies in an efficient manner. The most important viruses are PLRV, PVY, and PVX. Situations vary but generally PLRV and PVY occur together and are the most serious. This is true not only because the symptoms they produce are severe in almost all cases, but also because they are transmitted very easily by aphids. In effect these viruses are aerially disseminated. Very extensive infection of these two

viruses can occur even with low aphid populations especially if alate populations are arriving in the viruliferous state. PVX is of course contact transmitted and transmission can be prevented by planting arrangements and agronomic management. PVX usually produces milder symptoms and thus does not stand out as the highest priority, and as a result the resources at CIP are directed most intensively to producing adapted cultivars with resistance to PVY and PLRV.

Materials and Methods of Virus Resistance Breeding at CIP

The breeding effort at CIP has consisted of four main areas of work:

1. Genetic studies to determine the best way to handle the resistance in a breeding program where many other characters with complex inheritance must be manipulated simultaneously. This has included progeny testing to identify the best general combiners for PLRV resistance, and recurrent selection of PVY immune populations to increase the frequency of the Ry gene.

2. Screening seedlings at an early stage in order to reduce population numbers and have only resistant clones in advanced stages of evaluation.

3. Graft testing to confirm immunity to PVY and PVX in materials that have been selected for agronomic performance at least twice in the field. All clones with hypersensitive or susceptible reactions are eliminated at this point.

4. Replicated field trials to test advanced clones for resistance to infection by PLRV using PLRV-infected plants of *Datura stramonium* as sources of inoculum in the field.

Recurrent Selection to Increase the Frequency of PVY Immunity

The work of Muñoz et al.(1975) and Galvez and Brown (1980) led to the identification of monogenic immunity to PVY in the *andigena* and neotuberosum germplasm of CIP. By performing progeny tests of a number of different clones an initial group of clones was chosen. A population improvement program was started to increase the frequency of

the Ryadg gene and provide a diverse population from which we would be able to extract high frequencies of PVY immunity per se, duplex genotypes, in particular, and desireable agronomic characters. In table 1 the results of progeny tests are presented from the initial screenings. These individuals were intermated, the resulting progeny screened and only the resistant seedlings were intermated for the following cycle. The screening involved two mechanical inoculations and removal of plants showing visual symptoms. This process is schematically depicted in figure 1 and as can be seen was carried out three times. Remnant seed of cycles 0, 1, 2, and 3 have been screened in a single experiment with five replications and the results are shown in table 2.

Table 1. Selection of PVY immune parents on the basis of a progeny test (Immune x Susceptible).

Simplex Parents	Percent Healthy*
377967.4	41
377967.7	52
377968.1	44
377971.2	48
377985.2	52
377988.1	44
377988.2	50
Y18.8	50
Y20.6	45
Y20.11	47
XY13.14	58
XY14.7	45
Duplex parent	
Y23.14	80

* Progenies tested by ELISA after two mechanical inoculations in the seedling stage and one in the tuber derived plants

As is well known the genetics of autotetraploids is complex due to the diploid state of the gametes. As a consequence, the prediction of the effects of selection on single gene characters is best handled by making successive

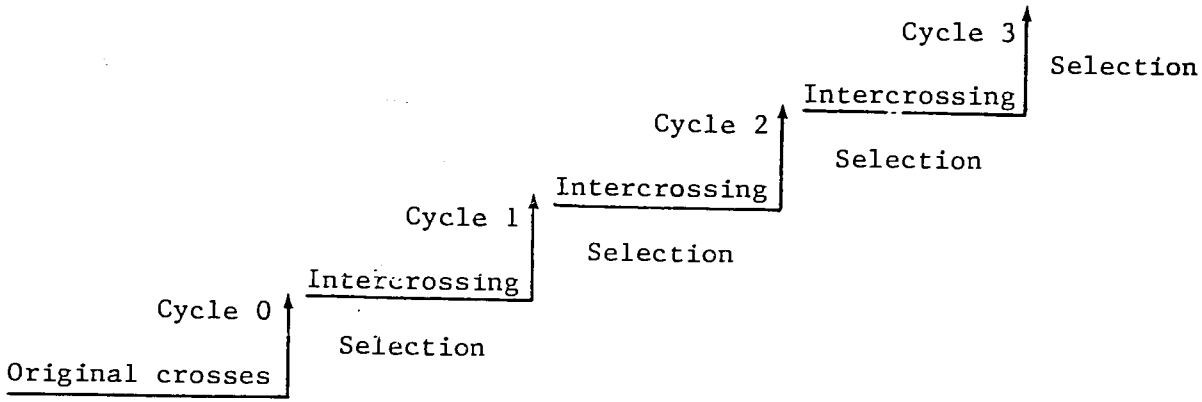

Figure 1. Scheme of recurrent selection for increase in PVY immunity (PVYR).

runs of the several equations necessary on a digital computer. This approach is superior to that of Hill (1971) in that it does not require the assumption of random mating equilibrium, which is a serious limitation of the applicability of his results to breeding populations of autotetraploids. The iterative method alluded to above was followed. For the sake of brevity the discussion below examines expected results relevant to the Ry gene of interest here.

Table 2. Frequency of PVY resistance in four cycles of recurrent selection.

CYCLE 0	PVY x Susceptible	
	PVY R-1	43.4%
CYCLE 1	PVY R-I x R-I Bulk	
	PVY R-II	60.2%
CYCLE 2	PVY R-II x R-II Bulk	
	PVY R-III	82.1%
CYCLE 3	PVY R-III x PVY R-III	
	PVY R-IV	91.3%

The phenotypic and gene frequencies, and the gene frequency change from one generation to the next are shown in figure 2. Cycle 0 consisted of crosses of simplex resistant by nulliplex susceptible parents. For the purpose of comparison the same parameters are shown for a diploid population in figure 3. It is most noteworthy that although the phenotypic frequencies differ very little over the course of six cycles of selection, the gene frequency of the diploid population reaches a much higher level. Thus for single gene characters similar changes in phenotypic frequencies are accompanied by much larger gene frequency changes in diploids than in autotetraploids. In figure 4, the dynamics of the different types of heterozygotes are shown. Here the most interesting pattern is that the

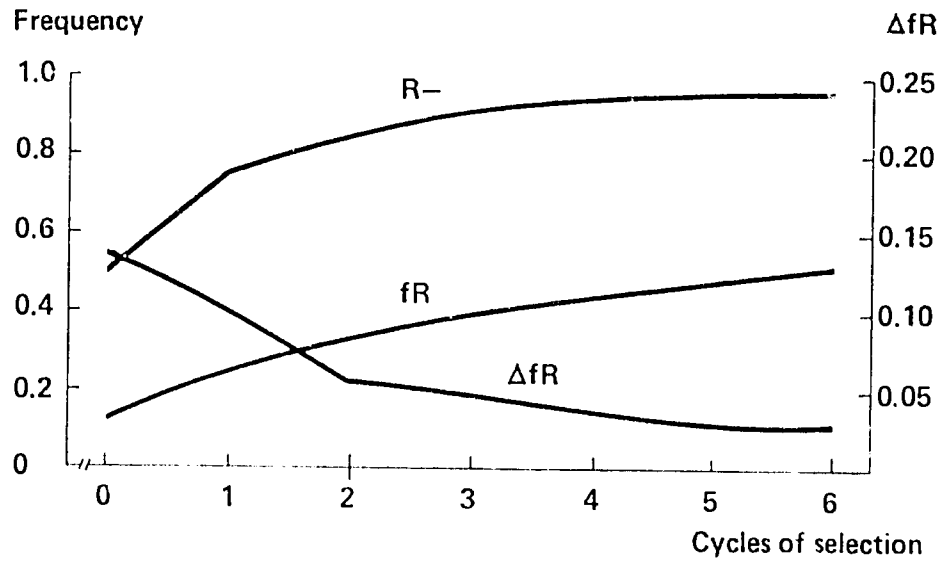

Figure 2. Phenotype (R-), gene frequencies (fR), and change in gene frequency (ΔfR) in autotetraploid population.

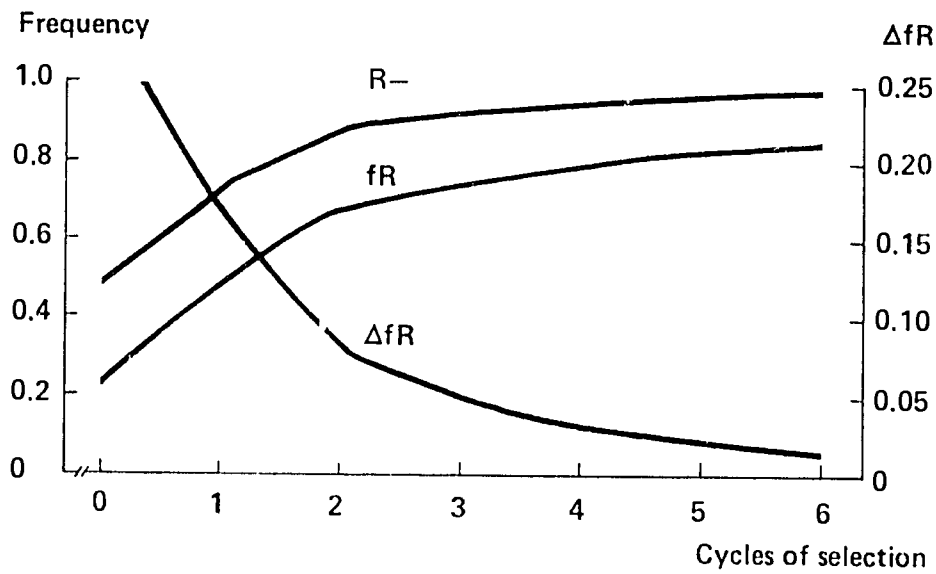

Figure 3. Phenotype (R-), gene frequencies (fR), and change in gene frequency (ΔfR) in diploid population.

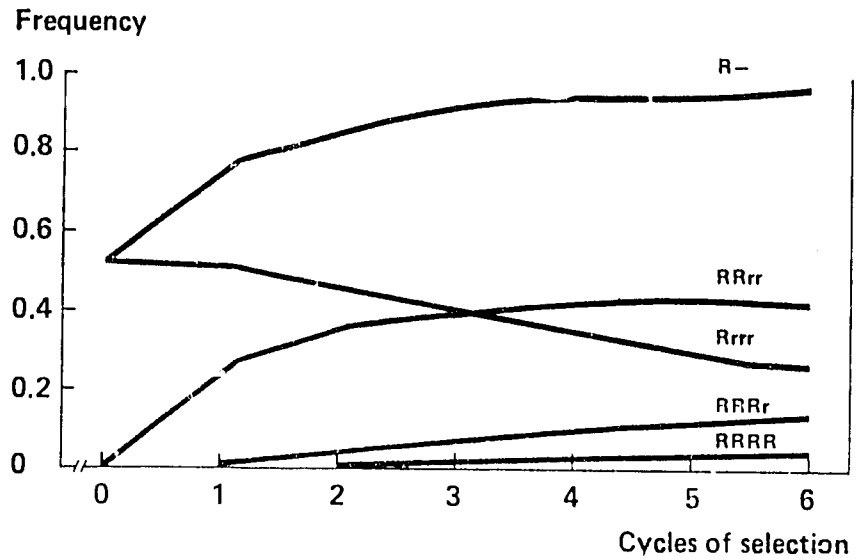

Figure 4. Frequency of resistant genotypes under selection in autotetraploid population.

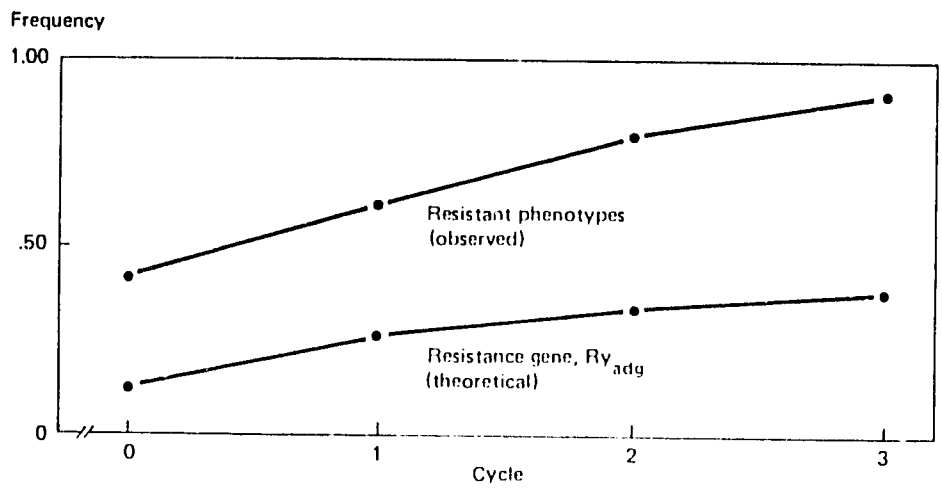

Figure 5. Observed frequency of resistant phenotypes and theoretical frequency for the RY_{adg} gene over 3 cycles of recurrent selection for PVY resistance.

population becomes dominated by duplex genotypes by the third cycle. Duplex genotypes, if they can be readily identified through progeny tests, and if they also are good parents in regards to agronomic characters, can be very useful components of other breeding programs where a high priority has been established for the incorporation of PVY. Progenies from crosses of duplex resistant parents with susceptibles will have between 5/6 and 7/9 resistant individuals these limits being established for random chromosome and maximum equational division, respectively (Burnham, 1962). In figure 5 the observed increase due to recurrent selection has been compared in a graph with expected gene frequency increase. Referring back to figure 4, it is expected that 40 per cent of the individuals in cycle 3 are duplex genotypes. As a postscript it is important to note that this type of recurrent selection program is not possible using the stoloniferum materials due to the male sterility caused by the interaction of stoloniferum cytoplasm and tuberosum nuclear genes. Fertility restoration is a rare and as yet unelucidated genetic phenomenon.

Essentially the same theory is relevant for any single gene character where the desired phenotype is controlled by a dominant allele. Although immunity to PVX has been given secondary importance, it is present in a large number of the PVY immune clones as a result of combined screening for PVY and PVX immunities (Brown and Fernandez-Northcote, 1981). It is considered of greatest importance in the Andean Region where together with potato virus S it is quite prevalent in the highlands.

Topcross Testing for High General Combining Ability for Resistance to Infection to PLRV

The resistance to infection for PLRV described above is known to be a polygenic trait with a low heritability (Brown, 1979). The low heritability and the lengthy process of field exposure and evaluation of tuber progeny for secondary symptoms due to uncertain expression of primary infection have led to a program of progeny testing of young seedlings in order to identify superior parents. A topcross method is being used for this purpose. Clones resistant to PLRV were crossed to a pollen composite and the progenies evaluated for resistance to PLRV as seedlings. This allows a

ranking of parental clones, that have been previously selected for several seasons in field exposure trials, according to the performance of their progenies resulting from crossing the clones as pistillate parents to a diverse pollen composite. Thirty-six progenies of such a topcross system were subjected to infection at a very early seedling stage. One month after inoculation, ELISA serology was performed on each plant. In table, 3 an analysis of variance for the performance of 36 pistillate parents is shown. Sixteen of the parents included in this analysis were PVY immune clones which had never been selected for PLRV resistance in an exposure trial although weak selection may have occurred during the course of field evaluation. An orthogonal contrast between the PVY and PLRV clonal groups was highly significant. The two groups separate neatly into two distinct populations. The distribution of the mean

Table 3. Analysis of variance of topcross progeny for resistance to PLRV. Orthogonal contrast shows significant difference between PVY immune and PLRV resistant groups. Arcsine transformation was used.

Source	d.f.	MS	F
Parents	35	786.42	5.01***
PVY vs PLRV	1	14891.48	95.46***
Error	72	156.99	

Table 4. Distribution of topcross parents in PVY immune and PLRV resistant groups. Scale is in arcsine transformed units of proportion of progeny health.

Groups	No. of parents in each category						
	0-15	16-30	31-45	46-60	61-75	76-90	$\overline{X}$
PVY	1	7	7	2	0	0	30.8
PLRV	0	0	5	9	4	1	54.3

performance of all parents is shown in table 4. The general combining ability effects are presented in table 5 for the highest performers down to and including the highest performing PVY immune clone. The two best combiners, LR 13.2 and 78 A 16.1, are clones selected from the breeding materials developed by Dr. Hans Ross of the Max Planck Institute. A West German commercial variety derived from these materials, cv. Pirola, ranks eighth in performance. We may conclude that certain components of tuberosum breeding materials constitute a germplasm resource for PLRV resistance.

Besides aiding in the choice of the best PLRV combiners this type of information is highly useful in selecting the PVY immune clones that will contribute the most PLRV resistance in the populations now being selected to combine the two resistances. One may avoid inclusion of PVY immune clones that confer extreme susceptibility to PLRV to their progenies, an important consideration in the management of a difficult and poorly heritable trait such as resistance to infection to PLRV.

Table 5. Topcross general combining ability effects of PLRV resistance in the highest eleven parents (arcsine transformation).

Clone	Pedigree	C_i
LR 13.2	MPI 63.613/35 x VD 4667	34.7
78A16.1	Datura x VD 4629	26.0
81A21.1	LR 19.4 x BP PS/PI	24.0
BR 63.15	Atzimba x (316.3 x Katahdin)	19.6
80N20.16	Serrana x 49.540/2	18.4
81A14.3	LR 12.7 x LR bulk	16.1
81A14.4	" "	14.8
Pirola	[(49.767/77 x Eva) x 185/14] x 175/28	13.2
80N20.3	Serrana x 49.540/2	10.5
81A51.6	B71.240.2 x BP PS/PI	10.4
80JA24.1	LR 12.6 x XY 14.7 (PVY immune)	8.4

S.E. $(\hat{C}_i) = 9.8$ S.E. $(\hat{C}_i - \hat{C}_j) = 13.8$

Durability of Immunities to PVY and PVX

The term durable resistance refers to host-pathogen systems that show stable pathogenicity-resistance profiles over time (Buddenhagen,1983). Resistance that is not durable is overcome by new virulence genotypes of the pathogen. In this regard a resistance that is absolute, such as immunity, is expected to give a strong selective advantage to pathogen virulence genotypes that can break it, provided that genetic variability for virulence exists in the pathogen population or will arise frequently by mutation. In general resistances that are controlled by single genes are candidates for instability because the pathogen is assumed able to evolve resistance breaking mechanisms with relatively high frequency.

The case of the potato-PVY host-pathogen system is perhaps an exception. The mutability of virus genomes is considered to be low in general in comparison to that of bacteria and fungi due to the small number of genes that make it up. The immunity of the gene from the andigena source has been found to be effective against a large range of isolates representing the total range of variability of PVY. However, this source is uniformly susceptible to PVA which is similar to PVY in characteristics and host range, though distinct serologically and is thought to have evolved outside the center of origin of the potato. Fortunately, this is not true for the stoloniferum source as it appears that it is a "stronger" gene. The gene from stoloniferum conditions immunity to all strains of PVY and PVA tested. We can therefore expect that the gene from andigena will be broken by PVA and might possibly be broken by highly deviant PVA-like strains of PVY. To determine this with greater certainty, the approach being taken is to empirically test this possibility by exposing immunity genotypes to diverse habitats and diverse arrays of PVY strains. CIP is starting an International Virus Resistance Stability Trial in 1984 to obtain data on this. Where tuberosum clones from European seed exporting countries have been grown to any great extent one can expect there to be a greater presence of PVA. For the long term the strategy is to preferentially deploy the Rysto gene in future breeding programs. The tetrad sterility which is associated with the interaction of stoloniferum cytoplasm and tuberosum genes is undesirable to breeders and should be eliminated by selection and use of restored genotypes exclusively as staminate parents in crosses.

Although no resistance-breaking isolate of PVY has been found, a different scene exists for PVX. An immunity-breaking strain of PVX was found by Moreira and Jones (1980) in Bolivia. This strain though serologically identical to other strains of PVX is quite different in its symptomatology in potato and on the indicator host *Gomphrena globosa* in which it does not produce local lesions but rather infects systemically and asymptomatically. However, immunity to this strain, called PVXHB, has been found in *S. sucrense*. Two accessions of PVXHB immune *sucrense* were crossed to a PVX susceptible clone, Maria Tropical. Two cuttings were made of each seedling and the cuttings graft inoculated separately with PVXHB and PVXCP (Cockerham's group 2). Both accessions are immune to PVXCP as well. The results of the segregation for the two resistances are shown in table 6. The segregation patterns are concordant with the tetrasomic segregation of two independent genes present in the duplex state in both of the accessions. The symbol RxHB is proposed to indicate the gene in *S. sucrense* controlling immunity to PVXHB. The immunity from *acaule* and *andigena* presently deployed may be sufficient. If PVXHB becomes an important pathogen, immunity to it is available in a fertile tetraploid gene pool completely crossable to cultivars.

Table 6. Segregation of immunities to PVXCP and PVXHB in two testcrosses of *S. sucrense*. Phenotypic codes explained in legend.

Cross	Phenotypic classes				χ^2 Mode of segregation	
	H+C-	H-C+	H+C+	H-C-	Random Chromosome 5:5::1:25	Maximum Equational 14:14::4:49
A	16	6	3	38	4.48 N.S.	4.60 N.S.
B	2	5	2	20	3.00 N.S.	2.38 N.S.
Pooled	18	11	5	58	5.25 N.S.	1.94 N.S.

Legend of Symbols

H+C- = PVX_{HB} susceptible, PVX_{C} immune

H-C+ = PVX_{HB} immune, PVX_{C} susceptible

H+C+ = PVX_{HB} susceptible, PVX_{C} susceptible

H-C- = PVX_{HB} immune, PVX_{C} immune

Selection of Advanced Materials with Virus Resistance

In an International Agricultural Research Center such as CIP, a considerable amount of the resources available must be directed toward short-term solutions. The clients, national programs in developing countries, need germplasm that has an opportunity to make an immediate impact. Therefore, a large part of the total work in the virus breeding program consists of producing and testing clones with a high yield potential and acceptable horticultural qualities. A list of clones that comprise the PLRV resistance component is presented in table 7. In groups 80N and 78C, it has been possible to obtain PLRV resistant materials also immune to PVY . Certain German varieties are PLRV resistant and PVY immune as well. The materials graft-tested for immunity to PVY are listed in table 8. Many of these clones are also immune to PVX. In groups 81B and 81C it may be noted that there is a special emphasis on the agronomic character earliness. This orientation will be even more pronounced in the future.

Table 7. Inventory of PLRV resistant advanced clones used as parents in the breeding program with indications of additional attributes.

Codes	Other Attributes	No. clones resistant
81A	Earliness	43
81F	Earliness, heat tolerance	2
79K	Earliness	2
80N	Earliness, PVY immunity	8
Polish var.	Tbr background	2
German var.	Tbr background, PVY immunity	6
78A	Earliness	2
78C	Earliness, PVY+PVX immunity	5
Pentlandfield clones	Tbr background	3
PTC clones	LB, BW resistance	13
	TOTAL	86

Table 8. Inventory of PVY immune advanced clones used as parents in the breeding program with indications of additional traits.

Codes	Other Attributes	No. clones immune
79D	Highlands adaptation, PVX immunity	11
TTY	Late Blight resistant	15
Y	Earliness	6
377 series	Good combiners with tbr	15
80JA+80J	High yielders, PVX immunity	36
79S	Late blight resistance	1
379 series	Earliness, heat tolerance	15
81B	Earliness,	31
81C	Earliness, PVX immunity	21
	TOTAL	151

No listing of materials would be complete without giving some indication of performance under field conditions. All the clones listed have undergone selection in three field sites representing the dry coast, the high Sierra, and the high jungle. Only a fraction of the clones show good adaptation over all sites, and these are of special interest for exportation to national programs, if their performance holds up during several years of multi-site testing. The yield performance of several resistant clones at the Highland site, Huancayo, is shown in table 9. The yields were quite high in this trial and the gratifying result is that a number of PVY immune clones have yields comparable to good commercial varieties. In table 10 results are presented for the dry coastal site, La Molina, for a different set of clones. Again yields are within the range of performance of the local best cultivars. The specific gravities indicate that in general the dry matter content is equal to or better than that of commercial cultivars included in this trial.

The above materials are the kind of clones being used as parents. Emphasis is placed on those parents which show good general combing ability for not only PLRV, but also yield and good tuber type. Those clones showing good agronomic performance as well as verified virus resistance are submitted to the Pathogen Tested Clone Program. They are passed through shoot-tip culture and multiplied pathogen free *in vitro* and can be exported to national programs. Four such clones were submitted in 1983 and are listed in table 11. Two clones combine PLRV resistance and immunity to PVY. Two provide the useful combination of PVY and PVX immunities.

CIP's germplasm must also show promise in the regions. The best examples we have are from the work being performed by the Turkish National Potato Program (TNPP).

Since 1977 segregating progenies have been sent to the Turkish National Potato Program for exposure to virus and selection for agronomic traits at their coastal site at Menemen. Each year the progenies were planted during the Spring season which extends from March to June, and is characterized by high populations of viruliferous aphids. A number of clones have reached the stage of regional testing. A sample of results from such testing are given in table 12. It may be noted that several clones are yielding

significantly more than the cultivar Isola which is the standard that has been used for comparison during the selection process.

Table 9. Commercial yield of virus resistant advanced clones and local cultivars in Huancayo, Perú (3,280 m.a.s.l.) 1983

Clone or cultivar	Virus resistance	T/Ha	Comments
80JA 24.6	PVY immune	70.0 a	s.g. 1.099
Cusco	Susceptible	69.5 a	
Ticahuasi	Susceptible	69.5 a	s.g. 1.078
80JA 3.1	PVY immune	66.2 a	s.g. 1.098
80N 38.7	PLRV resistant	64.1 a	
80JA 32.7	PVY immune	61.6 a	s.g. 1.097
Molinera	PVY susc.,PLRVr	45.2 b	

Values not sharing the same letter are significantly different at 5% level

Table 10. Yield of virus resistant advanced clones and commercial cultivars at La Molina, Perú (240 m.a.s.l.) 1983.

Clone or cultivar	Virus Resistance	T/Ha	Comments
Revolucion	Susceptible	27.8 a	s.g. 1.070
Rosita	Susceptible	26.7 a	
78C11.5	PVY+PVX immune, PLRV resistant	23.3 ab	s.g. 1.082
78A15.6	PLRV resistant	22.6 ab	s.g. 1.076
Serrana	PLRV resistant	21.8 ab	s.g. 1.071
Desiree	Susceptible	15.8 b	

Means with different letters are statistically different (5%).

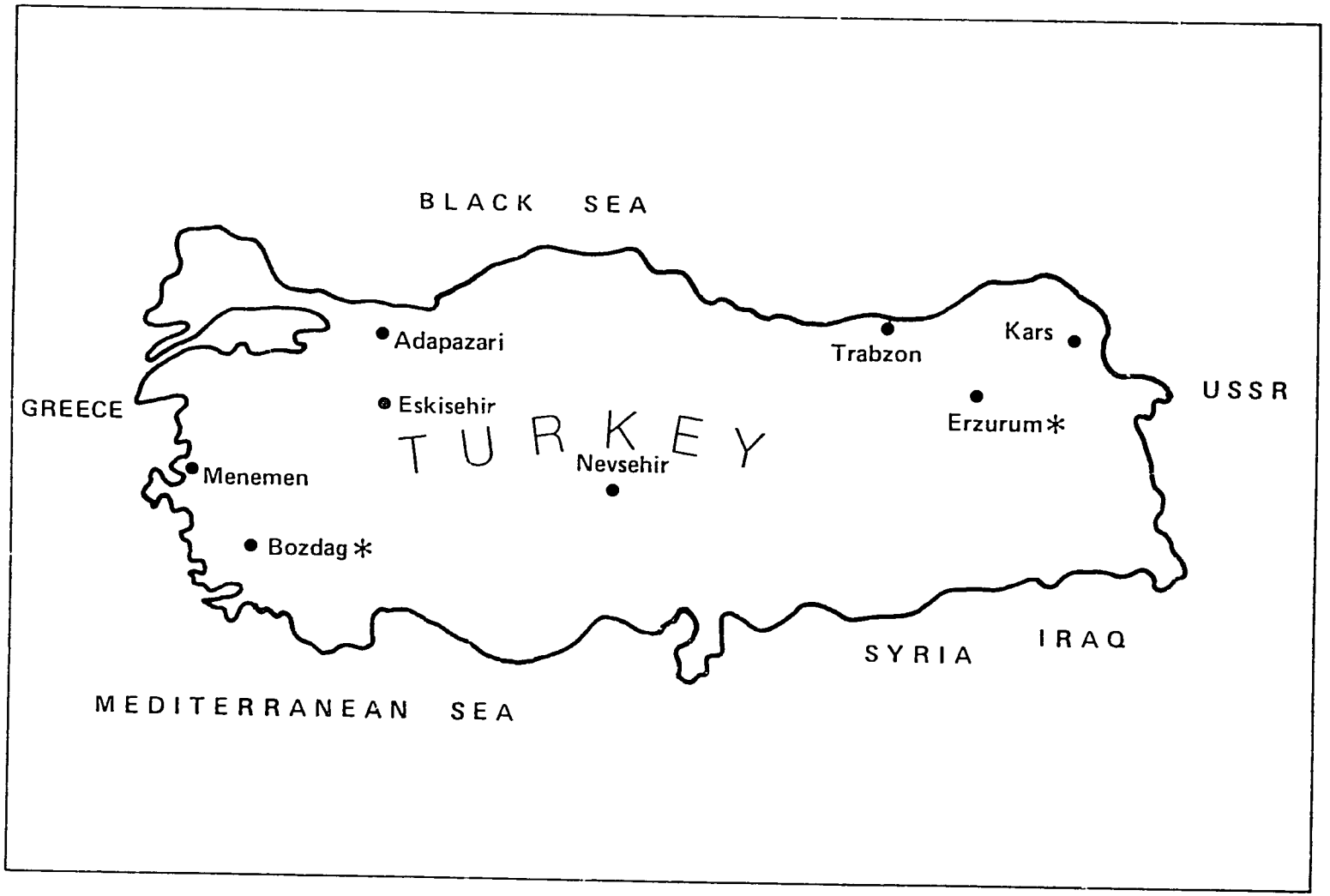

Fig. 6. Map of Turkey showing different potato growing sites and locations of Bozdag and Erzurum (*)

PG 295 derives its PVY immunity from stoloniferum, and its PVX immunity from acaule, while XY13.15 derives both immunities from andigena.

Table 11. Virus resistant clones submitted for multiplication in pathogen tested program.

Clone or cultivar	Resistance Attributes		
	PLRVr	PVYi	PVXi
78C11.5 1/	x	x	x
Pirola 2/	x	x	
PG295		x	x
XY13.15		x	x

1/ German variety
2/ Polish variety "Bzura"

Further encouraging results may be found in trials performed in Eastern Turkey near Erzurum. There an andigena x tuberosum hybrid is the highest yielder (Table 13). The location of these two sites is shown on the map in figure 6.

Table 12. Commercial yield of virus resistant advanced clones and commercial cultivars in Bozdag, Turkey 1983.

Clone	Pedigree	T/Ha
Granola	3333/60 x 267.40	60.4 a
81040.2	Serrana x XY 15.4	57.1 a
81055.11	PG 322 x XY 2.9	45.4 b
81035.15	Regina x XY 12.1	42.5 bc
81045.6	Serrana x XY 2.9	41.2 bc
81035.6	Regina x XY 12.1	37.1 c
Isola	(Sabina x Voran) x S. dem x Flora)	36.5 c

Values not sharing the same value are significantly different at the 5% level

Table 13. Yield trial from Hasankale near Erzurum, Eastern Turkey. June to October, 1983.

Identity	Pedigree	T/Ha
79055.1	702158 x I-1058	25.8 a
Granditolia	Clivia x Hansa	23.1 a
Granola	333/60 x 267.04	22.9 a
81044.9	Serrana x M. tropical	22.8 a
Isola	Sabina x Voran x [(S. dem. x tbr) x Flava]	21.3 ab
Cosima	(Sabina x Voran) x (MPI 41.969/377 x Flava)	17.4 b
Resy	SVP 50-2017 x MPI 19268	12.7 c

Values not sharing the same value are significantly different at the 5% level

New Sources of Resistance to PLRV from S. etuberosum and S. acaule

A very strong resistance to infection by PLRV was reported by Jones (1979) in S. brevidens. At first it was thought that the resistance was immunity, but after more sensitive testing virus was detected in graft-challenged plants. The Series Etuberosa is very difficult to cross with tuber-bearing species. However, Hermsen and Taylor (1979) succeeded in crossing another member of this Series, etuberosum, with S. pinnatisectum, a diploid Mexican wild species. The F1, designated as EP here, was sterile due to lack of homology between the genomes. Later Hermsen and Ramanna (1981) reported the production of fertile allotetraploids through induction in leaf explants. These EP materials were studied at CIP. True seed progenies of EP selfs and intercrosses were obtained from Hermsen. Extreme resistance to infection upon graft challenge was reported by Rizvi (1983). With these highly resistant materials as a starting point two projects were undertaken:

1) A study of the inheritance of the resistance in intra-EP crosses, and

2) The transfer of resistance to a tuberiferous gene pool, a work carried out as part of the doctoral dissertation of Rene Chavez (1984). The seedlings were infected by manually placing PLRV-infected aphids on the seedlings six weeks after sowing. Although ELISA serology was utilized this was shown to be insufficiently sensitive to detect infection in these materials. The final tabulation of resistance occurred after grafting the seedlings to D. stramonium and testing the stock tissue with ELISA. The results of the first project are summarized in table 14 where the proportions of each progeny infected are given for a complete diallel (except for one cross). In table 15 the means of various types of genetic crosses are shown.

Table 14. Proportion of EP progenies infected. Marginal female and male means indicated for diallel design.

	R_1	R_2	S_1	S_2	-means
R_1	.58	.79	.65	.60	.66
R_2	.56	.60	.50	.88	.64
S_1	.87	.87	.68	.65	.77
S_2	.61	.88	-	.64	.69
-means	.66	.78	.61	.69	

Table 15. Mean proportion of progenies infected in resistant x resistant (R x R), susceptible x resistant (S x R), resistant x susceptible (R x S), and susceptible x susceptible (S x S) crosses.

R X R	=	.63 ± .07
R X S	=	.66 ± .06
S X R	=	.81 ± .05
S X S	=	.66 ± .07

In summary, the material seems to be uniform genetically for susceptibility to infection by aphids. Similarly, it expresses in a uniform fashion a resistance to virus build-up in the tissues, which although infected, do not show the presence of virus when tested by ELISA. In table 16 the results of infection are shown for the original etuberosum and pinnatisectum accessions in relation to O.P. seed of the clone DTO 33, which is highly susceptible. The etuberosum would appear to be highly resistant to infection, while the pinnatisectum is quite susceptible. The EP hybrid may be an unfortunate combination of resistant and susceptible genotypes where the resistance to infection of one is suppressed by the susceptibility of the other. In any case, the strong resistance to virus build-up if transferable to a cultivated gene pool, could be an effective way to control the virus.

The results of Rene Chavez' thesis work indicate that hybrids of EP with acaule (AEP) and verrucosum (VEP) show the same pattern of high susceptibility to infection by aphid inoculation and resistance to virus build-up. These hybrids are tuber-bearing. An interesting hexaploid acaule-etuberosum-pinnatisectum (AEP-6x), which has apparently arisen from 2n eggs in acaule is male fertile, and produces abundant seed from selfing, is being explored as a bridging hybrid to extract the etuberosum factor(s) responsible for resistance to PLRV into a fertile quasi-acaule, which can serve as a vehicle for introduction of etuberosum chromosomes into a cultivated background. Hexaploid VEP hybrids provide an additional route for this introduction.

Table 16. Proportion of progenies infected of etuberosum and pinnatisectum accessions from which the original parents of EP were selected. DTO 33 included as a susceptible control.

245924 (S. etuberosum)	= 0.10
245139 (S. etuberosum)	= 0.05
230489 (S. pinnatisectum)	= 0.87
DTO 33 (Tbr x Phu)	= 0.95

A number of acaule accessions were screened for PLRV resistance. Several have been shown to have very strong resistance to PLRV infection by aphid inoculation. One accession was used as the starting point for the incorporation of this resistance into the cultivated gene pool. The scheme of this process is shown in figure 7. First the acaule was crossed to a hybrid between phureja and stenotomum to produce triploids. Because of the infertility problem always present with odd ploidy numbers it was necessary to try to overcome this by doubling the triploids to produce hexaploids. This was accomplished by treating germinating triploid seeds with a short exposure to colchicine, followed by rearing the seedlings on a special colchicine-shock-recuperation medium. In the end, the result necessary for further progress, i.e., doubling of the L-2 layer, was achieved in 16 out of 44 plants which survived and flowered.

