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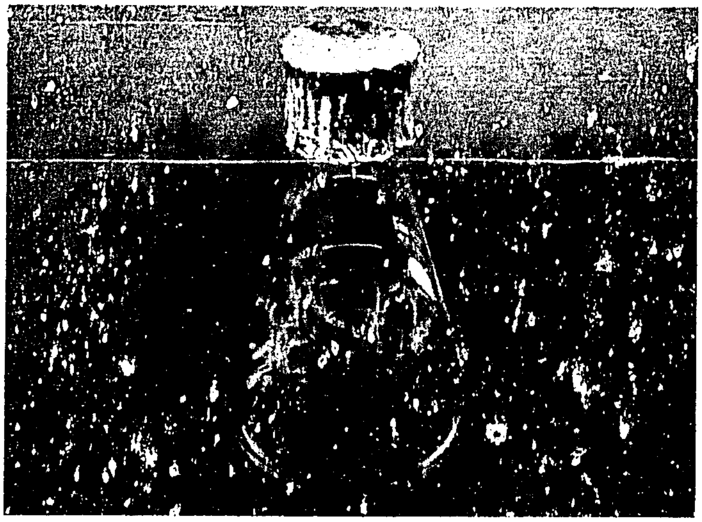

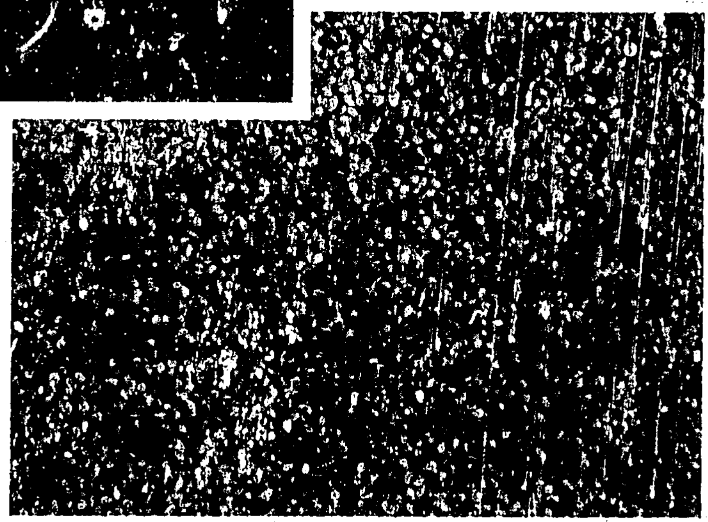

INNOVATIVE METHODS for PROPAGATING POTATOES

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Kneeling, left to right: Il Gin Mok, M. Upadhya, J. Bryan, J. Fernández, N. Van Uyen, A. Haverkort.

COVER PHOTOS:

Innovative propagation of potatoes by asexual (clonal) and sexual (seed) methods. In the flask, plantlets from nodal cuttings growing in vitro. At right, potato seeds (TPS).

INNOVATIVE METHODS for PROPAGATING POTATOES

REPORT OF THE XXVIII PLANNING CONFERENCE

December 10-14, 1984
LIMA, PERU

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FOREWORD

This is the 28th Planning Conference sponsored by the International Potato Center to develop guidelines for CIP research. The Report concerns research on true potato seed (TPS), tissue culture and rapid multiplication techniques. Previously, a Planning Conference on TPS was held in the Philippines, in 1970. Including this 28th Planning Conference, 283 participants from 42 countries have contributed to CIP's research program by presenting papers, discussing research strategy and formulating recommendations for future CIP research.

During 1984, 34 countries were conducting research on TPS at the experiment station level, 10 were involved in on-farm research while TPS was being used by farmers in Sri Lanka, China, Rwanda, Samoa and the Philippines. This is very good progress since CIP first reported on TPS research in 1978. Various practical rapid multiplication techniques have also been developed to aid national potato seed programs. Tissue culture techniques are playing an ever increasing role in a range of propagative activities. The gradual merging of tissue culture and other biotechnologies is providing background research for further improvements in potato production for developing countries.

O. T. Page

December 1984

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Opening Remarks-Planning Conference on Innovative Methods for Propagating Potatoes

R.L. Sawyer

Thanks to all of the invited participants for taking the time to help us here. Thanks to those board members who have stayed a second week to review what are the highest priority activities presently at CIP. The title Innovative Methods for Propagating Potatoes includes several major projects at CIP.

Seed production programs are a major bottle neck in use of the potato as a food in tropical countries or even in developing temperate countries. All of the topics for discussions this week are aimed at eliminating this bottle neck.

Is true seed going to take off now as has diffused light technology? How many countries will be using true seed as a standard practice in 1988? How do we get production of true seed in place? Who is going to be the producer? And what should CIP be doing to stimulate the private sector in true seed production? I hope questions such as these will be answered during this conference.

The number of possibilities for rapid multiplication of potato planting material has increased tremendously in recent years and is still increasing. How do we sort these various techniques out so that recommendations can be adjusted to fit specific conditions in national programs, and the levels of development encountered? Countries only recently starting to multiply some of their own planting material will probably need some direction and should not just be given an array of possibilities from which to choose.

The content of the agenda makes these discussions as important as any taking place last week during the Internal Review. In fact, we can consider this a continuation of the Internal Review.

RECOMMENDATIONS

The planning conference acknowledges the progress in TPS, tissue culture and rapid propagation. The conference, therefore, makes the following recommendations:

I Propagation with true potato seed

1. CIP and collaborating institutions will continue to produce and distribute to the regions TPS progenies with improved characteristics.

2. CIP should intensify its efforts in developing 4x and 2x parents with diverse sources of resistance to bacterial wilt, late blight and other important factors. The TPS progenies derived from parental materials should be tested in specific sites where the TPS has a high potential for farmer use.

3. The best locally adapted genotypes proving to be good parents should be utilized as parental materials for producing TPS progenies. These genotypes should produce large quantities of seed of high quality.

4. Superior TPS progenies identified in one region should be made available for testing in other regions.

5. Any TPS material for international distribution by CIP must continue to be tested for PSTV by the best available method.

6. Research efforts should continue on efficient methods for low cost production of TPS.

7. To ensure adequate seed supply CIP must be involved in stimulating the proper role of public and private sector in the production and distribution of TPS.

8. Evaluation of progenies and agronomic practices for alternative TPS utilization systems such as direct seeding, transplanting, seedling tubers and other methods should be emphasized.

9. Agronomic and pathological studies at CIP should emphasize those aspects related to seedling stand and survival.

10. In view of the accumulated progress on all aspects of TPS, the committee strongly suggests that this information not only go to refereed journals, but more important be made available through technical bulletins and manuals.

II. Integration of tissue culture and rapid propagation

1. The conference recommends further preparation of training materials to guide and facilitate integration of low cost tissue culture propagation with other rapid multiplication techniques, and further, that regional scientists include, where relevant, training on *in vitro* propagation in low cost facilities.

2. Through its regional network, CIP should assist where possible in supplying specific reagents for culture. CIP should investigate the feasibility of the production of ready-made complete propagation media.

3. Research should continue to investigate further simplification of methods for tissue culture and rapid propagation.

4. It is recommended, where applicable, tissue culture and rapid multiplication techniques be economically evaluated.

III. Stability and variation in culture and propagation

1. A more detailed analysis of the effect on genetic stability of the various conservation and propagation methods for potato germplasm should be made using the existing methods of electrophoresis and morphological measurements. CIP should seek contractual assistance in examining innovative methods for studying genetic stability/-variability.

2. Cryopreservation as a method of germplasm storage, is at present inadequate. However, further studies on the practicality and genetic stability of cryopreservation are encouraged.

3. CIP should carefully monitor the developing technologies of somaclonal variation, genetic manipulation and engineering. This could be accomplished in part by contractual research in promising areas. CIP should adopt relevant technologies when practical applications become apparent.

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 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 1 in a Neo-tuberosum population determined the genetic variability for 16 traits as well as the phenotypic and genotypic correlations amongst them. The results are presented in Tables 1 and 2, respectively.

These results indicated that there is no additive genetic variance for yield in the population studied. However, the estimates of additive variance for the component traits for yield, tuber number and size were relatively high. Therefore, selection utilizing additive genetic variance for increased tuber number and size should increase yield. Due to the high negative correlation between these traits, selection for an increase in one should decrease the other. Selection for increased tuber

Table 1. Additive genetic variance components and heritabilities for several traits in potatoes propagated from true seed (after Thompson, Mendoza and Plaisted, 1983).*

Trait	σ^2_A	$\sigma^2_{A \cdot E}$	$h^2_{(fs)}$
Perc. seed germination	104.1948 ±68.6792	1.4936 ±9.4504	0.53
Transplant survival	1.4224 ± 1.0864	0.8186 ±1.3386	0.30
Nursery vigor	0.5712 ± 0.2058	0.1996 ±0.1730	0.57
Nursery uniformity	0.2136 ± 0.1454	0.2418 ±0.1696	0.33
Vigor 60 days	0.4150 ± 0.2120	0.2248 ±0.2250	0.42
Uniformity 60 days	0.1216 ± 0.1362	0 2004 ±0.1784	0.00
Berry No.	0.1396 ± 0.0454	0.1234 ±0.0334	0.61
Days to maturity	19.0142 ± 6.7860	3.0136 ±6.8972	0.55
Tuber No.	0.0128 ± 0.0044	0.0106 ±0.0034	0.58
Tuber Size	0.0128 ± 0.0030	0.0006 ±0.0014	0.79
Yield	- 0.0048 ± 0.0076	0.0216 ±0.0116	0.00
Uniformity tuber color	0.7512 ± 0.8624	0.2784 ±1.0692	0.00
Uniformity tuber size	0.0964 ± 0.0724	0.0898 ±0.0736	0.08
Uniformity tuber shape	0.0055 ± 0.0970	0.0818 ±0.0668	0.00
Depth of eye	0.3568 ± 0.1280	0.1918 ±0.1022	0.56

* Heritabilities not included when σ^2_A estimate was smaller than its standard error.

Table 2. Genotypic and phentoypic correlations for several traits in potatoes propagated from TPS (after Thompson, Mendoza, and Plaisted, 1983).*

	Tran. surv.	Berry No.	Days to Mat.	Tuber No.	Tuber size	Yield+	Unif. size	Univ. shape+	Eye Depth
Transplant survival		.52	.04	.53	-.14		-.46		.41
Berry No.	.25		.53	-.19	.36		.72		.08
Days to maturity	-.02	.40		-.21	.63		.86		.38
Tuber. No.	.52	.01	-.04		-.94		-.36		-.82
Tuber size	-.06	.36	.46	-.62			.38		.68
Yield	.82	.40	.43	.56	.29				
Uniformity tuber size	-.09	.13	.19	-.03	.20	.19			-.24
Uniformity tuber shape	.06	.18	.13	-.002	.19	.20	.50		
Eye depth	-.09	.10	.31	-.38	.58	.16	-.26	-.09	

* Genotypic correlations above diagonal, phenotypic correlations below diagonal

+ Negative estimate of σ_A^2 = no genetic correlations size would be more desirable because of the positive correlations between tuber size and the quality related traits. In addition, the higher estimate of h^2 and lower estimate of $\sigma^2_{A.E}$ for tuber size indicate that the response to selection should be more rapid than for tuber number. Consequently, selection should be practiced for increased tuber size with sufficient weight being given to tuber number to maximize yield, since high yield results from the interaction of the two components.

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 clones 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 3 presents components of additive genetic variance, additive x environment interaction and narrow sense heritability.

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^2_A 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^2_A was found for uniformity of tuber size which means that variability must be introduced before improvement can be made by procedures requiring σ^2_A. The estimates of σ^2_A 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^2_A, being above 0.52 for all except uniformity of tuber shape and general appearance which were 0.37 and 0.39, respectively. The $\sigma^2_{A.E}$ 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.

Table 3. Additive 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 $\pm$0.0728	0.1068 $\pm$0.0256	0.63
Tuber number	0.0336 $\pm$0.0096	-0.0012 $\pm$0.0052	0.64
Tuber size	0.0104 $\pm$0.0032	0.0016 $\pm$0.0020	0.61
Yield	0.0208 $\pm$0.0064	0.0068 $\pm$0.0032	0.61
Tuber smoothness	0.5240 $\pm$0.1336	0.0092 $\pm$0.0504	0.71
Uniformity tuber color	2.1869 $\pm$0.5846	0.6913 $\pm$1.3816	0.65
Uniformity tuber size	-0.0421 $\pm$0.1107	0.0928 $\pm$0.0892	-0.06
Uniformity tuber shape	0.2419 $\pm$0.1038	0.1100 $\pm$0.0922	0.37
General appearance	0.3776 $\pm$0.1876	0.4240 $\pm$0.1736	0.39

Genetic and phenotypic correlations for the traits studied in this experiment were computed and are presented in Table 4.

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 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	"

Table 4. Genotypic and phenotypic correlations for several traits in potatoes propagated from true seed[1] (after Thompson and Mendoza, 1983).

	Tuber No.	Tuber size	Yield	Unif. color	Unif. size	Unif. shape
Tuber number		-0.24	0.74	-0.10		0.36
Tuber size	-0.13		0.45	-0.07		-0.23
Yield	0.75	0.73		-0.05		0.13
Unif.tbr.color	-0.10	-0.14	-0.09			0.64
Unif.tbr.size	0.57	0.26	0.48	0.37		
Unif.tbr.shape	0.22	-0.33	0.16	0.63	0.78	

Table 5. Mean values of traits measured in different family types propagated from TPS[1].

Perc. Germination

Tp. Fam.	Mean
Multiline	92.2 a
Cross	88.5 b
OP	87.7 b
Self	85.2 c
Bulk Cross	81.4 d

Transplant Survival

Tp. Fam.	Mean
Multiline	40.7 a
Cross	40.4 a
Bulk cross	39.8 b
Self	39.6 b
OP	39.5 b

No. Plants Harvested

Tp. Fam.	Mean
Multiline	28.9 a
Bulk cross	28.4 a
Cross	28.2 a
OP	23.4 b
Self	20.4 c

Yield (Log)

Tp. Fam.	Mean
Bulk cross	.7873 a
Multiline	.7678 a
Cross	.7516 a
OP	.5386 b
Self	.4545 c

[1]Mean separation by Duncan's .05

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 5 and 6. For the four traits presented in Table 5, it appears to be a clear cut advantage favoring the hybrids over the partially inbred progenies (selfed and open pollinated). In Table 6, 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 3). 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.

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.

The last part of the paper deals with the identification of progenitors which produce hybrid progenies with high yield, tuber uniformity, and earliness. Also, experimental work to determine the optimum experimental plot size and optimum number of replications to evaluate TPS population, is presented. Several experiments were carried out either utilizing genetic mating designs or just comparing the performance of a large number of progenies. In 1982, a N.C. Design I experiment was carried out at San Ramon including 16 males mated each to a random sample of six females. Table 7 indicates the average performance of some male clones.

Table 7, 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

Table 6. 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

Table 7. Average performance of some male clones from a Design I experiment carried out at San Ramón (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

a maximum benefit from this clone one should mate it to female clones which are early maturing and have a smooth shape. The 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 8. 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 significant 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.

Research was conducted to determine the optimum experiment plot size and adequate number of replications for evaluation of potato seedling populations. A population of seedlings grown from open pollinated seed of the clone DTO-33 was used. Uniformity trials were established in a San Ramon field. Plot sizes of 1, 5, 15, 30, 90, and 180 units were compared. Each unit had 4 seedlings. Statistical analysis was carried out as a split plot design. A purely statistical comparison of the

Table 8. 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*
R-268.1	-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

various plot size variances indicated that the optimum plot size is between 5 and 15 units, i.e. plots of 20 to 60 plants. Since loss of seedlings are frequent in this type of evaluations, it was considered that 40 seedlings per plot would be the adequate size. Also, it was found that the adequate number of replications was four.

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Utilization of Ploidy Manipulations in Breeding for TPS

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There are three main types of ploidy manipulations in breeding for TPS (16): 1) 4x progeny from 4x x 2x crosses, 2) 4x progeny from 2x x 4x crosses, and 3) 4x progeny from 2x x 2x crosses. The 4x parents are cultivars or advanced selections from breeding programs and the 2x parents are haploid-species hybrids that produce 2n pollen or 2n eggs. This paper will treat the essential characteristics, problems, and possible solutions associated with using the three types of ploidy manipulations.

The successful use of all three types of ploidy manipulations is dependent on two vital features, 2n gametes and Endosperm Balance Number.

<u>2N GAMETES</u>. (Gametes with the sporophytic, and thus, unreduced chromosome number). Three modes of 2n pollen formation have been described: second division restitution, SDR; first division restitution with crossing-over, FDR-CO (5, 13); first division restitution without crossing-over, FDR-NCO (14). Genetically, FDR-CO is superior to SDR in transmitting heterozygosity and epistasis to the progeny (11). Breeding results confirmed this superiority in that 4x progeny from 4x x 2x FDR-CO significantly outyielded the 4x progeny from comparable 4x x 2x SDR crosses (12). Preliminary evidence indicates that 4x progeny from 4x x 2x FDR-NCO are more uniform than progeny from 4x x 2x FDR-CO (Marc Masson, personal communication). They may also have higher tuber yields, but this needs to be evaluated with a large number of comparable 2x hybrid clones possessing either the FDR-CO or FDR-NCO modes of 2n pollen formation.

Our knowledge of the modes of formation and inheritance of 2n egg formation is more limited (3, 18). Both cytological and genetic evidence suggest that the most common mode of 2n egg formation is SDR (3, 18, 19). Other results indicate that 2n eggs can be formed by FDR-CO and FDR-NCO (4), but that the frequency of 2n eggs formed by these modes is too low for use in TPS production.

<u>ENDOSPERM BALANCE NUMBER</u>. Normal endosperm development is dependent on a 2:1 balance between the EBN from the female and male parents, respectively (6). When n gametes function in 4x (4 EBN) x 2x (2 EBN) crosses, the female to male EBN ratio will be 4:1, and following 2x x 4x crosses 1:1. These aberrant ratios usually lead to very abnormal

development of the endosperm, such that germinable seeds with triploid embryos are recovered at a very low frequency--the "triploid block." However, if 2n pollen functions following 4x x 2x crosses or 2n eggs after 2x x 4x crosses, the normal EBN ratio is established in the endosperm and normal seed development follows. The important point is that the progeny will be either almost exclusively or all 4x depending on the mode of 2n pollen or egg formation in the 2x parents. A similar situation in relation to the "triploid block" occurs following 2x x 2x crosses. The progeny will be almost entirely 2x and 4x if both n and 2n gametes function in the two, 2x parents. But if one of the 2x parents produces either only functional 2n pollen or 2n eggs, the progeny can be 100% 4x.

4x x 2x CROSSES. The characteristics of the 4x and 2x parents and the 4x hybrids are indicated in Figure 1. The 4x clone should be unrelated to the haploid parent of the 2x hybrid to avoid inbreeding. It should be adapted to the area the 4x progeny will be grown and have the tuber type appropriate for that area. Profuse flowering and good female fertility are necessary to obtain large numbers of fruit/plant and seed/fruit. A single plant should be able to produce 10,000 to 50,000 seeds/plant in order to produce TPS economically. Male sterility would also be desirable, so that if hand pollinations are used, emasculation would not be necessary. Further, if one can convince the bumblebees to take pollen from a highly male fertile 2x parent to a male sterile 4x parent, seed production costs for obtaining the 4x hybrids would be very low. The 2x male parent should have the proper maturity and tuber type, and flower profusely. It must produce a high percentage of 2n pollen by FDR-CO or all 2n pollen by FDR-NCO. It should also possess other desired traits that are needed in the 4x hybrid progeny. The concept involved here is to do the breeding of the 2x parents with disomic genetics rather than tetrasomic genetics. One could, for example, incorporate Y immunity into the 2x parent, and if the 2x parent formed 2n pollen by FDR-NCO, it would transmit this trait to all the 4x progeny (15). The 4x hybrids from 4x x 2x crosses possess seedling vigor, plant vigor, good uniformity, and large tuber yields. In our yield trials with TPS over the past several years, the hybrids from 4x x 2x crosses have had the best seedling vigor, uniformity and tuber yields when compared to TPS from other sources (8, 9, 17).

The major problem with the 4x x 2x approach is the cost of producing seed by hand pollination. The possible solutions to this problem include; doing the hand pollinations where labor is very inexpensive, finding out what attracts bumblebees to male fertile flowers and make use of this knowledge to trick the bees into regular visits of male sterile flowers, using tetrad sterility (1) in which normal exine is formed and the resulting pollen apparently attracts bees, but male sterility seems to result from a lack of separation of tetrads of microspores during pollen development, and particular genetic-cytoplasmic male sterilities in which the pollen is not functional but possesses normal exine.

4x Clone	x	2x Haploid-species hybrid
Unrelated to haploid		Proper maturity
Adaptation		Tuber type
Tuber type		Profuse flowering
Profuse flowering		High % 2n pollen, (ps)*-or only 2n pollen (sy3,ps)**
Male sterile		Other desired traits
Good female fertility		

4x Hybrids	
Seedling vigor	Plant vigor
Good uniformity	Large tuber yields

* ps-2n pollen by first division restitution with crossing-over.

**sy3, ps-2n pollen by first division restitution without crossing-over.

Figure 1. 4x progeny from 4x x 2x crosses.

A major asset of the 4x x 2x FDR-NCO approach is the high level of vegetative and tuber uniformity in many of the 4x progenies. This asset leads us to suggest that TPS could be used to produce certified seed tubers. It would appear to be an advantageous approach from both the disease and economic aspects. Biologically, the minimal uniformity needed would be for plant type, tuber shape, flesh color, skin color and specific gravity. This uniformity is available in particular families from 4x x 2x FDR-NCO crosses.

2x x 4x CROSSES. The major features needed in the parents to obtain 4x progeny from 2x x 4x crosses are listed in Figure 2. The 4x parent should have the proper adaptation and tuber type, and be unrelated to the haploid of the 2x hybrid. Profuse flowering and high male fertility are required to attract bees and provide bees with abundant pollen to use in pollinating the 2x clones. Round fruit in contrast to, for example, oval fruit on the 2x parent would be necessary to identify the fruit from the 2x parent; the 2x parent would be interplanted with the 4x parent to provide the bees the opportunity to make the 2x x 4x crosses.

2x Haploid-species hybrid	x	4x Clone
Maturity and tuber type		Adaptation
Self incompatible		Unrelated to haploid
Male fertile-no 2n pollen		Tuber type
High 2n egg frequency*		Profuse flowering
Profuse flowering-oval fruit		High male fertility
Attractive to bumblebees		Round fruit
Other desired traits		

4x Hybrids
Inexpensive seed production without emasculation and hand pollination

* 2n eggs by first or second division restitution

Figure 2. 4x progeny from 2x x 4x crosses.

The 2x parent would be selected for maturity, tuber type, profuse flowering, oval fruit, and other desired traits. Most important it must be highly male fertile (no 2n pollen), self incompatible, and produce a high frequency of 2n eggs; male fertile to attract the bees, self incompatible to prevent obtaining 2x progeny from selfing, and a large number of 2n eggs in order to obtain 30-100 seeds/fruit following bee transfer of pollen from the 4x clone.

The major advantage of this method is the inexpensive seed production accomplished without emasculation and hand pollination. We were able to obtain about 25 seeds/fruit from a 2x clone when we alternated it within the row with a 4x parent. However, two problems are evident; one, 25 seeds/fruit is not adequate, we need to identify 2x hybrids which produce 50 to 100 seeds/fruit in 2x x 4x crosses, and two, the 4x progeny from our bee mediated 2x x 4x crosses were not uniform and high yielding. This is not surprising, since cytological examination revealed that the 2n eggs were produced by SDR (18). Previous results with 4x x 2x SDR crosses were disappointing in yield and uniformity (12), so there was no reason to suspect that 2x SDR x 4x would be highly desirable. What is needed are 2x hybrid clones that produce high frequencies of 2n eggs by either FDR-CO, FDR-NCO, or apospory.

2x x 2x CROSSES. The requirements of 2x parents in order to produce 4x progeny from 2x x 2x are identified in Figure 3. Both 2x parents should be selected for maturity, tuber type, profuse flowering, other desired characteristics and attractiveness to bumblebees. The haploids and species parents of the 2x hybrids should be unrelated.

2x Haploid-species hybrid	x	2x Haploid-species hybrid
Maturity and tuber type		Maturity and tuber type
Unrelated haploid		Unrelated species
Profuse flowering		Profuse flowering
Male fertile-no 2n pollen		High % 2n pollen, ps or sy3, ps**
High 2n egg frequency*		No n eggs or fruit marker
Self-incompatible		Other desired traits
Other desired traits		Attractive to bumblebees
Attractive to bumblebees		

4x Hybrids
Inexpensive seed production
Near maximum genetic diversity
Good tuber yields

* 2n eggs by second division restitution.
** 2n pollen by first division restitution with or without crossing-over.

Figure 3. 4x progeny from 2x x 2x crosses.

The 2x male parent produces 2n pollen by FDR-CO or FDR-NCO. In order not to have fruit on the male parent or to be able to identify the fruit if they occur, it should either have no n eggs or a fruit marker. The 2x female parent is highly male fertile with no 2n pollen, self-incompatible, and produces a high frequency of 2n eggs. The inexpensive seed production plus near maximum heterozygosity and good tuber yields in the 4x progeny suggest this method is worth considerable investigation.

The signal problem is to identify 2x clones that produce high frequencies of 2n eggs. The mode of 2n egg formation is apparently not a problem, since 2x SDR x 2x FDR crosses result in high yielding 4x progeny (10). In essence, FDR and SDR may complement each other, since 100 percent of the parental heterozygosity from the centromere to the first crossover is transmitted to the progeny with FDR, and all the parental heterozygosity from the first to the seconod crossover is transmitted to the progeny with SDR. Further, although more tetra- and triallelic loci are present in 4x progeny from 2x FDR x 2x FDR as compared to 2x SDR x 2x FDR, no diallelic loci occur in the 4x progeny from 2x SDR x 2x FDR where the 2x parents are unrelated.

Recent research results add significantly to the potential value of the 2x x 2x approach. New haploid Tuberosum-wild species hybrids have been obtained that have very good tuber yields (2, 7). Many of these hybrids are also characterized by large, smooth tubers, low tuber set, and high specific gravity. Further, many of the haploid-wild species hybrids have 2n pollen and 2n eggs. These new materials provide the opportunity for further exploitation of any TPS breeding approach involving 2x hybrids.

OTHER PLOIDY MANIPULATIONS. Possible ploidy manipulations to obtain 3x, 5x, 6x and 8x progeny are listed in Table 1. The manipulations involving the sexual cycle are currently not appropriate for TPS production due to either lack of proper genetic material or to poor adaptation in the genetic stocks available.

Table 1. Possible ploidy manipulations to obtain 3x, 5x, 6x and 8x progeny.

3x	2x(2E) x 2x(1E)2n*	6x	3x(2n) x 3x(2n)
	4x(2E) x 2x(2E)		4x(4E) x 8x(4E)
			Cell fusion 3x + 3x
5x	6x(4E) x 4x(4E)		
	6x(4E) x 2x(2E)2n	8x	4x(4E)2n x 4x(4E)2n
	3x(2E)2n x 2x(2E)2n		Cell fusion 4x + 4x
	Cell fusion 3x + 2x		

* x-one set of 12 chromosomes, E-Endosperm Balance Number, 2n-2n pollen or 2n eggs.

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Hybrid vs. Open Pollinated TPS Families

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INTRODUCTION

The use of true potato seed (TPS) as an alternative means of potato propagation appears to be feasible as evidenced by results of research activities in many parts of the world. The adoption and use of this method may be particularily attractive in areas where clean seed tubers are not available.

The alternative ways of using TPS may involve transplanting, direct seeding, or production of seedling tubers. It is essential to select and use appropriate TPS progeny types with whatever method appears to be most suitable. The major types of TPS families that can be used for potato production are hybrid and OP progenies. The hybrids, produced by controlled pollination, can be derived either by the conventional method of intermating tetraploid cultivars and clones or by modified conventional breeding schemes employing meiotic mutants producing 2n gametes. In contrast, the OP's are the product of natural pollination and may be obtained from cultivars and advanced clones from the different kinds of hybrids. The seed of the OP progenies, which are mostly selfs, may contain some hybrids resulting from natural outcrossing. Results of evaluation trials conducted to date indicate that yield and other horticultural traits of the hybrids are, in general, superior to those of the OP progenies. However, since the cost of seed from open pollination is significantly lower than that of hybrid seed, it may be more economical to use OP progenies until a suitable method of producing low cost hybrid seed is available.

The purpose of this paper is to compare hybrid and OP progenies on the basis of current knowledge in areas of seed and seedling characteristics, seedling tuber production, tuber yield and uniformity, method and cost of seed production, and amenability for improvement.

Seed and Seedling Characteristics

Standability or seedling survival can be very important for the successful utilization of TPS for potato production. In order to achieve this requirement, TPS families must produce vigorous seedlings capable of rapid development and fast recovery from transplanting shock. Aspects of seed and seedling traits that may have some influence on these agronomic properties have been evaluated in hybrid and open-pollinated progenies.

There are some indications of differences in seed characters between the two major groups of TPS families. Seed traits that were observed to differ in the two family groups include seed weight and percent and rate of germination. The hybrid and OP families were also observed to differ in seedling vigor.

Seed weight: Results of seed analysis of four TPS families from 4x x 2x crosses and four families from open-pollination indicated a considerable difference in the weight of the seeds (Table 1). On the basis of the hundred seed weight, seeds from the hybrids were clearly heavier than the OP seeds. If this is a trait that is consistently expressed in all types of hybrid seeds, it may provide an opportunity to separate the hybrid seeds from the selfs in a batch of OP seeds.

Germination tests: Germination tests conducted in the laboratory and greenhouse revealed that hybrid seeds from 4x x 2x crosses had a higher percent of germination than seeds from open-pollination (Table 1).

Table 1. Hundred-seed weight, percent germination, and seedling vigor of hybrid and open pollinated true potato seed.

TPS families		Mean weight in milligrams*	Percent germination: Petri dish*	Percent germination: Jiffy mix*	Seedling vigor**
Hybrids					
W639 x W5295.7		62.2	92.0	86.0	4
W785 x W5295.7		87.2	97.5	94.0	4
W853 x W5295.7		57.1	95.5	94.5	4
Merrimack x W5295.7		66.9	93.0	89.0	4
	$\bar{x}$	68.4	94.5	90.9	4.0
Open-pollinated					
W639-OP		51.2	90.0	78.5	2
W785-OP		55.8	84.5	72.5	3
W853-OP		39.8	89.0	65.5	2
Merrimack-OP		45.9	85.5	78.5	2
	$\bar{x}$	48.2	87.3	73.8	2.3

* Average of three replications.
** Based on scale of 1 = least vigorous to 4 = highly vigorous.

The tendency of the hybrid seeds to germinate faster than the OP seeds was also evident in this trial. Differences in percent and rate of germination were particularly evident under the relatively less ideal conditions in the greenhouse than in the laboratory.

Seedling vigor: A very dramatic difference between the two groups of TPS families was in seedling vigor (Table 1). Seedlings from the 4x x 2x hybrids were exceptionally vigorous and uniform as compared to the seedlings from open-pollination. This important property of the hybrid seedlings may render competitive advantages to these seedlings at an early developmental stage. The superiority in vigor and uniformity of seedlings from 4x x 2x crosses has also been demonstrated in previous investigations (Macaso, 1983; Kidane-Mariam, 1985). In contrast, the seedlings from OP families are unthrifty and not quite as uniform as the hybrids. This may be due to the high rate of selfed progenies in the OP families.

Seed size and tuber yield: The observed seed weight differences between the hybrid and OP seeds prompted a follow-up study to examine the relationship between seed weight and tuber yield as well as some seedling traits. Seeds from eight 4x x 2x hybrid families and eight OP families were separated into large (> 1/17") and small (< 1/19") size categories. The hundred-seed weight of the larger seeds, on the average, was also higher than the hundred-seed weight of the small seeds in both groups of TPS families (Table 2), an indication that seed size and seed weight appear to be closely related.

Table 2. Hundred-seed weight of large and small seed classes in hybrid and OP TPS families.

TPS Families		Weight in mg	
		Large Seed (> 1/17")	Small Seed (< 1/19")
Hybrids			
1. New Haig x W5295.7		75.7	51.0
2. W639 x W5295.7		67.7	52.3
3. W853 x W5295.7		64.2	50.7
4. W760 x W5295.7		83.6	51.4
5. W231 x W5295.7		77.9	46.1
6. W744 x W5295.7		88.8	60.9
7. W785 x W5295.7		89.6	73.2
8. Merrimack x W5295.7		75.7	58.4
	$\bar{x}$	77.9	55.5
Open-pollinated			
9. New Haig-OP		61.2	46.2
10. W639-OP		56.2	43.8
11. W853-OP		50.6	39.9
12. W760-OP		81.6	57.5
13. W231-OP		58.1	48.0
14. W744-OP		55.4	43.1
15. W785-OP		73.2	42.8
16. Merrimack-OP		56.1	43.4
	$\bar{x}$	61.6	45.6

After evaluation of germination percentage and seedling vigor in the greenhouse, replicated yield trials were conducted in two locations during the summer of 1984. On the average, there was no strong evidence to suggest that the larger seeds in either family groups had higher germination or better seedling vigor. The tuber yields as well were not affected by the seed size/weight in this trial (Table 3). Thus, the positive correlation of seed weight and tuber yield reported by Dayal et al. (1984) were not evident in this trial.

The range of seed size may be an important factor in determining the relationship between seed size and tuber yield and other horticultural traits. It is possible that the reason we did not find any relation between seed size and tuber yield may have been partially due to a narrow range of seed sizes between the large and small seeds.

Table 3. Tuber yields in transplants from large and small seeds of hybrid and OP TPS families.

	Mean Yield (Tons/ha)			
	Hancock		Rhinelander	
TPS Family	Large Seed	Small Seed	Large Seed	Small Seed
Hybrids				
1. New Haig x W5295.7	40.9	37.9	15.7	20.9
2. W639 x W5295.7	35.1	34.6	11.6	13.8
3. W853 x W5295.7	29.5	31.9	28.0	24.0
4. W760 x W5295.7	34.6	42.7	19.4	25.2
5. W231 x W5295.7	28.9	37.7	17.5	13.4
6. W744 x W5295.7	31.9	34.7	18.7	19.1
7. W785 x W5295.7	35.1	27.9	10.0	13.7
8. Merrimack x W5295.7	35.3	29.5	17.8	13.7
$\bar{x}$	33.9	34.6	19.8	21.3
Open-pollinated				
9. New Haig-OP	13.8	13.2	3.5	4.2
10. W639-OP	18.0	21.3	5.3	9.2
11. W853-OP	16.6	17.3	9.2	10.4
12. W760-OP	24.5	25.0	8.1	4.8
13. W231-OP	21.0	23.1	12.9	14.1
14. W744-OP	23.1	24.3	8.1	4.2
15. W785-OP	19.2	26.6	9.0	7.2
16. Merrimack-OP	21.7	19.2	6.9	8.6
$\bar{x}$	17.3	18.5	8.5	8.1

Seedling Tuber Production

The use of TPS for the production of seedling tubers is one of the most desirable alternative approaches. Seedling tubers are tubers obtained from transplants and intended for use as seed tubers, either in the first tuber generation or after several cycles of multiplication. The desirability of this approach and the method of production have been extensively investigated by Wiersema (1982 and 1984) and other workers (Li, 1979; Devasabai, 1982).

In order to evaluate and compare the suitability of hybrid and OP progenies for seedling tuber production, seven TPS families from 4x x 2x crosses and seven families from open-pollination were evaluated in a nursery plot for yield and uniformity of seedling tubers in Hancock, Wisconsin during the summer of 1983. Sixty-four seedlings from each of the TPS families in each group were transplanted into a separate nursery plot of 0.49 m^2 (70 cm x 70 cm). The seedlings were planted about 10 cm apart within each plot. The trial was conducted in a randomized complete block design with 2 replications.

The results indicated that yields of first generation seedling tubers, in terms of both tuber number and weight, were significantly higher for the 4x x 2x hybrid progenies than for the OP progenies (Table 4).

Table 4. Seedling tuber yield and tuber size distribution of hybrid and open-pollinated TPS families.

	Mean yield/plot*		Tuber size distribution		
TPS families	Number of tubers	Weight in Kg	Small <2.5cm	Medium 2.5-3.5cm	Large >3.5cm
Hybrids					
W744 x W5295.7	189.0	6.4	42(29.0%)	54(30.2%)	73(40.8%)
W639 x W5295.7	150.5	5.7	32(19.3%)	70(42.2%)	64(38.5%)
W853 x W5295.7	180.5	4.9			
W760 x W5295.7	209.0	6.3			
W785 x W5295.7	189.5	5.8			
New Haig x W5295.7	147.5	5.3			
Merrimack x W5295.7	217.5	6.6			
$\bar{x}$	183.4	5.9			
Open-pollinated					
W744-OP	143.0	3.5	75(64.1%)	28(23.9%)	14(12.0%)
W639-OP	108.0	3.1	50(52.6%)	21(22.1%)	24(25.3%)
W853-OP	101.5	2.0			
W760-OP	190.0	3.5			
W785-OP	127.5	4.3			
New Haig-OP	121.0	2.7			
Merrimack-OP	142.C	3.1			
$\bar{x}$	133.3	3.2			

* Based on nursery plot size of 0.49 square meters

Another noticeable feature of the hybrids was their ability to produce more seedling tubers in larger size classes. Progenies from the 4x x 2x crosses produced higher proportions of uniform, large size tubers than the OP progenies.

The average increase of the 4x x 2x hybrids over the OP families was 38 and 84 percent in tuber number and weight, respectively. The OP progenies, in contrast yielded lower number of predominantly smaller tubers. An exception was the OP family from the 4x clone, W760 that yielded a total number of seedling tubers approximately the same as the mean of the hybrid families.

It should be pointed out that by rigorous selections against unthrifty or undesirable plants and/or tubers in the OP families, starting at seedling stage in the nursery and continuing at each cycle of multiplication of seedling tubers, it is possible to increase the proportion of desirable genotypes in the subsequent tuber populations of the OP families. Thus, by incorporating a high degree of selection pressure, the OP families could also prove to be adequate for the production of seedling tubers.

Tuber Yield and Uniformity

Several comparisons have been made between hybrid and OP TPS families for yield and other horticultural traits (Accatino, 1980; Bedi, 1979; Macaso, 1983; Kidane-Mariam et al., 1985). The results indicate that the hybrid families are superior to OP families in many of the traits studied.

Hybrid progenies which gave outstanding performances are from 4x x 2x crosses, one of the breeding schemes proposed by Peloquin (1983). This breeding scheme has been repeatedly demonstrated to be the most efficient, not only for TPS breeding, but also for clonal development (Macaso, 1983; Mendiburu and Peloquin, 1977; Kidane-Mariam et al., 1985).

In an experiment in 1983, transplants representing four groups of TPS families from 4x x 2x crosses and open-pollination were evaluated and compared on the basis of tuber yields and plant uniformity. The families consisted of six 4x x 2x hybrids and 24 OP progenies. The hybrid families were from crosses betweeen 4x clones and 2x Phureja-haploid Tuberosum hybrids producing 2n pollen by FDR. The OP families were from three categories of 4x parents, a) DTs - 4x derived from 4x x 2x crosses, b) advanced clones identified as highly male fertile, and c) advanced clones known to possess variable male fertility in which pollen stainability ranged from less than 3 percent to 10-30 percent during the flowering period (Arndt et al., 1985).

The results presented in Table 5 indicate a very significant difference between the mean tuber yields of the 4x x 2x hybrid families and the different groups of OP families. The average tuber yield of the hybrids was 28 percent higher than that of the highest yielding OP group, and about 48 percent higher than the combined mean yield of the three groups of OP families.

Table 5. Tuber yield (kg/plot)* and plant uniformity of transplants from different TPS family groups.

TPS family group	No. of families	Mean yield	Range	Plant Uniformity**
Hybrids (4x x 2x)	6	15.0a***	14.1-17.2	2.8
Variable male fertile 4x clones-OP	10	11.7b	8.5-15.7	2.2
DT (4x from 4x x 2x)-OP	6	9.4c	6.8-14.0	1.5
Male fertile 4x clones-OP	8	9.2c	5.5-12.3	2.2

* Based on a plot size of 5.40 square meters.
** Based on a scale of 1 = poor to 3 = good.
*** Means followed by the same letter are not significantly different at P=0.05. Unequal numbers of families was taken into account when calculating LSD values.

In 1982, Macaso also evaluated tuber yields and other horticultural traits of transplants from six groups of TPS families. These families were represented by one group from 4x x 2x crosses, three groups from 4x x 4x crosses, and 2 groups from open-pollination. The result of this trial is presented in Table 6. The mean yield from the 4x x 2x hybrid group was the highest among the hybrid, as well as the OP groups. Among the 4x x 4x hybrids, families from DT x DT gave higher tuber yields than similar families from 4x clone x 4x clone. It is also interesting to note that the OP's from DT's (4x clone from 4x x 2x crosses) gave higher tuber yields than inter-4x clone hybrids. However, if 4x clones with wider genetic base are used, the 4x clone x 4x clone crosses can also give good tuber yields with high level of uniformity. Results obtained from progeny evaluation trials of transplants from intermating 4x clones confirm this observation (Mendoza, 1979; Kidane-Mariam et al., 1985).

In the trial reported by Macaso (1983), the hybrid groups significantly outyielded the OP progenies of the 4x clone (Table 6). The low yield of the OP's from the 4x clones are probably the result of inbreeding depression due to reduction of interactions.

Table 6. Tuber yields in six groups of TPS families (Adapted from Macaso, 1983)

TPS Family Group	No. of families	Mean Yield (Tons/ha)
1. 4x x 2x	5	45.3[a]*
2. 4x x 4x (DT x DT)	5	34.1[b]
3. 4x x 4x (DT x Clone)	5	35.0[b]
4. DT - OP	4	31.1[bc]
5. 4x x 4x (Clone x Clone)	5	26.7[cd]
6. Clone - OP	7	23.4[d]

* Means followed by the same letter are not significantly different at P=0.05.

Considering overall performance, the families from 4x x 2x crosses were not only the highest yielding group, but also produced the most vigorous and uniform plants in the field. The outstanding performance of the progenies from 4x x 2x crosses can be explained on the basis of the FDR mode of 2n pollen formation. In this mechanism, more than 80 percent of the heterozygosity of the 2x parent is transmitted to the progeny.

Method and Cost of Seed Production

One of the greatest advantages of OP families over the hybrid families is the production of large amounts of low cost seed. With the aid of bumble bees, the only known pollinators of potato flower, very inexpensive OP seeds can be produced under natural conditions in the field. Peloquin (1979) estimated that seed yields of 10,000-50,000 per plant are possible. In contrast, the production of hybrid seed involves an added cost for hand emasculation and pollination. Furthermore, the amount of hybrid seed production per plant is only a fraction of the amount possible in OP seeds. Thus, with the present system of production, hybrid seeds would be considerably more expensive than OP seeds.

Because of the low cost of OP seed production, the use of TPS families from open-pollination can be viewed as an attractive alternative for raising an economical crop of potatoes.

One aspect of potato seed derived from natural pollination is the possibility that it can be mainly a product of self pollination. If this is the case, one can expect reduced tuber yield due to inbreeding depression from using OP TPS progenies. To test the validity of this assumption, we compared the tuber yields and plant vigor of three generations of OP progenies.

Six clones, 3 cultivars and 3 DT's (4x from 4x x 2x) were utilized as the initial source of seeds for the OP generations. Two reps. of 14 hills per OP generation of each clone were planted in a RCBD in Hancock in the summer of 1983. Two seedlings were planted per hill; hills were spaced 45 cm apart.

The mean tuber yields of each of the 3 generations from each clone is presented in Table 7. The results demonstrate that there is no marked difference in tuber yields between the different OP generations. Working with similar kinds of TPS families, Macaso (1983) also found no significant yield differences between OP-I and OP-II generations (Table 8).

Table 7. Tuber yields in different generations of OP TPS families.

Source Clones	Mean Yield (kg/plot*)		
	OP-I	OP-II	OP-III
DP 82	7.1	4.1	8.9
DP 52	8.0	6.1	8.0
DP 86	4.8	7.0	4.7
W744	6.7	3.7	7.3
Platte	3.7	5.2	3.7
New Haig	6.6	7.0	3.9
$\bar{x}$	6.2	5.5	6.1

* Based on a plot size of 5.40 m^2.

Table 8. Tuber yields of OP-I and OP-II TPS families (Adapted from Macaso, 1983).

Parentage	Mean Tuber Yields (Tons/ha)	
	OP-I	OP-II
DP-86	28.7	32.2
DP-82	30.0	36.2
W639	21.1	25.0
W231	19.1	25.4
W744	32.1	18.1
Merrimack	25.2	29.3
$\bar{x}$	26.0	27.7

A possible explanation for these results is that the individual plants serving as seed parents for the next generation may either be the most heterozygous of the selfed progeny or may be the product of outcrossing. This assumes that on the average, the plants that flower and produce OP seed represent the more heterozygous plants in the populations.

This explanation is supported by the findings of Arndt (1984 personal communication). Using a genetic marker for yellow tuber flesh, she was able to determine the occurence of some hybrids among the OP seed. Transplants from these hybrid seeds tended to produce more OP fruits and give higher tuber yields, thus suggesting that some level of heterozygosity may be essential to maintain reasonable yields of fruits and tubers in subsequent generations of OP families.

These results suggest a very simple and economical method of producing potatoes from true seed through the use of synthetic OP. The farmer would be provided with a mixture of OP seeds from, for example, four unrelated, highly male-fertile adapted clones. This would increase the opportunity for outcrossing compared to planting OP seed from one clone. Thus, it is possible that a farmer could continuously harvest and use OP seed from his plantings without significant reduction in tuber yield in subsequent generations. The total yield would be significantly less than that from hybrid seed, but the "economical yield" would be significantly higher.

Results obtained by Arndt et al. (1985) that may have bearing on synthetic or any type of OP seed production is the relationship of sterility to temperature. They noted that pollen sterility in some potato clones is closely related to unusually high temperature. Pollen stainability in some clones ranged from quite sterile (<3%) to highly fertile (20-30%) within a season. This property of the pollen does influence bee attraction, and thus affect OP seed production. It is suggested that deliberate attempts should be made to produce OP seeds in cooler areas with little or no extreme temperature fluctuations during the flowering period.

The use of OP seeds for commercial production of potatoes should be viewed as a stop-gap until a method is developed to produce inexpensive hybrid seed. To benefit from the yield and other superiority of hybrids, especially from the 4x x 2x crosses, the production of low cost hybrid seed should be one of the major research areas in the future. The biological mechanism of bee attraction, and the identification and use of clones incapable of selfing due tetrad sterility, self incompatability, or genetic-cytoplasmic male sterility should be explored in more detail.

Amenability for Improvement

With the advent of TPS technology, selection and breeding methods long used for the improvement of cereal and other sexually propagated crops may also be adopted for the improvement of TPS populations. A number of traits which have not been significant in potato cultivar development have to be considered in TPS breeding programs. Standability, seedling vigor, and capacity for fast recovery following transplanting are some of the major traits that are particularly important in the use of TPS for commercial production of potato. Yield and other horticultural traits, such as adequate resistance to diseases and insects, tuber and plant uniformity, earliness, and wide adaptation are equally important. To incorporate some or all of these important traits and derive desirable TPS populations would require genetic manipulations and utilization of breeding schemes that are new or have already been proven to be efficient. The use of genetic manipulations in order to develop and incorporate desirable traits in TPS progenies is more likely possible with the use of hybrid TPS progenies than with OP progenies. With high selfing rates in potatoes under natural conditions, the use of TPS progenies from open-pollination will have obvious limitations in achieving the yield and other potentials of hybrid TPS populations.

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Farmer Maintenance of TPS Varieties

Gary Atlin

Introduction

Farmers can either be provided with TPS from some institutional source, such as a commercial producer or a national program, or they can produce it themselves, by collecting seed resulting from natural pollination in their own fields. Hybrid progenies produced by specialized seed programs are undoubtedly superior to naturally pollinated populations which a farmer might produce. However, some farmers interested in taking advantage of TPS may have no alternative but to produce their own seed, as they do for many other crops.

Farmers without access to a seed distribution system may in fact be among those who can benefit most from the advantages of TPS. They may currently be using highly degenerated tuber seed, and may be faced with poor seed storage conditions. Such farmers may be rather undemanding with regard to tuber size, shape, and uniformity, and therefore not discouraged by the perceived disadvantages of TPS. Such farmers need to be given full consideration in CIP research strategy, which has tended to emphasize hybrid production and institutional seed distribution.

Several systems whereby farmers maintain and use their own TPS stocks can be envisioned. Farmers producing consumption potatoes from transplants might harvest seed each season from their own fields. Farmers using TPS to produce seedling tubers might renew seed stocks only once in three or four years. In both systems, however, the farmer's variety would be maintained through repeated cycles of natural pollination. This paper discusses the genetic consequences of this type of maintenance, and outlines some methods for maximizing yield potential of naturally-pollinated populations.

Factors affecting yield of farmer-maintained varieties

i) Mating system

An understanding of the potato mating systems is crucial to any description of how potato varieties will perform under long-term sexual multiplication.

Pollination of potatoes is both wind- and insect-vectored. Insect pollination is effected mainly by bees of the genus Bombus. These bees visit potato flowers only to collect pollen, since potatoes produce no nectar. To collect pollen, a Bombus bee alights on the flower and grips the anther cone. The weight of the bee causes the flower to hang down. The bee then vibrates the flower, shaking pollen out of the tubular anthers and onto its body, as well as onto the stigma, causing self-pollination. Cross-pollination results when foreign pollen is deposited on the stigma as a result of direct contact with the bee.

Thus, in environments where insect vectors are active, naturally-pollinated progenies consist of mixtures of selfed and crossed seed. Self-pollination predominates. Using a marker gene, Glendinning (7) estimated selfing rates in a Neo-Tuberosum population in Scotland, and obtained a selfing rate of 80%. Brown and Huamán (5) found that Andigena clones tested with a similar method at Huancayo, Perú, had selfing rates ranging from 55 to 90%. The bulk of the variability in outcrossing rate can probably be ascribed to differences in position of the stigma relative to the anther cone, and to differences in pollen fertility, among clones tested as females. In clones in which the stigma is never extruded beyond the anther cone, it seems unlikely that any outcrossing can occur. In clones which produce fertile pollen and which are characterized by extrusion of the stigma well beyond the anthers, selfing rates are likely to be intermediate. Some clones, such as Atzimba (under Peruvian conditions), have well-extruded stigmas, but produce pollen of low fertility. In such cases, pollen brought to the flower by the bee may be substantially more fertile than that shed by the flower itself, and insect pollination may result in a high level of cross-fertilization.

Bombus pollinators are apparently not universally distributed. They have been reported to cause substantial cross-pollination in potato plantings in Wisconsin (12), Scotland (7), the Peruvian highlands (5), and the Peruvian coast (2). Little, however, appears to be known about their activity in lowland tropic areas. Even in areas in which Bombus spp. populations are large, they can be fickle pollinators, ceasing suddenly to forage due to an environmental change or chemical application. However, when they work, they are prodigious. A bee pollinates a flower in 5 to 10 seconds. Individuals have been observed to visit as many as 120 flowers in a quarter of an hour (14).

Wind action has also been conclusively demonstrated to cause pollination in potatoes (1). It apparently causes only self-pollination. This conclusion is supported by the observation that emasculated or pollen-lacking flowers rarely set berries, even when adjacent to pollen fertile males (14).

Environments thus fall into two broad categories with respect to seed production: those in which insect pollinators are active, and those in which no insect pollination occurs. In the former, progenies resulting from natural pollination consist of mixtures of hybrid and selfed seed. In the latter, only selfed seed is produced. The two types of environments differ considerably in their consequences for farmer maintenance of TPS varieties. In both types, however, maintenance of varieties over several generations of natural pollination involves the accumulation of substantial inbreeding. Since the potato is a species which, in general, is severely affected by inbreeding, varieties maintained in this way are expected to decline in yield potential until an equilibrium inbreeding level is reached. This equilibrium depends on the selfing rate of the population, and on the number of parents which were included in the initial synthetic population. (A variety derived from a small group of selected parents through natural pollination is referred to as a "synthetic" by plant breeders.) Busbice (6) developed formulae by which the inbreeding level of a synthetic at equilibrium can be calculated, given the selfing rate and number of parental clones included.

The equilibrium inbreeding coefficient, F, is given for populations derived from 1, 2, and 4 parents at selfing rates of 90, 80, and 40% in Table 1. These calculated values are obtained with the assumptions that initial parents are non-inbred and unrelated, and that individuals at all levels of inbreeding set equal numbers of viable seed. It is clear from this table that selfing rate is the most important determinant of equilibrium inbreeding level. As selfing rate decreases, the importance of increased parent number is enhanced. At the intermediate selfing rate, increasing parent number from 1 to 4 results in a 16% reduction in equilibrium inbreeding. At the lower selfing rate an increase from 1 to 4 parents gives a 45% reduction in F.

The Busbice model also makes possible the calculation of the <u>rate</u> at which the equilibrium inbreeding level is approached over generations of multiplication of synthetics, under varying assumptions of parent number and selfing rate. The equilibria are approached asymptotically. At low selfing rates, the equilibrium is achieved earlier than at high selfing rates. In a synthetic with a large number of parents, a detectable fall in performance is likely to occur in each generation until the fourth or fifth when an 80% selfing rate is assumed. At a 40% selfing rate, little drop in performance is predicted after the third synthetic generation. At a 20% selfing rate, a comparison of the first and second naturally-pollinated generations might well fail to produce a significant difference.

No true synthetic populations have been produced in potatoes to date. Thus, it is not possible to compare the evolution of performance in populations of defined origins with theoretical prediction. Such comparisons are desirable, in that they provide a check of the main assumption required by the Busbice model: that individuals at all inbreeding levels are equally fertile. If this is not in fact the case, potato synthetics may perform as if they had selfing rates much lower than those generally estimated by studies using genetic markers.

There is some strong direct evidence that potato populations may be considerably more outbred than published outcrossing rates would lead one

Table 1. Inbreeding coefficients (F) of 1, 2, and 4-parent potato synthetics at equilibrium with 90, 80, and 40% self-pollination.

Self-pollination	Number of parents 1	2	3	4
%	F			
90	0.77	0.73	0.71	0.70
80	0.63	0.56	0.53	0.51
40	0.36	0.25	0.20	0.17

to expect. A Wisconsin study comparing first, second, and third-generation progenies resulting from natural pollination of 12 clones reported no significant difference among generations for yield (9). In a similar study, comparing yields of first and second generation progenies derived from natural pollination of 6 F_1 hybrid families at San Ramón, Perú, there was also no observable decline in yield from generation to generation (2).

The populations of clones making up each of these experiments may be considered to be roughly the genetic equivalent of a synthetic variety derived from a large number of parents. The observed absence of a decline in yield between the first and second generations of natural pollination in experiments conducted in such diverse environments points to the conclusion that potato populations behave as if they had a selfing rate of from 20 to 40%, rather than the 70 to 90% rate reported in tests of non-inbred clones. The finding indicates that inbred individuals are probably much less viable and fertile than individuals resulting from cross pollination.

Several conclusions regarding the formulation and performance of synthetic TPS varieties can be drawn from this discussion of the potato mating system. The most important is, that because of differential fertility and/or viability of heterozygous individuals in mixed populations, the performance of a potato synthetic is not likely to decline appreciably after the first generation of natural pollination. The second is that yield potential is likely to be enhanced by the inclusion of at least two, and preferably four, parents in the initial generation of a potato synthetic. The effect of increasing parent number above one is amplified at the "true" selfing rate of potato populations observed to date, which apparently is closer to 40% than the 80% average estimate obtained using genetic markers.

Even though TPS synthetics appear to accumulate less inbreeding than previously expected, at least in environments characterized by some insect pollination, advanced generations are still likely to be inbred at least to a degree equivalent to one generation of self-pollination. If insect pollination were to fail completely, a synthetic might accumulate much more inbreeding than this. In general, farmer-maintained TPS varieties are likely to be partially inbred populations consisting of more and less inbred individuals. The performance of a synthetic relative to that of similar, non-inbred material is a function of average inbreeding level, yield reduction of inbred individuals, and the interaction, if any, between inbred and non-inbred components (3). An understanding of the latter two factors is prerequisite to the development of methods to maximize the performance of farmer-maintained varieties.

ii) Performance of inbred progenies

Most reports indicate that inbred potato progenies are, on average, severely depressed relative to non-inbred parents. Krantz (8) reported that a group of clonally propagated, first-selfed-generation (S_1) progenies yielded only 83% as much as non-inbred parents. Selfing was continued in this material through 6 generations. By the S_6, only 4 of the original 66 lines still existed. The survivors yielded only 20% as much as the non-inbred parent clones. Severe yield reduction has also been reported in S_1 progenies under true-seed propagation (10,13).

Important exceptions to the general pattern of yield depression in inbred progenies are, however, sporadically reported. The frequency of occurrence of high-yielding S_1 lines may be surprisingly high. J. L. Marca and H. Mendoza (11) compared bulk hybrid with S_1 progenies of 50 Andigena clones in a field trial conducted at Huancayo, Perú, during 1984. Average S_1 yields were 15% lower than those of hybrids. However, approximately one inbred line in four equalled or exceeded the yield of its related hybrid.