Several hexaploids were graft challenged using PLRV-infected leaves of Datura stramonium. Symptoms were never observed in any of the materials, but virus could be detected after several months of grafting using ELISA in some of the clones, but not in others. For the purpose of determining whether the resistance can be transmitted into the equivalent of the the cultivated gene pool, two hexaploids one graft-resistant and one graft-susceptible were crossed to two PLRV susceptible cultivars, CGN 69-1 and DTO-33.

Figure 7. Scheme of incorporation of resistance to PLRV from *acaule* into cultivated gene pool.

S. acaule (A) x [*S. phureja* x *S. stenotomum*] (PS)

↓

APS (3x)

↓ colchicine

APS (6x)

S. tuberosum (T) x APS (6x)

↓

TAPS (5x)

TAPS (5x) x *S. tuberosum*

↓

TTAPS (4x +)

The pentaploid progenies were inoculated with PLRV-bearing *M. persicae* and tested for the presence of PLRV by ELISA two months after inoculation. The results of testing four progenies and the four parents are presented in table 17. The progeny of the hexaploid clone T3.1.33, showed virtually no infection, while the progeny of T2.17, the graft-susceptible hexaploid, showed nearly total infection. Both susceptible tetraploid parents included in the test for the purpose of comparison, were completely infected. This shows that the high resistance to PLRV present in the *acaule* can be transmitted to a fertile cultivated genetic background. The pentaploids that have resulted from this tetraploid x hexaploid crosses are equivalent to a synthetic *curtilobum* and are quite vigorous. Care has been taken to incorporate the PLRV resistance into the pentaploids using PVY immune tetraploids so that simultaneous selection for the two resistances may be carried out during the further nobilization of this genetic stock.

Table 17. Segregation of infected and healthy pentaploid progenies from 4x-6x crosses. Parents also indicated.

Cross/clone	Ploidy	ELISA 60-days after inoculation Number of Plants Infected	Healthy
CGN69.1 x T3.1.33	5x	2	17
DTO-28 x T3.1.33	5x	1	19
CGN69.1 x T2.17	5x	20	0
DTO-28 x T2.17	5x	19	1
DTO-28 parent	4x	10	0
CGN69.1 parent	4x	10	0
T3.1.33 parent	6x	graft-immune	
T2.17 parent	6x	graft-susceptible	

Conclusions

In the Reports of the Planning Conferences in Genetics and Virology, held in 1980 in Lima, Peru, resistance to PLRV was placed as the highest priority (CIP, 1980a, 1980b). Emphasis was placed on the need to look for better resistance in cultivated and wild species. Furthermore combining this with PVY resistance was considered essential as a breeding goal in order to provide varieties that can resist the highly damaging PVY-PLRV complex extent worldwide. Immunity to PVX was relegated to a secondary status although immunity to the resistance-breaking strain, PVX , was given a high priority.

HB

In the light of these recommendations it is useful to summarize the work now in progress at CIP in virus resistance.

1) New types of PLRV resistance from etuberosum and acaule have been studied and partially characterized. The resistance from acaule is available in a fertile pentaploid.

2) Immunity to PVY has been incorporated into a large set of families which are now available for export. Progress has been made in producing male-fertile populations with a high frequency of PVY immunity. Likewise, a set of PLRV resistant clones has been selected after extensive field testing. High general combining ability for PLRV resistance has been identified amongst the resistant clones. All breeding materials now screened are combinations between PVY immune and PLRV resistant parents.

3) It is projected that two to three clones with PVY immunity and/or PLRV resistance (preferably both) will be released annually for testing in the regions. Meanwhile, the best families for virus resistance and agronomic performance are produced as tuber families for testing and selection by National Programs. A number of clones from tuber families are in yield trials in Turkey.

4) Immunity to PVXHB has been found and is apparently monogenic.

Although progress has been made in finding better resistance to PLRV, and providing advanced materials with some level of PLRV resistance with PVY immunity, the successful introduction of virus resistant varieties into farmers' fields will depend on their agronomic performance. Therefore, future work plans will continue to emphasize the production of high yielding and broadly adapted clones. For this work to succeed we will need the capable collaboration of our national program counterparts. They in turn will need from us the best genetic recombinants that the germplasm and our manipulation of it can supply. Reviewing the progress made to date it is apparent that our common efforts are on the verge of diminishing damage caused by virus diseases in countries of the developing world.

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Breeding Bacterial Wilt (*Pseudomonas solanacearum*) Resistant Germplasm

Peter Schmiediche

Introduction

Systematic breeding for resistance to bacterial wilt started in 1967 at the University of Wisconsin. Rowe and Sequeira (1972) began this program by intercrossing a few resistant diploid clones of *S. phureja*, which had been found in the Central Colombian Collection (CCC), with *S. tuberosum* ssp. *tuberosum* germplasm. During field testing they found that resistance to late blight was also needed and that it would have to be combined with bacterial wilt resistance. The needed late blight resistance was obtained from Mexican varieties which are essentially ssp. *tuberosum* containing resistance genes from *S. demissum*.

In 1979, French, who was then with the Potato Program of the Ministry of Agriculture of Peru and the NCSU Agricultural Mission to Peru, received 369 clones from Wisconsin, representing 10 families. He started a screening program at Huambos in the Department of Cajamarca with his Peruvian collaborator, Isaías Herrera, a graduate student from the Universidad Nacional Agraria in La Molina. Herrera continued to collaborate with French as a research officer in the Ministry of Agriculture of Peru (Herrera, 1972). Herrera was soon transferred to another post, and by 1974 the material had changed hands three times, and of the 369 original clones seven had survived. On the basis of the assembled data, the variety Caxamarca (BR-63.74) was named in 1976 (Herrera *et al.*, 1977), and in the following year Molinera (BR-63.65 was named (De la Puente *et al.*, 1977).

French conducted a survey in Cajamarca in January, 1981 which revealed that local farmers had selected two more Wisconsin clones on their own and had named them Molinera-2 (thought to be BR-69.84 wich is now an official variety in Fidji) and Amapola (CIP 800950) whose identity has been lost. Molinera-2 had been selected for its culinary quality and Amapola for its high yield and resistance to late blight. Amapola had, for instance, been rejected by the National Potato Program because of its poor tuber shape.

Apart from sending 369 clones to Peru, the Wisconsin breeding program sent similar sets of material to 20 countries around the world. In the early years of this program, virtually no data about the performance of these clones was received from any of the participating

countries. After the foundation of CIP, the University of Wisconsin entered into a research contract with CIP and began to send more material. Much of this material is still in CIP's breeding program and is identified by codes like BR, MS, PSP, or PSW.

All of the bacterial wilt-resistant material that CIP sent to National Programs in the first seven years of its existence, came exclusively from the University of Wisconsin program. CIP's breeding program for resistance to bacterial wilt had the Wisconsin germplasm as its only source of resistance (CCC clones of S. phureja). Bacterial wilt resistant hybrids produced in the early years at CIP were coded MB.

The Breeding Program for Resistance to Bacterial Wilt at CIP from 1977 - 1980

In the beginning of 1978, every breeding program at CIP had to be completely reorganized because of the threat posed by potato spindle tuber viroid (PSTV). From that time, parental material had to be free of all known pathogens, in particular PSTV, before the material could be used for crosses that were made to produce hybrids (tuber families) for export. The cross itself had to be carried out under quarantine conditions in special quarters. This reorganization coincided with a new crossing plan for the bacterial wilt program. By 1977, data from National Programs in CIP's regions had clearly shown that the resistance coming from S. phureja was effective against all races and pathotypes of Pseudomonas solanacearum affecting potatoes, as long as the resistant material was grown under temperate highland conditions. Bacterial wilt was, for instance, eradicated in the highlands of the Department of Huanuco in Peru through a combination of measures, of which the use of the resistant variety Molinera was a very important one. Molinera continues to be cultivated in that area, however, not because of its bacterial wilt resistance but because of its resistance to late blight, which is a serious problem during the wet season.

When the cultivation of potatoes was slowly extended to the hot, humid lowland tropics, the bacterial wilt resistance so effective in the highlands tended to break down. Another disadvantage of this material, which had become obvious in the first eight years of its use, had been its relative lateness under tropical conditions (French, 1979).

The crossing plan of 1977 (Table 1) produced bacterial wilt resistant Group I. The two varieties Molinera (BR-63.65) and Caxamarca (BR-63.74) were crossed with a series of clones and varieties in order to introduce earliness into this material. The crossing plan for 1979 (Table 2) shows that the emphasis was on the incorporation of heat tolerance into the same material. The female parents are almost ex-

Table 1. Pedigrees of bacterial wilt resistant Group I, 1977

CIP No.	Pedigree
377830	BR-63.65 x N-574.1
377831	BR-63.65 x Katahdin
377835	BR-63.65 x Atlantic
377838	BR-63.65 x N-522.22
377847	BR-63.74 x Anita
377849	BR-63.74 x N-503.214
377850	BR-63.74 x DTO-28
377852	BR-63.74 x WRF-1923.1
377863	WRF-1919.2 x BR-63.65
377864	Anita x BR-63.65

clusively from Group I. The idea behind the latter set of crosses was that resistant genotypes would keep up their resistance if adapted to heat conditions. It was observed that resistance to bacterial wilt does not break down per se under such conditions but is a function of adaptation.

Confirmation of this hypothesis came at the beginning of 1983 from Sri Lanka where parts of the Group II material and another set of heat

Table 2. Pedigrees of bacterial wilt resistant Group II, 1979

CIP No.	Pedigree
376648	377835.3 x DTO-33
379666	377831 x Maria Tropical
379669	377835.12 x Maria Tropical
379673	377847.4 x Maria Tropical
379674	377847.5 x Maria Tropical
379676	377850.1 x Maria Tropical
379677	377851.3 x Maria Tropical
379679	377852.1 x Maria Tropical
379681	Serrana x CGN-69.1
379693	377835.12 x CGN-69.1
379695	377847.1 x CGN-69.1

tolerant x bacterial wilt resistant hybrids from the program of Dr. Mendoza were tested in the 1981-82 season under heat conditions. This material was tested in two sets of 105 and 147 clones, respectively. Of the set of 105 clones, 35 were selected as bacterial wilt resistant under heat conditions out of which 11 were also selected on the basis of yield. In a re-test the following year (1982-83 season), four clones were selected out of these 11 as once more completely resistant.

Of the 147 clones that contained parts of Group II, which was the result of the first set of crosses of bacterial wilt resistant x heat tolerant material, 76 clones were selected as resistant under lowland tropical conditions. The data of the re-tests were not available at the time of preparing this paper.

Table 3. Results of screening bacterial wilt resistant x heat tolerant hybrids in Sri Lanka, 1981-1983

ss	No. Clones Tested	No. of Clones BW Resistant Under Heat Conditions	No. of BW Resistant and Heat Tolerant Clones Selected
LT x BR bulk	105	35	11 first year 4 after re-test
Group II and LT x BR bulk	147	76	Not yet determined

LT = Lowland Tropical BR = Bacterial wilt resistant material

These results demonstrated beyond doubt that resistance to bacterial wilt must be viewed as only one element of a combination of factors of which adaptation is another integral part. Agronomic measures must also be added to the elements of resistance and adaptation in order to achieve lasting results as described in point number 5. The introduction of earliness and adaptation to heat into the bacterial wilt resistant germplasm is in part achieved through backcrosses. The pedigree of Molinera and Caxamarca is Atzimba x A-1 (316.3 (Phu 1386 x Phu 1339) x Katahdin). Molinera was, for instance, crossed to Katahdin for the generation of one family in Group I. Although these crosses achieved the desired results of bacterial wilt resistance under hot, lowland conditions, the proportion of bacterial wilt as well as late blight resistant progenies in the hybrid populations decreased markedly. The exact proportion of loss of resistance has, however, not yet been calculated.

When Group III was generated in 1979-80, CIP tried to restore some of the lost resistance by making crosses with bulks of late blight and bacterial wilt resistant material (Table 4). In the case of bacterial

Table 4. Pedigrees of bacterial wilt resistant Group III, 1979-80

CIP No.	Pedigree
379690	377835.9 x CGN-69.1
380572	Atlantic x PI/PS bulk
380576	377852.2 x PI/PS bulk
380577	377349.2 x PI/PS bulk
380579	377835.2 x CGN-69.1

wilt resistant bulk, it should be noted again that the crosses were genetically backcrosses, and that a degree of inbreeding had become unavoidable. This situation had come about because of the severe restrictions that had been placed on the breeding programs to prevent the use of potentially PSTV-infected parents. The parents used in these last two sets of crosses were the only ones known to be free of PSTV at that time.

The Breeding Program for Resistance to Bacterial Wilt at CIP from 1980 -1983

In mid-1980, the responsibility for the bacterial wilt breeding program was assumed by a new project leader. At the end of 1980 the tuber families of Group IV were generated (Table 5).

The most significant difference in the generation of Group IV when compared to the earlier three groups was the use of two new clones in the crossing program: Serrana INTA from Argentina and AVRDC-1287.19 from Taiwan. Serrana INTA introduced resistance to PLRV and PVY and immunity to PVX into the population as virus susceptibility was observed in Groups I and II. With AVRDC-1287.19 a new source of resistance to bacterial wilt was introduced that was not based on *S. phureja* but on other hitherto unused sources found in wild species such as *S. raphanifolium* and *S. chacoense*. Furthermore, AVRDC-1287.19 had the ideal combination of earliness and heat tolerance. Of the 8 families in Groups IV, five combine for the first time the traditional resistance derived from *S. phureja* with a new source of resistance.

It is too early to say anything about the performance of Group IV under field conditions in the regions. Some preliminary data, however, appear to indicate earliness, high yield and good resistance.

Table 5. Pedigrees of bacterial wilt resistant Group IV, 1980.

CIP No.	Pedigree
381046	377849.2 x AVRDC-1287.19
381047	Serrana x BR-63.5
381048	Serrana x 377831, 377852 bulk
381050	377835.9 x AVRDC-1287.19
381064	377852.1 x AVRDC-1287.19
381065	377835.2 x AVRDC-1287.19
381075	Serrana x AVRDC-1287.19
381077	Serrana x BR-69.84 + BR-63.5 bulk

For the production of Group V, which was generated in 1982, the idea of introducing new sources of resistance to bacterial wilt, late blight and earliness was followed again. The bacterial wilt resistant clones Cruza 148 from Mexico and the late blight resistant clone India 853 were used in crosses with the best of the bacterial wilt resistant material. With the use of these new clones earlier tendencies of inbreeding were counterbalanced and completely overcome. This had become possible because the list of pathogen-tested clones, which are potential parents, had become longer and longer in the last four years.

Selection Procedures Related to the Production of Tuber Families for Export

The strict quarantine measures for the production of export material require that crosses for the generation of seeds and the subsequent production of seedlings and tuber families are carried out in special quarantine quarters. Since tubers are produced from seedlings in small plastic pots, it is virtually impossible to make any selection based on yield and agronomic characters. The late blight resistance of this population was previously determined in a seedling screening test, and the survivors of that test are the ones used for the production of tuber families. This has not always been strictly carried through, as on two occasions limited space and broken down growth chambers prevented the late blight screening test prior to the production of tuber families. The families thus produced are, however, useful for those areas where

late blight is not present but where bacterial wilt is a serious problem. The frequency of bacterial wilt-resistant clones was, of course, also increased in these populations, as the blight test would have eliminated some of the bacterial wilt-resistant material. The wilt resistance of these populations produced under strict quarantine conditions is determined in stem cuttings taken from each seedling. This procedure, although labor-intensive, allows CIP to screen a given population against isolates of Pseudomonas solanacearum that are from specific countries or their regions. Group V was, for instance, screened against isolates from Brazil and Indonesia.

It might take years before the breeder sees his materials in the field, and performance data often do not come back from country programs or only many years after a tuber family has been sent out. In this case the breeder has no basis for further decisions. If data do come back, it is, for instance, possible to identify good general combiners for resistance and agronomic characters. This makes decisions on further cross combinations considerably easier allowing population sizes to be decreased. Two good combiners thus identified are Molinera and Atlantic. Many seedlings grown in quarantine produce only one or two tubers. In the past those clones were used only in Peru since their propagation in the screenhouse, which would have been necessary if they were to be exported, presented certain problems of timing and space. It is not known, however, whether the low number of tubers is a random phenomenom due to the highly artificial conditions of the quarantine quarters or whether it is a genetic character. An experiment to clarify this question is under way.

With the generation of Group V, sufficient material has become available for export as well as for observational trials in the field in Peru. This should enable the bacterial wilt breeding program to make preliminary selections on the basis of yield and agronomic characters in less than a year after performing the crosses. It is well recognized that the field data obtained in Peru cannot be the final and only criterion for the selection or rejection of a given clone. Two clones that were recently rejected on the basis of CIP field data from Peru had been selected in Brazil almost at the same time. This indicated once more the absolute necessity of close collaboration between regional research, national programs and source research.

Breeding for Resistance to Bacterial Wilt and the Importance of Agronomic Measures

As was mentioned before, resistance to bacterial wilt has to be combined with adaptation to adverse environmental conditions if potato cultivation continues to be extended into hot, humid areas. However,

the resistant adapted germplasm available at present will not withstand roo-knot nematode infestation and a continuous high level of Pseudomonas solanacearum inoculum in the soil. Recent observations in Central Africa demonstrated, for example, that in some countries volunteers are never eliminated after a potato crop and that they are able to maintain themselves for many years even if no other potato crop is grown in the same field. In one field, potatoes had not been cultivated for 10 years, and the potato crop then planted was almost completely wiped out by bacterial wilt. A close inspection of this field revealed an abundance of volunteers even ten years after the last potato crop. This and similar observations elsewhere indicate that resistance and adaptation alone will not control bacterial wilt completely. Resistance and adaptation have to be combined with agronomic measures such as the roguing of volunteers and careful measures of crop rotation and seed management. A long-term crop rotation experiment underway in Rwanda should help to answer some of the most important questions as to which type of rotation will keep the inoculum at manageable levels. Preliminary results indicate that the level of inoculum can be lowered through rotation so that even susceptible varieties can be grown with a low incidence of wilt. The combination of crop rotation measures and resistant adapted varieties should control the pathogen completely.

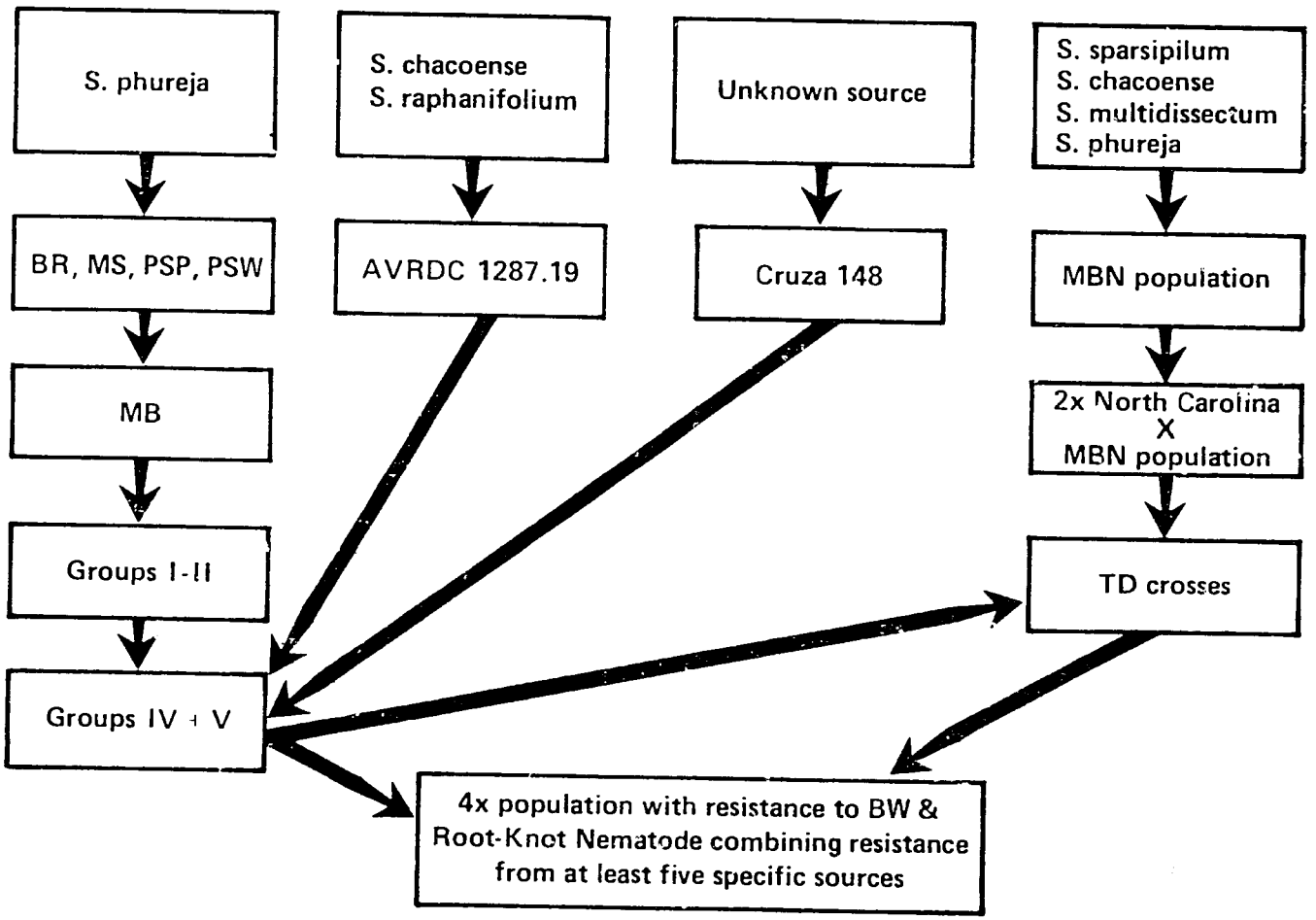
The interaction of root-knot nematode (Meloidogyne spp.) infestation and incidence of bacterial wilt is well documented (Jatala et al., 1975, 1977). There is very little Meloidogyne resistance of any consequence to be found in any of the tetraploid cultivated germplasm known. This means that at the moment there is no prospect of combining Meloidogyne with Pseudomonas resistance at the tetraploid level. Meloidogyne spp. infestation combined with the presence of bacterial wilt is, however, a serious problem in several potato-growing countries.

Resistance to Meloidogyne spp. has been identified in wild species whose use will be considered in the following chapter.

The Use of New Sources of Resistance

The complexity and highly specific pathogen-environment-host interaction of Pseudomonas solanacearum is well known, and efforts to respond to this versatility with more than one source of resistance have been in progress for some time (Schmiediche et al., 1982). Bacterial wilt-resistant accessions of S. sparsipilum, S. chacoense, and S. multidissectum were identified at CIP about five years ago. A population was established that was derived from the resistant accessions of these three species and from the traditional S. phureja source. This population consisted of 11,500 seedlings and was tested against four strains of races 1 and 3 of Pseudomonas solanacearum in a two-year screening

Fig. 1 **Sources of resistance to bacterial wilt and their utilization at CIP**



program. Only 40 clones belonging to eight families survived the rigors of this testing. These 40 clones were intercrossed in more than 200 combinations. Of the 100,000 seeds obtained, 10,000 seedlings were raised and tested against the particularly severe strain 013 of race 3. Some families showed survival rates of up to 67% which is a significant increase over the 30% recorded in the unselected base population when it was originally assembled and tested against the same pathotype.

From the survivors of this test, 167 clones from 42 families were planted and crossed with a bulk pollen from a diploid cultivated population. This population was received from Dr. F. Haynes at NCSU, who had assembled it from accessions of S. stenotomum, S. phureja, and S. goniocalyx. After it had been taken through seven cycles of recurrent selection under the hot summer conditions of North Carolina, this population had acquired good agronomic characters, a high dry matter content, and also a considerable level of resistance to bacterial wilt. Some families had 25 - 30% resistance when tested against strain 013 of race 3.

The idea behind this set of hybridizations was to combine high levels of resistance with advanced agronomic characters at the diploid level. The first 10,000 seedlings were planted early in 1983 and harvested in June of this year. The only selection criterion was agronomic characters and about 500 clones were selected out of 10,000. Their resistance is now being tested. This work is part of an M.S. thesis with the Universidad Nacional Agraria of Peru. The resistant survivors will be intercrossed for a further cycle of recurrent selection. At the same time some of these diploid clones will be crossed with the traditional and most advanced tetraploid material (Fig. 1).

In this way a tetraploid highly heterozygous population combining at least four sources of specific resistance to bacterial wilt will be generated. The available evidence from potatoes as well as other crops suggests that the alleles conferring resistance to a particular pathogen are not identical in all the species that have resistant accessions. The last step of CIP's breeding program for resistance to bacterial wilt attemps, therefore, to unite as many different resistance alleles from as many different specific sources as possible into one genetic population. Or, in other words, to put maximum genetic variability for the trait "resistance to bacterial wilt" into one population. This should equip this population with sufficient broad spectrum resistance as to meet practically any serious challenge in the field. We have several years to go to achieve this goal; the prospects are, however, quite realistic.

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Ploidy Level Manipulation Approach: Development of Diploid Populations with Specific Resistance and FDR 2n Pollen Production

M. Iwanaga

Introduction

There are three factors which provide unusual opportunities for potato improvement: 1) The wild and cultivated tuber-bearing relatives of the potato represent a large source of valuable germplasm, which is valuable in providing both specific desirable traits, such as disease and insect resistance, and for broadening the genetic base. 2) Haploids (2n=2x=24) of cultivars (2n=4x=48) can be readily obtained and used (Hougas and Peloquin, 1958). They offer the advantages of a direct approach to germplasm transfer from the numerous 24-chromosome, tuber-bearing relatives of the potato and simpler inheritance patterns (disomic vs. tetrasomic) which facilitate breeding work to combine desirable traits of the parents. They also provide us with a unique means of capturing genetic diversity. 3) The discovery of meiotic mutants that give rise to 2n gametes (gametes with the same chromosome number as the parents) provides unique and exciting opportunities to increase yield and genetic diversity. It is estimated that 2n gametes formed by First Division Restitution (FDR) transfer intact 80% of the genotype of diploids to their tetraploid progeny in 4x-2x and 2x-2x crosses (Peloquin, 1979). The meiotic mutant, parallel spindles (ps), is an FDR mechanism that accomplishes this transfer in the production of 2n pollen (Mok and Peloquin, 1975).

The ploidy level manipulation approach has been developed based on the analytic breeding scheme of Chase (1968), taking advantages of the above mentioned factors (Figure 1). Three steps are involved: 1) extraction of haploids from selected tetraploids, 2) hybridization between the haploids and useful 2x species and subsequent population breeding at the 2x level for multiple resistance, agronomic characteristics, adaptation and 2n pollen production in wide genetic backgrounds; and 3) transfer of the improved 2x germplasm into the 4x level via FDR 2n pollen.

There are several important implications of this strategy for CIP's worldwide responsibility. First of all, this strategy facilitates utilization of useful wild and cultivated species, which meets with the mandate of the Center. Second, the project has its main focus on breeding for multiple pest and disease resistances to meet with urgent needs of small farmers in developing countries who cannot afford expensive chemical controls of the pests. Potato cultivars with multiple resistances would be an essential component of technologies for potato production in developing countries. The present approach is effective

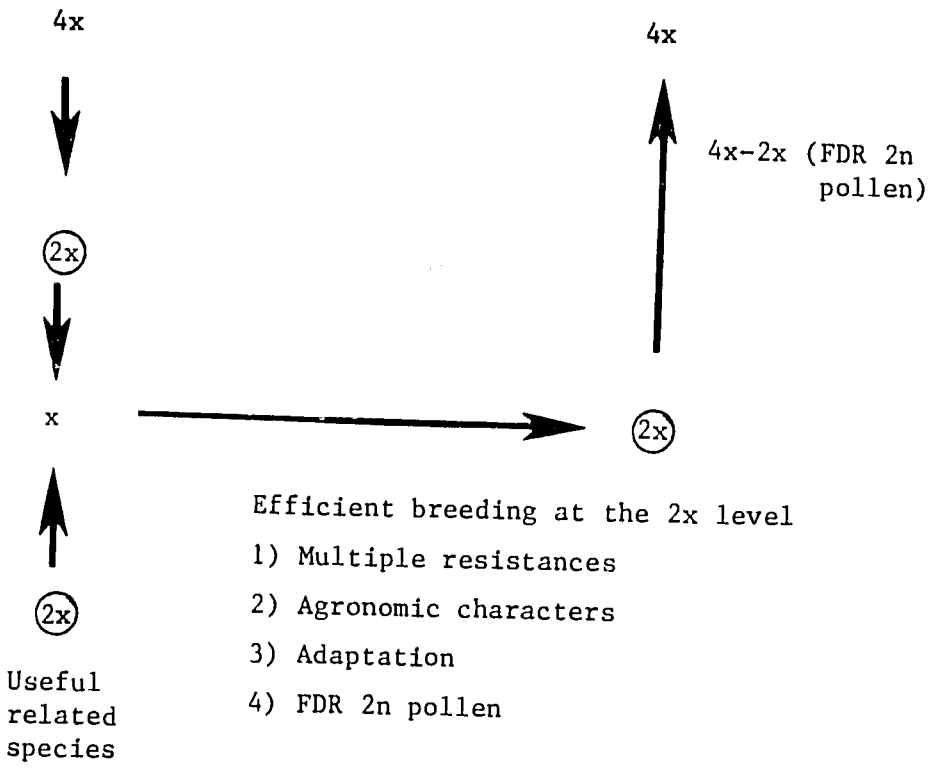

Figure 1. Scheme of ploidy level manipulations.

in incorporating multiple resistance into the breeding populations. Third, this fits well with CIP's worldwide responsibility to export germplasm to many national programs with different requirements for adaptation and consumer preference. This approach is meant to produce only 2x male parents with specific resistance(s) and FDR 2n pollen production which can be used for 4x-2x crosses to produce 4x progenies. By choosing local varieties of a country as female parents for the 4x-2x crosses, we can produce 4x progenies whose female parents contributes the horticultural type and adaptation desirable for the country. The 2x parents would provide important resistance(s). It permits reducing difficulties to incorporate important horticultural types of many different countries in our breeding populations. Fourth, the 4x progenies produced by 4x-2x crosses would be excellent not only for clonal selection program but also for utilization as true potato seed (TPS) cultivars. Uniformity in agronomic characters, high yield and multiple resistances are the most important traits for the use of TPS in developing countries. The present 4x-2x cross approach would produce better TPS families than normal 4x-4x crosses in regard to the important traits. Genetic homogeneity of FDR 2n pollen assure uniformity of the 4x TPS families and heterozygosity of FDR 2n pollen produced by heterozygous diploids would result in production of highly heterozygous and high yielding 4x progenies. Multiple resistances which are combined at the 2x level are efficiently transmitted to the 4x progenies by FDR 2n pollen. Finally, it is important to mention that the advantages of the present approach over breeding work at the 4x level do not mean that breeding work at the 4x level should be reduced. On the contrary, strong breeding work at the 4x levels complement the breeding work at the 2x level since final progenies will be produced by 4x-2x crosses.

Three conditions should be satisfied here before full potential of the approach using haploids and 2n pollen can be exploited. First, a large number of haploids with genetic background should be obtained, since intensive use of particular haploids results in narrowing the genetic bases. This might lead to coancestry and inbreeding which is highly undesirable, particularly for yield. Second, a good number of male fertile haploids should be utilized so that male sterility caused by interactions between tuberosum cytoplasm and nuclear genes of related germplasm can be avoided. This is very important to achieve efficient crossing work during the advancing generations of population improvement. Finally, identification of the parallel spindle (ps) gene in haploids extracted from tetraploids is crucial since the use of FDR 2n pollen by ps is essential to transfer improved 2x germplasm into the 4x level.

Table 1. Important traits and main sources of germplasm utilized.

Traits	4x spp*	2x spp
a) Disease resistance		
1) Late blight	adg, tbr**	
2) Early blight		phu, stn
3) Bacterial wilt		phu, spl, chc
4) Erwinia spp		phu, stn
5) PVX	adg, tbr**	
6) PVY	adg, tbr**	
7) PLRV	tbr**	
b) Pest resistance		
8) Potato tuber moth		stn, spl, tar
9) Root-knot nematodes		chc, spl, mcd
10) Cyst nematodes	adg	vrn
c) Environmental stress		
11) Heat	tbr, neo-tbr	phu, stn
12) Frost	adg**	
d) Agronomic traits		
13) Earliness	tbr, neo-tbr	phu, stn
14) High dry matter		phu, stn, gon

* Haploids have been obtained and utilized.
** Resistance has been transferred from related species.

Materials and Methods

Two different ecological zones within the tropics are considered. These are highland tropics and lowland tropics. The resistances or tolerances considered important for each zone are:

Highland tropics - PVX, PVY, cyst nematode, late blight and frost.

Lowland tropics - PVX, PVY, PLRV, bacterial wilt, Erwinia spp., late blight, early blight, root-knot nematode, potato tuber moth, and heat.

Extraction of haploids from selected tetraploids was done by 4x-2x crosses by using a diploid clone IvP 35, which was reported to be a good haploid inducer with embryo-spot marker (Hermsen and Verdenius, 1973). The seeds obtained from the crosses were checked for embryo spot. Observation of general morphology and count of chloroplast number of stomata were used to eliminate triploid or tetraploid plants grown from embryo spotless seeds. Pollen stainability, 2n pollen production and cytological mechanisms of 2n pollen were studied according to Marks (1954), Quinn et. al. (1974) and Mok and Peloquin (1975), respectively.

Results and Discussion

1) Extraction of a large number of haploids

A sample of 2,928 haploids with different attributes and genetic background was obtained from 76 tetraploids (Table 2). Extraction of a large number of haploids from late blight resistant tetraploids was emphasized, since only a small proportion of the haploids were expected to be highly resistant. Since the haploids were from many tetraploids having tuberosum, andigena, and phureja in their genetic background, it is expected that use of the haploids will not lead to inbreeding unless the number of useful clones becomes very small making mating between relatives difficult to avoid.

2) Male fertility of the extracted haploids

A sample of 501 haploids has been studied for pollen stainability. The haploids were classified into four groups according to pollen stainability. About one third of the haploids had less than 5% of pollen stainability and were considered to be male sterile, and 29% of the haploids had 5 to 30% of pollen stainability and were expected to have only low level of male fertility (Table 3). On the other hand, 14% of the haploids had 30 to 50% of the stainability and were expected to have good level of male fertility and the rest, 22% of the haploids, had more than 50% of the stainability and were considered highly male fertile. Thus, 36% of the haploids are expected to have an adequate level of pollen

Table 2. Numbers of haploids extracted from selected tetraploid

Resistance of tetraploid parent	Number of resistant parents	Genetic background	Number of extracted haploids
Late blight	21	tbr, adg	2,019
PLRV	10	tbr, adg	236
PVX / PVY	16	tbr, adg	312
Cyst nematodes	17	adg	-
Heat	12	tbr, adg, phu	243
	76		2,928

Table 3. Distribution of pollen stainability values in extracted haploids.

Pollen stainability (%)	<5	5-30	30-50	>50	Total
Number of haploids	179 (36%)	144 (29%)	70 (14%)	108 (22%)	501

Table 4. 2n pollen production of haploids extracted from Atzimba and 18 other tetraploids and estimated ps frequencies.

Tetraploid	No. of haploids studied	Without 2n pollen	With 2n pollen	ps frequency
Atzimba	213	106	107	0.71
18 tetraploids	109	45	64	0.77
	322	151	171	

fertility. Since pollen stainability is an indirect estimate of pollen fertility, real fertility value should be obtained from the amount of seed produced in 2x-2x crosses. Some of the haploids with more than 30% of stainability were used for 2x-2x crosses as male parents and high seed sets were obtained. The results are very encouraging, since use of haploids as male parents makes it possible to circumvent cytoplasmic-genic male sterility in hybrids with other 2x germplasm and to keep good level of male fertility during population improvement at the 2x level.

Male sterility is very common in haploids extracted from North American or European varieties (Ross et al., 1964). Carroll and Low (1976) reported that about 70% of their extracted haploids from four varieties had less than 10% of pollen stainability. The present study shows higher level of stainability in the haploids compared with those of Carroll and Low (1976). Use of many hybrids between tuberosum x andigena as a source of haploids was probably one of the reasons for the high stainability since chance of exposure of male sterile recessive genes is minimum from such heterozygous hybrids. Landeo and Hanneman (1979) attributed high level of male fertility of haploids from andigena clones to their heterozygosity. Another possibility is that many of the tuberosum parents utilized migh have demissum cytoplasm, but not tuberosum cytoplasm which results in male sterility interacting with nuclear genes. Since most of the tuberosum parents resistant to late blight have demissum in their genetic background, identification of cytoplasm donor for the tuberosum parents is important to know in order to avoid the accumulation of an increasing frequency of male sterility over several crossing and selection cycles. This is true, especially when modern day varieties and advanced clones stem from inter-specific crosses, e.g. involving demissum, acaule, stoloniferum, chacoense, vernei, etc. If the hypothesis is true, late blight resistant tetraploids which have demissum cytoplasm would be an excellent source of male fertile haploids.