Most inbreeding studies published to date have not continued beyond the S_1 generation. Because equilibrium inbreeding levels could be substantially greater than this in farmer-maintained synthetics, particularly in environments characterized by low vector activity, there is a need for more information concerning performance of more advanced selfed generations. There is also virtually no direct information available concerning the effect of inbreeding on reproductive characters in potatoes. Consequently, an inbreeding study was initiated at CIP with a view to generating information in these areas. Self- and sib-matings were attempted in a total of 37 F_1 hybrid progenies. Sufficient seed to test in replicated field trials was obtained in only 8 families. The 8 hybrids, together with the self (S_1) ($F = 0.167$) and sib ($F = 0.083$) progenies, were tested at Huancayo and San Ramón during Nov - April 1983-84. Mean yields for the two locations are presented in Table 2.

The S_1 progenies were severely depressed, yielding, on average, only 71% as much as the hybrids. Full-sib progenies, on the other hand, showed virtually no depression, in spite of being half as inbred as the S_1 families. The high yield of these progenies was unexpected, and may indicate that matings between relatives may be less harmful than self-pollinations. This finding requires confirmation in a larger sample of families.

There was no significant inbreeding level x location interaction observed in this study. Families, however, did interact strongly with inbreeding level. S_1 and full-sib progenies in some families showed very little depression, while in others inbred progenies performed very poorly.

Table 2. Mean yields* of F_1, sib-pollinated, and self-pollinated progenies in 8 families. San Ramón and Huancayo, 1983-84.

Progeny type	Family 1	2	3	4	5	6	7	8	9
					Yield				
F_1	8.02b**	7.06a	10.24a	4.13b	6.62a	13.45a	9.96a	9.38a	8.77a
Sib	10.72	6.82a	8.81a	9.26a	5.82a	11.92a	7.74a	9.00a	8.76a
S_1	6.80b	5.29a	7.21a	5.35b	4.69a	7.74b	7.18a	5.57b	6.22a

c.v. = 22.3%

* kg $plot^{-1}$ (20 plants, 0.3m x 0.9m)

** means within column followed by same letter not significantly different according to Duncan's New Multiple Range Test (p = 0.05)

This variability indicates that it may be possible, by choosing the proper parents, to construct TPS populations whose performance does not decline over generations of open-pollinations even at relatively high selfing rates.

Reliable seed yield data could only be obtained at San Ramón. Huancayo berries were infested with larvae of Rhagoletis flies, and had to be discarded. San Ramón data are presented in Table 3. Hybrid produced approximately 10 times as much seed as did S_1 progenies.

Sufficient S_2 seed to test in replicated trials was obtained in only 5 of the 8 S_1 families. Hybrid (F = 0), sib_1 (F = .083), sib_2 (F = .167), S_1 (F = .167), and S_2 (F = .306) generations in these families were planted in field trials in Lima and San Ramón during June-Nov , 1984. There was a significant inbreeding-level x location interaction, and significant heterogeneity of error among locations. The means for inbreeding levels within locations are thus presented separately in Table 4.

Yields in general were much higher at Lima than at San Ramón, where the material suffered from severe heat stress. At Lima, mean S_1 progeny yield was 87% that of the hybrids. S_2 progenies yielded only 54% as much the hybrids. Both types of sib-mated progeny again out-performed expectations. Yields at San Ramón were very low overall, and all inbred progenies were much more severely depressed. S_1's yielded 39% and S_2's only 20% as much as hybrids. Both sib_1 and sib_2 progenies exhibited yield decreases which were more than in proportion with their inbreeding coefficients. This result seems to indicate that inbreeding depression may be more severe under conditions of stress, causing even low levels of inbreeding to result in large yield declines.

The inbreeding level x family interaction was not significant at either location. Some variation among S_2 families did, however, exist. The same progeny was highest yielding among S_2's at both locations, producing 80% and 27% of the hybrid mean at Lima and San Ramón, respectively.

Analysis of seed yield data for these experiments was not complete at the time of writing. However, measurements of male fertility parameters made in cooperation with M. Iwanaga, are available (Table 5). S_1 and S_2 progenies produced less pollen per flower, and pollen of poorer quality, than did parental hybrids. This finding has twofold significance. First, it indicates that inbred parents contribute little to the pool of male gametes in a mixed population. Since, in tetraploids, inbred parents produce inbred gametes, this would tend to reduce the accumulation of inbreeding from its expected rate. Second, it suggests that inbred individuals may, in the presence of insect vectors, have higher outcrossing rates than hybrids. Both the seed yield and pollen production data support the hypothesis that potato populations are, under

Table 3. Mean seed yields* of F_1, sib-pollinated, and self-pollinated progenies. San Ramón, 1984.

Progeny type	Mean seed yield
F_1	2150 a**
Sib	1848 a
S_1	215 b

c.v. = 33.78 (log-transformed)

* per plot (7 plants, 0.3m x 0.9m)

** means followed by same letter not significantly different according to Duncan's New Multiple Range Test (p = 0.05)

Table 4. Mean yields* of F_1, sib_1, sib_2, S_1, and S_2 progenies in 8 families. Lima and San Ramón, 1984.

Progeny type	Yield	
	Lima	San Ramon
F_1	16.60 ab**	3.23 a
Sib_1	17.99 a	1.60 b
Sib_2	16.25 ab	1.24 bc
S_1	15.57 b	1.27 bc
S_2	9.03 c	0.50 c

* kg $plot^{-1}$ (30 plants, 0.3m x 0.9m)

** means within column followed by same letter not significantly different according to Duncan's New Multiple Range Test (p = 0.05)

Table 5. Pollen quantity and stainability of hybrid (S_o), first-generation inbred (S_1) and second-generation inbred (S_2) progenies in 5 families. La Molina, 1984.

Progeny type	Pollen Quantity* mg/flower	Stainability %
S_o	3.16 a*	68.5 a
S_1	1.53 b	46.1 b
S_2	0.81 c	27.9 c
c.v.	5.4%	16.4%

* Analysis of plot means, 3 replications, 5-20 flowers per replication.

** Means followed by some letter not significantly different according to Duncan's New Multiple Range Test ($p = 0.01$). Log-transformed (pollen quantity) and $\sqrt{\text{ARCSIN}}$-transformed values analysed.

conditions of natural pollination, largely allogamous in structure, if not in pollination mechanism.

iii) Performance of inbred-hybrid mixtures

Farmer-maintained varieties multiplied in vector-free environments (if these exist) are completely self-pollinated. To understand the behavior of such varieties, it is sufficient to study the performance of inbred progenies per se. However, varieties propagated in the presence of insect vectors are mixtures of selfed and crossed seed. In order to predict performance of such varieties, one must determine if inbred and hybrid components interact in some way, or if the yield of a mixed progeny is instead simply a linear function of the yields of the components. This knowledge permits a prediction to be made concerning the effect of measures which might be taken to reduce the proportion of inbred individuals in such a progeny.

The behavior of hybrid-inbred mixtures was observed in two experiments, each repeated at Huancayo and San Ramón during 1984. In one trial, bulk hybrid and S_1 seed was produced in each of two broadly-based population, for each population, a 1:1 mixture of hybrid and S_1 seed was made, and included as an entry in a field trial with the unmixed S_1 and hybrid progenies. The yield of the mixture was predicted nearly exactly by a linear function of the yields of the components (Table 6). A similar trial was undertaken with S_1 and full-sib progenies derived from four F_1 hybrids. Within each family, S_1 and full-sib seed was mixed in a 7:3 ratio, simulating the composition of a naturally-pollinated progeny harvested from a field planted to an F_1 hybrid. In this mix, the full sib individuals constituted the non-inbred component. Again, the yield if the mix was nearly equal to the prediction made on the basis of the yields of the components per se (Table 7). The linear relation between yield and proportion of hybrid individuals in a mix indicates that any measure which serves to increase the frequency of hybrids in a TPS synthetic will increase its yield potential.

iv) Conclusions

From the results of the experiments described above, it can be concluded that the performance of a TPS synthetic is a simple linear function of only two variables. These are:

1. The average inbreeding level of the population.
2. The degree of depression exhibited by inbred individuals.

Interactions between inbred and non-inbred individuals, whereby, for example, non-inbred plants compensate for the yield of adjacent inbreds, seem to be of little importance. A strategy for improving the performance of advanced generations of synthetic varieties must, therefore,

Table 6. Means of S_o, S_1, and mixed ($1S_o:1S_1$) progenies in two TPS populations. Huancayo and San Ramón, 1984.

Progeny type	Yield kg/plot*
S_o	12.24
S_1	9.11
$1S_o:1S_1$ (observed)	10.44
$1S_o:1S_1$ (expected)**	10.68

c.v. = 20.2%

* 24 plants per plot in San Ramón
30 plants per plot in Huancayo
Spacing: 0.3m x 0.9m

** Expected mixture yield = 0.5 x (Yield S_o + Yield S_1)

Table 7. Means of S_o, S_1, and mixed ($3S_o:7S_1$) progenies in 4 TPS families. Huancayo and San Ramon, 1984.

Progeny type	Yield kg/plot*
S_o	9.36
S_1	6.92
$3S_o:7S_1$ (observed)	7.60
$3S_o:7S_1$ (expected)*	7.65

c.v. = 20%

* 20 plants per plot
2 reps. per location
Spacing = 0.3m x 0.9m

** Expected mixture yield = (0.3 x Yield S_o x Yield S_1)

achieve at least one of two goals; it must reduce the average inbreeding level of the population, or it must reduce the degree of depression exhibited by inbred individuals. The choice of which goal to emphasize is dictated in large part by the pollination environment for which a variety is targeted; if vectors are absent, little can be done to reduce inbreeding, and the breeder must hope to reduce inbreeding depression through careful selection of parents. When vectors are present, both strategies are feasible. The remainder of this paper is devoted to a discussion of several practical methods by which breeders, seed producers, and farmers can achieve these goals.

Improving farmer-maintained varieties

i) Reducing inbreeding

In environments characterized by moderate to high levels of pollinator activity, the accumulation of inbreeding can be reduced either by reducing the selfing rate *per se*, or by eliminating seedlings resulting from self-pollination from the breeding population.

a) Reducing selfing rate

Recently, the suggestion was made that selfing rates could be permanently reduced in synthetic varieties by incorporating clones with cytoplasmic male sterility in the parental generation (3). To be effective in reducing selfing rates in populations, such clones must:

1. Produce abundant but non-functional pollen in order to attract bees;
2. Be highly female fertile;
3. Reliably transmit the sterility character to offspring.

There appear to be two types of sterility available in potatoes which fulfill these requirements. Clones carrying the type known as tetrad sterility self-fertilize very rarely, but can set large quantities of hybrid seed when bees are present (4). These clones are characterized by a failure of microsporogenesis such that pollen tetrads do not dehice to form individual pollen grains. Such clones can produce large quantities of pollen of low stainability. The trait appears to be reliably transmitted via the cytoplasm.

Another suitable form of sterility has been recently identified in the progeny of Atzimba x IVP-35. Both the F_1 and its OP progeny produce abundant, stainable pollen. This pollen, however, does not function well in fertilization (Table 8). The fact that both the F_1 and its OP progeny behave similarly is consistent with the hypothesis that inheritance is cytoplasmic.

Table 8. Berry and seed set on unrelated female clones following pollination by male sterile families and fertile check. La Molina, 1984.

	Flowers pollinated	Berry set %	Seeds per berry	Seeds per pollination
Atzimba x IVP35	28	88	148	130
(Atzimba x IVP35)OP	30	14	29	4.1
Fertile check	33	27	23	6.2

Either of these sterility methods may permit the construction of synthetic varieties with very low selfing rates. This could be done by initiating a synthetic population with a group of male-fertile parents and a large number of plants from a single male sterile clone. The mean selfing rate of the population would be reduced in proportion to the frequency of individuals carrying male-sterile cytoplasm in the initial generation. If male sterile individuals were to make up half of the population, the selfing rate would, of course, be reduced by 50% from the average level observed in the fertile parents. This reduction in selfing rate would result in a substantially reduced equilibrium inbreeding level in a TPS synthetic, and, consequently, improved performance. Assuming that male sterile individuals produce as much seed as do male fertiles, the reduction in selfing rate should be stable from generation to generation. This assumption is not unreasonable, since male sterile individuals in a naturally-pollinated population must be hybrids, and hybrids have been shown to set more seed than inbreds. The increased heterozygosity of male-sterile individuals should therefore, in synthetic varieties, offset the usual seed-set advantage exhibited by male fertiles.

It should be pointed out that the clone to be used as the CMS source need be selected only for high seed yield and a flowering period which coincides with other parents used to make up the population. This is because the frequency of nuclear genes contributed by a male sterile clone to a naturally-mated population is halved in each generation of sexual multiplication, and eventually declines to a level near zero. The equilibrium synthetic will resemble the male-fertile parents agronomically.

b) Selection against inbred seedlings

TPS systems usually involve some degree of overplanting followed by selection against weak seedlings. In transplant production, selection is applied when plants are transferred to the field. In production of seedling tubers in nursery beds, selection is applied when beds are thinned to the desired plant density. It seems likely that virtually any farmer able to raise TPS would be capable of applying this type of selection. If inbred seedlings are less vigorous than hybrids, selection would have the effect of reducing inbred frequency, and thereby would reduce the equilibrium inbreeding level of a synthetic population. A series of experiments was therefore conducted to determine if inbred seedlings could be effectively selected against in mixed populations, and to determine optimum stages for such selection.

First, the effect of inbreeding on germination rate was determined by observing germination of S_0, S_1, and S_2 progenies, derived from 10 Andigena clones, in petri-plates. Germination percentages were significantly higher for hybrid than for inbred progenies three days after seeds were incubated on moistened filter paper (Table 9). The result indicates that a farmer might be able to reduce the percentage of inbreds in a seedlot by germinating TPS in paper or in a small amount of nursery mix, and transplanting into a nursery bed or flat only the early-germinating seedlings.

The effectiveness of early selection in seedling flats was also examined. S_0, S_1, and S_2 seeds from four Andigena families were seeded in trays in a 1:2:1 ratio. This ratio was chosen to roughly approximate the expected proportions of the various inbreeding levels in a second-generation synthetic variety. Seeds were sown on a grid, and the inbreeding level of each seed recorded on a map. Simulated thinnings, with selection on the basis of plant vigor, were done 10 and 20 days after seeding. Selection intensity was 25%. Thinning at the second date was most effective in removing inbred individuals (Table 10). Virtually all S_2 plants were eliminated and the hybrid component was increased from 25% to 75% of the population.

A similar experiment was conducted in nursery beds at Huancayo and La Molina during 1984, in cooperation with S. Wiersema and J. Díaz. Hybrid and S_1 seed was produced in each of four families and seeded in mixtures in the manner described above. Three proportions of hybrid seed were examined: 25%, 50%, and 100%. The 25% hybrid mixture approximates a first synthetic generation, and only results pertaining to this mix will be described here. Selection of the most vigorous seedlings at thinning, using a 25% selection intensity, increased the hybrid proportion in the mix from 25% to 37.5% when averaged over both locations. Subsequent competition among plants continued to reduce the proportion of inbreds from thinning to harvest. When stems were cut, the percentage of hybrids had increased to 50% of the population.

The large increase in the final proportion of hybrids over their initial frequency at seeding observed in this experiment indicates that the yield potential of OP progenies and synthetic varieties can be substantially improved during the course of seedling-tuber production. This finding has important implications for national programs, as well as for farmers. Seedling tuber production schemes currently operating in Sri Lanka and under investigation in Rwanda operate as follows: OP seed is harvested from large plantings of a single, adapted clone. All of this seed results, genetically speaking, from self-pollination. The selfed seed is subsequently used to raise seedling tubers in beds. If a suitable pollen parent could be found with which the currently-used mother clone combines well, and always assuming a reasonable level of pollinator activity, a substantially hybrid progeny could be produced simply by interplanting the two clones, harvesting the OP seed set on both, sowing it at high density, and selecting intensely. A partially hybrid tuber progeny produced in this way would be expected to have a considerably higher yield potential than the completely selfed progeny harvested from a single clone.

Table 9. Germination rate and cotyledon expansion of non-inbred (S_o), S_1, and S_2 progenies in 10 andigena families

Inbreeding levels	No. germinated[1] (seeding + 3 days)	No. with expanded cotyledons[1] (seeding + 3 days)
S_o	18.1 a[2,3]	5.8 a[2,3]
S_1	15.7 b	5.7 a
S_2	5.7 c	1.0 b
c.v.[3]	11.4	20.1

[1] per 25-seed sample, with three replications.

[2] means followed by same letter not significantly different according to Duncan's New Multiple Range Test (p = 0.01). Analysis of Square-root transformed data.

[3] calculated on square-root transformed values.

Table 10. Effect of selection at 10 and 20 days after seeding on proportions of S_o, S_1, and S_2 individuals in mixed plantings*.

Date	Inbreeding level			χ^2
	S_o	S_1	S_2	
	%			
Seeding	25	50	25	
10 days	58	38	4	118.9**
20 days	72	27	1	233.0**

* 4 families seeded in plastic trays.

Original population/family = 192 plants
Selection intensity = 25%

ii) Reducing response to inbreeding

Considering the geographical range in which insect pollination of potatoes has been reported, it seems unlikely that useful production environments will be found in which potato flowers are always completely self-pollinated. However, it may be that in certain locations, cross-pollination rates will consistently be very low. Even in areas known to support large Bombus pollinations, little outcrossing occurs in some years. In such situations, inbreeding may accumulate rapidly in synthetic varieties. Such varieties must thus be specifically designed to "resist" inbreeding depression.

Strong evidence exists indicating that this can be accomplished for moderate inbreeding levels. Although the average effect of inbreeding in potatoes is severe, it was noted above that high-yielding S_1 progenies occur with some frequency. High yielding S_1 lines can be selected even in families which show strong average depression. This was confirmed in a selection study conducted at Huancayo and San Ramón during 1983-84. In each of 5 F_1 progenies, 7 S_1 lines were produced. The 35 S_1 lines were included in field trials with the 5 parental hybrids. The inbreds were, on average, severely depressed, yielding only 70% as much as the hybrids. There was, however, a two-and-a-half-fold variation in yield among S_1 lines. Yields of the 10 best entries in the experiment are presented in Table 11. Of the 5 highest yielding entries, 4 were hybrid. The 3 best S_1 lines, however, outyielded one of the hybrids, and yielded 80% as much as the best hybrid. Clones selected from high-yielding S_1 lines such as these can be intercrossed to produce populations which would perform well when multiplied in environments with moderate levels of insect vector activity.

Preliminary evidence indicates that extracting highly inbred lines which yield well relative to non-inbred material may be much more difficult. Selfing was continued in the more vigorous S_1 lines described above. Twenty-five S_2 lines were produced and tested together with the parental hybrids in a field trial at Lima during 1984. The best S_2 line in this study yielded only 62% of the hybrid mean. It thus appears likely that the production of material "resistant" to high levels of inbreeding will involve the screening of very large numbers of inbred lines.

Conclusions

The experimental results reviewed and presented herein lead to a number of recommendations regarding the formulation of synthetic TPS varieties, and their maintenance by farmers. The basis for these recommendations is that natural selection against inbred genotypes slows the accumulation of inbreeding in naturally pollinated TPS populations to such a degree that yield reduction has not been observed after the first generation of multiplication. Further improvements in yield potential can be made by:

Table 11. Yields of 10 best lines in trial with 5 hybrids and 7 inbred lines/hybrid. Huancayo and San Ramón, 1984.

Line	Progeny type	Yield kg $plot^{-1}$*
3	Hybrid	13.27
5	Hybrid	12.97
4	Hybrid	11.83
1	Hybrid	11.64
2.7	Inbred	10.58
2.3	Inbred	10.26
1.1	Inbred	10.23
3.7	Inbred	10.07
2	Hybrid	9.91
3.6	Inbred	9.87
c.v.		32%

* 20 plants, 0.3m x 0.9m

1. Including more than one parent in the initial synthetic generation;
2. Including a male-sterile parent in the initial synthetic generation;
3. Using parents which have been selected for performance in the inbred condition;
4. Applying artificial selection against inbred individuals during seedling production.

The first three recommendations fall within the province of the breeder. The fourth, however, can be carried out by farmers, involves little special knowledge, and no investment of capital beyond that normally involved in seedling production.

Finally, it should be stated that the key to reliable production of TPS through natural pollination is a better understanding of the ecology of the pollinators. We need to know more about the range of distribution of *Bombus* bees, and about why they sometimes fail to pollinate. This information may permit the development of techniques which increase the reliability of natural pollination. Such techniques could, in turn, make TPS a technology which is truly suited to the requirements of subsistence potato growers.

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Agronomic Management for Transplanting TPS Seedling

J. P. Malagamba

1. Introduction

Very few examples can be mentioned of a technology that has attracted as much research interest as true potato seed (TPS) has in developing countries in recent years. This high interest originates on the great flexibility offered by TPS in suiting different agro-economic conditions and by the rapid progress made in developing materials and techniques that could have applicability in many regions of the world. Indeed, a large proportion of the areas with high potential for adoption of TPS could already benefit of the high yielding, uniform materials developed at CIP and other institutions.

To our knowledge, the use of TPS is presently being investigated in more than 40 countries, most of them being developing countries. In several of those countries, alternative methods of TPS utilization are being evaluated not only in Experimental Stations but also under farmer field conditions. During 1984, pioneer farmers have produced potatoes commercially from TPS in three countries, without considering the People's Republic of China, where its extensive use by farmers is known for more than a decade.

Different surveys and results of on-farm work in several regions of Peru indicate that the potential for adoption of TPS technology will be especially favoured if the following general agro-economic conditions are present:

a. The climate must be suitable to potato production for a period greater than 3 months.

b. Potato yield in the area is low due to poor seed-tubers quality.
c. The cost of seed-tubers represents a high proportion of total production costs.
d. Skilled labor in vegetable growing practices is abundant and cheap.
e. The market price of ware potatoes is relatively high.
f. The market standards for tuber uniformity are not too strict.
g. The area dedicated to potatoes in the farms is small.

In general, these conditions can be found in two types of areas:

- Those presently cultivated with potatoes from seed tubers but where TPS could be a viable substitute that will produce a clear benefit. These areas may be characterized by higher market standards and, therefore, potatoes from TPS may not only have to offer economic advantages but also meet a competitive quality level. In substitution areas, high yield and uniform tuber quality are traits of particular importance.

- Those of potential expansion of the crop, i.e., areas where potatoes are not presently cultivated or have a secondary importance in the prevailing farming system. Vegetable growing areas where specialized labor is abundant, as well as small farms or farmyards in warm tropical zones, are in this category. At present, there are good evidences that in these areas TPS appears to have most immediate adoption potential.

According to the way TPS is utilized for potato production, two basic systems can be distinguished: direct sowing of TPS and transplanting seedlings to the field.

In direct sowing of TPS, characteristics of the seed and the normally low early vigor of the seedlings restrict the use of this method to small areas that can be managed more intensively. Therefore, this method has excellent application in seedling-tuber production in nursery beds where very high yields can be obtained, and in larger field areas only if field practices are properly managed. Because of the high multiplication rate in seedling-tuber production a small size nursery area at the initial stage can produce enough tubers for planting a large field the following season.

The system of transplanting seedlings to the field has shown, among other advantages, the possibility of producing vigorous seedlings in seed-beds and transplanting whenever conditions are appropriate. This permits an efficient use of the land and overcome the limitation derived from direct sowing TPS. When conditions are favourable for propagation by seed-tubers, part of the tubers produced from transplanted seedlings can also be utilized as planting material the following season. However, in areas of potential expansion of the crop, particularly those in warm tropical regions, ware potato production directly from transplanting appear as the most suitable system. Under those conditions, the possibility of using the seedling-tubers for planting the following seasons may be limited by the normally low aptitude of those areas for proper storage and maintenance of seed-tuber quality after field exposure to virus and other seed-tuber borne diseases.

2. Agronomic Research on Potato Production from Transplanted Seedlings

A. Seedling Production

When growing potatoes from transplants, the production of vigorous seedlings in a short time at the nursery is of great importance. The seed-bed characteristics, seedling management practices, and environmental conditions influence the quality of seedlings produced (1, 4).

Different seed-bed substrates for raising seedlings, using materials widely available at the farm level and common fertilizers have been investigated. In general, bigger seedlings that achieve transplant size earlier are obtained using substrates with a proper structure and fertility. Poor structured seed-bed substrates can be improved considerably by the incorporation of well decomposed organic materials. Composts made up of residues of different tropical crops and manure prepared at CIP's farm in San Ramón, either alone or mixed with soil and fertilizer, have provided excellent seedling growth (7).

In an experiment, a compost made of bean crop residue, manure, and leaf litter was used either alone or mixed with sand in different proportions (volume basis). Vigorous and early production of transplantable seedlings were obtained in 40 days after sowing when the compost was utilized alone and without fertilizers (Table 1).

Table 1. Fresh weight of 100 seedlings, 40 days after sowing in seed-beds of different proportions of compost and sand.

Percent of		Fresh Wt.
Compost[1/]	Sand	(g.)
100	0	249.6 a[2/]
50	50	159.7 b
25	75	95.4 b
0	100	6.7 d

1/ Compost analysis: N: 1.3%; P_2O_5: 30 ppm; K_2O: 0.05% Cond.: 1.3 Mmhos/cm; CEC: 12 meq/100 g.

2/ Values followed by same letter do not differ significantly (P = 0.05) by Duncan Test.

A well-prepared compost usually has enough nutrients to support adequate seedling growth prior to transplanting. When mixed with soil, sand or other materials, fertilizer application may be required(9). In this experiment, vigorous seedling growth was obtained when the compost was utilized alone. When the compost was mixed with sand, the addition of rock phosphate did not have significant effects on the response. Most probably deficiences of nutrients when the compost is mixed could be covered by fast release fertilizers.

Other organic materials that have been evaluated as soil amendments with good results include peatmoss, saw dust, natural forest litter, manure, and organic soil, among others. The improvement in substrate structure obtained with many of these

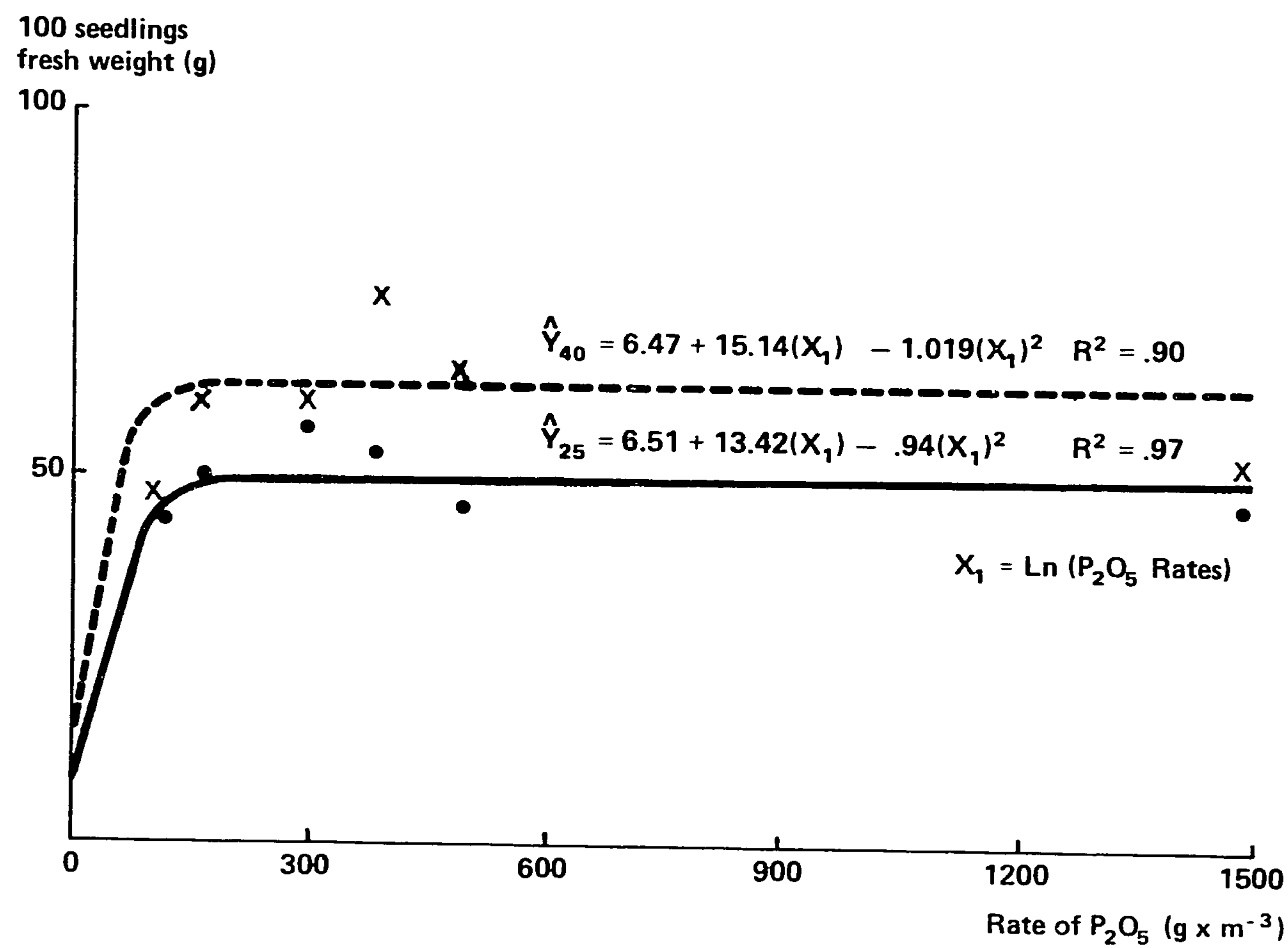

Figure 1. Effects of increasing rates of P_2O_5 on fresh weight of seedlings at 25 days ($\hat{Y}_{25}$) and 40 days ($\hat{Y}_{40}$) after sowing in a 1:1 peatmoss-sand substrate.

materials gives better distribution of moisture and aeration of the rooting zone with a subsequent improvement in seedling growth.

Other characteristics of the seed-bed substrate that influence the quality of seedlings are the substrate structure and fertility(6).In a study using soils of five vegetable growing areas of Peru, high conductivity and deficient structure were the main factors associated to poor seedling growth in two of the soils, whereas a high response to fertilizers was obtained in three of the soils. In this case, the fertilizers application was of 50-300-100 ppm of NPK, respectively, and manure was applied at a rate of 20 t/ha.

Phosphorus is a very important nutrient for vigorous early growth of the seedling. Using a 1:1 peatmoss sand mix by volume the optimum level of P_2O_5 at different seedling densities was investigated. It was found that at a density of 500 plants/m^2 rates of P_2O_5 slightly over 100 ppm produced highest seedling growth. At higher seedling densities, P_2O_5 requirements increased considerably for similar seedling growth than lower densities (Fig.1, and 2).

Potato seedlings are sensitive to the source of fertilizer utilized. In an experiment where a common rate of 100 ppm of N was provided by different proportions of urea and sodium nitrate, a very marked effect on seedling growth was obtained when increasing proportions of N were furnished in the nitrate form (Table 2). A similar effect has been observed with different sources of phosphate. In a soil of pH 5.2, diammonium phosphate and triple superphosphate produced sturdier seedlings than other P fertilizers at equivalent rates.

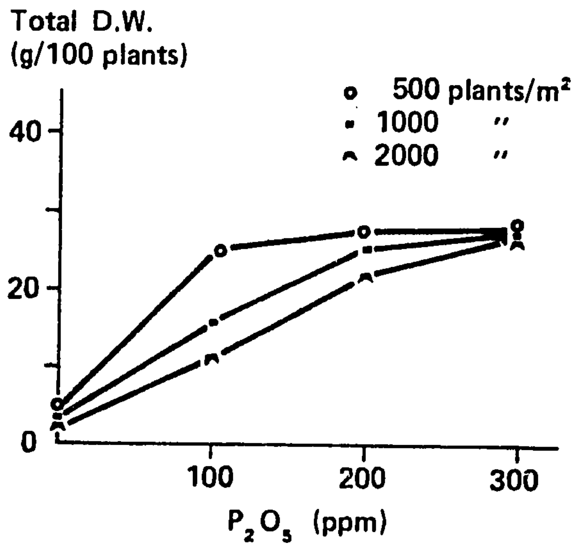

Fig. 2. Requirement of P_2O_5 for the production of vigorous seedlings 35 days after sowing at different densities (Lima. Progeny: DTO-33)

Table 2. Effect of different proportions of sodium nitrate and urea on fresh weight of a sample of 20 seedlings 42 days after sowing (Lima, Summer).

Nitrogen (ppm) $NaNO_3$	$(NH_2)_2CO$	Weight (g)
100	0	22.1 a[1/]
75	25	16.6 b
50	50	12.9 c
25	75	9.8 cd
0	100	7.5 d

[1/] Values followed by the same letter do not differ significantly ($P = 0.05$) by Duncan Test.

A proper source of N seems particularly important under hot environments where the nitrification process in the seed-bed may be affected by temperature.

Potato production from TPS seems to have a higher applicability within tropical farming conditions than the traditional potato grown from seed tubers. Many of the limitations of the latter in warm climates, such as seed tuber availability and cost, bulkiness and storage, are obviated by producing potatoes from seedlings. Also, potato seedlings can be grown and transplanted to the field whenever conditions are appropriate (1, 8).

The high temperature in low tropical areas normally cause a lack of uniformity in germination and growth of the seedlings. This results in extra field operations for the consecutive transplantings as seedlings reach the proper size, or trimming of the plants, or discarding a larger number of plants than those obtained in proper environments. By shading the seed-bed after sowing the temperature of the substrate can be significantly reduced and uniformized. However, excessive shading normally results in weaker seedlings. In San Ramon, shading periods of different length after sowing were evaluated for three consecutive seasons. The seed-bed was shadded to a level of 70% of transmitted total radiation at full sunshine. Best emergence and sturdier seedlings were produced when the seed-bed was shaded for a period not less than 14 days or longer than 28 days (Table 3).

Table 3. Emergence, height and stem diameter of seedlings shaded for different periods after sowing at San Ramon 1/.

Shade (days)	Emergence %	Height (cm)	Stem diameter (cm)
0	47.5 b 2/	4.3	1.50 c
7	60.9 b	5.0	1.57 c
14	80.1 a	5.2	1.87 ab
21	83.5 a	5.3	1.99 a
28	86.6 a	6.7	1.96 a
35	87.0 a	7.8	1.77 b

1/ Average values for three growing seasons.

2/ Values followed by same letter do not differ significantly ($P = 0.05$) by Duncan Test.

Seedling emergence and growth can also be impaired by low night temperatures. In experiments with several day/night temperature combinations, seedling emergence was significantly affected when night temperature was below 10°C (Figure 3). Low night temperature effect on seedling growth can be observed in the results of another experiment where seed of seven progenies was germinated for eight days at a common temperature of 20°C and then transferred to 30°C during the day and various night temperatures. Seedling growth was reduced by night temperatures of 10°C or lower (Table 4). Several seed-bed management practices that could improve seedling growing conditions in cool environments were evaluated. Significant increase in the minimum temperature, which resulted in earlier production and more uniform seedling growth was obtained by surrounding the seed-beds with black painted stones and covering them with clear polyethylene during the night (Figure 4).

Table 4. Effect of different night temperatures on potato seedling characteristics 35 days after sowing[1/].

Night Temp (°C)	Seedling Height (cm)	Internode length (cm)	Tops d.w. (mg/plant)	Root d.w. (mg/plant)
5	7.3	1.3	37.0 c[2/]	2.88 c[2/]
10	9.9	1.7	48.0 c	4.75 b
15	15.5	2.2	98.0 a	6.25 a
20	14.0	2.1	75.0 b	5.25 ab

1/ Common day temperature: 30°C. Average of 7 progenies. Seed germinated for 8 days at 20°C.

2/ Values followed by the same letter are not significantly different (P = 0.05) by Duncan Test.

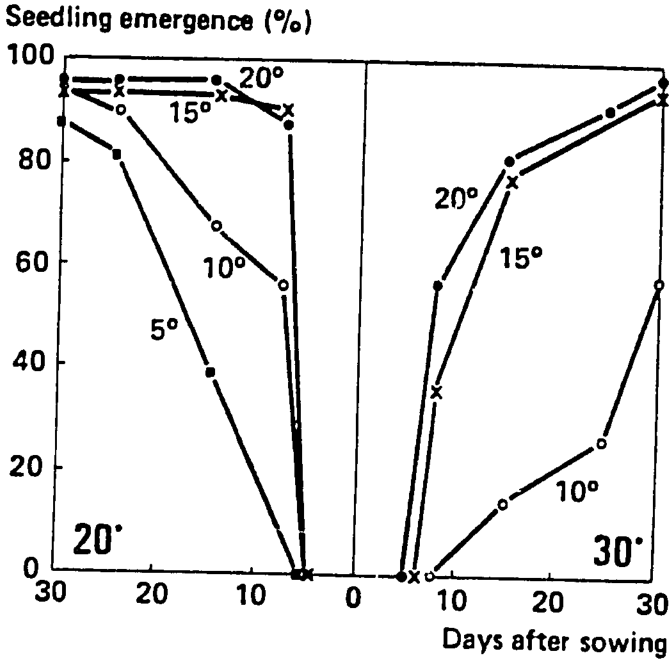

Fig. 3. Potato seedling emergence (%) under different night temperatures and 20° (left) or 30° (right) day temperatures. Averages for 8 progenies.

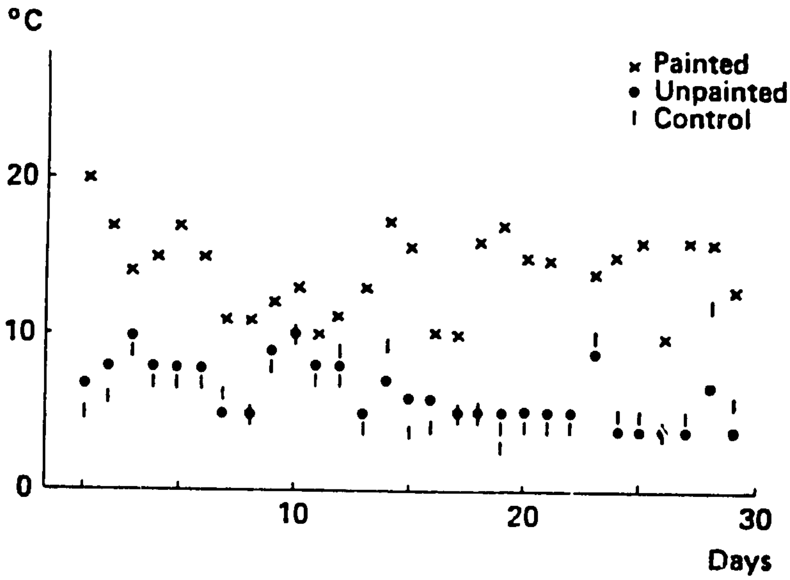

Fig. 4. Absolute min. temperatures (°C) on seed-beds heated by back-radiation from surrounding stones painted black (x), unpainted (.) and without stones (').

Potato seedlings must be ready for transplanting before a considerable number of tubers start to develop. Tuber formation in growing seedlings seems to be stimulated by sub-optimal conditions. When large numbers of tubers are initiated before transplanting, yield reductions usually occur.

The effect of night temperatures (10°C and 20°C) and daylength (12 and 18 hours) on tuber formation was studied in an early (DTO-33) and a late (69.47.2) progenies. High night temperature was observed to increase tuber formation at 12 hours daylength, but this effect was opposite at 18 hours daylength duration (Table 5).

Table 5. Proportion (%) of plants tuberized, with only stolons, and without stolon formation, 60 days after sowing as affected by different daylength and night temperatures[1/].

Progeny	Night Temp (°C)	Daylength (hours)	Plants (%) tuberized
DTO-33	10	12	35.6
DTO-33	10	18	33.3
DTO-33	20	12	55.5
DTO-33	20	18	-
69.47.2	10	12	2.3
69.47.2	10	18	-
69.47.2	20	12	16.7
69.47.2	20	18	-

[1/] Day temperature 20°C for all treatments. Seed was germinated for 8 days at 20°C constant temperature.

For large seed-bed areas, either for producing seedlings for transplanting or for seedling-tubers, the early vigor is particularly important for a rapid emergence and establishment(5). Stress and weed competition during the first few weeks after sowing may be serious limitations to proper seedling growth.

Also, the sowing process itself, because of the size and shape of the seed, is labor intensive and limits the scale of operation. Several methods for facilitating seed sowing in large field areas and enhancing early emergence and growth of the seedling were investigated. By fluid drilling pregerminated seed in a 4% Bentonite gel with fertilizer not only the sowing operation and time to emergence were significantly reduced, but also the growth of the seedling was greatly improved (Table 6). Although Bentonite is a relatively common product, other gels made out of substances of wider availability such as starch can also be utilized with good results (Table 7).

Table 6. Seedling emergence and characteristics 30 days after fluid drilling pregerminated TPS in fertilized Bentonite gel.

Treatment	Emergence (days)	Weight (g/pl.)	Height (cm)	Leaf diam. (cm.)
Bentonite 4% + Fert.	3	1.85	7.6	1.8
Bentonite 4%	4	1.55	5.7	1.4
Water	3	1.30	5.1	1.4
Control	8	1.20	4.9	1.3
LSD Test (P = 0.05)		0.28	1.50	0.27

Table 7. Germination (%) of DTO-33 o.p. seed after 6 days at 15°C in gels of different starch and concentrations

Gel	Conc. (%)	Germ. (%)	Consistency
Potato	1	92.5 a 1/	Poor
	2	90.5 a	Adequate
Cassava	1	82.0 de	Poor
	2	87.0 bc	Medium
	3	87.0 bc	Medium
Maize	1	91.0 a	Poor
	2	85.0 cd	Adequate
	3	79.0 e	Solid
Sw. potato	1	86.0 c	Poor
	2	93.0 a	Poor
	3	78.0 f	Poor
Control	-	93.5 a	--

1/Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

When gels of organic compounds are used, the addition of a fungicide such as Tiabendazole at a concentration of 25 ppm, to prevent fungal contamination has shown excellent results. A proper rate of fertilizer, basically phosphorus, incorporated into the gel and to seed-bed substrate so it is readily available to the germinating seed, has a very marked effect on seedling growth and establishment (Table 8).

In an experiment using different sowing methods, by fluid drilling seed to the field, either in clusters or in continuous rows, an early field establishment was obtained as compared to other sowing methods (Table 9). This early establishment was manifested not only in larger plants but by a greater weight of tubers per plant 75 days after sowing. Mulching of the soil in plots directly sown also helped significantly early field establishment of the plants.

Table 8. Effect of P_2O_5 incorporated to a 4% Bentonite gel for fluid drilling TPS and to the seed-bed substrate on potato seedling characteristics

Seed dispersant	Substrate	Weight (g/pl.)	Height (cm)	Leaf size (cm) Length	Leaf size (cm) Width
Bentonite + Fert.	Peat/sand + Fert.	2.73 a[1/]	10.0	2.9	2.0
Bentonite	Peat/sand + Fert.	2.57 b	9.0	2.6	1.8
Bentonite + Fert.	Peat/sand	0.93 c	6.9	1.9	1.5
Bentonite	Peat/sand	0.28 d	2.7	1.1	0.8
Water	Peat/sand	0.25 d	2.7	0.9	0.7

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

Table 9. Total and tuber fresh weight (g) per plant 75 days after sowing directly by different methods

Sowing method	Fresh weight Total	Fresh weight Tubers
Fluid drilling-clusters	47.7 a[1/]	23.20 a[1/]
Fluid drilling-cont. rows	42.9 a	23.23 a
Direct sowing-clusters, mulch	35.9 ab	15.20 b
Plug mix-clusters	30.5 b	15.37 b
Direct sowing-clusters	20.2 c	7.87 c

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05)

B. Transplanting and Seedling Establishment

Seedling establishment after transplanting is largely dependent on the progeny reaction to environmental conditions and to their ability to recover from the transplanting shock. Growth analysis of seedlings of the progeny DTO-33 o.p. before and after transplanting indicated that a considerable time was required for the seedlings to recover the original growth rate (Fig. 5).

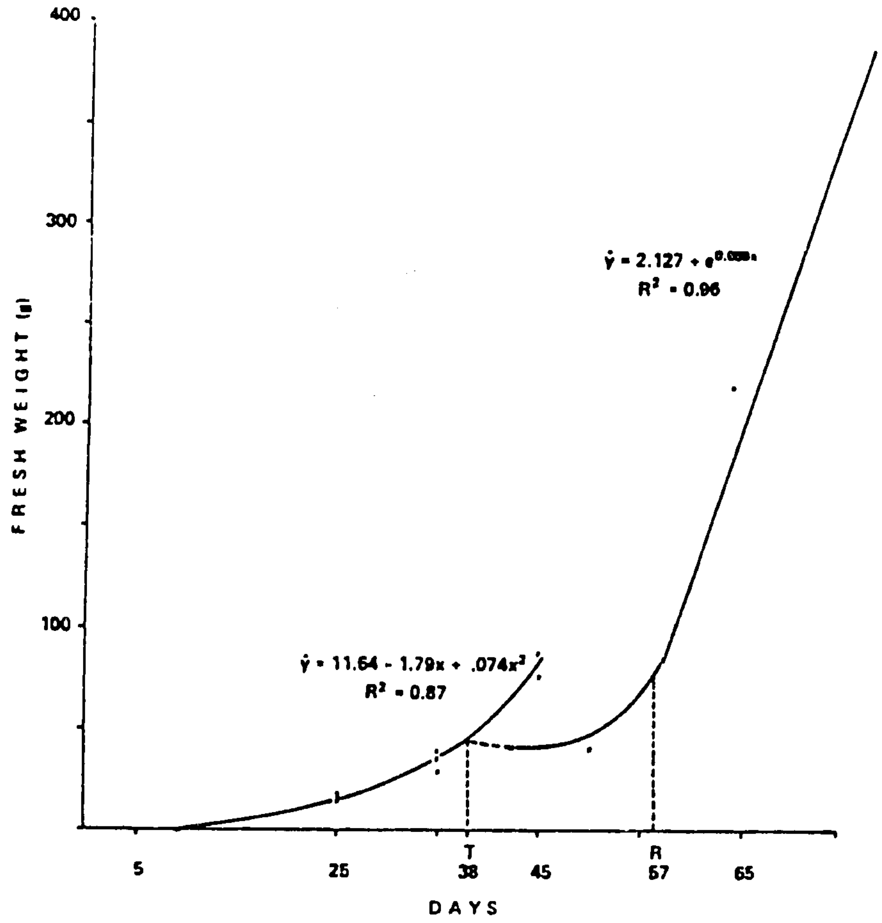

Fig. 5. Growth curve of DTO-33 o.p. seedlings in the nursery bed and after transplanting.

The calculated growth rate at transplanting (T), i.e., 38 days after sowing was recovered (R) at the day 57. In on-farm experiments conducted in several farms of a vegetable growing area at the coast of Peru, this progeny showed to be very sensitive to farmers' management practices resulting in very low seedling survival after transplanting. When a hybrid progeny, Atzimba x R128.6 was used and exposed to normal farmer practices excellent field establishment was obtained. When the growth of both progenies was experimentally analyzed in two growing seasons, these differences were clearly defined (Table 10).

Table 10. Growth rate of seedlings the day of transplanting (GRT) and time period needed to recover that rate (PRR) in two progenies and two seasons.

Progeny	WINTER GRT (g/days)	WINTER PRR (days)	SPRING GRT (g/day)	SPRING PRR (days)
DTO-33	0.14	19.4	0.17	13.2
Atz. x R128.6	0.12	12.7	0.13	10.3

In general, hybrids show a higher recovery rate than open-pollinated materials. In several experiments, conducted over a total of 30 progenies under controlled environment for seedling growth and using a substrate of even water distribution, seedling survival after transplanting was considerably better (by 20%) in hybrids than open-pollinated seed. The greater vigor of seedlings in improved progenies that allow for this ability to regrow fast after transplanting is an extremely valuable characteristic for the poorly prepared soils and management practices, normally present in developing regions, overcome competition of high weed population and to tolerate stress conditions.

In a set of progenies representing a range of response to transplanting a large number of seedling characteristics that could be associated to early recovery after transplanting were measured. It was found that the factor most highly responsible was the capacity of seedlings for immediate regeneration of an adventicious root system. New root formation in the period immediately after transplanting was found to account for 87% of the response in recovery after transplanting.

In hot areas management practices can be of great help to reduce the transplant shock. Under stress conditions, root blocks that are made by hand-operated machines used for other transplanted vegetables or by simple type of tools and materials available at any farm (Fig. 6) can significantly reduce the shock of transplanting (2). Root blocks of proper structure promote the growth of adventitious roots at the expense of the tops, which is a favourable characteristic for transplanting (Table 11)(3). Different materials have been evaluated and shown excellent properties for making root blocks, such as compost alone, or mixed with soil and fertilizers, and a 1:1 mix of peatmoss and sand with fertilizers. The favourable effect of using root blocks on seedling survival during the high stress season in San Ramon (Summer) can be observed in Table 12. Under farmer conditions, the use of root blocks has also shown great advantage at transplanting which reflected into higher yields (Table 13).

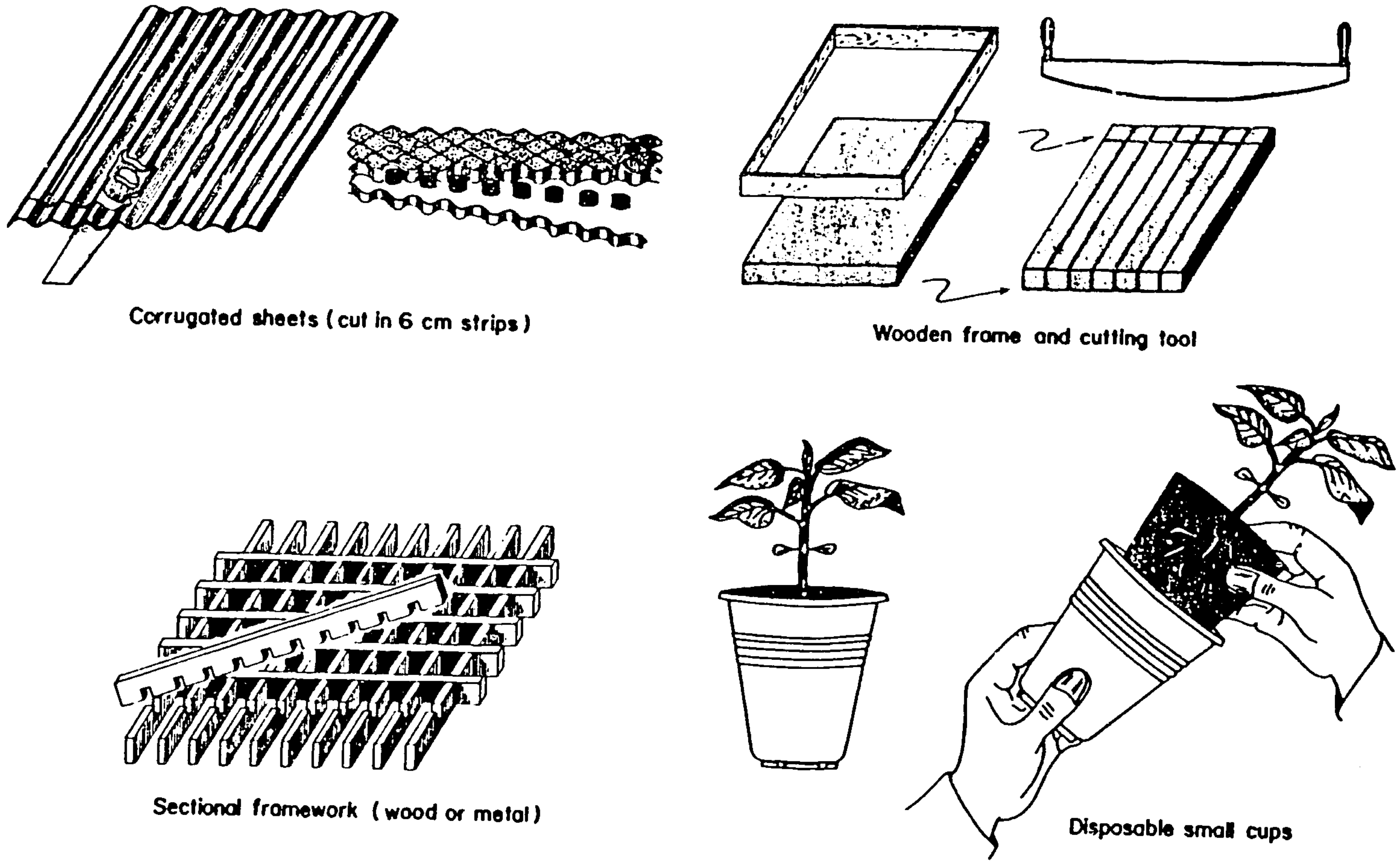

Figure 6. Materials for preparing root blocks.

Table 11. Differences in DTO-33 o.p. seedlings 45 days after sowing in root blocks and seed-bed of a fertilized peatmoss/sand substrate

Growing method	Height (cm)	Tops d.wt. (g/100 seedl.)	Roots d. wt. (g/100 seedl.)
Root blocks	7.98 b[1/]	7.93 b[1/]	0.93 a[1/]
Seed-bed	18.95 a	10.80 a	0.40 b

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

Table 12. Utilization of root blocks and their effect on DTO-33 o.p. seedling survival and yield under different stress conditions in San Ramon

Transplant method	Seedl. per block or hill	WINTER		SUMMER	
		Survival (%)	Yield (t/ha)	Survival (%)	Yield (t/ha)
Rooting blocks	2	89 a[1/]	14.4 a[1/]	90 a[1/]	9.9 a[1/]
Rooting blocks	4	90 a	14.7 a	92 a	11.2 a
Seed-bed	2	88 a	16.8 a	78 b	7.2 b
Seed-bed	4	92 a	15.5 a	81 b	7.9 b

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

Table 13. Yield obtained in large plots in a farmer field when root block and normal bare rooted seedlings were compared

Transplanting Method	Yield Total	(t/ha) Mktbl.
Normal	14.8	10.7
R. blocks	21.5	16.8

Several practices, including shading by associated crops, mulching, and others, for diminishing the effect of stress during transplanting have markedly improved survival and vigor of the transplanted seedlings and final yield in hot environments. A potato-maize crop association in San Ramon reduced soil temperature by about 5°C during the hottest part of the day with respect to a monocrop of potatoes, with a subsequent significant improvement of seedling survival (Table 14). However, the degree of light interception by the associated crop is directly related to the duration of such association without affecting potato yields. When potato seedlings were transplanted as a relay crop in between rows of mature maize plants, which were thinned to two levels of light interception, 30% and 60% of total radiation, and cut at three relay periods of 10, 25 and 50 days, seedling survival was improved by all combinations. Yield, however, was affected when the total radiation reaching the potatoes was reduced by 60% for a relay period longer than 10 days (Table 15).

Table 14. Soil temperature and potato seedling survival when transplanted in single or in two rows between maize or sunn hemp (San Ramon, hot-rainy season)

Crops	Number of potato rows	Soil temp (°C) 1/	Survival (%)
Potato-Maize	1	26.0	77.5 a 2/
Potato-Maize	2	27.3	73.0 a
Potato-Sunn hemp	1	28.2	64.5 a
Potato-Sunn hemp	2	29.7	62.0 a
Potato (Control		31.2	34.0 b

1/ Average soil temperature at noon at 5 cm for 15 days after transplanting.

2/ Values followed by the same letter do not differ significantly (P = 0.05).

Table 15. Effect of different relay periods and levels of intercepted radiation by a maize-potato association on seedling survival, yield and average tuber weight in San Ramon

Intercepted Radiation (%)	Length of Relay Period (ds)	Seedling Survival (%)	Yield (t/ha)	Ave. wt. of tubers (g)
30	10	78.7 a 1/	15.5 a 1/	18.4 a 1/
30	25	82.7 a	13.4 ab	15.3 ab
30	50	88.2 a	13.3 ab	15.8 ab
60	10	81.7 a	15.5 a	17.4 a
60	25	82.5 a	11.8 b	13.8 b
60	50	85.7 a	12.5 b	13.0 b
Control		66.0 b	15.9 a	17.0 a

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

In areas of high temperature and rainfall, to protect the soil surface after transplant may help in a faster seedling establishment. In some experiments carried out during the milder season in San Ramón and in Huancayo, mulching has not increased the usually high survival rate, after transplanting. However, the reduction on soil temperature and better maintenance of soil moisture associated to mulching, were probably responsible for the larger proportion of marketable size tubers produced in both experiments (Table 16).

Table 16. Effect of a straw mulch applied to transplanted DTO-33 o.p. seedlings on yield and tuber size distribution[1/] in San Ramon.

	Yield (t/ha)	Tubers >2.8 cm (%)
With mulch	12.5 a[2/]	81.2 a[2/]
Without mulch	10.4 b	73.5 b

1/ Average over treatments of different sources of fertilizer N.

2/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

Various insectices and application methods at transplanting were evaluated for two seasons in San Ramon, where insect activity during seedling establishment is greatly enhanced by warm temperatures (Table 17).

Table 17. Effect of different insecticides applied after transplanting on seedling survival in two growing seasons in San Ramon.

Insecticide	Method Application	Seedling Survival (%) 1982	1983
Furadan 75 WP	Sprayed to plant base	85.7 a [1/]	89 a [1/]
Lannate	Sprayed to plant base	89.0 a	97 a
Aldrin 2.5%	Powdered on the ridge	85.0 a	94 a
Sevin 20%	Powdered on the ridge	83.3 a	93 a
Ambush	Sprayed to plant base	72.3 b	96 a
Bait (Dipterex 20%)	Band application	69.7 bc	
Temik 10 G	Hole of transplant	61.0 c	
Sevin 80%	Sprayed to plant base	61.0 c	
Control		63.3 bc	62 b

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

Factors related to soil fertility have been observed to greatly influence seedling growth after transplanting and general performance of the potato crop from TPS. In San Ramon, in soils of low N content a hastened growth recovery after transplanting, which resulted in higher yields at harvest, was obtained with the application of 150 to 300 kg/ha of N applied half at transplanting and the other half at hilling (Table 18). Although manure application of 20 t/ha increased the final yield in this experiment, it did not affect significantly seedling vigor after transplanting.