3) Identification of FDR 2n pollen production in the extracted haploids

Screening work for 2n pollen production in 322 haploids with more than 5% of pollen stainability from 19 tetraploids resulted in finding of 151 haploids with 2n pollen (Table 4). Thus, about a half of the haploids were 2n pollen producers. Gene frequency of ps was estimated for the haploids from cv. Atzimba and for the haploids from other 18 tetraploids, assuming that ps was the only 2n-pollen production mechanism involved in the haploids. High ps frequencies were obtained in both populations, 0.71 and 0.77, respectively. This is in accordance with the high frequency of ps in tuberosum cultivars reported by Iwanaga and Peloquin (1982). Based on the segregation rate for 2n pollen production, genotype of Atzimba for ps was determined as Pspspsps. The present results clearly show that ps gene is common in haploids and extracted haploids are good source of ps gene.

4) Finding of a new synaptic mutant

During the study of meiosis of the extracted haploids, a new synaptic mutant was found in haploids of Atzimba. The mutant is inherited as a simple Medelian recessive, designated sy4 (Iwanaga, 1984). Usually, synaptic abnormalities result in male sterility due to abnormal chromosome distribution. However, when the mutant is combined with ps gene, fertile FDR 2n pollen with reduced genetic recombination can be produced. The genetic significance of this combination of the mutants is that it makes possible the transmission of increased level of heterozygosity and epistasis of the parent into the 4x offspring (Okwuagwu and Peloquin, 1981). Therefore, the finding of sy4 mutant along with ps in Atzimba haploids is extremely valuable for the present breeding approach, since desirable gene combinations bred at the 2x level can be transferred almost intact into the 4x offspring.

5) Screening of haploids for specific resistance

Evaluation of the haploids for field resistance to late blight has been done with collaboration of CIP pathologists, Dr. J. Henfling and Mr. H. Pinedo. A sample of 390 haploids was evaluated for field resistance to late blight by artificial innoculation in an environmental-controlled greenhouse. As expected, the level of resistance of some haploids was greater than that of their tetraploid parents, although average resistance of the haploids was approximately the same as that of the 4x parent. The haploids that are highly resistant to late blight were intercrossed to create a new population with a potentially greater average resistance than that of the 4x parents. Regression analysis showed that the level of resistance of haploids was strongly dependent on that of the parent (Figure 2), suggesting that additive gene effects are important for determining levels of resistance, and that significant progress can be made by phenotypic recurrent selection.

In 1983, a group of 503 haploids extracted from 32 tetraploids were evaluated for field resistance to late blight in a field condition at Molina, Huanuco. Eighty-seven haploids from 8 susceptible clones were also included in the experiment for comparison. Severe epidemic in the field killed almost all of the haploids from susceptible tetraploids while other haploid families from resistant clones showed segregation for the level of resistance and some haploids were found to be highly resistant (Table 5). Haploids of Atzimba, the largest group of haploids evaluated, showed especially clear segregation for the resistance. Seventy-six haploids with disease scores of 2, 3 or 4 were classed as highly resistant and will be a good source of resistance for further breeding work at the 2x level. As mentioned before, the Atzimba haploid family produces 2n pollen by sy4 and ps or ps alone with minimum segregation in the 4x progenies. Thus, the Atzimba haploids with high levels

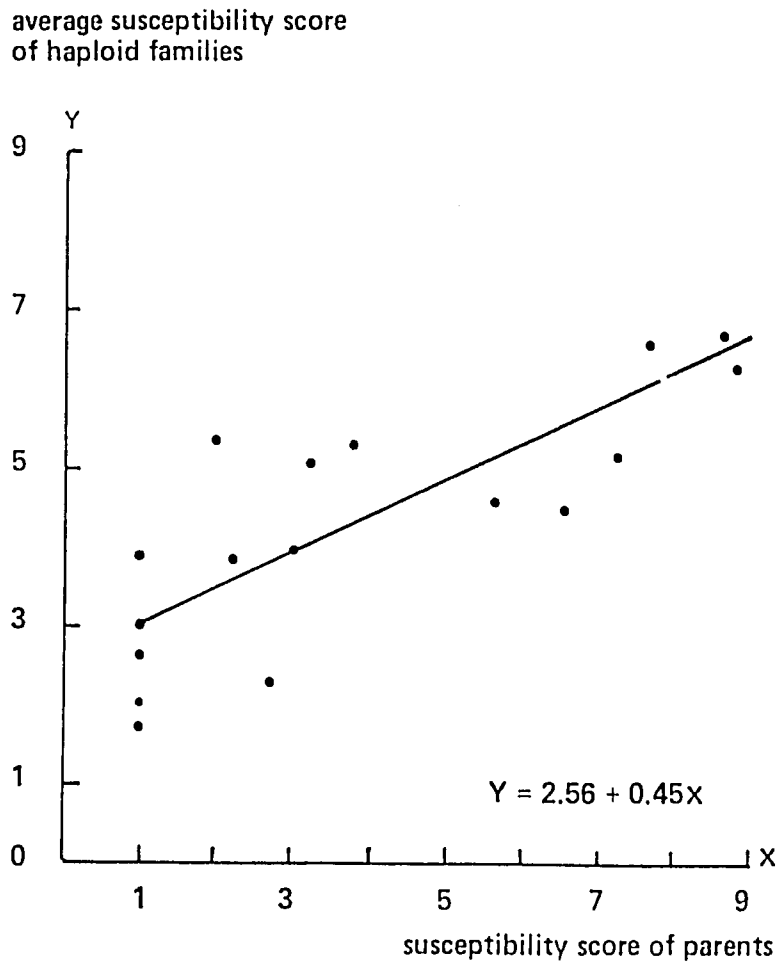

Figure 2. Linear regression of late blight susceptible score of haploid families on that of parents.

Table 5. Field evaluation of haploids for resistance to late blight (Molinos, Huanuco)

Source of haploids	No. of haploids for each score*									Total
	1	2	3	4	5	6	7	8	9	
Susceptible tetraploids	0	0	0	0	1	1	1	4	80	87
Resistant tetraploids	2	0	0	16	23	6	9	9	90	155
Atzimba (resistant)	29	17	11	49	57	21	11	12	54	261

* 1 = immune to 9 = highly susceptible

of resistance and production of 2n pollen are being used for 4x-2x crosses to transfer the resistance to the 4x level.

Preliminary screening work on a group of haploids for resistance to PVY and cyst nematodes resulted in finding of many haploids with the resistance and further evaluation to confirm the resistance is being done. Screening of some haploids for resistance to PLRV and heat was already initiated.

6) Development of diploids populations with specific resistance and FDR 2n pollen production

Many diploid populations with specific resistance to major abiotic and biotic stresses have been developed at CIP (Table 6). In case the source of the resistance is wild species, hybridization with cultivated germplasm was done to introduce good agronomic characters. A large number of male fertile haploids obtained at CIP in the last three years, facilitated the hybridization, avoiding serious problems of cytoplasmic-genic male sterility in the hybrids which is common when tuberosum haploids are used as female parent. Search for FDR 2n pollen production in the resistant diploids has resulted in its identification in many groups of the diploids with resistance to bacterial wilt, soft rot, late blight, early blight, root-knot nematodes, cyst nematodes, potato tuber moth, and heat. Crosses between 4x female parents and the resistant diploids with 2n pollen are being done to transfer the resistances to 4x progenies. On the one hand, intermatings of diploids with different resistances and screening work of the hybrids for combined resistances are in progress.

Table 6. Diploid groups selected for specific resistance: their genetic background and search for 2n pollen production.

Selected Resistance	Genetic Background*	Production of 2n pollen
Late blight	tbr, adg	Yes
Root-knot nematodes	chc,spl,mcd,phu,stn,tbr,adg	Yes
Bacterial wilt	chc, spl, phu, stn, tbr, adg	Yes
Erwinia spp.**	phu, stn	Yes
Early blight	phu, stn	Yes
Potato tuber moth	spl, phu, stn, tbr, adg	Yes
Heat	stn, phu, chc, tbr	Yes
Cyst nematodes	(1) spl,chc,phu,stn,tbr,adg	Yes
	(2) adg	Not studied
Potato viruses X and Y	tbr, adg	Not studied
Potato leafroll virus	tbr, adg	Not studied

*chc = *S. chacoense*; spl = *S. sparsipilum*; mcd = *S. microdontum*; phu = *S. phureja*; stn = *S. stenotomum*; tbr = haploids of *S. tuberosum* subsp. *tuberosum*; adg = haploids of *S. tuberosum* subsp. *andigena*

**obtained through the research contract at North Carolina State University, U. S. A.

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Inbreeding in TPS Progenies: Implications for Breeding and Seed Production Strategy

G. Atlin

Introduction

The potential advantages of botanical seed (TPS) over tubers for potato propagation include ease of storage and reduced disease transmission (Sadik, 1983). Available commercial potato germplasm is, however, rather poorly adapted to seed propagation. Because breeding systems for sexual progenies act only on that part of the genetic variance which is transmissable through gametes, while clonal selection acts on the entire genetic variance, and because family performance under clonal propagation is only weakly correlated with seedling performance (Howard, 1978), it is unlikely that populations adapted to the TPS production system can be derived directly from clonal breeding programs.

The objectives of this paper are to determine which types of TPS progeny are likely to prove most useful to farmers, and to propose strategies for their development and multiplication.

TPS progenies for production in developing countries

Ideally, the delivery of F_1 hybrid families should be the goal of TPS breeding systems. Non-additive gene action is important for many economic characters under both clonal and seed propagation (Thompson et al., 1983; Killick, 1977; Tai, 1976). Heterosis is also observed in crosses within and among taxonomic groups in *S. tuberosum* (Glendinning, 1969; Tarn and Tai, 1977). Such types of gene action are best exploited in hybrid progenies.

There is, however, little information available on the economics of production of hybrid TPS. Upadhya (1979) suggested that the cost of producing, by hand emasculation and pollination, enough hybrid seed to plant one hectare might be only \$5.00-\$6.00 in India, rendering a hybrid seed system feasible. His calculations, however, assume that production and distribution would be undertaken by a non-profit agency. They also assume that small farmers have regular access to a reliable seed distribution network. These assumptions are unrealistic in many developing countries where farmers generally maintain their own seed-stocks, purchasing only to obtain a new variety or when a shortfall occurs.

Because maize-breeding also has as its goal the delivery of heterozygous progenies, maize programs which have been successful in producing varieties for use in developing countries can serve, to some extent, as model systems for TPS breeders. The best F_1 maize hybrids are much more productive than their parental populations, yet the maize improvement

strategy of CIMMYT emphasizes the production of open pollinated varieties, citing "... the circumstances of the majority of farmers in the developing world and ... the weaknesses in the seed production and distribution systems in many collaborating countries." (Sprague, 1982). A similar philosophy guides maize improvement at IITA, several of whose open-pollinated varieties are produced over wide areas in West Africa (Kim et al., 1982). The same factors which impede the distribution of F_1 maize hybrids in developing countries make the early extension of a similar technology to potato producers unlikely. At least in the near term, if potato production from TPS is to be economically feasible, the progenies used by farmers will be obtained through open-pollination. Such varieties can be produced extremely cheaply. In Huancayo, Peru, OP seed yields have been estimated to be about 28 kg, or 36,000,000 seeds, per hectare (Malagamba, 1983). This amount would be ample for the seeding of 360 hectares.

Factors governing the performance of open-pollinated progenies

In order to devise a breeding and seed-production system geared to the delivery of OP progenies, the factors determining their performance must be understood. Two attributes of such progenies complicate the prediction of their performance from parental information.

The first problem in the prediction of OP varietal performance is that in autotetraploid species the allelic contents of gametes which unite to form the initial generation of a synthetic variety are dispersed through the population over subsequent generations. Thus, allelic interactions which may contribute to selection response in initial synthetic generations are rapidly lost, although such interactions may have been important in the selection of parents.

The performance of a tetraploid population may be conveniently described in terms of the components of Kempthorne's (1957, pp. 400-406) model, which express the value of an autotetraploid genotype $A_i\ A_j\ A_k\ A_l$ as:

$$A_i\ A_j\ A_k\ A_l = u+\alpha_i+\alpha_j+\beta_{ij}+\alpha_k+\beta_{ik}+\beta_{jk}+\gamma_{ijk}+\alpha_l+\beta_{il}+\beta_{jl}+\beta_{kl}+\gamma_{ijl}+\gamma_{ikl}+\gamma_{jkl}+\delta_{ijkl}$$

where u is the population mean, α_i is the effect of gene A_i, β_{ij} the effect of the interaction between genes A_i and A_j, γ_{ikj} the effect of 3 genes, and δ_{ijkl} the interaction of 4 genes. In a random-mating population, the expectations of α, β, γ, and δ are 0, and therefore, u is the expected value of $A_i\ A_j\ A_k\ A_l$. The genetic variance of the population, in the absence of epistasis, is partitioned as follows:

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_T^2 + \sigma_F^2$$

where σ_A^2 is the additive variance, σ_D^2 is the digenic variance, σ_T^2 the trigenic variance, and σ_F^2 the quadragenic effects. Only σ_A^2, the component due to the additive effects of genes, contributes to selection response in equilibrium generation (Wright, 1975), yet because the potato gamete is diploid and may carry interacting alleles at a given locus, it may be difficult to separate digenic from additive components of breeding value by conventional progeny-testing methods. If non-additive effects are large relative to σ_A^2, a tetraploid population whose parents have been selected on the basis of phenotype or general combining ability, and which expresses a selection gain in the first generation following recombination, will rapidly lose that part of the gain which was due to non-additive effects in subsequent generations. This phenomenon has been directly observed in potatoes. Sanford (1979) constructed two random-mating populations, each containing 18 parental clones. These populations were advanced through 7 cycles of forced random mating and evaluated as tuber families over two seasons. Specific gravity decreased slightly from C_1 to C_7, while mean tuber weight decreased 12%.

The second complicating attribute of OP potato varieties is that they are substantially inbred. In the first generation of open-pollination, this inbreeding results from selfing. In subsequent generations, inbreeding results from selfing and from matings between relatives.

Gallais (1967) extended Kempthorne's model to describe the mean of an inbred population. In such populations the effects β_{ii}, γ_{iii}, δ_{iiii}, and δ_{iijj}, absent in non-inbred populations, occur. These effects do not have an expected value of 0, and therefore, the mean of an inbred generation is expressed as follows:

$$u_I = u + 6\,F\beta_{ii} + (4P_0 + P_1)\,\gamma_{iii} + P_0\,\delta_{iiii} + P_2\,\delta_{iijj}$$

where u_I is the mean of an inbred generation, u is the mean of the non-inbred population, F is the probability that two alleles drawn from an individual are identical by descent, P_0 is the probability that $A_1 = A_2 = A_3 = A_4$, P_1 is the probability that $A_1 \neq A_2 = A_3 = A_4$, and P_2 is the probability that $A_1 = A_2 \neq A_3 = A_4$. The mean of an inbred population is therefore seen to be determined by three genetic factors:

i) the mean of the non-inbred parental population;

ii) the frequencies of the homo-allelic interactions;
iii) the magnitude of the homo-allelic effects.

The first component is self-explanatory. The second is a complete description of the degree to which a population is inbred, and the third dictates the population response to inbreeding. The breeder's aim is clearly to determine the breeding and seed-increase methods which maximize the mean of the non-inbred population and minimize inbreeding and its negative effects in the populations which are to be used by farmers.

It is important to note that the non-inbred population referred to is an equilibrium population. Efforts to improve inbred generations by improving the mean of the parental population will be rewarded to extent that genetic advance is due to additive effects. In practice, effective methods for improving the population mean and for reducing homo-allelic interactions are likely to be identical. Gene frequencies cannot be changed without changing the frequencies of interactions and the techniques which effectively measure the additive value of a genotype are the same as those which expose deleterious homo-allelic interactions. Some methods for minimizing inbreeding and improving inbred populations are discussed below.

Avoiding inbreeding

The level of inbreeding in a synthetic population is a function of outcrossing rate, the number of original parents, their inbreeding level, and their degree of relationship (Busbice, 1969). Some of these parameters may be manipulated to reduce the accumulation of inbreeding as populations are advanced. This discussion will consider only synthetics derived from non-inbred, unrelated parents. The relative importance and ease of exploitation of the other two parameters, parent number and outcrossing rate, are reviewed herein.

The effect of parent number and selfing rate on the inbreeding coefficient, F, in synthetic generations 1 to 5, is illustrated in Figure 1. Selfing rate is the major determinant of inbreeding level. As was pointed out by Brown and Huaman (1983), additional parents only marginally reduce inbreeding levels at high rates of self-pollination. At lower rates of selfing, parent number has a more marked effect. There appears to be little advantage to synthetics containing more than 4 parents at selfing rates above 50%. Selfing rates in tetraploid potato populations have not been fully characterized, but estimates range from 55-90%, varying considerably with genoytpe and environment (Brown and Huaman, 1983; Glendinning, 1976). If 70% can be taken as a rough mean value it is apparent that efforts to reduce selfing rate will have a greater impact on the inbreeding level of a synthetic population than the inclusion of additional parents.

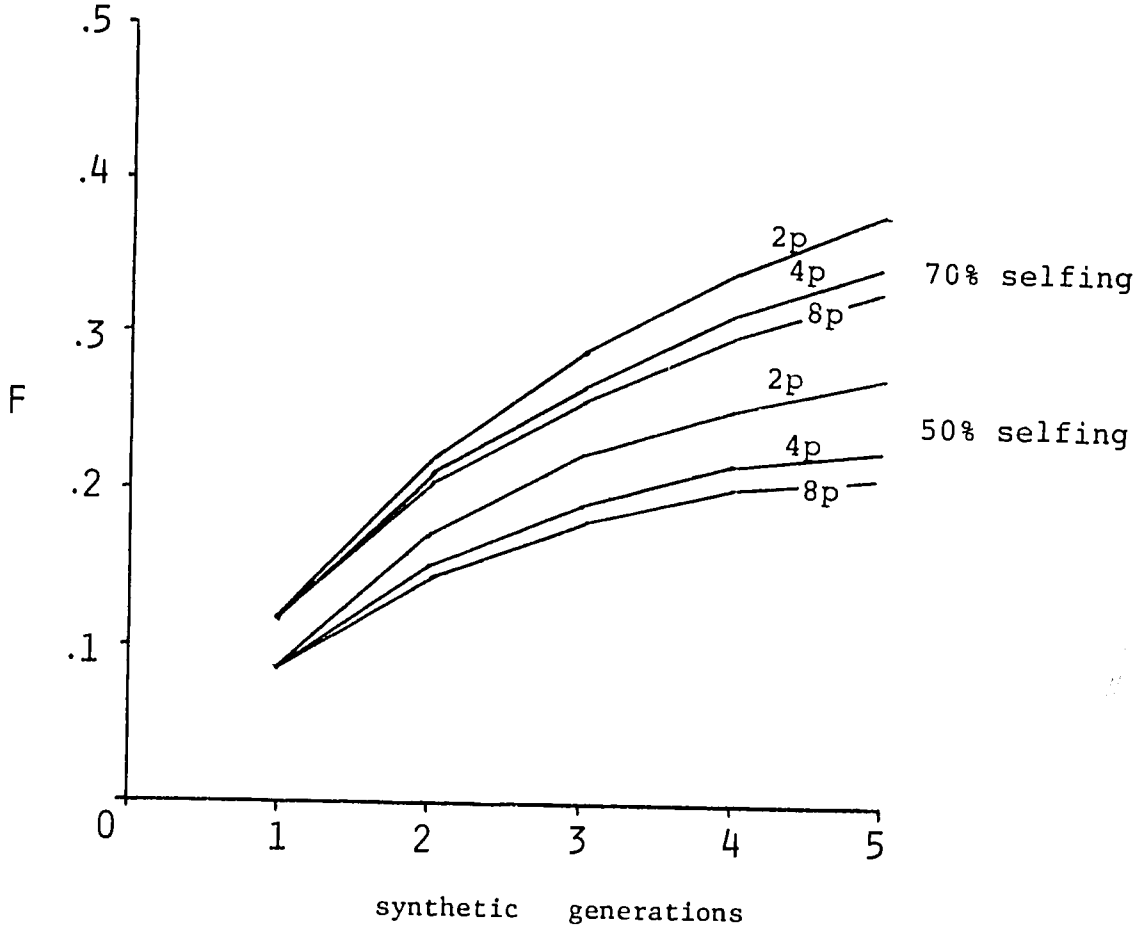

Figure 1. Inbreeding coefficients of 2, 4, and 8 parent synthetics with 50% and 70% self-pollination.

Two genetic approaches to the manipulation of selfing rates are available to breeders. Brown and Huaman (1983) have suggested that selection be undertaken to increase the outcrossing rate of TPS populations. There indeed appears to be substantial variation for the character, but its measurement is difficult and its inheritance unknown. Another method by which selfing rates in synthetic populations might be reduced is the inclusion of male sterile parents in the initial generation. Male sterility is relatively easily identified and is usually simply inherited.

Male sterility abounds in the tetraploid potato germplasm but is usually associated with an absence of pollen or production of malformed pollen unattractive to bees. Steriles of this sort set virtually no seed, since both self- and cross-fertilization in potatoes result almost exclusively from insect visits (White, 1983). However, some types of sterility have been identified which are characterized by the formation of bee-attracting but non-functional pollen. A tuber family combining the latter trait with prolific field production of berries was identified at CIP in 1983. Inheritance of the trait remains to be determined.

Mode of inheritance greatly influences the effect of male sterility on the selfing rate, and hence on the inbreeding coefficient, of a synthetic. Sterility conditioned by a single dominant gene reduces inbreeding only in early synthetic generations, since, in the absence of heterozygote advantage, the frequency of such a gene would be halved in each generation. Cytoplasmic male sterility would, on the other hand, permanently reduce the self-pollination rate, since mixtures of cytoplasms do not change their proportions within large populations in the absence of selection. Genic-cytoplasmic sterility would similarly have a permanent effect.

Inbreeding co-efficients were computed for generations one to five of 4-parent synthetics with selfing rates of 70% under the hypothesis of male sterility conditioned by a dominant gene (Figure 2), and under that of cytoplasmic male sterility (Figure 3).

In synthetics with 2 genically male sterile parents constituting 70% and 2 fertile parents constituting 30% of the Syn 0 generation, inbreeding co-efficients in Syn 1, Syn 2 and Syn 3 are 30%, 76% and 90% of those expected in an equivalent synthetic composed of 4 fertile parents. By Syn 5, no difference in inbreeding co-efficient remains between the "sterile" and "fertile" synthetics. The second synthetic generation, composited as described above, has F = 0.16, as compared to F = 0.21 in an equivalent fertile synthetic, and F = 0.17 in the OP_1 generation from a clone produced in a large plot. Thus the main benefit of genic male sterility is that it permits a second seed-increase generation to be undertaken, yielding a progeny which is still no more inbred than a conventional OP.

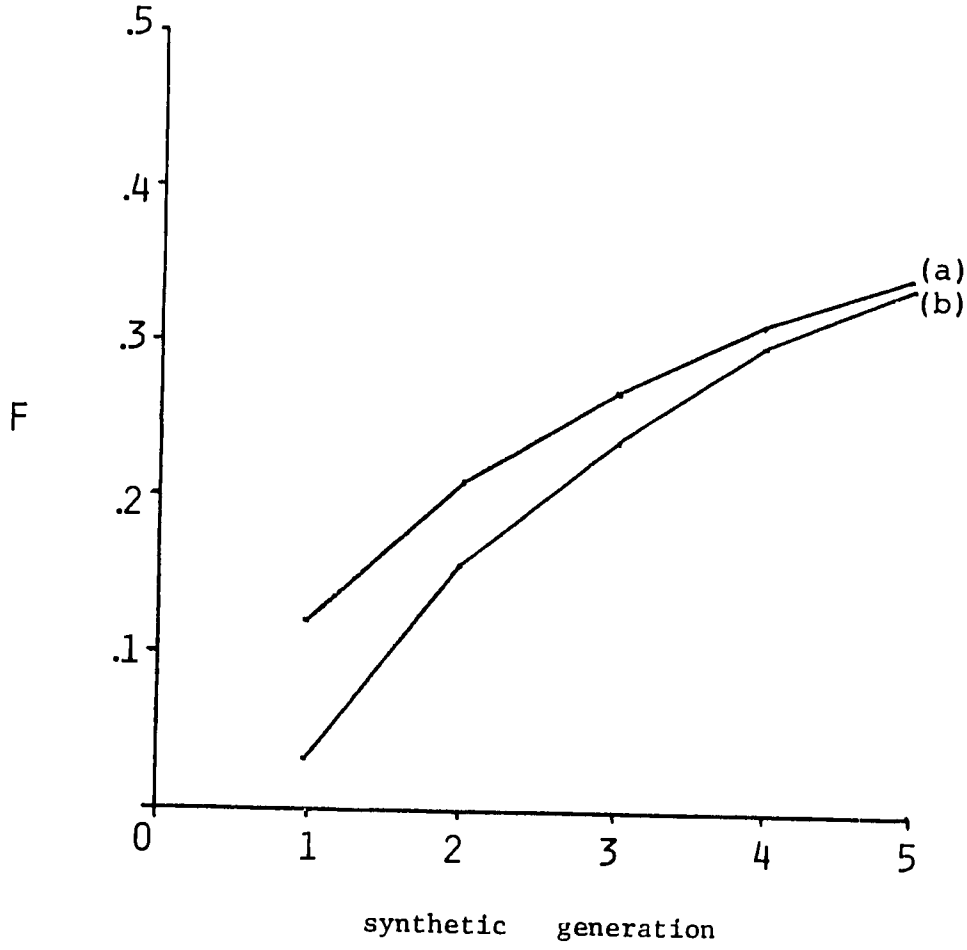

Figure 2. Inbreeding coefficients (70% selfing) of 4-parent synthetics with: a) 4 fertile parents;
b) 2 fertile parents comprising 30% and 2 genically male sterile parents comprising 70% of Syn 0

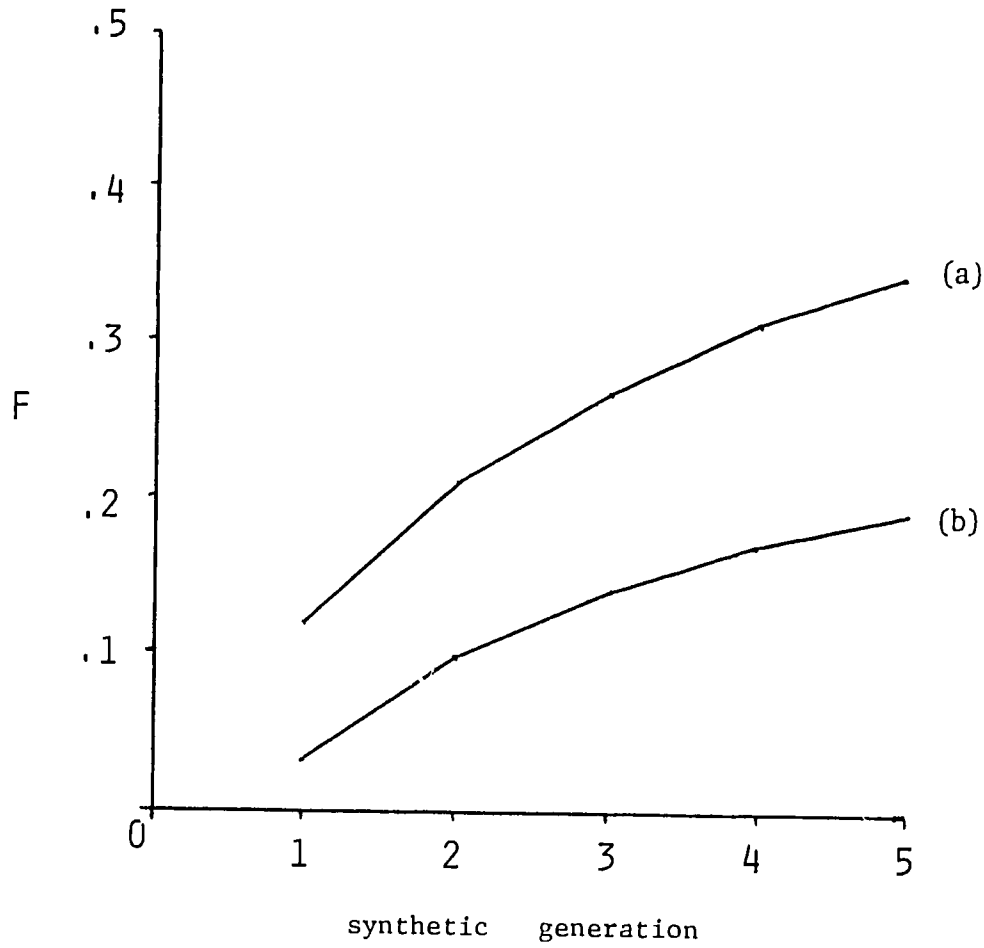

Figure 3. Inbreeding coefficients (70% selfing) of 4 parent synthetics with: a) 4 fertile Syn 0 parents and b) 3 fertile parents comprising 30% and 1 cytoplasmically male sterile parent comprising 70% of Syn 0

Cytoplasmic male sterility, on the other hand, can confer a lasting reduction in inbreeding upon a population. The fifth generation of a synthetic originally derived from one male sterile and three fertile parents, with the male sterile comprising 70% of Syn 0, has F=0.18, as opposed to F=0.34 in Syn 5 of a four-parent, completely fertile synthetic. The inbreeding co-efficient of a population carrying a genic-cytoplasmic sterility system would settle at an equilibrium somewhat above that expected for cytoplasmic sterility, and would depend largely on the frequency of the nuclear sterility factor in the population.

It is apparent that opportunities for substantially moderating the accumulation of inbreeding in TPS population exist, regardless of the mode of inheritance of male sterility.

Improving inbred generations

The above discussion indicates that the types of TPS progeny likely to be available to farmers will have been increased through several generations of open pollination and thus will be inbred to a degree equivalent to at least one generation of selfing. In such progenies, homo-allelic interactions occur with substantial frequency, and, because recombination has destroyed parental genotypic structures, the additive values of alleles assume great importance. The general effect is a marked reduction in the performance of inbred potato progenies. Krantz (1951) reported that a group of clonally propagated S_1 progenies yielded only 83% as much as their non-inbred parents. Mendoza and Haynes (1973), in a re-analysis of data reported by Rowe (1967), observed that an increase in inbreeding co-efficient from 0.11 to 0.33 led to a 50% reduction in yield of several clonally propagated tetraploid families. Selfed and OP progenies propagated by true seed have also been reported to yield, on average, considerably less than non-inbred progenies (Macaso, 1983), although reported results confound effects of genetic background with inbreeding level.

There is ample evidence to suggest, however, that it may be possible to improve the performance of inbred generations. Although the average effects of inbreeding are quite severe in potatoes, there is considerable variation in the performance of individual S_1 lines, some of which approach the yields of good F_1 hybrids (Bedi, 1979; Glendinning, 1976). This type of variation has been observed and exploited in other species. In an examination of response to inbreeding in lines derived from six alfalfa clones, Hill (1976) reported that some unselected progenies exhibited little or no depression for the monitored traits over four generations, while others were quite sensitive. Such differences can reflect real differences in the breeding value of autotetraploid progenies. Pfeiffer and Bingham (1983) conducted inbreeding with selection in the progeny of two doubled-haploid alfalfa clones. The original clones were estimated to have an inbreeding coefficient of 0.33, and to carry no more

than two alleles per locus. Four generations of sib mating were undertaken with visual selection for vigour. Advanced generation clones in the progeny of one parent outyielded that parent, while the crosses between advanced generations outyielded the parental cross substantially. The advanced generations also out-performed parents in crosses to an unrelated tester. The authors observed that, since little heterozygosity existed in the advanced inbred generations, improvement under selection was due entirely to "... the accumulation of favourable alleles already present, due to recombination within linkage blocks." It is apparent that inbreeding permits effective selection in autotetraploids on that part of genotypic value which acts additively, because the confounding effects of intralocus interactions are largely eliminated. Further support for this hypothesis is found in the work of Rotili and Zanone (1976). They produced 4-clone alfalfa synthetics derived from parents which had been selected under two generations of selfing. The Syn 2 generation of these populations outyielded synthetics of identical parent number and genetic background but derived from non-inbred parents.

The principal drawback to the use of selection under inbreeding in producing parents for OP varieties is that the final product is, of course, inbred. If heterozygous parents with good breeding value could be identified by means of a progeny test, they could be combined to form a synthetic population which would have a lower equilibrium inbreeding coefficient than one formed from inbred parents, yet which might show reduced depression on inbreeding. It is clear that a test of selfed progeny is required to identify such parents. Tests of heterozygous progeny do not assay homo-allelic interactions, and confound allelic with digenic effects to varying degrees.

In diploids, a test of S_1 progeny may be sufficient to identify parents with good breeding value, because, in such progenies, at least 50% of loci are homozygous. Selection based on selfed progeny testing has been reported to reduce inbreeding depression in selfed maize populations (Goulas and Lonnquist, 1977; Horner et al., 1973; West et al., 1976). In autotetraploids, however, one generation of selfing may be insufficient to assay the value of a parent. This is because early selfed generations deriving from a heterozygous tetraploid individual still contain high frequencies of multi-allelic loci. Allelic interactions at such loci may obscure the additive value of genes carried by the parent. Demarly (1963) gives recurrence relations for calculating the frequency of such loci. Values of generations S_1-S_3 are presented in Table 1 for a locus which was tetra-allelic in S_0. In the S_1, 5/6 of the offspring are either tri- or tetra-allelic. The frequency of tri- and tetra-allelic loci is not reduced below 0.5 until the S_2. It is therefore likely that a test of S_2 or S_3 progeny would be required in order to identify the value of a parent for inclusion in an OP potato population.

Table 1. Frequencies of genotypic structures resulting from selfing at a tetra-allelic locus $A_1A_2A_3A_4$

Generation	Frequency				
	P_0	P_1	P_2	P_3	P_4
S_1	0	0	0.1667	0.6667	0.1667
S_2	0.0278	0.2222	0.2778	0.4444	0.0278
S_3	0.1111	0.3333	0.3102	0.2408	0.0046

where: P_0 is the probability that $A_1 \equiv A_2 \equiv A_3 \equiv A_4$

P_1 is the probability that $A_1 \not\equiv A_2 \equiv A_3 \equiv A_4$

P_2 is the probability that $A_1 \equiv A_2 \not\equiv A_3 \equiv A_4$

P_3 is the probability that $A_1 \equiv A_2 \not\equiv A_3 \equiv A_4$

P_4 is the probability that $A_1 \not\equiv A_2 \not\equiv A_3 \not\equiv A_4$

Conclusion

Breeding and seed-increase methods designed to deliver inexpensive TPS progenies to farmers in developing countries should be geared to the production of OP varieties. The mating system of the tetraploid potato is such that these varieties are likely to be inbred to a level equivalent to at least one generation of selfing.

Modern potato breeding research has concentrated on optimizing the genotypic structure of individual clones or hybrid families. This approach cannot be used to improve OP populations, since specific structures can only be maintained through controlled matings. Progress in the improvement of OP populations thus can only result from accurate identification of the value of alleles carried by parents. Two approaches to the problem of selecting parents for such populations merit testing. A program of selfing with selection may be undertaken within high-yielding TPS families. Such a program would expose deleterious homo-allelic effects and permit selection based on allelic value. Parents produced in this manner would, however, have to restored to a heterozygous condition through a program of intercrossing prior to use as synthetics.

Alternatively, parents could be selected on the basis of the performance of their S_2 or S_3 progeny. Such testing is also expected to assay the true breeding value of parents, but would not permit the extensive recombination which might occur in a program of selection in inbred lines.

Both the methods described above are time-consuming, and should only be adopted if they prove substantially more effective than less expensive measures based on phenotypic performance or general combining ability. The test criterion should be the performance of advanced generation synthetics derived from each method rather than that of the initial recombined populations.

Once parents have been selected for OP varieties, methods must be devised to reduce to a minimum the inbreeding accumulated during their multiplication. It seems likely that male sterility will prove the most useful tool for achieving this end. A systematic effort to screen germplasm for sources of useful sterility, and a program for its incorporation into good parents, could significantly improve the potential performance of synthetic varieties and might eventually provide the basis for a hybrid TPS system.

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Selection of Uniform Progenies to Use TPS in Commercial Potato Production

H. A. Mendoza

Introduction

The research in the area of utilization of true potato seed as means of producing seed or ware potatoes or both has received a great deal of attention since 1977. The first efforts consisted on studying the genetic nature of the variability for several traits relevant to TPS. No previous genetic information about traits such as seed germination, seedling vigor, transplanting survival, uniformity or tuber color, shape, and size, etc., was available. Therefore, it was indispensable to acquire this knowledge to define a strategy and methodology to utilize CIP's breeding populations. Mendoza (1979) and Thompson (1980) provided research results about the early efforts to characterize the nature of the genetic variability for a number of traits important to the utilization of TPS. In the following areas this research continued providing more genetic information and on that basis an intensive selection was initiated to identify superior progenitors with sufficient general combining ability (GCA) to produce high yielding and uniform progenies. A number of these progenitors has been identified and are available for distribution to national programs of potato improvement. Also, several progenies adequate to the use of TPS for potato production have been selected and distributed. All the research work carried out so far has been within the population breeding approach utilized at CIP (Mendoza, 1980). The results reported in this paper have been obtained in the period 1980-1983 and are related to estimation of genetic parameters in the breeding population, comparison of various TPS progenies and identification of parental stocks with high GCA for yield, earliness, and uniformity of tuber characteristics.

Estimation of Genetic Parameters

In previous work (Mendoza, 1979), utilizing a N.C. Design I, found no additive genetic variance for tuber yield in a Neo-tuberosum population. Later, (Thompson, Mendoza, and Plaisted, 1983) utilizing the same N.C. Design I in a Neo-tuberosum population determined the genetic variability for 16 traits. Once again, no additive genetic variance was found for tuber yield, hence the narrow sense heritability, $h^2=0$. Heritabilities for other characteristics were of a variable magnitude, i.e. percent germination, .53; transplant survival, .30; days to maturity, .57; number of berries, .58; tuber number, .60; tuber size, .79; etc.

In 1980, genetic experiments were carried out to determine genetic parameters for 11 traits in one of CIP's breeding population represented by S. tuberosum, spp. tuberosum and andigena, hybrids amongst them as well as tuberosum x phureja hybrids. A random sample of 4 ones was designated females and each female was mated to a randomly taken sample of four clones designated males in a N.C. Design II mating plan. Each 4 x 4 mating, i.e. 16 progenies, constituted a set and the experiment had 10 sets with a total of 160 progenies. Details of this research are reported by Thompson and Mendoza, 1983.

Table 1 presents components of additive genetic variance, additive x environment interaction and narrow sense heritability.

Table 1. Genetic variance components and heritabilities for several traits in potatoes propagated from true seed. (After Thompson and Mendoza, 1983)

Trait	σ^2_A	$\sigma^2_{A \cdot E}$	h^2 (fs)
Berry number	0.2092 ± 0.0728	0.1068 ± 0.0256	0.63 ± 0.18
Tuber number	0.0336 ± 0.0096	- 0.0012 ± 0.0052	0.64 ± 0.18
Tuber size	0.0104 ± 0.0032	0.0016 ± 0.0020	0.61 ± 0.19
Yield	0.0208 ± 0.0064	0.0068 ± 0.0032	0.61 ± 0.19
Tuber smoothness	0.5240 ± 0.1336	0.0092 ± 0.0504	0.71 ± 0.18
Uniformity tuber color	2.1869 ± 0.5846	0.6913 ± 1.3816	0.65 ± 0.17
Uniformity tuber size	- 0.0421 ± 0.1107	0.0928 ± 0.0892	0.06 ± 0.15
Uniformity tuber shape	0.2419 ± 0.1038	0.1100 ± 0.0922	0.37 ± 0.16
General appearance	0.3776 ± 0.1876	0.4240 ± 0.1736	0.39 ± 0.19

To remove scale effects from genetic variance component estimates the data for berry number, tuber number, tuber size, and yield were transformed to logarithms. Estimates of σ_A^2 were less than their standard errors for germination percentage and transplant survival. Those traits are not included in Table 1 where the estimates for all remaining traits are presented. The means were 89 and 96 for percent germination and percent transplant survival respectively, which were high enough that those would not be characters to include for improvement in a breeding program. No σ_A^2 was found for uniformity of tuber size which means that variability must be introduced before improvement can be made by procedures requiring σ_A^2. The estimates of σ_A^2 were larger than the standard errors for all other traits observed. The estimate of non-additive variance was significant for berry number, yield, tuber smoothness, uniformity of tuber color and uniformity of tuber size.

Heritability estimates were high for most of the traits exhibiting σ_A^2, being above 0.52 for all except uniformity of tuber shape and general appearance which were 0.37 and 0.39, respectively. The $\sigma_{A \cdot E}^2$ estimates were either below their standard errors or slightly above for all traits except berry number, yield and general appearance.

Notice that in this population the narrow sense heritability for yield was high, i.e. $h^2 = .61$, while in research previously mentioned no additive genetic variance was found, i.e. $h^2 = 0$. For other traits like berry number, tuber number, tuber size, etc., there was a high degree of coincidence of the estimates of this experiment with the N.C. Design I experiment mentioned earlier.

Genetic and phenotypic correlations for the traits studied in this experiment were computed and are presented in Table 2.

The positive correlations between berry number and yield were desirable since selection for increased yield should increase seed production. As expected, tuber number and size were negatively correlated; however, the values were relatively low, especially when compared to the correlations between each of those traits and yield. Since the estimate of σ_A^2 for uniformity of tuber size was negative, there was no genetic correlation between it and any other trait. The correlations between uniformity of tuber color and shape, and the yield related traits were low.

From the results presented the following inferences can be made. The high estimates of heritability for most of these traits suggest that individual plants selection should be considered rather than family selection. Since genetic correlations were high between some of the yield related traits, and since improvement in tuber uniformity and

Table 2. Genetic and phenotypic correlations for several traits in potatoes propagated from true seed[1]. (After Thompson and Mendoza, 1983)

	Berry No.	Tuber No.	Tuber Size	Yield	Tuber Smoothness	Unif. Color	Unif. Size	Unif. Shape	General Appear.
Berry Number		0.48	0.002	0.39	0.04	- 0.07		0.04	0.43
Tuber Number	0.35		- 0.24	0.74	- 0.23	- 0.10		0.36	0.16
Tuber size	0.003	-0.13		0.45	- 0.01	- 0.07		- 0.23	1.00
Yield	0.37	0.75	0.73		- 0.58	- 0.05		0.13	0.83
Tuber Smoothness	0.01	- 0.24	- 0.01	- 0.43		0.60		0.42	- 0.38
Uniformity Tuber Color	- 0.08	- 0.10	- 0.14	-0.09	0.56			0.64	- 0.12
Uniformity Tuber Size	0.48	0.57	0.26	0.48	0.09	0.37			
Uniformity Tuber Shape	0.12	0.22	- 0.33	0.16	0.36	0.63	0.78		0.16
General Appearance	0.25	0.27	0.70	0.76	- 0.29	- 0.06	0.59	0.31	

[1] Genetic correlations above diagonal, phenotypic correlations below diagonal

smoothness would be desirable, a selection index would be effective. For example, tuber number X_1, general appearance X_2, and yield X_3 would be desirable characters to use in an index for yield improvement because the genetic correlations between them were high. Tuber smoothness X_4, uniformity of tuber color X_5, and shape X_6 would also be included in the index for improvements in those characters. The index constructed using those characters with economic weights of 1, 1.5, 3.0, 0.5, 0.2, and 0.3 for X_1 through X_6, respectively was

$$I = 4.77X_1 + 0.62X_2 - 5.79X_3 - 0.09X_4 - 0.20X_5 + 1.16X_6$$

The expected gain in yield when the index is used for selection and the superior 5% of the progeny is selected equals 1815 kg/ha which is 34% higher than the expected gain when selection is practiced for yield alone.

These results and predictions indicate that improvement in yield, tuber smoothness, uniformity of color and shape should be rapid when selection is practiced at the locations used in this study.

In 1980, (Thompson and Mendoza, unpublished results) experiments were carried out combined with the N.C. Design II previously discussed, to compare the performance of the following types of progenies:

Single crosses	160	progenies
Bulk crosses	20	"
Multiline	10	"
Open pollinated	20	"
Selfed	20	"

Parental clones used in the Design II were also selfed, allowed to open pollinate, and bulk pollinate to derive additional family types. Multilines were created by mixing equal numbers of seeds of four of the crosses made in each set.

Data from the comparison of the performance of these families of diverse origin for eight characteristics were recorded and are presented in Tables 3 and 4. For the four traits presented in Table 3, it appears to be a clear cut advantage favoring the hybrids over the partially inbred progenies (selfed and open pollinated). In Table 4, for tuber number there is the same trend as in the previous table, i.e., the hybrids are superior to the partially inbred progenies. However, in weight per tuber there was no difference amongst progenies. The relationship amongst these two yield components is explaining the differences in yield (Table 1). At equal tuber weight, the hybrids are yielding higher due to a larger tuber number. For the characters

tuber uniformity and eye depth the partially inbred progenies, either OP or self, are equal or better than the hybrids. These results are understandable considering that the progenitors utilized in these experiments were taken at random. Therefore, a wider range of variability was expected for these traits, particularly in the bulk cross and multiline hybrids.

Table 3. Mean values of traits measured in different family types propagated from TPS[1]

Perc. Germination		Transplant Survival	
Tp. Fam.	Mean	Tp. Fam.	Mean
Multiline	92.2 a	Multiline	40.7 a
Cross	88.5 b	Cross	40.4 a
OP	87.7 b	Bulk Cross	39.8 b
Self	85.2 c	Self	39.6 b
Bulk Cross	81.4 d	OP	39.5 b

No. Plants Harvested		Yield (Log)	
Tp. Fam	Mean	Tp. Fam.	Mean
Multiline	28.9 a	Bulk Cross	.7873 a
Bulk Cross	28.4 a	Multiline	.7678 a
Cross	28.2 a	Cross	.7516 a
OP	23.4 b	OP	.5386 b
Self	20.4 c	Self	.4545 c

[1] Mean separation by Duncan's .05

The results presented on comparison of progeny types would indicate that hybrids are the most adequate progeny type for the use of TPS for commercial potato production. Uniformity and eye depth, traits in which the hybrids were equal or inferior to the partially inbred progenies can be easily improved by a careful selection of progenitors. To make a final decision on the utilization of hybrid vs OP progenies it would be necessary a careful economical analysis to find out if the average differences in yield could justify the greater cost of hybrid seed compared to the minimum cost of the OP seed.

Table 4. Mean values of traits measured in different family types propagated from TPS[1]

Tub No. (Log)		Weight per Tuber	
Tp. Fam.	Mean	Tp. Fam	Mean
Bulk Cross	2.4342 a	Cross	.0243 a
Multiline	2.3939 a	Multiline	.0243 a
Cross	2.3721 a	Bulk Cross	.0231 a
OP	2.2371 b	Self	.0220 a
Self	2.1267 c	OP	.0204 a

Uniformity[2]		Eye Depth[2]	
Tp. Fam.	Mean	Tp. Fam.	Mean
Self	6.06 a	Self	6.27 a
Cross	5.89 a	OP	6.06 ab
OP	5.84 a	Cross	5.94 bc
Multiline	5.46 b	Multiline	5.82 bc
Bulk Cross	5.42 b	Bulk Cross	5.70 c

[1] Mean separation by Duncan's .05

[2] Rated using values 1-9 where 1 = least desirable and 9 = highly desirable

The last part of the paper deals with the identification of progenitors which produce hybrid progenies with high yield, tuber uniformity, and earliness. Several experiments were carried out either utilizing genetic mating designs or just comparing the performance of large number of progenies. In 1982, an N.C. Design I experiment was carried out at San Ramon including 16 males mated each to a random sample of six females. Table 5 indicates the average performance of some male clones.

Table 5, is showing that there are parental materials adequate for the use of TPS as means of potato production. The clone 7XY.1 in this experiment was, on the average, the highest yielding. Its tuber uniformity was acceptable but its progenies were from medium to medium late. From this experiment as well as from others it was observed that to obtain a maximum benefit from this clone one should mate it to female clones which are early maturing and have a smooth shape. The

Table 5. Average performance of some male clones from a Design I experiment carried out at San Ramon (1982).

Male Clone	Yield (gr/plant)	Earliness*	Tuber Uniformity**
7XY.1	696	4.0	6
DTO-28	625	2.4	5
377250.7	577	3.7	6
14XY.4	561	4.3	3
LT-7	508	3.8	6
377888.8	431	2.2	4
Atlantic	406	2.7	5
India 1035	362	2.6	6

* Earliness 1 early 5 late
** Tuber uniformity 1 lack of uniformity 9 completely uniform

clone DTO-28 transmits on the average, high yielding and early maturing progenies. However, one should mate it to female progenitors which have a smooth shape, i.e., Atzimba or Katahdin. LT-7 is a clone which transmits an adequate yield, acceptable uniformity and early to medium growing period. This combines well with a wide range of female clones. The clone 377888.8, Atlantic, and India 1035 in spite of their modest average yield transmit earliness to their progenies and a variable degree of uniformity. One of the most outstanding TPS progenies identified at CIP is Atlantic x LT-7 which is easily confused with a variety due to the high degree of uniformity in tuber color, shape, depth of eyes, and yield per hill.

In 1982 a sample of 20 high yielding clones was mated to two testers and evaluated in three environments for the traits yield, tuber uniformity, and seedling survival. General combining ability effects for these clones are presented in Table 6. One can notice a great deal of variability in the behaviour of the female clones. There are some which are very good to transmit yield and seedling survival but their tuber uniformity is very poor, i.e. 377885.15 and 377922.30. The most valuable clone in this group was 377891.19 which had significant GCA estimates for all characteristics. The clones N568.4 and 377887.76 in spite of having GCA estimates for yield not significant but larger than the s.e. ($\hat{g}_i$), had significant GCA estimates for tuber uniformity. Therefore, they could be utilized in a TPS program by mating them to high combiners for yield like LT-7 or DTO-28. In these trials, the performance of Atzimba was erratic and its progenies appeared to interact a lot with the environment as it was noticed by the highly signif-

Table 6. General combining ability effects ($\hat{g}_i$) for a sample of twenty females mated to two testers.

Female	Yield	Tuber Uniformity	Seedling Survival
377885.15	5.02**	-.22	3.62**
377922.30	4.48**	.03	2.62*
377891.19	3.96**	.70**	2.54*
377935.27	3.11**	.45*	.54
N568.4	2.61	.53*	1.37
377871.28	1.95	.20	-.13
65-ZA-5	1.62	-.27	-.80
377892.7	1.61	-.55*	-.55
377888.17	1.56	-.05	.29
377877.9	1.48	-.72*	2.12
377887.76	1.44	.37*	3.95**
3XY-2	.02	-.80	4.50**
377887.17	-.12	.28	1.37
MS-17.3	-1.23	.12	-.13
377882.27	-2.53	.12	-1.46
377964.3	-2.94*	-.21	-4.30*
Atzimba	-3.39*	.37*	-3.05*
R268.1	-5.66**	-.05	-1.05
India 832	-5.83**	-.38*	-3.88**
LT-1	-7.13**	.03	-7.63**
s.e. $\hat{g}_i$	1.35	.16	1.11
s.e. $(\hat{g}_i - \hat{g}_j)$	1.91	.23	1.57

icant interaction female x environment in the analysis of variance. Its GCA estimate for yield was negative but it still showed its ability to transmit uniformity to its progenies.

As a result of many experimental data a number of parental clones have been identified to produce progenies adapted to TPS utilization. Amongst the best are LT-7, 378015.13, 378015.16, Atzimba, Katahdin, and 7XY.1. Also DTO-28, R-128.6, 377904.10 for their high GCA effects for yield, provided that a careful choice of female parents is made to obtain tuber uniformity. Atzimba and Katahdin have proven to be adequate partners for these clones.

In summary, we feel that in the 1980-1983 period, a great deal of genetic information has been gained permitting the breeding work to be carried out on a solid base.

Also the comparison of progeny types appears to indicate a superiority of hybrids, particularly single crosses, over the partially inbred progenies resulting from open pollination. Finally, a number of progenitors have been identified to produce progenies well adapted to potato production from TPS.

The future efforts will be directed to continue selecting superior progenitors to be made available to National Programs. Also, an international TPS progeny performance trial will be shortly initiated not only to provide superior progenies but also to learn more about progeny x environment interactions which might be very important in the proper choice of genetic materials for these purposes. Research on the use of synthetics, effects of inbreeding and open pollination in progeny performance are also under way.

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Apomictic Approach to Introduce Uniformity and Vigour into Progenies from True Potato Seed (TPS)

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Introduction

Apomixis can be applied in certain crops for identical or near-identical seed propagation in spite of the crop itself being highly heterozygous and polyploid. Breeding can only be carried out through facultative apomictic or non-apomictic relatives. Classical examples are Poa pratensis and many tropical grasses.

In another group of crops, to which e.g. the sugar beet belongs, apomictic varieties do not (yet) exist, but apomixis occurs in related species. e.g. in polyploid species of the section Corollinae of the genus Beta.

In Solanaceae apomixis has never been detected unambiguously. The question then may be raised whether there are perspectives for inducing apomixis into potato and using it for apomictic seed production. Some researchers (Savidan, 1982) consider apomixis as being one single phenomenon based on one or more genes. In their view apomixis is either present or absent and cannot be subdivided into components. Or the other way around: apomixis cannot be built up by uniting so-called "elements of apomixis", as has been suggested a.o. by Petrov (1970). Indeed, where apomictic seeds originate from aposporic embryosacs, like in Panicum maximum, or from adventitious embryo formation like in Citrus, Savidan may be right, the more so because he found apospory in Panicum to be controlled by one gene. As neither apospory nor adventitious embryony has been proved to occur in potato, Savidan's ideas are not relevant to potato breeders, whereas Petrov's conception on elements of apomixis stimulates thinking on pathways that may lead to apomictic or near-apomictic production of true seeds in potato.

There are obvious reasons for a potato breeder to hunt after apospory. It is a mechanism leading to fully identical true seed progeny and complete independence of heterozygosity of parents because meiosis and thus recombination of genes is fully absent. In addition, genetic control may be relatively simple.

However, at this stage of research it seems realistic to assume that nature has not created the treasure of apospory in potato. It may be questioned whether potato breeders are more clever than nature. Therefore, it seems wise to nurse the ideas and knowledge which we have at present and which are developing in a way to give promise of success.

In discussing elements of apomixis the following questions are relevant to a potato breeder and will be treated in this paper.

1. Which meictic processes lead to 2n gametes and in particular to 2n eggs?

2. Which processes are the basis of the second element of apomixis: identity or near-identity of all 2n gametes?

3. An important question for practical breeders is treated in a separate chapter: how can the relevant processes or mechanisms be transferred from an unimproved clone having such mechanism into an advanced parental line lacking any restitution mechanism?

4. How can a breeder induce and utilize the third element of apomixis: the development of an unfertilized 2n egg into a plant being identical or near-identical to the parent?

Synapsis and Desynapsis with an Otherwise Normal Meiosis

Synapsis implies homologous pairing, normal gene recombination and balanced distribution of chromosomes at anaphase I resulting into reduction from 2n to n chromosomes. The final result is a balanced tetrad with tetraedral arrangement of four functional microspores and a basically linear arrangement of four megaspores, of which as a rule only the chalazal one is functional.

Desynapsis implies visually normal pairing of homologous chromosomes at first prophase of meiosis, predominantly univalents at diakinesis and metaphase I and unbalanced distribution of chromosomes at anaphase I. The final result is an unbalanced tetrad with non-functional aneuploid spores.

Ramanna (1983a) studied pairing at pachytene, diakinesis and metaphase I of meiosis in 51 desynaptic mutants from the crosses mentioned in Table 1. He analysed 66-198 pollen mother cells per mutant and found complete homologous pairing at pachytene and an overall average of 2.4

bivalents per cell at metaphase I. In individual mutants desynapsis is clearly partial. These results are not in agreement with the supposition by Okwuagwu and Peloquin (1981), and Peloquin (1982), that there is no gene recombination at all in the mutants from the crosses USW 5295-7 X USW 5337-3 and reciprocal.

One gene, Ds/ds controls the difference between synapsis and desynapsis, the latter being the recessive character (Ramanna, 1983a). A homogeneous series of 3:1 ratios was obtained from all six crosses investigated (Table 1). These results show that there is only one locus segregating in the crosses analysed.

Table 1. Homogeneous 3:1 ratios for synapsis: desynapsis in six crosses, both parents being heterozygous at the ds locus (Data from Ramanna, 1983a).

	Crosses	Number of Plants Normal	desynaptic	χ^2	P
1.	USW 5337-3 x USW 5295-7	45	15	0	1
2.	USW 5295-7 x USW 5337-3	54	12	1.636	0.30-0.20
3.	USW 5295-7 x 2102-37	47	15	0.022	0.90-0.80
4.	USW 5337-3 x 2102-37	10	6	1.333	0.30-0.20
5.	USW 7589-2 x 2102-37	44	16	0.089	0.80-0.70
6.	2102-37 x USW 7589-2	30	12	0.286	0.70-0.50
	Total	230	76	3.366	
			χ^2 totals	0.009	0.95-0.90
			χ^2 homogeneity	3.357	0.70-0.50

Okwuagwu and Peloquin (1981) studied microsporogenesis in the crosses 1 and 2 in Table 1 and identified the desynaptic mutant sy3. Iwanaga and Peloquin (1979) reported in the same crosses a desynaptic mutant sy1, which according to the authors affected only megasporogenesis.

On the other hand, Jongedijk (1983), using Ramanna's results (1983a) on microsporogenesis, studied megasporogenesis in plants from the six crosses in Table 1 and found that in all of them micro- and megasporogenesis were either both normal (17 plants) or desynaptic (29 plants). Later results obtained by Jongedijk (unpubl. data) with 23 normal and 22 desynaptic plants from the crosses 1 and 2 in Table 1 were in complete agreement with the earlier findings: not one exception

could be detected. This is strong evidence for the conclusion that in the crosses studied only one gene for desynapsis (Ds/ds = Sy_3/sy_3) is involved affecting both micro- and megasporogenesis.

Remark: The symbol ds was used prior to sy and thus should be adopted also in potato.

Synapsis and Meiotic Nuclear Restitution

As pointed out before, synaptic plants normally display homologous pairing, reduction of 2n → n chromosomes and produce reduced or n-gametes. Occasionally after reduction (2n → n) restitution of the 2n number is observed in a certain proportion of spore mother cells leading to unreduced or 2n-gametes. This proportion is usually low, but may vary greatly dependent on genotype, environment and physiological age of the plant. Up to now only few clones with a relatively stable and high proportion of 2n-gametes have been detected although the number is steadily increasing.

Restitution mechanisms are known to occur since the early twenties in many plant genera. In the potato, restitution was rediscovered in the late sixties and more importantly its potential value for potato breeding was recognized and investigated in a systematic way. It is right to pay tribute to Peloquin and his associates for their pioneering work in this field. They distinguished two different ways of 2n gamete formation, viz. First Division Restitution (FDR) and Second Division Restitution (SDR). FDR and SDR have in common that normal gene recombination and reduction occurs. However, restitution of 2n proceeds in different ways. The basic difference is that with FDR the two chromatids of each chromosome are included in different 2n-spores leading to maintenance of parental heterozygosity in the gametes formed, whereas with SDR the chromatids of each chromosome are included in one 2n-spore thus leading to increased homozygosity in the gametes formed. Crossing-over does not invalidate this principle, but only modifies the genetic outcome for certain groups of loci. The details, genetic implications and relevance to potato breeding and evolution have been published at length and need not be repeated here.

According to Ramanna (1979) it is not unusual that in synaptic plants FDR and SDR occur simultaneously. The plant then produces a mixture of n-gametes, FDR-gametes and SDR-gametes. In synaptic plants FDR during microsporogenesis is caused primarily by fused spindles at second division of meiosis; parallel spindles may or may not lead to FDR-spores. This mechanism cannot occur in megaspore mother cells,

because the reductional cell wall is formed before the start of the second meiotic division. This explains why in synaptic plants 2n-eggs as a rule originate through SDR (Jongedijk, unpubl.). SDR occurs both during micro- and macrosporogenesis after completion of normal first division as a result of equational division of chromosomes not being followed by equational cell wall formation.

As mentioned before, 2n-sporogenesis is under genetic control and thus can be manipulated in potato breeding. The simple monogenic recessive control (e.g. genotypes psps for FDR) as reported by Mok and Peloquin (1975) is controversial for several reasons. Intercrossing psps clones (the crosses 1, 2, 3 and 4 in the tables 2 and 3) leads to a considerable proportion of F_1 offspring with fully normal meiosis and less than 1% big (2n) pollen (Ramanna, 1983a and Wagenvoort, personal comm.). Furthermore, the genetic analysis made by Mok and Peloquin (1975) is based on 5% big pollen discriminating between psps and Ps.plants. This criterion is too arbitrary and too variable for being used in genetic analysis. Iwanaga and Peloquin (1982) even used the criterion of at least 1% big pollen in their genetic analysis of varietal genotypes. Also 3% has been used by some authors. The implications of such criteria are shown in the Tables 2 and 3. Table 2 presents data from Ramanna (1983a), from which the percentages and ratios in Table 3 were calculated.

From these tables it may be clear that the percentage of big pollen is not useful in genetic analysis of meiotic nuclear restitution. On the other hand, the occurrence of big pollen is an indication of 2n-gamete formation and to that purpose it may be used for roughly screening populations of potato species. But even in that case one has to be cautious in drawing conclusions.

The system of synapsis and reduction in conjunction with 2n-restitution has some obvious advantages for the analytic breeding procedure at the diploid level. As mentioned before, it can be manipulated in potato breeding owing to its genetic control. Furthermore, FDR and SDR clones generally produce also some n-gametes. This is crucial for breeding at the diploid level, because diploid-diploid matings always give rise to a certain proportion of diploid offspring. And this is a must for continuing breeding at the diploid level. In addition, n-gametes are largely prevented from contributing to the progeny of tetraploid-diploid matings owing to the non-viability of tetraploid or pentaploid endosperm which is associated with triploid hybrid embryos. On the other hand, the occurrence of diploids in progenies from 2x - 2x matings makes the system under discussion unsuitable for the production of tetraploid TPS material through bilateral sexual polyploidization. In this respect the system discussed in the next chapter is more promising.

Table 2. Classification of F_1 plants on the basis of percentage of big pollen (> 25 μ ø) in the total (normal + desynaptic) progeny of 6 crosses (Ramanna, 1983a).

	Crosses*	# of plants in each percentage class							Total plants
		0	1	3	5	10	20	100	
1.	USW 5337-3 x USW 5295-7	13	6	5	7	9	21		61
2.	USW 5295-7 x USW 5337-3	11	13	7	1	8	23		63
3.	USW 5295-7 x 772102-37	5	4	2	5	4	25		45
4.	USW 5337-3 x 772102-37	0	1	1	2	1	11		16
5.	USW 7589-2 x 772102-37	16	3	3	2	6	9		39
6.	772102-37 x USW 7589-2	7	4	4	1	2	1		19

* According to the literature the crosses 1 through 4 are psps x psps and the crosses 5 and 6 psps x not reported genotype.

Table 3. Percentage of psps plants and ratio psps:Ps. in the 6 crosses in Table 2 on the basis of the criteria for psps:5%, 3% and 1% big pollen respectively as used in the literature.

Cross	Percentage of big pollen as criterion for psps- genotype					
	%psps-plants			Ratios psps:Ps.		
	>5%	>3%	>1%	>5%	>3%	>1%
1	60.7	68.9	78.8	1.5:1	2.2:1	3.7:1
2	50.8	61.9	82.5	1.0:1	1.6:1	4.7:1
3	75.6	80.0	88.9	3.1:1	4.0:1	8.0:1
4	87.4	93.7	100.0	6.9:1	14.9:1	1:0
5	43.6	51.3	59.0	0.8:1	1.1:1	1.4:1
6	21.0	42.3	63.2	0.3:1	0.7:1	1.7:1

Desynapsis Associated with Restitution

Desynapsis in association with meiotic nuclear restitution has been described before long, but its potential for potato breeding has only recently been recognized. This system has been rediscovered almost simultaneously by Okwuagwu (Okwuagwu and Peloquin, 1981) and by Ramanna (Hermsen and Ramanna, 1981; Ramanna, 1983a). Ramanna (1983a) was the first to demonstrate clearly, that its value mainly derives from the fact, that it invariably leads to functional FDR-gametes only, because both reduced gametes and SDR-gametes are eliminated in a natural way as a consequence of aneuploidy. This holds true in both micro- and megasporogenesis (Jongedijk, 1983; Ramanna, 1983b).

FDR restitution in desynaptic clones may be caused either by fused spindles on the male side only, or by pseudohomotypic division both on the male side (Ramanna, 1983) and female side (Jongedijk, unpubl. data). Only FDR-restitution leads to functional fertile gametes. Consequently, male and female fertility of stable desynaptic clones are a good measure for degree of restitution in micro- and macrosporogenesis respectively and provides an efficient means for detecting the degree of restitution. So male restitution in dsds clones can be estimated by the percentage of stainable pollen; female restitution by seed set after crossing dsds clones with 2-pollen-producing male parents (Jongedijk, 1983). In synaptic plants (DsDs and Dsds) FDR restitution usually occurs only on the male side and is detectable by cytological assessment of meiosis only.

An other aspect of desynapsis and restitution deserves attention. Okwuagwu and Peloquin (1981) and Peloquin (1982) have reported that desynapsis would prevent gene recombination. So in association with FDR restitution it would have the potential of transferring fully intact parental genomes to the offspring. As mentioned before, Ramanna (1983a) observed in all desynaptic mutants complete homologous pairing at pachytene and even, though limited to an average of 2.4 bivalents per cell, at metaphase I. This statement is important from a basic point of view. For practical breeding purposes it only means, that the diploid genome of a stable desynaptic FDR clone may be transferred to the progeny somewhat more intact than that of a synaptic FDR clone. Therefore in this respect the potential of desynaptic clones should not be over-estimated.

As explained before, in desynaptic clones basically only FDR 2n-gametes are fertile, both on the male and female side. This has some direct implications for breeding. On the one hand, it enables the breeder to apply FDR successfully in bilateral sexual polyploidization, BSP,

(diploid-diploid matings → tetraploid progeny) as well as in unilateral sexual polyploidization, USP, both ways, tetraploid x diploid and diploid x tetraploid. This is a highly promising aspect also in relation to TPS technology. On the other hand, breeding at the diploid level is complicated, because after crossing diploids the diploid level cannot be maintained. Therefore, for continued breeding at the diploid level the breeder is forced to work either with heterozygous Dsds-clones, or with partially desynaptic dsds-clones in which also some reduced gametes may be functional. We return to these breeding aspects in the next chapters.

Before discussing breeding procedures using FDR-restitution it may be useful to summarize first some relevant features and implications of meiotic nuclear restitution in synaptic and desynaptic clones.

(i) Mechanisms leading to FDR 2n-gametes

* synapsis: fused/parallel spindles (fs/ps) and only on the male side;

* desynapsis: fs/ps on the male side only and pseudo-homotypic division both on the male and female side.

(ii) Functional gametes (omitting clones with 100% n-gamete formation)

* synapsis: - n + 2n (FDR) only on the male side
  - n + 2n (FDR) + 2n (SDR) only on the male side
  - n + 2n (SDR) both on the male and female side,

  hence : only mixtures and no FDR 2n-eggs.

* desynapsis: only 2n (FDR) both on the male and female side,

  hence : no mixtures and no functional n- or 2n (SDR)-gametes.

(iii) Applicability of FDR in TPS technology

* synapsis: only USP of the type 4x ♀ . 2x ♂ → 4x

* desynapsis: - USP both ways 4x.2x and 2x.4x → 4x

  - BSP = 2x.2x → 4x

(iv) Loss of gametes (effect on degree of fertility and seed set)

* synapsis: male n-gametes due to defective endosperm associated with triploids ('triploid block') from fusion of 2x + x gametes;

* desynapsis: n and 2n(SDR)-gametes, both male and female, due to aneuploidy (→ sterility).

Hence: with desynapsis only 2n(FDR)-gametes give rise to tetraploid progeny both with USP and BSP, whereas with synapsis both 2n(FDR) and 2n(SDR) may lead to tetraploid progeny from 4x.2x-matings.

(v) Feasibility of breeding at the diploid level

* synapsis: feasible because of functional n-gametes (both male and female);

* desynapsis: not feasible because sterility of n-gametes leads either to polyploidy (both parents producing 2n-gametes) or to no seed set (only one or none of the parents producing 2n-gametes).

(vi) Genetic control of mechanisms

* desynapsis: monogenic recessive;

* fused/parallel spindles: monogenic recessivity reported, but in fact more complicated with either several recessive genes or even polygenes involved;

* pseudo-homotypic division: genetic basis unknown.

(vii) Ease of detection of FDR-restitution

* synapsis: preliminary screening for percentage of big pollen followed by cytological screening for fused spindles frequency;

* desynapsis: male and female fertility determinations as explained before.

Breeding Procedure Using FDR-Restitution: General Outline

Breeding at the diploid level using dihaploids and diploid related species may result into advanced diploid parental lines. These lines

are to be included into a final programme of sexual polyploidization and selection of the best combiners. However, before starting that final programme the parental lines need to be tested for occurrence of recessive ds-alleles (Dsds, dsds) and FDR-restitution. If Dsds parental lines are found, dsds progeny can readily be produced by intercrossing such heterozygotes. The dsds segregants allow eventual detection of FDR-restitution by scoring male and female fertility. Fertility means that the way is open to USP or even BSP and determination of combining abilities.

If only DsDs-lines happen to occur, such lines may produce big pollen indicating either FDR- or SDR-restitution. The restitution mechanism has to be established by studying meiosis. If predominantly fused spindles are found, further breeding through USP (4x.2x) is feasible. Although the occurrence of functional SDR-gametes cannot be excluded, such clones are useful in practice as has been amply demonstrated by several groups of researchers.

On the other hand, if all parental lines would turn out to be synaptic DsDs without any indication of restitution (no big pollen), an additional backcross programme has to be carried out using synaptic or partially desynaptic FDR-clones as sources of genes for FDR-restitution and the parental lines (recurrent parents) as sources of genes for desirable agronomic traits. Such backcross programmes will be explained in the next chapter.

The Introduction of FDR-Restitution and/or Desynapsis into Diploid Parental Lines without Restitution and Homozygous Dominant at the Locus for Desynapsis

The presently available clones with a high-level FDR-restitution, either or not associated with desynapsis, are of little direct agronomic value. FDR-restitution and/or desynapsis can be introduced from such clones into advanced diploid material via backcrosses.

a. FDR-restitution (without desynapsis)

Be SR the code for synaptic clones with stable high-level FDR-restitution but little agronomic value and RP (= recurrent parent) the code for synaptic clones without restitution but with high agronomic value. Then assuming either recessivity or polygenic control of restitution the following breeding scheme may be appropriate (scheme 1). The sibcrosses are included either to obtain recessive segregants for restitution or to raise the level of polygenic restitution in each generation.

Scheme 1. Backcross procedure for introducing recessive FDR-restitution into synaptic advanced diploids. For SR and RP: see text.

	Growing seasons	Breeding procedure	
Cycle 1	1	SR x RP ↓ F_1	
	2	sibcross ↓ sib-F_1 progeny	Selection*
Cycle 2	3	selected plants x RP BC1	
	4	sibcross ↓ sib-BC_1 progeny	Selection*
Cycle 3	5	selected plants x RP ↓ BC2	
	6	sibcross ↓ sib-BC_2 progeny	Selection*
Etcetera			

* Selection in successive sib-generations comprises screening for agronomic traits, preliminary screening for production of big pollen followed by meiotic screening for fused spindle formation.

As pointed out before, synaptic diploid parental lines with FDR-restitution through fused spindles are to be included into a final programme of unilateral sexual polyploidization (4x.2x) for selection of the best combining tetraploid and diploid parents. Such parents produce the uniform, vigorous and also otherwise valuable populations, that may be used either directly for TPS-technology or as sources for selection of clonal varieties.

b. FDR-restitution with desynapsis

Be Dsds the initially unimproved, non-desynaptic clones with a genotype for high FDR-restitution and one recessive allele for desynapsis, and DsDs the advanced diploid clones to be used as recurrent parent (RP) in the backcross programme.

Scheme 2 gives the general backcross procedure.

Scheme 2. General backcross procedure for introducing desynapsis into advanced diploids.

Generation	Backcross series	Resulting genotypes (generation)
F_1	Dsds (P) x DsDs (RP)	DsDs (F_1) + Dsds (F_1)
BC1	Dsds (F_1) x DsDs (RP)	DsDs (BC1) + Dsds (BC1)
BC2	Dsds (BC1) x DsDs (RP)	DsDs (BC2) + Dsds (BC2)
BC3	Dsds (BC2) x DsDs (RP)	DsDs (BC3) + Dsds (BC3)
Etcetera		
Final step:	Dsds x Dsds a.o. dsds	

Explanation to Scheme 2.

Per generation a sufficient number of plants (to be discussed in the next paragraph) are backcrossed with the recurrent parent and at the same time testcrossed with a good Dsds-plant from the previous or other generations to identify DsDs and Dsds. After identification, the backcross progenies from DsDs x DsDs (RP) are removed, whereas those from Dsds x DsDs (RP) constitute the next backcross generation.