Table 18. Yield of potatoes from transplanted seedlings of DTO-33 o.p. as affected by increasing rates of N and manures, San Ramon.

N Rates (kg/ha)	Manure application 0	Manure application 20 t/ha	Mean Yield (t/ha)
0	19.1	24.3	21.7 b[1/]
150	22.1	27.8	25.3 a
300	21.5	26.8	24.2 a
450	20.5	23.1	21.8 b
Mean Yield (t/ha)	20.8 b[1/]	25.5 a	

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

A similar response of increased seedling vigor to N was obtained in another experiment where different methods of N application were compared. Those treatments in which N distribution was closer to the rooting zone, e.g. in bands below the transplant or applied to the transplant hole and covered, showed higher yield and also a larger proportion of marketable tubers (Table 19).

Table 19. Effects of a common rate of N (150 kg/ha) applied by different methods on yield of potatoes from transplanted DTO-33 o.p. seedlings in San Ramon.

N Application Method	Total Yield (t/ha)	Marketable tubers (%)
Hole of transplant	15.9 a[1/]	68.2 a[1/]
Bands below transplant	15.8 a	62.4 a
Incorporated into ridge area	13.9 ab	54.8 b
Control	12.4 b	50.1 b

1/ Values followed by same letter do not differ significantly by Duncan Test (P = 0.05).

C. Agronomic Evaluation of Selected Progenies

Hybrid and open-pollinated progenies from selected clonal material are continuously evaluated in different environmental conditions represented by the three major CIP Experimental Stations (Lima, Huancayo and San Ramón). In these evaluations, the agronomic practices are similar to those normally utilized for raising other crops that are also transplanted. Recently, the evaluation is also including the agronomic performance of selected progenies using seedling-tubers.

Progress in terms of yield and average size of tubers of the best 10 hybrids and open-pollinated progenies during the period 1980-1983 is shown in Fig. 7.

Although controlled hybrids offer best potential for improvement, some open-pollinated progenies have also shown acceptable yield and general agronomic characteristics. Possibly, many of the latter come from clones of high outcrossing rate. This fact is being investigated at present. Hybrids, either manually made or natural hybrids evidently offer a great advantage over open-pollinated materials. Higher yield, uniformity, earliness, and possibility to incorporate resistance to major diseases are enough reasons to support and pay for the production of hybrid seed. Hybrids also have other general advantages of extreme importance: They produce seedlings of high early vigor, they have a higher ability to recover from the transplanting shock possibly due to their hybrid vigor, and they overcome the general tendency to produce a small average size of tubers, a character of high heritability (Fig. 8).

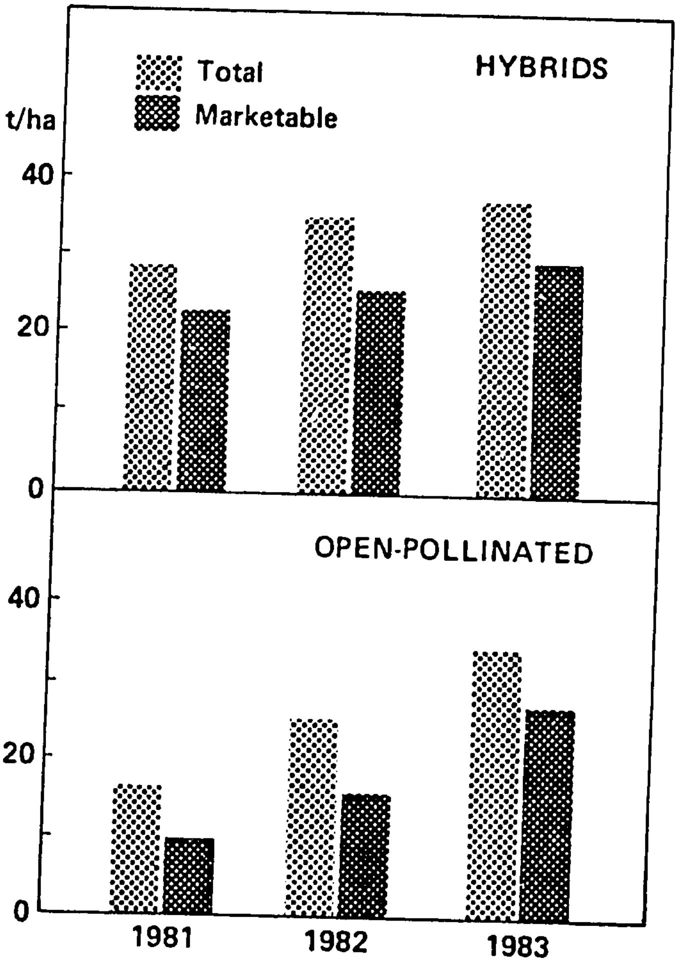

Fig. 7. Average total and marketable yield in 10 selected hybrids and open-pollinated progenies from 1980 to 1983.

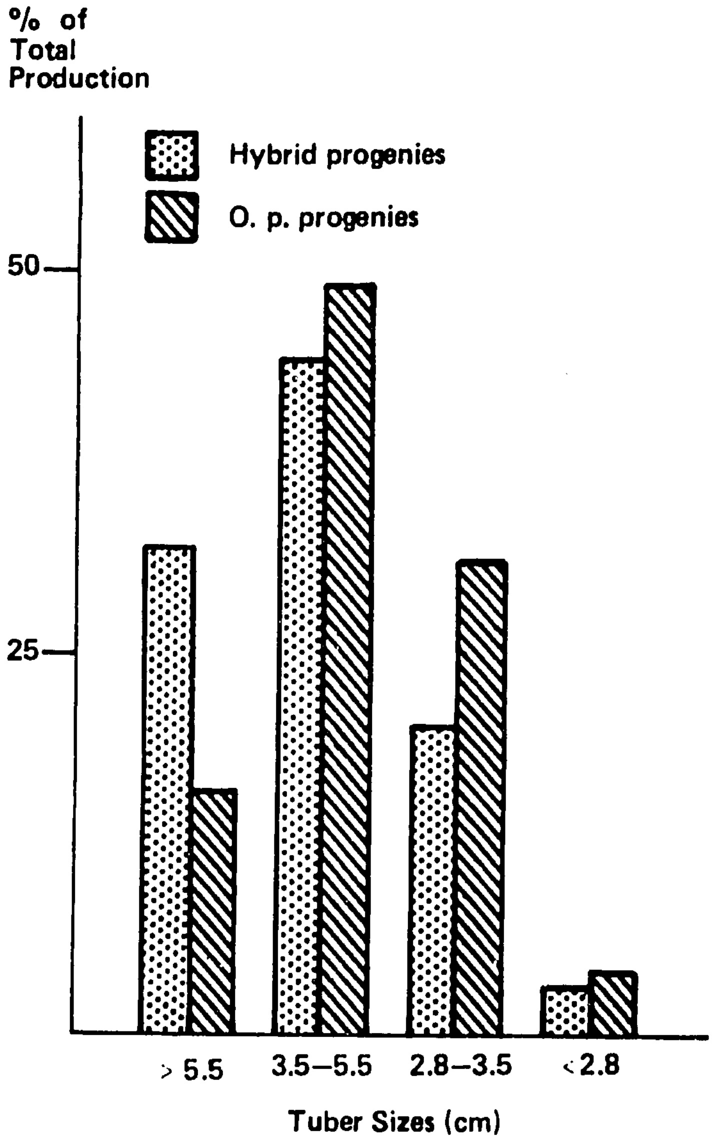

Fig. 8. Proportion of different size tubers produced by three highest yileding hybrids and open-pollinated progenies.

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Production and Utilization of Seed Tubers Derived from True Potato Seed (TPS)

S. G. Wiersema

INTRODUCTION

Poor seed tuber quality and high cost of healthy seed tubers may hamper potato production in many developing countries. Potato production from seed tubers derived from TPS (seedling tubers) potentially combines the rapid plant development from seed tubers with the high health standard and low cost of TPS. In 1980 research was started at the International Potato Center (CIP) to develop methods of producing seedling tubers and evaluate their performance. This research has concentrated on the intensive production of seedling tubers in densely planted beds rather than in normal field rows. In beds, production is concentrated in a small area, which makes it relatively easy to control growing conditions. This permits the creation of suitable conditions for direct TPS sowing or seedling transplanting, and at the same time tuber-transmitted diseases and their vectors can be more effectively controlled. Also, in some areas, this system permits the production of seedling tubers during the off-season thereby reducing the storage period.

This paper reviews briefly the techniques of production of first generation seedling tubers in beds, the utilization of seedling tubers, and the application of this system of producing planting material in developing countries.

PRODUCTION OF SEEDLING TUBERS IN BEDS

Beds for the production of seedling tubers may be located in a field with suitable soil conditions or alternatively, when field conditions are not suitable for the production of healthy seedling tubers, in a nursery using a prepared growing medium. At CIP, the principles of seedling tuber production in beds were studied in a nursery using beds, 1 m wide and 25 cm deep, filled with a prepared growing medium (substrate). Beds were separated by a 65 cm wide pathway.

Type of Substrate and Substrate Fertility

Excellent yields were obtained when a 1:1 mix of sand and shredded peatmoss or sand and plant compost was used (Table 1).

Table 1. Total weight and number of useable seedling tubers produced in different nursery substrates 1/

Substrate	Tubers >1 g 2/ kg/m^2	No./m^2
50% sand, 30% peatmoss, 20% soil 3/	5.70	712
50% sand, 50% peatmoss	7.84	725
50% sand, 30% compost, 20% soil	5.78	736
50% sand, 50% compost	7.42	839
50% sand, 30% manure, 20% soil	4.34	686
50% sand, 50% manure	2.67	474
LSD = 0.05	0.95	135
CV (%)	23.0	22.0

1/ 100 plants per m^2
2/ Mean of 2 experiments
3/ Field soil: 46% sand, 42% silt, 12% clay

When 20% field soil was added to these substrates, total tuber weight decreased significantly. The manure used in these experiments contained a high salt concentration and appeared not to be suitable as a source of organic material. These and other experiments have shown that early seedling growth responds negatively to high salt concentrations (Fig. 1). Since most N and K_2O fertilizers substantially increase the electrical conductivity of the soil solution (Rader et al, 1943), the application of these fertilizers at sowing gave detrimental effects on emergence and subsequent seedling growth, especially at high temperatures when salts tend to concentrate near the soil surface. Excellent results have been obtained when N or K_2O fertilizers were not applied at or before sowing in beds (Wiersema, 1984). On the other hand, seedlings respond favourably to P_2O_5 (Malagamba, 1983) and no negative effects on growth have been found when amounts as high as 80 g P_2O_5 per m^2 bed were applied at sowing (Wiersema, 1984). Based on several experiments the optimum regime of fertilizer application, in a 1:1 mix of peatmoss and sand, was found to be the supply of P_2O_5 at sowing (40-80 g per m^2) and N and K_2O (40-60 g per m^2) in split doses (4-8 applications) after plant establishment.

Planting System

Alternative planting systems are direct sowing in beds followed by thinning, or raising seedlings elsewhere to be transplanted to the beds. In the comparison of these planting systems the TPS for both

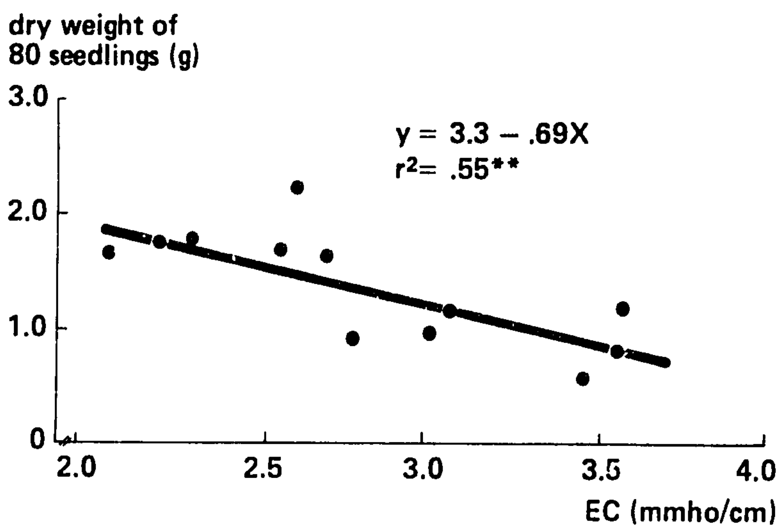

Fig. 1. Relationship between electrical conductivity (EC) of the substrate and seedling dry weight 30 days after sowing.

systems was sown on the same date. Fig. 2 shows that both planting systems gave a similar tuber yield at maturity. The plots with transplants matured 15 days later than the direct-sown plots mainly due to stagnating growth of the seedlings after transplanting.

An important criteria when choosing between planting systems is the length of the growing period in the beds, especially when several crops can be grown each year. Under conditions as those in Lima the growing period in beds was 110 days following direct sowing, and (125-35=) 90 days following transplanting. Thus, the total growing period in the beds was only 20 days more with direct sowing than with the transplanting system.

Direct sowing would seem an attractive labour saving method to produce seedling tubers, although the application of this method may be limited by the temperature requirement for germination of TPS. Optimum temperatures for germination are reported to be between 15°C and 20°C, while above 25°C or below 10°C germination is inhibited (Stier and Cordner, 1937; Steinbauer, 1957; Sadik, 1979; White and Sadik, 1983). In a nursery area, however, measures such as shading and frequent irrigation can be applied to manipulate soil temperature. Seedling emergence during the summer in Lima, with a monthly mean maximum air temperature of 29.5°C, a monthly mean minimum air temperature of 21.8°C and a monthly mean day temperature of 25°C was delayed by 2-3 days compared to that during the winter, but the percentage of final emergence was similar. Another possibility to permit direct sowing at sub-optimum soil temperatures is the use of pregerminated seed by applying techniques such as fluid drilling (Currah *et al*, 1974).

Plant Population

Increasing plant population in beds showed an increase in the number and weight of useable tubers (Table 2). Tubers larger than 1 g were defined as useable since those could be successfully multiplied under field conditions of the Experimental Station at CIP. The number of tubers in all size grades increased as a result of increasing plant population, except the number of tubers larger than 40 g which was similar in all plant populations. Although in some experiments a further increase of plant population to 150 or 200 plant per m^2 gave a further increase in the number of useable tubers, practices such as hilling prevented the application of such high plant populations. According to our experiences, 100 plants per m^2 spaced at 10 x 10 cm appeared to be an optimum plant population with suffcient space between plants to permit hilling by the application of additional substrate.

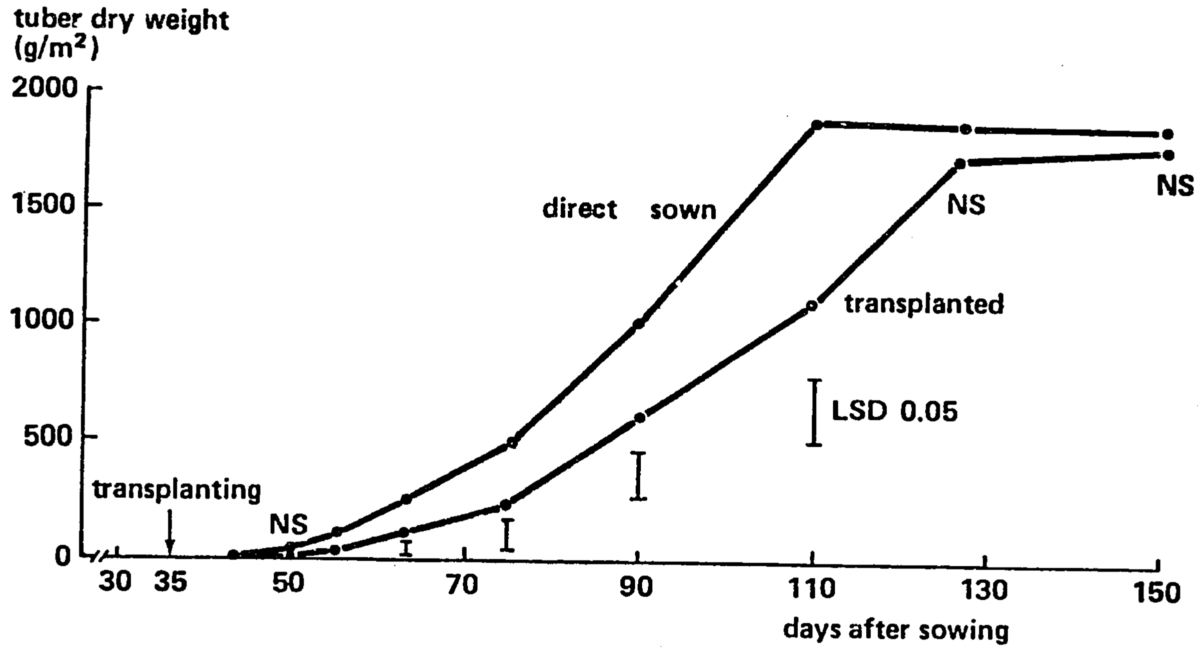

Fig. 2 Effect of planting system on total tuber yield and length of the growing period. (NS = not significant)

Mean of 2 progenies: DTO-33 o.p. and Atzimba x 7 XY.1
Plant population : 100 plants per m^2.

Table 2. Effect of plant population on yield and size distribution of seedling tubers in nursery beds

Plants /m² [2/]	Spacing (cm)	Tuber Yield [1/] <1 g No./m²	1-5 g No./m²	5-10 g No./m²	10-20 g No./m²	20-40 g No./m²	>40 g No./m²	Total >1 g No./m²	Total >1 g kg/m²
6	40.8x40.8	75	133	61	59	40	23	316	4.25
12	28.9x28.9	119	175	73	89	53	19	409	4.99
24	20.4x20.4	183	268	116	116	68	21	589	6.58
48	14.4x14.4	283	390	145	161	73	22	791	7.92
96	10.2x10.2	434	550	160	175	74	18	977	8.76
LSD 0.05		71	53	31	28	14	NS	88	1.04
CV (%)		27	14	23	19	19	26	12	23

1/ Mean of two progenies: DTO-33 o.p. and Atzimba x DTO-33

2/ After thinning.

The positive response of yield to increasing plant population would seem to be largely associated with the slow ground cover after seedling emergence (Table 3). Although 48 plants per m² is a rather high density, an increase to 96 plants per m² decreased the period from sowing to 100% ground cover by 7 days. The significant response of yield to increasing plant population has important consequences for the production of consumer potatoes from TPS by either direct sowing or transplanting methods. Seedlings for the production of consumer potatoes are usually transplanted at approximately 12 plants per m². Table 2 shows that the yield at this plant population was only 57% of that produced at 96 plants per m². Therefore, it would seem that total yields from seedlings transplanted to the field could be increased when higher plant populations are realized. This practice, however, would increase tuber number and consequently decrease tuber size. It would, therefore, seem difficult to obtain high yields from transplanted seedlings and at the same time a high proportion of large tubers, unless progenies are identified with fewer tubers per stem.

Table 3. Effect of plant density on rate of ground cover in nursery beds 1/

Plants 2/ /m^2	Days from sowing to 100% ground cover
6	73
12	70
24	62
48	56
96	49

1/ Mean of two progenies: DTO-33 o.p. and Atzimba x DTO-33

2/ After thinning

Plant Selection

The plants grown in beds were subjected to artificial and natural selection pressures. The artificial selection took place during thinning when weak and deformed seedlings were removed, and natural selection resulted from intense plant competition. The proportion of plants eliminated by plant competition increased with increasing plant population (Table 4). Many of these plants produced a few small tubers before they died, and the question arose as to whether these small tubers would represent early maturing types or perhaps inferior genotypes. If the early dying plants were to represent such genotypes then the smaller tuber grades at harvest would represent a larger proportion of these genotypes than the larger tuber grades. In order to establish as to whether all grades of first generation tubers represented similar genotypes, the genetic yield potential of four size grades of first generation seedling tubers was determined (Fig. 3). The four grades of first generation seedling tubers were obtained from nursery beds and multiplied in separate blocks in the field. At harvest 20-60 g (second generation) tubers were selected at random from each multiplication block. The four groups of second generation tubers produced similar yields with no significant difference in tuber size distribution (Fig. 3). In addition, no difference in length of the growing period between the four groups was observed. These results indicate that all four seedling-tuber grades of the first generation represented similar groups of genotypes in terms of yield potential and maturity class, and would be equally suitable for multiplication into seed-tubers destined for the production of consumer potatoes.

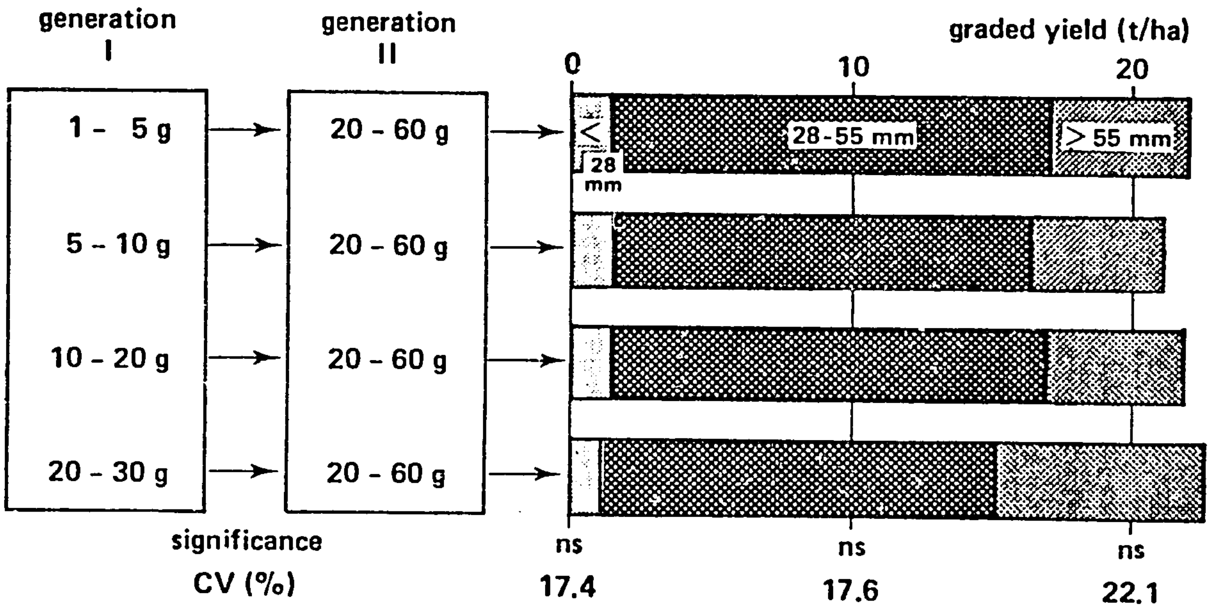

Fig. 3. Graded yields from second generation tubers originating from different sizes of first generation seedling tubers (DTO-33 o.p.).

Table 4. Effect of plant density on plant survival (%) in nursery beds 1/

Plants 2/ /m2	Days after sowing			
	55	80	100	120
6	100	100	91	84
12	100	100	77	70
24	100	100	75	66
48	100	98	64	46
96	100	96	40	37

1/ Mean of two progenies: DTO-33 o.p. and Atzimba x DTO-33

2/ After thinning

Hilling

When TPS is sown direct in beds, all stolons are borne above ground. Hilling was carried out by the application of additional substrate after plant establishment. Table 5 shows that the application of 2-3 cm substrate significantly increased the number of useable tubers compared with no hilling, while application of more substrate did not further increase tuber number. Observations on stolon formation showed that with 2-3 cm "hilling", about 80% of the total number of developing stolons was covered by substrate, while these stolons showed a strong tendency to grow diageotropically. Hilling levels of more than 3 cm would only seem to be required in situations where a limited amount of tuber greening cannot be accepted. With 3 cm "hilling" the proportion of green or partly green tubers did not usually exceed 4% of the total tuber number.

Table 5. Effect of hilling on the number and weight of useable seedling tubers [1/] produced in direct sown nursery beds

Quantity of substrate applied (cm)[2/]	Summer		Winter	
	No./m^2	kg/m^2	No./m^2	kg/m^2
0	319	3.62	607	8.74
2-3	385	4.07	772	9.74
6-7	409	4.21	691	9.92
LSD 0.05	42	NS	56	0.78
CV (%)	12.4	21.7	9.6	9.7

1/ Tubers >1 g

2/ Measured after several irrigations

Application of B-nine

In densely planted beds, plants became etiolated due to mutual plant shading. Two applictions of B-nine at a concentration of 5 g/l, resulted in shorter and sturdier plants with shorter internodes compared with the control (Table 6), but no positive effect on total tuber weight or number was found. When plants received three applications of B-nine, tuber malformation was observed.

Table 6. Effect of B-nine application [1/] on seedling development 59 days after sowing

	Control	B-nine	LSD .05	CV (%)
Stem length (cm)	51.6	34.1	4.8	11.7
No. nodes	22.7	22.7	NS	4.9
No. stolons/plant	6.9	5.9	0.6	10.2
Longest stolon (cm)	15.9	9.5	3.3	26.8

1/ Two applications at a concentration of 5 g/l.

In addition to reducing stem length, B-nine reduced the number and length of stolons. Therefore, when seedlings are planted for the production of consumer potatoes, the application of B-nine may have a positive effect on transplant survival through sturdier seedlings while the size of tubers may increase as a result of fewer stolons per plant. The application of B-nine would not seem to offer advantages in seedling tuber production, except perhaps with progenies that produce very long stolons which might grow above ground.

Evaluation of TPS Progenies

The number of useable tubers produced by selected progenies in nursery beds ranged from 807 to 1365 tubers per m^2 bed, with a total tuber weight ranging from 7.82 kg to 16.15 kg (Table 7). When the tuber yields are expressed as weight per unit of total nursery area, including pathways, yields ranged from 4.7 kg to 10 kg per m^2. This shows that very high yields from TPS can be obtained when growing conditions are adequately controlled. The average yield of the open-pollinated progenies did not differ significantly from that of the hybrid progenies, either in total weight or in tuber number. From a progeny evaluation point of view, the yield potential of the seedling tubers is more important than the progeny performance in nursery beds. Seedling tubers derived from DTO-33 o.p. were multiplied and compared with low virus seed tubers from the advanced clone DTO-33. Results of five experiments in Peru (Lima and San Ramon) showed no significant difference in total tuber yield obtained from both types of seed tubers. This demonstrates that although DTO-33 o.p. performed poorly when direct sown or transplanted (Table 7), its seedling tubers were able to compete with high yielding clonal material.

Generally, the desired traits of progenies to be used for seed tuber production are perhaps different from those of progenies selected for direct production of consumer potatoes by either direct sowing or transplanting methods. When progenies are selected for the production of seedling tubers, traits such as seedling vigor and transplant survival are of minor importance, since first generation seedling tubers can be produced under more optimum conditions in a nursery when field conditions result in poor transplant survival. The requirement for low tuber number per stem, in order to produce larger tubers, is important when consumer potatoes are directly produced from transplanted seedlings, but for seedling tuber production tuber number per stem is a far less critical trait. One reason is that the number of tubers produced in beds can be easily manipulated through plant population. Another reason is that plants from seedling tubers produce usually fewer tubers per stem than transplanted seedlings of the same progeny (Wiersema, 1984), which results in larger tubers.

Table 7. Evaluation [1] of progenies for the production of seedling tubers in nursery beds [2]

TPS progeny	Tubers >1 g kg/m^2	No./m^2	No. growing days from sowing
CKF.69.1 x 4179 DI	16.15 a[3]	963 c	120
Anita o.p.	15.62 a	1355 a	120
Atzimba x R128.6	14.95 a	933 c	120
4179 DI o.p.	14.63 a	833 c	140
Atzimba x DTO-33	13.48 a	1070 b	105
Atzimba x 7XY.1	13.13 ab	1365 a	105
Participación o.p.	12.69 ab	1019 c	120
Murca o.p.	12.26 abc	1306 a	140
Tomasa Cond. o.p.	9.68 bc	1064 bc	140
DTO-2 x TS-1	8.85 cd	807 c	105
DTO-33 x DTO-28	7.89 d	895 c	90
DTO-33 o.p.	7.82 d	808 c	90

1/ Evaluation during the winter in Lima

2/ Plot size: 1 m^2, bordered at 2 sides by a pathway of 65 cm
Plant population: 100 plants/m^2

3/ Duncan's Multiple Range test at P = 0.05.

UTILIZATION OF SEEDLING TUBERS

Health Standard

Since the growing medium used in beds can be easily disinfected before sowing, infection of seedling tubers by soil-borne pathogens is considered to be of little importance compared to that by virus diseases. Observations on virus incidence in seedling tubers produced in Lima concentrated on PVY and PLRV since these are the most important viruses in terms of yield reduction (Beemster and Rozendaal, 1972). The combined incidence of these viruses in first generation seedling tubers was 5.3% and 15.2%, in two different growing periods (Table 8). These data indicate that seedling tubers of a reasonable health standard can be produced in an environment where the degeneration rate is known to be relatively high. When there is a much higher infection pressure of insect transmitted viruses, the beds might have to be covered by an aphid-proof screen, since systemic insecticides do not prevent the spread of PLRV by viruliferous aphids from surrounding fields, nor reduce the spread of stylet-borne viruses such as PVY (Schepers, 1972).

Table 8. Virus incidence in seedling tubers produced in nursery in Lima 1/

Growing period	N° tubers tested	Virus incidence 2/ PLRV	PVY	%
Early summer	150	-	-	94.7
		+	-	3.3
		-	+	1.3
		+	+	0.6
Winter	112	-	-	84.8
		+	-	9.8
		-	+	4.5
		+	+	0.9

1/ Progeny: DTO-33 o.p.

2/ Determined by Dr W. J. Hooker using ELISA serology.

Size of Seedling Tubers

The small size of tubers produced in beds has a positive effect on the total number of sprouts or potential stems produced per unit area, which in turn favours the multiplication factor (Wiersema, 1983). Since the size of seedling tubers is smaller than those normally used for field planting, sprout and plant growth of these tubers were evaluated. Tubers of a similar dormant period were selected for observations on sprout growth in a diffused-light store. At two months after the end of dormancy, sprout length increased with increasing tuber weight (Fig. 4) indicating slower sprout growth in smaller tubers. To study early plant growth, single-sprout tubers of different sizes were planted in pots and the total dry weight of the plants was determined 24 days after planting. Simultaneously, single-sprout tubers were planted in the field to determine the rate of ground cover. The results indicated a decreasing plant dry weight (Fig. 5) and a decreasing rate of ground cover (Fig. 6) with decreasing seed tuber weight. This suggests a negative effect of a decreasing amount of tuber reserves per stem on early plant growth.

Several researchers have suggested that the amount of reserves per stem can affect growth and, sometimes, yield (Bremner and El Saeed, 1963; Rozier-Vinot, 1971, 1972; Iritani et al, 1972). These authors indicate faster emergence and more rapid shoot growth with an increasing amount of tuber reserves per stem. As pointed out by Allen and Scott (1980), in plants from tubers above 20 g, differences in growth due to the amount of tuber reserves per stem are relatively small and not noticeable in the tuber yield at mature harvest. With tubers smaller than 20 g, however, the amount of reserves per stem appear to affect growth. We found that yields per main stem were significantly lower in plants from 5-20 g tubers than in plants from 40-60 g tubers, throughout the growing period (Wiersema, 1984). Since these differences seemed to arise largely from lack of ground cover during the initial stages of plant growth, it can be assumed that stems from small tubers are potentially capable of producing the same total yield as those from larger tubers, provided that their growing period is sufficiently long. However, when the growing period is relatively short, crops from tubers smaller than 20 g may require a higher number of stems per unit area, in order to compensate for slow initial plant growth.

As a consequence of slow initial growth of plants from small tubers, the spatial arrangement of these plants affects the rate of ground cover. Preliminary results of experiments on different spatial arrangements showed that particularly in plants from 1-10 g tubers a less rectangular spatial arrangement increased early soil cover significantly. A less rectangular plant arrangement can be achieved by narrow row spacings or alternatively by the use of multiple row beds or flat beds where plants

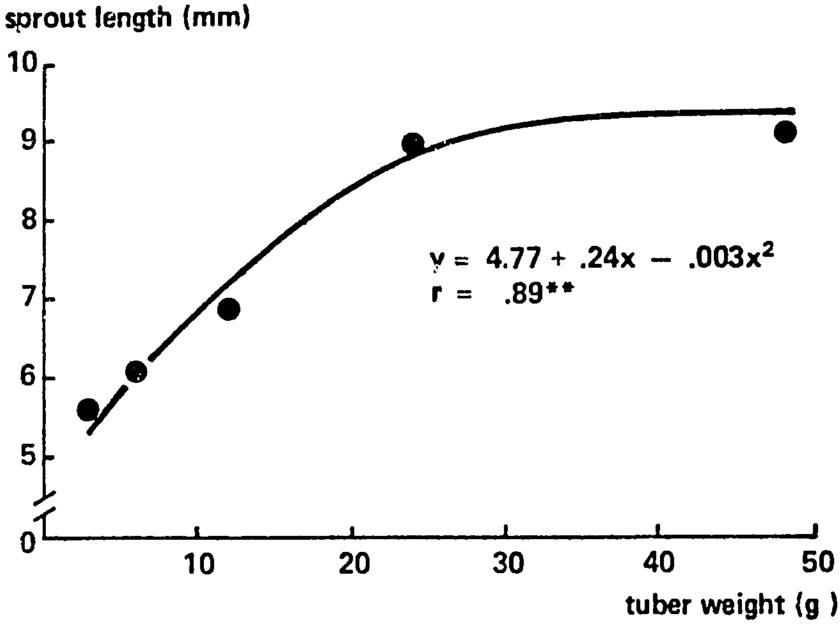

Fig. 4 Effect of tuber weight on sprout length of single-sprout tubers.

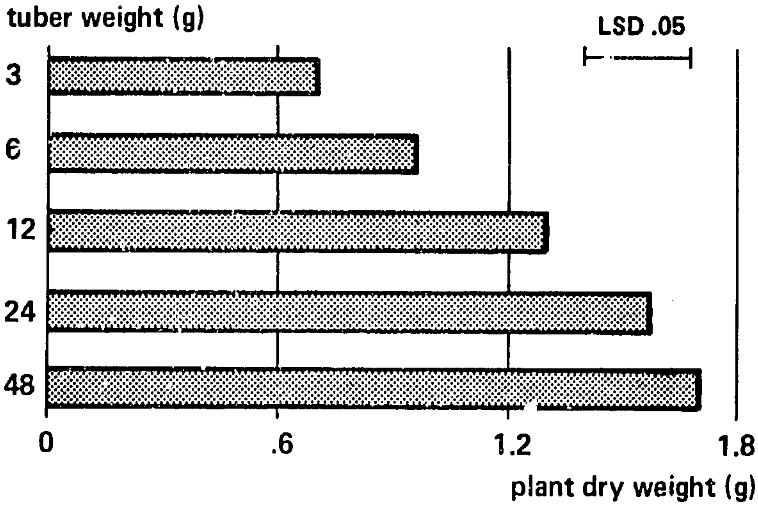

Fig. 5. Dry weight of plants from single-sprout tubers in pots, 24 days after planting.

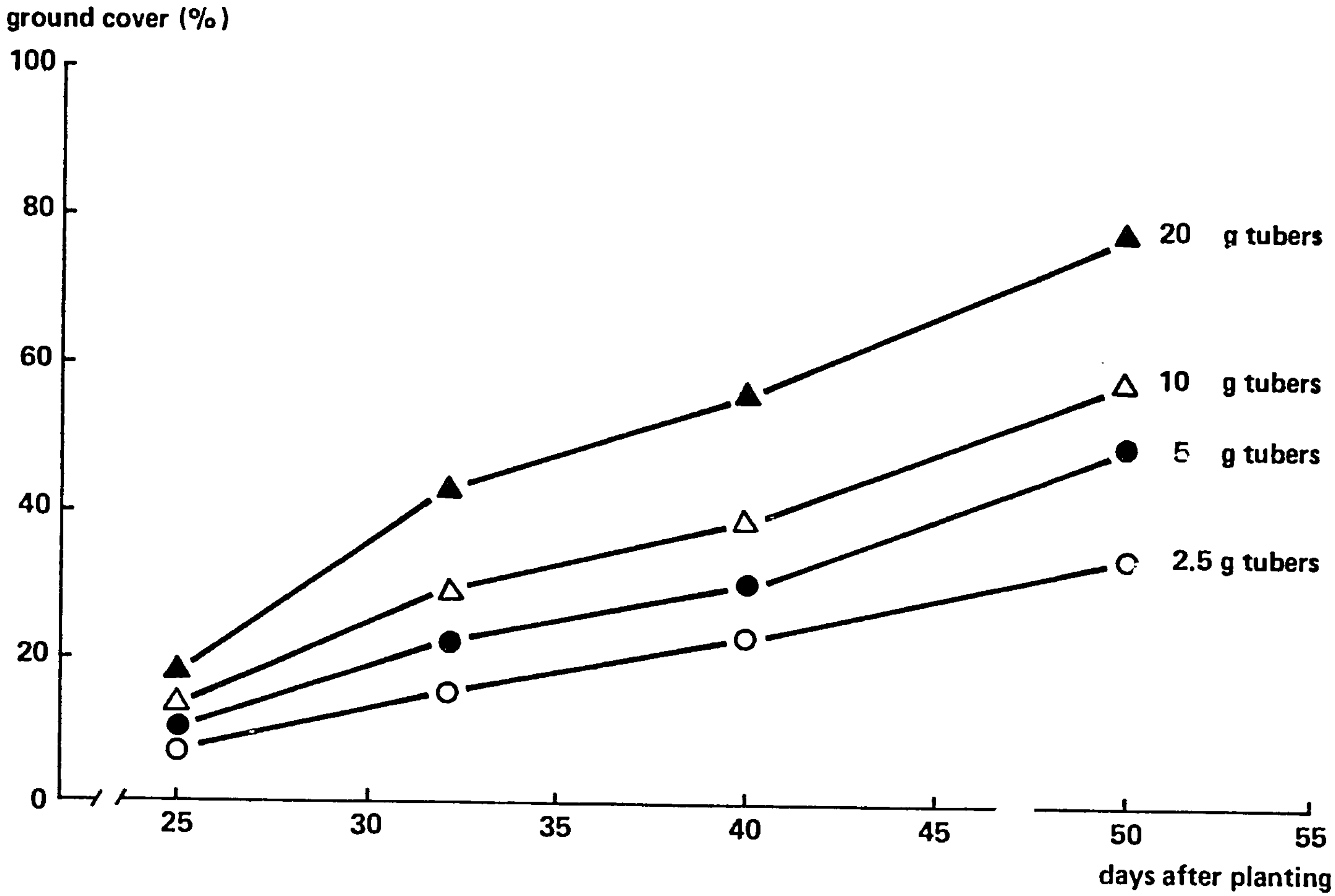

Fig. 6 Effect of weight of single-sprout tubers on rate of ground cover.

are grown in a square pattern. A comparison of different methods to multiply small tubers (Table 9), using similar management practices, shows that yields in the field plots tended to increase with a plant arrangement of decreasing rectangularity. Although plants were planted in a square pattern in field beds and nursery beds, higher yields were obtained in nursery beds due to the use of a sand and peatmoss mix.

Table 9. Yields (kg/m^2) 1/ from small seedling tubers in different planting methods 2/

Seedling tuber weight (g)	PLANTING METHOD 3/			
	1 m wide nursery beds 4/	1 m wide field beds 4/	Single field rows at 40 cm between rows	Double field rows at 80 cm between rows
1-2	7.50	4.44	3.71	4.15
2-5	8.10	5.09	4.57	3.82
5-10	9.59	5.45	5.50	4.86
MEAN	8.41 a 5/	4.99 b	4.59 bc	4.28 c

1/ Yields per unit of total area, including pathways between beds

2/ Progeny: Atzimba x DTO-33

3/ Mean of 11, 16 and 25 plants per m^2

4/ Square plant arrangement

5/ Duncan 0.05

ALTERNATIVE SYSTEMS TO PRODUCE AND MULTIPLY SEEDLING TUBERS

First generation seedling tubers produced in beds are of a sufficient health standard to permit at least one field multiplication. Table 10 shows that one tuber multiplication reduces considerably the nursery area required for the production of first generation seedling tubers. This would reduce managerial requirements since the production of first generation seedling tubers is perhaps more delicate than subsequent multiplications. One tuber multiplication would also take advantage of the high multiplication factor associated with small tuber size. In addition, several experiments have shown that the mean tuber size considerably increased after the first multiplciation. Therefore, it would seem that this system of producing seed tubers from TPS would be more effective when first generation seedling tubers are multiplied at least once.

Table 10. Nursery area (m^2) required to produce 1 ton of seedling tubers

N° multiplications[1/]	Yield in nursery beds 4 kg/m²	6 kg/m²	8 kg/m²
0	250	167	125
1	16.6	11.1	8.3
2	2.2	1.5	1.1

1/ Multiplication factors:

- first generation seedling tubers: 15
- second generation seedling tubers: 7.5

Figure 7 shows three examples of alternative systems of production, multiplication, and distribution of seedling tubers. In alternative 1, individual farmers produce and multiply their own seedling tubers independently. In this scheme the production of seed tubers is completely decentralized with no problems associated with seed tuber transport. In alternative 2, seedling tubers are produced and multiplied by specialized farmers or cooperatives, who then supply seed tubers to producers of consumer potatoes. In this scheme the production of planting material can be more efficiently organized and should result in better quality seed tubers. This scheme requires some distribution of seed tubers. In alternative 3, high quality seedling tubers are produced by a large national institution to be used as basic seed tubers. This scheme requires a rather extensive distribution system for seed tubers. These examples show that with TPS as a source of healthy planting material various systems of seed tuber production can be realized.

CONCLUDING REMARKS

Although the techniques to produce seedling tubers were studied in nursery beds, the production principles also apply to field beds using suitable field soil. In addition, many of the production techniques described here can also be applied to rapid multiplication in beds of vegetative materials such as *in vitro* plantlets, stem cuttings, and sprout cuttings. Similarly, the information on multiplication of small seedling tubers can be directly applied to multiplication of small tubers resulting from rapid multiplication methods.

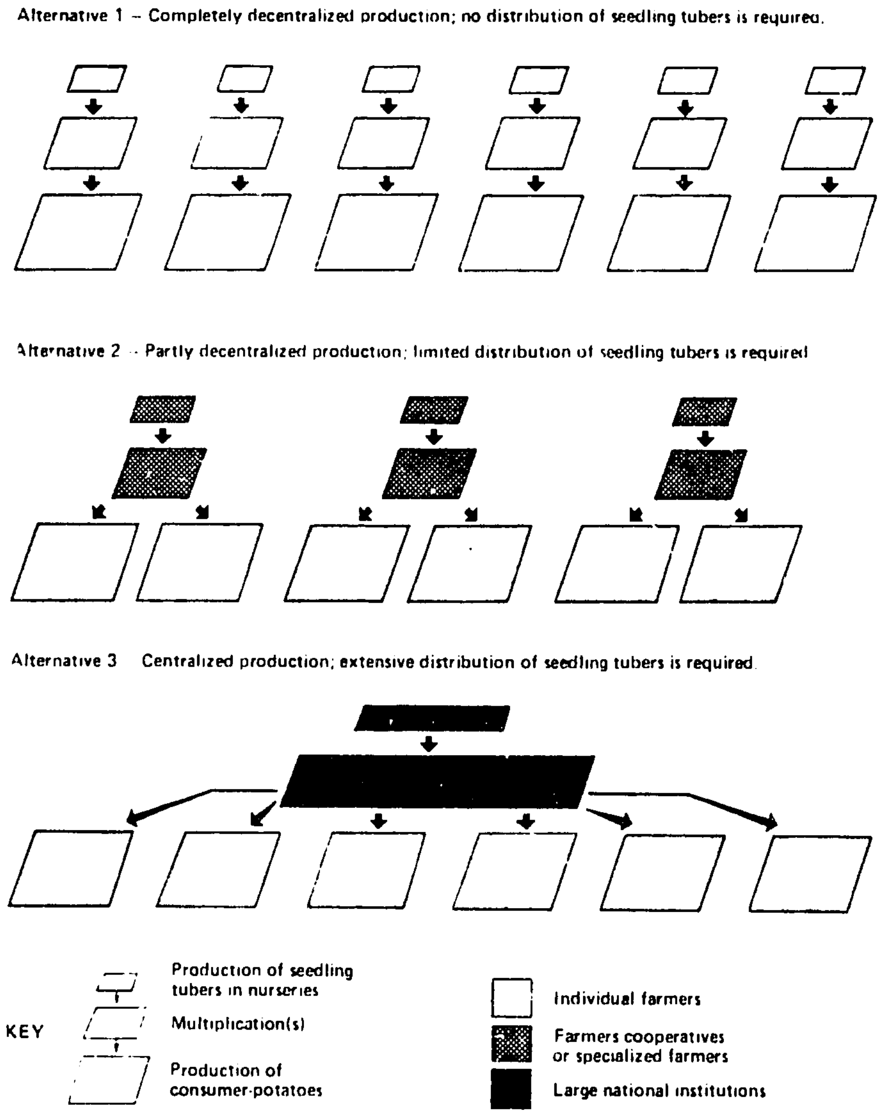

Fig. 7 Three alternative systems of production and distribution of seedling tubers.

Although, in this paper I have concentrated on the production of seed tubers from TPS, the production of consumer potatoes in beds would also seem attractive because of the high amount of dry matter produced per unit area. This may be of particular interest in areas where food preparation practices do not exclude the use of small tubers.

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True Potato Seed Production: Flowering, Quality, and Economics

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INTRODUCTION

Some of the most important components in the success of true potato seed technology for commercial potato production are flowering characteristics of parental material, the quality of TPS produced, and the economics of TPS production. Various reports on potato production from true seed have emphasized that the success of this technology depends largely on the quantity and quality of TPS produced at relatively low cost. These two factors will ensure that farmers of the developing world, who are expected to benefit from this alternative method of potato production, not only get the TPS cheap but are also assured of maximum production.

In this paper, we present our data on flowering, quality, and economics and discuss them in relation to information available in the literature.

FLOWERING

In 1933, Stevenson and Clarke reported that an additional photoperiod was a requirement to aid in potato breeding. Later, Clarke and Lombard (1939) established a definite relationship of day length to flowering and seed production in potato varieties. Their results showed that long photoperiods of 14 to 18 hours are optimal for maximizing flowering and berry set in the genotypes studied. They further reported in 1942, that the number of buds developing in the first inflorescence of the potato is influenced by variety, size of seed piece, and light treatment. Fewer buds were initiated in plants from 5 g seed pieces than from 25 g and 45 g pieces. However, there were no significant differences between 25 g and 45 g pieces for the number of buds initiated.

In the subtropics and tropics where the potato is grown at high altitudes during long summer days (photoperiod varying between 12 to 15 hours), most of the varieties bloom and fertile genotypes set berries. Under conditions in India, both Andigena and Tuberosum varieties, bloom and set berries, when grown during summer (long days), in hills at altitudes between 900 to 3000 m. When grown during the winter (short days) in the plains, however, only a few genotypes bloom and set berries.

Earlier studies (Upadhya, 1983) have shown that among 38 genotypes studied, the number of flowers/inflorescence varied from 6 to 26. Heritability differences and genetic advance (as percent of mean) among the genotypes were 89.93 and 61.82, respectively. This indicated substantial additive genetic effects and also that selection can be effective.

Methods generally used by potato breeders for obtaining flowers and preventing bud abscission are grafting onto tomatoes (Carson and Howard, 1944) and growing on brick (Thijn, 1954).

Studies using three genotypes compared the effect of planting on brick to that of normal planting. The number of flowering bunches/plant and number of flowers/bunch when grown under long days at 2521 m were considered in these studies, the data are presented in Table 1. The three genotypes differed among themselves and planting on brick only increased the number of flowering bunches/plant as compared to normal planting. However, the number of flowers per bunch was not affected in the three genotypes. These observations substantiate other earlier findings (Upadhya, unpublished) that the mean number of flowers/bunch in a genotype is genetically determined. The planting on brick only increases the number of flowering bunches/plant. Subsequent studies have, however, shown that a higher number of flowering bunches/plant increases the competition for the nutrients among them. This adversly affects TPS quantity as well as quality.

Although data on the above two characters (number of flowering bunches/plant and flowers/bunch) were not taken on tomato grafts, observations on average berry weight were recorded in the three genotypes grown by the three methods described above. Observations showed that there is a differential behavior of the three genotypes to methods of growing with regard to average berry weight. Only TPS-5 showed a significant increase in berry weight, when grown on brick or grafted on tomato as compared to normal planting (Table 2).
Bardyug (1981) reported that in the tomato the first truss contributes 30.5% of the total seed yield and the 6th truss only 6%, and that the first four inflorescences were the most productive. Clarke and Lombard (1942) in potato, had shown that single stems from seed pieces weighing 25 to 45 g produced a maximum number of buds in the first inflorescence.

In order to compare berry set and berry size on plants grown from single eye tuber pieces (25-30 g) with plants grown from whole tubers, a trial was conducted by us in the hills (2521 m) during the summer of 1984. The stems were trimmed to retain the first flower bunch only, and the

TABLE 1: EFFECT OF NORMAL AND BRICK PLANTING ON FLOWERING IN THREE GENOTYPES

Method of Planting	Characters		TPS-3	TPS-5	TPS-12
Normal planting	No. of flower bunches per plant	Range	4 to 18	3 to 18	22 to 32
		Average	6	7	27
	No. of flowers per bunch	Range	4 to 35	3 to 23	4 to 18
		Average	16	11	10
Brick planting	No. of Flower bunches per plant	Range	6 to 26	9 to 25	34 to 48
		Average	14	16	43
	No. of flowers per bunch	Range	3 to 39	3 to 24	4 to 26
		Average	16	11	12

bunch was trimmed to retain only the first 6 buds. Observations on plant habit and blooming showed that the majority of single eye pieces gave single thick stems which did not require staking. Whole tubers produced an average of 3-4 stems which required staking. Thus the cost of stakes and labor was reduced. The trimming of flower bunches (retaining 6 buds), induced synchronous flowering, as well as increased bud and flower size. The period between blooming of the first flower and the 6th flower is reduced to 48 hours instead of 4-6 days.

An initial experiment was conducted by us in 1984,to compare the percent berry set in MF-I, when flowers were pollinated thrice with TPS-3 pollen in untrimmed bunches and bunches that had been trimmed to retain 6 flowers/bunch. The results showed that the percent berry set in untrimmed bunches was 69.81%, whereas trimmed bunches gave 86.67% berry set. Also, the average berry weight from trimmed bunches was significantly higher than that from untrimmed bunches. Similarly, self-pollinated berries collected from trimmed and untrimmed bunches of TPS-3 were analyzed for berry weight, number of seeds/berry, as well as TPS quality parameters. The data (Table 3) further confirmed that trimming the flower bunch significantly increased the berry weight and number of seeds/berry. These observations clearly indicated that trimming the flower bunch reduces competition between fruits.

Based on the above observations, therefore, data were taken in the three crosses involving MF-I as female parent raised from cut-seed pieces and whole tubers. The data presented in Table 4 indicate that (1) the percent berry set and average berry weights were similar in the two treatments and, (2) the percentage of berries 2 cm diameter was higher in plants grown from whole tubers than from cut pieces in two out of three crosses. These results thus convinced us so that the stems of all female parents were trimmed to retain a single bunch/stem and 5-6 flowers/bunch for all crosses attempted during this season of summer of 1984.

Studies are being carried out on the stigma characteristics and duration of receptivity. Initial results obtained from Scanning Electron Microscopic studies of the stigmatic surface of TPS-3, at different stages of development, revealed papillae-like structures. At the very young stage (closed bud), the papillae have no stalk-like structures (Fig. 1). These develop during bud maturation and at the time stigma is most receptive, the papillae had developed erect stalks, become enlarged, and looked fully filled as well as bent to one direction (Fig.2). At later stages (2 days after anthesis), these papillae became highly shrivelled (Fig. 3). The shape and size of the

TABLE 2: EFFECT OF NORMAL AND BRICK PLANTING AND GRAFTING ON TOMATO, ON THE AVERAGE BERRY WEIGHT IN THREE GENOTYPES

Genotypes	AVERAGE BERRY WEIGHT (g)		
	Normal planting	Brick planting	Grafting on tomato
TPS-3	16.505	17.331	14.550
TPS-5	5.271	7.980	10.983
TPS-12	5.520	8.149	5.422

TABLE 3: EFFECT OF TRIMMING OF STEM AND FLOWER BUNCH IN TPS-3 ON BERRY SIZE, NUMBER OF SEEDS/BERRY AND OTHER TPS QUALITY PARAMETERS.

Treatment	Percent of berries in 2cm class	Average berry wt. (g)	Average seeds/ berry	Average 100 seed wt. (mg)	Proportional of different embryo types				Seeds in 1/18" class
					A	B	C	D	
Trimmed (stem and bunch)	94.5*	16.54*	293	63.3*	35.2	58.7	0.4	5.7	79.7*
Untrimmed (bunch only)	46.3	12.27	292	57.2	26.7	66.9	0.4	6.0	57.5
Bulk Harvest	22.5	10.84	175	52.6	22.9	72.9	0	4.2	29.7

* Significant at $p = 0.05$

TABLE 4: EFFECT OF RAISING THE MOTHER PLANT FROM WHOLE TUBERS OR SINGLE EYE PIECE ON BERRY SET, BERRY SIZE AND WEIGHT, NUMBER OF SEEDS/ BERRY AND TPS PARAMETERS.

Cross	Type of Planting material	Percent berry set	Percent of berries 2 cm class	Average berry weight (g)	100 seed weight (mg)	Average number of seeds/ berry	Percent of browned seeds	Proportion of seed types (%) A	B	C	D	Seeds in 1/18" class (%)
MF-I X TPS-7	CP	54.9	8.2	13.3	53.7	323	0.04	61.7	34.7	0.2	3.4	53.7
	WT	55.6	13.4	14.1	55.0	366	0.62	65.0	32.3	0.2	2.5	44.2
MF-I X TPS-13	CP	65.4	10.9	14.7	57.5	357	0.91	59.3	36.3	0.4	4.0	47.6
	WT	71.1	7.2	14.9	62.9	278	3.71	69.3	28.2	0	2.5	69.8
MF-I X TPS-29	CP	60.0	6.5	12.2	51.7	348	2.70	58.7	35.9	0.1	5.3	37.7
	WT	78.8	12.9	14.6	58.7	307	2.17	58.7	37.8	0	3.5	68.7
LSD (0.05)				1.41	6.11	52.4						

CP = Single eye pice
WT = Whole tuber

Material was planted in the field on flat beds. Single flowering bunch was per stem with only six flowers was retained. The flowers were emasculated followed by pollination thrice.

Fig.-1

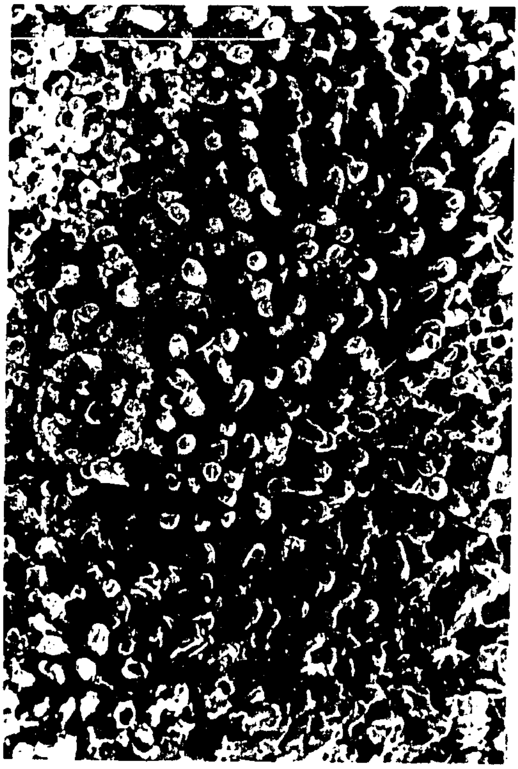

Fig-2

Fig-3

Figs.1 to 3: Scanning Electron Micrographs of the stigmatic surface of potato genotype TPS-3 at three stages of development:

Fig-1: At closed bud stage.

Fig-2: At fully receptive stage before anthesis showing fully developed papillae like structures.

Fig-3: At 2 days after anthesis showing complete abortion of papillae like structures.

papillae seem to have a direct relationship to the receptivity of the stigma. Preliminary calculations indicate that the duration of the receptivity phase of the stigma in TPS-3 lasts about 18-36 hours, from the time the papillae are fully developed to the time when shrivelling starts.

The potato belongs to the group of plant species that have a multiovular gynoecium. Initial counts of the number of ovules per gynoecium indicate about 1000 to 1200 (Fig. 4). But a maximum of only 30% to 40% of them get fertilized and develop into seeds. This proportion of fertilized ovules is always toward the styler end of the fruit. The unfertilized ovules are toward the peduncle end, which later gets covered by the developing placental tissue and show as a compacted mass (Figs. 5 and 6).