The question about the number of plants that need to be tested per generation is easy to answer for desynapsis (about 16), but hard

to answer for FDR-restitution, because of lack of exact knowledge about its nature and inheritance. DsDs- and dsds-plants (synaptic) can be tested for restitution by determining the percentage of big pollen and, in plants with a high percentage of big pollen, the frequency of fused spindles at meiosis. In dsds-plants only male and female fertility need to be scored for estimating FDR-restitution during micro- and megasporogenesis respectively. Following scheme 2 dsds-plants segregate from the testcross and their agronomic value increases with each generation as illustrated in scheme 3.

Scheme 3. Origin of dsds-segregants from successive testcrosses.

Dsds (F_1)	x	Dsds (P)	→	dsds (F_1/P)
Dsds (BC1)	x	Dsds (F_1)	→	dsds (BC1/F_1)
Dsds (BC2)	x	Dsds (BC1)	→	dsds (BC2/BC1)
Dsds (BC3)	x	Dsds (BC2)	→	dsds (BC3/BC2)

If at the start of the backcrosses different well-combining DsDs clones are available, the backcross procedure can be applied on each of them separately. If it would be possible to select during the procedure besides genotypes with male FDR-restitution also some genotypes with female FDR-restitution, then we may end up with improved Dsds-clones and possibly - via the testcrosses - improved dsds-clones, some of them producing 2n (FDR)-eggs, others 2n (FDR)-pollen and some (probably rare) producing both.

The Dsds-clones could be intercrossed deliberately for determining combining ability and possibly for further improvement. From the progenies obtained Dsds- and dsds-plants can be selected: the heterozygotes for continued breeding and the dsds-plants for being included in USP and BSP. As a matter of course the degree of restitution has to be established also at these breeding stages.

Although much research still needs to be done, the application of desynapsis and FDR-restitution in producing single and double crosses via USP and BSP is no longer a utopian scheme and therefore promising also for TSP-technology.

Apomixis

Tetraploid hybrid seed obtained via BSP from diploid desynaptic parents with FDR-restitution may be used directly for TPS purposes because of agronomic value, uniformity and easy seed production if one parent combines female restitution with cytoplasmic male sterility. In addition it might also be possible to select outstanding tetraploid genotypes and to release them as clonal varieties. So the BSP-approach using desynapsis and restitution is a flexible system as opposed to somatic hybridization, because the latter does not have any potential for recombination and further selection.

Potato clones with desynapsis and restitution can be maintained as clones. An approximately identical reproduction is feasible in desynaptic clones with female restitution via pseudogamic induction of seed development using marked S. phureja as pollinator (pseudogamic apomixis). Up to now only low levels of female restitution have been found in the desynaptic material studied so far (Jongedijk, 1983). On the male side much higher levels of restitution have been detected in similar material. A near-identical reproduction of such material might be feasible through anther culture, thus offering a new method of freeing plants from tuber-borne diseases.

Future Research

Basic research on desynapsis and restitution has to be continued at places where the know-how is available and a considerable number of highly fertile desynaptic clones have been obtained.

The usefulness of such clones for testing the breeding value of advanced tetraploid parental lines and cultivars is being tested in a cooperative programme between the Department of Plant Breeding and the Foundation for Agricultural Plant Breeding where two scientists who just graduated in plant breeding at the Agricultural University, have started a project in which different methods of determining the breeding value are compared. Masson from Clause breeding company in France and former student of Peloquin has joined this group.

The feasibility of the breeding procedure, explained in the previous chapters, has to be investigated. In relation to efficient TPS production an evaluation of cytoplasmic male sterility for use in large scale seed production by insects has to be continued.

Also related to TPS and apomixis is the development of S. phureja genotypes combining high-level induction of parthenogenetic seed with homozygosity for embryo colour, e.g. from S. brevidens.

A logical topic for future research is that breeding of agronomically superior diploid material should be continued by all scientists who are already involved in it. The more so because new tools have become available for producing vigorous and uniform tetraploid hybrid populations from diploids.

Finally: varieties and other valuable tetraploid material should be investigated thoroughly for their genotype of desynapsis and occurrence of female FDR restitution by crossing with desynaptic fertile diploids an analyzing the progenies in the way as explained before. The results of such investigations must be of great value for breeding tetraploids with desynapsis and female restitution. These tetraploids might be maintained or in the future even propagated as true seeds through pseudogamic apomixis.

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Utilization of Wide Crosses in Potato Breeding

J.G.Th. Hermsen

Introduction

Recent research on wide crosses at the Plant Breeding Dept., Wageningen, has been focussed predominantly on two groups of wild species: (i) Mexican species mainly as new sources of late blight resistance, but also as bridging species in wide crosses. (ii) Chilean non-tuberous species, mainly as new sources of PLRV resistance.

Table 1 summarizes the species used and their potential contributions to potato breeding.

Three long-term projects have resulted in advanced populations tracing back to series of wild species which are highly valuable, but hardly accessible to potato breeding, viz. Bulbocastana and Pinnatisecta from Mexico and Etuberosa from Chile. The breeding procedures and results will be presented and discussed.

Breeding Procedures and Results

The schemes 1, 2 and 3 illustrate the breeding pathways applied in the development of three populations built up of four Solanum species each. Apart from some characteristic differences which will be explained below, the pathways have in common the following features:

(i) The use of two species which in addition to their bridging function have the potential to contribute greatly to the value of the final populations (see Table 1).

(ii) Doubling the chromosome number at one or two stages where sterility was blocking further progress.

(iii) Only conventional crossing making use of previous experience and optimal environmental conditions.

(iv) The occurrence of one narrow bottle neck, mostly at the stage where S. tuberosum is being included for the first time.

Table 1. Summary of the Solanum species used in wide crosses. The abbreviations are used in the text; the letter codes in the following schemes.

Species	Abbre-viation	Letter code	Potential sources of resistance to	Geographic origin
S. bulbocastanum	blb	B	Late blight, insects, viruses	Mexico
S. verrucosum	ver	V	Late blight	do
S. stoloniferum	sto	S	Late blight, PVY, insects	do
S. pinnatisectum	pnt	P	Late blight	do
S. jamesii	jam	J	Pseudomonas, late blight	do
S. etuberosum	etb	E	PLRV, PVY, frost	Chile
S. brevidens	brd	B	PLRV, PVY, frost	do
S. acaule	acl	A	Frost, PVX, PLRV	Andes
S. phureja	phu	P	Pseudomonas, late blight	do
S. tuberosum	tbr	T	Many diseases and pests	do

Once that bottle neck has been overcome through extensive crossing during a long period of time there are no more barriers to further breeding. It is very crucial to prevent narrowing the genetic base and loss of desired genes of such stages of the breeding procedure.

A. The First Population: New Source of Late Blight Resistance

Scheme 1 illustrates the pathway leading to the population indicated by ABPT. It aimed at breeding new material for resistance to late blight derived from *S. bulbocastanum*.

Scheme 1. Origin of ABPT.

4x - *S. acaule* (A) x 2x - *S. bulbocastanum* (B) ..... Step 1

↓

3x - AB (4x - AB not useful)

↓ doubling ..... Step 2

6x - AB x 2x - *S. phureja* (P) ..... Step 3

↓

Bottle neck! ±4x - ABP x 4x *S. tuberosum* (T)..... Step 4

↓

4x - ABPT

5½x - ABPT x 4x - *S. tuberosum* ..... Step 5

6x - ABPT

Explanatory Remarks

(1) The F_1 - plants 4x - AB were sterile and remained so after doubling the chromosome number to the octaploid level.

(2) The two bridging species are *S. acaule*, because of its direct crossability with *S. bulbocastanum*, and *S. phureja*, because direct crosses of 6x - AB with cultivars led to loss of late blight resistance. This was predicted on cytological grounds by Hermsen and Ramanna (1973) and confirmed experimentally in Toluca valley, Mexico, by Turkensteen (CIP).

(3) The bottle neck in scheme 1 is step 4; 20,000 pollinated flowers produced many berries but only 40 ABPT hybrids with different ploidy levels as indicated in the scheme and described in detail by Hermsen and Ramanna (1973).

(4) The 5 1/2 x and 6x - ABPT hybrids were the most resistant ones and originated from unreduced gametes of 4x - ABP in step 4.

The ABPT clones were sib-mated and crossed readily with potato cultivars. From the progenies well-yielding clones with stable high-level late blight resistance were detected in Toluca valley, Mexico, by Turkensteen. They have been included in a breeding programme in which late blight resistance was combined with resistance to PVY and PVX.

Recently some new ABPT genotypes were produced and crossed with potato cultivars. The results are presented in Table 2.

The new clones will be tested for resistances and earliness in 1984.

b. <u>The Second Population: New Source of Resistance to Late Blight, PVY and PVX.</u>

Scheme 2 illustrates the pathway leading to the population indicated by VBST. This procedure was initiated as an alternative to the ABPT approach in order to avoid the serious bottle neck indicated in scheme 1.

Scheme 2. Origin of VBST. TV^5 = *S. verrucosum* with *S. tuberosum* cytoplasm.

```
2x - TV5 (V) x 2x - S. bulbocastanum (B) ............... Step 1
             ↓
         2x - VB
             |
             | doubling ............................... Step 2
             ↓
         4x - VB x S. stoloniferum (S) ............... Step 3
             |   ↓
             | 4x - VBS x 2x - FDR clone 2102-37(T).... Step 4
             ↓
         3x - VBST
             |
             | doubling ............................... Step 5
             ↓
         6x - VBST x S. tuberosum cvs ................ Step 6
                 ↓
               5x - VBSTT
```

Table 2. Results from crosses between five ABPT genotypes and five cultivars. pf = pollinated flowers; b = berries; s = seeds. Results 1982. Alcmaria: early; PVX - inmune; Gloria: early; PVY and PVX resistant; Ob. Frühe : mid-early, some late blight resistance; (Ntb x Alcm)87 : PVX and PVY.

Crosses	Number of ABPT genotypes	Total number of			Hybrids grown in 1983	Average	
		pf	b	s		b/pf	s/b
ABPT x Alcmaria	5	183	97	775	63	0.53	8.0
ABPT x Gineke	3	187	109	1082	80	0.58	9.9
ABPT x Gloria	4	78	52	428	91	0.67	8.2
ABPT x Ob. Frühe	4	113	24	352	59	0.21	14.7
ABPT x (Ntb x Alcm).87	5	23	18	80	43	0.78	4.4
Total	5	584	300	2717	336	0.51	9.1

Explanatory Remarks

(1) Crosses between pure S. verrucosum and S. bulbocastanum gave 54% berry set, but only 0.3 seeds per berry. In addition the hybrids were cytoplasmically male sterile and remained sterile after doubling (Hermsen and Ramanna 1976). Therefore the cytoplasm of S. verrucosum was substituted by that of S. tuberosum via a backcrossing programme leading to TV^5. Crossing TV^5 by S. bulbocastanum gave male sterile diploid progeny. However, this male sterility was not under cytoplasmic control but due to structural differences between the parental chromosomes. Therefore fertility could be recovered by chromosome doubling.

(2) Crossability of TV^5 with S. bulbocastanum is sufficient to produce large enough F_1's and the same holds true for the crosses of steps 3 and 4.

(3) In step 4 an FDR clone was used producing both n and 2n gametes. As all progeny was triploid, only the n male gametes were functional.

(4) Step 6. Ten late blight resistant 6x - VBST clones were crossed with five cultivars. Five clones were not successful, one clone gave few seeds and four clones gave a reasonable berry and seed set leading to nearly 5500 seeds in 1982. Part of these were grown in 1983 and 1314 tuber producing hybrid seedlings were harvested (Table 3). Testing for resistance to late blight, PVY and PVX and for earliness and other agronomic characters will be carried out in 1984.

Products of step 4 in scheme 2 (= 3x - VBST) were tested in 1982 for late blight resistance in the field at Wageningen using the method described by Tazelaar (1981), and for PVY resistance in the greenhouse.

Table 4 presents the late blight scores: 23 out of 28 clones were highly resistant and were continuously distributed over all classes.

Five clones showed a disease reaction reminding of R gene resistance, although only one clone appeared fully susceptible with a score similar to that of susceptible Bintje. The hexaploid VBST parents used in the crosses with cultivars all had a low score (0.04 - 0.79).

Table 3. Results from crosses between five 6x - VBST genotypes and five cultivars. pf = pollinated flowers; b = berries; s = seeds. Ntb = Neotuberosum. For cultivars see caption of Table 2.

Crosses	Number of VBST genotypes	Total number of				Average	
		pf	b	s	hybrids harvested	b/pf	s/b
VBST x Alcmaria	5	658	138	2623	276	0.21	19.0
VBST x Gineke	5	320	39	284	180	0.12	7.3
VBST x Gloria	5	209	65	1220	304	0.31	18.8
VBST x Ob. Frühe	5	391	59	1054	312	0.15	17.9
VBST x (Ntb x Alcm).87	4	33	13	296	242	0.39	22.8
Total	5	1611	314	5477	1314	0.19	17.4

Table 4. Frequency distribution of 23 VBST genotypes for late blight score in 1982 at Wageningen.*

	Classes of weighted means of late blight scores												
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.3
Number of clones	5	3	4	1	2	2	1	1	1	1	0	1	1

* In addition five clones got the scores 1.93, 2.25, 2.61, 2.96 and 5.04. Control varieties: Bintje 5.87, Libertas 2.50. Scores $\leq$ 2.50 are considered resistant.

A preliminary greenhouse test revealed the presence of PVY resistance among the VBST clones, probably introduced via the accession PI.160226 of *S. stoloniferum*.

C. The Third Population: New Source of PLRV Resistance

Scheme 3 illustrates the pathway leading to genotypes which according to the four species involved can be indicated as VEPT or TVEP.

Scheme 3. Origin of VEPT and TVEP. TV^5 = *S. verrucosum* with cytoplasm of *S. tuberosum*.

2x - S. etuberosum (E) x *2x - S. pinnatisectum* (P) .....Step 1

↓

2x - EP

↓

doubling .....Step 2

2x - TV^5 (V) x 4x - EP ..... Step 3

↓

3x - VEP

↓

doubling ..... Step 4

cultivars (T) x 6x - VEP x *cultivars* (T) Bottle neck!..... Step 5

↓ ↓

5x - TVEP 5x - VEPT

Additional Information about Scheme 3

(1) The steps 1, 2, 3 and 4 have been explained in detail by Hermsen and Taylor (1979). Hermsen et al. (1981 a, b), Ramanna and Hermsen (1979, 1981, 1982). Steps 3 and 5 were made possible by doubling the chromosome number of the completely sterile hybrids 2x - EP and 3x - VEP respectively. Testing for PLRV resistance was carried out at CIP: Rizvi (1982) selected extremely resistant genotypes from 4x - EP populations which were obtained through selfing and sibbing of 4x - EP hybrids.

(2) There is one severe bottle neck in the breeding scheme, i.e. step 5 (Table 5).

Table 5. Results from reciprocal crosses between four hexaploid TV^5 x 4x - EP clones (coded VEP and numbered 1, 3, 6 and 9 in Table 6) cultivars pf = pollinated flowers; b = berries; s = seeds. Results 1982.

Crosses	Total number				Average	
	pf	b	s	hybrid plants (1983)	b/pf	s/b
VEP x Alcmaria	59	27	1	1	0.46	0.05
do x Gineke	1478	642	54	10	0.43	0.08
do x Schwalbe	205	147	3	0	0.72	0.02
Total VEP ♀	1742	816	58	11	0.47	0.07
Alcmaria x VEP	41	0	0	-	0	-
Gineke x do	539	3	12	1	0.006	4.0
Schwalbe x do	12	0	0	-	0	-
Bintje x do	63	0	0	-	0	-
Burmania x do	34	0	0	-	0	-
(Ntb x Alcm.) x do	310	2	29	0	0.006	14.5
Total VEP ♂	999	5	41	1	0.005	8.2

Table 5 shows that using VEP as female parent with cultivars as males gave a good berry set, but an extremely low seed set. At least 93% of the berries were empty. In the reciprocal crosses (VEP male) berry set was extremely low, 5 per 999 pollinated flowers, but seed set relatively good. The seeds obtained germinated badly (17%) but those which germinated gave rise to 12 hybrids, one TVEP and 11 VEPT. These hybrids were vigorous. All except one had deep purple stems and flowers. Some plants had flowers which besides a normal calyx had double corollas. The hybrids were pentaploid, as expected, and produced a reasonable amount of stainable pollen. Tuber production was good (Fig. 1).

In 1983 all twelve hybrid seedlings were backcrossed with varieties and nearly 7500 seeds could be harvested from ten of them (Table 6).

It may be concluded, that breaking the breeding barriers between S. etuberosum and potato cultivars has been achieved. (The extremely high seed set obtained on the only TVEP - hybrid can not yet be explained). On the other hand it has to be admitted, that for practical reasons testing for PLRV resistance in successive generations has not been carried out. This fact, in addition to the severe bottle neck at step 5 justifies the suspicion that probably no PLRV resistance from S. etuberosum is present in the 12 clones. Nevertheless the twelve hybrids warrant testing for resistance to PLRV and the results of these tests will determine whether the extensive backcross material has any direct value for the PLRV programme.

Figure 1. Tubers harvested from 12 seedlings of the crosses VEP x cultivars and reciprocal. Measurements of the labels are 9.5 x 5 cm.

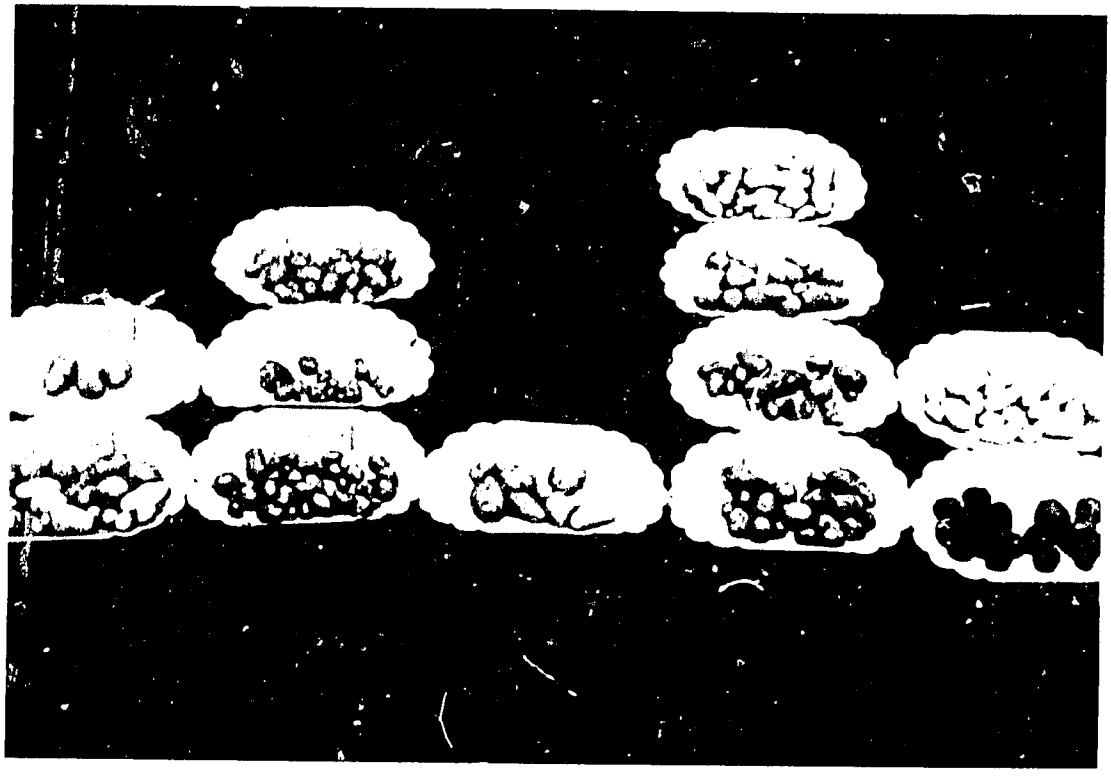


Table 6. Summary of results from backcrosses of pentaploid VEPT and TVEP with cultivars of S. tuberosum. Figure codes of female parents: 1st figure = seedling number of VEP (1982)
2nd figure = seedling number of VEPT (1983).
Figures codes of male parents ('cultivars'):
cv 1 = Alcmaria cv 3 = Gineke cv 5 = (Ntb x Alcm.). 87
cv 2 = Gloria cv 4 = Ob. Frühe cv 6 = (do).105
cv 7 = (MPI 75-14 x (Ntb x Alcm.).87) clone nr 4.

Female parents	Male parents "cultivars"							Total number			Average	
	1	2	3	4	5	6	7	pf	b	s	b/pf	s/b
VEP x Gineke												
1 - 1	x	-	-	-	-	x	-	5	3	13	0.60	4.3
1 - 2	x	x	-	-	x	x	-	13	10	23	0.77	2.3
3 - 1	x	x	-	-	x	x	x	36	12	118	0.33	9.8
3 - 2	x	x	x	-	x	x	x	40	15	120	0.38	8.0
3 - 3	x	x	-	-	x	x	x	76	25	244	0.33	9.8
6 - 1	x	-	-	-	-	x	-	11	1	0	0.09	0
9 - 1	-	x	-	-	-	-	x	27	6	39	0.22	6.5
9 - 2	x	x	-	-	x	x	x	33	22	273	0.67	12.4
9 - 3	x	x	-	-	-	-	x	20	15	54	0.75	3.6
9 - 4	x	x	-	-	-	-	-	5	0	-	0	-
Total								266	109	884	0.41	8.1
VEP x Alcmaria												
6 - 1	x	x	-	x	x	x	x	177	89	1005	0.50	11.3
Gineke x VEP												
seedling 1	x	x	x	x	x	x	x	88	32	5600	0.36	175.0

New Broadly Based Starting Material

Apart from spare seed of selfed and sibbed 4x - EP from which the resistant genotypes were selected by Dr. Rizvi, new broadly based material

has been produced in 1982 and part of it doubled in 1983. This programme included three accessions of both S. brevidens and of S. etuberosum on the one hand and the bridging species S. jamesii, S. pinnatisectum and TV^5 on the other. The pooled crossing data are presented in Table 7.

It is apparent that crossability of S. jamesii with S. etuberosum and S. brevidens in terms of berry set and even more so in terms of seeds per berry is better than that of S. pinnatisectum. TV^5 crosses well with only a few specific genotypes: three out of 28 S. etuberosum plants and one out of 28 S. brevidens plants produced seeded berries in spite of the large number of pollinated flowers per plant (8th column in Table 7).

Table 7. Summarized results from crosses of three accessions each of S. etuberosum (etb) and S. brevidens (brd) with S. jamesii PI 275265 (jam), S. pinnatisectum PI 230489 (pnt) and TV^5. TV^5 = S. verrucosum with S. tuberosum cytoplasm. pf = pollinated flowers; b = berries; s = seeds.

Species ♀	Species ♂	Female plants with Total	berries	Total number pf	b	s	Averages pf/♀	b/pf	s/b
etb*	jam	17	13 (77%)	67	29	614	3.9	0.43	21.2
	pnt	26	20 (77%)	274	120	278	10.5	0.44	2.3
	TV^5	28	11 (39%)	1282	29	356	45.8	0.02	12.3
brd**	jam	28	25 (89%)	196	109	2763	7.0	0.56	25.4
	pnt	27	26 (96%)	325	107	174	12.0	0.31	1.6
	TV^5	28	6 (21%)	1082	11	13†	38.6	0.01	1.2

* accession numbers GLKS 31/2, PI 245924, PI 245939
** accession numbers PI 218228, PI 245763, PI 245764
† all 13 seeds from one berry.

Part of the seeds obtained were sown in 1983. Hybridity of the seedlings could be established. Nearly 50 hybrid seedlings were successfully doubled and will be included in the breeding programme in 1984. This new starting material has to be tested simultaneously for PLRV, PVY and late blight resistance at least before large-scale

crossing with deliberately chosen cultivars (step 5 in scheme 3) will be carried out. A thorough discussion with the CIP staff about the way how to proceed and how to continue cooperation in this extensive programme, has taken place.

Discussion

Basically the question about the accessibility of species from series Bulbocastana, Pinnatisecta and Etuberosa has been answered: it is possible along the ways outlined in the schemes presented. Obviously other pathways are possible, as will be explained below for the Etuberosa species. It has been shown that with the ABPT and VBST approach (schemes 1 and 2) late blight resistance could be transmitted into advanced material. However, for PLRV resistance (scheme 3) from Etuberosa species it still remains to be demonstrated.

ABPT and VBST Compared

It may be assumed that late blight resistance in ABPT derivatives traces back to S. bulbocastanum. The VBST approach is different in that the source of late blight resistance is the gene pool VBS with three species contributing to the final resistance. This approach is not in agreement with the principle of building resistance columns (Hermsen, 1974).

The Use of Bridging Species and the Choice of Cultivars

The use of bridging species is unavoidable when working along conventional lines. Although this method is laborious and genes may get lost on the way to nobilization, the bridging species may contribute several additional valuable characters to the progeny. A clear example is the late blight resistance which is lacking in Etuberosa species, but present in the bridging species S. pinnatisectum and TV^5. The choice of cultivars is also crucial, because apart from general agronomic characters they may contribute earliness and resistances that are missing in the wild species.

Somatic hybridization might replace the classical approaches described before. However, it may be questioned firstly whether short cuts are desirable (no contributions of bridging species, hardly any chances for gene recombinations), secondly whether short cuts will indeed be realized, for after somatic hybridization, only few hybrid genotypes

are obtained and these hybrids may well offer barriers to further nobilization, which then have to be broken yet along conventional lines.

Alternative Approaches for Etuberosa Species

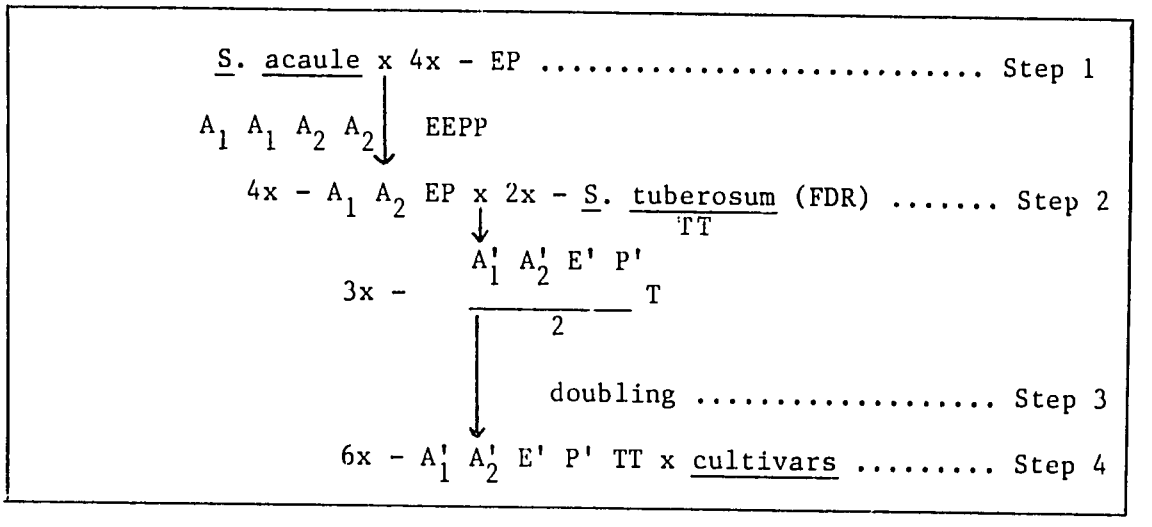
It has been shown (table 7) that S. jamessi has a better crossability with Etuberosa species than S. pinnatisectum. It may be expected that scheme 3 can basically also be applied with S. jamesii as first bridging species. TV^5 has the disadvantage that its crossability with Etuberosa species is restricted to few genotypes. This drawback can be overcome by extending the number of Etuberosa plants in step 1 of scheme 3. In addition TV^5 is closer related to S. tuberosum than Pinnatisecta species and consequently scheme 3 might be simplified.

Johnston and Hanneman (1982) produced a triploid hybrid between S. brevidens and S. chacoense. Ehlenfeldt and Hanneman (1984) obtained one seed with a pentaploid embryo from a cross between this triploid hybrid and tetraploid S. tuberosum AG 231. This pentaploid apparently had originated from a triploid 2n gamete of the triploid female parent and a reduced gamete of AG 231. Crosses between this pentaploid as female with 8 potato cultivars were successful. A basic advantage of such approach is the relatively large contribution of S. brevidens to the progeny and the need of only one bridging species, S. chacoense, being closely related with S. tuberosum and comprising many valuable characters.

Schmiediche and Chavez (unpubl. results) from CIP found S. acaule to be crossable reciprocally with our 4x - EP material. So S. acaule could serve as a second bridging species instead of TV^5 (scheme 3). However, it has the disadvantage that the contribution of 4x - EP to the progeny is lower than with TV^5: 50% vs 67% in the crosses with 4x - EP and 25% vs 33% after the next step, i.e. crossing with cultivars. If crosses of S. acaule x 4x - EP with cultivars would be more feasible than 6x - (TV^5 x 4x - EP) with cultivars, the bottle neck in scheme 3 might be less narrow or even absent.

It is interesting to compare the S. acaule approach of Schmiediche and Chavez with scheme 2 for VBST from the point of view of genomic composition. In this context a suspectedly successful pathway of the S. acaule approach may run parallel with the VBST scheme as follows (cf scheme 2)

Scheme 2



Step 4 may be expected to be successful.

However, there are some differences with VBST.

(i) The genomes of S. acaule are closely related. Although, preferential pairing in 4x - A_1 A_2 EP might be expected (only bivalents) and no crossing over between S. acaule and EP, multivalents and univalents may occur.

(ii) In VBST three species contribute to late blight resistance. In A_1A_2 EP only S. etuberosum provides the PLRV resistance, unless PLRV resistant S. acaule accessions are chosen as bridging species.

(iii) S. etuberosum is characterized by a strong structural hybridity. This may increase the speed of nobilization, but also the loss of resistance genes.

Late blight Resistance

Because of the importance of late blight all over the world there is an urgent need of a broadly based resistance. Mexican sources have been explored and are available in a nobilized form in ABPT and VBST derivatives. The resistance detected in South American species has to

be further exploited and transferred into nobilized material. Then attempts to combine resistance from both sources may be highly promising. However, it is still an open question, whether such approach will lead to material combining high level late blight resistance with earliness, which is a pious wish of several generations of potato breeders.

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Use of Meiotic Mutations in Breeding

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Introduction

Inherited variations in the meiotic process have been found in many plant species. They provide unusual materials for investigations in cytology, genetics, evolution and breeding. Several recent reviews (1, 2, 8) surveying the past, present and future research activities with meiotic mutants emphasize their value in cytological, developmental and biochemical investigations of the meiotica process. Strikingly, no reference is made to the use of meiotic mutants in breeding and germplasm transfer. The meiotic variants found in the tuber-bearing Solanums are uniquely valuable in breeding, germplasm transfer, and half-tetrad analysis: This report will consider inherited variations that affect microsporogenesis and megasporogenesis, and their utility in breeding and germplasm transfer.

There are several features of meiotic mutants which should be noted: 1) Almost all of those reported are controlled by a single locus; 2) The mutations are invariably recessive; 3) Sometimes the character usually associated with a certain genotype fails to appear -- that is penetrance is not always 100%; 4) The number of meiocytes that express the parental genotype can vary from 1 to 100%. Variable expressivity is the rule not the exception with meiotic mutants; and 5) Expression is significantly modified by genetic, environmental, and developmental factors.

Mutants affecting microsporogenesis

The first meiotic mutants were found while evaluating a breeding method involving 4x-2x crosses. Twenty 4x cultivars were crossed with more than 100 2x hybrids (Group Phureja x haploid Tuberosum). The progeny were mainly 4x, very vigorous, and the mean tuber yield of many 4x families exceeded that of the cultivar parent (3). Several 2x parents were exceptional in that many rather than few seeds/fruit were obtained when they were used as pollen parents, and more than 99% of the progeny were 4x. These results suggested the possibility of a systematic method of 2n pollen formation. Among the exceptional 2x clones, there were two types based on characteristics of the progeny. One type gave the highest yielding and more uniform progeny.

Cytological analyses of microsporogenesis provided the clues to explain the breeding results (10). In the normal sequence the first meiotic division is not followed by cytokinesis, two spindles in the second division are oriented so that their poles define a tetrahedron, and cytokinesis results in a tetrad of four n microspores.

In three exceptional clones the first division is normal but in some sporocytes second division spindles are parallel, and following cytokinesis two 2n microspores are formed. A modification of this result occurs when second division spindles are tripolar, and one 2n and two n spores are formed following cytokinesis. The parallel orientation of second division spindles is inherited as a simple recessive, ps (11). The second type of exceptional 2x clone gave 4x progeny that were quite variable and relatively low yielding. Cytological observations revealed a different meiotic variation. The first division, in many sporocytes, was followed by cytokinesis, the second division does not occur, except that chromatids fall apart, and a dyad of two, 2n spores is formed. This variation, premature cytokinesis, pc, is also simply inherited (11).

The significance of the meiotic mutants resides in their genetic consequences (16). Parallel spindles is essentially a First Division Restitution (FDR) mechanism. All loci from the centromere to the first crossover, that are heterozygous in the parent will be heterozygous in the gametes, and one-half the heterozygous parental loci between the first and second crossovers will be heterozygous in the gametes. In contrast premature cytokinesis is genetically equivalent to a Second Division Restitution (SDR) mechanism. All the loci from the centromere to the first crossover will be homozygous in the gametes, and all loci between the first and second crossover, that are heterozygous in the parent, will be heterozygous in the gametes. The percent heterozygosity transmitted by the gametes in roughly estimated to be 80% with FDR in contrast to less than 40% with SDR. A large fraction of the epistasis is also transmitted with FDR. Breeding results support the superiority of FDR in that progeny from 4x x 2x FDR outyielded those from 4x x 2x SDR by 30-50% (12).

The breeding scheme of obtaining 4x progeny from 4x x 2x FDR crosses is effective due to large tuber yields of the progeny, its wide application, and for the production of potatoes from true seed where the seedling vigor, yield and uniformity of the progeny are important assets (16, 22).

This breeding scheme is also an effective and efficient method of germplasm transfer from cultivated and wild, 2x species to 4x cultivars (16, 20). Haploids play a major role in this germplasm transfer. Most introductions of the cultivated 2x and almost all introductions of wild 2x species do not tuberize before frost in north temperate regions. Selection adaptation is very effective in the cultivated 2x, but not in the wild 2x. However, when particular haploids are crossed to the wild species, S. chacoense, many F_1 hybrids produce large tuber yields, some approaching the yield of cultivars. Further, the hybrids varied greatly in maturity, and several were as early as the early cultivars (9).

Recently, hybrids were obtained between haploids and the wild species S. berthaultii, S. boliviense, S. canasense, S. infundibuliforme,

S. microdontum, S. raphanifolium, S. sanctae-rosae, and S. tarijense (Hermundstad, unpublished). Again the particular haploid used in the cross had a highly significant effect on tuberization in the hybrids. Almost 100% of the hybrids tuberized when particular haploids were the female parent in contrast to 0% tuberization in other families where a different haploid was the parent. The tuber yield of many hybrids, particularly with S. berthaultii, S. microdontum, and S. tarijense greatly exceeded that of the haploid parent. This unique ability of particular haploids to put wild germplasm in a usable form represents an outstanding example of "germplasm enhancement" (22). More important, it should facilitate the incorporation of valuable traits of wild 2x species into 4x cultivars.

Combinations of meiotic mutants

Several other recent findings add to the value of the 4x x 2x and 2x x 2x breeding schemes. First, a new meiotic mutant, sy3, was discovered (17, 18). It is characterized by lack of chiasmata (thus probably no crossing over) in some sy3 clones, only univalents at "metaphase I," and the univalents are distributed randomly to telophase I nuclei. This would ordinarily lead to almost complete male sterility, since the chance of the telophase I nuclei receiving a haploid set of chromosomes is $\frac{1}{2}^{12}$. However, when the synaptic mutant is combined with the parallel spindles mutant, a high frequency of functional 2n pollen is formed. No matter how unequal the distribution of chromosomes is in the first division, parallel spindles in the second division ensure the incorporation of two sets of 12 chromosomes in each pair of 2n microspores. The genetic significance of this combination of meiotic mutants resides in the opportunity they provide the incorporation of the intact genotype of the 2x parent into all the 2n male gametes. Thus, 100% of the heterozygosity and epistasis of the parent can be transmitted to the progeny, a powerful breeding tool (18, 21).

The clones homozygous for both sy3 and ps are of particular value in that, 1) They transmit the intact genotype of the parent to the offspring; 2) They provide a homogeneous sample of heterozygous 2n gametes to test the parental value of 4x clones; 3) Only 4x progeny result from 2x-2x (sy3, ps) crosses; 4) Highly uniform progeny for production of potatoes can be obtained from 4x x 2x crosses; 5) Doubled clones of sy3, ps, would make it possible to screen for 8x progeny following 4x x 4x (sy3, ps) crosses (18).