Since the potato has a multiovular gynoecium, an optimum pollen load is necessary for maximum seed set. To find the optimum amount of pollen load that gives the highest number of seeds, we pollinated emasculated flowers (6 flowers/bunch) one, two and three times during the receptivity period of the stigma. Table 5 presents the percentage of berry set, mean berry weight, and number of seeds/berry. The data clearly indicate that three pollinations resulted in the highest berry set, the largest berries, and the highest number of seeds/berry. Therefore, as a routine for all crosses, the female flowers are now pollinated three times.

A preliminary survey was done on 10 genotypes for their open-pollinated (OP) berry size, number of seeds/berry, and average 100-seed-weight. There was a highly significant and positive correlation ($r = +0.72$) between average berry weight and number of seeds/berry; positive but non-significant value ($r = 0.23$) for berry weight and 100-seed-weight, whereas negative and significant value ($r = -0.6$) for 100-seed-weight and number of seeds/berry (Table 6).

Efforts have also been made to study the effect of increased N and P applications on the fertility of the flower as indicated by the average berry weight and number of seeds/berry. Single eye pieces (25-30 g) of TPS-3 were planted in flat beds, and six combinations of N and P doses were applied at the rate of 120, 240, and 360 kg/ha of N, and 80 and 160 kg/ha of P. When the plants began to flower, only the first flowering bunch was retained per stem and the bunch was trimmed to keep the first six buds. Self-pollinated berries were collected at the mature green stage, and berry weight was taken from each treatment to calculate the average berry weight. The seeds from individual berries were extracted

Fig-4

Fig-5

Fig-6

Figs.-4 to 6: Photomicrographs of dissected overy of potato at different stages of development.

Fig-4: Exposed placenta showing ovules one day before opening of the flower.

Fig-5: Stage of development of fertilized and unfertilized ovules 6 days after pollination.

Fig-6: Stage of development of fertilized and unfertilized ovules 25 days after pollination.

TABLE 5: EFFECT OF ONCE, TWICE AND THRICE POLLINATION OF EMASCULATED POTATO FLOWERS ON BERRY SET, AVERAGE BERRY WEIGHT AND NUMBER OF SEEDS/BERRY. (TPS-3 POLLINATED WITH ITS OWN POLLENS)

Pollination	Percent berry set	Range of berry weight (g)	Mean berry weight (g)	Average number of seeds/ berry
Once	27.5 b	1.5 - 4.5	2.86 c	35 c
Twice	33.75 b	3.0 - 7.0	5.56 b	80 b
Thrice	71.00 a	8.0 -11.0	9.52 a	217 a

Duncan's multiple range test significant at 1%.

TABLE 6: RELATIONSHIP AMONG AVERAGE BERRY WEIGHT, NUMBER OF SEEDS/BERRY AND 100 SEED WEIGHT (GROWN AT 2521 m).

Genotype (OP)	Average berry weight (g)	Average number of seeds per berry	Average 100 seed weight (mg)
TPS-3	11.53	210	66.80
TPS-6	5.76	206	56.25
TPS-7	6.29	86	83.75
TPS-10	6.72	235	52.30
TPS-12	3.50	109	58.20
TPS-13	7.15	111	63.50
TPS-14	4.67	176	50.80
TPS-30	3.63	220	28.80
MF-I	11.93	341	52.80
MF-III	4.22	93	68.00
	X	Y	Z

Correlation coefficients (r): $_{XY} = 0.72^{**}$, $_{XZ} = 0.23$ ns, $_{YZ} = -0.6^{*}$

* Significant at P = 0.05 ** Significant at P = 0.01

after ripening them, and the average number of seeds/berry was calculated. Statistical analysis of the data, using Bartlett's test, showed that the differences among the treatments for average berry weight and number of seeds/berry were nonsignificant.

TPS QUALITY

The quality of a seed is determined by seed vigor which is generally defined as the superior performance of a genotype after planting, compared to the same genotype or other genotypes under defined experimental conditions (McDaniel, 1973). Several reports indicate the effort of research workers working on TPS to define the components of seed quality.

Dayal et al. (1984) have shown that there is a positive and significant correlation between 1000 seed weight and tuber yield/plant and tuber number/plant, and a positive but nonsignificant correlation between 1000 seed weight and average tuber weight. Efforts have therefore been directed towards understanding the variability in seed weight of TPS and the proportion (percentage) of different classes of seed sizes within and between populations. Such an approach is expected to yield results that could be used to define two of the components of TPS quality; namely, average size and weight of seeds in a TPS sample.

Data taken on different berry sizes (according to their diameters) in OP berries of TPS-36 showed a direct relationship of berry size with number of seeds/berry, 1000 seed weight, and proportion of bolder seeds (Table 8). Based on these observations, we decided to sieve out berries of 2 cm diameter and only use berries 2 cm size for TPS extraction and studies of quality parameters in hybrid and OP populations.

Observations in different crosses were also taken to work out the correlations between berry weight with the number of seeds/berry and 100-seed-weight. The data on 18 crosses made in the plains (112 m) are presented in Table 9. A positive and highly significant value of r = +0.71 was found for the correlation between berry weight and number of

seeds/berry. A positive but nonsignificant correlation (r = 0.47) was found between berry weight and 100 seed weight. However, unlike the OP berries, where a strong negative correlation had been found between the number of seeds/berry and 100 seed weight (Table 6), no correlation could be established between these two characters in berries from 18 crosses (Table 9). We therefore, assume that in the case of hybrid TPS, these two characters are independent and that the female parent and the

TABLE 8: EFFECT OF BETWEEN BERRY SIZE ON SEEDS/BERRY AND TPS CHARACTERISTICS IN TPS-36 (OP) GROWN UNDER NORMAL PLANTING AT 1219 m.

Berry size (diameter)	Average seeds per berry	Average 100 seed weight (mg)	Percentage of seeds in 1/18" class	Proportion of different embryo type seeds (%)			
				A	B	C	D
1 cm	47	37.2	0	9.6	70.2	0	20.2
1 cm - 1½cm	169	38.4	32.3	7.0	72.5	1.2	19.3
1½cm - 2 cm	139	49.3	28.5	10.2	67.0	1.2	21.6
2 cm	250	43.2	26.5	29.1	53.3	1.5	16.1

Average values of 15 berries in each size class

TABLE 9: RELATIONSHIP AMONG AVERAGE BERRY WEIGHT, NUMBER OF SEEDS/BERRY AND 100 TPS WEIGHT IN 18 CROSSES MADE IN PLAINS (112 m)

Cross	Average berry weight (g)	Average number of seeds/berry	Average 100 seed weight (mg)
MF-II x MF-III	8.73	224	67.3
MF-II x TPS-3	7.82	241	73.3
MF-II x TPS-13	5.16	140	72.7
MF-III x TPS-13	6.74	306	74.7
TPS-3 x MF-III	9.03	385	59.5
TPS-4 x TPS-3	4.37	137	53.2
TPS-6 x MF-III	3.59	97	49.5
TPS-6 x TPS-3	9.86	318	71.4
TPS-6 x TPS-13	2.54	91	49.3
TPS-7 x MF-III	7.18	246	68.5
TPS-7 x TPS-3	5.92	193	74.5
TPS-7 x TPS-13	5.64	189	65.0
TPS-12 x MF-III	5.56	185	64.8
TPS-12 x TPS-3	4.59	200	56.3
TPS-12 x TPS-13	5.02	208	49.1
TPS-13 x MF-III	7.05	191	72.0
TPS-13 x TPS-3	5.43	156	60.1
TPS-37 x TPS-3	8.65	127	52.7
	X	Y	Z

Correlation coefficients (r):

r_{XY} = 0.71** r_{XZ} = 0.47 ns, No correlation was found between Y and Z.

** Significant at P = 0.01

male parents determine the number of seeds/berry indepently of 100 seed weight. Furthermore, it also shows that in crosses a high number of seeds/berry having a high 100 seed weight can be obtained.

The data given in Table 10 also show that male and female parental lines show variation in their genetic potential in affecting the number of seeds/berry and 100-seed-weight. To further substantiate this characteristic of female and male parental lines in another environment, observations were recorded in crosses made at high altitude (2521 m) during summer (long days) (Table 10). The observations on the number of seeds/berry, 100-seed-weight, and proportion of seeds in 1/16" class clearly indicate that the three characters are highly dependent on not only the female parent, but also on the male parent.

Six crosses were made in the hills (2521 m) during natural long days and in the plains 112 m during winter with an additional photoperiod to observe the effect of the two contrasting environments on the above-mentioned three characters (seeds/berry, 100-seed-weight, and 1/16" seed fraction) (Table 11). In general, the values for the three characters were higher for the high altitude than the low altitude. The higher value of the two quality components (100 seed weight and proportion of bolder seeds) showed that the hybrid TPS produced at high altitudes under natural long days were better than those produced at low altitudes with additional photoperiod from artificial light. However, extensive selection efforts for suitable cross combinations would allow the production of good quality hybrid TPS at low altitudes by giving additional photoperiod, provided the temperatures are conducive during fertilization, berry and seed maturation.

Upadhya et al. (1981) described an additional quality component of TPS concerning the type of embryo shape of the seed. Later, data were presented to show that A-type seed with a cercinnate embryo give a higher percentage of germination than B-, C- and D-types, and also that the seedlings from the A-type are more vigorous and have higher fresh and dry weights than other types (Upadhya, 1983). To further evaluate the differences among different embryo type seeds, 100 seed weights of A-, B- and D-type seeds of medium size (1/16"-1/18") from TPS-7 (OP) were taken. Analysis of the data showed significantly higher 100-seed-weights of A- and B-types, than the D-types (Table 12). The A- and B-types, however, did not differ significantly, though the mean value of the B-type was found to be lower than that of the A-type. The lengths and dry weights of A- and B-type embryos were taken after dissection from seeds of TPS-2 (OP). Statistical analysis of the data (Table 13) showed that though the lengths of A- and B-embryos do not differ significantly, the dry weight of A-type embryos is significantly higher than that of the B-type.

TABLE 10: EFFECT OF MALE PARENTS ON THE TPS CHARACTERISTICS OF HYBRID SEEDS FROM SINGLE FEMALE PARENT GROWN UNDER NORMAL PLANTING AT 2521 m AND POLLINATED THRICE AFTER EMASCULATION

Female parent	Male parent	Average seeds per berry	Average 100 seed weight (mg)	Seeds in 1/16" class (%)	Proportion of different embryo type seeds			
					A	B	C	D
MF-I	MF-III	179	73.0	43.0	70.1	25.5	0.5	3.9
	TPS-3	203	75.3	46.5	49.1	48.4	0.4	2.1
	TPS-5	151	69.8	-	59.1	39.1	0	1.8
	TPS-6	236	74.0	-	54.7	43.3	0.7	1.3
	TPS-7	345	45.0	-	41.7	52.1	0	6.2
	TPS-11	180	68.8	40.0	55.0	40.4	0	4.6
	TPS-13	163	64.5	14.5	66.2	31.0	0	2.8
	TPS-29	317	56.7	10.0	47.1	51.9	0	1.0
	TPS-30	285	52.8	50.2	28.0	65.5	0	6.5
	OP	413	49.3	14.6	54.1	40.2	0	5.7
MF-II	MF-1	84	83.6	52.9	67.5	30.5	0	2.0
	MF-III	69	96.3	95.6	60.4	36.9	0	2.7
	TPS-3	250	85.6	40.5	59.7	38.9	0.6	0.8
	TPS-11	192	87.2	83.9	74.8	24.4	0	0.8
	TPS-13	53	91.5	87.5	78.6	21.4	0	0
	TPS-30	224	90.5	86.5	44.8	50.1	2.1	3.0

TABLE 11: COMPARISON OF DIFFERENT TPS PARAMETERS IN HYBRID TPS PRODUCED IN HILLS (2521 m) AND IN PLAINS (112 m).

Cross	Location	Average number of seeds/ berry	Average 100 seed weight (mg)	A type seed fraction (%)	Seeds in 1/16" class (%)
MF-II X MF-III	Hills	169	96.3	60.4	95.6
	Plains	224	69.0	54.5	58.9
MF-II X TPS-3	Hills	251	85.6	60.1	88.5
	Plains	167	74.6	32.8	71.4
MF-II X TPS-13	Hills	53	91.5	78.6	87.5
	Plains	140	76.3	60.0	74.2
TPS-7 X MF-III	Hills	222	72.2	46.9	67.6
	Plains	246	67.0	37.2	34.1
TPS-7 X TPS-3	Hills	212	79.5	24.5	85.5
	Plains	193	66.5	30.7	62.5
TPS-7 X TPS-13	Hills	222	71.0	45.0	71.9
	Plains	189	68.5	48.2	30.3

TABLE 12: COMPARISON OF AVERAGE 100 SEED WEIGHTS OF DIFFERENT EMBRYO TYPE SEEDS OF TPS-7 (OP) TAKEN FROM MEDIUM SIZE (1/16" - 1/18") GROUP

Type	Average 100 seed wt. (mg)
A type	72.0 a
B type	69.7 ab
Bulk	68.5 b
D type	60.7 c

Duncan's Multiple range test significant at 5%.

TABLE 13: COMPARISON OF EMBRYO LENGTH AND DRY WEIGHT FROM MEDIUM SIZE A AND B TYPE SEEDS OF TPS-2 (OP).

Embryo type	Length (mm)	Dry weight (25 embryos) (mg)
A	3.07	1.27
B	3.38	0.85
t test	non-significant at P = 0.01	significant at P = 0.01

Biochemical analysis was carried out for certain TPS constituents such as carbohydrates, soluble proteins, total lipids and phospholipids. The A-, B-, and D-type seeds, as well as different sizes of seeds, were analyzed using standard analytical procedures (Tables 14 and 15). The results showed that, in general, the bolder seeds had higher contents of these constituents. A- and B-type seeds did not differ in their total lipid and phospholipid content; whereas, the A-type generally had higher soluble proteins, and the D-type showed the lowest amount of total sugars and soluble proteins.

The data presented in tables (Tables 3 to 11 and 16), also have a column showing the proportion of different embryo type seeds as percentages of the TPS sample studied from a treatment.

In evaluating the effects of applying higher doses of N and P to the mother plants given in Table 7, TPS quality parameters were also considered. The increased doses of N and P reduce the proportion of A type seeds, but the treatment of 240 Kg/ha N and 160 Kg/ha of P increased the proportion of A type over the base dose. These observations show a positive interaction of N and P with this TPS quality component. The effects on 100 seed weight due to doses of nitrogen applied and interactions between N and P doses were found to be significant at the 5% level; whereas the effects on 100 seed weight, due to P doses alone, were found to be highly significant at the 1% level when the data were subjected to Bartlett's test. Therefore, higher doses of N and P seem to have a detrimental effect on 100-seed-weight.

Similarly, the size of the berry also shows a relationship with the proportion of different embryo-type seeds. As is shown in Table 9, the percentage of A-type seeds increases with the increase in berry size. Therefore, the reduction in berry size occuring due to nutritional imbalance affects the proportion of A-type seeds. Or if berry size is purely the result of number of ovules fertilized, as reflected in the number of mature seeds/berry, then the proportion of A-type seeds is also affected. However, if both nutritional imbalance and the number of ovules fertilized are having an interaction, then too the proportion of A-type seeds is affected.

The data presented in Table 11 also give a clear indication that in hybrid TPS, the interaction between female and male genotypes, as well as the genetic constitution of zygotes, determines the percentages of different embryo-type seeds.

TABLE 7: EFFECT OF INCREASED N AND P TO THE MOTHER PLANTS OF TPS - 3 ON BERRY WEIGHT, NUMBER OF SEEDS/BERRY AND TPS PARAMETERS.

Treat-ments	Average berry weight (g)	Average number of seeds per berry	Average 100 seed weight (mg)	Proportions of different embryo type seeds (%)				Seeds in 1/18" Class (%)
				A	B	C	D	
N_1P_1	14.37	271	70.4	28.5	63.3	0.8	7.4	82.2
N_1P_2	14.47	295	63.9	19.4	71.5	0.7	8.4	73.8
N_2P_1	12.67	251	63.6	21.4	71.5	0.2	6.9	77.4
N_2P_2	13.40	223	64.1	32.7	61.4	0	5.9	69.3
N_3P_1	13.20	252	66.8	16.4	72.0	0.4	11.2	69.3
N_3P_2	12.60	238	58.1	22.7	63.7	0.4	13.2	58.6
LSD (0.05)	2.27	57.6	4.52					

N_1 = 120 kg/ha, N_2 = 240 kg/ha, N_3 = 360 kg/ha

P_1 = 80 kg/ha, P_2 = 160 kg/ha

Single eyed tuber pieces planted in flat beds at 10 x 60 cm spacing. One flowering bunch per stem with six flowers was retained.

TABLE 14: BIOCHEMICAL ANALYSIS OF TPS FOR CARBOHYDRATES AND SOLUBLE PROTEINS (MG/G DRY WEIGHT OF SEEDS) IN TPS-2 (OP) SEEDS

Size	Total sugars	Total reducing sugars	Total starch	Soluble proteins
Medium	40.3	22.7	0.027	22.75
Small	34.25	18.9	0.024	15.75
t test	ns	ns	ns	ns
Medium				
Type A	16.0	10.1	0.016	36.0
Type B	15.0	14.0	0.012	23.0
Type D	10.0	10.0	0.012	20.0

(Average of 4 years sample of seeds)

TABLE 15: ANALYSIS OF TPS FOR TOTAL LIPIDS AND TOTAL PHOSPHOLIPIDS CONTENTS (MG/G DRY WEIGHT OF SEEDS) OF TPS-7 OP SEEDS

Components	Medium size		Different seed sizes				
	A type	B type	Extra Large 1/14"	Large 1/14"- 1/16"	Medium 1/16"- 1/18"	Small 1/18" 1/20"	Very Small 1/20"
Total Lipids	29.70	31.18	21.30	24.60	9.66	8.23	-
Total phospholids	4.690	5.172	3.412	3.307	1.841	1.391	0.144

The altitude at which the mother plant is grown for hybrid TPS production also affects the proportion of A-type seeds (Table 11). Hybrid TPS produced in the hills (2521 m) showed in general, a higher proportion of A-type seed fraction than those produced in the plains (112 m). This effect, however, depends on the female and male parents involved in a cross.

TPS from TPS-3 (OP) were analyzed for the proportion of different embryo-type seeds produced from plants grown under normal planting, planted on brick, and grafted on tomato (Table 16). The data show that the percentage of the seeds of different embryo types is affected by these methods. Normal planting gives a higher percentage of A-type than planting on brick or grafting on tomato.

In the experiment for evaluating effects of trimming vs. nontrimming of flowering stem and flowering bunch on the quantity and quality parameters of TPS, the data are presented in Table 3. The results show that along with other parameters, trimming also improves the proportion of A-type seeds. This effect can be attributed to the fact that trimming of the stem to retain a single flowering (and retaining only the first six buds) evidently increases the supply of nutrients to the buds, flowers and berries during development by reducing the competition from other bunches and among berries. Thus, this single operation contributes to improving the proportion of large berries, higher 100-seed-weight, higher proportions of bolder seeds and A-type seeds.

In the hybrid TPS produced for comparing the effects of growing the mother plant from either a single eye piece or a whole tuber, observations were also made on the proportion of different embryo-type seeds (Table 4). Data show that growing the mother plant from single eye pieces as compared to plants grown from whole tubers increases the proportion of C- and D-type seeds and slightly reduces the proportion of A-type seeds, depending on the male parent used.

During the screening of hybrid and OP TPS from various crosses and genotypes, we observed that there were differences among different genotypes and crosses with regard to the percentage of browned seeds. These browned seeds are of two types:

a) those empty seeds where the embryo aborts at an early stage when very little endosperm development has taken place, leading to the browning of tissues inside (Fig. 7);
b) those seeds that have well-developed embryo and endosperm, but during maturation some event triggers a reaction that leads to various degrees of browning of either endosperm tissue or of embryo or both (Fig. 8).

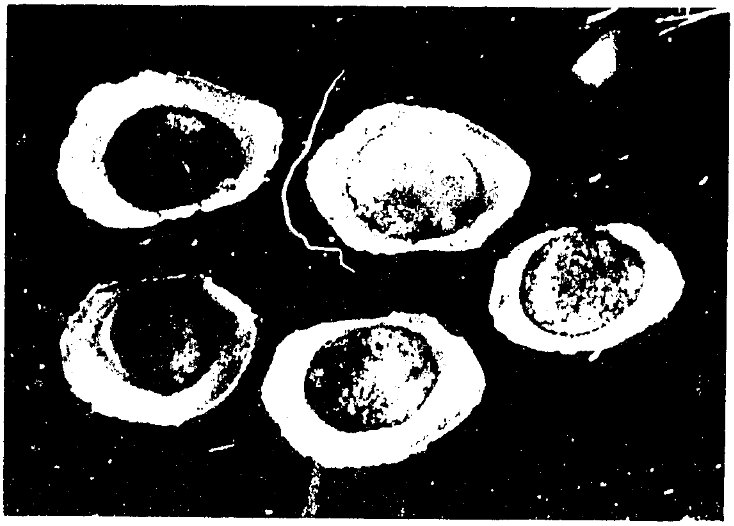

Fig-7

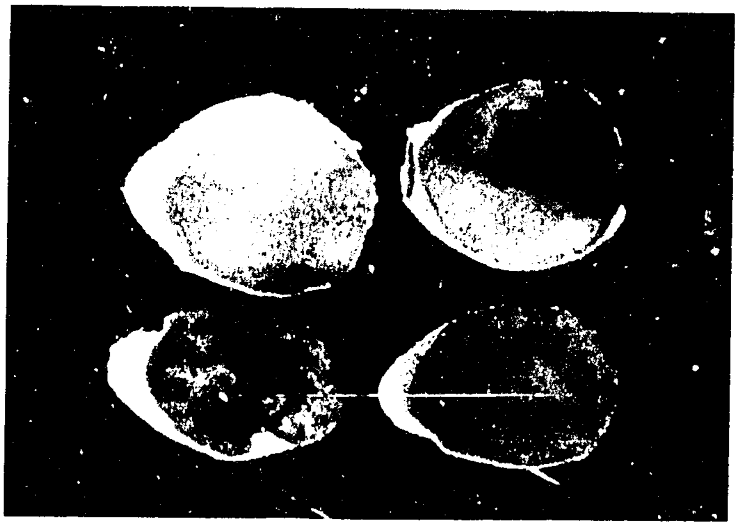

Fig-8

Figs.- 7 and 8: Photomicrographs of two types of browned seeds

Fig.-7 : Browned seeds with very little endosperm and in which embryo had aborted at a very early stage.

Fig.-8 : Browned seeds with full endosperm and embryo development.

TABLE 16: COMPARISON OF THE PROPORTION OF DIFFERENT EMBRYO TYPE SEEDS IN TPS-3 (OP) WHEN RAISED UNDER THREE METHODS.

Methods	Proportion of different embryo type seeds (%)			
	A	B	C	D
Normal planting	5.2 a	87.8 a	0	7.0 b
Planted on brick	6.4 a	86.0 a	0	7.6 b
Grafted on tomato	1.4 b	87.1 a	0	11.5 a

Duncan's Multiple Range Test significant at 1% level.

When these two types of seeds were kept for germination, the first category of seeds did not germinate. But the seeds in the second category either did not germinate or showed little emergence of the radicle which was followed by death. These observations suggest that the proportion of such browned seeds would affect the overall quality of a TPS sample by reducing percentage germination.

Data were recorded on the presence of browned seeds in hybrid TPS, using MF-I as the female parent, grown either from single eye pieces or whole tubers, and pollinated with three pollen parents. Table 4 shows that plants grown from whole tubers produce in general, a higher percentage of browned seeds. Also, TPS-29 as male parent produced a higher percentage of browned seeds in hybrid TPS; and TPS-7 when used as male parent produced the lowest percentage of browned seeds. Hence, in the future, TPS samples should also be routinely analyzed for the proportion of browned seeds when they are being analyzed for the effects of different treatments and crosses for assessing overall quality.

The seedlings raised from A- and B-type seeds from five crosses were transplanted in a trial to evaluate the differences of plant characteristics and yield potential. The data (Table-17) indicate that the A-type seedlings attained more height and higher number of leaves than the B-type, and that the average yield/plant as well as number of tubers/plant from the A type seedlings were higher than in the B-type.

There is a paucity of published literature on the effects of various factors influencing the quality and quantity of TPS. The data in this report are presented as a directional step toward first defining some of the components of TPS quality and second, in evaluating the effects of various factors both intrinsic (including genetic) and environmental which determine the quality of TPS.

COST OF HYBRID TPS PRODUCTION

It is essential to have estimated cost of TPS production to work out the cost of producing a commercial potato crop. An estimate will enable this alternative technology to be evaluated in comparison with the use of seed tubers.

Studies during the last two years have indicated that for the commercial exploitation of hybrid TPS production, the production of 100 berries after emasculation of female flowers before pollination needs about 3½ times more number of man-hours, when compared to pollination without emasculation. The above conclusion is based on the following calculations:

TABLE 17: AVERAGE SEEDLING HEIGHT AT FLOWERING, NUMBER OF LEAVES ON THE MAIN STEM, TUBERS/PLANT AND YIELD/PLANT OF A AND B TYPE SEEDS (MEDIUM SIZE) OF FIVE CROSSES GROWN IN MID HILLS

Genotype	TPS-19 X TPS-5		TPS-2 X TPS-6		TPS-2 X TPS-5		TPS-4 X TPS-5		TPS-18 X TPS-5	
Character	A	B	A	B	A	B	A	B	A	B
Seedling height at flowering (cm)	80.22	74.17	73.83	56.97	76.33	63.57	87.68	80.33	78.77	77.00
Leaves on main stem (number)	29.90	27.30	26.70	23.00	27.70	24.60	29.20	27.40	27.80	26.40
Tuber/plant (number)	12.67	9.00	10.67	8.67	12.67	12.00	12.33	10.33	14.00	11.33
Yield/plant (g)	399.30	303.30	457.30	375.20	466.30	356.00	408.30	303.00	360.70	313.00

1) Average total time taken in male flower collection and pollen extraction:
   a) collection of 100 flowers - 15 minutes
   b) pollen extraction from 100 flowers:
      - manually - 20 minutes
      - using mechanical vibrator - 16 minutes

2) Time in man-hours from male flower collection, pollen collection, and thrice pollination of unemasculated flowers of TPS-2 with TPS-3 pollen:

Total number of female plants used	= 12
Total number of flowers pollinated	= 478
Total number of berries set	= 341
Total man-hours spent	= 6½ hr
Percent berry set	= 71.3
Man-hours spent on producing 100 berries	= 1 hr and 55 min

3) Time in man-hours from male flower collection, pollen extraction, emasculation and thrice pollination of TPS-7 with TPS-3 pollen:

Total number of female plants used	= 12
Total number of flowers pollinated	= 255
Total number of berries set	= 240
Total man-hours spent	= 17½ hr
Percent berry set	= 94.1
Man-hours spent on producing 100 berries	= 7 hr and 20 min

Therefore, production of hybrid TPS by resorting to pollination of flowers without emasculation, appears to be a very promising method. There are, however certain prerequisites for adopting this time-saving method. The most important one for present consideration is that the female parent should be a producer of OP progenies, which are reasonably vigorous, fairly homogenous for plant and tuber characters, and comparably good yielders. This is desirable because even though there is a certain percentage of selfed seed among the hybrid seed, the production and quality of the produce would not be severely affected.

These conclusions are substantiated by recorded observations on the average number of seeds/berry and three of the TPS quality components as affected by three pollinations of emasculated and non-emasculated flowers of TPS-7 with TPS-3 pollen, in the hills (2521 m) under normal planting (Table 18).

TABLE 18: EFFECT OF EMASCULATION AND NON EMASCULATION OF FLOWERS ON SEED SET AND QUALITY PARAMETERS IN HYBRID TPS (TPS-7x-TPS-3) PRODUCED AT 2521 m UNDER NORMAL PLANTING.

Flower Treatment	Average seeds/ berry	Average 100 seed weight (mg)	Percentage age of seed in 1/16" class	Proportion of different embryo type seeds			
				A	B	C	D
With Emasculation	212	79.5	85.8	24.5	58.0	2.4	11.0
Without Emasculation	175	89.6	78.4	26.0	68.0	1.6	4.4

For the purpose of calculating the amount of hybrid TPS which can be produced from one hectare, two assumptions are made based on the practical experience gained during the last two years: 1) a minimum of 200 seeds/berry having an average 100-seed-weight of 100 mg are obtained, and 2) five berries/bunch are harvested from a single stem. Based on these two assumptions, the total amount of hybrid TPS that can be produced from one hectare would be:

One berry produced 200 seeds = 200 mg
Single bunch/stem produces 5 berries
Total amount of seeds from 5 berries = 1 g
Desirable stem density of female parent for hybrid TPS production has been found to be 150,000/ha at stem density of $15/m^2$.
150,000 stems 5 berries/stem will produce 7.5×10^5 berries.
7.5×10^5 berries will give $\frac{1}{5} \times 7.5 \times 10^5$ or
150 kg/ha.

Cost of hybrid TPS production in US$ under Indian conditions of labour and land:

	With emasculation	Without emasculation
Labor (man days/ha) (for emasculation and/or pollination, berry collection and seed extraction)	6,000.00	2,200.00
	US $	US $
Labor cost 1.5 US$ per day	9,000.00	3,000.00
Cost of cultivation of one hectare of female parent	900.00	900.00
Cost of cultivation of ¼ hectare of male parent	225.00	225.00
Total cost in US$	10,125.00	4,425.00
Returns from the sale of 25t of potato from 1¼ ha for consumption (60 US$ per ton)	1,500.00	1,500.00
	8,625.00	2,925.00

Cost per kg of hybrid TPS if 150 kg/ha is produced/ha	57.50	19.50
Cost of 100 g of hybrid TPS (required for planting one hectare)	5.75	1.95

The estimated cost of production show that for 100 g of hybrid TPS, enough for planting one hectare of transplanted seedling crop, would be US$5.75 if emasculation of female flowers is carried out and US$1.95 if the flowers are pollinated without emasculation.

The ratio between the costs of 100 g of hybrid TPS following the two methods is 1: 2.95. In other words the quantity of 100 g of hybrid TPS would be 1/3 times cheaper if pollination without emasculation is used instead of emasculation of female flowers.

SUMMARY

Studies have been conducted to understand the effects of altitude (climate), photoperiod and manipulation of the female parent on berry set, size of berry, number of seeds/berry and size of seeds as well as on some quality components of TPS. The results show that there is a differential response of the female parent depending on the genotypes.

However, in general, high altitude, cooler climate and 14-16 hours photoperiod favour better blooming, higher berry set, larger size of berries with higher seed number and bolder seeds along with higher proportions of A-type seeds.

These parameters are also affected by the doses of nitrogen and phosphorous applied to the mother plant, the degree of competition within and between flowering bunches, and the genetic make up of the female and male parents.

Observations reported also indicate that suitable parents can be selected which can produce higher quantity and quality of hybrid/open pollinated TPS. The quantity and quality of TPS can be improved by suitable doses of N and P as well as by reducing the competition within and between flowering bunches if the first truss per stem, with only first six flowers, is retained. Resorting to the use of single eyed pieces instead of whole tuber not only reduces the quantity of tuber seeds used per hectare, but also results in the production of plants with single thick stems which do not require staking. This reduces the cost of stakes and labour.

Potato has a multiovular gynoecium containing 1000 to 1200 ovules out of which only 30 to 40% of the ovules generally get fertilized and produce seeds. The stigmatic surface shows changes in its surface structures in relation to the receptivity. The pollen load directly determines the quantity and quality of TPS produced. Hence pollination of stigma for three times within the receptivity period of 18-36 hours produced largest number and size of berries as well as highest quantity and quality of TPS.

A positive and significant correlation was found between the berry size and quantity and quality of TPS. Results indicate that seed extraction from berries larger than 2 cm diameter gives highest quantity and quality of TPS.

The calculations based on the present information showed that under Indian conditions of land and labour, the cost of production of 100 g of hybrid TPS would be US$5.75 if emasculation of female flowers is carried out and US$ 1.95 if the flowers are pollinated without emasculation. Therfore, the cost of producing hybrid TPS would be reduced to about 1/3 if pollination without emasculation is practiced.

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Research on the Physiology of Potato Sexual Seed Production

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Introduction

The emergence of true potato seed (TPS) technology as an alternative for potato production in developing nations is the result of research by breeders who have identified superior progenies and agronomists who have developed suitable field practices. A recent experimental yield report (Peru) from transplanted seedlings of a hybrid progeny is close to 70 tons per hectare (Malagamba, personal communication). Tuber size and uniformity also continue to be improved. Additional research will further enhance the potential for adoption of this new technology.

In some locations in Peru, on-farm trials have demonstrated a clear economic benefit from the use of TPS when compared to the traditional methods of potato cultivation. For extensive adoption of TPS technology there is an immediate need for developing efficient techniques on seed production.

Several major constraints can be identified that cause difficulties in seed production:

1. The shy flowering incidence and low fertility of most early and tropically adapted progenitors of TPS.

2. The lack of parental flowering synchrony for production of superior hybrid progenies.

3. Inefficient seed production methods.

CIP (International Potato Center) has recently placed emphasis on obtaining practical information that would be of use in large seed production schemes. This paper is a progress report of the methodologies and research directions employed toward this objective and includes a brief discussion of the encouraging results obtained during 1984.

Methods to Assess Potato Gamete Fertility

Germination of pollen in vitro is widely used as a standard technique to evaluate its potential in vivo fertility and to test the adequacy of staining methods (2, 5, 10, 19, 28). Limitations of this technique (i.e., false positives, false negatives, and erratic behavior) are generally recognized by investigators. The ultimate test to determine pollen fertility is its seed set capability; however, in vivo fertility, as measured by seed set, is a time-consuming process. In addition, environmental effects (i.e., wheather and mineral nutrition) and/or physiological factors (i.e., stigma receptivity and gamete fertility) can also influence seed set.

In 1976, Janssen and Hermsem (12), using a relatively simple media, associated potato pollen germinability with seed set (correlation coefficient 0.68 at P 0.05). They did not present data on seed quality or number of seeds produced per berry. When the objective is seed production rather than plant breeding, the effectiveness of pollen viability tests must be more critically evaluated.

A systematic search at CIP for the causes of erratic behavior of pollen germinability has resulted in a method that yields highly reliable data. Experiments are being conducted to determine the potential of this method and others described below to determine a pollen samples' seed production capability under field conditions. Preliminary results suggest that pollen handling could be an important factor influencing seed quality.

Description of Methodology for in Vitro Pollen Germination

1. Germinating medium: Distilled water containing 20% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca_3(NO_2)$ $4H_2O$, 200 ppm $MgSO_4$ $7H_2O$, 100 ppm KNO_3, Tween 80, 0.4%, at a pH 5.5 adjusted using 0.1 N KOH.

2. Large pollen samples are collected into gelating capsules or glass vials from potato flowers by using a vibrator. The pollen samples are thoroughly mixed using a "Vortex Genie" vibrator mixer.

3. Twenty drops, 5 microliters each, of germinating medium are placed on plastic petri dishes. With a needle, minute pollen samples are deposited gently on top of each drop. Petri dishes are then incubated for two hours at a constant temperature (20°-25°C) over moist filter paper. The hanging-drop technique described by Stanley and Linskens (29) is used, which permits direct microcospic observation.

4. Data is recorded by counting through a microscope at 100X magnification on 10 drops selected to contain samples of 50-250 pollen grains. Total and germinated pollen grains are counted. A pollen grain is considered germinated when its pollen tube is equal or larger than its diameter. Petri dishes can be stored two days at 5°C for future evaluations.

Simpler methods of pollen stainability are used on the potato (10, 11, 12, 29). Usually, these methods reflect the presence of callose (aniline blue) or other cytoplasmic constituents (acetocarmine) in pollen grains. However, they often show poor correlation with pollen viability. More rigorous staining techniques are being investigated which test for the activity of oxidation enzymes (nitro-blue tetrazolium stain) (11) and the presence of an active esterase plus the integrity of the plasmalema (Fluorochromatic reaction) (10, 17).

Studies on ovule fertility, essential to seed production, have been difficult due to laborious sectioning and paraffin embedding procedures. Stelly et al. (30) developed a fast method of high resolution and contrast for whole ovule observations. This technique, developed for cytogenetic and cytological research, is being investigated at CIP for potential use in evaluating female fertility.

Effects of Pesticides on Potato Gametes

Dramatic infertility was found at CIP's experimental station in Huancayo (central part of Peru) during the 1984 season. This was evidenced by the lack of pollen germinability, low berry formation in the field, a concomitant lack of natural pollinator (Bombus spp.) activity, and the failure of viable seed formation from the berries produced by controlled pollinations--all of which prompted an investigation to determine the cause. An intensive survey in the Mantaro Valley (where Huancayo is located) comparing the clones Tomasa Condemayta and Yungay revealed that this unusual infertility occurrence was not particular to the experimental station, but was generalized in all commercial fields visited. Unknown and unsuspected evironmental effects were suspected, until a small (approximately 600 m^2) family plot which had never received any pesticides was discovered in Sicaya. The pollen of the two clones was proven viable as determined by in vitro germinability tests. Large numbers of open-pollinated (OP) berries also contained viable seed and many bumblebees were observed actively harvesting pollen.

As a result of these observations, an experiment was performed in CIP-Lima to determine the effect of commonly used pesticides on potato gametes. Negative effects of chemicals on gamete fertility have been demonstrated by workers for other species (2, 5, 6, 13, 18, 31). A randomized complete block design with three replications of twenty plants each, with a guard row separating each treatment, was used to apply the insecticides. Two pesticides rates were applied seven days apart during bloom at recommended concentration rates for a 50% and 80%

pest incidence level. Flowers were collected three days after application and pollen was immediately extracted for subsequent evaluations of in vitro germinability. Results in Table 1 show that all pesticides used, with the exception of Dithane M-45 and Morestan both at the 50% pest-incidence dosage, were effective in significantly reducing pollen germinability. The combination of Lannate (Methomyl) and Dithane M-45 was dramatic to the point of consideration as a potential gametocide. Berries collected from most pesticide treatments were visually inferior as compared to the controls. The potential deleterious effects of pesticides on female (ovule) fertility is also being investigated. The data in Table 1 support the need for continuing research on the potential damaging effect of pesticides on potato gamete fertility.

Table 1 Effect of recommended rates of insecticides and fungicides for controlling two pest incidence levels (50% and 80%) on in vitro pollen germinability of DTO-33.

Treatment	Pest incidence level	
	50%	80%
Control	29.3 a	29.1 a
Morestan + Dithane	28.0 a	20.7 b
Dithane	26.1 ab	20.5 bc
Monitor	23.7 bc	19.0 bcd
Ambush + Dithane	24.1 bc	14.5 e
Monitor + Dithane	21.2 cd	14.8 e
Ambush	19.6 d	17.1 de
Lannate	18.0 d	10.4 f
Lannate + Dithane	10.4 e	7.0 g
CV (%)	12.16	14.43

Means followed by the same letter are not significantly different at $P < 0.01$.

Organo phosphates have been blamed for the decrease of bumblebee activity in many parts of the world. In Canada they were shown to be responsible for the complete elimination of bumblebees, but now, safer insecticides have been identified to aid in their reintroduction (21). These observations and ours point to the considerable research still needed on how to manage wild bumblebee populations, especially when seed production schemes are dependent on their activity. We recommend to avoid pesticide applications during bloom.

Nevertheless, gametocides are promising tools in reducing the cost of hybrid seed production, and research, in this regard, will be aimed at investigating the potential of Methomyl. Additionally, the presence of bumblebees might be a factor in self-seed-contamination when producing hybrid seed, and insecticides might be necessary to control these bumblebees.

Promotion of Flowering

Presently, most of the promising progenies that produce either ware or seed tuber potatoes from TPS are crosses whose male parents (e.g., DTO-28, DTO-33, LT-2) are CIP selections for the lowland tropics. These clonal selections have deficient flowering under short-day conditions. In contrast, most female TPS parents selected by CIP readily flower during short days and are not considered the principle subject of flowering treatments.

Eguchi,an agronomist at CIP determined (personal communication) that flower differentiation takes place during tuber sprout formation. It is safe to assume, therefore, that competition for assimilates by other developing organs such as roots, tubers, and vegetative buds could limit floral development, especially under the non-inducive environmental conditions (i.e. decreased daylength and light intensity) found commonly in the tropics during the potato growing season. The low flowering incidence of TPS progenitors with superior combining ability has prompted research to investigate the potential of flower promoters. These methods are reported to increase the sink strength of the differentiated floral organs, thereby encouraging flower development and/or preventing premature abscission.

Gibberellin-like activity decreases in potato leaves with decreasing daylength (25). Gibberellic acid (GA_3) has been reported to stimulate flowering in potatoes (23). Inadequate concentrations of this hormone could be partly responsible for the decreased ability of the flower bud to compete for the necessary nutrients. Other plant hormones and growth factors could also play an important role. Benzyladenine (BA) and GA_3, when applied simultaneously, were found to be particularly effective in developing the inflorescence of tomato (Lycopersicon esculentum Mill.) plants under adverse light conditions (17).

The potential of growth regulators to prevent flower bud drop in potatoes was clearly demonstrated during the winter of 1984 in Lima with the clone DTO-33. A complete randomized block design was used with three replications of 20 plants each, and a guard row between each treatment. Chemicals were applied once with a surfactant, by spraying the developing flower truss at first macroscopic visualization. Results are presented in Table 2. All treatments, except BA at 20 ppm, were effective in increasing total flower and pollen production as compared to the controls. All treatments, except that of BA at 20 ppm and GA_3 at 200 ppm applied alone, significantly increased in vitro germinability of pollen. The increase was 100% when BA 20 ppm + GA_3 50 ppm was applied to the plants.

Table 2 Number of flowers and pollen characteristics of DTO-33 as affected by different growth regulator treatments.

	Flower characteristics		
Treatment	Total # flowers	Total Pollen weight (mg)	Germinability in vitro (%)
BA 20 ppm	20	20.9	16.6
GA_3 50 ppm	105	200.3	22.2
GA_3 200 ppm	352	232.9	20.1
BA 20 ppm + GA_3 25 ppm	96	91.4	23.5
BA 20 ppm + GA_3 50 ppm	187	143.2	32.1
BA 40 ppm + GA_3 100 ppm	299	226.5	26.3
Control	37	62.9	16.3
LSD 0.05			4.01

Within the same experiment, one inflorescence within each treatment was pruned to two flowers of uniform maturity per replication and pollinated with a viable, fresh pollen tester of the clone R128.6 (Table 3). Ovule fertility apparently was not influenced by most treatments (Table 3). Seed number per berry extracted from OP berries (flowers which escaped collection) treated with GA_3 alone, was decreased. Almekinders (personal communication) observed increased stigma exertion in GA_3-treated potato flowers--a possible explanation for this result.

Table 3 Number and weight of berries produced by potato plants as affected by different growth regulator treatments.

	DTO-33 x R128.6		DTO-33 OP	
Treatment	1	2	1	2
BA 20 ppm	0	0	6	224.7
GA_3 50 ppm	3	132.0	18	167.16
GA_3 200 ppm	2	170.5	11	161.17
BA 20 ppm + GA_3 25 ppm	4	131.3	26	186.29
BA 20 ppm + GA_3 50 ppm	5	146.5	13	209.86
BA 40 ppm + GA_3 100 ppm	0	0	23	190.71
Control (H_2O) + Tween 20, 0.1% conc.	2	181.0	14	197.57

1 = number of berries
2 = average number of seeds per berry

Plants from the clone DTO-28 were also induced to retain a higher proportion of flower buds with increased pollen production than the respective controls by applying the combination of Kinetin 20 ppm and GA_3 40 ppm (Table 4). Further investigations are necessary before practical recommendations for hormonal application can be suggested, particularly since environmental effects on GA_3 sensitivity are numerous in the literature (27, 30). In addition, results at CIP indicate that the timing of hormone application is critical and that some varieties are not affected.

Table 4 Flowers collected, weight of pollen produced and number of pollinations with pollen produced by DTO-28 plants.

Treatments	Flowers collected (%)	Weight of pollen (%)	Number of pollinations (%)
Control (100%)	100	100	100
Kinetin 20 ppm + GA_3 40 ppm	339	206	204

Polyamines (spermine, spermidine, and putrescine) and other related nucleosides are important in cell growth, possibly because of their polycationic association with nucleic acids and other anionic macromolecules (16). These substances which are the subject of recent research as inhibitors of senescence (19), are ubiquitous constituents of most developing organisms. They occur in organelles (i.e., ribosomes) and are known to stimulate replication and transcription (15). Evidence indicates their importance during the early stages of pollen germination in the Petunia, and after ovule fertilization in wheat (14). Polyamines have been reported to substantially increase flower bud formation and fruit set in apples, especially under unfavorable environmental conditions that reduce endogenous levels (3).

Polyamines (1 ppm) were applied with a surfactant approximately one week before bloom to plants of the clone DTO-33 at CIP-Lima the winter of 1984. Experimental design consisted of a randomized complete block with three replications (20 plants each) and a guard row separating each treatment. Results showed a significant increase of in vitro pollen germinability by the application of polyamines as compared to the control (Table 5). The practical application of polyamines merits further experimental evaluation, especially in regard to the cheaper spermidine. OP berries were also collected from these plots and seed quality is being quantified from all experiments mentioned.

Table 5 Effect of polyamines on in vitro germinability (%).

	Days after spraying		
Treatment	12	19	21
Control	15 a	27 a	23 a
Putrescine	30 b	30 b	41 b
Spermine	29 b	29 b	38 b
Spermidine	40 b	38 b	37 b

Means followed by the same letter are not significantly different at $P < 0.01$.

Hormones and other chemicals might not be readily available in developing nations, or costs might be prohibitive. Methods such as artificial light to supplement the inherent short days could be more appropriate. Fifty plants of DTO-33 were grown in the screenhouse at CIP-Lima in a mixture of 1:1 sand and peatmoss in 19 cm diameter plastic pots placed next to each other. Daylength was increased to 16 hours by a 200-watt bulb and applied continuously as a supplement to available daylight. When flowers and pollen produced by these potato plants were compared to those of 60 field grown plants during the same period, flower retention and pollen yield were increased four times by daylight extension (Table 6). The possible confounding effect of the screenhouse environment has to be determined before practical recommendations can be made. The practicality of this technique as a strategy to circumvent the decreased pollen production ability of superior male progenitors for TPS production under limiting conditions seems promising but deserves further work and more critical evaluation. Daylength could also be increased by extending the natural daylength instead of the continuous conditions from which our observations were derived. Even more attractive is the possibility of promoting flower development by short night time interruptions of light.

Table 6 Flower and pollen production on the clone DTO-33 as affected by artificial light.

Treatment and daylength	Number of flowers	Pollen weight per flower (mg)	Total pollen production (mg)
Field (60 plants)			
12 hours	180	2.67	480
Artificial light (50 plants)			
16 hours	745	2.85	2122

The promotion of fruit development for optimum seed quality is a separate research topic from 'Promotion of Flowering' and work is currently in progress 1) to evaluate effects of auxins in preventing premature berry abscission, and 2) to determine the possible relationship of fertilizers on seed production and quality.

Effects of Fertilizer Regime on Flowering

The mineral nutritional needs of potatoes grown for tuber production have been the subject of extensive investigation. Potato grown for TPS production deserves a proper investigation of its possible specific fertilizer needs. It is believed, for example, that environments conducive to tuber bulking could be detrimental to flowering and perhaps to high-quality seed formation. A contract between Dr. Swen Villagarcía (Universidad Agraria of La Molina) and CIP to determine the fertilizer needs of TPS prdocution has been set up and considerable research is now under way in Peru. Preliminary results are herein reported.

Increasing levels of nitrogen (N) increased flower production and pollen germinability in the clone DTO-33, but decreased flower production in DTO-28 (Table 7). Increased N fractioning was superior in inducing flower production and pollen germinability for both DTO-33 and DTO-28 (Table 8). Waiting until flowering to apply N as a sidedress appears disadvantageous. since a smaller percentage of flowers was retained in both clones and pollen germinability was observed to decrease slightly in DTO-33.

Table 7 Effect of N level (ppm) on flowering of two potato clones* and pollen in vitro germinability of DTO-33.

N level (ppm)	No. open flowers		% germinability
	DTO-33	DTO-28	DTO-33
100	100	100	100
200	104	85	98
300	137	60	190

Table 8 Effect of N fractioning on flowering and pollen germinability in vitro (A = 100%).

Nitrogen application	Total number of flowers		% Pollen germination DTO-33
	DTO-33	DTO-28	
A	100	100	100
B	124	114	110
C	118	101	93

A = 1/2 at planting, 1/2 at hilling
B = 1/2 at planting, 1/4 at hilling, 1/4 at flowering
C = 1/2 at planting, 1/2 at flowering

Data in Table 9 indicate that pollen germinability could be increased with N and K applications, while phosphorus alone had the opposite effect. These data also indicate that flower production is influenced by macronutrient availability and more detailed data will be obtained in 1985.

Table 9 Effect of N-P-K fertilizations on flowering and pollen germinability of DTO-33*.

N-P-K	Number of flower buds	Number of open flowers	% in vitro germinability
0-0-0	100	100	100
200-0-0	158	205	134
200-200-0	168	179	176
0-0-100	151	211	155
0-200-100	219	230	126
0-200-0	158	258	74

* Values relative to those of NPK rate of 0-0-0.

Seed Quality

Seed quality for most vegetable crops is measured by a variety of parameters: cleanliness, high germination, freedom of seed-borne disease, and of weed seed, reasonable genetical purity, high vigor and an ideal moisture content. Seed from potatoes has not received a rigorous analysis of the factors that can be used as measures of quality (i.e., high vigor), due to its recent introduction and limited use. Seed size, germinability, and number are the parameters that have been used as possible considerations. The data available on seed size in the TPS literature, however, reports conflicting results and is thereby inadequate as a base for sound conclusions (4, 32, 35,*). Harrington stated that for most vegetable crops "medium and large seed are more vigorous than small and extra-large seeds, and that seed of higher density is more vigorous than seed of lower density" (8). Recent work in India by Uphadya (31) has associated seedling performance with potato seed embryo formation as evaluated through a dissecting microscope. Further evidence is needed to determine the appropriate parameters to judge the quality of seed with reference to its potential for producing potatoes.

* See elsewhere in this report of Planning Conference. Hybrid versus Open Pollinated TPS families. H. M. Kidane-Miriam <u>et al</u>.

Detailed data have been obtained in 1984 from the hybrid Atzimba x R128.6, produced in Huancayo on the effect of number of pollinations and berry size on seed quality measured as seed number, weight, and embryo type. Seed number and weight (per 100 seeds) per berry increased with berry size (Table 10). Similar data were obtained with other hybrids and by other workers. When these data were analyzed statistically, however, the results were difficult to interpret, especially when seed number per berry was considered. Seed weight was significantly increased only when compared to small berries pollinated once. The importance of the statistical significance analysis is in question and more research is still needed.

Data on Table 10 indicate that pollinations on two or three occasions improved significantly the number of seeds per berry as compared to a single pollination. Similar results were observed for average seed weight. Multiple pollinations did not affect significantly the proportion of superior embryo types (A-C). Data in Table 11 show that if the embryo type is important as a parameter of performance, larger seed size could be used to increase the proportion of superior embryo types. Although additional experiments are needed to elucidate the most appropriate pollinating scheme, the apparent advantages of multiple pollinations are evident.

Table 10 Effect of the number of pollinations and berry size on seed number/berry and seed weight (100 seeds) of Atzimba x R128.6.

Berry size	1 Pollination		2 Pollinations		3 Pollinations	
	1	2	1	2	1	2
Large	122 a	89.9 a	173 a	124.5 a	175 a	101.8a
Medium	77b	92.3 a	141 a	118.8 a	121 b	96.2a
Small	38 b	55.8 b	-	-	52 c	80.5a

1 = Number of seeds per berry
2 = Weight of 100 seeds (mg)
Means followed by the same letter are not significantly different at $P < 0.05$.

Table 11 Effect of seed size on the proportion of seed with A-B embryo type (Atzimba x R128.6).

Size of Sieve	% of seed embryo A-B
1/15"	89.90 a
1/17"	79.43 b
1/20"	59.53 c
C.V. (%)	18.72

Means followed by the same letter are not significantly different at $P < 0.05$.

Seed Extraction

Fermentation of mashed berries was studied as a possible method to assist in seed extraction. When large samples of berries were harvested properly (mature but not over ripe) and fermented, this method proved a potential tool to facilitate seed extraction (Table 12). Four replications of 100 seeds each were treated with a 10% of chlorox solution (10 min.) followed by a 1500 ppm GA_3 solution (24 hours) and germinated on moist filter paper in closed petri dishes at 20°C. The means from three experiments, using seed (OP) of DTO-33, showed that the fermented treatment of 96 hours at 30°C was significantly higher, faster, and more uniform in its germination rate than the control.

Table 12 Potato seed (DTO-33 OP) germination (%) at 2, 4 and 8 days fermented for 96 hours at 30°C.

Treatment	Days after start of germination		
	2	4	8
Fermentation	37 *	87 **	97 **
Control	18	63	85

* = $P < 0.05$
** = $P < 0.01$

Data were also obtained with OP berries of Renacimiento, 79D-10-9, 104-12LB, and bulk samples from Huancayo and San Ramon, and are supportive of the result presented here. These encouraging data were obtained under ideal laboratory conditions and their significance under field conditions will be ascertained. The importance of uniform emergence is emphasized in using TPS to produce potato tubers, especially when direct field seeding is considered, to avoid the dangers of soil crusting and weed competition during early seedling development.

Methods for Hybrid Seed Production

Emasculation and pollination studies conducted at CIP-Lima have provided data to predict manpower needs for a large-scale scheme on hybrid seed production. By closely monitoring four workers during a three-week project to emasculate and pollinate flowers of seven different varieties, it was estimated that one person can pollinate approximately 2,000 flowers in 3-1/2 work hours and emasculate 1000 flowers (Fig. 1).

Data is also being obtained on the possibility of using stored pollen in large field production schemes to circumvent the lack of synchrony in flowering between intended parents and to facilitate labor needs at peak use. Preliminary experimental results showed that dry (silica gel) and cold pollen (-20°C to 5°C) could be stored for up to two months and still retain its fertility to produce good quality seed.

A practical method of vine training to prevent berry rot has been adopted from the tomato to prevent berry contact with the soil. In Lima, *Rhyzoctonia* was found in approximately 25% of control berries. The possibility of this disease being carried with the seed is also being investigated in collaboration with CIP's Pathology Department. Initial findings showed that *Rhyzoctonia* and other fungal pathogens were present on seed extracted from diseased berries.

Conclusions

The preliminary data presented in this paper demonstrate clearly that potatoes grown for TPS production have specific needs that differ from those grown for commercial production. The data also show that solutions are available to overcome most of the major constraints to growing potato for TPS production. Furthermore, that continued research on TPS production is paramount.

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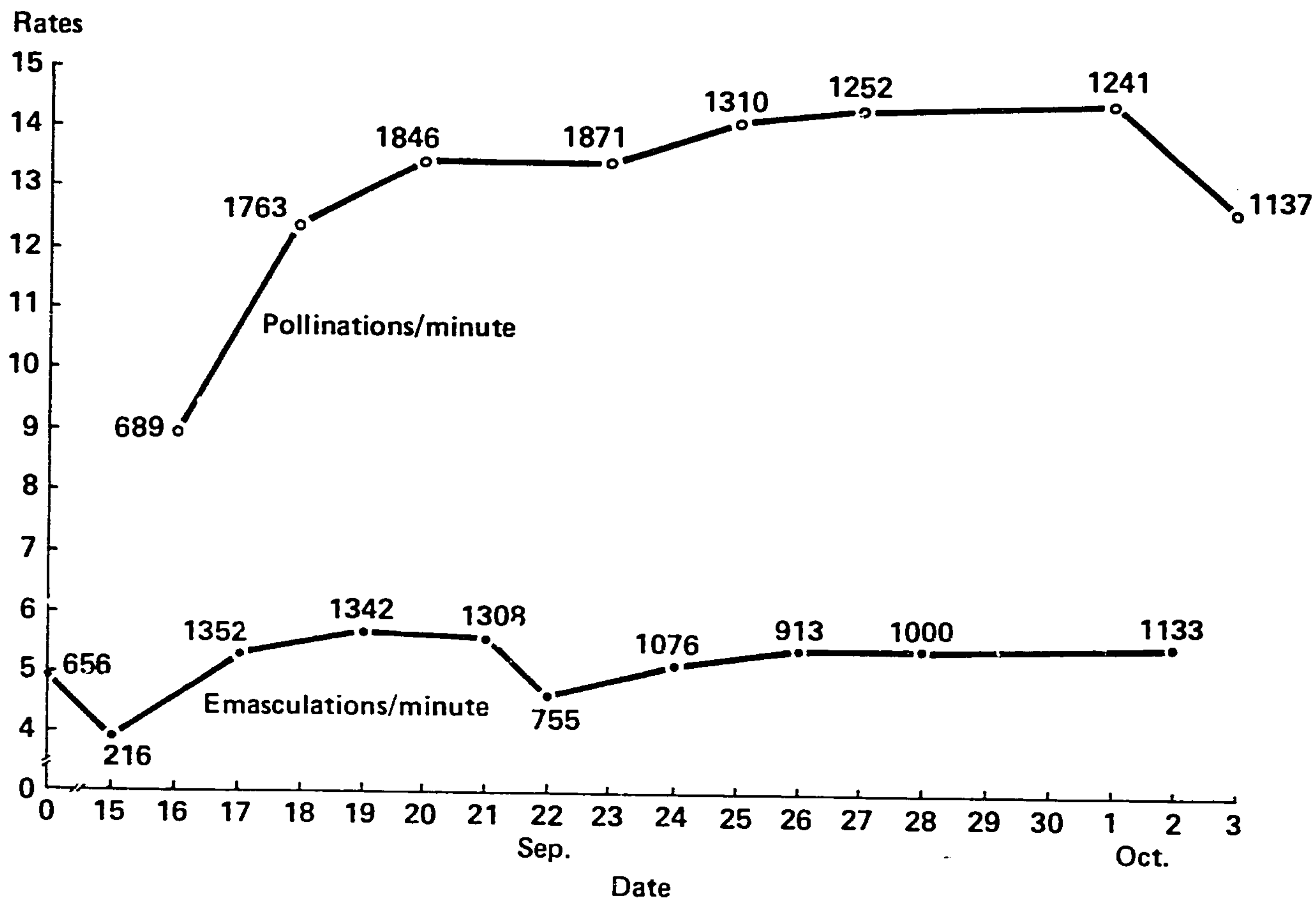

Fig. 1. Rates of pollinations and emasculations per minute at CIP Lima, winter 1984, using five clones (CEX69.1, 65ZA.5, I 931, Molinera, Atzimba). Note low rates of emasculations due to difficulty of the task. Also, note lower rates within a task as number of flowers decreases.