Iwanaga (5) has recently reported a very important finding. He has extracted haploids from the Mexican cultivar Atzimba and determined that approximately one-half of the haploids are ps, ps, and that about one-half of these in turn are homozygous for a new synaptic mutant sy4. This mutant is characterized by poor pairing at pachytene and a high univalent

frequency at diakinesis. The good vigor and fertility, plus the late blight resistance present in many Atzimba haploids further enhances Iwanaga's discovery of sy4. This material will be invaluable in further breeding in general and for late blight resistance in particular.

Another meiotic mutant, sy2, was discovered in S. commersonii (6). It is characterized by all univalents in the first division, and very poor fertility. A low frequency of stainable 2n pollen occurs probably as a result of the formation of restitution nuclei in the first division followed by a normal second division. Crosses are being made in an attempt to obtain plants homozygous for both sy2 and ps, since they are expected to have a high frequency of fertile 2n pollen. While determining the inheritance of sy2, a new meiotic variation was detected in a few plants (Hanneman, unpublished). Again, only univalents occur, but not even an abortive spindle is formed, so there is no chromosome movement. All 24 chromosomes appear to be included in the nucleus of most microspores (monads). It is obvious from the previous discussion that we have a wealth of variations in microsporogenesis in the tuber-bearing Solanums to utilize in genetic and breeding investigations.

Schroeder (24) determined the parental value of 2x, 2n pollen clones in 4x x 2x crosses through use of parent-family correlations for 11, 4x selections, and 26, FDR 2x selections. Family means for maturity were highly correlated with 2x parent or midparent means, but not with 4x parents. Parent-family correlations for depth of eye gave similar results. No significant correlations existed between means of parent total yield and family yields. Thus, phenotypic selection among 2x materials should be efficient for maturity and tuber appearance. However, selection of 2x parents to increase progeny yield should be based on progeny testing. The minimal progeny testing would be to cross a potential 2x parent with one early in maturing and one medium to late maturing 4x clone. Improved 2x parents in the above traits have been identified.

Similar meiotic variations in other plants

Meiotic variations, similar to those in potatoes, have been found and are being used in other crop plants. Several clones of diploid alfalfa were found to have 2n pollen and produce good seed set in 4x x 2x crosses. The cytological basis of 2n pollen formation is parallel orientation of metaphase II spindles, and inheritance studies indicate control by a simple recessive (13). The use of FDR, 2n pollen in breeding highly heterozygous 4x, and in germplasm transfer from the 2x to the 4x level is being pursued (13).

Progress with meiotic variations in red clover has been very rapid. Clones of 2x red clover have been identified that produce 2n pollen, and the cytological mechanism is parallel spindles at the second division. However, one clone was found to have only functional 2n pollen. Cytolo-

gical examination revealed univalents in the first division and parallel spindles at the second division similar to the sy3, ps clones of potato. Even more interesting are the crossing results. Hybrids between 4x red clover (from colchicine doubling) and the 2x clone with only 2n pollen were significantly more vigorous than the 4x from colchicine doubling. The discovery of these meiotic variants in red clover appear to provide unusual breeding opportunities (19).

The functioning of 2n pollen in 4x x 2x crosses in many other plants has been noted. Further, in some fruits and ornamentals (for example blueberries and azaleas) obtaining 4x progeny from either 4x x 2x or 2x x 4x crosses where 2n gametes function is a recognized breeding method.

The inherited variations discovered in potato, alfalfa and red clover were found in breeding and genetic populations of these species and their relatives. Induction of mutations affecting the meiotic process has been successful for example in corn (2) and rice (7). It is not surprising that most induced mutations had an effect on either pairing and/or chiasma formation, and they were recessive.

Meiotic variation in megasporogenesis

Several variations in megasporogenesis that result in 2n eggs have been reported. Iwanaga and Peloquin (4) found a meiotic mutant, sy1, which only affects megasporogenesis. It is characterized by a fairly high univalent frequency and irregular distribution of chromosomes in the first division leading to high female sterility. Restitution nuclei at the first division occur at a low frequency, and when followed by a normal second division would give rise to FDR 2n eggs. Limited genetic evidence from half-tetrad analysis indicates the 2n eggs are probably formed by FDR.

The mutant sy3 is expressed in megasporogenesis as well as microsporogenesis (23). A high frequency or all univalents occur at prophase I, and the chromosomes are apparently randomly distributed at anaphase I. Complete female sterility is expected unless restitution nuclei are formed in the first division and a normal second division follows. Crossing sy3 clones as females with both 2x and 4x clones provides some information. No 2x progeny were obtained, so either no or a very low frequency of n eggs are formed. However, some 4x progeny were obtained from 2x (sy3) x 4x crosses, so functional 2n eggs probably are formed as a result of restitution in the first division and a normal second division -- an FDR mechanism. The frequency of 2n eggs per ovary (2-20) is low, but probably can be improved.

Stelly (25) carried out extensive investigations on the frequency, mode at origin, and genetics of 2n eggs in 2x hybrids using, 2x x 4x crosses plus genetic and cytological analyses. The inheritance of 2n eggs appears

qualitative with two or three systems each controlled by recessive factors. The results of half-tetrad analysis from Y/y and yyyy crosses (Y-yellow flesh) with most clones fit that expected if 2n eggs are formed by SDR. The results with a few clones deviated significantly from those expected on either SDR or FDR 2n egg formation. It is possible that they form some 2n eggs by SDR and some by FDR.

An important advance for studying the cytology of 2n egg formation was made by Stelly (26). He developed a stain-clearing technique with whole ovules that gives excellent preparations. It is fast, four ovules per minute can be analyzed for presence of 2n eggs, and allows for easy manipulation of ovules. Analysis of meiosis in clones that form a high frequency of 2n eggs indicates the first division is normal, but no second division occurs, so 2n eggs are formed by SDR.

Meiotic variations that result in FDR 2n eggs are necessary for full exploitation of the breeding scheme based on obtaining 4x progeny from 2x - 2x crosses. This scheme involves the development of superior, unrelated, 2x hybrids. These hybrids are selected for adaptation, other desired characteristics and 2n gamete formation by FDR. Tetraploids, with near maximum heterozygosity, plus other valuable characteristics are obtained from intermating selected 2x hybrids. The 2x clones, producing 2n pollen by parallel spindles or a combination of ps and sy3 are available. But it is important to identify unrelated 2x clones that produce high frequencies of 2n eggs by FDR.

Bilateral sexual polyploidization with meiotic mutants

Several years ago 4x were obtained from matings between 2x hybrids that produced 2n eggs and 2n pollen (15). The 4x progeny were more vigorous and outyielded their 2x "full-sibs" by 30-50% if the 2n pollen was formed by FDR. The mode of 2n egg formation was not known at the time of these results. Recently, it was determined by Stelly's stain-clearing technique, that the 2n eggs were formed by FDR. Thus, SDR 2n eggs combined with FDR, 2n pollen produced highly heterotic progeny in regard to yield. Similar results have been obtained by Chujoy (unpublished). He obtained 4x and 2x progeny from crosses between haploid Tuberosum x S. chacoense hybrids and Phureja x haploid Tuberosum hybrids. Again the 4x progeny greatly outyielded the parents and 2x progeny, and the yields of several 4x clones exceeded that of the cultivars entered in the yield trial. The significant point is that the 2n pollen was formed by FDR and the 2n eggs by SDR.

The two previous results demonstrate that SDR x FDR can result in vigorous, high yielding progeny. From the standpoint of transmission of heterozygosity, it is possible to envision how FDR and SDR can complement each other. For example, with FDR 100% of the heterozygosity from

the centromere to the first crossover and 50% from the first to second crossover is incorporated into the gamete; in contrast with SDR, 0% of the heterozygosity from the centromere to the first crossover and 100% from the first to second crossover is present in the gamete. Thus, from the centromere to the first crossover FDR x FDR is superior to SDR x FDR, but from the first crossover to the second crossover SDR x FDR is better than FDR x FDR. If possible then, "valuable genes" located between the centromere and first exchange should be heterozygous in the FDR parent, and "valuable genes" past the first exchange similarly heterozygous in the SDR parent for maximum benefit from SDR x FDR crosses. It is important to compare progeny from FDR x FDR with those from SDR x FDR to evaluate the a priori assumptions.

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Population Breeding Applied to Improvement of Unadapted Solanum Cultivated Species

Robert L. Plaisted

Population breeding is any system that treats selected plants in bulk rather than as individuals. Two examples of these systems are mass selection and recurrent selection. In contrast is a pedigree system which makes crosses between selected plants. With cross pollinated crops population breeding is more apt to be a routine where the final variety form is a synthetic or open pollinated variety than where the final variety is a hybrid or a pure line. In the latter, population breeding is often employed to create sources for developing new inbreds or pure lines. Potato breeding in the countries with suitable seed production programs is largely a pedigree system with limited use of population improvement programs. In most cases, parental lines are developed by judicious combination of parents in a pedigree system. Instances of population breeding applied to unadapted pools of germplasm are the programs in the U.S. and Europe to develop populations of S. phureja and tuberosum subspecies andigena adapted to Northern latitudes. There are few parallel programs in other crops. As a rule, once a crop has become adapted in a new region, further introductions tend to be used for specific genes rather than the broad base of genetic variability. Adaptation is so valuable and so complex, breeders of varieties of all crops tend to avoid going far afield for germplasm. A good example is soybeans, a rather new crop for the U.S. Delannay (1983) surveyed the ancestral lines of North American varieties. He found that the genetic base changed little from the 1950's to the present. Recently there has been a greater use of new introductions, but mostly these have been used in backcrossing programs as sources of single genes.

Expectations of Population Behavior

The expectations of genes in populations can be defined in special cases as well as the probabilities of gene segregation in specific pedigrees. The special case that can be best defined is a random mating population. In such a population, each individual genotype has a probability of functioning as a parent equal to its frequency in the population. Random mating populations with equal survival of progeny will eventually reach a state of equilibrium where the relative frequency of the different genotypes will remain constant from one cycle of mating to the next. It is this type of population that behaves in predictable ways.

One of the first questions to be considered is how quickly will a given random mating population achieve equilibrium. The answer depends on the nature of the population. If the population is a diploid, and only a single locus is considered, equilibrium is reached in one generation of random mating. If two loci are considered, then the extent of disequilibrium is reduced by (1-c) each generation of random mating where c is the frequency of recombination. In effect, it takes 3 or 4 generations to achieve equilibrium for 2 independent loci and much longer for linked loci (Figure 1). Polyploidy has a similar delaying effect. The degree of disequilibrium for a single locus in an autotetraploid is reduced by random mating by $\frac{1-\alpha}{3}$ each generation, where α is the measure of chromoatid segregation. In effect, it takes 2 or 3 generations of random mating for an autotetraploid to reach equilibrium (Figure 2). Another way to look at this is to consider the approach toward equilibrium of a population that initially is totally simplex for a given gene. Again it shows 2 generations of random mating are reasonably effective in reaching equilibrium (Figure 3). The point of this is that rarely do we initiate population studies or selection programs with populations in which all the genetic combinations are at equilibrium frequency. An exception was recently reported by Sanford and Ladd (1983) in which the base population for a leaf hopper resistance study was random mated for 7 generations.

In most of our work with populations, we are not content to just create a pool of germplasm with certain characteristics, but we wish to modify it through selection. If we start with random mating populations at equilibrium, it is possible to derive equations which express the rate of change in gene frequency which will arise due to the selection. Consider diploid populations first. If q is the frequency of the recessive allele where dominance is complete, the decline in the frequency of the recessive allele following elimination of all homozygous recessive individuals will be at the rate of $\frac{q^2}{1+q}$. If the detection of the homozygous recessive is incomplete, by an amount of 1-s where s is the efficacy of selection, then the rate of decline in the frequency of the recessive allele will be $\frac{sq\ (1-q)}{1-sq^2}$. These equations are plotted in Figure 4. If the alleles express no dominance, then the rate of change in gene frequency due to selection against one of them is $\frac{sq\ (1-q)}{2-2sq}$. This equation is plotted in Figure 5. The points to make about these figures are that any degree of imcompleteness in the selection process will seriously reduce the rate of change of gene frequency. Further, the rate of change becomes very slow at either very high or very low gene frequency.

FIGURE 1. EFFECT OF LINKAGE ON RATE OF APPROACH TO RANDOM MATING EQUILIBRIUM.

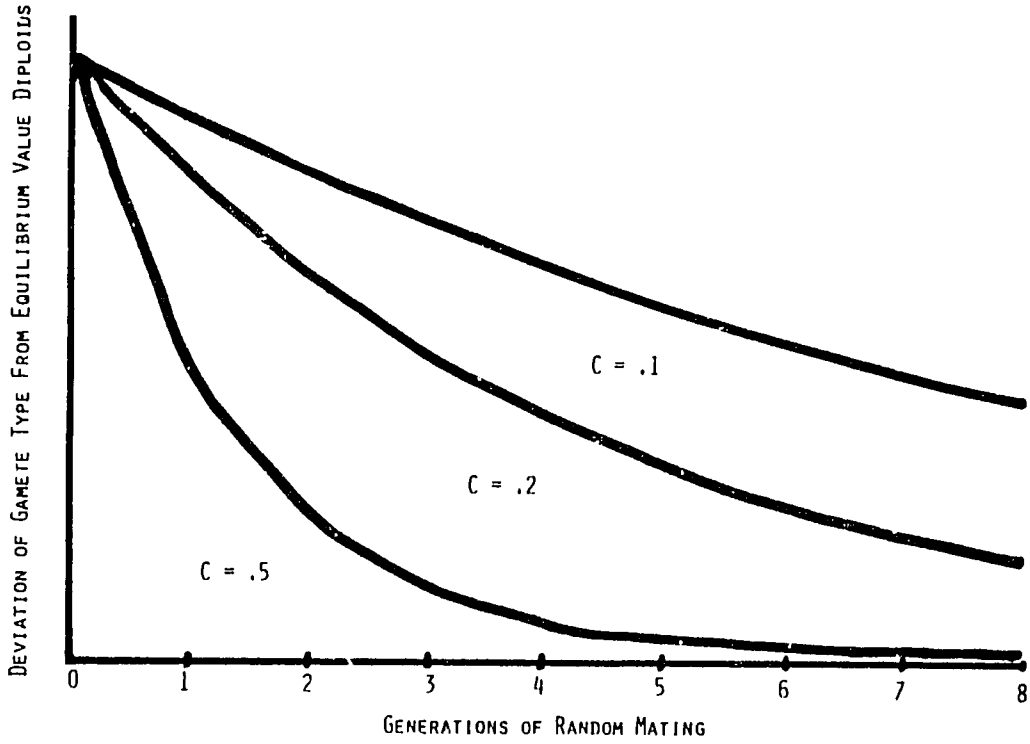


FIGURE 2. TETRAPLOIDS. APPROACH TO RANDOM MATING EQUILIBRIUM.

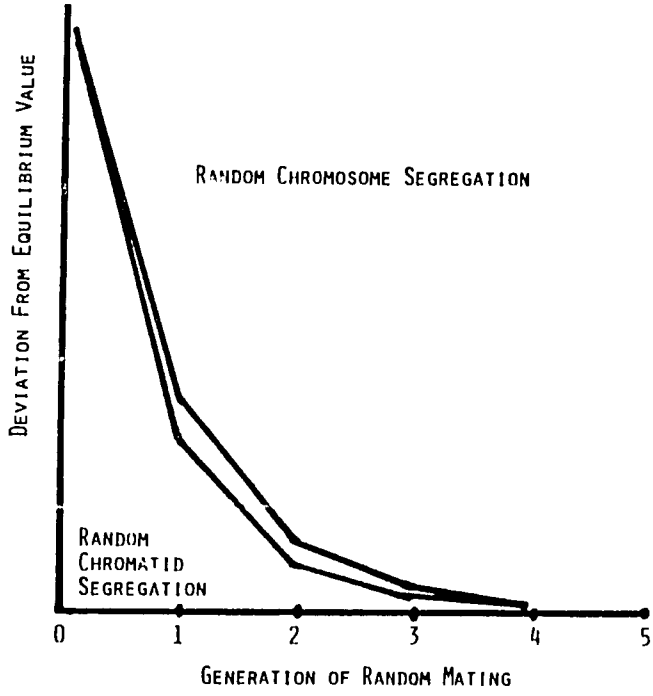


FIGURE 3. RANDOM MATING OF TETRAPLOIDS.

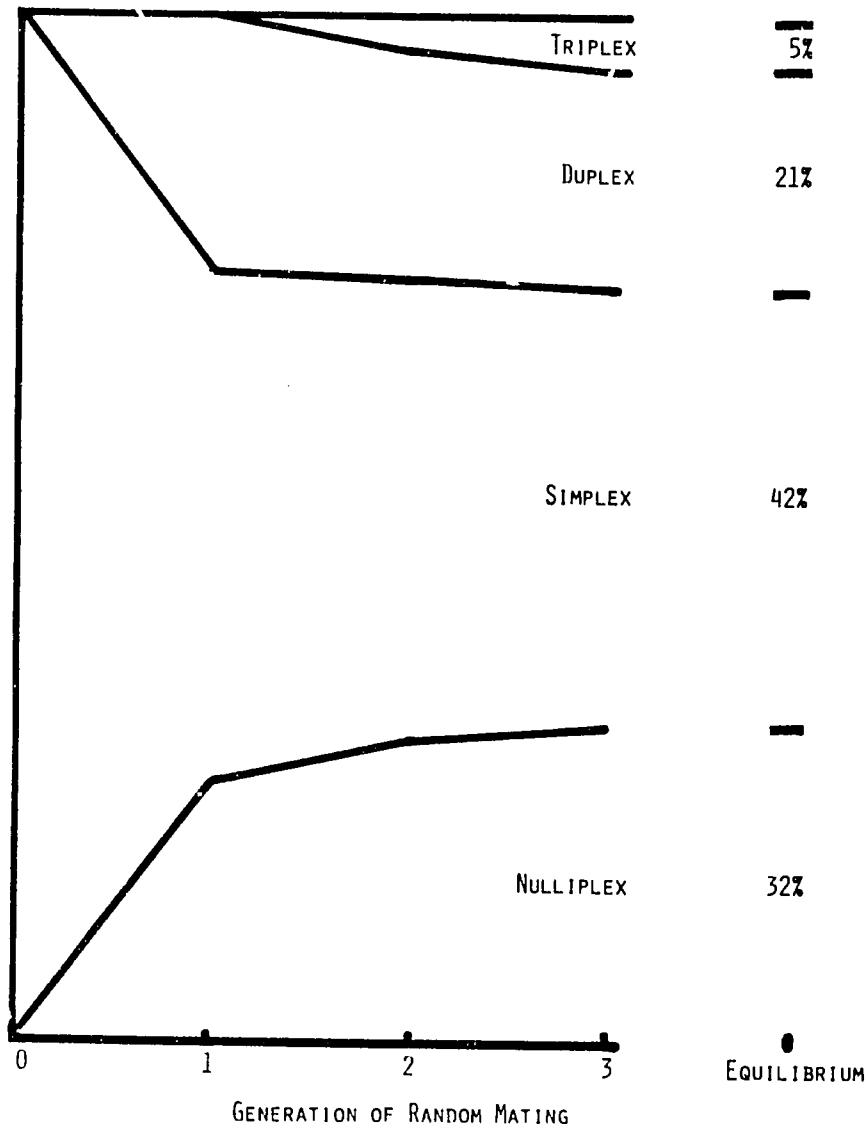


FIGURE 4. SELECTION AGAINST A RECESSIVE WHEN DOMINANCE IS COMPLETE.

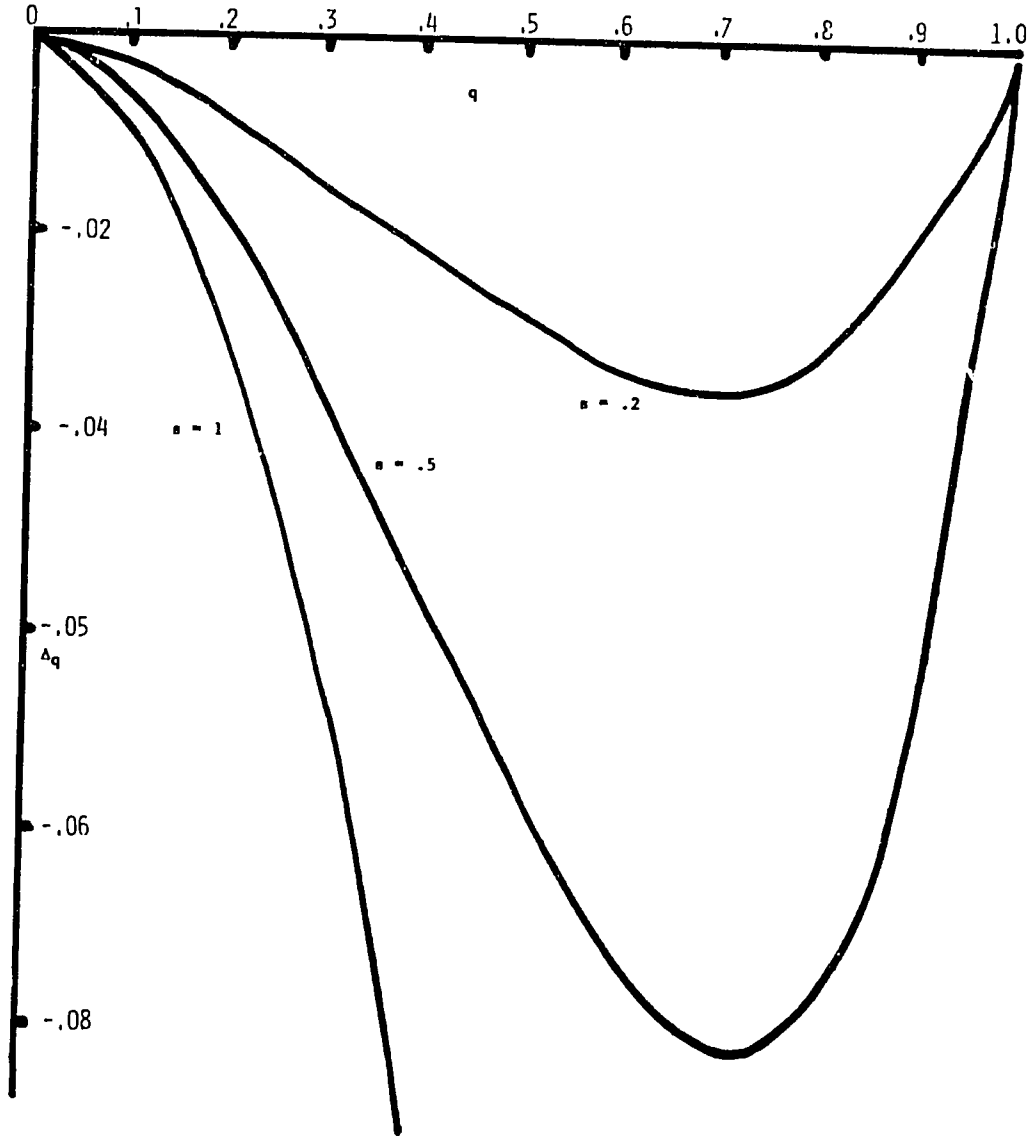
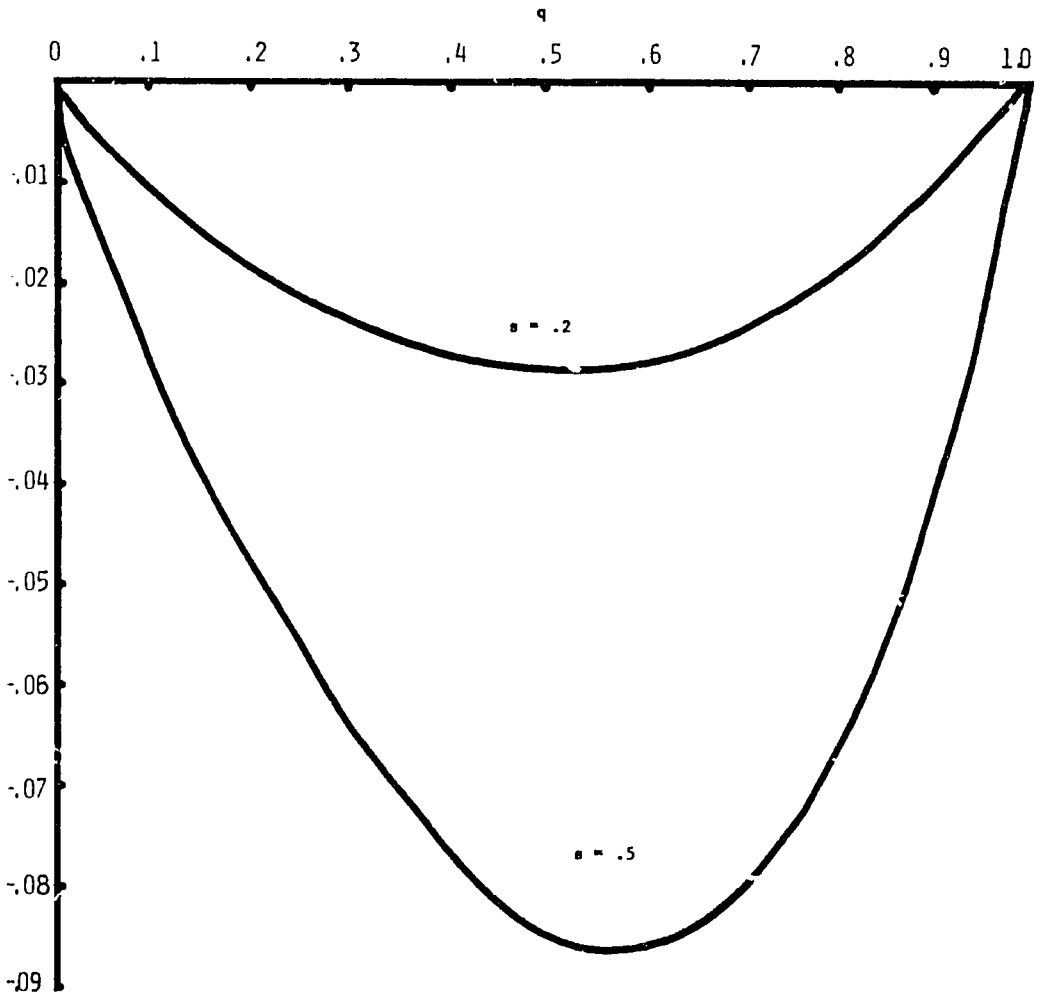


FIGURE 5. SELECTION AGAINST ONE ALLELE IN THE ABSENCE OF DOMINANCE.



Another way to look at this is to ask the question: How many generations of elimination of the homozygous recessive individuals will be required to reduce the gene frequency by a specified amount? The answer is in the equation $t = \frac{q_o - q_t}{q_t q_o}$ where t is the number of generations and q_o and q_t are respectively the original and final gene frequencies. To achieve a shift in gene frequency from .5 to .01 would require 98 generations. A shift from .5 to .1 would require 8 generations. Again, the point is that outside the middle ranges of gene frequency, changes occur slowly without a pedigree system to identify heterozygotes.

As you might expect, the situation with an autotetraploid population is somewhat more complex and extreme. Busbice, Hill, and Carnahan (1972) presented formulas for the rate of change is gene requency when there is total selection against the mulliplex genotypes. If selection is complete, the response is $\frac{(1-q)q^4}{1 - q^4}$ where q is the frequency of the recessive allele. If selection is incomplete and s proportion escape elimination, then the response is $\frac{(1-q)(1-s)q^4}{1 - (1-s)q^4}$. These equations are not very revealing, but if they are plotted as in Figure 6, they show that tetraploids are more conservative to changes in gene frequency than are diploids and that when gene frequencies approach .4 to .5, even complete elimination of the mulliplex individuals produces very slow additional changes in gene frequency. Another way to illustrate this is in Figure 7. This illustrates the shift in genotypes which occur when an initial population is all simplex and thereafter all the mulliplex are discarded after random mating. The proportions of mulliplex and quadriplex do not change rapidly.

One of the challenges in a population improvement program is to maintain a satisfactory level of genetic diversity while making substantial improvements through selection. The two are opposing forces. Several factors can influence the loss in heterozygosity.

A factor of considerable importance is the chance for selfing to occur instead of random mating. This is a factor of special importance in considering the use of open pollinated seed in tetraploid populations. Estimates of selfing under these conditions are given by Jeffrey White (1983) to be in the range of 90%. Bennett (1968) has presented examples of genotypic frequencies to be expected with a mixture of selfing and random mating (Figure 8). This shows the importance of making controlled pollinations in these populations to avoid unnecessary inbreeding.

FIGURE 6. RATE OF CHANGE IN GENE FREQUENCY FROM SELECTION AGAINST HOMOZYGOUS RECESSIVE INDIVIDUALS.

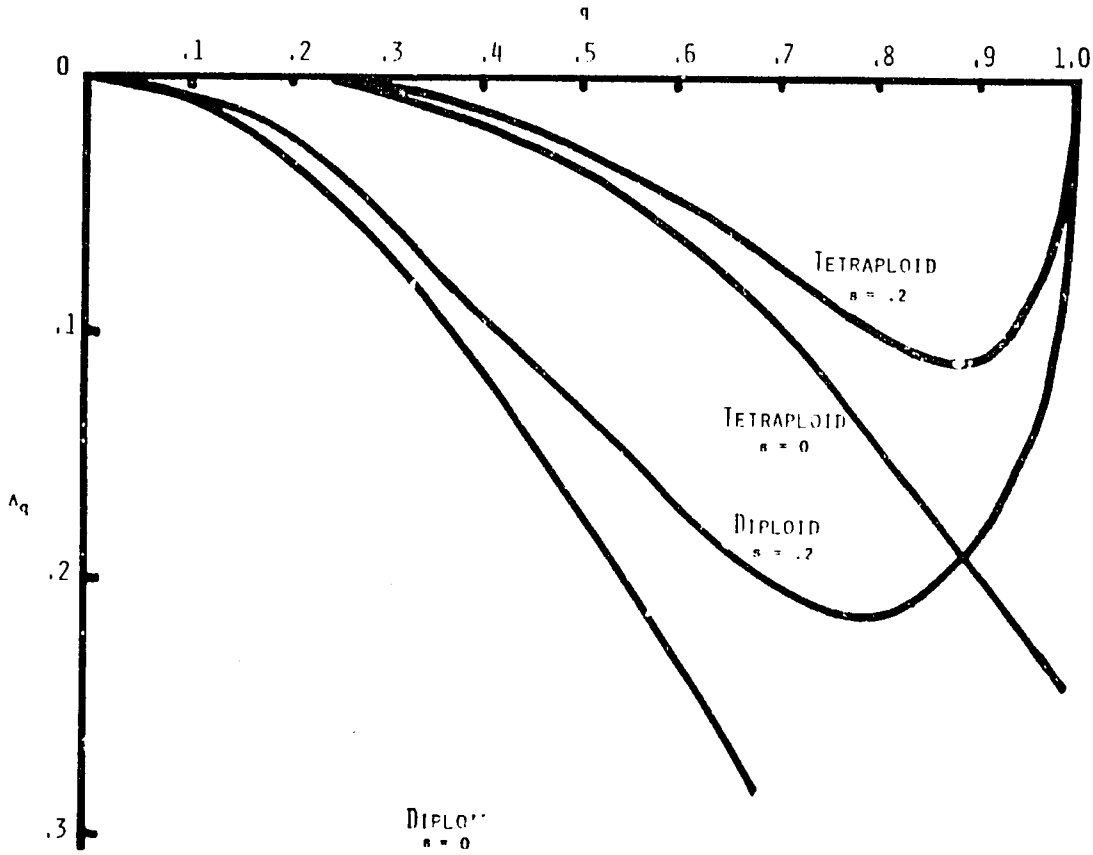


FIGURE 7. THREE GENERATIONS OF RANDOM MATING WITH SELECTION AGAINST THE NULLIPLEX INDIVIDUALS.

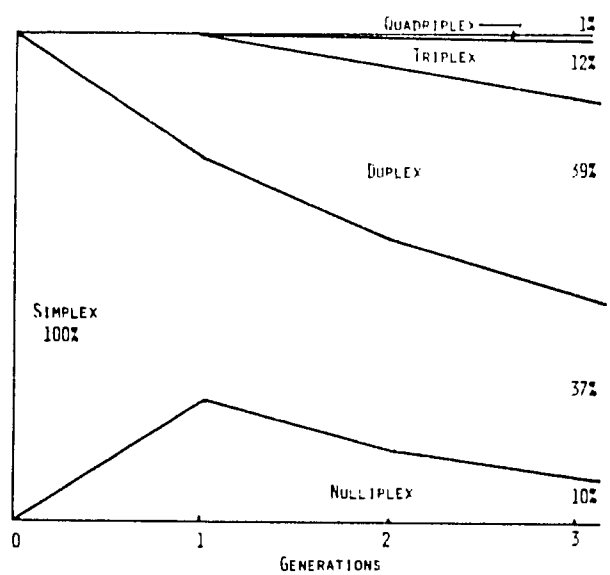
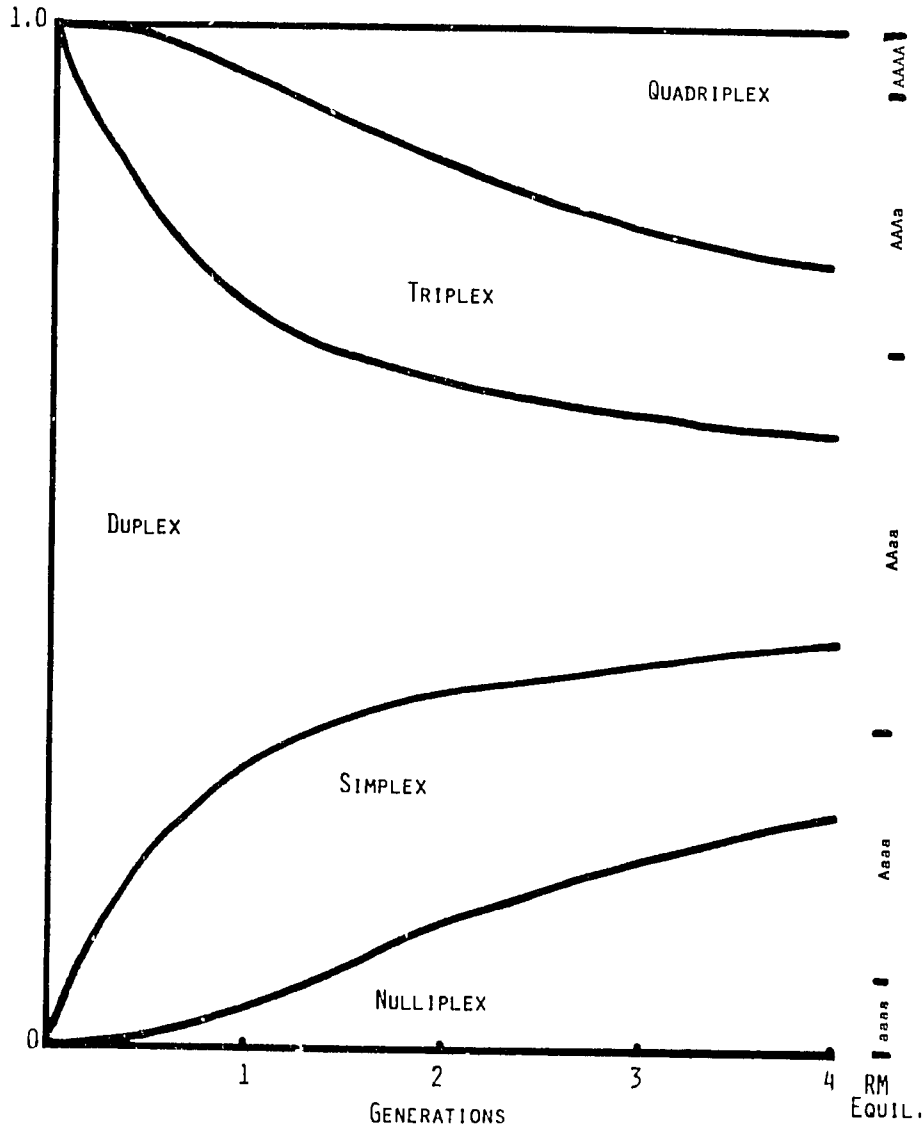


FIGURE 8. POPULATION EVOLUTION WITH 10% RANDOM MATING AND 90% SELFING.



A second major factor is simply the number of clones which function as parents for the next cycle. Obviously the smaller the fraction of individuals that are selected, the more intensive is the selection pressure. However, reducing the number of parents too much sacrifices future gains. In looking at changes in gene frequency due to selection, we observed that the conservative character of tetraploids was a disadvantage. In the context of conserving heterozygosity, the same effect is an advantage. Figure 9 presents the panmictic index (P), a measure of heterozygosity, for diploids and tetraploids when 6 or 25 individuals are used to propagate the population. The number of tetraploid parents can be surprisingly small before serious inbreeding occurs.