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Inbreeding and True Potato Seed Production

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Introduction

The successful introduction of TPS technology in developing countries, particularly where the potato is not a traditional crop, demands a reappraisal of conventional potato breeding strategies, not only in terms of breeding procedures, but also of the end-product developed through breeding, in order to exploit fully the potential of the potato as a sexually propagated crop. As a contribution to TPS research, a joint project between the University of Birmingham and the Plant Breeding Institute, in collaboration with the International Potato Center, and funded by the U.K. Overseas Development Administration has been developed to evaluate the potential of inbreeding for the production of TPS. Inbreeding as a strategy has received relatively little attention from potato breeders. In this paper we shall put forward some ideas in support of using an inbreeding strategy for producing true seed potatoes.

The Uniformity of Potatoes from TPS

Uniformity is the norm when potatoes are grown from tubers. In contrast seedling generations exhibit considerable heterogeneity because of the great heterozygosity which is 'locked-up' in the potato as a result of vegetative reproduction. Several approaches to breeding the genetic uniformity necessary for TPS have been evaluated in recent years. These approaches have included the selection of tetraploid parental materials (Mendoza, 1979; Peloquin, 1979; Kidane-Mariam et al., 1984), the use of dihaploids and diploids which produced diplandroids and diplogynoids (Mendiburu & Peloquin, 1971, 1977; Mok & Peloquin, 1975; Peloquin, 1979) and their use in 4x-2x crosses. These methods are compromises between maximizing heterozygosity, maintaining the necessary level of gametic uniformity, and relative ease of seed production. Inbreeding, which achieves the highest level of gametic uniformity, may be exploited either through the production of F_1 hybrids between inbred lines (Yashina & Pershutina, 1971), a strategy in which heterozygosity is favoured at the expense of ease of seed production, or by the use of autogamous inbred lines in which gametic uniformity is due to homozygosity. The choice between these approaches is dependent on whether heterosis must be equated with heterozygosity.

Inbreeding Depression vs Heterosis

In proposing an inbreeding strategy we agree with Jinks & Lawrence (1983) in questioning the widespread belief that the best phenotypes, particularly for yield, are produced by heterozygotes rather than homozygotes. In outbreeding crops breeders attempt to avoid inbreeding depression, which is expressed as a reduction in vigour, fertility and yield. The usual explanation of inbreeding depression is that it is due to the fixation of unfavourable or deleterious recessives. Inbreeding increases the frequency of loci which are homozygous, some of which will become homozygous for these deleterious recessives. Concerning the genetical basis of inbreeding depression, Jinks & Lawrence (1983) have indicated that it is due to the fact that the characters of interest are determined by genes with non-additive effects, of which those whose effects are deleterious are but a subset of such genes. These genes will segregate upon inbreeding.

Heterosis is the converse of inbreeding depression. Heterosis has been utilized as a productive breeding strategy in a variety of crop species, but its genetic base is still the subject of some debate. The two main genetic models of heterosis are the overdominance model (Hull, 1945), and the dominance model (Williams, 1959; Sinha & Khanna, 1975). The overdominance model, which proposes that heterozygosity is intrinsically advantageous, has been expanded to a multilocus model by Li (1967), and to include multialleleic effects by Mendoza & Haynes (1974). Equally, in support of the dominance model, it has been shown that heterosis may result from additive x additive and additive x dominance effects at a few loci (Seyffert & Forkman, 1976), or from linkage and linkage disequilibrium (Sved, 1972; Arunchalam, 1977). However, in recent theoretical work on the genetical basis of heterosis Jinks (1981, 1983), has argued that heterosis is not dependent on heterozygosity per se, but on the genic content of the individual, and therefore that heterosis may be 'fixed' in homozygous recombinants produced through inbreeding. Although they do not dispute that overdominance may occur, they argue that there are few substantiated cases of major genes showing overdominance.

The quantitative model developed by Jinks, Pooni and co-workers at Birmingham (Jinks, 1981, 1983) argues that heterosis results from the dispersal of dominance and non-alleleic interactions by linkage and linkage disequilibrium, and that, if these genetic parameters are estimated for a given cross using the triple test cross mating design, then the frequency of important transgressive segregants in inbred lines derived from the F_2 by single seed descent can be predicted.

What are the Effects of Inbreeding in Potatoes?

The effects of inbreeding in cultivated tetraploid potatoes have been examined by a number of authors (Krantz, 1924, 1946; Krantz & Hutchins, 1929; Guern, 1940; Neciporcuk, 1949; Hagberg & Tedin, 1951; Feistritzer, 1952; Gowen, 1956; Engel, 1957; Rudorf, 1958; Deshmukh & Verma, 1960; Pushkarnath, 1960; Mullin & Lauer, 1966; Zadina, 1973). In general, severe reductions in vigour, yield and fertility with successive generations of inbreeding were observed. However, several researchers including Krantz & Hutchins (1929), Krantz (1946), and Pushkarnath (1960), have noted differences between the responses of different lines to inbreeding. More recently, Trinkler et al. (1976) have stated that they were able to select inbred lines which showed little or no inbreeding depression. Trinkler *et al*. (1980) compared the performance of secondary inbred lines (I_1 - I_4) derived from crosses of inbred lines (I_1xI_1, I_2xI_2, and I_3xI_3), and found no inbreeding depression. The secondary inbred lines had yields of up to 50% greater than the initial F_1. Budin & Soboleva (1978) obtained I_1 to I_3 lines from spontaneously-doubled F_2 hybrids of dihaploid *S. tuberosum* ssp. *andigena* with *S. phureja*, *S. stenotomum*, and *S. pampasense* which showed no significant inbreeding depression.

Similarly, at the diploid level, inbreeding depression has been observed. De Jong & Rowe (1971) found that I_2 inbred lines derived from dihaploid Group Tuberosum x Group Phureja and Group Stenotomum hybrids had an average yield in the I_2 of only 36% of the I_o, but that the four families did respond differently to inbreeding. Abdalla (1970) found that the responses of different lines of self-compatible *S. verrucosum* to selfing varied considerably.

The Effects of Inbreeding in Other Outbreeding Crops

Considerable variation in response to inbreeding has been observed in a number of other predominantly outcrossing species. Ockendon & Currah (1982) found that seven of 11 inbred lines of runner bean (*Phaseolus coccineus* L.) showed no significant inbreeding depression. Inbred clones of *Trifolium pratense* L. have been produced which equalled or excelled the parental clones in all characters examined (Taylor *et al*., 1970). Schuster & Michael (1976) produced I_7 and I_8 generation lines of *Brassica napus oleifera* with higher yields that the parental material. In alfalfa (*Medicago sativa* L.) Hill (1975, 1976) found clones which showed little or no inbreeding depression, Melton *et al*. (1969) and Melton (1970) observed a wide range of responses to selfing, and Panella & Lorenzetti (1966) observed that the I_3 generation was more vigorous than the I_2.

The Attainment of Homozygosity

There are several ways in which homozygosity can be achieved, amongst which anther culture and single seed descent are two important strategies, and which will be used in this project. Anther culture techniques to produce dihaploids from tetraploids, or even monohaploids as proposed by Wenzel *et al.* (1979) can be used to produce homozygous lines. It is apparent, however, that no general anther culture protocols are available, and that different potato genotypes differ in their 'tissue culture ability'. Single seed descent is a breeding strategy in which selection is deferred until a suitable level of homozygosity is achieved.

We propose to attempt to inbreed diploid potatoes. The reasons for this are primarily logistical. Achieving the desired degree of homozygosity at the diploid level will require far fewer generations of single seed descent, and much less material than would be required at the tetraploid level. Nevertheless inbreeding at the diploid level does present other problems. The tuber-bearing Solanums have a gametophytic system of incompatibility, but polyploids are self-fertile probably due to competitive interaction or mutual weakening of S-alleles (Dodds & Paxman, 1961). As a general rule, most diploid species are self-incompatible, but there are notable exceptions among the wild species, including *S. verrucosum*, *S. etuberosum*, *S. brevidens*, *S. morelliforme* and *S. polyadenium* (Hawkes, 1958, 1963). While some species are strictly self-incompatible, self-compatible genotypes are found within others such as *S. phureja* (Dodds, 1956; Cipar, 1964; Hermsen & Sawicka, 1979). Olsder & Hermsen (1976) have also found self-compatible dihaploids derived from *S. tuberosum*. Self-compatible genotypes have been identified in diploid populations developed at the Scottish Crops Research Institute-Pentlandfield (C.P. Carroll, personal communication). In several crops, such as brassicas (Taylor, 1982), sweet potato (Sood *et al.*, 1982) and rosaceous tree fruits (Visser, 1981; Visser & Verhaegh, 1980; Visser & Oost, 1982), successful methods at breaking self-incompatibility barriers have been developed. These include pollen irradiation, 'pioner' and 'mentor' pollen, heat treatments and enhanced CO_2 atmospheres. Several of these approaches will be used in an attempt to break self-incompatibility, although the identification of self-compatible genotypes at an early stage would be advantageous. A study of incompatibility in diploid potatoes is the subject of a separate research project at Birmingham.

A TPS Ideotype

What sort of a plant do we expect to produce by inbreeding at the diploid level? Yield comparisons between diploid and tetraploid potatoes are usually made under a conventional production system. Undoubtedly tetraploid potatoes do exhibit a yield advantage over diploids (Mendoza & Haynes, 1976; Mendiburu & Peloquin, 1977), but this advantage does not appear to be due to polyploidy per se (Rowe, 1967; De, Maine, 1984). While Mendoza (1979)) has argued that the yield advantage of tetraploids is due to multiallelic interactions and heterozygosity, based on the overdominance model of heterosis (Mendoza & Haynes, 1974, 1976), Sanford & Hanneman (1982) have suggested that there is a possible heterotic threshold of yield in potatoes.

Jinks & Lawrence (1983) have pointed out that the effect of selection in cultivated species will be to raise the frequency of genes for favourable expression of the selected character(s), that is, those genes which display dominance in the desired direction, and therefore, when such material is inbred, the resultant inbreeding depression is proportional to the response that has previously been obtained by selection. Jinks & Lawrence (1983) further argue that although in such populations heterozygotes, on average, may be superior in fitness terms to homozygotes, because of dominance, the objective of plant breeding is identify individuals whose performance is well above average, and that these individuals, in the absence of overdominance, are as likely to be homozygotes as heterozygotes. That is, the apparent correlation between yield and heterozygosity is specious.

Despite the potentially greater yield in tetraploid cultivars, elite diploid lines have been identified. Carroll & De, Maine (1981) found that four out of ten diploid hybrids obtained by crossing Tuberosum dihaploids were not significantly different in yield from the top yielding control cultivar cv. Desiree. Swiezinski (1984) has produced several diploid families in Poland which have yields greater than the average for tetraploid families, even though the mean of the diploids was lower.

One can question the validity of comparisons of yields obtained from plants grown from TPS and those grown from tubers. A TPS seedling is a one-stemmed plant. Perhaps the ideotype we should aim to select is one which produces only one (or just a few) tubers of marketable yield per plant. Such an ideotype would channel all its productivity into this small number of tubers, rather than into many, of which only a proportion would be of marketable yield. Under such circumstances the apparent yield disadvantages of diploid potatoes would become less important as long as each plant yielded in this manner. We envisage that TPS will be used directly for the production of a ware crop rather than for a seed tuber crop. Consequently marketable yield per unit area is of greater importance than total yield per plant. The sizes of tubers acceptable as marketable yield varies from country to country. Variation in tuber size in potatoes affects both total and marketable yield and is due to several factors, including (i) space per plant; (ii) stems per plant; (iii) size of stems; (iv) date of emergence; and (v) tuber sizes on one stem. When TPS is utilized directly, especially when seedlings are transplanted, the first four of these factors would be reduced or eliminated. The only factor of significance would be due to differences between different genotypes in producing a range of tuber sizes, and could be reduced in importance by the choice of suitable parental material.

In many developing countries the potato is utilized as a vegetable and not as a stable food. Consequently it is unrealistic to imagine the potato replacing important staple crops in the near future. The inclusion of the potato into new cropping systems, or its introduction into new environments, might be achieved more readily if we no longer continue to think of it being grown in the traditional way as from tubers on ridges, but look for new ways in which it may be cultivated. One can visualize that the potato may be adopted as a vegetable crop, with seedlings transplanted to the field in raised beds, as is typical with vegetable production in many countries. The manipulation of planting densities would then allow the achievement of acceptable yields. Such a system would be suited to the needs of small farmers in developing countries of the tropics. The successful adoption of TPS technology in many parts of the developing world will ensure that the potato becomes a valuable dietary supplement.

Conclusions

We have outlined some arguments in favour of an inbreeding strategy for TPS production, even though we recognize that important practical constraints will be faced. Once a sufficient level of homozygosity has been achieved, it can be exploited in the interim through the production of F_1 hybrids, although the production of inbred lines is the ultimate aim of the research project.

Inbreeding in potatoes presents a considerable challenge, but one for which the potential rewards are great. This is an area of TPS research which has received no emphasis until now. It is our hope that this project will make a contribution to the utilization of TPS in developing countries.

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Field Experimental with TPS in Bangladesh

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ABSTRACT

The studies were conducted to explore the feasiblity of using true seed as an alternative to the traditional seed tuber. TPS hybrid progenies were superior to the open-pollinated ones A few of them gave comparable yields with tuber-propagated commercial cultivars displaying satisfactory standards for plant and tuber characteristics. Marketable yields ranged from 95.4 to 97.4 per cent in the potential TPS hybrids and 90 per cent in K. Jyoti (OP) as against 96.8 per cent in Patrones, raised asexually.

Seedling tubers (F_1C_2) from promising TPS progenies, despite their small size, were productive and compared favourably with imported seed for yield and health standards. Procedures are discussed for a commercially viable system of harnessing seedling tubers as substitute to costly basic seed for subsequent clonal multiplication. This will help in regularly supplying clean seed that the small farmers can afford.

Open-pollinated progenies of 3 traditional cultivars showed promising results with mean yields ranging from 18.30 to 24.05 t/ha It is suggested that some of these cultivars with proven adaptability could be rejuvenated by developing their inbreds.

Preliminary agronomical studies revealed that the number of seedlings transplanted in a unit area should correspond to the number of main stems of the crop raised from tubers for high production of tuber yields.

It seems necessary that commercially acceptable TPS progenies evolved for the tropics should have inherent ability to profusely flower and set berries for producing locally viable seed.

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INTRODUCTION:

Research at the International Potato Centre (CIP) has focused on the feasibility of using true seed as alternative to the traditional seed tuber. This is a means of virtually starting with virus free material and thus providing an economical but effective technology because of high investments on the costly tuber seed. Use of TPS is also convenient for handling and long distance transportation with no rick of deterioration during transit and storage. Apart from freedom from common viruses it also does not carry many of the seed borne diseases like late blight and bacterial wilt. Such a technology seems to be more feasible for developing countries where the holdings are small and the farmers have scarce resources. On the otherhand, the farmers of these countries are good horticulturists familiar with raising vegetables that are transplanted from nursery to field and would make this technique a successful venture. In this context, research on different aspects of TPS in Bangladesh in the past three years have been carried out to study feasibility of the production of the potato from botanical seed instead of tubers. This short presentation describes results of the relevant studies during 1981 to 1983.

MATERIALS AND METHODS:

Several hybrid and open pollinated seed progenies from CIP, region VI, the Templetion Plant Breeders in New Zealand and Bangladesh were evaluated at the Potato Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Dhaka during 1981-83. The seed was sown in the raised nursery beds by 2nd half of October containing seed bed mixture of 2 parts of organic manure, 1 part of ash and 1 part of topsoil from the field. Growing seedlings were provided with shelter against direct sunlight during 10.00 to 15.00 hours. Emergence was obtained between 7 to 10 days after sowing. One month old seedling were transplanted in the field with half of the nitrogen being applied at the time of transplanting and the remaining half after the seedlings were fully established and started active growth. Full dose of P_2O_5 and K_2O was applied at the time of transplanting. Rate of fertilizers was same as recommended by PRC for potatoes raised asexually. Two seedlings per hill were provided and spacings adopted were 60 cm from row to row and 25 cm in the rows. Irrigation was applied immediately after transplanting. In all, 5 irrigations were applied as against 4 to the crop raised conventionally. Harvesting was done at full maturity and the tubers from TPS (F_1C_2) were cold stored.

Comparison of F_1C_2 tuber progenies carried forward from 1981-82 pertaining to 5 TPS hybrids and one open pollinated progeny was carried out during 1982-83 against imported basic seed. The trial received all the agronomic treatments with regard to fertilization and other cultural practices as recommended by PRC.

RESULTS AND DISCUSSIONS:

Yield potentiality of TPS populations:

Results (Table 1) indicate that hybrid progenies in general, were superior to open-pollinated ones during 1981-82. Among the hybrid progenies, Kufri Chandramukhi X JEX/B-689 had the highest yield and was closely followed by K2500 X CP1406, DTO 28 X Atzimba, N2-48 (a) X JEX/B-689, and Kufri Sindhuri X JEX/B-689. The yields ranged from 16.48 to 21.60 t/ha in 87-90 days and it compared favourably to the crop raised from the tubers. Produce from TPS hybrids DTO-28 X Serrana and N2-48 (a) X JEX/B-689 was quite uniform and commercially acceptable. DTO 28 X Atzimba and Kufri Chandramukhi X JEX-689 though produced large size tubers, were less uniform phenotypically.

In Kufri Jyoti, an open-pollinated progeny, yield potential of 12.42 t/ha had been obtained against the national average yield of 10 t/ha. In another observational trial, yield to the order of 14.6 t/ha in 80 days after transplanting was reported. Best yield was 24.5 t/ha. Another important observation is that the growing crop of Kufri Jyoti from TPS was highly uniform in both plant and tuber characters. Also it showed a useful degree of field resistance to late blight both in foliage and tubers.

Table 1: Performance of TPS (F_1C_1) during 1981-82

Hybrid/O.P. Progenies	Area Transplanted (m²)	No. of Plants Harvested	Total Yield (kg.)	Yield* (t/ha)	Maturity
1. K. Joyti (OP) (i)	43.2	234	48.70	11.27	81
(ii)	54.0	292	73.30	13.57	97
2. DTO: 28 X Serrana	13.5	80	17.00	12.59	90
3. DTO: 28 X Atzimba (i)	10.8	64	20.20	18.70	95
(ii)	10.8	56	15.80	14.63	90
4. $N_2$48 (a) X JEX/B-689	21.6	115	37.80	17.13	90
5. K. Sindhuri X JEX/B-689	10.8	63	17.80	16.48	87
6. K. Chandramukhi X JEX/B-689	8.1	47	17.50	21.60	87
7. K 2500 X CP 1406	8.1	38	15.20	18.76	87
8. CP 1406 X JEX/B-723	10.8	57	5.80	4.63	88
9. Dhankri X CP-1406	8.1	45	8.90	10.98	81
10. Waitney (OP)	-	No Plant Establishment			

* 1 = Highly hetrozygous

10 = Extremely uniform

During 1982-83, comparison of one hybrid and two open-pollinated progenies showed that CP-1406 X JEX/B-723 gave better performance than the open pollinated progenies (Table 2).

Table 2: Evaluation of TPS during 1982-83

Hybrid/OP Progenies	Area Transplanted (m)	Yield (t/ha)	Maturity (days)	Range of Tuber Size	Uniformity of tubers
CP-1406 X JEX/ B-723	9	14.16	79	Medium to large	8
CIP 800226	3	11.50	79	Small	9
Kufri Jyoti	38.5	9.80	79	Medium to large	10

1 : Highly uniform with high consumer acceptance

1 : Highly segregating

During 1983-84, two hybrid progenies and one open pollinated progeny were evaluated against variety Patrones, raised asexually at BARI, Joydebpur, Potato Sub-Station Munshiganj and Regional Agricultural Research Station Hathazari, Chittagong. Their performance reflecting yield, growth and yield components have been presented (Table 3). TPS populations produced higher yield than the open pollinated progeny of K. Jyoti. It is also noteworthy that DTO-33 X R-128.6 and Atzimba X R-128.6 gave comparable tuber yield with tuber propagated commercial cultivar Patrones. Marketable size of the tubers ranged from 95.4 to 97.4 per cent in hybrid progenies and 90 per cent in K. Jyoti (OP) against 96.8 per cent in Patrones raised conventionally. All TPS progenies displayed satisfactory standards for plant and tuber characteristics. Ground coverage was remarkably better in both the hybrid progenies than that of Patrones. Results of the evaluation at the three locations representing different ecological regions were almost similar and indicated the potentiality of potato production by TPS.

Table 3: Evaluation of TPS Progenies during 1983-84

A. Potato Sub-Station Munshiganj

Progenies	Area (m2)	No.of Hills	Yield (kg.)	Size Distribution (mm) Above 35	28-35	Below 28	Mean Tuber Weihgt (g)	Uniformity of plant and tubers
PTO-33 X R128.6	16.2	86	28.00 (17.02)	73.7	23.3	3.0	24	9
Atzimba X R128.6	16.2	87	26.00 (16.02)	66.8	28.6	4.6	22	9
K. Jyoti	9.0	50	7.00 (7.77)	77.2	13.6	9.2	16	8
Patrones (Check)	16.2	86	27.00 (16.64)	76.6	20.1	3.1	33	10

1 Tuber yield in t/ha in shown in parenthesis

2 Uniformity rating: 10 = Highly uniform;
1 : Highly segregating

B. Regional Agricultural Research Station, Hathazari, Chittagong (Adopted from A. Quasem)

Progenies	Height Per Plant (cm)	No. of Tubers Per Plant	Weight of Tubers Per Plant (g)	Dry Matter Percentage	Yield t/ha
Atzimba X R128.6	40.95 b	7.7 b	434.8 c	22.18 a	15.17 b
DTO-33 X R128.6	43.45 a	15.0 a	606.2 b	18.32 b	19.04 a
K. Jyoti	28.39 a	8.2 b	220.2 a	22.95 a	6.13 c

Mean not having a letter in common are significantly different at 5% level according to Duncan's multiple range test.

Table 3 (Continued ...)

Progenies	Area (m^2)	No. of Hills	Yield: Per Plot of 100 m^2 (kg)	Yield: t/ha
Atzimba X R128.6	18	100	28.00	15.50
DTO 0.33 X R128.6	18	100	21.45	11.92
K. Jyoti (OP)	18	100	19.30	10.70
Patrones (Check from tubers)	18	100	28.65	15.90

The foregoing results from the use of TPS indicate potential of the new technology. The comparative higher yields in hybrid progenies is because of heterotic effects. It is reported that hybrids of tetraploid - diploid combination crosses or diploid crosses are uniform vigorous and high yielding than inter-tetraplids and OP progenies (4) which is attributed to large amount of heterozygosity transferred by the diploid parent to 4X progeny via FDR (2, 3). The present level of potato yield from the inter-tetraploid progenies could be also improved through a cycle of intercrossing followed by recurrent selection. Choice of suitable parents with wide genetic differences is also essential to develop potential hybrid progenies with the inherent capacity for marked vigor and high yield due to heterotic effects. Similarly productive open-pollinated progenies could be obtained by sib-mating of vigorous plants within its population rather than selfing or from self set berries. It would create a population with enhanced frequency of desirable genes for adaptation and yield. Genetic heterogeneity in varietal composition has vital biological advantages. It provides more efficient utilization of ecological resources by variable plant height, rooting systems and good protection against the incidence of diseases and pests.

Evaluation of TPS (F_1C_2) progenies (seedling tubers):

Comparison of F_1C_2 tuber progenies for their yielding ability and health gave commendable yield (Table 5). Yields were comparable with imported seed but excelled once grown seed by significant margin.

Table 5: Performance of F_1C_2 progenies during 1982-83

F_1C_2 Progenies	Total Yield (t/ha)	Seed Used (t/ha)	Net Yield (Total Yield-Seed)	Growth Mark*
DTO 28 x Serrana	31.33	3.37	27.96	10
DTO 28 x Atzimba	24.83	3.64	21.19	10
K 2500 x CP 1406	23.89	2.60	21.29	10
$N_2$48(a) X JEX-B-689	23.61	2.78	20.83	8
K. Jyoti	23.28	3.40	19.88	9
K. Sindhuri X JEX-B-689	23.17	3.42	19.75	8.5
K. Chandramukhi JEX-B-689	20.67	3.00	17.67	8
Dhankri x CP-1406	20.06	2.69	17.37	7
CP 1406 x JEX-B-72[illegible]	17.11	1.32	15.79	7
Mean net yield	-	-	20.19	-
Imported Basic Seed Mean of 4 Standard Varieties	26.56	4.80	21.76	9
Mean of 4 Standard Varieties (Once grown seed)	19.56	4.00	15.56	7

* 10 = Highly Vigorous

1 = Very Poor

In another observational trial, performance of seedling tubers (F_1C_2) confirmed the above observations (Table 6) seedling tubers from potential TPS progenies despite their small size were productive and compared favourably with imported seed.

Table 6: Performance of F_1C_2 progenies (1982-83)

TPS Progenies	Area Planted (m^2)	Yield (t/ha)	Plant Height (cm)	No. Tuber Per Plant
CP-1406 X JEX-B-723	2.4	16.60	40.4	12
N2-48 (X) X JEX-B-689	5.4	18.40	48.8	14
DTO-28 X Serrana	21.6	22.37	52.0	15
DTO-28 X Atzimba	21.6	19.31	55.1	68
K. Chandramukhi X JEX-B-689	21.6	19.53	62.8	19
K. Jyoti (OP)	18.9	21.19	58.8	22
Mean Yield	-	19.57	-	-
Imported Basic Seed (Mean of 4 Varieties)	2.4	21.91	-	-

Since most of the common viruses and tuber borne diseases are not transmitted via TPS, the farmers can with advantage utilize tiny seedling tubers as propagating material and thus providing a low cost and low risk technology because investment on costly tuber seed is no longer a limiting factor. The system of seed tuber production from TPS can provide potato growers in developing countries with simple but promising device of production of healthy seed tubers in their own environment (5). The Chinese through necessity have already commercially established the viability of the production of tuber seed from TPS in seedling beds as early as 1967 (1). Further investigations are however, needed to standardise seed multiplication scheme starting with TPS to workout that how far the seed stocks thus developed, could be maintained in their health. Studies on these aspects are underway at PRC, BARI.

Agronomy of TPS:

The results of the preliminary studies on production technology of TPS are summarized as follows:

Raising seedlings:

TPS sown on raised beds of 2 x 0.90 meters and about 30 cm high and provided with shelter of palm mats during 10.00 to 17.00 hours, had maximum germination ranging from 60 to 70 per cent. Germination was observed after 7 days of seeding and completed 60 to 70 per cent germination in viable seed within another one week. The seedling made rapid growth and were sturdy. Seed raised in seed boxes under net house were weak, etiolated and elongated and plant establishment in fields was poor. Such weak and dilapidated seedlings are prone to damping of.

For obtaining normal germination, seed whould be evenly broadcast and covered with fine layer of well rotton farm yard manure. Insecticidal dust should be applied as insurance against seed being carried away by insects.

A fertilizer mixture of 100 kg N, 200 kg P_2O_5 and [illegible] kg K_2O/hectare applied before final seed prep[illegible]tion was found to be optimum dose. Subsequently, 2 foliar sprays at interval of 6 days with 2 per cent urea was found to improve growth of the seedlings. Care must be exercised to ensure proper moisture in the seed bed for proper growth of the seedlings.

Transplanting and soil management:

Transplanting of seedlings (one month old) resulted in better plant establishment and growth. In the older seedlings, tuber initiation was observed. Short day conditions and abundant sunshine induce early tuber set.

Transplanting of seedlings on ridges gave better plant establishment and higher yield than planting on flat.

Comparison of seedling density suggested 2 seedlings per hill at spacing of 60 x 22.5 cm was optimum under tropical conditions for obtaining a quick and adequate ground cover and high production of tuber yield. Since one true seed produces single seedling which corresponds to one main single stem, number of seedlings should be proportionate to number of main stems of the crop raised from tubers per square meter for a high production potential.

Optimum planting time in central and southern regions is 15th November to 5th December whereas transplanting of seedlings is as late as 31st December. For better yields, the seedlings needs to be transplanted by 15th November so that crop raised from TPS get prolonged growing season and favourable cool season for tuberisation.

In general, the TPS raised crop matured later than its corresponding control plants.

Flowering and fruit set:

Most of the tuberosum varieties usually do not flower under short day conditions. Certain cultivars that flower; either the flowering is sparse or duration of flowering is too short unlike in temperate conditions. Preliminary studies have shown that flowering and berry setting in such genotypes can be greatly improved by planting on bricks and removing the tubers. Another observation is that late planting by early December was conducive for flowering than the early planting. It is also important to create a cool microclimate for profuse flowering for prolonged duration. Equinox conditions are more favourable to flowering than short day conditions.

CONCLUDING REMARKS:

Problems inherent in the use of TPS, such as identification of potential parental lines with profuse flowering and high fertility under tropical environment, incorporation of resistances for major diseases of the region into TPS progenies and reasonable degree of uniformity in plant and tuber characters and vigor need to be considered before adoption of TPS on a commercial scale. Agronomical and physiological aspects of TPS for adoption by small farmers in the tropics are important and need to be perfected.

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Experimental and Farmers' Use of Botanical Seed and Seed Tubers in Rwanda: Production Increase

A.J. Haverkort and A. Devaux

Introduction

Rwanda grows about 42,000 ha of potatoes per year (7) in two seasons corresponding with two rainy seasons. Production takes place mainly at altitudes above 2000 masl. Farms are small (about 0.85 ha) and besides labour and privately owned land, the only other input is seed potatoes kept from one season to the next. Changing seed or varieties used to be rare.

Before 1960, varieties were introduced from Europe; in the sixties, some varieties came from Uganda, and in the early seventies a few Mexican varieties were introduced in research stations.

Extensive screening of germplasm, seed production and distribution started in 1979 with the creation of PNAP, a collaborative effort of ISAR and CIP. PNAP's objective is to increase national potato production. With its breeding and seed production programs, the Rwandese National Potato Program (PNAP) overcomes two main constraints on potato production in the country: the unavailability of varieties resistant to diseases, mainly Phytophthora infestans, and the unavailability of seed tubers free of diseases such as Psedomonas solanacearum and other seed borne pathogens.

Two systems to improve national potato production are the subject of research in Rwanda: production and distribution of seed tubers of improved varieties and production and utilization of botanical seed. In 1980 research was initiated on the use of botanical seed. The two systems are here compared with the aid of a simulation model. This provides insight into some requirements which the use of botanical seed should fulfil inorder to contribute to increases in national potato production and in order to compete with or complement the use of seed tubers of resistant varieties.

Materials and methods

PNAP is a research program that aims to augment potato yields on farms. It is organized in 7 divisions (1): breeding, pathology, agronomy, storage, seed production, agro-sociology and on-farm research. To produce and distribute seed tubers, the breeding, seed production and on-farm research divisions work closely together.

The seed production division depended in the early stagges of the program on local varieties such as Muhabura and Bufumbira selected from the best farms. Small quantities of introduced varieties such as Sangema and Montsama were introduced on the basic seed production farm. Since 1983, four selected varieties such as Gahinga and Kinigi have been multiplied and distributed (3). More varieties were named in the breeding division in 1984. Every year about 5000 genotypes are screened, mainly tuber families from CIP and from local crosses (2). All advanced breeding material in its final stages of selection is tested by the on-farm research division in nummerous on-farm trials (5) before being released as a variety.

The quality of the seed of new varieties multiplied is maintained is maintained through a system of positive and negative selection (6, 8).

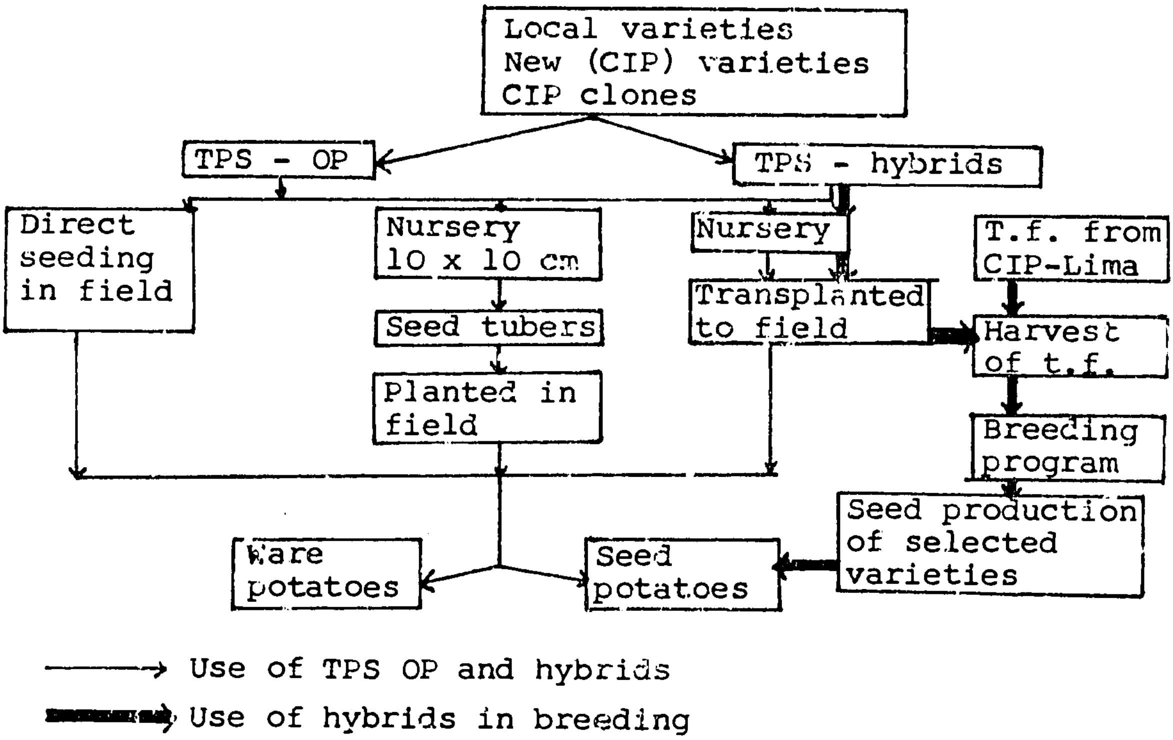
Seed produced on the seed farm is distributed to about 15 rural development projects throughout the country. These projects multiply the seed once or twice before distributing it to seed producing farmers' cooperatives or individual farmers (3).

The breeding, agronomy and on-farm research divisions collaborate in the search for the best means of improving production using botanical seed. Breeding selects the best OP and hybrid progenies from the point of view of vigour of seedlings, yield and resistance to diseases, both in the nursery or field and when used as seedling tubers in further multiplications. Agronomy tests their optimal use in the seedbed and transplanting (fertility and blight control), and the use of seedling tubers with and without selection in further multiplications (4). The on-farm research division tests the best progenies and techniques in on-farm trials. Figure 1. schematically represents the different ways TPS is being used at PNAP to obtain new varieties and improved (seedling tuber) seed on farms.

Results of on-station TPS trials

From 1980 until 1984, PNAP implemented dozens of trials using TPS. Subject to trial were sowing and transplanting methods, the use of fertilizers, manure and fungicides in the nursery and in the field, the use of seedling tubers and the

Figure 1. Scheme indicating the utilization of botanical potato seed in Rwanda



comparison of open pollinated (OP) and hybrid progenies. The following results are representative samples of those obtained at the PNAP station in Ruhengeri (1850 masl) on volcanic soils, mean temperatures: 18°C (Tables 1. to 5.).

Table 1. Comparison of different systems utilizing Gahinga-OP

	Yield (t/ha)	Tuber weight (gr)
Gahinga OP transplanted from trays	13.2	36.3
Gahinga OP transplanted from seed bed	8.8	34.3
Gahinga OP direct seeding	9.4	31.3
Gahinga OP seedling tubers planted	28.7	81.7
Gahinga variety tubers planted	40.8	164.0
LSD 0.05	4.5	13.6

This trial was implemented under optimal fertilizer and late blight control conditions and showed that both transplanting and direct seeding gave unsatisfactory results.

Table 2. Field trials with Gahinga OP transplanted from a nursery

Treatment	t/ha	Treatment	t/ha
Control	5.0	Control	12.7
500 kg/ha DAP	24.7	Fungicide 1x/2 weeks	17.9
1000 kg/ha DAP	18.8	Fungicide 1x/week	29.1
40 t/ha farm manure	15.6	Fungicide 2x/week	26.7
LSD 0.05	9.3	LSD 0.05	7.2

The fertilizer trial was carried out with a weekly fungicide spray and in the fungicide trial, 500 kg DAP was applied. The trials showed that with adequate application of fungicides and fertilizers, good yields can be obtained from transplants.

Table 3. Nursery trials with Gahinga OP, 100 seeds per m^2, harvested at maturity

Treatment	tub/m^2	kg/m^2	Treatment	tub/m^2	kg/m^2
Control	91	0.7	Control	251	2.6
50 g/m^2 DAP	355	4.1	Fungicide 1x/2 weeks	495	5.7
150 g/m^2 DAP	570	7.4	Fungicide 1x/week	426	4.8
4 kg/m^2 manure	243	3.1	Fungicide 2x/week	383	6.5
LSD 0.05	167	1.3	LSD 0.05	161	1.2

The fertilizer trial was sprayed once a week with a fungicide and 50 g/m^2 DAP was applied in the fungicide trial. With adequate inputs, good yields can be obtained in the nursery.

Table 4. Comparison of different transplanted progenies with clonal Sangema (no l.b. control)

Progeny	t/ha
2 hybrids from Lima like Atzimba x DTO33	12.8
3 hybrids from PNAP like 720055 x Atzimba	23.8
4 Open pollinated progenies like Sangema OP	11.5
1 Clone (variety Sangema)	15.8
LSD 0.05	4.3

This trial showed that hybrids of which both parents are late blight resistant, give the highest yields. Even higher than clonal Sangema in this trial. It also appeared that some local OP's gave higher yields than certain hybrids resulting from local crosses.

Table 5. Yields obtained from seedling tuber crops planted with (S+) or without (So) positively selected seed

Progeny	Yield (t/ha)		Δ Yield (%)
	S+	So	
720055 OP	30.1	28.4	8
Atzimba OP	19.5	18.7	4
720055 x Atzimba	38.9	28.8	34
Atzimba x Montsama	34.6	24.1	44
720055 x Montsama	34.6	26.0	33
LSD 0.05	2.8	2.8	

Selection of the best clones in the first crop planted with seedling tubers increased yields up to 44 % and was more effective in the hybrids than in the OP progenies.

The results of the trials on the station were sufficiently succesful to begin on-farm trials as of 1983. It should be noted, however, that the high yields obtained on the station with transplants, were due to the high levels of inputs, normally unavailable for farmers in Rwanda.

Results of on-farm trials using **TPS**

In 1983 and 1984, 20 on-farm trials using botanical seed were carried out on farms in Rwanda. Transplanted seedlings from a nursery and seedling tubers produced in the nursery were compared with the farmers' own seed or seed mixture. Care was taken only to change one practice of the farmer in these trials (5): transplants or seedling tubers in stead of the farmers' seed. Tabie 6. gives the results.

Table 6. Results of on-farm trials using TPS in Rwanda

Type of trial	Number of trials	Δ Yield (%)	Δ Tuber weight (%)
Seedling tubers	11	+119	-10.5
Transplanted seedlings	9	+ 45	-51.1

Farmers' average yields were 11.0 t/ha. The progenies used were Nseko OP and Kinigi x 378676-6.

On-farm nurseries of 1 - 3 m^2 were established to obtain seedling tubers from the hybrid in 1983 and 1984. Table 7. gives the results obtained in Kinigi (volcanic soils) and Cyeru (laterite). In these nurseries, 2 seeds were placed at 10 x 10 cm distances and the plantlets were allowed to mature.

Table 7. Results of on-farm nurseries in Rwanda, production of seedling tubers with Kinigi x 378676-6

Trials	Number	tub/m^2	kg/m^2
1983, volcanic soil, rainy season	4	432	9.80
1984, volcanic soil, dry season	7	205	2.05
1984, laterite soil, dry season	8	405	3.70

Presently another 22 seedbeds are installed on farms in the North of the country. All seedling tubers produced will be planted in the next season and their yields compared with those from the farmers' traditional seed.

Results of on-farm trials using seed from improved varieties

The yield improvements obtained using botanical seed need to be compared to those obtained with national programme seed in order to be able to judge the impact either system may have on national potato production.

In 1980 - 1984 about 200 on-farm trials with program seed were installed, Table 8. Farmers' average yields with their own seed were 10.7 t/ha.

Table 8. Results of on-farm trials with national program seed in Rwanda

Seed source	Years	Number	Yield increase (%)
Local varieties	80-81	8	23
Introduced varieties	81-82	72	40
Selected varieties	82-84	106	112

Varieties selected and named in Rwanda more than doubled farmers' yields. PNAP now distributes about 250 tons of improved seed per year to rural development projects. These projects multiply the seed once before distributing it to seed farmers or seed producing cooperatives. It is estimated that already over 25 % of poatoes grown in the country originate from the national program.

Discussion

The national production increase (NPI) in a given season that results from distribution of national program seed depends on the yield increase per hectare of the seed originating from the national program (after a number of multiplications over the farmers' traditional seed, and the hectareage with improved seed (HIS) grown by the farmers:

NPI = YI x HIS

YI in a certain season depends on the initial yield increase (IYI) of the seed just leaving the national program and the degeneration rate (DR) due to accumulation of seed borne diseases and loss of resistance:

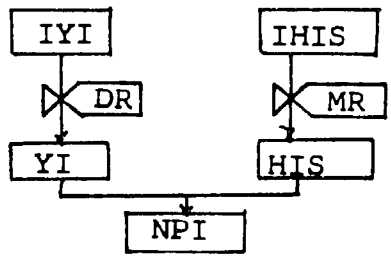
YI = IYI x DR

HIS in the same season depends on the initial hectareage with improved seed (IHIS) leaving the program each season and its multiplication rate during a number of seasons:

HIS = IHIS x MR

The model is schematically represented in Figure 2.

Figure 2. Relational diagram describing the factors contributing to the increase of national potato production



In a formula: NPI = $\int$((IYI,DR) x (IHIS,MR)), the following assumptions are made:

IYI is estimated at 7 t/ha (doubling national yields of 7 t/ha) based on Table 8., selected varieties.

IHIS is estimated at 66.7 ha/season based on the output of improved seed of the Rwandese national program.

DR = -0.583 t/ha/season when it is assumed that it takes 12 seasons (8 years) for the variety to degenerate to yields comparable to the national average of 7 t/ha.

MR is composed of a Replanting Component (RC) and a Distribution Component (DC): MR = RC + DC and is estimated at 1 + 4 at seed multiplication projects and DC diminishes at an estimated rate of .40 % per season in subsequent multiplications on farms because gradually less of the seed is used for distribution to neighbours and more and more will be consumed or sold. Thus:
MR = 1 + 4 x 0.6(EXP(s-2))

The formula for the accumulated national production increase for 12 seasons becomes:

$$NPI = \sum_{1}^{12}\left[\int((7,-0.583) \times (66.7,(1+4x0.6(EXP(s-2))))\right] = 802369 \text{ t.}$$

The accuracy of this figure may be disputed, but when the same methodology is followed to calculate how botanical seed should be used, then the weaknesses of the model will apply to both systems. The following assumptions are made as to how seedling tubers should perform in order to contribute as much to national production increase as seed tubers from the national program:

IYI from seedling tubers on farms equals 7 t/ha, again doubling yields as Table 7. shows to be possible.

MR = 1 + 4 x 0.6(EXP(s-2)), assuming the same multiplication rate and distribution rate for seedling tubers as for the new varieties.

DR = -0.583 t/ha/season, assuming the same degeneration rate for seedling tubers as for the new varieties.

From this it follows that in order to obtain the same national production increase from seedling tubers as from the new varieties, IHIS also should be 66.7 ha/season. With 1.5 seasons per year and 2 t/ha planted (the same as for the new varieties), 200 t of seedling tubers should become available annually.

When assuming nursery yields of 5 kg/m^2, 40,000 m^2 of seed beds are needed per year or 20,000 m^2 per rainy season, or 5000 farmers with 4 m^2 seed beds. When sowing 200 seeds per m^2, 8 million seeds need to be produced by the national program or by farmers themselves per year. In order to shift from seed tuber production to botanical seed, the national program would need less land and labour to produce this quantity of TPS per year: 1 ha with 40,000 plants producing 200 seeds per plant would suffice, instead of about 20 ha needed for the production of 200 t seed tubers.

If farmers were to produce their own OP botanical seed, each season 5000 farmers would need about 800 seeds to sow 4 m^2 seed beds. In this case the national program would have to supply 5000 farmers with a variety, Nseko for instance, that yields ample berries with sufficient botanical seed to produce seedling tubers that double yields in comparison to their own varieties.

Conclusions

Trials on farms in Rwanda show that both seedling tubers and seed tubers of improved varieties can double yields. To increase national production to the same extent as the production and distribution of new varieties do, botanical seed should be used in the following ways:

About 10,000 farmers per year would have to produce 20 kg of seedling tubers. The botanicalseed they would need should come from the national program or the national program would have to supply them with a few tubers of a proper variety producing OP botanical seed to be extracted by the farmers themselves.

The role of the rural development projects multiplying tuber seed would remain as instrumental for the multiplication and distribution of botanical seed as it is now for seed tubers of improved varieties.

Before the national program shifts noticeably to botanical seed production, more research will have to be done to compare the different possibilities that exist. Study will be needed with regard to farmers' willingness to introduce botanical seed in their farming systems, and the feasibility and eventual cost-saving aspects of the shift at the national program level, at the level of the rural development projects and at the farmers' level.

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Socioeconomic Research on the Production and the Utilization of True Potato Seed in Peru

Anibal Monares

The research on the production and the utilization of true potato seed (TPS) is still at the beginning stage. Most of the research on TPS has concentrated on genetic and physiological aspects related to known systems of TPS utilization (Accatino and Malagamba, 1983; Hermsen, 1981; Peloquin, 1981; Wiersema, 1983). Research on the production and use of TPS by farmers is scarce and quite recent (Kim et al, 1983; Li and Shen, 1979; Monares et al 1983; Upadhya, 1979).

TPS has been used extensively by breeders for obtaining new potato varieties. It has also been used by farmers to improve or occasionally replace the quality of degenerated potato varieties in locations where seed tuber certification programs do not exist. Several cases of this applied use of TPS have been reported in some isolated areas of the Andean countries, such as Colombia, Ecuador, Peru and Chile. There is evidence indicating that some native varieties from the Andean region of Latin America were created by the farmers' use of TPS technology (Monares, 1981; Salaman, 1970). In China, TPS has been used since 1972 as a first stage of some regional programs on basic seed tuber production (Bo Fu, 1984). More recently, commercial farmers in other Asian countries (Sri Lanka, Rwanda, and West Samoa) have started to use TPS on a small scale.

This paper presents the preliminary results of a socioeconomic research study on the production and utilization of TPS in Peru. The study was conducted by the International Potato Center (CIP) during 1982-1984.

I. Production of TPS

One of the challenges that faces TPS technology for future farmer adoption in developing countries is the need to develop production methods and institutional delivering systems that make quality seed available to farmers at a low price. CIP has been investigating these methods in Lima as well as throughout its regional research network.

In a socioeconomic study, calculations were made on the cost of producing 1 kg of TPS through different agronomic methods and of two progenies, Atzimba x DTO-28 and Caxamarca x R 128.6.* Table 1 shows the cost of producing 1 kg of Atzimba x DTO-28 seed and the relative importance of the main technical activities in which the total cost can be broken down. Total cost per kg was US$307, a surprisingly low figure for a production process done under experimental conditions. The main cost component was emasculation, representing about 60% of total production cost. Cultivation and pollination followed next as main cost items.

Table 1. Cost of producing 1 kg of hybrid TPS by activities at CIP, Lima, 1984

Activities	Cost (US$)	%
Production of parental clones	55	18
Flower and pollen collection	13	4
Emasculation	181	59
Pollination	30	10
Berry harvest	6	2
Processing and seed extraction	22	7
TOTAL	307	100

The same cost figure can be disaggregated by production factor (Table 2). The variable cost is less than 1/3 of total production cost and labor represents only 8%. The outstanding cost item is the technical management factor, which attempts to capture the cost elements associated with scientific knowledge and the managerial

* Only 100-150 g are needed to cultivate 1 ha of potato.

ability needed to carry out on the production process in an efficient and practical way. A private company producing TPS would certainly include this factor in its general cost balance.

Table 2. Cost of producing 1 kg of hybrid TPS by production factor at CIP, Lima, 1984

Production factor	Cost (US $)	%
Variable costs		
Seed	14	5
Labor	25	8
Fertilizers	4	1
Pesticides	22	7
Other chemicals	8	3
Materials	1	-
Equipment	17	6
Fixed costs		
Interest on capital	7	2
Rent on land	1	-
Technical management	208	68
TOTAL	307	100

Table 3 presents a summary of the production cost of TPS obtained with and without emasculation and the cost of open-pollinated (OP) seed for the two selected progenies. From the production standpoint Caxamarca x R 128.6 was a more efficient progeny than Atzimba x DTO-28. The production cost diminished dramatically without emasculation. This reduction was even greater for OP seed. Furthermore, for producing 1 kg of TPS with emasculation, only 110 m^2 of land and no more than 15 man/day were needed.

Table 3. Cost of producing 1 kg of TPS of two progenies at CIP, Lima, 1984

	Atzimba x DTO-28		Caxamarca x R 128.6	
	Prod. cost (US $)	%	Prod. cost (US $)	%
Hybrid seed				
With emasculation	306.2	100	234.6	100
Without emasculation	125.5	41	110.2	47
Open-pollinated seed	82.9	27	66.8	28

This brief economic analysis suggests that TPS could be produced by a private company or by a national agency at a relatively low cost. The market price of the hybrid TPS would probably be less than the price of tomato seed currently used in most developing countries. In this case, interested farmers would spend in seed per hectare less than 5% of the amount of money presently being spent.

Another important consideration is that in many developing countries most farmers do not produce the seed of the vegetable crops they cultivate. Seed for them is a typical purchased input, as is fertilizer and pesticides. This implies that most small potato producers from market-oriented areas will probably prefer to buy hybrid TPS --instead of producing their own OP seed-- if the hybrid seed proves to be a more productive planting material. Open-pollinated seed will probably be the preferred choice in subsistence farming areas with favorable ecological conditions for potato flowering and fruit setting.

II. Utilization of TPS

A low farm price for TPS is a necessary but not a sufficient condition for the existence of an economically viable seed industry. Also required is a significant and stable demand force coming from farmers who perceive the potential profits derived from using of TPS under their particular ecological and socioeconomic conditions.

In 1982 a multidisciplinary group of scientists from CIP, Lima, started investigating the potential use of TPS at the farm level in Peru through a network of on-farm experiments conducted under diverse farming conditions. The general purpose of these experiments was to appraise the gap between the technology's present stage of development and what would be needed for farmer adoption.

Objectives of On-Farm Experiments

The experiments focused on two specific objectives:

1. Identify the key elements of TPS technologies that influence their economic performance and acceptability at the farmer level.

2. Identify agroeconomic conditions under which TPS technologies have the greatest potential for farmer adoption.

Research Sites

In the last three years, we have conducted more than 30 TPS experiments under farmer conditions in one highland and two coastal zones of Peru (Table 4). Similar areas to these three zones can be found in many developing countries. The progenies Atzimba x R 128.6 and Atzimba x DTO-28 were used in most experiments.

The first selected zone, Tarma, is a traditional potato area (about 3,000 m) with only one potato growing season. Rainfall is irregular, but irrigation is available during the dry season, and there is a low rate of seed tuber degeneration. It is a vegetable crop area with mostly small farms and farmers skilled in transplanting, but labor is relatively scarce.

The second selected zone, Cañete, is also a traditional potato area where potatoes are grown in two different seasons. It is located in the semi-arid coast of Peru where there is no rainfall, but irrigation is available. The rate of seed tuber degeneration is rapid, and the main crops competing with potatoes are cotton and maize. Vegetable crops are unimportant, farmers are not familiar with transplanting, and labor is abundant.

Table 4. Agro-economic characteristics of TPS research sites (Peru)

	Tarma	Cañete	Callao
Agro-ecological characteristics			
Traditional/non-traditional potato zone	Traditional	Traditional	Non-traditional
Altitude	Highlands	Lowlands	Lowlands
Rainfall pattern	1000 mm (irregular)	No rain	No rain
Irrigation availability	Yes	Yes	Yes
Seed tuber degeneration rate	Low	High	High
Quality seed tuber availability	High	Low	Low
Crops competing with potatoes	Vegetables	Cotton, Maize	Vegetables
Socio-economic characteristics			
Farm size	Small	Small, medium	Small, medium
Farm potato consumption/total production	30%	10%	2%
Labor availability	Low	Intermediate	High
Potato yield level	15 t/ha	12 t/ha	18 t/ha
Price of ware potato	200 $/t	220 $/t	250 $/t
Cost of seed/cost of production	30%	40%	50%

The third zone is Callao, a non-traditional potato area close to Lima, where potatoes are also grown in two seasons. It is an area with irrigated vegetable crops, abundant labor, farmers skilled in transplanting, and the rate of seed tuber degeneration is rapid.

Experimental Results

In Tarma, results from on-farm experiments showed that the TPS transplanting system generated an acceptable yield, but was less profitable than farmer's seed tuber technology (Table 5).

Table 5. Profitability of TPS and farmer technologies in Tarma, Peru, 1982

Economic variables	Farmer practice	TPS technology
Marketable yield (t/ha)	25	23
Output price ($/kg)	.13	.11
Labor use (man/day)	163	234
Gross return ($/ha)	3,250	2,530
Variable cost ($/ha)	1,258	1,107
Net return ($/ha)	1,992	1,423
Rate of return (5/4)	1.6	1.3

In Cañete, TPS seedling tubers were evaluated in both the late and early seasons (Table 6). System 1 compared farmer's good quality seed from the highlands with TPS in nursery bed. System 2 evaluated highland seed and seedling tubers produced in seedbeds the preceding season. System 3 tested farmer's coastal seed and seedling tubers, both produced by the farmer in the late season.

Table 6. Use of TPS technologies in three potato production systems in Cañete, Peru

Production systems	Planting material		
	Early season	Late season	Early season
System 1			
Farmer practice		Highland seed	
TPS technology		TPS in bed	
System 2			
Farmer practice		Highland seed	
TPS technology	TPS in bed →	Seedling tubers from seed	
System 3			
Farmer practice		Highland seed →	Coastal seed
TPS technology		TPS in bed →	Seedling tubers from seedbed

Table 7 indicates that TPS technology was more profitable than farmer seed only in the early season when farmers used the virus-infected seed produced locally the preceding season (System 3).

In Callao, the TPS transplanting system was evaluated in the late and early seasons (Table 8). System 1 compared farmer's highland seed with transplants from TPS. System 2 tested farmer's coastal seed against seedling tubers coming from transplants. System 3 evaluated coastal seed and transplants from TPS.

In the three systems evaluated in Callao, TPS technology showed higher yields and net returns than conventional seed tuber technology (Table 9). Nevertheless, the profit was larger in System 3, when farmers used the virus-infected seed produced on the coast.

Table 7. Profitability of TPS and farmer technologies in three potato production systems in Cañete, Peru, 1982-1984

Economic variables	System 1		System 2		System 3	
	Farmer practice	TPS technology	Farmer practice	TPS technology	Farmer practice	TPS technology
Marketable yield (t/ha)	24.0	40.0	24.0	18	14.8	18.8
Output price ($/kg)	.26	.26	.26	.26	.24	.24
Labor use (man/day)	105	312	105	120	95	110
Gross return ($/ha)	6,240	10,400	6,240	4,680	3,552	4,514
Variable cost ($/ha)	2,905	10,174	2,095	2,189	1,989	2,126
Net return ($/ha)	3,335	226	3,335	2,491	1,563	2,388
Rate of return (5/4)	1.2	.0	1.2	1.1	.8	1.1

Table 8. Use of TPS technology in three potato production systems in Callao, Peru

Production systems	Planting material	
	Late season	Early season
System 1		
Farmer practice	Highland seed	
TPS technology	Transplants from TPS	
System 2		
Farmer practice	Highland seed ⟶	Coastal seed
TPS technology	Transplants from TPS ⟶	Seedling tubers from transplants
System 3		
Farmer practice	Highland seed ⟶	Coastal seed
TPS technology		Transplants from TPS

Preliminary Conclusions from On-farm Experiments

On the basis of on-farm experiments conducted by CIP in the last three years in Peru, some preliminary conclusions can be made. They should, however, be taken with caution since the number of trials was relatively small and the selected research sites and cooperator farmers might not be representative of the areas and farmers most suitable for TPS adoption. More research is needed before any conclusive statement can be presented.

1. Labor use

On-farm trials conducted in Peru indicated that TPS technology increased the use of labor compared to traditional seed tuber technology. The labor requirements of the transplant systems were 50% higher per hectare, but they were no more than those of other vegetable crops which require transplanting such as celery, onions, and beets. The two-stage use of seedling tubers from beds increased labor use up to 15%. As better progenies become available, simpler and more practical agronomic techniques will be developed. Farmers

Table 9. Profitability of TPS and farmer technologies in three potato production systems in Callao, Peru, 1982-84

Economic variables	System 1		System 2		System 3	
	Farmer practice	TPS technology	Farmer practice	TPS technology	Farmer practice	TPS technology
Marketable yield (t/ha)	19.1	24.0	14.9	19.8	14.9	22.3
Output price ($/kg)	.20	.26	.26	.26	.24	.24
Labor use (man/day)	110	160	105	118	105	160
Gross return ($/ha)	3,820	4,080	3,576	4,752	3,576	5,352
Variable cost ($/ha)	2,524	2,145	2,329	2,151	2,329	2,096
Net return ($/ha)	1,296	1,935	1,247	2,601	1,247	3,256
Rate of return (5/4	.5	.9	.6	1.2	.5	1.6

will become more familiar with TPS techniques, and significant reductions in labor requirements can be expected. In densely populated potato-producing areas, the adoption of TPS could help to alleviate rural unemployment.

2. Production costs

The cost of producing consumer potatoes from TPS was, in general, lower than the cost of producing potatoes from seed tubers on per hectare basis as well as per kilogram of output.

Two main factors influenced primarily the magnitude of the cost reduction: (1) the cost of seed tubers replaced by TPS, and (2) the additional cost of labor and other inputs (such as pesticides) required for using TPS.

In areas where the cost of seed tubers is a large proportion of production costs and minimum additional inputs are required for using TPS, the potential cost saving from TPS technologies can be even larger than in Peru.

3. Net return

TPS technology was less profitable than seed tuber technology in highland areas, such as Tarma, where farmers can obtain inexpensive low-virus seed. Nevertheless, in lowland areas, particularly non-traditional potato zones, such as Callao, where off-season production is possible TPS technology performed as well as or better than seed tuber technology. In these areas, TPS technology was relatively more profitable in the early plantings than the use of low-cost but highly degenerated seed saved from the previous harvest. Later in the season, more expensive but higher-yielding seed tubers from the highlands produced higher net returns.

On-farm trials conducted in the early season in Callao indicated that the transplanting system and producing tubers from seedbeds were equally profitable. Most farmers from this zone, however, were more interested in the transplanting system because it adapted better to their farming conditions. This is a vegetable-growing area and farmers are familiar with the transplanting operations used in other crops.

In Cañete, a traditional lowland potato area where vegetable crops are unimportant, farmers expressed more interest in the use of seedling tubers produced in a seedbed or in a directly sown plot.

In Peru, where consumers select for tuber size, flavor, and color, the average price of potatoes from TPS was between 10-20% lower than that of a crop from seed tubers. This price difference became smaller and even disappeared in seasons of insufficient potato supply and high market prices. In other developing countries, where consumer discrimination does not exist, the price of potatoes from TPS should be similar to the price of potatoes from traditional varieties.

Concluding Remarks

The knowledge and experience derived from TPS agroeconomic studies in Peru up-to-date indicate that farmers with difficulties in obtaining healthy seed tubers are the most likely candidates for using TPS in the near future. The system of using TPS, either by transplanting for ware production or by seedling tubers, will depend on such factors as labor availability, climate, and water supply.

In areas where farmers use seed tubers of acceptable quality, the potential for TPS use seems limited. In this case, the cost saving due to use of a cheaper planting material is small. Exceptions are those areas that use costly imported seed tubers from abroad or from distant production zones. However, TPS may still be an economic attractive alternative if progenies with desirable characteristics such as earliness, late blight and bacterial wilt resistance are introduced to compete with the commercial varieties that do not exhibit these traits.

For the near future more research effort should be placed on the potential use of TPS in subsistence farming areas where the potato is grown under limiting environmental and socioeconomic conditions. Backyard garden farmers in non-traditional potato areas seem to be a promising target group. Small farmers located in traditional potato-growing areas, because of their need to periodically renovate planting material, may also benefit from the use of TPS. Finally, it is certainly worthwhile to explore the use of TPS in urban home gardens as a low cost alternative that would contribute forward to alleviating the increasing food shortage affecting low income people in developing countries.

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True Potato Seed in Mexico, Central America, and The Caribbean

J. Fernández E., M. Villarreal G.

In the region of Mexico, Central America, and the Caribbean, in ten potato producing countries around 100 thousand hectares of potatoes are grown; 10% of this potato production area corresponds to Mexico. As shown in the following figures, potato production per capita exceeds ten kilograms in only three countries, while in six of them production is below five kilograms.

Per Capita Potato Production

Country	Kg.
Cuba	15.3
Costa Rica	12.4
Mexico	11.1
Panama	6.4
Guatemala	4.8
Dominican Republic	4.6
El Salvador	3.9
Honduras	1.7
Haiti	1.5
Nicaragua	1.0

Potato is a high nutrient value crop that can have an important role in regions where the basic diet is cereals and other roots and tubers, in order to increase the nutrition content. To achieve this objective, it is necessary that the production of food crops be appropriate for the potato producers.

Potato in the region of Central America, Mexico, and the Caribbean is not a basic crop, as maize, wheat, rice, and beans. However, potato production contributes significantly to the income of most of those countries.

Potato production problems within the region are similar, although they have distinct priorities. There is a coincidence in the first and principal limiting factor, the lack of healthy seed tubers. The cost of potato production is very high due to many factors, the major of which is the cost of seed-tubers. It represents around 40-50% of total potato production cost. Potato yields are very low due to use of deficient technology. Although improved technology is available, this is not used due to socio-economic factors, such as lack of agro-chemicals as well as healthy seed tubers, and an inappropriate credit system. In addition, an important factor is the unstable potato market which represents a risk to the potato grower. For these reasons, he does not adopt the improved, but expensive technology. Most of the available technology comes from the developed countries and in many cases this technology is not appropriate for our countries' local conditions.

If we are concerned about the agronomical requirements to increase the potato yield or to produce a better potato crop in any aspect, we should also think of the selection, modification or generation of techniques that can be adopted by the potato grower and do not represent an economical risk.

For many years we have been growing potatoes in the region. This cropping was initiated under a complete dependence of receiving seed tubers from European countries; that seed dependence started changing, little by little, by a development towards self-sufficiency. In some countries of the area, this self-sufficiency has already been reached, and in others, big efforts are being made to do the same using different methods. The principal method to reduce the volume of potato import is the use of healthy seed-tubers, and second is the use of rapid multiplication techniques.

Recently, results of research done at the International Potato Center (CIP) on a modern alternatives technology for potato production from True Potato Seed (TPS), have been evaluated in the region. These evaluations are done by planting the materials sent to us by CIP, and observing their performance at the research station level.

Mexico is the only country in the area that has a Breeding Potato Program. This program has been using TPS as a potato propagation technique, basically for experimental purposes. At the same time, and with the knowledge of the use of TPS as a source of seed, we have been observing the performance of the progenies from several crosses for hybridization of different genotypes. We are looking for promising material to obtain new potato varieties or crosses for selection of progenies with a minimal segregation, especially in tuber color shape, and size uniformity.

We believe that the use of TPS should have a wide acceptance as an alternative to obtain tubers to be used as seed. The use of TPS for ware potato production is still remote although in some countries of the area tuber color or shape is of little importance commercially. In Costa Rica, for example, tubers with white and red skin are mixed in the same sack for their consumption, while in Mexico, we buy at the same market the tuberosum and andigena type of potatoes. In Cuba, the main concern is to obtain high yields.

However, we think that the use of potato seed tubers from TSP can have a wide acceptance at the plateau of Guatemala or in the Mexican highlands as a source of healthy material, because in these areas typical farmers are small having around 1 hectare.

In El Salvador or the Caribbean Islands, the use of TPS can be good due to their warm, humid tropical weather conditions.

Potato production from TPS may, as well, be adopted by the largest vegetable producers in the region, due to their experience in vegetable production. However, they have enough economical resources to continue with the traditional potato production method obtaining high yields and economical profit.

At present, the National Institute for Agricultural Research (INIA), in Mexico, is conducting a project for the selection of both open pollinated and hybrid progenies with minimal segregation in tuber color, skin, and shape. This project is based on materials provided by the National Potato Program. The results are being compared with those of TPS materials from CIP and others provided by a commercial company in the United States.

The transfer of technology for the use of TPS in the region, has occurred through formal training courses in CIP, as well as in the National Potato Program of Mexico. Today, in all the countries in this area, TPS technology is known and considered a good alternative for the obtention of good quality seed-tubers for yield increase. However, this alternative technique is not considered a short-term solution to potato production.

TPS Research in Korea

- Method and Economics of TPS Production -

I. G. Mok

Introduction

Potato production from TPS has been investigated since 1980 at the Horticultural Experiment Station. The results of research projects are summarized as follows.

Breaking TPS dormancy GA 250 ppm treatment provided low germination rate compared with GA 750 and 1,500 ppm treatments. However, it increased germinability as much as those two treatments (>80%) when Ethrel 100 ppm was additionally treated. It means commercial GA for agricultural use, which provides 250 ppm as the maximum concentration, could easily be used for breaking dormancy in TPS (2).

Obtaining healthy seedlings It was recommended to use the mixture of ½ soil and ½ carbonized rice hull as nursery media. Leaf spray of urea or Hyponex stimulated seedling growth. Seedings grown for 35 days were the most suitable for transplanting to the field (2).

Selection of high yielding crosses Yield and tuber uniformity of TPS progeny have been determined by the use of several hundreds crossed and open-pollinated families. TPS families produced higher yields in fall than in spring (1,3). The crosses C047, H82154 and H82199 gave tuber yield of 20 tons per ha over 3 years while a leading cultivar, Dejima 15 tons. The tuber shape and uniformity of those crosses were acceptable.

Utilization of seedling tubers Less than 1 percent of virus infection was observed from the plot planted with seedling tubers. The yield of the plot was relatively low, although C047 was used in the trial. The selection of high yielding family at the second clonal generation is required. It is estimated that about 600 m^2 of nursery is necessary to produce enough seedling tubers for planting 1 ha.

In the present study, the methods of TPS production were compared in terms of TPS yield and cost.

Materials and Methods

Parental clone An early maturing variety Irish Cobbler and a medium-late maturing variety Alamo were used as female parents. A diploid Group Phureja haploid Group Tuberosum clone, W5295.7 which produces 2n pollen was used as a male parent.

Treatment There were four treatments with different spacing and cultivation method : 1) 70cm between rows and 30cm between hills with hilling when plants were about 20cm in height, 2) the same space without hilling practice, 3) 110cm between rows and 50cm between hills without hilling, 4) 70cm between rows and 30 cm between hills under a net-house where the potato was grown on bricks and developing tiny tubers were regulary removed. Plots for the treatment 1,2 and 3 were located at the open field and irrigation was not practiced. The treatment 4 was done under a net-house of which roof was covered with plastic film to prevent rainfall, and furrow irrigation was practiced whenever required. Sticks were used to support plants upright. Net covered the whole frame provided protection against wind and aphids.

Pollen collection and pollination Flowers were collected and dried over night at room temperature. A vibrator was used to shed pollen from anthers to gelatin capsules. The percent of viable 2n pollen in the male parent, W5295.7 was about 50% as determined by microscopic observation after aceto-carmine staining.

Every flower bloomed was pollinated without emasculation. Pollinated flowers could be easily distinguished by observing stigma so that flowers were pollinated just once. Irish Cobbler is male-sterile but Alamo is male-fertile. At field condition, neither of them bears even a single berry without artificial pollination.

Results and Discussion

<u>Flowering, fruit setting and seed productivity</u> The number of flowering as affected by planting density and cultivation method in each cross was presented in Table 1. The number of flowering per plant was greatly increased with the wider spacing. By the on-brick cultivation under net-house, the number of flower per m^2 was increased 6 times in Irish Cobbler and twice in Alamo compared with the average of field plots. But the number of flowering per m^2 was decreased with the wider spacing of 100 x 50 cm due to less plant number per unit area. Irish Cobbler was more sharply responded to the hilling treatment and to the on-brick cultivation. The flowering period of Alamo was later and longer than that of Irish Cobbler.

Fruit setting, as shown in Table 2, varied in the range of 43.9 - 79.3 % in Irish Cobbler x W5295.7 and 4.3 - 76.1 % in Alamo x W5295.7. The highest fruit setting was obtained with the on-brick method, especially in Alamo x W5295.7 the increase was more than 10 times as compared with the mean of field plots. The weight of seed per fruit was also increased by means of the on-brick method by 60 percent in Irish Cobbler x W5295.7 and 30 percent in Alamo x W5295.7. As a result, the amount of seed produced per m^2 was drastically increased with the on-brick method : 17 times in Irish Cobbler x W5295.7 and 29 times in Alamo x W5295.7 (Table 2). It is evident that regular irrigation and the removal of nutritional competition between berry and tuber increased the number of flowering, percent fruit setting, and seed yield. Among the field plots, the 70 x 30 cm without hilling treatment gave the highest seed productivity per unit area.

Evidences from a crossing plot located next to this experimental site indicated that early sprouting increased flowering, fruit setting, and the number of seed per fruit as presented in Table 3. Pre-sprouted plants, 5-7 cm in height with 2-3 leaves, were transplanted to the field together with the normal tuber planting as control. In comparing the pre-sprouted with the normal planting, Irish Cobbler did not differ in percent fruit setting and the weight of seed per fruit, while a mid-late variety Dejima gave a great difference in those variables. Flowering began 5 and 11 days earlier at the pre-sprouted plot in Irish Cobbler and Dejima, respectively. Also, the number of flower was almost doubled as planted with the pre-sprouted tuber.

As illustrated in Figure 1, the minimum temperature began to increase from June 13th. The flowering of Irish Cobbler was terminated well before the night temperature increases in both planting method. On the other hand, the flowering of Dejima lasted till

Table 1. Number of flower as affected by planting density and cultivation methods.

Crosses Treatments	# of plant used	Total # of* flowering	# of flower per plant	# of flower per m^2	Flowering period
Irish Cobbler x W5295.7					
70 x 30 cm hilling	240	1,721	7.2	34.1	June 5-14
70 x 30 cm no hilling	240	2,703	11.3	53.6	"
110 x 50 cm no hilling	90	1,546	17.2	30.7	"
70 x 30 cm net-house on bricks	10	555	55.5	264.3	May 31-June 14
Alamo x W5295.7					
70 x 30 cm hilling	240	2,942	12.3	58.4	June 8-27
70 x 30 cm no hilling	240	2,635	11.0	52.3	"
110 x 30 cm no hilling	90	1,567	17.4	31.1	"
70 x 30 cm net-house on bridks	10	188	18.8	89.5	June 4-13

* Every flower was pollinated.

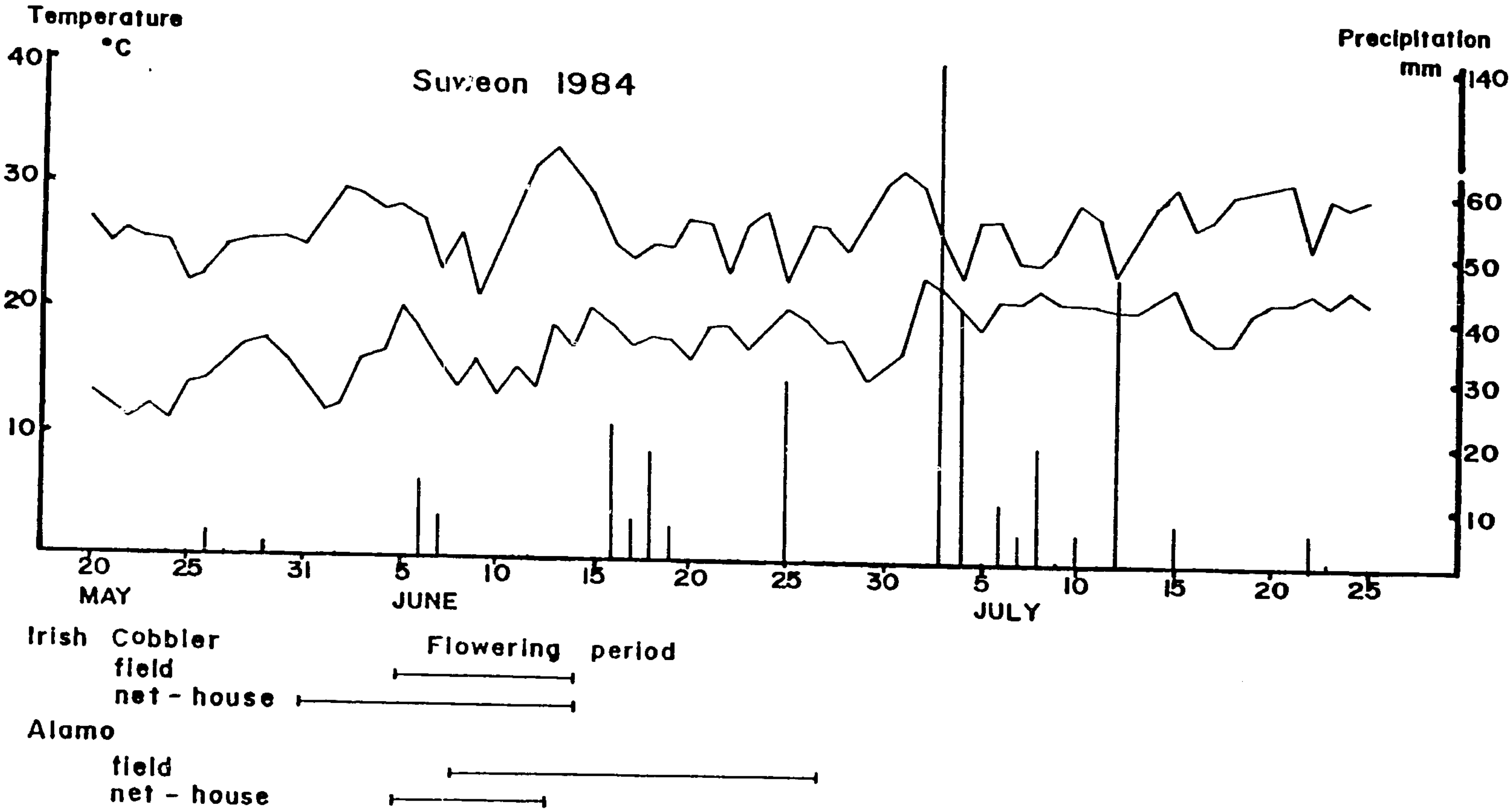

Fig 1. Maximum (upper line), minimum temperature (lower line) and precipitation (vertical bars) during flowering and fruit setting.

Table 2. Number of fruit and weight of seed harvested as affected by planting density and cultivation methods.

Crosses Treatments	Total # of fruit harvested	Wt. of seed harvested	% fruit setting	Wt. of seed per fruit	Wt. of seed per m^2
		g		mg	g
Irish Cobbler x W5295.7					
70 x 30 cm hilling	756	41.3	43.9	54.6	0.82
70 x 30 cm no hilling	1,421	80.3	52.6	56.5	1.59
110 x 50 cm no hilling	1,026	61.5	66.4	59.9	1.22
70 x 30 cm net-house on bricks	440	42.2	79.3	95.9	20.10
Alamo x W5295.7					
70 x 30 cm hilling	127	13.3	4.3	104.7	0.26
70 x 30 cm no hilling	217	22.3	8.2	102.8	0.44
110 x 50 cm no hilling	154	14.3	9.8	92.9	0.28
70 x 30 cm net-house on bricks	143	19.6	76.1	137.1	9.33

Table 3. Effect of pre-sprouting on flowering,fruit setting and seed yield.

Crosses Planting method	No. of flower per plant	% fruit setting	Wt. of seed per fruit	Wt. of seed per plant	Flowering period
			mg	mg	
Irish Cobbler x W5295.7					
Pre-sprouted	11.2	58.5	81.7	534	May 31-June 7
Control	5.8	78.6	72.0	335	June 5-8
Dejima x W5295.7					
Pre-sprouted	12.5	8.0	80.0	80	May 28-June 7
Control	6.4	1.0	42.5	3	June 8-20

June 20 in the control plot while finished by June 7 in the pre-sprouted plot. It is likely that the lower minimum (night) temperature during flowering would be favorable to fruit setting. Besides irrigation and the removal of tubers, the favorable night temperature during the flowering period contributed, at least partially, to the tremendous increase of seed yield obtained by the net-house cultivation. It seems that net-house hastened the growth and development of young plant when the night temperature is still too low out in the field. In general, the earliest planting date is limited to March 25 in Suweon area. Therefore, to obtain the highest seed yield, on-brick cultivation under a net-house and planting with the pre-sprouted tubers would be recommendable, especially for the mid-late and late maturing varieties such as Alamo and Dejima.

Since 1,000 seed weight of TPS is roughly 500 mg, the number of seed in a single fruit was estimated as 100-200 in Irish Cobbler and 200-250 in Alamo. The result proved the fact that the use of a diploid 2n pollen producing clone as a male parent also guaranteed practically enough number of seed per fruit.

Time requirement and cost   Time required for each step of hybrid TPS production, i.e., flower and pollen collection, pollination, berry harvest, and seed extraction, was measured and converted to 10a (one lengh of ha) basis as presented in Table 4. Only one field plot, 70 x 30 cm without hilling and the net-house cultivation were compared, since these methods produced the higher seed yield. The calculation of number of flower for pollination was based on the actual flowering data (see Table 1), and it was assumed that one half of total area is effective for net-house to allow space and paths. For the field trial, most time was spent for pollination (35 percent in Irish Cobbler x W5295.7 and 60 percent in Alamo x W5295.7) ; for the net-house method, seed extraction required most time (66 and 59%, respectively for each cross). The time consumption for seed extraction can be reduced by introducing a simple seed extractor.

In Table 5, material and labor cost for hybrid TPS production were presented on the basis of 10a. Since the number of planting was one half for the net-house cultivation, the cost of seed tuber, fertilizers, compost, insecticides, and other materials were also halved compared with the field plot. The cost for net-house included house frame (usable for 10 years), net (for 2 years), sticks for plant support (for 5 years), bricks (for 10 years), and labor cost for the construction. Labor cost for general management of net-house was 4 times higher than that of field plot, since the additional input such as planting on bricks, binding to sticks, removal of tubers, and irrigation were required. Labor cost for seed production was calculated from the total time presented in Table 4. Thirty percent suspended time for preparation and breaks was included.

Table 4. Time required for each step of hybrid TPS production per 10 a.

Crosses Treatments	Estimated number of flower for pollination	Flower and pollen collection	Pollination	Berry harvest	Seed extract & drying	Total
		— hour (percent) —				
Irish Cobbler x W5296.7						
70 x 30 cm no hilling	53,600	44 (21)	75 (35)	19 (9)	74 (35)	212 (100)
70 x 30 cm net-house on bricks	132,100*	111 (12)	179 (19)	32 (3)	635 (66)	957 (100)
Alamo x W5295.7						
70 x 30 cm no hilling	52,300	47 (22)	126 (60)	16 (8)	21 (10)	210 (100)
70 x 30 cm net-house on bricks	44,800*	44 (12)	99 (26)	12 (3)	222 (59)	377 (100)

* The calculation is based on the assumption that one half of total area is effective for planting.

Table 5. Material and labor cost for hybrid TPS production per 10a.

Crosses Treatments	Materials			Labor		Total
	Tuber seed	Fertillizer & miscellaneous	Net-house	General management	Seed production**	
			1,000 won (USD)			
Irish Cobbler x W5295.7						
70 x 30 cm no hilling	57.5 (72)	73.5 (92)	-	225.6 (282)	162.1 (203)	518.7 (649)
70 x 30 cm net-house on bricks*	28.7 (36)	46.2 (60)	317.2 (396)	451.2 (564)	730.9 (914)	1,574.2 (1,970)
Alamo x W5295.7						
70 x 30 cm no hilling	57.5 (72)	73.5 (92)	-	225.6 (282)	160.6 (201)	517.2 (647)
70 x 30 cm net-house on bricks*	28.7 (36)	46.2 (60)	317.2 (396)	451.2 (564)	288.2 (360)	1,131.5 (1,416

* The calculation is based on the assumption that one half of total area is effective for planting.
** Thirty percent suspended time is included to caculate the cost.

Total cost was about 2 and 3 times higher at the net-house than at the field plot in Alamo x W5295.7 and Irish Cobbler x W5295.7, respectively.

To obtain the net cost of hybrid TPS production per 10a farmer sale price of the potato harvested at the field was deducted out of the total cost per 10a. The net cost divided by seed yield gives the net cost for 10 grams of TPS which is quite enough to grow seedlings for 10a. The net cost for 10 grams of TPS was given in Table 6. The cross Irish Cobbler x W5295.7 required less net cost when produced at the field. On the contrary, the cross Alamo x W5295.7 required less net cost at the net-house where the seed yield was sharply increased.

The farmer price of TPS was calculated by assuming that he would anticipate 625 USD per 10a as net income from his farm business. The net income is higher than that of the farmer producing certified potato seed and is lower than that of the farmer producing vegetables in plastic houses. Although the higher input was given to construct the net-house, it paid back by reducing the estimated farmer price, compared with the field plot, to 60 and 20 percent in Irish Cobbler x W5295.7 and Alamo x W5295.7, respectively. The use of TPS, according to the result, will cost less than one tenth in purchasing seed compared with the tuber seed planting in Korea.

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Table 6. Estimated price of hybrid TPS.

Crosses Treatments	Total cost per 10 a (A)	Sale price of potato harvested (B)	Net cost per 10 a (A-B)	Seed yield per 10 a	Net cost for 10 gr of TPS	Estimated farmer price for 10 gr of TPS*
	—— 1,000 won (USD) ——			gr	—— won (USD) ——	
Irish Cobbler x W5295.7						
70 x 30 cm no hilling	518.7 (649)	470.3 (588)	48.4 (61)	1,593	304 (0.4)	3,443 (4.3)
70 x 30 cm net-house on bricks	1,574.2 (1,970)	-	1,574.2 (1,970)	10,048	1,567 (2.0)	2,064 (2.5)
Alamo x W5295.7						
70 x 30 cm no hilling	517.2 (647)	354.1 (443)	163.1 (204)	442	3,690 (4.6)	17,265 (21.6)
70 x 30 cm net-house on bricks	1,131.5 (1,416)	-	1,131.5 (1,416)	4,667	2,424 (3.0)	3,496 (4.4)

* As farmer's income, 500,000 won (USD 625) per 10a was added.

True Potato Seed (TPS) Research in Egypt (1)

by

C.Engels(2), S.Sadik(2) and R.A.El-Bedewy (3)

INTRODUCTION

Among vegetable crops in Egypt, the potato is considered as one of the important crops for export and local consumption. About 1.2 million tons are produced annually, of which 900,000 tons are locally consumed and about 150,000 tons are exported.

Egypt imports annually 50,000 tons of seed tubers from Europe at a cost of nearly 25 million U.S. dollars. This is planted in December to February during the Spring season. More than 100,000 tons are saved from the Spring crop in refrigerated and traditional non-refrigerated stores for planting in September to November during the Autumn season. In general, yields achieved during the Spring season are superior to those obtained in Autumn because of the poor physioligical age and health quality of planting materials used in Autumn.

Any improvement by reducing the amounts of imported seed tubers through local production of healthy seed tubers or through production from TPS will have a tremendous impact on local production.

Research in Egypt by CIP was started in 1984 to test the feasibility of introducing potato production from TPS for local consumption as a supplement to the traditional method of using seed tubers as planting material. The main objective of introducing the TPS system of potato production is to produce more potatoes at a lower cost to meet some of the food demands of the expected 30 million more people that are expected to be borne by the year 2000. It is also hoped that the TPS system of potato production can be transferred to other contries of the Middle East from Egypt if successfull.

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FLOWERING AND TPS PRODUCTION

Studies on floral induction and berry set were conducted in the field using European cultivars. The studies were conducted in two locations :

1. Kafr El-Zayat near Tanta which is located in the center of the Nile Delta at 30° 40'N.
2. St. Catherine which is located in the southern part of the Sinai desert at 28° 30'N.

At kafr El-Zayat,, 11 cultivars were planted in the field on February 26,1984 in 20 cm deep furrows filled with sand and peatmoss mixture to enable the removal of stolons and tubers as they develop, thus simulating the well known " tuber milking " and " brick " methods for floral stimulation. Stolons and tubers were removed from plants periodically. In an adjacent field the same cultivars along with others were planted as usual, and stolons and tubers were not removed. All cultivars that flowered did so regardless of whether stolons and tubers were removed or not. The only possible exception was cv. Baraka which flowered and set berries in greater amounts when stolons and tubers were removed.

Flowering and berry set of European cultivars was studied in Kafr El-Zayat. Certified (class A) tubers of 34 cultivars were planted on January 29,1984. Observations on flowering were made periodically during the growing season and berries were harvested on May, 20. .About half of the cultivars flowered in varying degrees. Some cultivars such as Isna flowered profusely, however most flowers abscised. Other cultivars such as Alpha flowered profusely , however the flowers failed to open and therefore no berries were obtained. Most berries were above average in size and contained good quantities of seed with high viability rates (Table 1).

In St. Catherine, 11 European cultivars and one TPS progeny from CIP Atzimba x 7 X Y.1 were planted on April 18,1984. The location was selected because of its cool climate during days increasing in length. By and large flowering of all cultivars was poor,, but it was interesting to note that cultivars that flowered at St. Catherine were those that flowered at Kafr El-Zayat. Because of the early onset of hot summer winds, flowers abscised and no berry set was observed.

Table 1 : TPS production of European cultivars and clones in Kafr El-Zayat, Egypt 1984.

Cultivar or Clone	Berries from 250 plants	Total Seed Weight (gm)	Seed Weight per berry (mg)
Pent. Ivory	337	45.3	134.4
Granola	11	1.5	136.4
Vittorini	29	3.7	127.6
Cara	250	31.1	124.6
# 284	124	15.2	122.5
Mirka	38	4.3	113.1
# 649	26	2.7	103.8
Domina	290	29.3	101.0
Drayton	31	2.9	93.5
Grata	14	0.8	57.1
Sahel	8	0.4	50.0
Isna	37	1.7	45.9
Diamant	3	0.13	43.3
Total	1448	170.2	117.5

NURSERY MANAGEMENT

The objective of work in Egypt on managememt of nurseries is to produce good quality transplants and high yields of seedling tubers taking into consideration farmers capabilities and constraints.. To achieve this objective, research has been focused on simplifying seed bed construction, cooling by shading or other means, reducing or eliminating the need for peatmoss in seed bed by using other available organic residues, irrigation and fertilizer application, and control of seedling damping-off.

A - Transplant Production

1. Seeding substrate :

The use of the 1:1 peatmoss/sand mix augmented with nutrients have been successfully used by several workers for producing potato transplants and seedling tubers in ground beds . The incorporation of even small amounts of soil in the mix (20 %) had negative effect on seedling growth and seedling tuber production (1, 2). The use of peatmoss / sand mix is costly and sometimes impossible to use because of unavailability of peatmoss to farmers and therefore, in most cases, can not be recommended to farmers. Consequently investigations in Egypt have been conducted to identify a substrate mix that contains minimal amounts of peatmoss or a substrate that contains other organic sources available to growers as substitutes for peatmoss.

Six mixes of peatmoss, soil and sand augmented with 100,300,100 ppm NPK respectively were evaluated for their suitability for transplant production in 15 cm deep wooden flats (Figure 1).

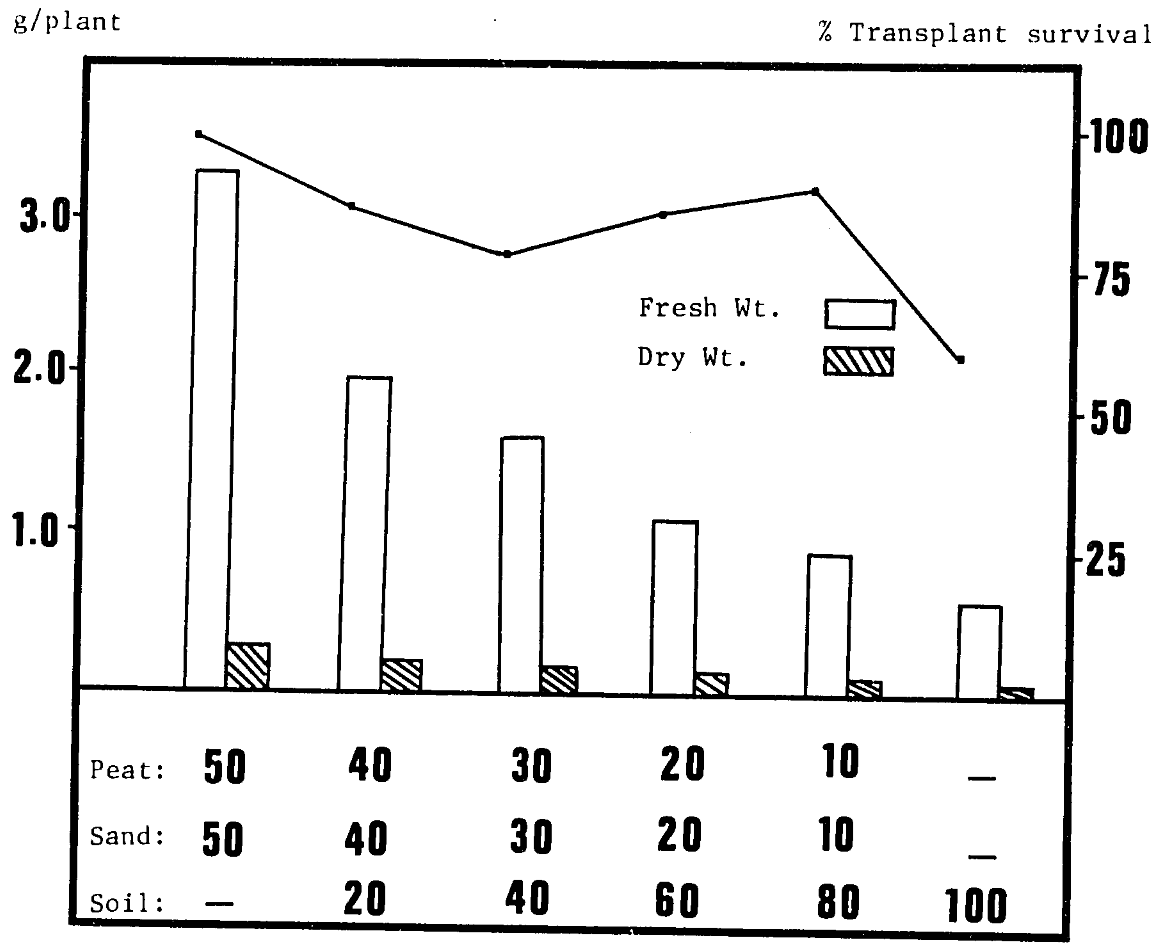

Figure 1: EFFECT OF SEEDING SUBSTRATE COMPOSITION ON SEEDLING GROWTH AND SURVIVAL AFTER TRANSPLANTING

At the time of transplanting, seedling growth was best in the peatmoss/sand mix and decreased as the soil portion of the mix increased. Transplant survival in the field was best in mixes that contained the least amounts of soil because of difficulties in obtaining a coherent " earth ball " around the root system. Substrate mixes containing high portions of soil had surface crusting, possible bad gaseous exchange and high weed populations, factors that adversely affected early seedling growth and ultimately growth and survival in the field after transplanting.

In another experiment, several types of mixes including 100% soil were compared in ground beds. It was observed that most of the problems in ground beds containing 100% soil were related to poor early seedling growth. Later plant growth in ground beds containing 100% soil was not significantly different from that obtained in the 1:1 peatmoss/sand mix. This observation suggested the possibility of concentrating the peatmoss in the top 5 cm. layer of the ground bed instead of mixing large quantities of peatmoss throughout the soil in the ground bed. This localized mixing was achieved firstly by mixing a 2 cm layer of peatmoss with the total amount of soil in a 30 cm deep ground bed, and secondly by mixing a 1 cm layer of peatmoss with the top 5 cm soil layer. Through this procedure the upper 5 cm layer of the ground bed had a peatmoss content of about 30%, while the bottom layer had a peatmoss content of about 10 %. This localized surface application of peatmoss saved a significant amount of peatmoss.

An experiment was conducted to evaluate seed germination, seedling emergence in ground beds with and without localized peatmoss application. The treatments were the following :

1. 50% peatmoss and 50% sand
2. Soil with localized peatmoss application.

The experiment is still in progress, however 20 days after sowing, there were no apparent significant differences between both treatments in regard to percentage of emerging hills or number of seedlings (Table 2).

Table 2 : Effect of substrate composition in ground beds, 20 days after sowing on seedling emergence of Atzimba x R 128.6.

Substrate	Emerged Hills (%)	Emerged Seedlings (%)
50 : 50 peatmoss/sand	93 ± 3.5	75 ± 3.5
Soil with localized peatmoss application .	94 ± 4.7	70 ± 9.8

Another experiment was conducted in 15 cm. wooden flats to test the hypothesis that a localized modification of the upper layers of the soil is suitable for seedling production. The flats contained the following substrates :

1. 50% peatmoss : 50% sand.
2. 90% soil/peatmoss with localized peatmoss application.
3. 90% soil/peatmoss with localized application of wood shavings.
4. 90% subsoil/peatmoss with localized peatmoss application.

In this experiment the peatmoss and wood shavings were localized in the upper 5 cm layer of the flat as described earlier. The results of this experiment are presented below.

Table 3 : Effect of substrate composition in wooden flats on seedling emergence and fresh and dry weights of plants of Atzimba x R 128.6.

Treatment*	% Emergence 15 days after sowing	gm/plant 38 days after sowing +	
		Fresh Wt.	Dry Wt.
1	74 ± 9.0	1.29 (a)	0.107
2	77 ± 5.0	0.95 (c)	0.084
3	83 ± 5.0	---	----
4	84 ± 5.0	1.16 (b)	0.097

* see above for explanation of treatments.
\+ calculated mean of 30 plants.

Seedling emergence in all treatments 15 days after sowing was satisfactory, however, plant growth until transplanting was significantly better in the 50 : 50 peatmoss/sand.. None the less the seedlings obtained from all treatments except where wood shavings were applied were considered of good quality for transplantings. The application of wood shavings resulted in weak, stunted and chlorotic seedlings that eventually died. This may have resulted from the inhibitory effect of preservatives injected in imported lumber from Europe.

In effort to eliminate the need for using peatmoss in substrate mixes for transplant and seedling tuber production we are considering the possibility of using inexpensive organic farm residues in composted form such as chopped whole maize plants, maize cobs, sugar cane bagasse, saw dust and hyacinth plants. Most of these organic sources are being composted and their potential use will be evaluated.

2. Irrigation of nurseries :

Previously, flats and ground beds at CIP's research station in Egypt were watered daily by watering can or a fine spray gun connected to a watering hose. These gentle watering methods are necessary during seed germination and early seedling emergence. Watering of ground beds by these methods proved to be time consuming and did not provide enough water distribution especially in low soil layers of ground beds. Investigations to find better methods of watering, showed that watering ground beds by flooding every three days after seedling emergence gave better water distribution throughout ground beds. This simplification of water application is the same method used by Egyptian farmers for producing vegetable transplants.

3. Fertilizer application to nurseries :

The recommended schedule of fertilizer application of Wiersema(2) for ground beds for seedling tuber production was simplified by reducing the N and K quantities and their frequency of application. Good results have been obtained by applying 80 gm P_2O_5 / m^2 in one dose to mixes before seed sowing only. Eighteen gm/m2 each of N and K_2O were applied in three doses (in water solution) at 14 days after seedling emergence, after the second hilling (~ 30 days) and when the ground bed were covered with foliage (~ 40 days). These simplifications reduced labor inputs and cost of fertilizer without yield reduction.

Under conditions in Egypt Atximba x 7 XY.1 is a late progeny and it was found that its haulm growth was excessive and its maturity was delayed significantly and seedling tuber yields were reduced by a schedule of nitrogen application similar to that of Wiersema (Table 4).

Table 4 : Effect of nitrogen application on plant growth and seedling tuber yield of Atzimba x 7 XY.1 in 1:1 peat/sand mix.

Total applied N in 9 doses (gm/m^2)	Fresh weight of haulm at harvest (kg / m^2)	Seedling tuber yield(> 1 gm)	
		Weight (kg/m^2)	Number /m^2
7.7	4.97 ± 0.44	3.70 ± 0.24	513 ± 25
19.2	5.43 ± 0.34	2.96 ± 0.27	423 ± 22
38.3	7.59 ± 0.38	2.63 ± 0.21	340 ± 17

4. Seedling damping-off:

Damping-off of seedlings in flats, ground beds or after transplanting has been a serious problem that resulted in the destruction of several experiments. Fungicides such as Benlate and Rizolex have given partial protection. In order to make the TPS technology an acceptable method for farmers, the pathologist is concentrating his research to produce effective and appropriate methods to control damping-off.

The method of using subsoil in ground beds to control damping-off has been found useful (Figure 2).

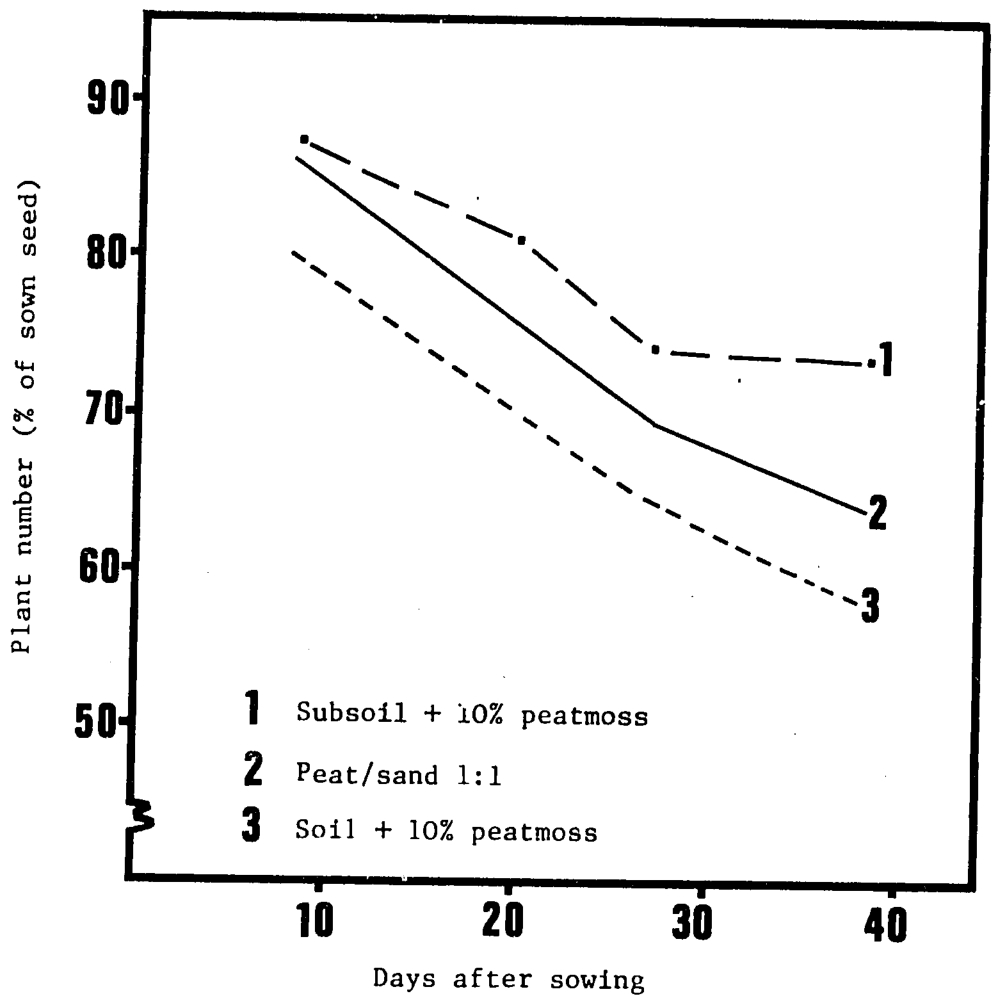

Figure 2: EFFECT OF SEEDING SUBSTRATE ON SEEDLING SURVIVAL

5. Transplant shock and survival :

Several experiments were conducted to reduce transplant shock and to improve transplant survival in the field. Seed of Atzimba x 7XY.1 was sown in ground beds on September 7,1984, and transplanted to the field on October 8, 1984. Seedling emergence after two weeks from sowing was 85 % and seedling survival after 1 and 2 weeks from transplanting in the field were 95% and 93 % respectively. Seed of Atzimba x Katahdin was sown on September 7, 1984 and transplanted on October 10,1984. Seedling emergence after two weeks from sowing was 90% and seedling survival was about 97 %.

During transplanting of both progenies,, maximum and minimum temperatures of day and night were over 35 and 18°C respectively. Despite these high temperatures, transplant survival was very high. It is worth noting that the survival rate of seedlings of progeny Atzimba x R 128.6 transplanted in Norember was lower than that of Atzimba x 7XY.1 and Atzimba x Katahdin transplanted on October when day and night temperatures were higher than in November.

B - Seedling Tuber Production

1. Strategy :

There could be an important potential for the use of seedling tubers from TPS by **farmers in** Egypt and most countries of the Middle East provided that the following important conditions are fulfilled :

(a) The ability to produce seedling tubers 2-3 months before planting to ensure that dormancy is broken.

(b) Availability of adapted early maturing TPS progenies.

(c) Availability of TPS.

In Egypt, for example, there are two main seasons for potato production :

1. Spring season : Seed tubers are imported annually from Europe and planted between December and February. The crop is harvested in March / April for export and May/June for local consumption, and for planting during the Autumn season.

2. Autumn season : Seed tubers obtained from the Spring crop are planted between September and November. The health and physiological qualities of planting materials used in this season are not satisfactory.

Egypt exports annually about 150,000 tons (12.5 % of total production) to Europe and Arab markets, while local consumption is about 900,000 tons (75% of total production). Egypt is anticipated to continue its interest in the export market, therefore importation of certified seed tubers of cultivars accepted in European markets will continue. CIP's strategy therefore is to provide a technology of producing seedling tubers to produce ware potatoes for local consumption during the Spring and Autumn seasons.

Seedling tuber production for planting in the Spring will largely depend on our ability to obtain well sprouted seedling tubers for planting in January and February.. If we take into account that it takes 4 months to produce seedling tubers and 2 months of storage to break dormancy naturally before planting, then TPS must be sown in early August.. During that time day and night temperatures are above optimum for seed germination and seedling growth. Therefore methods for cooling nursery beds such as shading, frequent irrigation and others, must be used..

An ideal solution to the above problems would be the availability of TPS progenies that have heat insensitivity during germination and early seedling growth, early maturity, and short tuber dormancy.. The availability of such progenies can make it possible to produce seedling tubers for the main potato crop in Egypt during the Spring season and result in a tremendons impact on potato production especially for local consumption.

Short of a TPS progency with the above attributes our strategy is to produce seedling tubers for the Spring by sowing TPS as early as possible in August under protective conditions and to harvest at the end of November, thus allowing 2 months for breaking seedling tuber dormancy before planting.

For Autumn planting the strategy is to use first or second generation seedling tubers :

Second generation seedling tubers originate from plants that were exposed to virus infection and thus are of lower health quality. Therefore the use of first generation seedlings is more desirable.

2. Nursery management in summer :

In 1983, TPS was sown on August 5 in ground beds shaded for 2 weeks with rice straw and supplied with frequent irrigation. Seed germination was above 90% and adequate seedlings were obtained and transplanted on September 15. This was possible because of the unusually mild temperatures during August.

In 1984, TPS was sown in shaded ground beds on August 6 and germination was 80 - 90 %. For shading, cheese cloth allowing 60 % light transmission or bamboo mats allowing 30 % light transmission were used and proved adequate. Damping-off was serious and early seedling growth was characterized by slender, tall seedlings with long internodes. This type of growth made it necessary to hill earlier than usual to give seedlings some support. During September, seedling growth improved as day and night temperatures decreased. Early seedling growth in August was improved in ground beds by mulching with shredded maize stocks or paper. The experiments are still in progress, but by all indications, good seedling tuber yields are expected in late November. Seedling tubers from these experiments will be planted in February 1985, provided that the dormancy can be broken.

3. Storage of seedling tubers :

First generation seedling tubers of 2 TPS progenies produced in the Spring season were harvested at different dates and stored for different periods in a Nawalla (non-refrigerated store) and in a cold store at 4^{o}C.. The objective of the experiment was to evaluate the storability and subsequent suitability of the stored seedling tubers for ware potato production.

Table 5 shows that the number of decayed tubers even after 150 days of storage in the Nawalla was surprizingly low and is similar to figures obtained during storage of local cultivars stored under the same conditions.

In the same experiment above the effect of storage in the Nawalla on the loss of seedling tubers of different grades was also evaluated and again the amount of losses of even small sized seedling tubers were found acceptable (Table 6).

Table 5 : Effect of storage on losses of seedling tubers of 2 TPS progenies (1984) .

Harvest date		TPS Progeny	Type of store	Storage days	% # Decayed tubers	% Total weight loss	% Physiological loss
April	28	Atz. x Kat	Nawalla	150	15	38	24
May	10	Atz. x 7XY.1	Nawalla	138	5	21	18
June	9	Atz. x 7XY.1	Nawalla	108	3	18	16
June	6	Atz. x Kat.	Nawalla	105	4	11	9
June	26	Atz. x 7XY.1	Nawalla	89	2	10	9
June	26	Atz. x 7XY.1	5^oC	89	10	13	3

Abbreviations : Atz. = Atzimba ; Kat. = Katahdin

Table 6 : Losses of different grades of seedling tubers of Atzimba x Katahdin during 105 days of storage in Nawalla .

(%)	Weight grade (gm) > 35	20-35	5-20	1-5
Number of tubers	0	6.0 ± 2.8	4.0 ± 1.2	5.2 ± 1.7
Total weight loss	6.2 ± 1.41	14.6 ± 2.5	13.4 ± 1.7	11.4 ± 1.5
Physiological weight loss	6.2 ± 1.4	10.8 ± 1.2	10.4 ± 0.9	7.6 ± 2.0

4. Field emergence :

Seedling tubers that were stored in the Nawalla were presprouted in diffused light and planted one tuber per hill at 25 cm within rows and 75 cm between rows. The number of emerging hills and stems per hill were recorded 19 days after planting (Table 7).

Table 7 : Plant emergence and stem number of several grades of seedling tubers of 2 TPS progenies.

TPS Progeny	Storage (days)	Grade (g/tuber)							
		> 20		5-20		1-5		< 1	
		A	B	A	B	A	B	A	B
Atz. x Kat.	150	-	-	97	3.0	90	1.7	76	1.3
Atz. x 7XY.1	138	-	-	93	2.3	93	1.8	-	-
Atz. x 7XY.1	108	99	3.5	95	2.4	83	1.7	-	-
Atz. x Kat.	105	99	3.4	96	2.4	92	1.8	-	-
Atz. x 7XY.1	89	98	3.0	96	2.1	93	1.5	77	1.2

Abbreviations : Atz. = Atzimba ; Kat. = Katahdin ; A = % emergence;
B = stems/plant ; - = no tubers available .

As expected larger tubers had better emergence rate and stem number per plant. However, it is interesting to note that tubers weighing less than 5 grams emerged well and produced adequate number of stems per plant after 3 to 5 months in non-refrigerated stores.

The above results suggested another experiment, in which seedling tubers of Atzimba x 7XY.1 , stored in a Nawalla for 105 days were classified into two weight grades : 1-5 and 5-20 gm/tuber. Of each grade 1 to 4 tubers were planted per hill on October 18, 1984. Plant emergence and number of stems per hill were recorded 19 days after planting (Table 8).

This experiment is still in progress, however it is apparent from the data that an adequate stem number per unit land area can be achieved by increasing the number of small tubers per hill or by planting small tubers at closer spacing within the row.

Table 8 : Plant emergence and stem number of two grades of seedling tubers planted at different rates per hill (Atzimba x 7XY.1).

Grade g/tuber	Tubers/hill	Emergence %	Stems / hill
1 - 5	1	95	1.6
	2	99	2.7
	3	100	4.1
	4	100	5.4
5 - 20	1	96	2.2
	2	100	4.0
	3	100	6.1
	4	100	7.9

RECOMMENDATIONS

Based on experience in Egypt, several areas of research need the attention of those working on potato production from TPS.

1. Breeding of TPS progenies suitable for various environments with emphasis on :
   - Adaptation for day length..
   - Less sensitivity to high temperatures during germination and early seedling growth..
   - Early maturity preferably after 90 days.
   - High yields with higher proportion of marketable tubers.
   - Short dormancy.
2. Availability of TPS in sufficient quantities to meet farmers needs at reasonable cost.
3. Development of simple control methods for damping-off.
4. Development of simple and safe methods for breaking tuber dormancy.
5. Identification of pre- and post- emergence herbicides.

ACKNOWLEDGMENTS

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The Commercialization of TPS - The Pan-American Seed Experience

E. Leue

The concept of potatoes from true seed as a Pan-American Seed product was first suggested in 1976 by Alan Arrowsmith, our Executive Vice President. The initial interest in TPS in Pan-American was exclusively as a home garden product; they were unaware of other research in TPS ongoing at that time. It was felt that TPS would fit very well into the Pan-American product line, for several reasons. First, Pan-American and its closely allied sister company, Linda Vista in Costa Rica, have had a long tradition of offering varieties from seed of products that are traditionally vegetatively propagated. The most notable example of this was the Carefree Geraniums, introduced in 1968, which became the first seed geraniums with significant commercial impact. Many ornamental bedding plants, including impatiens, double petunias, coleus and forcing snapdragons, were also largely vegetatively propagated at that time, and Pan-American Seed varieties represented major advances. More recently, Pan-American has successfully introduced an everbearing strawberry and a cut flower anemone from seed. Traditionally Pan-American Seed has earned the reputation of being the innovators of the garden seed industry.

It was also thought that a potato from true seed, though not suitable for large scale potato production in the United States, might be uniquely suited to home garden needs. There is some demand for small, 3-5 cm tubers, used for gourmet dishes. Although the volume for this product isn't high, the price can be \$2 to \$3/kg. Consumers associate small tubers with new potatoes and often assume they are fresher, or better quality than standard grade A potatoes. It was also felt that yield <u>per se</u> may not be of prime importance to the home gardener, as compared to tuber number, perceived quality, convenience, novelty and so on.

A small market research study was conducted to determine consumer's attitudes and expectations concerning growing potatoes. Results showed that only 25% of gardeners grew potatoes. People expected a yield of 1-1.5 kg. per plant, composed of 6-8 tubers. It was felt that TPS transplants might be attractive to some of the nonpotato growers as well as capturing a portion of the 25% of gardeners who currently use seed tubers.

Another reason why Pan-American Seed became involved in TPS is simply that it would be a new product for us and for our ultimate customer, the bedding plant grower, to sell. It would allow plant nurseries to offer a full line of garden vegetable transplants to their customers. Many nurseries do not deal in seed tubers; if a gardener wishes to grow potatoes, he must visit a more farm oriented nursery, or plant tubers from the store which may be treated with maleic hydrazide, or order tuber eyes from a catalogue. Tuber eyes currently cost up to 15 cents each from a mail-order catalogue, include a minimum of starch reserves, and are often too dried out to sprout. In our experience, emergence from these prepared tuber sets is only about 70%, and yield is not significantly better than from good true seed transplants. It was also felt that the availability of potato plants in garden centers, at a time when the casual gardeners were buying other bedding plants and garden vegetables, might stimulate impulse sales.

Finally, a significant part of Pan-American's business is with color packet and mail-order packet seed companies. These markets are always interested in new products, even if only for novelty value.

The initial concept of TPS for Pan-American Seed therefore was as a new vegetable in the bedding plant market in the U.S., Canada and possibly northern Europe. It is a competitive, quick moving industry, where the average lifespan of a new variety is 3-5 years before it is outdistanced by competitors or replaced by the company that produced it.

Soon after Pan-American began the initial investigations into TPS they realized that the potential went far beyond the bedding plant market. Contact with several university potato researchers in the United States, and with the Internationl Potato Center, alerted Pan-American to the fact that TPS might have special advantages to potato growers in some tropical and substropical countries. Marketing experts in the company and the parent corporation, Geo. J. Ball, Inc., estimated that the potential market in the bedding and packet seed segments was very large, and for international use, potentially enormous.

In 1980, efforts in both research and marketing were stepped up considerably. Over a dozen university and USDA research locations were visited by Pan-American staff for the collection of information on potatoes and TPS and to obtain samples of TPS from commercial varieties and experimental clones. Three staff members visited the International Potato Center and returned with extensive information on the use of potatoes worldwide and on the projected value of TPS to countries which do not have adequate certified seed programs. Two people also visited Dr. Anoop Bedi, in New Zealand, to look over his TPS breeding program.