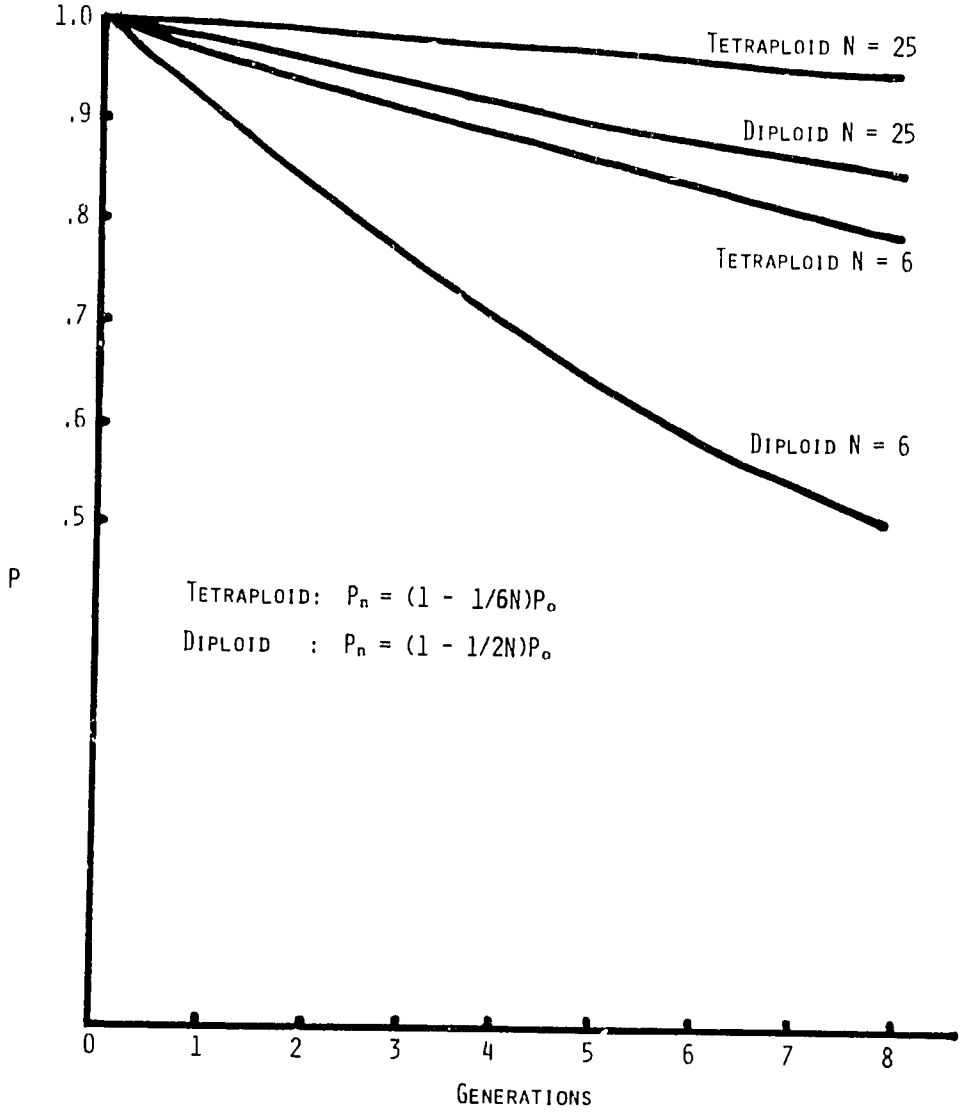
If an unequal number of clones function as males and females, this too will reduce the effective population size. In a diploid, the effective population size is $\frac{4\ Nf\ Nm}{Nf + Nm}$ where Nf is the number of females and Nm is the number of males. For example, if for reasons of convenience or limited pollen production a population of 40 has only 10 males, the effective number is not 40, but 32. Exclusion of the amount of selfing that would occur in the random mating frequency will not greatly influence the effective population number as long as the number of parents is not very small. The effective number with the exclusion of selfing becomes N + ½.

The decay in heterozygosity due to a limited number of parents presented in Figure 9 assumes the same number of parents at each cycle. Often in practice this does not occur. Sometimes the initiation of a population improvement project starts from a small base because of unavailability of a large number of suitable parents, or the start is a tentative one, small because its future is not certain. Sometimes an epiphytotic or other severe happening will wipe out a large portion of one generation. At any rate, the result is an unequal number of parents in successive cycles. Again, for diploids the effective number is $Ne = \frac{t}{[1/N_1 + 1/N_2 + \ldots 1/N_t]}$. The generation with the smallest number has the greatest effect on the effective number. For example, consider a population which starts with 20 individuals the first cycle and 100 the next 2. The effective population size is 43.

Applications of Population Improvement to Potato Breeding

I now want to review with you aspects of my experience with population improvement that illustrate the points I have just made about the expectations of genes in populations.

FIGURE 9. LOSS OF HETEROZYGOSITY IN RANDOM MATING DUE TO SMALL NUMBERS OF PARENTS.



If I had to condense all my impressions about population improvement into one statement, it would be that this method is a powerful tool for developing germplasm resources into useful breeding resources. The evolution of the andigena population we introduced 20 years ago has been very striking. I dwelt in detail on this project at the 1980 planning conference (Plaisted, 1980). Two points deserve attention. First, population improvement is impressively effective if good genetic material is the base and selection pressure is steadily applied over a period of time. Second, a valuable range of diversity can be maintained that would be more difficult by pedigree methods. Let me try to substantiate my impression about the power of the method. At the 1980 conference, I presented a table with the production characteristics of the 7th cycle selections. Since then some of those clones have been retested. To illustrate the useful diversity I would like to give first attention to the frequency of PVY immunity. This was a single locus, dominant gene discovered in the 2nd and 3rd cycles at a low frequency. If it had not been for this discovery, it seems certain we would have lost this population to PVY. For this reason we put high priority on its presence and began insuring that all crosses had at least one resistant parent. At each subsequent generation, all the seedlings were inoculated and clones with virus symptoms were eliminated. This procedure has not penalized the advancement of the population. The simple inheritance of the trait, the ease of screening, and the absence of any pleiotropic effects have certainly contributed to the apparent change in gene frequency. I use the word "apparent" because we have not made an effort to measure the actual change in gene frequency. Certainly it is now greater than .25 and likely less than 0.5.

Second, I would like to comment on the resistance to late blight in these clones. Recall that andigena long had a reputation among plant breeders in the U.S. and Europe as being too late and too susceptible to late blight to be useful in variety development. With only seven cycles of selection, the quality of this trait has improved to where a few clones such as A146-9 and A158-1 can grow the full season under epiphytotic conditions at Ithaca, NY and show only slight defoliation. The population as a whole is now more resistant than any of the U.S. varieties. In three years' trials, in September, Katahdin was 90% defoliated while the two selected clones were less than 5% defoliated and the whole set of clones selected for all qualities was 25% defoliated (Table 1). Now when we use this population in crosses, we consider it a source of resistance to late blight, rather than a source of susceptibility. This population has proven to be a valuable resource for several other traits. Even without a special selection pressure, there is a good frequency of clones resistant to PVX and wart. A158-4 has been outstanding for specific gravity. Over 4 years

it has averaged 1.098, about .026 units above Katahdin. In the past 3 years Huarte (1983) has shown that the vine type can be quickly shifted toward types indistinguishable from tuberosum. The question of whether this material has really become a neo-tuberosum is still debatable. It is not yet comparable to present day tuberosum for yield of large tubers and tuber appearance, but clones in it are comparable to sketches of potato varieties in a Hiram Sibley seed manual published in Rochester, NY in 1883. So it seems that in 20 years we have come from 1583 to 1883, and have only 100 years more to go. That may take another 20 years for the population as a whole, but a few clones are at that point now. The best we have is A231-1. This clone which appears to be PVY immune, contrary to the 1980 report, has looked attractive enough these past three years that next year we are entering it into our variety selection program.

Table 1. Seven cycles of selection for resistance to late blight.

	Mean Score 1980-82	Approximate % Defoliation
A146-9	2.2	<5%
A158-1	1.8	<5%
Population Mean (25 clones)	.4.0	25%
Katahdin	7.1	90%

These gains have arisen in spite of our violation of some of the principles described earlier. First the initial genetic base was limited and our population size was small, largely because it was a tentative start, without strong conviction in the mid 1969's that anything worthwhile would come of it. Hence the first cycle had only 631 clones and 23 selections. We maintained a record of the female parent, but made no attempt to use this information in the selection process. From this information the 20 seventh cycle survivors can be traced back to 4 original clones. Part of the reduction in base is due to the constriction arising from the PVY epidemics in the second and third cycles. Another way we violated the principles of random mating was to use OP seed. In our desire at that stage to shorten the cycle of selection for two generations we used open pollinated fruits as a source of seed. This produced a degree of inbreeding that restricted improvement in the next two cycles.

Based on these experiences, in 1969 we started anew with over 1000 accessions of andigena from several sources. It would have been much simpler if we were to start today and could use the CIP collection. This time we dictated that we would do our selection within accessions to avoid their loss entirely. In spite of this, our current cycle relates to 308 of the original 1000. The remainder were lost because of poor seed germination in the original accessions, or failure to produce any tubers or seeds in the early generations.

I believe that this early erosion of accessions in such a population improvement program should prompt one to consider an initial cross to an adapted population followed by a few generations of random mating without selection. Even this number of 308 which seems small relative to the andigena collection at CIP, taxes our capability. After the first generation, we have tried to avoid the use of open pollinated fruits. We try to make at least two selections in each accession to be able to discard the PVY susceptible clones and to reduce the risk of selecting clones that will not produce seeds. Seven hundred clones are about the limit of the space we can allocate for crossing this material. We like to grow about 100 seedlings from each accession and 30,000 is about the limit of our space for these. Physical limitations quickly affect one's decisions in a population improvement program.

We did not want to risk the erosion of this population due to PVY that occurred with the first population, so in the 3rd cycle and 4th cycles we made one outcross to the PVY resistant clones of the more advanced population. It did not seem sensible to go through the same slow process of selecting in this population for blight resistance that already existed in the advanced population. So the 5th cycle was produced by one more outcrossing to the advanced population, this time using the most blight and PVY resistant clones of the advanced population with one more generation of selection. My point in this is that I think it is more important to keep in mind the goals for the population than it is to preserve some sanctity of origin and to be willing to outcross to another population to gain something important as long as the original diversity is not seriously diminished.

Another population we are putting together demonstrates the use of several sources of desirable germplasm. We are trying to produce a heat tolerant population with resistance to root-knot nematodes, bacterial wilt, late blight, and PVY. To put this together we obtained two hybrid sparsipilum phureja families from Dr. Mendoza, three phureja-tuberosum hybrids with bacterial wilt resistance from Dr. Sequeira at Wisconsin, and combined these with our andigena. This is the population Pedro Gomez (1983) reported on the root-knot and bacterial wilt resistance at CIP's 10th Anniversary symposium. It

appears the disease resistance base, with the exception of resistance to Meloidogyne hapla, is in place, the population is at the tetraploid level, and tuberizes under manageable day length. This has taken only 6 years. I doubt we could have achieved as much and maintained as much diversity thru a pedigree system.

We have a third population we have managed differently and given us some experience worth sharing. That is a population created to transfer glandular trichomes from S. berthaultii to cultivated potatoes (Mehlenbacher, 1983). We started by crossing a few selected diploid clones with a few tetraploid clones in a pedigree fashion. This introduced the trichome attributes into an agronomically manageable background, one with the H1 gene for Globodera rostochiensis resistance. The next set of crosses was with bulk pollen from the andigena population to add the gene for PVY resistance. This was essential to protect the plants during their field evaluation for aphid resistance. The susceptible plants would be dwarfed by virus before the full season of aphid counts was accomplished. The selected offspring of these crosses were pollinated with bulk pollen from F_2's of andigena x berthaultii or of the tuberosum hybrids backcrossed to andigena. This stage of crossing added to the tetraploid genetic base and accommodated the recessive nature of the trichome expression. The quality of the trichome expression was further enhanced by a generation of sib mating. Now after 4 years, we have tetraploid clones with reasonable tuberization and segregating a density of trichomes and exudate quality approaching the original berthaultii clones. The PVY gene is in high frequency and the H1 gene in lower frequency. From now on we expect to be able to alternately backcross to tuberosum clones in a pedigree system and sibmate as in a population system. Each generation the parents are tested for PSTV and PVY and the offspring are inoculated with PVY and PVX. These are rogued for virus symptoms and selected for tuber type. The survivors are then screened for the browning reaction as a measure of exudate production, followed by a visual count of trichome density and a measure of exudate droplet size. The survivors are judged for vine type and crosses are made. The seedlots used for the next generation are finally based on the results of the field test of the parent clones under heavy aphid pressure.

Like the andigena population, this one illustrates the serendipitous discoveries that can occur when the genetic base is a good one and diversity is not seriously diminished during selection for adaptation. In this case we are finding clones with good resistance to the Colorado Potato Beetle. We think the resistance is independent of the trichome traits useful for aphid resistance and not due to high glycoalkaloid production.

Population Improvement in the Perspective of Variety Development

The telling testimonial for a breeding method or the product of it is whether it's being put to effective use in producing new varieties. I expect you to measure what I have said about population improvement by the use we have made of it in our variety selection program.

Essentially all US and Canadian varieties are the result of pedigree type crossing programs. The selectivity exercised in the choice of parents can be measured by the frequency that named varieties enter into the pedigrees. Of 135 varieties released since 1950, 12 are the product of crossing two varieties and 73 have one variety as a parent. Only 50 resulted from crossing two breeding lines. In our own breeding program, I find myself increasingly selective in the choice of breeding parents, emphasizing a small number which have proven their worth in previous progenies. Also we try to make large quantities of seeds so that we can resow any progenies that perform well in the first sampling. Using bulk pollen does not lend itself to repeated use as a parent, but it generally is efficient in producing large quantities of seeds. Increasingly I am using clones selected for specific attributes from the breeding populations for use in hybrid combination with other clones.

U.S. and Canadian Varieties
Released 1950-1983

No parents were named varieties	50
One parent was a named variety	73
Both parents were named varieties	12

The only data we have that can be used to document the impact of the andigena parents on the variety selection program is to look at the percentage of clones which survive after different stages of selection. The crosses made in 1981 and selected as single hills in 1983 present the best comparisons in terms of being crosses to common parents (Table 2). The data are the consequence of the selection system and so are not from replicated or randomized trials. The two fields should be considered separately as they are almost 300 miles apart on different soil types. At Willsboro, the bulk pollen from adg males selected for late blight (LB) resistance produced about half as many selections as NY66, a tuberosum clone used by us frequently because it has proven to

TABLE 2

1981 CROSSES SELECTED ONE GENERATION

WILLSBORO FIELD

FEMALES		NY66 (TUB)	ADG LB	ADG CHIP	Q155-3 (TUB)
5 TUB CLONES	ORIG. NO.	3085	2055		
	% SELECTED	22	12		
4 TUB x ADG CLONES	ORIG. NO.	9256	1760		
	% SELECTED	19	9		

MT. PLEASANT FIELDS

		NY66 (TUB)	ADG CHIP	Q155-3 (TUB)
3 TUB CLONES	ORIG. NO.	5613	9176	5351
	% SELECTED	12	7	14
5 ADG CLONES	ORIG. NO.	2076		
	% SELECTED	/		

be a good parent. The adg (LB) clones tend to produce late maturing offspring. The cross between NY66 and the adg (LB) bulk produced as many selections (24%) as the tub x NY66 progenies. A less biased comparison is that of the Tub x Adg hybrids compared to the tub clones crossed to the two males. The 9 clones are on a par with each other as far as clonal selection is concerned. It appears the tub x adg hybrids were only slightly less effective in producing first generation selections. At Mt. Pleasant, the adg clones also produced about half as many selections as the tuberosum clones. However, in the 5 adg chosen as females, there was more extreme variation than among the 3 tub used in comparison. Three of the 5 adg averaged 2% selected and the other 2 averaged 15%. One of these was the clone A231-1. The 3 tub selection percentages were 11, 11, and 15. Given comparable selection pressure in selecting for horticultural type in the adg or tub x adg hybrids, there is no selection penalty in using them as parents and they contribute other desirable traits such as disease resistance.

In 1980, we crossed 11 tuberosum, 4 andigena, and 4 hybrid clones to the same tuberosum clone, NY66. The number of seedling hills was not large, being 3307, 1604, and 855, respectively for the 3 types of crosses. After two stages of selection, all phenotypic based on single plots each year, the tuberosum and hybrid parents yielded 11% selections and the andigena yielded 6% (Table 3).

Table 3. 1980 crosses with NY66 (Tuberosum) which survived 2 generations of single plot selection.

Female	No. of Progenies	% Clones Selected
Tuberosum	11	11
Tuberosum x Andigena	4	11
Andigena	4	6

The 1979 data are not usable because a flood lost most of that generation as seedling hills. The 1978 crosses are useful because that year we made many tub x adg hybrids. Also, those progenies have now been subjected to 4 years of selection, the last being a yield trial at two locations (Table 4). The percentage of selected clones after two stages of selection was similar for the three types of crosses. In the third stage, again an unreplicated plot with 24 hills, the tuberosum by tuberosum crosses began to increase in relative percentage selected. In the fourth stage, a replicated yield trial, the tub x tub crosses performed considerably better than either the tub x adg hybrids or the backcrosses to tub. The factor that discriminated most against the hybrids was the smaller size distribution of the crop. I think that further selection of the adg population for larger tuber size will correct this deficiency.

TABLE 4

1978 CROSSES AFTER FOUR GENERATIONS OF SELECTION.

	TUB x TUB	(TUB x ADG)TUB	TUB x ADG
NO. OF ORIGINAL HILLS	2489	1743	35,555
MEAN % AFTER SINGLE HILL SELECTION	7.8%	12.1%	13.2%
MEAN % AFTER 4 CUT TUBER UNIT SELECTION	1.1%	1.4%	1.4%
MEAN % SURVIVING AFTER THIRD STAGE SELECTION	.32%	.17%	.27%
% SURVIVING AFTER 1ST YEAR YIELD TRIALS	.12%	0	.02%

Earlier I described our experience of increasing the frequency of the gene for PVY resistance in the andigena population. In that case, by giving the gene a high priority in selection, through mass selection we are able to bring the gene frequency to near the limit in about 10 years. We have had two related experiences in the pedigree type variety development program. The first is with the H_1 gene from andigena for resistance to the golden potato cyst nematode. The second is with the andigena gene for PVY resistance. Nematode resistance became a serious component of the breeding program in 1950 and a primary component about 1960. It has taken an additional 20 years to achieve the objective of having the majority of our parents used in crossing to be at least simplex for the H_1 gene. Some of our objectives still require the incorporation of susceptible clones as parents, so the percentage of resistant offspring is not yet at 75%. Thus, even though the caracteristic has been emphasis equal to that given the PVY gene in the andigena population, the need to consider other attributes has reduced the rate of change in gene frequency.

In the decade of the 1970's, PVY was our most serious virus problem in the variety program. This motivated us to build this gene into our crossing program comparable to the use of the H_1 gene. Progress is slow. The F_1 hybrids between the andigena and tuberosum tend to be lost in the final stages of variety selection and clones at this stage of selection are most desirable for use as parents. The resistance is of limited value to farmers in choosing a variety, so little accommodation can be made in other marketable attributes. I predict that it will take another 20 years to bring this gene to the same frequency as the H_1 gene now is in the program even though the donor parent was much better adapted. Changes in gene frequency of specific genes are much more rapid in a population improvement program than in a clonal development program.

Summary

I believe that population improvement methods have been under utilized in potato breeding, but that they should not be used in exclusion to traditional pedigree methods. Population methods are ideally suited to developing broadly based pools of germplasm which can be used as sources of breeding parents. When the need arises for progenies with attributes from these populations, specific clones can be selected for hybrid combination with the best adapted clones. Where a broader range of variability is desired, bulk pollen from the population can serve as the parent. Where speed in reaching adaptation is of prime importance, an early generation can be by crossing to representatives of the adapted clones. Where the original accessions are so unadapted that their progenies suffer marginal survival, the same decision might be in order. Where the initial level of adaptation is not limiting the

survival of progenies and a greater assurance of germplasm preservation is desired, then the introgression of the adapted source can be delayed. Where the population is to serve diverse regions of adaptation, then it may be better to maintain its isolation. The efficiency, the flexibility, and the capability of preserving a broad range of heterogeneity are the prime attributes of population improvement methods.

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Use of CIP's Germplasm in National Program: The Brazilian Program

Fermín De la Puente C.

Introduction

The potato is the most important vegetable crop in Brazil. In general this crop can be characterized as follows:

- About 200,000 ha are grown every year;
- Total production is approximately 2,000,000 ton/year;
- Average yield of 10 ton/ha is indicated;
- Growing and production areas are more concentrated in the South (Paraná, Santa Catarina and Rio Grande do Sul) and Southeast (Minas Gerais and Sao Paulo);
- Almost the entire all crop is treated in Sao Paulo, Rio de Janeiro and Minas Gerais;
- Mainly yellow skinned, cream or yellow-fleshed cultivars are being grown, except in Rio Grande do Sul were red-skinned potato is accepted;
  Highly dependent on foreing cultivars and imported basic seed from Netherlands (Bintje and Radosa), Germany (Achat) and Sweden (Bintje and Delta);
- Boiled and fried potatoes are mainly consumed;
- Non-producing areas are also consuming this food, such as the northern region (Amazonas, Pará, etc);

Main problems can be indicated as follows:

- Diseases: brown rot, late blight, early blight, dry rot, soft rot, PLRV and rhizoctonia;
- High production cost;
- Low soil fertility often accompanied by aluminum toxicity;
- Unfavorable climate: high temperature, frost (in the South), irregular rain distribution;
- Sophisticated marketing.

Potato Breeding in Brazil

Potato breeding was initiated in 1947 at the Instituto Agronômico de Campinas-IAC (Dr. Olavo José Boock) and Instituto de Pesquisa Agropecuária do Sul-IPEAS (Drs. Delorge Motta da Costa and Mozart Teixeira Liberal). In 1971, the potato program of the Instituto Agronômico de Minas Gerais (Dr. Aquira Mizubuti and Francisco Severo Guglielmelli Filho) was re-activated.

At the present time, the following breeding programs are in activity:

- CNPH (Brasília, D.F.);
- EMCAPA (Espírito Santo);
- EMGOPA (Goiás);
- EPAMIG (Minas Gerais);
- IAC (São Paulo);
- CNPFCT (R. Grande do Sul)

The Potato Breeding Program at the National Vegetable Research Center-EMBRAPA

This program was formally initiated in 1980. Its main objetive attempted to select new potato cultivars adapted to Brazilian conditions.

Three different strategies were considered. The objective, methodology and goal for each one are described bellow:

Short-term (4-5 years)

Objective: Selection of new cultivars for non-traditional areas.

Methodology: Selection of new cultivars from advanced genetic material from CIP and other Institutions.

Goal: To avoid the concentration of potato production in only a few traditional areas.

Medium-term (6-8 years)

Objective: Selection of new cultivars with some specific characteristics.

Methodology: Selection from specific advanced genetic material.

Goal: To attack specific biotic and/or abiotic limiting factors of potato production.

Long-term (more than 8 years)

Objective: Selection of new cultivars for traditional areas.

Methodology: Selection of new cultivars from advanced introduced genetic material and from our breeding program.

Goal: To avoid the extremely high dependency of this area on foreign cultivars.

Human Resources and Facilities

To achieve the main objectives of this program it was necessary to develop the basic human resources and facilities as indicated in the following table.

Table 1. Human Resources and Facilities, CNPH-Potato Breeding Program

	1979	1981	1983
Human Resources			
- Researchers	1	3	8
- Technicians	1	1	1
- Field laborers	3	5	12
Facilities			
- Screen & green houses	1 ($200m^2$)	1 ($200m^2$)	5 ($720m^2$)
- Cold storage	-	1 ($200m^3$)	1 ($200m^3$)
- Tissue culture lab	-	1	1
- Phytosanitary labs	-	5	5
- Computers	-	-	1
- Sprouting house	-	1	1

Genetic Resources

Two different kinds of germplasm for selection was considered in our program. First, the introduced germplasm (clones and segregating families) and secondly the material from our own crosses. The introduced germplasm for selection was mainly from the International Potato Center (CIP, Lima-Peru), Wisconsin University (USA), Cornell University (USA), Agriculture Canada Research Station - Fredericton (Canada) and Templeton Plant Breeders (New Zealand). The type of introduced germplasm of Table 2 concerns mainly the development of our program at CNPH regarding its Human Resources, facilities and experience.

Table 2. Development of type of germplasm introduced at CNPH - Potato Program for Selection

1980	Clones and tuber families.
1981/82	Segregating families and specific clones.
1983	Secregating families and parental materials.

The main characteristics of these materials were: earliness, wide adaptation, resistance or tolerance to biotic or abiotic factors. The advanced populations introduced into our program for selection are reported in Table 3.

Table 3. Genetic populations introduced under selection.

Introduction dates	Clonal families (n°)	Genotypes (n°)
01-05/80	100	1,564
04-07/80	81	2,046
05-09/80	42	4,563
02-06/81	24	2,059
09-01/81/82	59	7,862
12-04/82/83	249	10,275
TOTAL	555	28,369

The selection of these populations was mainly for type of plant and tuber, apparent yield, earliness and reaction to bacterial wilt (Pseudomonas solanacearum), early blight (Alternaria solani), leaf roll virus (PLRV), leaf miner (Liriomyza huidobrensis), tolerance to hot temperature and aluminum toxicity.

Results

The percentage and number of genotypes selected initially from these genetic segregating populations are indicated in Table 4. About 12.4% (3,510 of the clones) were selected in this primary attempt. Some of these clones listed (Table 5) yielded significantly higher than the controls and they also showed a desirable type and vigor of the plants. Thanks to the high heterozygous and heterogeneous populations tested, some outstanding genetic combinations were identified from which a large number of clones were selected. Among these combinations are: Kufri Jyoti x M206, Raritan x DTO-28, Atzimba x CCC-107 71C127 x Alemaria, 71A102 x Sirtema, R 293.33 x Katahdin, Delta x Pamir, Tondra x Hydra, and Cosina x Hydra. Some parents included in these introduced populations were found with a high frequency in the pedigree of the selected clones. The most important ones are: Kufri Jyoti, Sirtema, Atzimba, Alemaria, Raritan, Atlantic, Platte, Hudson, Superior, Utlantan, W639, W231, W5295.7, W533.7, NY-61, NY-59, Hydra, Baronesa, Tondra, Desirée, Pamir, etc. Also, some genetic combinations and clones were identified from this material with a high degree of resistance or tolerance to potato leaf roll virus, early blight, leaf minor, hot temperature and aluminum toxicity. References about these are reported in tables 6, 7, 8, 9, 10, 11 and 12. Also some progenies from hybridization and open pollinated seed showed a hight phenotypic uniformity (tubers and plants) for the TPS approach. These progenies are indicated in Table 13.

In a brief summary the results which were obtained following the three indicated strategies can be pointed out as follow:

Strategy-I (short-term)

- 20 selected clones are now in the stage of seed cleaning and rapid multiplication for release.

Strategy-II (medium-term)

- 10 clones with specific characters have been selected and sent to CIP to be used as parents for future breeding work.

- Some clones have been identified with resistance or tolerance to PLRV. Early blight, leaf minor and hot temperature. They also show a good agronomic characteristics.

Strategy-III (long-term)

- Some genetic combinations and parents have been identified, which will be used for future breeding work.

- Experiences on the utilization of TPS, 2n-gametes, neo-tuberosum and wild species are under way.

Conclusions

The following conclusions can be pointed out from our experience with the utilization of CIP's germplasm.

Two approaches could be followed for better utilization of CIP genetic material:

a. As source of new cultivars, and/or

b. As parental material for breeding purposes.

The two approaches are highly dependent on:

a. Strategies of the national programs, and

b. The proper interaction among national and CIP scientists.

TABLE 4. Results of the preliminary selection from the introduced genetic populations

TESTING DATES	Nº OF GENOTYPES EVALUATED	Nº AND PERCENT OF GENOTYPES SELECTED
01.05/80	1,564	340(21.7)
04.07/80	2,046	341(16.7)
05.09/80	4,563	518(10.0)
02.06/81	2,059	208(11.5)
09.01/81/82	7,862	903(11.5)
12.05/82/83	10,275	1200(11.6)
	28,369	3510(12.4)

Table 5. Main characteristics of some advanced clones.

CLONES CNPH Nº	PEDIGREE	PLANT TYPE	YIELD t/ha*
103209	Lt 18.41 x Phy bulk	Very good (VG)	45.5
103609	ATZIMBA x INDIA 1058 B	VG	39.5
103309	LT-1 x NEOTUB	VG	35.7
119609	ATZIMBA x NEOTUB	GOOD (G)	35.0
028509	71A 102 x SIRTEMA	G	32.9
097409	LT 18.15 x Phy bulk	VG	32.2
118709	B 5052.9 x RARITAN	VG	31.9
DESIRÉE	CONTROL	G	29.1
BINTJE	CONTROL	G	26.3

* Average of 4 trials

TABLE 6. Genetic combinations from which high percent of clones with resistance to PLRV were obtained.

CIP	- SERRANA	x M. TROPICAL
	371240.2	x CFK 69-1
	573272	x 14XY-4
	SERRANA	x XY 15-4
	WEIMA	x XY-9
	DTO-2	x 14XY-4
BRAZIL	- JACY	x 656.70
	JACY	x 652.64
	ARACY	x 652.64
	SOUTH ESK	x MPI 62.47
	ARACY	x ABNAKI
	ABAETE	x 656.70

Table 7. Best selected clones for yield and PLRV resistance.

CLONE CNPH Nº	PEDIGREE	PLANT VIGOR	YIELD t/ha*
100544	SERRANA x 14XY	VERY GOOD (VG)	59.2
25-2	JACY x 652.64	VG	52.8
31-1	JACY x 656.70	VG	50.4
19-1	ARACY x 644.98	GOOD (G)	44.8
310466	SERRANA x 14XY	VG	40.6
310551	PG 281 x XY 18.4	G	38.2
77-1	DELTA x MPI-49540	REGULAR (R)	37.5
31-2	JACY x 656.70	VG	34.3
310402	B 71240.2 x 14XY-4	G	34.0
19-[illegible]	ARACY x 644.98	VG	33.6
62-1	ARACY x IAC 5752	G	31.2
ACHAT	CONTROL	R	9.5
BINTJE	CONTROL	R	8.2

* Yield after 4 cicles of field infection

Table 8. Genetic combinations which showed high tolerance to L. blight.

MAINE 40	x	DTO-28
MAINE 40	x	PVX BULK
6.5 (PVX)	x	MAINE 40
Y 22.3	x	XY BULK
XY 13.8	x	M TROPICAL
377212.15	x	PVY 23.14
XY 2.9	x	37 7999.1
SERRANA	x	ATZIMBA

Table 9. Best selected clones for yield and early blight resistance.

CLONE Nº CNPH	PEDIGREE	PLANT VIGOR	PRODUCTIVITY
016008	67.104/16 x VDT 6895	R/G	G
019808	Kufri Jyoti x M206	G	G
025308	72/India-1035 x R 268.1	VG	VG
026608	Atzimba x CCC-107	R/G	G
030508	67.104/6 x VDT 6895	R/G	G
038808	Raritan x DTO-28	G	R/G
063209	Tondra x Hydra	R	R/G
080909	LT 10.32 x Bulk Pseudo.	VG	VG
085409	LT 18.41 x Bulk Pseuod.	G	G
118709	B 5052.7 x Raritan	R/G	VG
142609	Coyota x 2.37	G	G
144509	Yungay x 1.5	R	G
610002	F 74.123	VG	G
610003	F 74.123	G	G
610047	F 74.123	R	G

NOTE: R = Regular G = Good VG = Very good

Table 10. Selected clones with resistance to leaf miner (*Liriomyza huidobrensis*)

CLONE CNPH Nº	PEDIGREE	PLANT VIGOR
015908	BL-2-9 x 378200	G
019008	72/INDIA 1035 x R 268.1	VG
076409	UTLANTAN x PVY BULK	VG
118909	WRF 1919.2 x LOPEZ-6	G
101409	ATZIMBA x NEOUTB	G
131609	NY-59 x ADJ (PVX)	R
117309	WRF 1919.2 x PHY BULK	R
104409	LT-1 x CCC-107	G

Table 11. Selected clones with high tolerance to hot temperature.

CLONE CNPH N°	PEDIGREE	PLANTA VIGOR	YIELD t/ha*
085409	LT 18.41 x Pseudo Bulk	VERY GOOD (VG)	13.4
111809	LT 22.6 x Pseudo Bulk	VG	10.4
103609	ATZIMBA x INDIA 1058 B	GOOD (G)	8.4
079109	ATZIMBA x INDIA 1059 B	G	7.6
131109	NY 59 x AD 6 (PVY)	G	7.4
ULLA	CONTROL	G	6.0
ARACY	CONTROL	G	3.5
BINTJE	CONTROL	REGULAR	0.9

* Yield obtained summer - 1983 (Rio de Janeiro)

Table 12. Genetic combinations, cultivars and wild species with tolerance to Aluminum toxicity.

GENETIC COMBINATIONS	- KUFRI JYOTI	x	M206
	LT 2449	x	BULK PSEUDO
	1923.1	x	INDIA 952
	TUBEROSUM	x	BF-2
CULTIVARS	- BARONESA (BRAZIL)		
	BINTJE (HOLLAND)		
	ELVIRA (HOLLAND)		
WILD SPECIES	- *S. orophilum* (OCH-12076)		
	S. bukasovii (OCH-13858)		
	S. ambosinum (OCH-11299)		

Table 13. Best genetic progenies for the TPS approach.

ATZIMBA	x	DTO-28
BARAKA O.P.		
71.0088 (TONDRA x HYDRA) O.P.		
MINEIRA O.P.*		
ATLANTIC	x	DTO-33
XY 13.8	x	M. TROPICAL
W 760	x	W 5295.17
W 884 O.P.		
MERRIMACK	x	DTO-17
W 774	x	W 5295.17
W 785	x	W 5295.17
E-59 O.P.		

* Open pollinated Progeny.

Microspores, Protoplasts, and Somatic Hybridization as Tools in Potato Breeding

G. Wenzel, B. Foroughi-Wehr, F. Kohler, and R. Schuchmann

Introduction

The last status report on the incorporation of in vitro techniques into potato breeding was concluded by the sentence: It has to be stated that not a single clone, which has been produced via an analytical synthetic breeding scheme - incorporating partheno- and androgenesis together with protoplast fusion - exists (Wenzel et al. 1980). At the same time we were optimistic that we could construct such a clone within the next years. Although we do have now the next years, such a clone still does not exist. As strongest obstacle has turned out the selection step after protoplast fusion. The other steps work more or less satisfactorily; this is in particular the parthenogenetic reduction from 4x to 2x and the androgenetic step from 2x to homozygous doubled monohaploids. The period of the last three years between the two program conferences is marked by quiet trials to broaden the applicability of in vitro techniques and to open them to the field. At the last conference Hermsen forecasted that another part of plant biotechnology, genetic engineering (see Fig. 2), would become extremely attractive. This proved true and not only under the scientific but increasingly also under the financial aspect. So people start to reckon tissue culture approaches already to the classical part of plant breeding and leave it to the practical breeders to adopt these techniques according to their direct needs. In Table 1 e.g. data are summarized for a program of dihaploid production finanzed by the private breeders. During the last season they started to select the fertility amongst these nearly 6000 primary dihaploids and to produce interdihaploids. Similar approaches are now in progress with cereals, especially with barley, where breeders in Canada produced already varieties via parthenogenetic haploids and in Germany programs are fixed for androgenetic haploid production.

In the following paragraphes we will mention the last development of the use of microspores, cells, and protoplasts in our institute's program, and further we will review somatic potato fusion work.

Table 1. Summary of three years of dihaploid extraction from tetraploid clones of private potato breeders in Germany.

Breeder	Starting clones	Fertilizations	Seed balls	Seeds without seed spot	2x plants	Tuber bearing plants
I	38	7 976	4 377	8 206	2 637	1 969
II	22	2 661	1 543	2 909	907	709
III	39	7 326	3 503	7 619	2 265	1 758
IV	34	4 218	2 093	5 520	1 775	1 415
	133	22 181	11 516	24 254	7 584	5 851

1. Microspore Culture

The concept of a stepwise reduction of the ploidy level with the advantage of a simpler inheritance is generally accepted for qualitatively inherited characters. Along this line we concentrate on clones carrying the extreme resistance to PVX, which has been extracted from *Solanum acaule* (Ross 1954), and on the extreme resistance to PVY extracted from *S. stoloniferum*. Besides those, we use for these two viruses as well as for PLRV field resistances. They have been extracted by the late Dr. Baerecke from *S. tuberosum* clones, preferably the clones 44.1016/10; 49.540/2 and 55.957/54.