The process of developing a new commercial product turned out to be very involved. Every step, from production and handling of the seed, through detailed cultural recommendations to growers, to full instructions to home gardeners, had to be verified or developed. A variety of techniques for grinding potato berries, and for separating pulp from seeds, were tried. Different fermentation times were tested, and their affect on seed appearance and germination noted. A variety of seed cleaning equipment was used before a process was determined. Many combinations of treatments were tried to break dormancy, and experiments were run with fungicide treated and pelleted seed.

Each step of seedling growth was examined using materials and conditions with which bedding plant growers were familiar. Soil temperature, depth of sowing, timing of fertilization, time and depth of transplanting and crop time were established. TPS seedlings were considered to be too stringy to be attractive, so a program of treatment with the growth retardant B-9 was worked out, which gave compact plants with thicker stems and darker green leaves.

Considerable effort also went into working out cultural recommendations for home gardeners. Trials were conducted regarding time of planting, spacing of hills, number of plants per hill, planting in a variety of containers, fertilizer rates, effects of mulching and hilling, effects of rates and types of herbicides, effects of B-9 on yield and effects of premature tuberization on yield. Direct sowing with either raw or pelletized seed was compared to transplants and to pregerminated seeds in hydrogel or plug mix. A large trial was grown in West Chicago comparing 64 open pollinated and hybrid TPS lines with clonal varieties with six replications. Portions of this trial were replicated at two other locations in northern Illinois, and samples of from four to twelve lines were sent to over 100 universities, government institutions, customers and cooperators around the world.

Introduction of Explorer took place in the fall of 1981, accompanied by considerable advertising and fanfare. In retrospect, the introduction may have been premature, but it was stimulated by a variety of false or misinterpreted reports of competitors' activities at the time. The International Plant Research Institute (IPRI) was reported to have a high yielding F_1 hybrid "ready to go" in 1981. Sahin, an independent seedsman in Holland, was sampling a series of TPS lines to some of our customers, presumably in anticipation of introduction. Dr. Bedi had a named variety, 'Waitangy', and was threatening to sell directly to our customers. There was concern that some European companies that are already potato breeders could quickly move into true seed. There was even a report that Arco Ventures had "20 to 30 Ph.D. breeders working on TPS". Therefore, Pan-American decided that it couldn't risk delaying introduction.

Although first year sales were good, Explorer achieved only limited success, for a number of reasons. It proved to be more difficult to grow for bedding plant growers than anticipated. It was more attractive to white fly and aphids than the plants they were used to. It was often grown at higher temperatures than recommended, producing pale, spindly plants. Growth regulators, used to improve seedling appearance, also tended to promote tuberization in the flat, if daylength was short.

Even well grown plants sometimes didn't sell well. Gardeners didn't recognize the plants--they had to be pointed out and promoted much more than other bedding plants. Even when consumers were made to understand what the seedlings were, they were suspicious that the plants would not produce potatoes. Also, there was a general lack of interest in growing any kind of potato--they are too inexpensive a vegetable, perceived as taking too much work and garden space. Also, the plants at the nursery were priced fairly high, at or above that of a hybrid tomato, because of the longer crop time. After one experience with TPS, most growers decided that they would rather put their greenhouse space into crops that sold themselves, such as Impatiens.

In the garden, Explorer gave mixed results. It was not as widely adapted as had been hoped, performing reasonably well in the north, but very poorly in California and most of the southern United States. The transplants are more sensitive to stress than many garden vegetables and can respond by going into premature senescence that is practically irreversible. Also, gardeners who were unfamiliar with potatoes were unprepared for the higher fertilizer requirements, and for Colorado potato beetles and other common pests and diseases.

Consumers who bought packets of seed found the young seedlings difficult to nurse along, as compared with more robust seedlings of tomato, pepper, cabbage and cucumber. Some ignored the directions and sowed directly outdoors, usually with unsatisfactory results.

Explorer did not, therefore, become a long term success for two reasons. First, the product itself didn't live up to expectations. Second, the real demand for a home garden TPS variety was significantly overestimated.

First attempts at international sales also ran into problems. Pan-American does a lot of business in flower seeds in Europe and expected to sell TPS there as well. EEC quarantine restrictions came to their attention during the sampling phase, so no sales were made to Common Market countries.

Evaluation of hybrid and OP TPS for tropical adaptation was also problematic. Because breeding was based in West Chicago, Illinois, Pan-American had to depend heavily on reports of trials from other countries. Linda Vista provided valuable information on adaptation to Costa Rican conditions at 1400 and 2100 meters, but because of logistical problems and natural disasters, less than half the experiments initiated there yielded results. Of the trials sent to customers, cooperators and institutions worldwide, reports were received on perhaps 15-20% of them. Of these, about half did not provide valuable feedback because of mishaps, or because the trial was not conducted or evaluated properly. Also, most of the samples sent out had poor tropical adaptation, which discouraged cooperators from helping us in later years.

Another major obstacle was encountered in attempting to import germplasm for breeding purposes. Efforts to import _in vitro_ clones from CIP were initiated in 1980 and renewed in 1982. Permission to import was finally granted in 1983 from the USDA quarantine service, and clones were received. To date, this material is still restricted to the greenhouse and laboratory, as clearance has not been issued from the USDA to grow it in the field.

Preliminary trials with hybrid TPS lines have shown that great improvements over Explorer are possible. Hybrids have been identified with two to three times the yield of Explorer with much better appearance and uniformity. One hybrid has been selected with very good performance as well as relatively good seed production characteristics in the parents.

Because of import restrictions, any hybrid TPS that might be used in the U.S. market must be produced in the U.S. We have identified appropriate parental materials and production techniques for production of hybrid seed in northern Wisconsin, in cooperation with the research program of Dr. S. J. Peloquin at the University of Wisconsin. However, a decision to produce and market a hybrid TPS variety has not been made at this time. This is in part because of the extreme variability in seed yield from season to season, as can be expected in an uncontrolled field environment. In the years that Pan-American has been involved in small scale seed production in Wisconsin, 1981 was well above average, 1982 was below average, 1983 was perhaps the worst season in Dr. Peloquin's 25 year experience, and 1984 was somewhat above average. Although the same hybrid combinations were not made each year, so that an exact comparison cannot be made, the variance between 1981 and 1983 was at least a 5-fold, and possibly a 10-fold, difference in seed yield. This makes hybrid TPS a high risk under these conditions, because cost of farming plus hand labor would vary relatively little depending on the season.

In conclusion, the Pan-American Seed Company administration has decided that further breeding work in Illinois for home garden TPS varieties is not justified by current sales of the product. However, they feel that the TPS concept is still viable, and the Ball corporation is very interested in maintaining activity in TPS development. The emphasis, however, will shift from breeding in Illinois to seed production research in some of the lower cost seed production facilities owned or contracted by the corporation. We will be carrying out production research in cooperation with Linda Vista, at their higher altitude location at 2100 meters in Costa Rica. We will also be trying small scale production in some or all of the production facilities of Petoseed Company, Inc., another of our sister companies. Peto currently has production in Baja, Mexico, Chile, Taiwan, mainland China and Thailand. Finally, we are considering shifting TPS breeding to Costa Rica, where screening for tropical adaptation will be the main emphasis.

Methods of Rapid Multiplication for Seed Potato Programs[1]

J.E. Bryan[2]

Use of the so called Rapid Multiplication methods in potato seed certification schemes has been a dramatic and fast moving phenomenon during the past one and half decades.

Ever since the removal of viral diseases from seed stocks became a reality, certification associations have been under pressure to remove other diseases and to shorten the time needed to multiply small seed lots up to commercially viable numbers in a short time. This has been accomplished. Techniques have been developed to not only remove non-systematic diseases but also to allow high quality seed to become available to ware growers before the seed stocks become reinfested to a significant degree.

One of the first methods utilized was stem cuttings, Cole and Wright (2). It was shown that eight plants derived from one tuber could produce 5000 rooted cuttings in six months by cloning and re-cloning. The first practical use of stem cuttings seems to be more related to removal of non-systematic diseases such as Erwinia and Phoma spp., Hardie (6), with rapid multiplication of secondary importance.

Single node cuttings were initially used to produce rooted plantlets for nematode resistance screening in breeding programs. Senka (10), Franco (3). Later, Goodwin (5) and Bryan etal (1), found that an infinite number of cuttings could be produced by re-cloning the cuttings and when transplanted to the field, these cuttings yielded 0.5 Kg. or more.

Use of entire sprouts as planting material seems to be almost as old as potato cultivation. Many indigenous farmers in the Andean countries still utilize sprouts, abet to a minor degree. Hamann (7) describes a layering procedure to maximize production from a single tuber. The basis of this system is maximizing sprout growth and cutting the sprouts into tiny pieces each having a single node. He was able to obtain an increase ratio of up to 1:7,600.

1) Prepared for XXVIII Planing Conference: Innovative Methods for Propagating Potatoes.

2) Sr. Seed Production Specialist, International Potato Center, Lima, Peru.

Tiny tuberlets produced from leaf-bud cuttings produced up to 0.5 Kg. of tubers per tuberlet in field trials. Mother plants, depending on size, produced from 37 to 288 leaf-bud cuttings, almost all of which produced tuberlets ranging from 0.5 to 3 cm. diameter. Lauer, (8).

Other techniques such as stolon cuttings, "milking" small tubers from mother plants and variations of these four principal methods have also been described. Shading of cuttings, day length, use of hormones, special fertilizers, rooting media, etc. have been the subject of many studies.

The removal of viral diseases by tissue culture techniques instigated the development of rapid multiplication methods. Therefore, it is only fitting that tissue culture techniques are at present enjoying a reputation of being the ultimate in rapid multiplication. Roca etal (9) describes a fifty-fold increase meristem tips in shaker culture. Other publications followed on in-vitro propagation. One of the more interesting, Van Uyen and Vander Zaag (13) describes the use in-vitro propagation by Vietnamese farms for ware potato production. Bryan, unpublished, showed that by using in-vitro plantlets in beds at high densities, 350 to 580 useable tubers can be produced in 1 m^2 .

Bryan etal (1) describes an integrated rapid multiplication scheme for potatoes that emphasizes the combined use of two or more methods. This system allows for varietal and climatic differences when a single technique doesn't work well. In-vitro production can be fitted into this scheme.

Goodwin (5) gives an excellent justification for the use of rapid multiplication techniques over the normally propagated tubers. He divides these techniques into two groups:

1) glasshouse techniques which includes sprout, stem, single node, leaf-bud and similar methods, and
2) tissue culture or in-vitro techniques. He makes a case in favor of the glasshouse methods over the tissue culture methods. However, the Vietnamese model of using "Rustic" tissue culture, combined with apical cuttings contradicts the need for a sophisticated tissue culture lab. At the same time the Vietnamese model reinforces Bryan' integrated system of combined techniques. It remains to be seen how practical the rustic type tissue-culture methods are. All indications to date show they can be adapted in some areas.

In summary, we can say that Rapid multiplication techniques, including tissue culture are causing a revolution in potato seed multiplication programs. They are limited to use in the first generation of a program. They are costly, especially in terms of manpower and minimum facilities. On the other hand, they are cheap in terms of decreased years needed to produce certified seed and in the increased health of that seed. Almost all programs in developing countries utilizing rapid multiplication techniques have opted for an integrated system, utilizing more than one method. In the more successful programs, tissue culture or in-vitro methods of propagation is one of the combined techniques utilized.

There has been and will continue to be a proliferation of publications on the subject as more and more national programs adapt the described techniques to local varieties, climatic conditions, facilities and most of all, to local needs.

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From Test Tube to Transplants in Vietnam

Nguyen Van Uyen

Abstract

In vitro plantlets of new P. infestans-resistant cultivars were received in Dalat, Vietnam, from the International Potato Center (CIP), in 1980. Farmers identified three superior cultivars, but due to a lack of seed tuber were trained in rapid multiplication techniques. Ten farmers established such centers (satellite) and maintained in-vitro material and rapidly multiplied it both in-vitro, as well as by taking cuttings. By let 1983, 90% of the 400 ha grown in Dalat was of these three new cultivars, a result of the rapid multiplication by farmers. Details of the methodology and future possibilities in Vietnam are discussed.

Introduction

The potato is playing an increasingly important role in providing food to more than 60 million people in Vietnam (Ho et al., 1983). Half a million hectares in both the Red River Delta (RRD) and the Mekong River Delta (MRD) are potentially feasible areas for growing potato in rotation with rice.

The major limiting factor in potato production has been the supply of good seed material. Tissue culture propagation of newly introduced potato varieties, combined with non-sterile cutting propagation, has proved to be an efficient method for rapid propagation of potatoes in Dalat, situated in the central highlands of Vietnam (1500 m) (Uyen and Vander Zaag, 1983-1985). This report discusses the results of four years of development of this procedure and its future possibilities for improving of potato production on a larger scale in both the RRD and MRD.

I. Advantages of tissue culture techniques in potato seed production. The "Inter-Center Seminar on IARCS and Biotechnology" held at IRRI (April, 1984) strongly recommended the use of tissue culture in the conservation of germplasm and rapid propagation on all IARC mandated crops, including the potato. Research conducted at CIP (1983 Annual Report) and other national institutions have emphasized the role of tissue culture in potato breeding, virus cleaning, conservation of healthy materials, and rapid propagation.

A. Rapid propagation. The first shipment of test tube plantlets of CIP cultivars to Dalat was received at the end of 1980. We have received up to the present 60 cultivars in vitro, most of with resistance to *Phytophthora infestans*. Among these, 3 varieties--Atzimba, CFK-69.1, and B 71.240.2--have been well accepted by Dalat farmers. By the end of 1983 more than 90% of the potato growing area at Dalat (400 ha) had been planted with these new varieties.

This dramatic increase in area of these 3 new varieties was established, not by massive importation of seed tubers, but by applying a new method of propagation practiced at the farmer level. The propagation procedure first proposed in 1980 and later improved, consists of five steps (Fig. 1).

This procedure was adopted by farmers who were called "satellites". These farmers (initially 10) had diverse backgrounds with only one having formal training in laboratory techniques. On-the-job training and a keen interest resulted in successful adoption and utilization of the tissue culture phase. A stepwise explanation follows:

1. In-vitro propagation. A total of 200-300 test tube plantlets are needed by one satellite in one growing season. Farmers prefer to do this themselves, by purchasing five to ten original test tubes from the State Station and propagating the plantlets in vitro in a family-size laboratory. The plantlets should have 4 to 5 internodes (30 to 40 days), before cuttings are made for the next step.

2. Stock mother tray. In a wooden tray (50 x 90 x 12 cm) filled with a mixture of fine subsoil and/or sand and cow manure (3:1), 800 single node cuttings from the in vitro plants are rooted. The plants are watered twice daily and protected from direct sunshine.

   After 20 to 30 days (depending on season and variety), apical buds are harvested at one-week intervals. Each harvest gives an average of 1600 apical buds. In 80 to 90 days the number of apical buds from the mother tray amounts to over 15000.

1	"In vitro" plant in test tube from research station
↓	- multiplication taking five cuttings/tube, two times (2 months).
25	In vitro plants in test tubes
↓	- 1 month growing period.
100	Single node cuttings rooted in sandbed at $1000/m^2$
↓	- harvested weekly during 3 months
1,000	Apical and axillary bud cuttings rooted in subsoil-manure medium in beds at $1000/m^2$
↓	- harvested weekly during 2 months
10,000	Apical and axillary bud cuttings rooted as plantlets in pots; after 15-20 days transplanted to the field for commercial production
↓	- 3-4 months growing period
tubers:	Large used for consumption, small for seed

Figure 1: Rapid Multiplication Procedure Used by Farmers (From Uyen and Vander Zaag, 1983).

Special care is taken to prolong the life of the mother plants in the juvenile state. When necessary, the tray is moved to a safer place (e.g., inside the house during strong winds or rain).

3. Seedbed propagation. The apical buds harvested are rooted in subsoil beds. The composition of substrate is as mentioned in the second step; however, most satellites use subsoil and manure. Subsoil is considered to be generally free of pathogens and has a good structure which provides good aeration. During one potato growing season, these seedbeds provided 200 to 300 thousand apical cuttings for potting in the next step.

4. Potting. Banana leaves are generally used for making small pots (2.5 x 5 cm). The pots are filled with a subsoil manure mixture and used for rooting the apical cuttings taken from seedbeds. Duration of these steps 15 to 20 days.

5. Field planting. Farmers prefer to plantlets derived from tissue culture potato at a normal density of 30,000 plants/ha. Since the potato is an important cash crop, they prefer to have large tubers for the market and use the small tubers as seed material. It is generally accepted by farmers that the first tuber "generation" from cuttings when replanted gives the highest yield. Yields begin to decrease after the third "generation" of seed tubers coming from the in vitro plantlets. At that time farmers return to use cuttings. Presently about 6% of the total area in Dalat is grown from cuttings. This is providing enough seed tubers to satisfy all the farmers (Uyen and Vander Zaag, 1985).

   Thus, from 200-250 test tubes, one satellite can produce about 300,000 plants for planting on about 10 hectares. This produces at least 70 tons of small seed (tubers) for the next season.

B. Cost of cutting production. The success of this system is partially due to the economic benefits obtained by the farmers who do the work. In Table 1 a summary of the expenses of a typical satellite operation is given. The inputs are minimal and simple and have been adapted by using locally available materials such as old gas cylinders and bamboo for autoclaving

Table 1. Cost analysis of an average satellite farmer who produces 200,000 potato plantlets annually

Equipment	Cost USD
Test tubes (500)	10.00
Sterile box (UV light) (1)	25.00
Autoclave (old cylinder) (1)	30.00
Plastic sheets, Bamboo mats, wooden frame, alcohol lamp and other items	70.00
Total	135.00
Equipment cost (amortised over 2 years)	67.50
Materials for 200,000 plantlets	
Chemicals for in vitro culture	5.00
Manure, soil, other items	11.00
Banana leaf pots	30.00
Total	46.00
Labor (2 persons)	240.00
Total cost to produce 200,000 plantlets	353.50
Total cost (1000 plantlets)	1.77
Selling price (1000 plantlets)	5.00
Profit / year	646.00

and shading. A farmer who sells 200,000 cuttings can make a net profit of $646 USD. The labor input; however, is generally provided by the family, so the return to labor becomes $886 USD. This is a very high income by normal Vietnam standards.

C. Present number of satellites. Due to the saturation of the new varieties, and the preferred use of the tubers from cuttings as seed, the demand for cuttings has decreased, resulting in only three satellites remaining (Uyen and Vander Zaag, 1985). These three produced over 600,000 plantlets in 1984. This is enough to provide quality seed tubers for all of the potato production in the Dalat region.

D. Maintenance of germplasm. Dalat farmers have observed that the yield of potato plants from cuttings and subsequent "generations" remains relatively stable after four years of using the same variety. This was not the case for formerly introduced varieties such as Greta, Cosima, and Desirée, which required new importation of healthy seeds after 3 to 4 years. In vitro conservation of germplasm minimizes the degeneration of a good variety due to exposure to vectors during propagation by traditional methods. It also reduces greatly the cost of field maintenance and transport and the danger introducing new pests and diseases to a given area.

Tissue culture is the most efficient method of international and inter-regional exchange of germplasm. The National Potato Program of Vietnam is giving more attention to the use of this novel technique in increasing potato production.

II. Future plans for this propagation procedure in the RRD and MRD

The use of tissue culture for potato seed production is already well established at Dalat. Its expansion requires more research and on-farm evaluation.

1. RRD: Potato is grown in the RRD from mid November to February, which coincides with the winter season in North Vietnam when minimum and maximum temperatures are (15°C and 25°C, January and February). Land preparation and planting must be done during a brief period so that the potato crop will not delay the rice crop that follows.

A resort area at Tam Dao (700 m), situated in the RRD, could provide a large number of cuttings by mid-September (when night temperatures are 20°C) for farms cooperatives in the RRD to practice seedbed propagation. This approach requires good training and organization.

Another possibility for seed tuber production, is to grow a late potato crop from February to April. High-density planting with cuttings of day length insensitive varieties should be considered. Tubers of the late crop have better seed quality since storage time is reduced.Potatoes are stored in diffused light at high temperatures for 8 to 9 months (Feb./Nov.).

2. MRD: The southern provinces of Vietnam have much warmer climatic conditions than the RRD, with the minimum temperature never going below 20°C during the coolest months of the year. Areas are suitable for growing a potato crop from mid-November to February. It has been successfully attempted in the MRD, on an experimental scale during the past two years with yield up to 17 t/ha using tuber seed (Ho et al. 1983).

   Seed tubers from the MRD could be provided from Dalat. Preliminary results also show that apical cuttings could be transferred, at low cost, from Dalat to MRD to be potted on-site in November and December.

III. Conclusion

Rapid propagation of the potato by tissue culture, combined with non-sterile seedbed propagation will be an efficient alternative for commercial seed production in Vietnam. The Dalat experience has shown that this goal can be achieved without much financial investment. The farmers themselves can actively take part in the rapid diffusion of a super variety.

In vitro germplasm conservation shows great promise as a low cost method for long-term maintenance of virus-free, healthy clones. The in vitro multiplication and cutting production has formed the basis of an informal but highly effective seed production program by Dalat farmers. This should serve as a good example of what could be done else where in the developing world.

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Rapid Multiplication of the Potato (Solanum spp.): Research and Use in the Philippines

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Abstract

Rapid multiplication has been on-going since 1980 in the national seed program as a means of quickly multiplying basic seed. Numerous other agencies are now also using various rapid multiplication techniques for multiplying germplasm. Farmer adoption of these techniques has so far met only limited success. The problem of allowing mother plants to become "physiologically old" and *P. infestans* have been the major constraints. Several on-farm trials have given good yields from cuttings. Research work on agronomic and physiological aspects are discussed. Problems still to be resolved are presented.

Introduction

The advantages of rapid multiplication are well known. A seed program can rapidly multiply and maintain clean nuclear stocks. Farmers can plant cuttings and save on seed tuber costs. New cultivars can be rapidly multiplied after their release for commercial production. With all these advantages there are numerous constraints that still need to be resolved prior to the widespread adoption of rapid multiplication techniques.

In this paper we will give a summary of the results of rapid multiplication by the national seed program, the use of cuttings for tuberlet production, research results on the use of cuttings for commercial production both at the research station and in the farmers' field. A description of the 5 locations where this work has been done is given in Table 1.

Philippine - German Seed Potato Program

In 1980, the use of stem cuttings from large mother plants was started as the method of rapidly multiplying virus free (indexed)

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Table 1. Description of 5 environments where rapid multiplication work has been conducted.

Location	Elevation (m)	Lattitude (°N)	Temperature (°C) Nov-Feb max	Temperature (°C) Nov-Feb min	Temperature (°C) Mar-Oct max	Temperature (°C) Mar-Oct min	Rainfall (mm) Nov-Feb	Rainfall (mm) Mar-Oct*
Benguet	2200	15	15	8	17	11	50	2000
Baguio	1300	15	22	11	24	16	30	1700
Mindanao	1100	8	26	16	27	17	500	2000
Mt. Banahaw	800	14	23	16	28	19	800	2800
Canlubang	150	14	29	21	33	23	80	900

* Most of this rainfall comes from June to September.

material (Jordens-Rottger, 1984). The stem cuttings were rooted in the screenhouse at Baguio and once rooted, transplanted to the field on the seed farm in the highlands. This method has been used successfully for the past years producing approximately 100,000 cuttings annually (Jordens-Rottger, 1984). Yields were from 500 to 800 g/hill. This method has been and will continue to be used primarily for the cultivars Greta and Conchita and also Cosima.

In vitro multiplication was started in 1982. Nodal cuttings are multiplied in vitro. Plantlets from in vitro are transplanted into sterilized soil placed in small plastic bags (5 cm in diameter). After 2 to 3 weeks apical cuttings are taken and rooted in the sterilized soil in plastic bags. These rooted cuttings are transplanted to the field, to high density beds or into pots. These yields approximately 400 g/plant (Jordens-Rottger, 1984). Presently 2,000 plantlets are produced weekly. This method is used primarily for Granola, Fina and R. Pontiac.

Tuberlet Production at the Regional Germplasm and Training Center (RGTC) of CIP, Mt. Banahaw

The RGTC, which has existed for the past 2.5 years, has as a primary objective the rapid multiplication of new cultivars. These come as in vitro plantlets. A simple laboratory was constructed with two glass walls to allow natural light to enter. No electricity is available for the facility. After in vitro multiplication they are placed in a media consisting of a 1:1:1 mixture of coarse sand, peat moss and compost in trays with 0.5 g P/plantlet added. These are grown in the screenhouse. Foliar spraying with a liquid fertilizer is done weekly after the harvesting of cuttings.

Sprouts also have been used to establish mother plants. Sprouts are cut into single node section, rooted on moist tissue paper and then transfered to the media described above. Cultivars respond differently to this method. Only those cultivars for which large number of mother plants are needed has sprout cuttings been used. LT-2 and DTO-2 have both responded well to this methodology.

Apical cuttings are taken from the mother plants every 5 to 7 days. They are then soaked in a rooting hormone and rooted in a medium textured sand in trays. After 2 weeks these are well rooted and ready for transplanting.

In an aphid proof screenhouse, beds were made using 15 cm wide boards, making beds of 1 x 3 m2. Fumigated or sterilized compost was placed to a depth of 7 cm in these beds. One kg of chicken manure plus 35 g P was added/m2. Rooted cuttings are placed in these beds at a density of 100/m2 in 1983 and 33/m2 in 1984. The population density was reduced because extreme competition resulted in many plants not producing tubers.

During the growing period compost was added twice to make the bed 15 cm deep. Nitrogen was either sidedressed at the rate of 10 g/m2 or sprayed at 30 days after transplanting. Irrigation using a sprinkling can was done as necessary.

Using this methodology, yields have ranged from 1.1 to 4.1 kg/m2 for beds of cuttings grown during the dry season and early wet season (Table 2). The growing period was normally 90 days from transplanting to harvest (Graza and Vander Zaag, 1985).

Tuber number varied with the cultivar ranging from 112 to 214 tubers/m2, equivalent to 3.4 to 6.5 tubers/transplant. There was no effect on yield or tuber number by reducing the plant density from 100 to 33/m2. The major constraint to higher yields is the lack of solar radiation and relatively warm temperatures (Table 1).

This method has worked best from February to June when daylength is longer and temperatures are warmer. Lower rainfall and higher solar radiation also are favorable during this season. Mother plants grow faster, tuber initiation on mother plants and on cuttings is later and rooted transplants grow rapidly and tuberize later after a good canopy has developed. The November-February season is being used successfully by adding 1 hour of light during the night provided by a small generator.

On-farm Evaluation of Rapid Multiplication Techniques

Mother plant and cutting production: In the traditional highlands, 10 lady farmers have been trained in Baguio on rapid multiplication techniques in March 1984. They returned with mother plants. They were unable to keep the mother plants physiologically young and late blight was severe resulting in a loss of all the mother plants at all ten locations. These have been retrained and a new attempt is planned.

In Mindanao, a total of 10 junior farmers were trained on rapid multiplication techniques. During 1983 several of them were able to produce a large number of cuttings. Eventually the same 2 problems as mentioned earlier resulted in the loss of the mother plants.

In July 1984 one of the authors went to a village to live with an interested family for 100 days, who previously had had training. Using sprouts (cvs. Cosima, Greta and Conchita) and in vitro material of Sangema about 4 m2 of mother plants was established in 2 months. Subsequently, 2,000 cuttings/week were being harvested and these were rooted in another 4 m2 of space. This operation is now fully operated by the family using local soil and compost. Rooted cuttings were first used for 5 on-farm trials to compare these to farmers local seeds. The subsequent cuttings were being planted by the farmer.

Table 2. Yield of tubers from plantlets transplanted to beds in the screenhouse at the Regional Germplasm and Training Center at Sta. Lucia, Philippines.

Cultivar	Source (I/S*)	Yield (kg/m2)	Total	Tuber Size >30g	15-30g	5-14g	<5g
			#/m2				
100 transplants/m2							
LT-2	I	2.3	152	9	36	63	44
LT-2	S	2.2	159	9	26	60	64
DTO-33	I	3.5	152	54	50	33	15
DTO-33	S	2.3	146	13	14	74	45
LT-4	S	2.5	149	25	58	55	11
Average		2.6	152	22	37	57	36
33 transplants/m2							
MS-1C.2	I	2.7	214	14	57	84	59
LT-2	S	1.1	177	2	34	45	96
P-3	I	3.9	130	40	43	31	16
N-503.31	I	2.7	139	18	49	43	29
LT-4	I	1.8	112	8	28	40	36
I-822	I	2.2	202	3	35	68	66
CFK-69.1	S	1.8	112	11	22	52	27
Sangema	I	2.2	144	6	28	48	62
ASN-69.1	I	2.9	207	8	32	70	97
B-71-240.2	I	4.1	159	14	34	69	42
Average		2.5	160	13	37	56	54

* I - in vitro origin mother plants
S - sprout origin mother plants

Yields obtained using cuttings in farmers' fields: In the highlands, trials were conducted at 4 locations using cuttings coming from the RGTC. P-3 and Serrana both gave consistently high yields of more than 600 g/plant under late blight controlled conditions. These were grown during the longer day early rainy season. The farmers were surprised and content with their harvests.

In Canlubang under very hot conditions (see Table 1), DTO-2 and LT-2 yielded over 22 t/ha in 70 days with a high % of marketable sized tubers. Although these are good yields, the management input was high in the form of labor and watering especially.

Research Results Obtained at the RCTC

A series of experiments were conducted at the RGTC from March to August 1984. In all trials 2 or more cultivars were tested. The cultivars LT-2 and DTO-2 are both heat tolerant and adapted to longer day conditions. P-3 and Cruza 148 are both poorly adapted to longer day and warm growing conditions. Serrana was also used in some trials and is similar to LT-2 in adaptation. In all trials 1 x 6m plots were used with a double row of cuttings with 40 transplants/treatment which was split into 2 cultivars. In all experiments except when it was a variable, 20 t of chicken manure, 1000 kg 18-46-0 and 400 kg 0-0-60 were broadcast on a hectare basis and incorporated prior to transplanting. Another 1000 kg 0-18-0 were banded around the transplants at transplanting. Sidedressing of 50 kg N/ha using ammonium sulphate was done at two to three weeks after transplanting. Fungicide and insecticide spraying as well as irrigation was done as needed.

All cuttings were produced in mother beds of 3 m2 containing more than 100 mother plants/m2. Apical shoots were harvested at 5 to 7 days interval and rooted in trays of sand and after 2 weeks was transplanted to the field.

A brief summary of the results are the following:

1) Rooting media did not effect the development of cuttings with pure sand, volcanic cinder, coir dust/sand (1:1), peat moss/sand (1:1) and sawdust/sand (1:1) all gave similar results. It appears that whatever medium is locally available and well enough aggregated to permit aeration for root development is suitable. In our case Bacterial Wilt (BW) is present so top soil should be avoided.

2) Rooting hormone did not significantly improve rooting, survival or final yield of cuttings compared to no hormone. Two levels of IBA (10 and 50 ppm), "best grow" (10 and 50 fold dilution) and the CIP hormone were tested.

3) Shading of cuttings during the rooting phase is necessary when solar radiation is high. The use of coconut leaves, mesh and partial shade for part of the day all proved to be equally effective in assuring a good survival of the cuttings even after transplanting.

4) Addition of P fertilizer during the rooting phase at 0.2 to 0.4 g P/plantlet increased growth of the cuttings, plant height and canopy cover during the first 5 weeks after transplanting, but did not improve plant survival in the field.

5) The age of cuttings harvested from mother plants (larger (older) versus new shoots) showed that rooting was equally good but the transplant survival and canopy development and tuber yield were always higher with larger older cuttings. The generally adverse weather conditions are the cause of the loss of smaller cuttings.

6) The age of the transplant 0 (no roots), 2, 3 and 4 weeks old (from the date harvested from mother plants), statistically, had no effect on transplant survival and crop growth and yield, however, the 2 and 3 week old cuttings tended to give a superior performance and are recommended.

7) Banana leaf potlets versus bare root transplanting did not differ in transplant survival, canopy development and in all components of yield. In an environment such as Mt. Banahaw it is recommended not to use potlets.

8) The removal of the banana leaf (potlet) from the transplant at transplanting compared to its remaining intact did not improve a transplant survival but improved yields for P-3 but not for LT-2 (statistically not significant). It is recommended to remove the leaf if potlets are used.

9) Pruning of transplanted cuttings at transplanting, 2 and 4 weeks did not effect survival compared to the control. The 2 week pruning gave the highest yield and also had the greatest canopy development although statistically not significant.

10) At higher plant densities (60, 80 and 100,000 plants/ha) yields were improved. At least 60,000 plants/ha is recommended, however, tuber size may be somewhat reduced.

11) The planting system study showed that raised beds were superior to flat beds, that shallow transplanting requires hilling-up while deeper transplanting did not.

12) On this volcanic soil the various N and K levels did not improve yields while P at 280 kg/ha was essential for optimum yields. The relatively low yield (<15 t/ha) did not permit a proper assessment of the fertility requirements.

In all of the above results, LT-2 and DTO-2 cultivars developed smaller canopies (60 to 80% of maximum) and had high harvest indexes of more than 0.80 and generally yielded 10 to 20 t/ha depending on the experiment and the planting date. In sharp contrast the P-3 and Cruza 148 had large canopy development, tuber yields of less than 10 t/ha and very low harvest indices and were late in maturity. Serrana was intermediate for all parameters. Yields decreased markedly with later planting. This past season was exceptionally overcast and wet with more than 750 mm of rain from April to July and almost 600 mm in August.

These results are preliminary and are now being repeated at Canlubang (November-March) and at the RGTC (February-June) to substantiate these preliminary findings.

Major problems with rapid multiplication for farmer adoption

1) Mother plants and cuttings of different genetic sources have responded adversely to short photoperiod and cool or hot temperatures. European tuberosum cultivars are particularly sensitive to short days (early tuberization) and cool temperatures (slow growth). The cultivars selected for late blight resistance in Mexico are less sensitive. Most cultivars with S. Andigena or S. Phureja do well under short days and often fail to tuberize in the field under longer days. Figure 1 is a simplified summary of how various cultivars and species of the potato respond to temperature and daylength. A combination of warm minimum temperatures (17-20°C) and a photoperiod of more than 12 hours is the ideal. Either warm temperatures or longer days alone will also bring success with numerous cultivars which cool temperatures and short days results in only a few cultivars being able to produce good cuttings and transplants. So far only LT-2, LT-5 and DTO-2 have grown well at warmer minimum temperatures than 20°C. Photoperiod has little effect under such growing conditions.

   Large areas of S. E. Asia do or can grow potatoes during the short day cooler months of the year. With the traditional S. Tuberosum cultivars there have been numerous failures during this season. A prime example is the northern part of Vietnam Hanoi (21°N latitude) when photoperiod and temperatures drop sharply by December (Fig. 2). Here cuttings tuberize even before transplanting for cultivars such as Ackersegen and Mariella and these then fail to develop a canopy of more than 10 cm in height. To successfully use cuttings, not only are less photoperiod sensitive cultivars necessary but one needs to add artificial light, increase temperatures (through the use of plastic, locating mother plants on sunny side of house, windbreak, etc.) and try to locate cooler locations to establish mother plants when the temperatures are still too hot (Fig. 2).

2) A high degree of P. infestans resistance is required to grow mother plants in highland areas.

3) Rooting of cuttings using locally available media still appear to be a major obstacle. Especially in the highlands and mid elevation areas (BW).

4) Proper irrigation or reliable rainfall is a major barrier to the adoption of cuttings.

5) Labor input is greater and often the farmer is not willing to do this except in the case of a new cultivar.

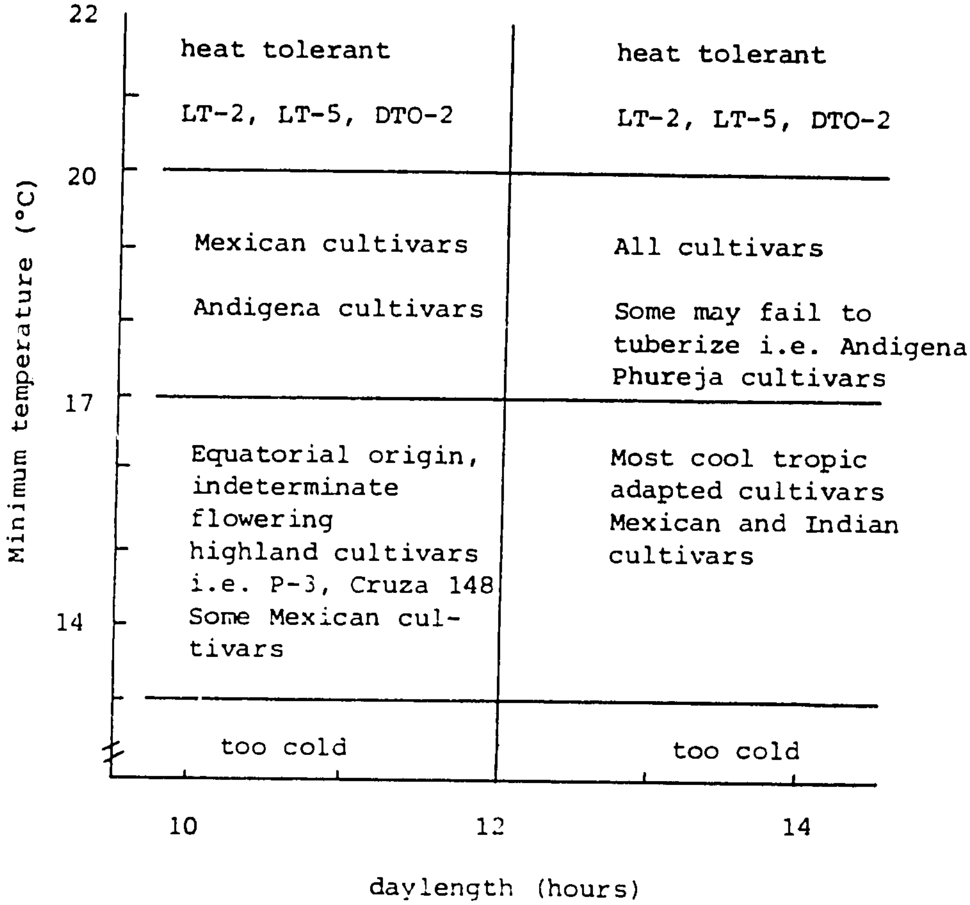

Fig. 1. Temperature and daylength conditions which favor good growth of mother plants, cuttings and transplants.

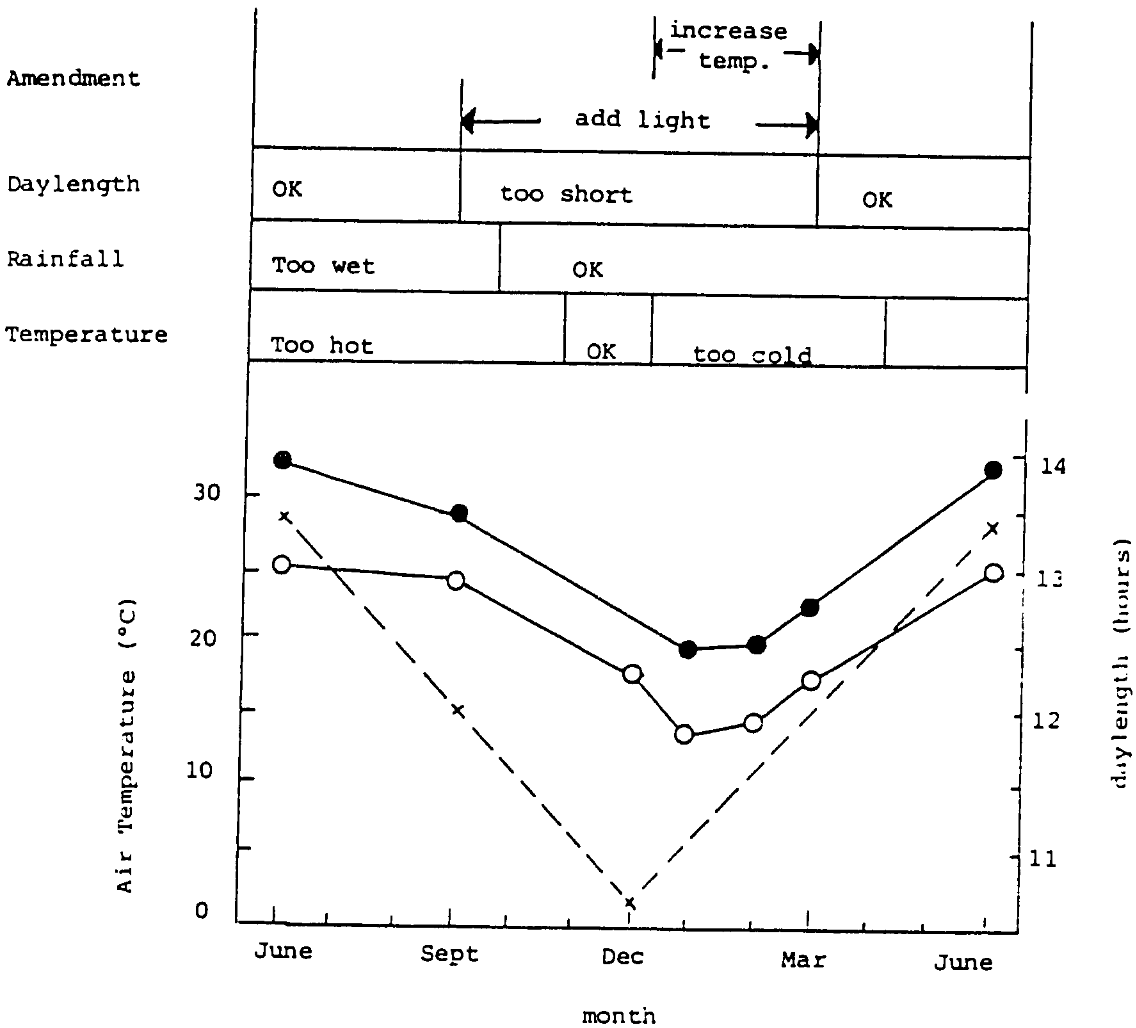

Fig. 2. Minimum (o) and maximum air temperature (●) and daylength (x) at Hanoi. Assessment of temperature, rainfall and daylength and amendments that are needed throughout the year for successful production of mother plants, cuttings and transplants.

Conclusion

Rapid multiplication will gain importance primarily by government agencies in multiplying promising cultivars. Farmers will primarily adopt rapid multiplication techniques to obtain a new promising cultivar or when he is in a bad financial situation that he can not afford to purchase even small poor quality tuber seed. After the technology has been refined, outstanding farmers with a small land holding may be the one to produce cuttings for sale to neighbouring farmers. This ould serve as an informal yet innovative seed program as has occurred in Vietnam (Uyen and Van.der Zaag, 1985).

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Use of Rapid Multiplication Techniques by the Peruvian National Program

Ing° César Vittorelli

The basic seed production project in Perú has adopted two techniques to produce pre-basic seed. The first is to obtain stem cuttings from virus free tubers by transplanting cuttings to beds within the greenhouse. This tubers receive the name of pre-basic seed. This two production systems are conducted in six production centers throughout the country.

The seed production squeme starts with the virus free material coming from in-vitro plants or from tubers obtained from CIP. In the future the National Potato Program will be able to obtain its own virus free material in the laboratories at the experimental station of La Molina, and Los Baños in Cajamarca.

This material is utilized to produce mother plants which produce basic seed. Mother plants are replaced with in-vitro material but could be replaced by cuttings or tubers from mother plants. Cutting production - from beds and their tuber production are what we call pre-basic material, which after one generation in the field will become basic seed. We don't know how many generations of basic seed can be maintained. This will depend on the production area or location and on the management that will be given to the crop. Presently we are planning on having two generations under our control then the seed will be handed to farmers to continue the sequential process until seed for the commercial growers is obtained.

For mother plants production we are utilizing single node cuttings - sprout cuttings. For the production of cuttings from beds and from mother plants we use the stem cuttings. To insure the quality of the material serological testing of every mother plant is made. In the beds a random sample is taken for this purpose. To the present all testing have been negative. For the stem cuttings that are obtained from the beds, they are excised at a standard size of 7 cms. approximately. Treated with a rooting hormone and then placed in the rooting bed, which is compacted coarse - sand at a density of 1000 plants/m^2, under the weather conditions prevalent at La Molina, cuttings are ready to be transplanted after 12 to 15 - days in the highlands. The process takes longer because of the lower temperatures. Rooting periods differ among varieties.

The most important problem we had initially was dumping-off in the - rooting beds resulting from excess of moisture. It was more severe with - small size cuttings.

At the begining we had up to 10% losses, today losses are less - 2%. The problem was solved by raising the cutting beds and placing a - screen underneath with the purpose of improving drainage with very tiny cuttings the first node is underground necessarily and this results in - the formation of a tuber. This type of cutting is not convenient in - transplanting to the field because it would produce only two or three tubers and die.

To avoid this the cuttings are excised at approximately 2 cms. below the last node also they are taken from physiologically young plants. Older plants approaching flowering are not good for stem cutting production. One worker can prepare from 700 to 1000 stem cuttings daily.

The cuttings are fertilized 4 to 5 days before transplanting to the field. The cuttings that are transplanted to beds don't receive previous fertilization.

The soil, sand, peat mixture for the beds is sterilized by heat or chemicals before transplanting. Several materials could be used to built the beds. We use bricks or bambu cane. The soil underlying is desinfected with Basamid a chemical to control nematodes and weeds. Then a layer of pebbles is placed at the bottom of the bed and convered with sterilized mixture. Then follows phosphate application and transplanting. Fertilizer is applied in solution. Sometimes nematicides are utilized. The - mixture is utilized only twice because of fertilizer accumulation. In - Puno where bricks and wood are difficult to find adobe has been utilized after previous desinfection with a solution of calcium hipochlorite. Planting density in the beds is 100 cuttings/m^2.

Production in beds is in order of 500 tubers per square meter. This tubers are stored under cold until they are sent to the highlands before planting season. They are transported in carboard boxes. They are stored under diffuse light for greening and uniform sprouting. The large tubers are cut in two pieces. Planting starts with the very small tubers placing two or three together following with the large tubers up to 90 - grams, then the cut tubers are planted by tuber units so as to facilitate rouguing.

Our first experience with direct transplanting of cuttings to the field was with small farmer in Yauyos. In dry soil the fertilizer was placed at the bottom of the furrow and covered with soil, then the stem cuttings were transplanted and irrigated inmediately afterwards. We found that not all the workers planted at the same depth, some cuttings were placed too deep in the ground, close to the fertilizer and this resulted in salt damage and high mortality. Moreover, even when the field was irrigated inmediately after transplanting, the water took too long to reach the roots because the soil was dry, producing stress and high losses. After this, we decided to broadcast the fertilizer and plowing and rowing the field with a good moisture content in the soil just before planting. Later it was decided to plant without fertilization. Fertilizer was applied after the cutting was implanted and survival was insured. Fertilizer dosis were splitted and hilling was done several times as the cutting developed. It is essential to transplant with good moisture in the soil. This is achieved by irrigating the field a few days before and rowing the same day of transplanting.

It is necessary to irrigate after transplanting. The frequency of irrigation is part of the agronomic management and depends on the weather and soil conditions of a particular location. Nematicides and fungicides are utilized at planting sometimes.

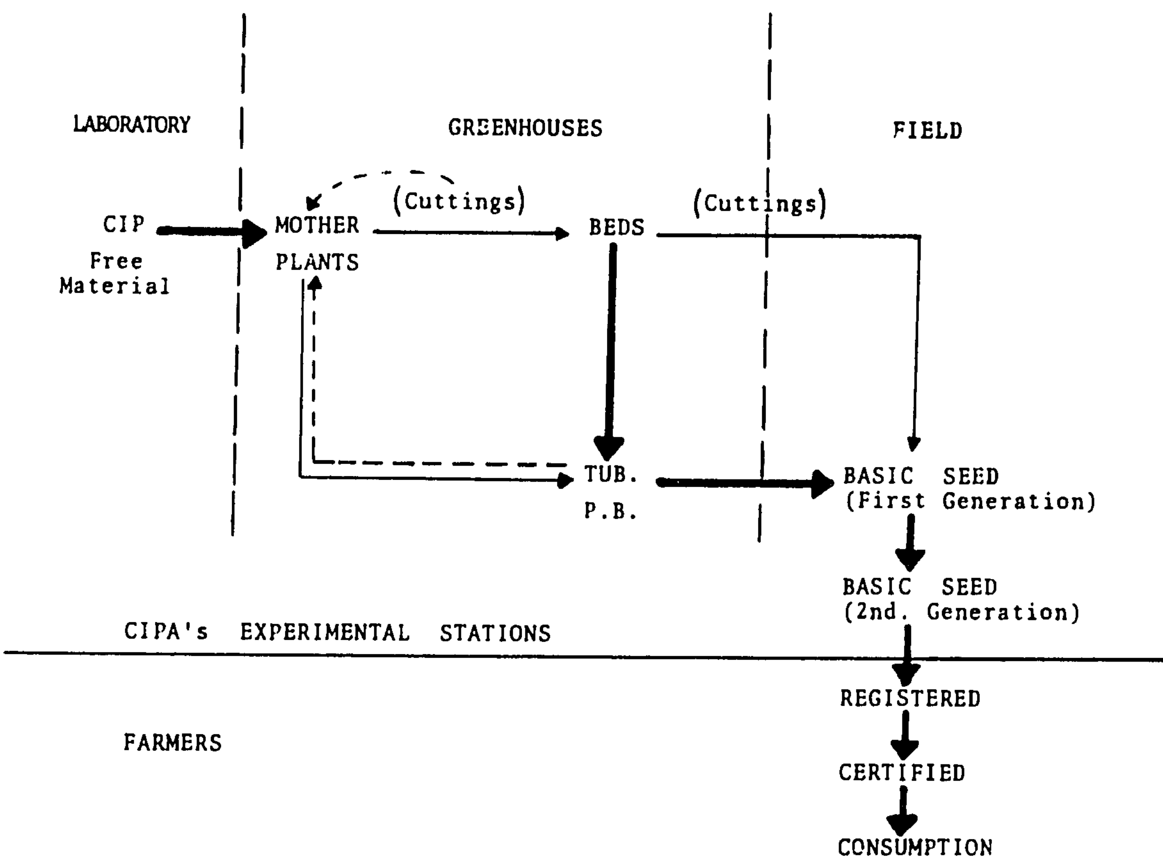
The planting density is 100,000 cuttings per hectare. This is to say 50,000 hills with two cuttings per hole at 20 cms. between hills and 1 mt. between rows. The survival rate is more than 90% and productivity is more than 0.5 kg. per hill. Yields are approximately 25 tn/ha. tubers produced from cuttings are frequently deformed.

Early plantings in the highland is adventageous since frost occurs at maturity time and there is no need for chemical defoliation. Later planting have to be protected at the end of the season to avoid frost damage, also high potasium fertilization and night irrigation are utilized to protect the plants.

Seed plots are surrounded with wheat for isolation. The wheat is not fertilized and yellow flowered weeds are left to grow with it, in order to serve as yellow traps for aphids. These are then controlled with systemic insecticides.

Rapid multiplication in-vitro can be alternatevely used instead of mother plants.

For transplanting the in-vitro plantlets to beds they are removed from magenta and the agar medium washed from the roots with a jet of water. Transplants in several locations has been succesfull and will probably replace mother plants for the initial steps of rapid multiplication in the near future.



Use of Rapid Multiplication Techniques by the Korean National Programs

I.G. Mok, S.Y. Kim and K.K. Kim

Basic seed production by tissue culture

Tissue culture techniques are being used for the production, rapid multiplication and maintenance of virus free plant material. The scheme is as given in Fig. 1.

Tubers for initial material are treated with GA_3 100 ppm for 1 hr and placed under dim light at 20°C for etiolated sprouting. Etiolated sprouts have the advantage of elongated meristem for easy dissection. Dissected meristems, 0.2 - 0.4 mm long, are transferred into 50 ml conical flasks with 10 ml of medium for meristem culture and placed on an oscillating shaker. After the meristem has developed into shoots about 1 - 2 cm long, these shoots are transferred into 100 ml conical flasks with 20 ml of medium for multiplication. One part of the shoots obtained from the 1st multiplication are rooted, grown into plants and tested for viruses. The other part of the shoots are used for the 2nd multiplication. Shoots from the meristem culture proved to be virus free are used for further multiplication, or for *in vitro* tuberization for the maintenance of virus free material. Tuberlets induced under *in vitro* conditions, which are free from pathogens, are stored at 3 - 5°C and can be used whenever multiplication is necessary. It takes about 200 days from sprouting to tuber harvest and the multiplication rate is 10^5 provided that facilities are available.

Medium for rapid multiplication

To establish an efficient method for rapid multiplication, a series of experiment had been conducted during 1978 - 1980 that included the search for the best combination and concentration of sucrose, gibberellic acid, cytokinin and auxin (1). Each step of *in vitro* multiplication requires different concentration of gibberellic acid and kinetin as indicated in Table 1. It has been suggested that gibberellic acid supresses callus formation during shoot elongation, and kinetin stimulates the development of adventitious buds (1,6).

Acclimation

Acclimation of rooted young plantlets is undertaken as transplanting to Jippy pot contained carbonized rice hull and sand muxture. Then the pots are placed in a 500 ml beaker covered with a watch glass to give high humidity and kept under 500 lux, 20°C condition. A week later, young seedlings are transplanted to large pots in a greenhouse. The procedure has been proved successful, as it gives almost 100 percent survival rate.

Serological test

Plants growing in a greenhouse after *in vitro* multiplication are subjected to serological test twice. Presently, precipitation test on slide glass is used. The introduction of ELISA method is in progress. For basic seed production, already tested clean tubers are used as initial material. Thus, virus test during *in vitro* multiplication is usually omitted.

The result of serological test of greenhouse grown basic stocks are presented in Table 2. Plants obtained from meristem-tip culture gives much less infection rate comparing with stem cutting or reuse of basic stocks. The reason of the infection is unknown, probably due to improper management practice in the greenhouse.

Eradication of viruses

The elimination of PVX from infected plants is relatively easy by applying meristem-tip culture as shown in Table 3. However, the elimination of PVS requires repeated heat treatment in combination with meristem-tip culture (3,4,7). *In vitro* plantlets are subjected to 37 - 39°C heat treatment for 3 - 6 weeks and their meristem-tips are immedately dissected for the subsequent culture. It has been successful to obtain virus free plants from an infected variety Kang-weon No. 6 which was bred at the Alpine Experiment Station (2).

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Table 1. Liquid medium for *in vitro* rapid multiplication.

Multiplication step	Basic medium	Sucrose	GA	Kinetin	IAA
		%	ppm	ppm	ppm
Meristem-tip culture	Murashige & Skoog (5)	3	0.1	0.1	-
1st multiplication	"	3	0.1	0.5	-
2nd & 3rd multiplication	"	3	0.01	0.5	-
Rooting	"	1	-	-	0.1

Table 2. Result of serological test of leaves from meristem-tip culture, stem cutting and tuber planting in cv. Dejima.

Material	Number of plant tested	Number of plant infected		
		PVS	PVX	PVS + PVX
Meristem-tip culture	245	-	-	2
Stem cutting	252	5	1	5
Tuber planting (basic stock)	153	4	1	4

Table 3. Comparison of several techniques on eliminating viruses.

Treatment	Result of virus test	
	PVS	PVX
Control	+	+
Heat treatment	+	+
Meristem-tip culture	+	-
Heat treatment & meristem-tip culture	+	-
Repeated heat treatment & meristem-tip culture	-	-

from Kim, et al (1983)

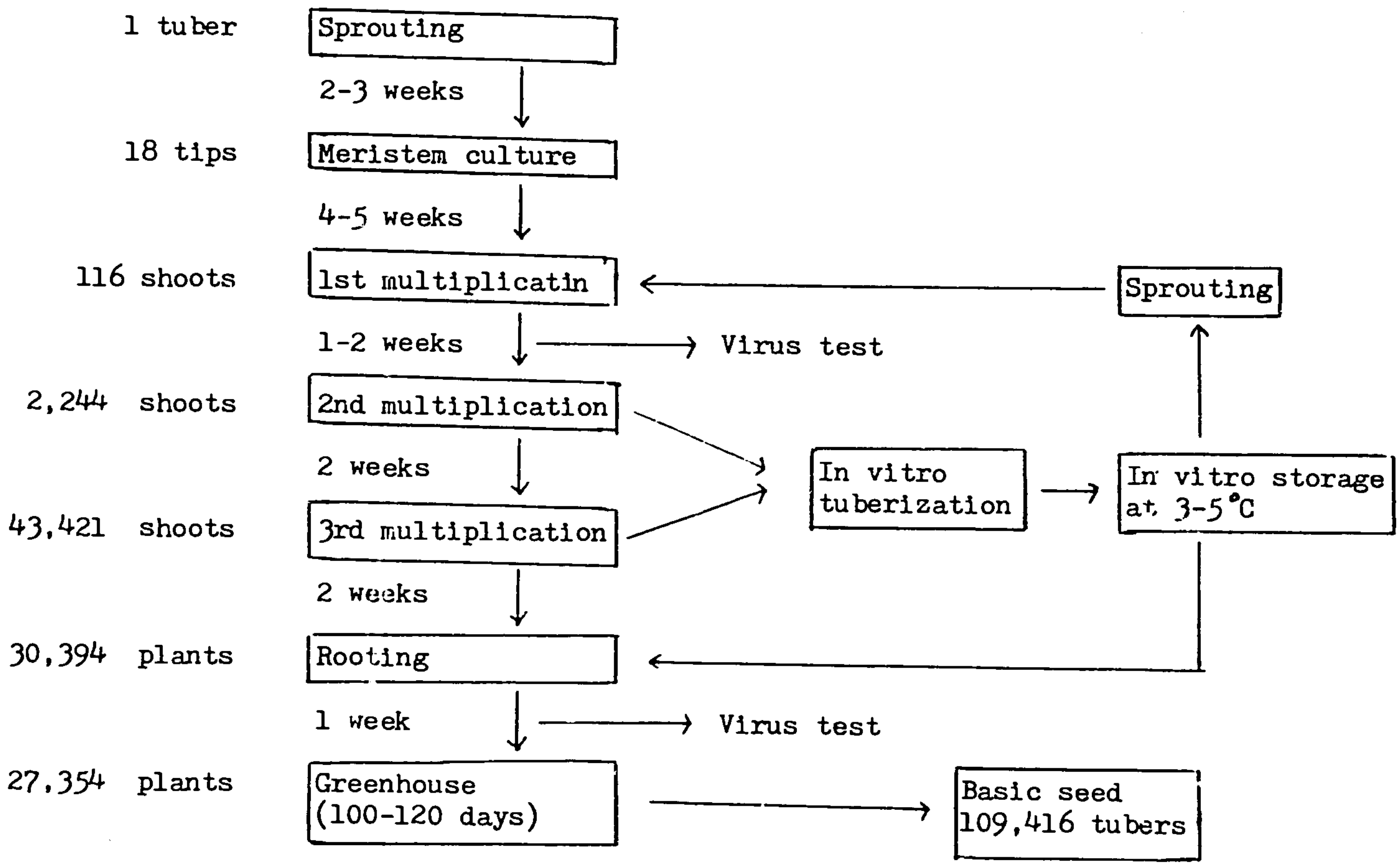

Fig 1. Radid multiplication and maintenance of virus free plant material in basic seed production.

Tissue Culture Propagation of Potatoes:

Advantages and Disadvantages

John H. Dodds

A. Introduction

Although no longer a particularly novel field, the area of plant tissue culture has attracted much interest in recent years. Among the wide range of *in vitro* techniques available, micropropagation is without doubt the technique which has attracted the most commercial interest. The use of rapidly proliferating shoot cultures as a way of obtaining large numbers of apparently uniform progeny has become a reality for a number of crop plants.

The potato has often been described as a model crop plant for tissue culture studies. It manifests an extraordinary plasticity of development and yields positive results to almost all *in vitro* techniques.

This article will review the wide range of *in vitro* techniques which can be applied to propagate the potato. An analysis will then be made of the advantages and disadvantages of *in vitro* methods over conventional propagation systems, together with some suggestions that may help solve some of the problems, and exploit the advantages.

B. Micropropagation Techniques

1. Single Node and Shaken Shoot Cultures

These two methods are the standard micropropagation methods currently in use at CIP. The basis of this type of propagation system is the outgrowth of axillary buds to form a new plantlet (Table 1). For single node cuttings the *in vitro* plantlets obtained from CIP's pathogen-tested collection are cut into single node segments, small leaves are removed and the small nodal segments are transferred to fresh micropropagation media. The axillary bud of the single node then begins to develop and a new shoot is formed, in 3-5 weeks. This new plantlet can be subdivided again into single node segments and recultured.

For shaken cultures a liquid media is used in place of agar solidified media and the medium has a different composition (see CIP Specialized Technology Document). Whole shoots from in vitro plantlets are layered into the liquid medium in an erlenmeyer flask and gently aggitated. After 3-4 weeks the axilliary buds along the whole length of the shoot have released their dormancy and several new plantlets have formed, which can be excised and reinoculated for another phase of rapid micropropagation.

The growth of the in vitro plantlets in these two systems are dependent on dormancy release and the outgrowth of already formed buds. The system is extremely rapid and can be used for the production of large numbers of plantlets.

These two systems have been adopted as part of a propagation programe by a wide range of institutions and companies.

2. Regeneration of Plants from Single Cells

For this presentation the term single cells has been taken to encompass both protoplasts (with cell wall removed) and true single cell suspension cultures.

The regeneration of a whole intact plant from an isolated single cell was the first demonstration of the theory of the totipotency of plant cell (Steward, 1958). Perhaps too large an extrapolation has been made here and I feel some caution should be exercised over considering this as evidence that all plant cells are totipotent.

However, in the case of potato, reports have indicated the relative ease with which whole plants can be regenerated from single cells and protoplasts (Shepard, 1980; Thomas, 1981). Normally, after a period of new wall synthesis, cell division begins and the isolated cells begin to form small cell aggregates. The formation of plantlet can then be induced either through an organogenic or an embryogenic pathway as described in more detail below.

Although in the early stages of plant propagation a lot of emphasis was placed on this technique for propagation, it has now been shown that the amount of variation to be expected in the propagules can be high. More details are given on this later.

3. Regeneration from Callus

Callus can be defined as a disorganised proliferation of cells. The callus may have a variety of origins, it may have come from isolated cells or protoplasts or it may have been induced on a cultured explant of a plant organ.

The classical experiments of Skoog and Miller (1957) using tobacco showed that, by appropriate modification of the hormonal balance of the culture medium it is possible to induce organ differentiation. This technique can be applied easily to potato callus to initiate the production of roots or shoots and eventually a whole plant.

Another feature, which must be kept in mind when working with callus, is the problem of loss of morphogenic potential (Chandler and Dodds, 1983). After several transfers in culture, the cells of the callus lose the ability to regenerate plants.

As will be discussed in more detail later, the major problem with this technique is that the plantlets formed have suffered significant genetic variation during the callus phase due to a high frequency of chromosome abnormalities during the mutotic divisions.

4. Regeneration from Tissue Explants

The potato shows remarkable plasticity of development. Regeneration of whole plants has been possible from the culture of a number of different explant sources.

Leaf discs are a very convenient starting material for this type of study (Webb et al, 1982). Once in culture, the first stage of development is normally the proliferation of small nodules of callus on the cut edges of the tissue. After a few more weeks given the appropriate hormone stimulus the regeneration of plantlets begins.

In the last few months, we have been successful in the regeneration of plantlets from cultured potato roots. The plantlets formed from these explants do not appear to go through a callus stage. The significance of this will be discussed later.

5. Formation of Somatic Embryos

In the history of tissue culture, there are a number of classical experiments. One of these was the experimental induction of somatic embryos in cultured single cells of carrot, thus demonstrating the totipotent nature of those cells. Similar techniques were applied recently to cassava (Stamp and Henshaw, 1982) and we are currently looking at the induction of somatic embryoids in potato. The induction is triggered by a hormonal change in the media and under some circumstances can be well synchronized (Komamine, 1978).

It is hoped that further experiments will lead to the defining of optimal conditions for the regeneration of somatic embryos in potato, at which point a more detailed analysis of genetic stability/variation in the system can be carried out.

6. Induction of In Vitro Tubers

The conventional methods of propagation of the potato is by growth of tubers. It has been shown recently that by the appropriate stimulus it is possible to induce the formation of small 'tubers' in an *in vitro* system. From the point of stimulus to the harvesting of the tubers takes about 4-5 weeks. A 250 cm^3 conical flask of shoots can produce about 14 tubers. Preliminary experiments show that 95% of these tubers, even extremely small ones, can be used to produce new plantlets. The tubers formed *in vitro* when produced from pathogen-tested and sterile plants may form excellent material for international germplasm exchange.

Summary to Methodology

The above listed *in vitro* methods constitute a wide variety of forms for producing new propagules. However, a number of features are of importance not only the number of propagules produced, but also the genetic status of the tissue and the cost effectiveness of the technology. Let us now look at some of these features in CIP's system for *in vitro* maintenance, propagation and distribution.

C. Advantages of In Vitro Methods

1. Pathogen-tested Status

The material maintained in CIP's pathogen-tested collection (*in vitro*) has been through thermotherapy and meristem culture followed by rigorous testing for the presence of virus and other pathogens. *In vitro* material can then be maintained in the pathogen-tested state, with no risk of reinfection.

This material is also available for international exchange all the year round, either in the form of small *in vitro* plantlets or possibly in the future in the form of sterile *in vitro* tubers.

2. Rates of Propagation

A comparison of the rates of propagation for the various *in vitro* systems described previously is shown in Table 1. A number of these systems theoretically appear to be highly favorable for rapid propagation and can be used to generate an almost infinite number of plantlets through regeneration from individual cells. There are however problems of genetic variability in these systems, so the benefit of large number may be offset. The two methods currently in routine use at CIP appear to strike a good balance between producing large numbers of plantlets with good genetic stability of the material. It may be that improved results could be obtained with the *in vitro* system and further experiments here would be most useful.

3. Storage for Exchange of Germplasm

A key advantage to the use of *in vitro* material is the relative ease with which material can be stored and retrieved. The pathogen-tested material can also be exchanged internationally either in the form of *in vitro* plantlets or tubers.

4. Manpower and Facilities

It has been shown recently (see previous session) both in national program studies and at CIP, that a minimum of training is required for someone to carry out routine subculture work under rather simple conditions. It is important for people to realise that for simple propagation work (i.e. single node cultures) the facilities required are few and relative inexpensive. We should dispel the notion that tissue culture techniques are sophisticated and high cost.

D. Disadvantages of In Vitro Methods

1. Manpower Requirements

The techniques outlined previously are almost all capable of producing large numbers of plants, however, the cost and availability of manpower can be a limiting factor. It is possible using the single node cultures or shaker cultures to produce about 200,000 plants per person per year. The type of conditions and the skill of the labor force have a marked effect on this output. Although it has been shown that tissue culture transfers can be made under rather rustic conditions, it is important that the operator is adequately trained to keep losses due to infection, especially of stock plantlets to a minimum.

2. Handling of Material

The small plantlets produced by tissue culture methods are obviously more difficult to handle than conventional material. A number of handling stages are required. Firstly, each of the *in vitro* transfer stages followed by (i) removal from the culture vessel, (ii) removal of agar from the roots by rinsing in stream of water, (iii) placement into compost and maintenance of a humid environment for several days.

All of these operations are time consuming, and in areas where labor costs are high will add significantly to unit cost of the *in vitro* plantlets However, if labor costs are low, and a well-organized system is set up, the unit production cost can be highly competitive.

3. Genetic Stability

The genetic fidelity of *in vitro* propagated material is a highly controversial area at the present time. It has long been known that the mitotic divisions of callus cultures are not particularly stable and a large number of variants arise (D'Amato, 1974). Variation has also been shown in potato plantlets derived from isolated protoplasts (Shepard, 1981, 1982) and it is proposed that these 'somaclonal variants' may have use in improvement programmes (Scowcroft and Larkin, 1983; Jones, 1983).

Putting to one side the question as to whether these variants may or may not have use for improvement, there is a need to be able to maintain genetic stability of vegetatively propagated material both for clonal propagation and for the maintenance of germplasm.

Experiments to date indicate that if organised tissue sources are used (i.e. propagation through axillary meristems) the systems appear to be stable; however, in single cell or callus forming systems variation will be generated. The methods used for detection of variation, for example, electrophoresis, are high unsatisfactory, however, with the present 'state of the art' they are probably the best available. Much more research is needed in this area to better define the problem and possible solutions.

E. Conclusions and Comments

As may be expected, there is a balance of positive and negative features to *in vitro* propagation. Each case should be taken on the basis of the problems and advantages that exist at that site. For example, labor cost and availability may make feasible *in vitro* propagation in some areas but not in others. The numbers of plants that are required to make a significant impact are staggering and cannot probably be produced at the institute level. It is probably necessary to decentralise the system and have the producer or the specialist nurseryman producing their own plants on a local area basis. The previous session has devoted special emphasis to this area. Tissue culture is important, however, for maintaining a nucleus of clean material for introduction into conventional propagation programmes. Much more information is required about the genetic stability of *in vitro* material.

The areas of tissue culture currently employed at CIP are probably the most beneficial, however, much more effort should be directed towards more detailed studies on *in vitro* tuber formation to complement present routine work on maintenance, propagation and export. Many of the techniques outlined here have great applicability at the National Program level for improved seed production schemes.

In the future plant tissue culture studies may be of fundamental importance in the further development of such areas as pollen culture and mutant selection; somaclonal variation and varietal improvement; genetic manipulation and gene insertion.

The problems of these technologies must be appreciated and the benefits fully employed to develop an integration of these techniques with other disciplines to work for improvement of quality and yield of potato prodution.

Table 1. Comparative features of various *in vitro* propagation systems

System	Culture time	Number of propagules	Type of facilities	Genetic stability	Comments
Single node	4-5 wks	x 10	minimal	good	Already used at CIP (routine)
Shaker cultures	3 weeks	x 15	need shaker	good	Already used at CIP (routine)
Single cells	16 weeks	Almost infinite	fully equipped laboratory	poor	Produces large amount of variation
Callus	8 weeks	large numbers	fully equipped laboratory	poor	Produces large amount of variation
Regeneration from explants	8 weeks	large numbers	reasonable laboratory	depends on system	Experiments in progress at CIP with leaf discs & roots
Somatic embryos	8-12 wks	large numbers	fully equipped laboratory	unknown	Experiments in progress at CIP. Little known about level of variation
In vitro tubers	4 weeks	0.5 tubers (ave. 8 sprouts)=4	reasonable laboratory	good	Experiments in progress at CIP. Although not perhaps the best, has many advantages

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PROTOPLAST AND SOMACLONAL VARIATION RESEARCH ON POTATO

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INTRODUCTION

Tissue culture techniques applied to potato are now widely employed for a variety of purposes. The main applications are listed in Table 1. In addition to these applications, novel techniques that may play an increasingly important role in future genetic modification of potato cultivars are also listed.

Table 1. Current and future applications of tissue culture to potato

Current applications	Micropropagation
	Meristem culture (virus elimination)
	Embryo rescue (e.g. wide crosses)
	Anther culture (2n→n)
	Increasing ploidy (n→2n→4n)
	Germplasm storage/transportation
Future applications	Modification by somaclonal variation
	Gene transfer by protoplast fusion
	Gene introduction by *Agrobacterium* (transformation).

Emphasis in this paper will be on some of the techniques covered in the future applications of Table 1. In particular, modification of potato by somaclonal variation and the techniques of gene transfer by protoplast fusion will be discussed, and the present status of transformation by *Agrobacterium* will be outlined.

Work in these areas relies on basic tissue culture techniques of regeneration of plants either from excised tissues or from protoplasts. Since the merit of some applications depends on the techniques involved, these will be outlined briefly.

REGENERATION TECHNIQUES

Explant culture

Plants may be regenerated from cultured surface-sterilized explants, such as pieces of leaf, stem, rachis or tuber. Regeneration normally involves the production of adventitious shoots following callus induction. Shoot regeneration has been achieved using either a single medium for callus induction and shoot production (Roest and Bokelmann, 1976; Jarret *et al.* 1980) or a simple two-stage procedure in which callus is induced on an initial medium containing auxin and cytokinin and this is followed by shoot production on transfer to medium with cytokinin and gibberellic acid (Webb *et al.* 1983; Wheeler *et al.* 1985; Karp *et al.* 1984). Callus usually forms by 2 weeks at the cut surfaces of the tissue, and after about 6 weeks large numbers of shoots may be produced from each explant. Shoots can be excised, rooted, transplanted to soil and then grown to produce mature plants. Regeneration has been achieved for many potato cultivars, dihaploid and monohaploid lines (Wheeler *et al.* 1985; Karp *et al.* 1985), by this relatively simple technique.

Protoplast isolation and culture

Protoplasts are obtained by enzymatic removal of the plant cell wall. To obtain viable leaf protoplasts, careful control of leaf growth is required. This may be achieved by growth in environmental

Table 2. Some potato cultivars, clones and Solanum species from which plants have been regenerated from protoplasts

Cultivar, clone or Solanum sp	Reference
Russet Burbank	Sheppard and Totten, 1977
Katahdin	Sheppard, 1982
Bison	"
Atlantic	"
Maris Bard	Thomas, 1981
Majestic	Creissen and Karp, 1985
Fortyfold	Nelson, 1983
Pentland Crown	Creissen, 1984
King Edward	D. Foulger (unpublished)
Desiree	"
Maris Piper	" ; Gunn and Shepard, 1981
Feltwell	Gunn and Shepard, 1981
Foxton	"
Bintje	Bokelmann and Roest, 1983
Dihaploids	Binding *et al.* 1978
	Wenzel *et al.* 1979
S. brevidens	Nelson *et al.* 1983
"	Barsby and Shepard, 1983
S. dulcamara	Binding and Nehls, 1977
S. phureja	Creissen, 1984
S. chacoense	Butenko *et al.* 1979
S. nigrum	Binding *et al.* 1982

cabinets, where control of light, humidity and nutrients is necessary, or by growth of 'shoot' cultures _in vitro_. Various protocols for potato protoplast isolation, culture and regeneration have been reported (Karp _et al_. 1985). These involve culture of protoplasts in liquid media for about 3 weeks, followed by transfer to solid media where protoplast-derived callus is produced that is then transferred to differentiation and shoot emergence media. Shoots can be excised, rooted and transferred to soil. Between 5-10% of potato protoplasts initially plated out may yield plants, and the whole procedure takes 4-6 months. Successful culture of protoplasts requires careful attention to details such as plating densities and timing of transfer to different media. Some cultivars, clones and wild species that have been regenerated in this way are given in Table 2.

CONSEQUENCES OF A CALLUS PHASE

In those culture techniques where the organisation of meristematic and axillary buds are maintained, regenerated plants are normally stable. However, when the cell growth becomes disorganised under the influence of an imbalance of plant growth regulators with the production of a callus phase, then the stabililty may be lost, and regenerated plants can exhibit somaclonal variation. The production of variant plants in this way for potato is now a well established phenomenon.

Potato plants can be regenerated either from explants or from protoplasts, as already outlined, and information is available from starting material that is tetraploid (2n=4x=48), dihaploid (2n=2x=24) or monohaploid (2n=x=12). A description of the variation obtained may conveniently be separated into observed cytological and morphological changes, and the type and extent of the variation must be related to the state of the starting tissues (ploidy, genotype and tissue source) and the method of plant regeneration.

CYTOLOGICAL (NUMERICAL AND STRUCTURAL) CHANGES

Regeneration from tetraploid tissues

Plants regenerated from explants via a short callus phase from leaf, stem, rachis and tuber of the cultivars Desiree, Champion and Myatts Ashleaf were found cytologically to be relatively stable, and 87% of regenerants were euploid (Wheeler _et al_. 1985). Of these, plants from tuber explants appeared least stable, with 4 out of 7 being aneuploid. In aneuploid plants, with one exception, the chromosome range was 48 ± 1 (Wheeler _et al_. 1985).

Plants regenerated from protoplasts of tetraploid potatoes pass through a longer callus phase. Although earlier work with cv Russet Burbank indicated no chromosomal changes in regenerants (Shepard et al. 1980), detailed cytological studies of British potato cultivars has indicated considerable variation in chromosome numbers. For example, 4% of plants of cv. Maris Bard (Thomas, 1981), 30% of cv. Fortyfold and 57% of cv Majestic regenerated from protoplasts were euploid (Karp et al. 1982; Creissen and Karp, 1985). The aneuploid Maris Bard regenerants were characterised by high chromosome numbers apparently resulting from chromosome doubling followed by loss (2n=70-95), but aneuploid Fortyfold and Majestic regenerants more commonly either gained or lost a few chromosomes at the tetraploid level (2n=48 ±1-3). Clear evidence of three structural chromosome changes in regenerants of Majestic were obtained (Creissen and Karp, 1985). The status of regenerated plants may be affected by genotype and culture conditions.

Regeneration from dihaploid/diploid tissues

Of 50 plants regenerated from dihaploid leaf pieces (2n=2x=24) and examined cytologically, 38% remained dihaploid and 60% doubled to the doubled dihaploid constitution (2n=4x=48). No aneuploids were observed, and one plant was mixoploid (Karp et al. 1985). In contrast to results obtained from tetraploid protoplasts, Wenzel et al. (1979) found that of 48 dihaploid protoplast-derived regenerants, only two aneuploids were present and the remainder had doubled up to the tetraploid level. Similarly, Nelson (1983) found that protoplast-derived plants of the diploid S. brevidens were 24% diploid, 52% tetraploid and 24% aneuploid.

Regeneration from monohaploid tissues

Plants regenerated from monohaploid leaf pieces (2n=x=12) almost all doubled up to 24 chromosomes, with occasional plants remaining at the monohaploid or redoubled to the tetraploid levels (Karp et al 1985). Data for monohaploid protoplast regeneration are not available.

These observations demonstrate that it is particularly important to check the chromosomal constitution of regenerants from tetraploid tissues, especially those derived from protoplasts, but regeneration from explants of monohaploid or dihaploid tissues can be used as a rapid and efficient method of increasing the ploidy levels.

MORPHOLOGICAL CHANGES/SOMACLONAL VARIATION

Shepard and his co-workers sparked off the wide interest in somaclonal variation of potato by reporting that plants regenerated from protoplasts of tetraploid Russet Burbank differed in various ways from parental plants in such characters as tuber shape, yield, maturity date, photoperiod response and morphology (Shepard 1980; Shepard et al. 1980; Secor and Shepard 1981); and in responses to challenge by fungi or filtrates of early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) (Matern et al. 1978, Shepard et al. 1980). In this work obviously aberrant regenerants were discarded. These were probably aneuploid. In work at Rothamsted morphological variation has similarly been observed in protoplast-derived regenerants that, in part, relates to chromosome numbers. All

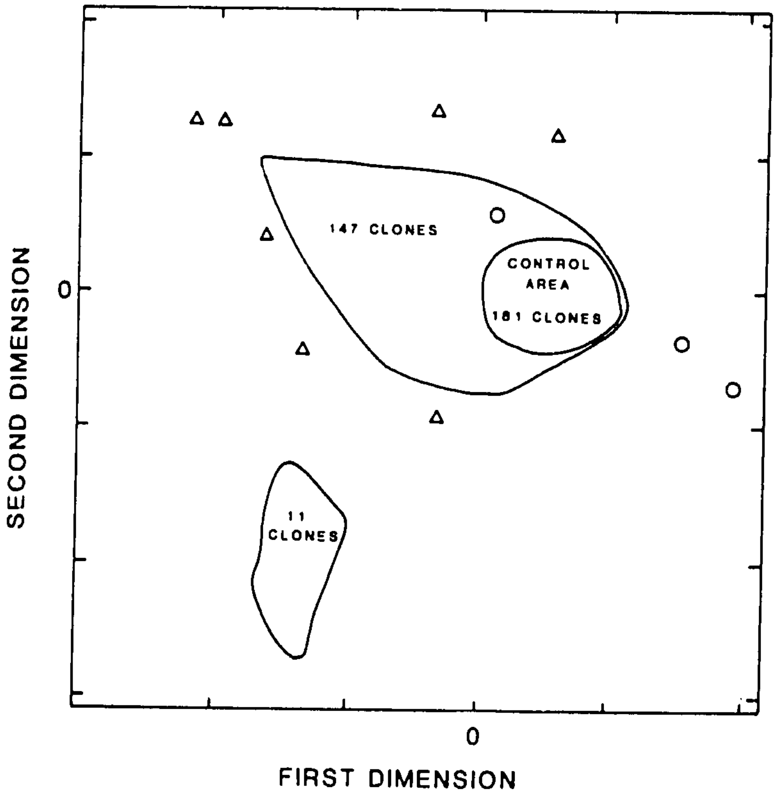

Fig. 1. The distribution of 346 regenerated clones of cv. Desiree along the first two dimensions after principle component analysis of about 30 different measurements. 181 regenerant clones were within the control area, 147 regenerants were in the area indicated, and 11 white skinned tuber variants were in a separate group. Outlying control (O) and regenerant clones (Δ) are also indicated (Evans et al. 1985).

those plants with high chromosome numbers (doubled up then some lost) were grossly aberrant, but plants with 48 ±1-3 chromosomes were either indistinguishable from controls or showed limited variation (Nelson, 1983; Jones et al. 1983; Creissen and Karp, 1985). Similarly, Sree Ramulu et al. (1983) reported that of 620 protoplast-derived plants analysed, 64% resembled the parental material and 36% showed variations. Of the variants, 82% were aneuploid.

Potato clones regenerated from explants, as already described, rather than from protoplasts, also exhibit potentially useful variation (Karp et al. 1985). As might be expected from the low proportion of aneuploids, few grossly aberrant plants are produced. An important point is that apparently cytologically normal plants can show somaclonal variation. A detailed analysis of explant-derived regenerants from leaf, rachis or stem pieces of cultivar Desiree has demonstrated altered tuber yield, colour, number and extent of infection by scab, (*Streptomyces scabies*) and changes to flower colour, habit and leaf morphology (Wheeler et al. 1985; Evans et al. 1985). Field data from replicated plots, evaluated by principle component analysis (Fig. 1), has indicated that about half the 346 clones evaluated differed from control plants. The variants (eg. in tuber colour) examined have been essentially stable so far. In the

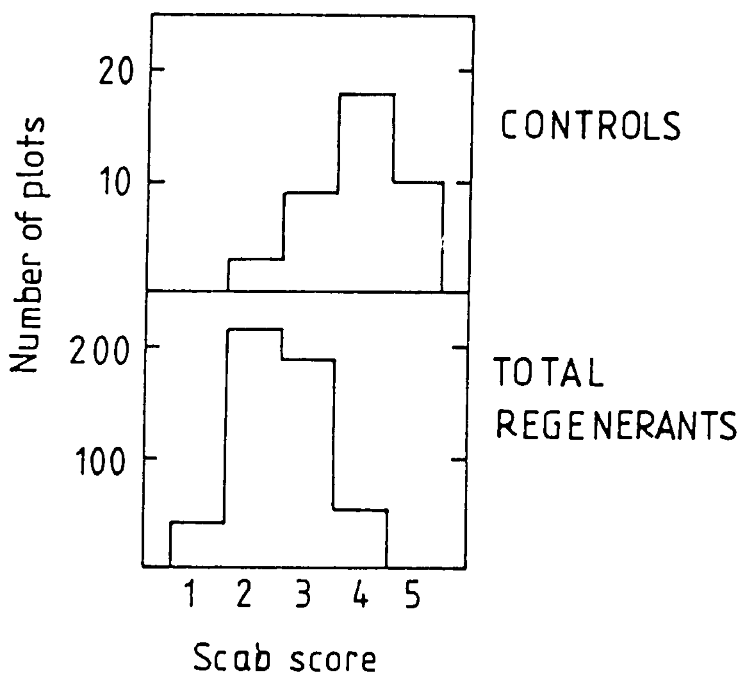

Fig. 2. Tuber scab score distribution of field grown Desiree regenerants. Percentage of surface affected between 1 = 0% and 5 = 75%.The regenerants originated from both leaf and rachis explants, the controls were grown on the same site over 2 years (Evans et al. 1985).

case of scab, the scores for the whole regenerant population were more resistant (Fig. 2), and this implies an epigenetic change that might be unstable with time. Although many characters may vary, it cannot be stated that all agronomic characters can be altered by somaclonal variation. Thus no resistance to *Globodera pallida* was obtained in this population of regenerated Desiree, and such changes as tuber colour from white to coloured is limited to one isolated report (Rossignol, personal communication).

In contrast to plants regenerated from tetraploid tissues, plants regenerated from dihaploid potato protoplasts (regenerants essentially tetraploid) were uniform in morphology (Wenzel *et al.* 1979), and similarly plants regenerated from monohaploid or dihaploid potato explants, or from the diploid wild species *S. brevidens*, were uniform for the morphology typical of the ploidy level. For example, leaflets of dihaploid potatoes or diploid *S. brevidens* are typically narrower than those of respective tetraploid plants.

Somaclonal variation and crop improvement

That variation in regenerated potato plants does occur, and at relatively high frequencies, is now well established. In many cases the technique offers a way of improving a well-established cultivar that is not available by conventional means. Interest is such that in both government and private breeding companies in the U.K. substantial effort is being invested to evaluate the application of somaclonal variation to potato improvement. Two factors that have promoted this approach are (1) the long time lag (8-15 years) between a conventional sexual cross and the selection that results in a new cultivar, and (2) the resistance in the market-place to adopt new cultivars. On the basis of the results described it is recommended that breeders who wish to create new variation with a minimum of grossly aberrant (and

Table 3. A comparison of the techniques of explant and protoplast regeneration in potato (Jones et al. 1984)

Explant Regeneration
- Many plants regenerated by simple procedure
- Chromosome number relatively stable
- Variation in useful characters

Protoplast Regeneration
- Technically intricate and labour intensive
- Considerable chromosomal changes
- Useful and useless variation
- Necessary to investigate the potential of protoplast fusion and transformation

therefore useless) plants, should use the tissue explant regeneration cycle for the reasons summarized in Table 3, and regeneration from protoplasts should be used for gene transfer by protoplast fusion or transformation (Jones et al. 1984).

However, it should be noted that more information is required on a number of aspects of somaclonal variation before a clear picture of its value in potato improvement can be made. These aspects include the fact that without directed selection, the variation that arises is fortuitous, and not all changes occur with equal probability: perhaps some desired changes are not possible. The stability of variants also needs to be tested. Until we have a better understanding of the underlying events of somaclonal variation, it will not be possible to provide all the answers.

POTATO PROTOPLAST FUSION

With the regeneration of plants from protoplasts, it is now potentially possible to produce new hybrids by fusing together different protoplasts, that (1) cannot be produced sexually, (2) allows the asexual combination of complete or partial genomes and (3) does not require detailed knowledge of the genetic and molecular basis of the characters to be combined (Jones et al. 1984; Karp et al. 1985).

Two methods of fusion are available - chemical and electrical. The chemical procedure involves adhesion of protoplasts with polyethylene glycol followed by fusion with Ca^{2+} ions at high pH, and has been well described (Kao et al. 1974; Evans, 1983). This procedure is characterised by a relatively low and variable fusion frequency, and little control of fusion partners is possible. More recently, electrical methods of fusion have been developed (Zimmermann, 1982). Protoplasts can be aligned in chains by a high frequency alternating electric field by 'dielectrophoresis'. When in contact, fusion is induced with a short direct current pulse. By control of protoplast density, method of introduction and fusion pulse conditions, a relatively high frequency of one-to-one fusions of different protoplast can be obtained with production of viable heterokaryons (Tempelaar and Jones, 1985a). The procedure has been scaled up for mass fusions (Tempelaar and Jones, 1985b) with regeneration of plants. This technique therefore offers a real advance in the production of novel somatic hybrids.

To date, application of fusion techniques to potato has been limited, but several groups world-wide are now applying them more rigorously and more results can be expected.

Protoplasts of potato have been fused with the production of hybrid plants with tomato (Melchers *et al.* 1978; Shepard *et al.* 1983), *S. chacoense* (Butenko and Kuchko, 1980), *S. nigrum* (Binding *et al.* 1982, *Nicotiana tabacum* (Skarzhinskaya *et al.* 1982), *N.sylvestris* (Kueh *et al.* 1984) and *S. brevidens* (Barsby *et al.*1984; S. Austin pers. communication). For the taxonomically more distant fusions, problems of chromosomal stability have been encountered, but most of the work was done before the cytological status of plants regenerated from potato protoplasts was well understood. However, in the last case, both dihaploid and tetraploid potato protoplasts have been fused with diploid *S. brevidens*, with the regeneration of plants with additive chromosome numbers, and these have been evaluated in field trials (S. Austin, personal communication). This result is promising for fusions between *Solanum* spp and between established potato cultivars.

The approach adopted at Rothamsted has been either to combine complete genomes at the dihaploid/diploid level, to yield tetraploids (cytologically most such hybrids can be expected to be stable); or to fragment the genome of a donor (tetraploid or diploid) with useful properties and fuse with a tetraploid recipient, with the aim of limited genome transfer. This approach may allow introduction of useful characters without other undesirable traits. The potential of the technique is for transfer of characters whose genetic basis is unknown or complex (eg. polygenic). For practical applications, many hybrid plants will have to be produced and screened for the desired traits and for chromosomal stability.

POTATO TRANSFORMATION

The aim of transformation is to introduce and stably integrate specific genes into potato cells, and to regenerate intact plants in which such genes are expressed in a controlled way that will lead to specific modifications.

There is as yet a shortage of identified and characterized genes that may be introduced in this way, but recent work has established that of the crop plants, potato is particularly amenable to such techniques. Although it is becoming apparent that direct uptake of genes by protoplasts is possible (Shillito *et al.* 1984), the emphasis so far has been on using the bacteria *Agrobacterium tumefaciens* and *A. rhizogenes* as vectors for gene introduction (Hooykaas and Schilperoort, 1984). On infection of wounds, or by mixing, with protoplasts ('cocultivation'), a section of DNA ('T'-DNA) coding for a few genes can be transferred from the bacteria and integrated into plant chromosomes. When wild type strains are used, the 'T' DNA codes for excess hormone production with the formation of galls (A. tumefaciens) or 'hairy roots' (A. rhizogenes). The genes in the 'T'-DNA can be modified, removed or replaced with other desired genes.

The potential of this approach for potato has been shown by the production of plants, via culture, of commercial cultivars derived from infections with 'shooty' mutant (excess cytokinin) strains of A. tumefaciens (Ooms et al. 1983). Here, modified potato plants containing bacterial genes exhibited altered morphology and development in characters such as photoperiod responses and early tuberisation. Similarly, Ooms et al. (1985) have regenerated potato plants containing genes from A. rhizogenes that have crinkly leaves, extensive root systems and produce tubers with prominent eyes. The latter resemble some of the potatoes only normally found in South America. Undoubtedly specific single genes will be introduced by this technique that will in time contribute to the production of new cultivars.

CONCLUSIONS

Potato is amenable to a range of tissue culture techniques, some of which are routinely in practical use.

The newer techniques of potato modification - somaclonal variation, protoplast fusion and transformation - in general require more expertise, knowledge and laboratory facilties. However, breeders in Europe and North America are begininning to investigate and apply some of these techniques to potato. The simplest of these, somaclonal variation, can be developed at any standard tissue culture facility, but fusion and transformation require increasingly more back-up. The decision of how much effort should be apportioned to the techniques still requires more information, but it should be stated clearly that these techniques do not replace conventional breeding in any way. Rather they should supply or make available new sources or combinations of germplasm or genes for breeders to use, evaluate and select improved potato plants. They should also speed up the production of new cultivars.

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Anther Culture and its Uses in Potato Breeding

A. Sonnino

A. Introduction

It is only twenty years since the first successful report of haploid production by anther culture in *Datura* appeared (Guha and Maheshwari, 1964). Since then, many experiments have been successfully performed to extend this technique to a large number of species and genera. As a result of this intense research activity, anther culture has become a routine breeding tool for a few crops (Maheshwari *et al*, 1983).

This paper will briefly review the possible uses of anther culture in breeding and will analyze some prerequisites for practical application of anther culture in potato breeding.

B. Uses of Anther Culture

1. Uses in Breeding and Genetic Studies

Some possible uses of haploid plantlets are summarized in Figure 1. Some applications are important for fundamental studies and are beyond the aim of the present article, others, however, have a direct application on plant breeding. Haploids can surely be useful tools to increase our still poor knowledge of potato genetics.

The production of homozygous lines is probably the most promising application of anther culture. It allows the production of pure lines in self-pollinated and in self-compatible allogamous plants without passing through several cycles of inbreeding, thus, saving several years of work in bre ding programs. The release of new varieties of tobacco, ric and wheat (Zeng, 1983) are good examples of the potential of this technique.

Anther culture provides a novel way of obtaining pure lines in self-incompatible and in heterogametic dioecious plants.

2. Applications of Anther Culture in Potato Breeding

In potato, reduction of ploidy level from 4x to 2x can be routinely performed by female pathenogenesis (Hermsen and

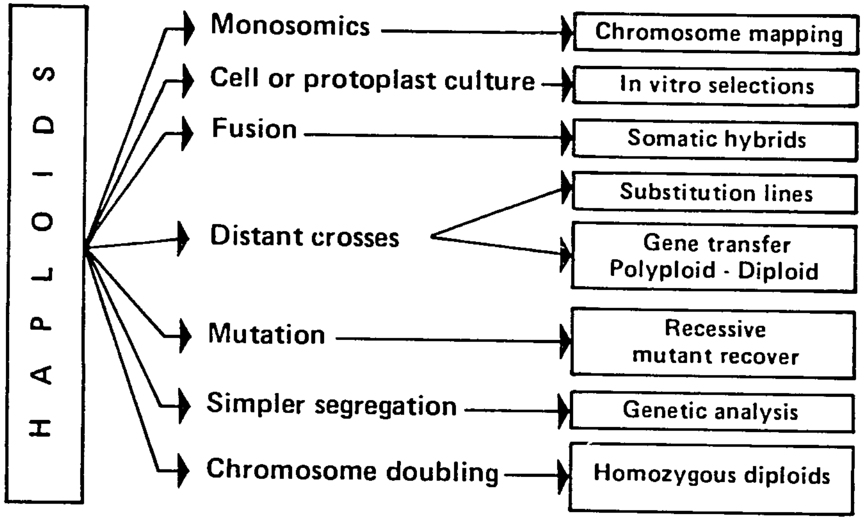

FIG. 1

Main possible applications of haploids in plant breeding and genetics

Verdenius, 1973), thus producing large numbers of diploid plants. However, this technique is only of small significance for obtaining monoploids (Van Breukelen et al, 1977). As diploids, commonly self-incompatible, can not be selfed, anther culture is a suitable method of obtaining completely homozygous clones.

Homozygous diploid lines with different genetic attributes such as disease resistance and other desirable horticultural traits could speed up the accumulation of desired traits in a single genotype. Diploids with a high number of favourable genes can afterwards be reconducted to the tetraploid condition either by somatic hybridization (Wenzel et al, 1979) or by meiotic doubling (Peloquin, 1982). Evaluation of lines in homozygous condition should allow us to separate additive from non-additive effects and thus select lines with high parental value.

Furthermore, homozygous diploids can be easily doubled by adventitious bud technique, given appropriate conditions (see below). The resulting homozygous tetraploids should be self-incompatible and consequently may represent good female parents for production of hybrid TPS (True Potato Seed).

Culturing irradiated anthers may give rise to stable, homozygous mutants (Przewoźny et al, 1980), as there is no dominance in monoploids, even recessive mutations can be recovered in such a way. This technique may be usefully applied to induce meiotic mutants, to obtain genetic markers or to improve horticultural traits.

C. Prerequisites for Practical Application of Anther Culture

In order to apply anther culture as a routine technique within a breeding program, the following criteria should be met:

1. Production of a large number of doubled haploids of many different genotypes should be practical and reproducible.

   Unfortunately, not all potato genotypes respond well to anther culture (Irikura, 1975; Simon and Peloquin, 1977; Wenzel et al, 1979). Wenzel and Uhrig (1981) reported that this "tissue culture ability" (TCA) is strongly dependent on the genotype, and can be transferred between genotypes via sexual breeding. Results obtained at CIP by Tanaka (1984) and by myself fully supported their report. Thus, it seems possible that TCA can be introduced into different useful breeding lines, which currently lack that potential.

Since spontaneous chromosome doubling occurs during development of plantlets from anther (Wenzel et al, 1980), there should be no problem in doubling chromosome number. Nevertheless, the yield of doubled haploid plantlets via anther culture is still too low for routine practical application.

2. Doubled haploids should be cytologically stable and phenotypically normal.

3. Interesting characters should be transmitted from the parents to the doubled haploids.

The last two points do not apparently represent any problem in potato anther culture. Transmission of single-gene determined characters, as well as of polygenically inherited traits, was demonstrated by Wenzel and Uhrig (1981). Only selection against lethal or sublethal factors seems to occur (Wenzel et al, 1980; Uhrig, 1983).

D. Factors Affecting Anther Culture in Potato

1. Tissue Culture Ability

The importance of genetically determined TCA and the possibility of its transfer by sexual combination breeding have been stressed above. Figure 2 shows results obtained by Dr Tanaka (1984) at CIP. Tanaka tested 20 progenies resulting from a cross between a high TCA clone (provided by Dr Wenzel) and CIP clones 381320.23, resistant to root knot nematode, but without TCA.

The majority of the hybrids showed some degree of response to anther culture, some hybrids even showed a TCA higher than the best parent. The wide range of continuous variation among the progenies leads us to discard the hypothesis that TCA is controlled by one major gene (Tanaka, 1984). That is in agreement with the results of Wenzel and Uhrig (1981).

2. Growth Conditions of the Donor Plants

Dr Tanaka (1984) reported that plants grown in Huancayo (3,280 m.a.s.l.) showed a better response to anther culture

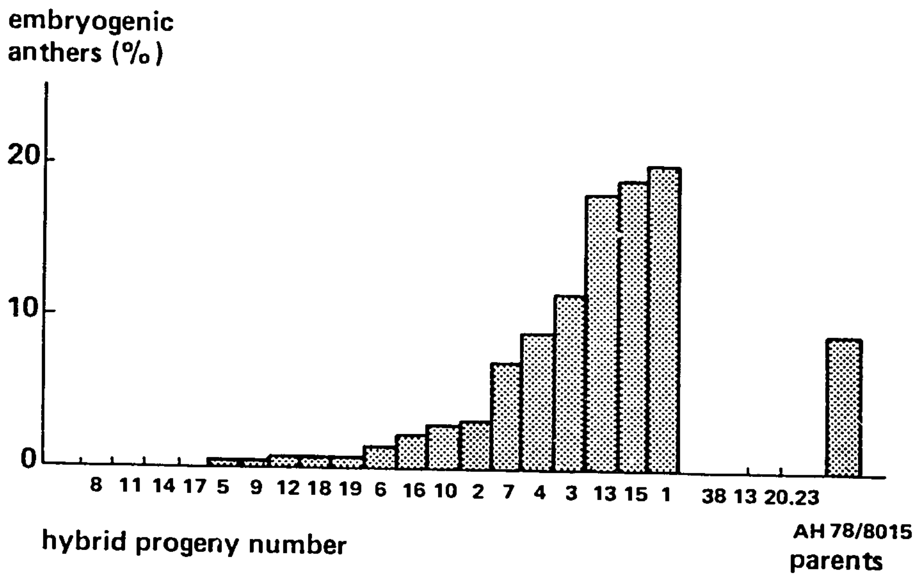

FIG. 2

Percentage of embryogenic anthers in 19 F_1 hybrid progenies and in their parents compared to plants grown in La Molina (240 m.a.s.l.) (Table 1). In a similar comparison, repeated this year, some genotypes responded better when the donor plants were grown in Huancayo, while other genotypes performed more efficiently when the donor plants were grown in Lima (La Molina) (Table 2). The discrepancy between the results obtained in 1983 and in 1984 are probably due to different growing conditions of the donor plants in Huancayo (open field in the former year, greenhouse in the latter). The different responses of plants of the same genotype cultivated in the two environments may be attributed to the different growing temperatures. In fact, flowering plants were exposed in Huancayo to an average maximum of 28.1°C and to an average minimum of 4.0°C, while in Lima the average maximum was 26.6°C and the average minimum 16.2°C (temperatures recorded in greenhouses). Considering the different reactions to the temperature of the genotypes tested, it is possible to infer that genotype-environment interactions also affect the production of pollen-derived plants. Preliminary observations also indicate that photoperiod may be important.

All these factors, as well as their interactions, should be experimentally investigated by additional experiments, performed in controlled environments.

Table 1. Response to anther culture of plants grown in Huancayo and in La Molina (1983)

	HUANCAYO		LA MOLINA	
Line	Anthers plated (no.)	Embryogenic anthers (%)	Anthers plated (no.)	Embryogenic anthers (%)
FI.12	50	6.0	133	0.8
FI.13	73	16.4	375	18.1
FI.14	66	4.5	75	0.0
FI.15	84	27.4	260	18.8

Table 2. Response to anther culture of plants grown in Huancayo and in La Molina (1984)

	HUANCAYO		LA MOLINA	
Line	Anthers plated (no.)	Embryogenic anthers (%)	Anthers planted (no.)	Embryogenic anthers
FI.1	115	11.3	750	6.0
FI.3	152	1.3	347	2.0
FI.4	62	2.7	190	4.7
FI.13	272	23.5	531	11.9
FI.15	77	3.9	627	13.9

3. Pollen Stage

Apparently the stage of pollen development is not critical to production of haploid embryos with successful results obtained from grains ranging from early to late uninucleated stage (Figure 3).

4. Pretreatment of Anthers

Pretreatment of flower buds at 8°C for two days has been found to be effective in increasing haploid production, possibly due to prolonged pollen viability.

5. Culture Medium

According to Wenzel and Uhrig (1981) the type of culture medium is less important than plant genotype, since the best genotypes will develop on a wide range of media. P59 medium (Jacobsen and Sopory, 1977) commonly used is a MS basal medium supplemented by IAA and BAP (both at a 1 mg/l concentration) charcoal (0.5%) and sucrose (5%). P59 medium was compared to a medium of the same composition, but constituted by two layers (DL). The lower layer was formed by solid P59 medium with 0.5% charcoal, while the upper layer was formed by liquid P59 medium, without charcoal. This combines the advantages of charcoal with liquid media, effective in removing inhibitory compounds released by the anthers (Johanson *et al*, 1982).

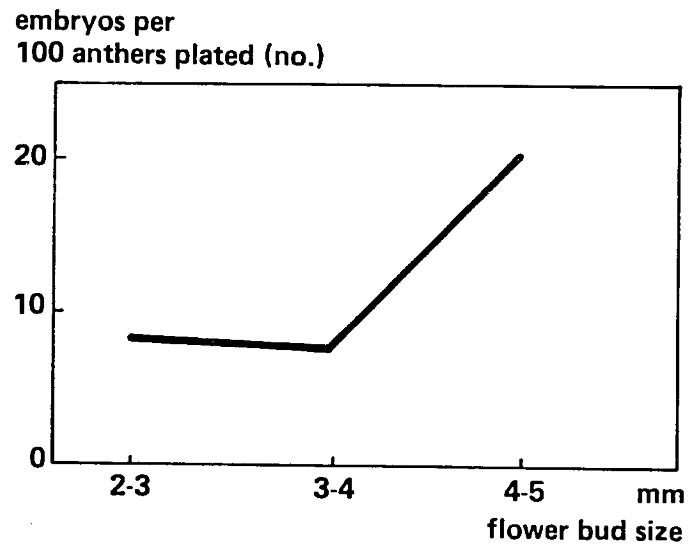

FIG. 3

Embryo formation in anthers plated at different stages

Table 3 shows results obtained by culture of anthers at different developmental stages and belonging to eight different clones and hybrids. In general, the double layer medium did not improve the efficiency of anther culture.

An increase of embryo production was observed in anthers of advanced stages cultivated on DL as compared to the anthers of the same stage cultivated in P59 solid medium (Figure 4). In fact, when anthers in late stages floated on the liquid phase of the DL medium, pollen-derived material was released into the medium and developed either as embryos or as calli. The increase of embryo development can be then due to avoidance of space limitations, that hampers embryo growth inside the anthers cultivated on solid medium. Adjustments of hormonal content at the liquid phase of the medium, balancing the lack of the charcoal action, can further enhance the efficiency of the method (Figure 5).

Table 3. Response to anther culture of eight clones and hybrids in two culture media

Medium	Anthers plated (no.)	Embryogenic anthers (%)	Embryos per embryogenic anther (no.)	Embryos per 100 anthers plated (no.)
P59	2818	8.23	2.94	24.24
DL	1967	6.71	3.27	22.56

6. Proembryo and Embryo Management

In some cases, plantlets arise directly from anthers and they can be transferred straight onto a normal propagation medium. More frequently, small embryos develop within the anther and nearby from released microspores. Even transferring them on a regeneration medium, only a few are able to develop directly into plantlets, or to regenerate from calli. This is one of the most critical constraints of potato anther culture, since it drastically reduces the yield of pollen-derived plants.

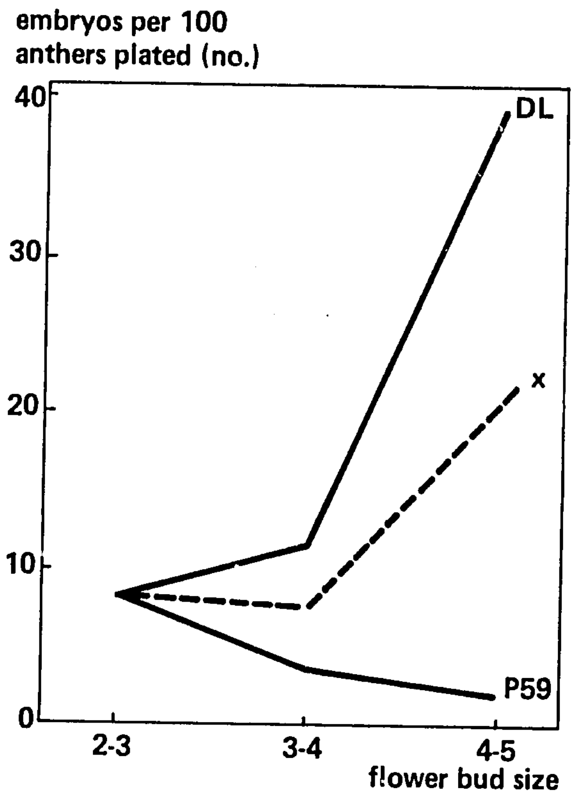

FIG. 4

Embryo formation in anthers of different stage cultivated on two media

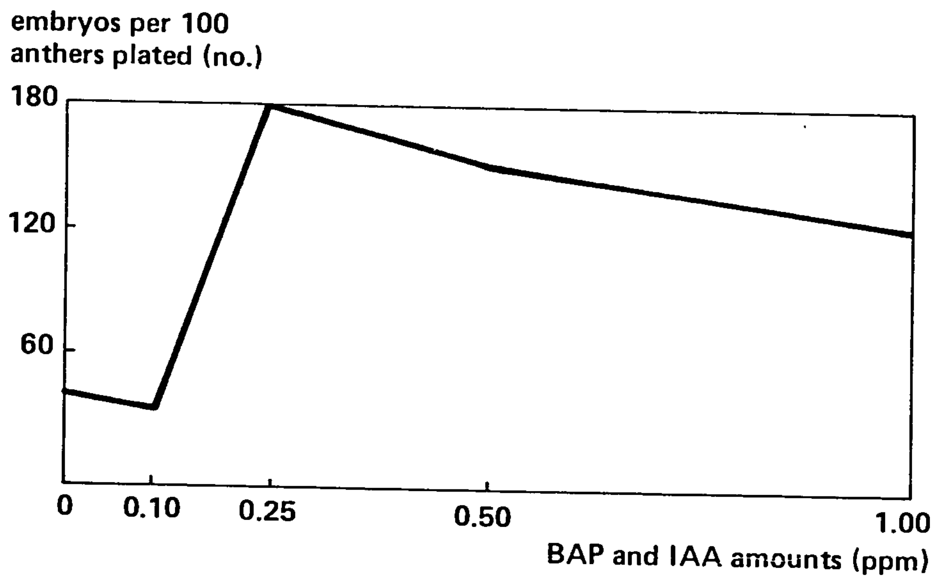

FIG. 5

Embryo formation in anthers cultivated on DL medium with different hormonal content in the liquid layer

As mentioned above, the presence of lethal or sublethal genes, working only at haploid level, represents an important factor of this scarce survival (Wenzel *et al*, 1979). However, the same problem occurs when anthers of anther-derived plants, that should be free of lethal factors, are cultivated. Physiological factors should therefore be taken into account.

7. Haploid Doubling

Almost all the potato anther culture-derived plants were found to be diploids (Wenzel *et al*, 1980), therefore, the question arises whether these plants regenerated from pollen grains or from anther somatic tissues. Although the mode of embryo formation from anthers and the degradation of anther wall indicate an androgenetic origin of the embryos, the question should be experimentally verified. Electrophoretic studies of the regenerated plants will provide useful information about their origin (Zamir *et al*, 1981).

E. Doubling Homozygous Diploids

To obtain heterozygous tetraploids, both somatic fusion (Wenzel *et al*, 1979) and meiotic doubling (Peloquin, 1982) techniques are available. So far, somatic fusion appears to be more difficult than meiotic doubling.

Homozygous tetraploids from homozygous diploids can be obtained by the use of colchicine (Dionne's technique) (Ross *et al*, 1967) or adventitious bud technique (Hermsen *et al*, 1981). The latter technique has the advantage of avoiding chimera formation although the possible induction of variability should be carefully taken into account. Our results indicate importance of genotype even for somatic regeneration (Table 4).

The frequency of leaf, stem and petiole explants with regeneration and the frequency of tetraploids among the regenerants of AH-79/7878, a clone with high TCA were significantly higher than those of two other clones without TCA. This suggests that TCA is related to capability of explants to regenerate in general. If this is the case, the production of homozygous tetraploids from homozygous diploids via adventitious bud technique can be easily achieved due to TCA of homozygous diploids.

Table 4. Frequency of somatic regeneration and of tetraploids among the regenerants in three diploid clones

Clone	Inoculated explants (no.)	Explants with regeneration (%)	Regenerants checked for ploidy level (no.)	Tetraploids (%)
378950-74 x BW 43-1	152	3.95	22	0.00
381318-11	200	2.00	36	8.33
AH-79/7878	88	34.09	20	85.00

F. Conclusions

Before anther culture can be routinely applied in potato breeding programs, its efficiency must be improved. In particular the following topics need more research:

1. The mechanism of TCA is still completely unknown. TCA may be due to high embryogenic pollen quantity (Heberle-Bors, 1983), high regeneration capability of the embryogenic pollen, or to high chromosome doubling ability of the embryos.

   An understanding of the TCA mechanism may permit regeneration from difficult genotypes, avoiding time consuming crosses, or methods to enhance regeneration in "good" genotypes. For instance, in tobacco it was possible to increase embryogenic pollen quantity and pollen plant production by chemical treatments of the donor plants (Heberle-Bors, 1983). This possibility will be checked in potato.

2. Management of developing embryos represents so far the major constraint in potato anther culture. The presence of lethal factors can explain only partially the poor survival rate of the embryos, as mentioned above. A better understanding of the reasons for this phenomenon is needed, in order to increase the overall efficiency of the technique for haploid plant production.

In conclusion, anther culture still needs adjustments and refinements before it can be usefully applied in potato breeding programs. However, when well established and combined to classical breeding procedures, it opens exciting possibilities for speeding up and for making more efficient potato improvement.

Acknowledgments

I am fully indebted to CIP, for providing me with an excellent opportunity and work facilities to carry out the research presented in this paper. It is impossible to mention individually all people who offered me help, friendship and assistance. To all of them my sincere gratitude.

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Planning Conference

on

Innovative Methods for Propagating Potatoes

A G E N D A

The purpose of the Conference is to evaluate techniques such as true potato seed (TPS), tissue culture, and various methods for the rapid propagation of potatoes under developing country conditions.

Monday, December 10

Chairman, O.T. Page

09:00	Welcoming and introductory remarks. R.L. Sawyer Director General
09:15	Seed tuber propagation in developing countries. J. Bryan
09:35	Seed tuber and TPS propagation in Rwanda. A. Haverkort
10:00	Coffee

PROPAGATION WITH TRUE POTATO SEED (TPS)

10:30	Breeding of TPS populations. H. Mendoza
11:00	Utilization of ploidy manipulations in breeding for TPS. S. Peloquin
11:30	In-breeding and TPS production. M. Jackson
12:00	Discussion - Future breeding plans for TPS.

12:30 Lunch

Monday afternoon

Chairman, H. Mendoza

14:00 Agronomic management for transplanting TPS seedlings.
P. Malagamba

14:20 Propagation by seed tubers derived from TPS.
S. Wiersema

14:40 Flowering, TPS quality, and economics of TPS production.
M. Upadhya

15:00 Coffee/tea

15:20 Studies on sexual reproduction in potato.
N. Pallais

16:00 Discussion - Future CIP research on TPS, agronomy, and utilization.

Tuesday, December 11

TPS IN NATIONAL PROGRAMS

Chairman, P. Accatino

08:30 Field experimentation with TPS in Bangladesh.
L. Sikka

09:00 Experimental and farmer use of TPS in Rwanda.
A. Haverkort

10:00 On-farm trials with TPS in Sri Lanka.
S.P.R. Weerasinghe

10:30 Coffee/tea

11:00 TPS in Mexico and Central America.
M. Villarreal

11:30 TPS research in Korea.
Il Gin Mok

12:00 Lunch

Tuesday afternoon

Chairman, P. Malagamba

14:00 Development of TPS research program in Egypt.
S. Sadik

14:30 Hybrid vs. Open pollinated
H. Kidane

15:00 Coffee/tea

15:30 Discussion - Future of production and use of TPS in developing countries.

Wednesday, December 12

RAPID MULTIPLICATION TECHNIQUES FOR POTATO PROPAGATION

Chairman, M. Upadhya

08:30 Various methods of rapid multiplication for seed programs.
J. Bryan

09:00 From test tube to transplants in Viet Nam.
Nguyen van Uyen

09:30 Rapid multiplication research in the Philippines.
P. Vander Zaag

10:00 Coffee/tea

10:30 Use of rapid multiplication techniques by the Peruvian National Program.
C. Vittorelli

11:00 Use of rapid multiplication techniques by the Korean National Programs.
Il Gin Mok

11:30 Discussion - Potential for on-farm use of rapid multiplication technology.

12:15 Lunch

Wednesday afternoon

TISSUE CULTURE

Chairman, S. Sadik

14:00 Tissue culture propagation of potatoes: advantages and disadvantages.
J.H. Dodds

14:30 Protoplast and somaclonal variation research on potato.
M.G.K. Jones

15:00 Anther culture and its uses in potato breeding.
A. Sonnino

15:30 Coffee

16:00 Discussion - Germplasm maintenance, in vitro variation stability/variability.

Thursday, December 13

A small Committee will formulate recommendations arising from discussions on TPS, tissue culture and rapid multiplication.

Friday, December 14

09:00 Final discussion and approval of recommendations during the day.

All participants.