Testing the virus resistance after ploidy level reduction, we found both: clones showing extreme resistance, reacting hypersensitive, and clones with field resistance only, being susceptible after grafting on infected tomato stocks. We also found alterations of the resistance type: either primarily field resistant clones became extreme resistant or extreme resistant ones showed only field resistance. Besides these still resistant clones, completely susceptible clones segregated as expected.

Since we have data now on the maintainance of such resistances over a five years period, we can state that it is probable that field resistance to PVY and PLRV is expressed and maintained throughout the process of ploidy level reduction and doubling. From 84 PVY and PLRV field resistant clones 26 are after growth under high virus pressure in the field since three years healthy.

This means that both qualitatively as well as quantitatively inherited resistances and probably other characters are maintained during the haploidization process. As they have passed the haploid level, a quantitative resistance has become homozygous and can be transferred, at least to the next generation like a qualitative trait. For PVX or PVY this might not be too crucial but for PLRV, where no qualitative resistance is available, this should be important.

A second point, which turned out to be rather useful in the work with haploids, is the fact that it is possible to gain more information about the starting clones. Especially under the aspect of parent selection for crosses, the haploids uncover characters which are not visible in the parental material. Further, it should be stressed that via a haploid step genomes are purified from sublethal allels, which either segregate to wild type or to lethal. Consequently, it is not too surprising that doubled haploids show rather good vigour despite their inbreeding nature.

2. Somaclonal Variation

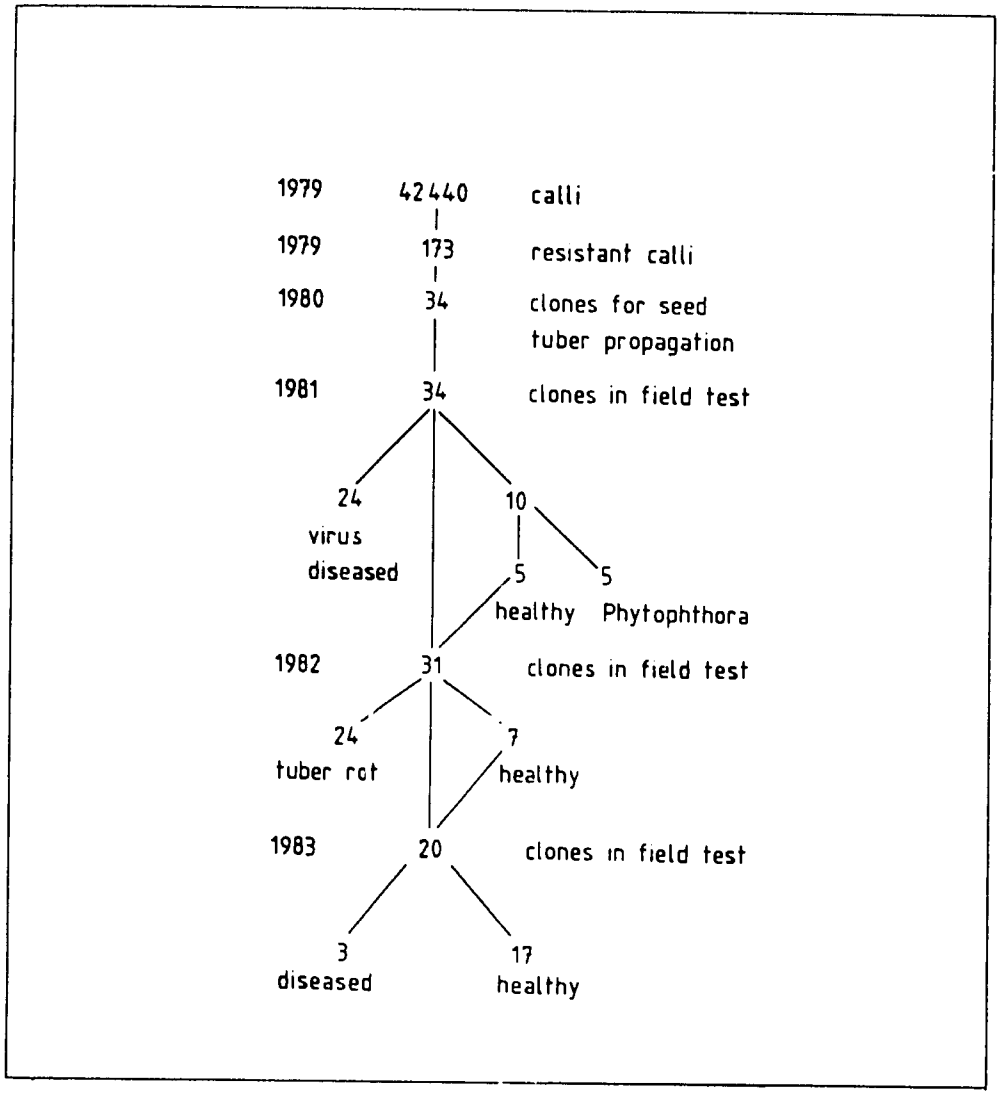
For the last chromosome doubling we proposed protoplast fusion. Although this has not yet been achieved, we would like to spent a few words on a phenomenon of new variability in tissue culture and protoplast derived material, respectively. It is now under discussion how far this variability can be successfully used in potato breeding. Shepard (1982) found such a tremendous variability in clones from 'Russet Burbank' that he recommended the use of such protoclones for intracultivar improvement. We regenerated more than 3000 clones from dihaploid protoplasts and found also some striking aberrants, but most of these turned out to be aneuploid without any practical use. Having discarded these, the rest was uniform and revealed no phenotypic differences, or other variability e.g. in the total protein pattern after electrofocussing. On the other hand aneuploids are rather common and also stable in potato. In a cross e.g. of 3x x 2x they can be produced in large numbers and some are even vigorous (Stabel 1982). It is generally accepted now that the amount of variability is dependent on the genotype. Probably we find in tissue culture a comparable situation to genotypes being rather plastic to environmental stresses: clones with a high amount of plasticity react plastic against in vitro stress and show consequently a high degree of variability while in other clones variants don't survive.

One advantage of somaclonal variation in comparison to mutation breeding is the chance to apply a selective pressure directly during the in vitro phase. Using such an approach a most direct way for screening to resistance is opened up for those pathogens, which attack their host by toxins. In potato this is true e.g. for Phytophthora infestans and several Fusarium species. These pathogens can also be grown on defined liquid media, in which the toxin is excreted. The toxin can be separated from the fungal growth medium and added as selective pressure to the potato cultures, shoots, seeds, calli, cells, or protoplasts. Behnke (1979) started such experiments, using 5 different dihaploid potato clones. Callus pieces of 3 mm size have been plated on potato growth media containing three times the concentration of the toxin. In recent experiments defined toxin fractions can be used, obtained from Stolle and Schöber (publication in prep.). The action of the toxin can easily be measured by the increase of fresh weight of shoot cultures. The killing rate used in the experiments was about 95% of plated calli. Surviving calli were transferred several times on toxin containing and toxin free media before regeneration was induced. On the plants regenerated from resistant or tolerant calli the diameter of lesions after mechanical spore inoculation was significantly reduced. The number of sporangia formed did not differ, which means that only the infection efficiency was reduced and not the fungal growth rate. The numbers of this experimental series are given in Fig. 1. As the starting material was selected only for a high regeneration capacity, the majority of the regenerants was lost in the field because of other diseases. In order to get some idea of what might have happened during in vitro selection, we tested protein patterns of selected resistant and susceptible clones (Wenzel et al. 1984). It was not possible to correlate one specific alteration to a specific behaviour during selection.

A similar selection system is now developed for isolated microspores, combining the advantage of high individuum numbers with haploidy. This does not work for potato at the moment, where the development of the isolated microspores stops at the microcallus stage. Therefore, we would like to mention some data from our barley isolated microspore system. As in potato, also in barley some microspores start to develop and stop at the 8-12 cell stage. In barley we could now condition the culture medium by an undefined compound produced from the anther wall but also from ovules. In such conditioned media the development continues and plants can be regenerated. We are trying to identify this conditioning factor, and as we know already that it is not

specific only for spring barley, but also helps in winter barley, experiments are in progress to determine if it is beneficial in other cereals or even in potato. With this factor the regeneration of isolated microspores of barley works to such an extent, that we could start to select for toxin resistance. We are using fusaric acid and could select already androgenetic calli which grow on 0.2mM fusaric acid, a concentration which is toxic for unselected material.

Fig. 1. Selection for Phytophthora infestans resistant potato clones starting from dihaploid calli.



At the moment we are trying in a parallel set of experiments to select in protoplast suspensions of dihaploid potato clones for resistance against Fusarium oxysporum, F. sulphureum and S. coeruleum and Phytophthora.

3. Work on Protoplasts

The technical details on protoplast isolation and fusion have been reported already (Wenzel et al. 1980). The most critical step in potato protoplast work is the somatic hybridization. And here the selection heterocaryotic hybrids turned out extremely difficult, particularly between S. tuberosum and S. tuberosum without clearcut markers. In Table 3 the general selection procedures for somatic hybrids are summarized. For potato (x) potato hybrids we started with method f, (Table 2), which uses hybrid vigour as the selection criterium.

Table 2. Experiments using hybrid vigour as selection procedure for somatic hybrid isolation.

Wild type	(x)	Wild type	Heterosis	Ref.
Solanum tuberosum	S.	tuberosum	callus	Wenzel et al. 1980
Datura innoxia	D.	quercifolia	shoots	Schieder et al. 1982
Brassica oleracea	B.	campestris	plants	Schenck et al. & Röbbelen et al. 1982

Table 3. Summary of techniques for somatic hybrid identification (modified from Schieder 1982).

I. Complementation			
a.	auxotroph *Ab*	(x) auxotroph *aB*	wild type *AaBb*
b.	albino *Ab*	(x) albino *aB*	wild type *AaBb*
c.	resistant R_1r_2	(x) resistant r_1R_2	double resistant $R_1r_1R_2r_2$
d.	phenotype *A*	(x) phenotype *B*	phenotype *C*
e.	no regeneration	(x) no regener.	regeneration
f.	haploid	(x) haploid	vigorous diploid
g.	diploid	(x) diploid	heterotic amphidiploid
II. Mechanical procedure			
a.	single culture of hybrid protoplasts		
b.	nurse culture		

The clones used in such early experiments were selected according to their regeneration capacity. Unfortunately amongst 3000 clones regenerated from the most vigorous calli none turned out to be a true somatic hybrid in the further tests. Probably the few clones used, did not complement in such a favorable way that they expressed real hybrid vigour. This may be also due to the failure of the fusion itself. Now we are extending the spectrum of clones, which can be regenerated from protoplasts, and hope that some of them will express good combining ability after fusion visible by hybrid vigour.

But as long as this does not work, we have to look for other possibilities to identify hybrids. The most elegant way of combining two different resistances, e.g. two exotoxins, cannot be used in potato, as such clones are not regenerable as in Nicotiana sylvestris (White and Vasil 1979) or in Daucus carota (Harms et al. 1981). Butenko and Kuchko (1980) had, like others (Table 4), the good luck that fusion products grew on a medium the parents would not grow on. For S. tuberosum (x) S. tuberosum this cannot work. Therefore, we concentrated on the use of albinos either as mutants (Prezewosny et al. 1980) or on a reversable bleaching procedure using the herbizide SAN 6706 (Uhrig 1981). In both methods the protoplasts from the two parents are visually distinguishable and the fusion bodies can be microscopically

identified. The fusion bodies may either be marked in a semisolid medium or they are fished by a micropipette. For tobacco it turned out beneficial, to grow such isolated hybrid cells in the presence of a nurse culture (Hein et al. 1983). Experiments to transfer this system to S. tuberosum (x) S. tuberosum fusions are in progress at the Max-Planck-Institute in Cologne.

Table 4. Somatic hybrid selection by the use of specific media for parental and hybrid protoplasts (Schieder 1982).

wild type	(x)	wild type	wild type on selective medium	Ref.
Nicotiana glauca		*N. langsdorffii*	plants	Chupeau et al. 1978
Petunia hybrida		*P. parodii*	plants	Power et al. 1977
Solanum tuberosum		*S. chacoense*	plants	Butenko & Kuchko 1980

If one is not so much interested in such narrow hybridizations, the main advantage of which is the combination of e.g. quantitative characters without any meiotic mixing and chromosome doubling at the same time, then one can use selection procedures, which couple e.g. albinism of parents with morphological characters of a wild type parent (Table 5).

The most prominant example is the somatic hybrid between potato and tomato of Melchers et al. (1978). In the following paragraph the present situation of these experiments shall be reviewed.

Somatic hybrids between potato and tomato exist from three experimental series at Tübingen. In all experiments protoplasts of potato were isolated from dihaploid clones with cross fertility and with selffertile 24 chromosomed tomatoes. From the first series all regenerated hybrids, even one with 48 chromosomes, are completely sterile (Melchers 1983).

Table 5. Successful examples for the identification of somatic hybrids by fusion albino mutant parents with wild type parents (modified from Schieder 1982).

albino	(x) wild type	= green	Reference
Daucus carota	*D. capillifolius*	plants	Dudits et al. 1977
Lycopersicon escul.	*Solanum tuberosum*	plants	Melchers et al. 1978
Datura innoxia	*Atropa belladonna*	shoots	Krumbiegel & Schieder 1979
Nicotiana tabacum	*N. rustica*	plants	Nagao 1978
Nicotiana tabacum	*N. glutinosa*	plants	Nagao 1979
Nicotiana tabacum	*N. alata*	plants	Nagao 1979
Nicotiana sylvestris	*N. tabacum*	plants	Evans et al. 1983
Nicotiana otophora	*N. tabacum*	plants	Evans et al. 1983

Some of the hybrid lines give the impression that they are mixed chimeras not between potato and tomato but rather between hybrids with mixed chromosome numbers. Some other hybrids had chromosome numbers around 72 probably 4 chromosome sets from potato and 2 from tomato, like the ones Shepard described in 1983. These plants never showed normal growth in soil (Melchers 1983). From a later series of experiments with a clone as potato parent, which also was very successful in anther culture (H_3-703), new hybrids exist, which have again chromosome numbers around 48, and some even 48 (Melchers pers. communication). The first plants of these are now growing in soil. It is not clear yet, if they will give fertile amphidiploid or amphiploid hybrids. According to the results obtained up till now by Melchers and Shepard there is only little hope to achieve such plants.

So at the moment, the potato (x) tomato hybrids have succeeded besides their scientific importance only some credit in a side line. Investigations of Smillie et al. (1979) demonstrated that some hybrids have cold resistance intermediate between resistant potato and sensitive tomato.

The very varying chromosome number of the pomatoes and the topatoes point in one direction, which also should be focussed, as it opens up a new possibility in somatic hybridization. Probably quite often during culture of somatic hybrids chromosomes are lost. Table 6 documents some of such cases where after protoplast

fusion, from one parent only specific characters were present in the hybrid. Probably, due to chromosome elimination, other characters were lost. Such chromosome elimination can be studied when the chromosomes of the parent are rather different in size. In the case of potato and tomato it is nearly impossible but here it happened that from a hybrid with its odd shape, suddenly a rather normal shoot developed, probably due to chromosome loss of the tomato parent (Melchers and Wenzel unpublished).

The phenomenon of chromosome elimination in intergeneric fusion products seems to be useful for the transfer of some genetic information from one plant to another. Dudits et al. (1979) fused protoplasts of a nuclear coded albino mutant of Daucus with green wild type protoplasts of Aegopodium, which had never undergone divisions. Their selected green plantlets possessed the chromosomes of Daucus, but several chemical studies proved that also genetic information of Aegopodium was expressed.

Table 6. Summary of experiments, where after somatic hybridization only parts of the genome of one parent were expressed (modified from Schieder 1982).

Species fused	plants	Reference
Daucus carota (x) Aegopodium podagraria	+	Dudits et al. 1979
Daucus carota (x) Petroselinum hortense	+	Dudits et al. 1980
Arabidopsis thaliana (x) Brassica campestris	+	Gleba & Hoffmann 1979
Vicia faba (x) Petunia hybrida	-	Binding & Nehls 1978
Datura innoxia (x) Atropa belladonna	+	Schieder & Krumbiegel 1981
Solanum tuberosum (x) Nicotiana tabacum	+	Skarzhynskaya et al. 1983
Solanum sucrense (x) Nicotiana tabacum	+	Skarzhynskaya et al. 1983
Atropa belladonna (x) Nicotiana tabacum	+	Gleba et al. 1983
Solanum nigrum (x) S. tuberosum	+	Binding et al. 1982
Lycopersicon esculentum (x) Solanum tuberosum	+	Melchers & Wenzel (unpubl.)

Besides the transfer of a few, or even single genes, by somatic fusion between species and genera, today genetic engineering is opening up a new approach towards a programmed transfer of genetic

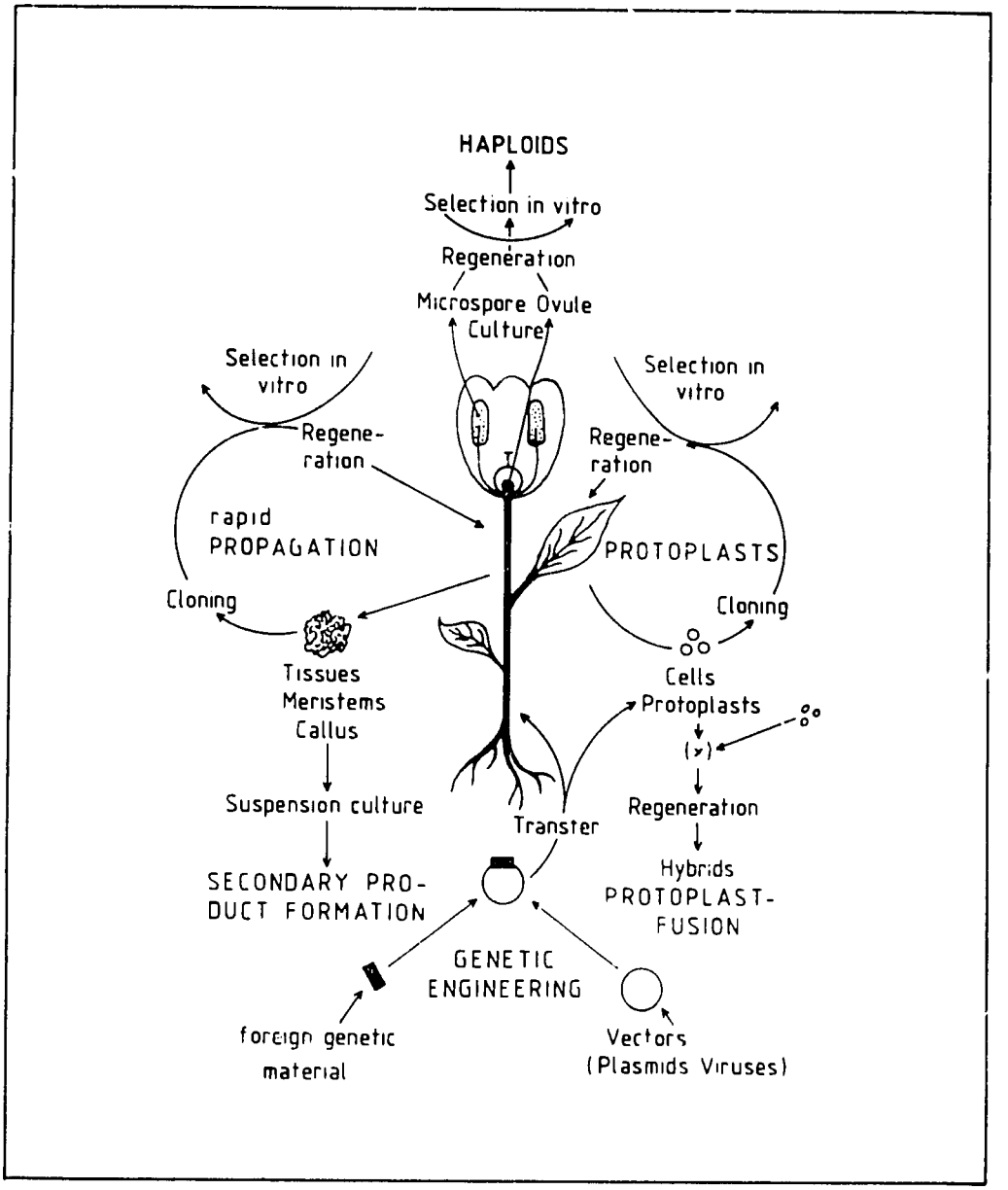
information. Petunia and tobacco express already bacterial genes and for potato the first promotors are isolated which specifically can switch on gene activity either in tubers, stems or leaves (Willmitzer pers. communication). I would like to stress, however, that most of the genetic engineering techniques demand cell culture steps, making continuation especially of regeneration necessary.

Conclusions

We shall try to answer a final question: Has potato been improved by biotechnology? What we understand under biotechnology are all the techniques summarized in Fig. 2. We think that we can answer with Yes. Especially during the last three years the reproducibility of in vitro techniques has been increased to such an extent that practical breeders make use of tissue culture techniques. We don't have to discuss about the use of rapid propagation or meristem culture, it's already a classical breeding step. Also the use of dihaploids is generally accepted today. But the more spectacular techniques, like direct selection for disease resistance in vitro, or the new somatic amphidiploid potato variety, are still missing. The influence of the genotype on the regeneration capacity is here so strong that today these techniques are not widely applicable. We are following the line of incorporating the character tissue culture or regeneration capacity into our clones, as we found it more economic to broaden the plasticity, than to optimize a medium. This strategy is meanwhile generally accepted and from experiments with barley, we know that the mode of inheritance is not too complicated (Foroughi-Weir et al. 1982).

We think there is not so much a danger in wasting money by further exploring cell culture applications in potato, but by switching over too rapidly to genetic engineering. Tissue culture techniques are, according to the equipment needed, rather cheap, and therefore the potentiality opened up in an institute like the CIP is enourmous. Whether it is necessary to do in the CIP the next step towards genetic engineering is, however, doubtful.

Figure 2. Summary of biotechnological procedures applicable to potato.



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A Perspective View of the Potato Variety Improvement Programme of India

N.M. Nayar

India grows potato in about 7, 50, 000 ha and produces about 10 million tonnes annually. In area India ranks fourth in the world after the USSR, Poland and China and in yield sixth after the USSR, Poland, USA, China and East Germany. The per hectare production is about 13 tonnes (against 14 tonnes world average). The entire quantity is used for human consumption. In India, potato is used mostly as a vegetable.

Potato was introduced into India in about 1615 about 40 years after its introduction into Europe, but its cultivation seems to have picked up only after another 150 years. In India, the area under potato accounts for about 0.5% of the total cropped area only. If we look at the trends in area and production over the last three decades, we can see that the area under potatoes increased about three-folds during this period, while the production increased about six times. In terms of increase in productivity, potato ranks second among all the cultivated crops in India after wheat, with rice coming third. This can be considered quite significant, because unlike as for wheat, rice and other food crops, potato has not received any special incentives from the State by way of minimum support price, etc.

Potato is grown in India in almost all the States and under very diverse conditions. It is possible to see a standing crop of potato round the year in one part of the country or the other. At the same time, two-thirds of the potato is grown in the autumn and winter months of October-November to March-April in the vast Indo-Gangetic plains, and about one-fifth of the area in the Himalayan areas during the summer and rainy seasons of March-April to September-October.

Potato research was begun in India in 1935 at Kufri, Simla hills under the Indian Agricultural Research Institute, New Delhi. It was put on a permanent footing in 1949 with the establishment of the Central Potato Research Institute. The first cultivars were released by the Institute in 1958. So far, 24 improved cultivars suitable for all the climatic conditions have been released.

Research on the potato in India is carried out at the Central Potato

Research Institute and under the All India Coordinated Potato Improvement Project (AICPIP) also located in the CPRI. In the Institute, the potato variety improvement work is carried out in eight of its research stations located all over India. The All India Coordinated Potato Improvement Project (AICPIP) is a cooperative programme between the Indian Council of Agricultural Research and the State agricultural universities. Under this, potato work is under way in 16 universities. Each CPRI station is manned by a multidisciplinary team of 5-20 scientists and each university centre by 2-6 scientists.

For purposes of breeding work, India can be identified into four major zones and three special areas. These are: 1) the western north Indian plains; 2) the central north Indian plains; 3) the eastern north Indian plains; and 4) the north Indian hills.

The special areas are the north Bengal hill region covering Darjeeling district, where wart disease is a problem, the south Indian tropical hills in the Nilgiris where the cyst nematode is a problem, and peninsular India where potato cultivation is becoming increasingly popular, and it is grown under relatively warmer conditions.

The characteristics and breeding objectives of these zones are discussed below:

1. The western north Indian plains. This region is spread over Punjab, Haryana, Rajasthan and the neighbouring areas and covers about 10% of the area under potato cultivation in India. It is possible to grow here two crops of short duration (90-110 days) from September-October to January (autumn crop) and January to March-April (winter crop). However, most of the crop is grown as autumn crop. On a rough estimate, more than half of the crop produced here is sold as seed for planting in peninsular and eastern India and the remaining quantity is used for table purposes. The variety requirements of this zone are short-period of maturity, a degree of resistance to frost, photoinsensitivity and tolerance to warmer conditions. The latter two characteristics are important as the produce is used as seed in peninsular India.

   One of the newer priority objectives is increased tolerance to heat. In this region, potato is presently planted in early October when maximum temperatures are 30-32°C, and the objective is to be able to plant the crop about 15-20 days earlier than this, when the prevailing maximum temperatures are about 33-35°C. Earliness is another desired character, in that if the crop could be harvested at 65-75 days, the farmer would benefit in two ways: (i) his

produce would fetch about 40% more price than if he harvests and sells the produce at the normal time; (ii) he would get enough time to prepare the land properly and raise a normal crop of wheat. In this region, late blight appears only once in about three years, and then also, in only mild form. However, at present a higher level of resistance to late blight is desired as more than half of the produce goes as seed to other parts of the country where late blight can be a problem. Resistance to the virus diseases X, LR and Y are necessary and important.

2. The central north Indian plains. This zone consists of western UP and MP. It covers about 25% of the total area. This is the area of maximum yield potential, since a crop of about 110-120 days duration can be raised here. Frost is unlikely to be a problem and so is late blight. Viruses X, LR and Y are important.

3. The eastern north Indian plains. The region consists of eastern UP, Bihar plains, Bengal plains and Orissa. It covers about 30% of the total area. The growing period is about 100 days, late blight appears more regularly, delayed harvesting may cause charcoal rot. The farmers have a decided preference for red-skinned and smaller sized tubers. Resistance to the virus X, LR and Y are as important as in the other regions.

4. The north Indian hills. This region consists of about 15% of the total area. As in Europe, potato is grown here during the long days of spring and summer. The growing period is about 135-145 days, late blight is serious, soil is acidic, and in the central Himalayan region (western UP hills) - which consists of 10% of the total area - brown rot is an added problem.

The western Himalayan hills, which includes HP, has been the traditional seed producing and supplying region for the entire country until about 20 years ago, until the Institute showed the feasibility of raising healthy seed stocks in the north Indian plains under aphid-free or low aphid conditions.

At present, both the HP hills and the north-western plains together constitute the main seed producing region for peninsular and eastern India. Of late, the central north Indian plains have also begun to produce seeds, but the use of this seed is now largely confined to within that region only. For this reason, the cultivars grown in the western Himalayan region under long day conditions and those grown in western north Indian plains under short day conditions need to possess in addition to the attributes

given above, another important attribute, a wide range of adaptability and photo-insensitivity.

In eastern India, potato is grown in about 75,000 ha. Here, the temperatures are mild -- ranging from near-zero to 29°C -- and the rainfall is heavy -- 2000-3500 mm are common, and it may go up to 4500 mm --. Potato can be grown in two seasons, March to July and September-December. Here, late blight and brown rot are both serious problems. In fact, it has been suggested that the complex of LB races that occur in eastern India may be even more numerous and the intensity of its occurrence more acute than that available in Toluca Valley in Mexico. In the early years of the Indian breeding programme, the CPRI used also to send its material to Toluca Valley for screening purposes.

In north Bengal, in the area adjoining Nepal and close to Bhutan, is Darjeeling district where wart disease was observed 30 years ago. This area lies in the eastern Himalayas, and receives heavy rainfall -- more than 3000 mm annually. There are two potato growing seasons, January to August in the higher hills and September to December in lower altitudes. In this region, the temperatures are mild, but the minimum will go below freezing point and late blight is serious. In addition, wart and brown rot are also important problems. It appears that fortunately, both the intensity of infection and extent of occurrence of wart are not increasing. Cultivars possessing wart immunity and good resistance to late blight, are available. In addition, several lines possessing red tubers, another desired attribute for this region, are in advanced generation trials.

The peninsular India including Gujarat, Maharashtra and Karnataka accounts for about 7% of the area under potatoes in India. Potato is grown in two seasons here, from July to September and October to February. This area offers good scope for extending the area under cultivation, and potato cultivation has been increasing steadily in this region in the last two decades. Here, the average prevailing temperatures are higher than those in the north Indian plains. Brown rot is the most important plant protection problem. This region is now totally dependent on the western north Indian plains and west Himalayan hills for its seed requirements. The Institute has now initiated a study to determine the feasibility of raising seeds within the region during the aphid free or low aphid period. If and when this becomes possible, we then shall have to develop cultivars specifically adapted to this region. They will have to be more temperature tolerant and possessing resistance to bacterial wilt in addition.

Contiguous with this area and towards its south are the Nilgiri hills. In Tamil Nadu, potato is grown in about 13,000 ha and three-fifths of this area are in the Nilgiri hills. These are tropical hills rising up to 2200 m about MSL, the temperatures remain mild -- zero to 29°C -- and annual rainfall is over 1400 mm. Here potato is grown round the year, but generally in three main crops. Late blight is a serious problems in addition to the cyst nematodes. Both Globodera rostochiensis and G. pallida occur here. Brown rot is also prevalent in some areas. Cultivars resistant to late blight have been released for this region already, and recently one line (SON-110) possessing combined resistance to cyst nematodes and late blight has been identified and is under adaptability yield trials now.

In the foregoing account, a general account of the potato growing zones of India, and the characteristics and breeding objectives of each of them have been given. A general account of the overall breeding objectives of the Indian potato breeding programme is now given below:

1. Yield remains and will continue to be the overriding consideration. In fact, the attainment of high yield will take into account really the realization of many other objectives such as resistance to biotic and abiotic factors, earliness and so on. The present day improved cultivars are able to give 30-35 tonnes/ha in 100-110 days by adopting the package of practices developed in the institute. We visualize that we shall be able to raise this to 60-70 tonnes/ha within the next 15-20 years.

2. Insensitivity to varying photoperiods is another desired attribute. In India, potato is grown under a variety of situations - the long day conditions of north Indian hills, short days of the north Indian plains, the lengthening days of again the north Indian plains when potatoes are grown from January to March-April, and the near day-neutral conditions of south Indian hills and peninsular India.

3. Ability to tuberize under high thermoperiods will be one of the highest priority programmes. Already, most Indian improved cultivars possess a fair to good degree of heat tolerance. This is now sought to be improved further, with a view to extending potato cultivation in peninsular and south-eastern India.

4. Another high priority objective will be to have in varieties good keeping quality and resistance to degeneration. After potato is harvested during December to March, the summer sets in, when the maximum day temperatures go up to 42-44°C. The farmers would normally prefer to keep their produce in their own premises in improvised country stores rather than in cold stores for a variety of reasons - particularly, those meant for sale. Storage under cold conditions results in the accumulation of reducing sugars, which reduces its consumer acceptability. The attributes of a variety having good keeping quality will then consist of resistance to degenerative losses, resistance to soft and dry rots and a lowered rate of reducing sugar accumulation. The relationships between these attributes and dormancy, resistance to viral diseases, etc. are currently under study.

5. An improved responsiveness to fertilizer application is another objective. The concept of an ideal plant type and breeding for better responsiveness to applied fertilizers have become accepted ways for cereal breeders since well over two decades - and this is what has paved the way for the green revolution in cereals. An analysis of incremental responses to major nutrients carried out by us recently showed that the present day cultivars are slightly better in this regard than the early improved cultivars, through concious selection had not been done for this. We are now currently trying to understand the components of an ideal plant type in the potato and the factors that contribute towards increased fertilizer responsiveness. This will include also an attempt to see if some economy could be achieved in the requirements of P and K particularly, since both these major nutrients are nonrenewable resources.

6. Resistance to diseases. Certain present-day Indian cultivars possess good degrees of resistance to late blight. Several of the advanced generation hybrids possess even better levels of resistance that the present-day cultivars. With these and the fungicidal sprays, we feel that we shall be able to contain the disease for the time being.

Brown rot has lately been elevated as the N°1 disease problem. Our past efforts in understanding the nature of the disease and breeding for resistance to it have not been particularly successful. We are now reorganizing the entire programme on this disease.

Resistance to degenerative diseases, as we have already noted, will receive more attention in future. In our preoccupation with the late blight, yield and heat tolerance, this aspect could not probably get adequate attention so far. It has now been decided to develop programmes for immunity to PVX and PVY and resistance to PLRV.

I have already mentioned about our breeding programmes against wart and cyst nematodes. We propose to intensify our work on cyst nematodes in all their aspects. Root-knot nematodes have been recorded from the potato in India since about 25 years. Currently, we have initiated a study to assess its importance in potato production. Until this is completed, no breeding work is contemplated.

Breeding for quality has been one of the projects of the Institute since a long time. Presently, some lines possessing up to 25% dry matter are under advanced generation trials. In future, this will not be a separate programme, but all the advanced generation hybrids will be assessed for certain quality parameters as dry matter content and dormancy in the 5th-7th vegetative generations. It is, however, realized that in future, the potato will find increasing use in food and processing industry in India, and then cultivars having high dry matter content and lower rates of carbohydrate conversion will get a preference.

There are in addition some special breeding programmes like the True Potato Seed (TPS), rapid multiplication, tissue culture, haploidy breeding, etc. Their potentialities are recognized and the research projects covering these programmes have been streamlined and put in proper perspective. In tissue culture, no immediate fall-out is expected -- other than research publications! A few lines developed through haploidy breeding are in advanced generation trials. In the TPS, our present emphasis is on working out the cultural or agronomic requirements of the seedlings, particularly for raising them successfully under relatively high temperatures. The potential usefulness of this in improving potato cultivation in certain parts of the country is recognized. In this context, it is interesting to record that the first Director of CPRI, Dr. Ramanujam had as early as in the early fifties, visualized the potentialities of TPS, and accounts of his studies have been published in the annual reports of the Institute for those years. Unfortunately, for various reasons, including possibly professional jealously, the work was not then continued.

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UTILIZATION OF GENETIC RESOURCES IV

A G E N D A

Monday, December 12

Chairman, morning session
O. T. Page, Director of Research

08:30	Welcome and Opening Remarks. R. L. Sawyer Director General
08:50	Brief Review of Recommendations presented in "Utilization of Genetic Resources of the Potato III", November 1980. O. T. Page
09:05	Selection of parental stocks for heat tolerance and earliness. H. A. Mendoza
09:30	Breeding for combined resistance to potato leafroll virus (PLRV) and potato virus Y (PVY). C. Brown
10:00	Coffee
10:30	Breeding bacterial wilt (*Pseudomonas solanacearum*) resistant germplasm. P. Schmiediche
11:00	Development of a diploid population with multiple resistance to diseases and pests. M. Iwanaga

11:30 Discussion

12:30 Lunch

Monday afternoon

Chairman, afternoon session
K. J. Brown
Director, Regional Research & Training

13:30 Effects of inbreeding on true potato seed (TPS) progenies.
G. Atlin

14:00 Selection of uniform progenies to use TPS in commercial potato production.
H. Mendoza

14:30 Apomictic approach to improve uniformity in TPS progenies.
J. Hermsen

15:00 Coffee

15:30 Discussion

Tuesday, December 13

Chairman, morning session
J. Valle-Riestra

08:30 Utilization of wide crosses in potato breeding.
J. Hermsen

09:00 Use of mutation in plant breeding.
S. J. Peloquin

09:30 Population breeding applied to improvement of primitive *Solanum* cultivated species.
R. L. Plaisted

10:00	Coffee
10:30	Use of CIP's germplasm in national program: the Brazilian program. F. de la Puente
11:00	Discussion
12:30	Lunch

Tuesday afternoon

Chairman, morning session
P. Malagamba

13:30	Somaclonal variation as a tool for plant improvement. L. Schilde
14:00	Protoplast fusion as a breeding tool. G. Wenzel
14:30	Discussion
15:00	Coffee
15:30	Development of preliminary recommendations.

Wednesday, December 14

Moderator, morning session
H. Mendoza

09:00	Discussion and establishment of final recommendations.