COMPARISON OF CYTOPLASMIC MALE STERILITIES IN PROGENIES OF TUBEROSUM × ANDIGENA AND TUBEROSUM × NEO-TUBEROSUM CROSSES[1]

Francisco L. Vilaró[2], R.L. Plaisted[3], and R.W. Hoopes[4]

Abstract

The genetic base of the Tuberosum germplasm pool is believed to be quite narrow. To broaden it, the Cornell potato breeding program has been adapting to North American conditions a population based on Andigena introductions made in the 1960's. The adapted population has been termed 'Neo-Tuberosum'. When individuals from the early Andigena population were used as males in crosses to Tuberosum, most of the offspring were male sterile. This did not occur in the reciprocals. In the transformation of this population from Andigena to Neo-Tuberosum, significant unexpected changes occurred in its ability to induce this cytoplasmic male sterility (CMS).

Various Tuberosum x Neo-Tuberosum progenies had much greater male fertility than Tuberosum x Andigena progenies. CMS effects were absent in some progenies. This may have resulted from improved expression of restorer genes that counteract the CMS effects. There may be very few restorer genes, or only one acting in a dominant way, but they appear to need an appropriate genetic background, additively controlled, to be expressed.

This increase in reproductive ability of Tuberosum-Neo-Tuberosum progenies over Tuberosum-Andigena progenies occurred at a fairly constant rate with each cycle of selection for tuberization under long days. This would indicate pleiotropic or linkage effects among genes controlling tuberization and modifier genes for fertility restoration. High levels of male fertility within those hybrid progenies, similar to those expected with intra-Tuberosum crosses were attained by the 5th cycle of selection. Approximately at this generation, tuberization ability and tuber appearance of this Neo-Tuberosum population resembled Tuberosum more than Andigena.

High levels of fertility and flowering ability occur in the Neo-Tuberosum population itself, evidence that an enhancement of tuberization does not impair reproductive ability. By selecting appropriate Neo-Tuberosum clones,

[1]Paper No. 748. Dept. of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853. This research was supported by a contract between Cornell University and the International Potato Center, Lima, Peru.

[2]Estacion Experimental Granjera "Las Brujas", CIAAB. CC 33085, Las Piedras, Canelones, Uruguay.

[3]Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853.

4 Frito-Lay, Inc. Rhinelander, WI.

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hybrids with selected Tuberosum clones and cultivars could be made at the 4x level and haploids could be derived to make crosses at the 2x level. Progenies from these hybrids have the advantage of the broader genetic base of the Neo-Tuberosum population while avoiding most of the unwanted CMS effects.

Compendio

Durante el desarrollo de dos poblaciones de Neo-Tuberosum en Cornell, a partir de varias introducciones de Andigena, cambios significativos fueron puestos de manifiesto en la población mejorada, en factores que inducen macho esterilidad citoplásmica. Varias progenies a partir de cruces de Neo-Tuberosum sobre Tuberosum mostraron niveles de fertilidad masculina varias veces más grande que cuando las introducciones originales de Andigena fueron usadas como padres polinizadores. Además, el grado de floración fue correspondientemente incrementado. Se pudo demostrar que seleccionando clones particulares de Neo-Tuberosum a través de tests de progenie, los efectos de la macho esterilidad citoplásmica pueden ser completamente anulados en híbridos interespecíficos con Tuberosum. Una explicación probable para este incremento en los niveles de fertilidad masculina en las progenies de Neo-Tuberosum puede ser la expresión mejorada de genes restauradores de la fertilidad los cuales contrarrestan los efectos de macho esterilidad citoplásmica. Estos genes restauradores pueden ser muy pocos, tal vez sólo uno actuando en forma dominante. Sin embargo, para ser expresados estos genes parecen requerir un background genético apropiado el cual es controlado aditivamente.

Este incremento en la habilidad reproductiva de las progenies Tuberosum-Neo-Tuberosum sobre las progenies Tuberosum-Andigena ocurrió a una tasa bastante constante con cada ciclo de selección para tuberización para días largos. Esto indicaría efectos pleiotrópicos o de ligamiento entre genes que controlan la tuberización y genes modificadores para restaurar la fertilidad. Altos niveles de fertilidad masculina dentro de estas progenies híbridas, similar a aquellas esperadas con cruces intra-Tuberosum, fueron alcanzados por el quinto ciclo de selección para tuberización de la población de Neo-Tuberosum. Aproximadamente en esta generación, la habilidad de tuberización y la apariencia de los tubérculos de esta población de Neo-Tuberosum semejaban más a Tuberosum que a Andigena.

Los altos niveles de fertilidad y de habilidad de floración en la población de Neo-Tuberosum y en sus progenies híbridas, dio evidencia de que un mejoramiento en la tuberización no perjudica la capacidad reproductiva. A través de seleccionar clones Neo-Tuberosum apropiados, se podrían producir híbridos al nivel 2x y 4x con clones y cultivares Tuberosum seleccionados. Las progenies de estos híbridos tendrían la ventaja de la base genética más amplia y al mismo tiempo evitando la mayoría de los efectos no deseados de esterilidad citoplásmica.

Introduction

The main difference between the Tuberosum and Andigena Groups of *Solanum tuberosum* is their photoperiod reaction. Tuberosum has been selected for a longer critical photoperiod than Andigena. It is a common experience that when Andigena clones are used as males to pollinate Tuberosum clones, most of the resulting hybrids are male sterile (3, 8). Reciprocal crosses do not show this response; thus cytoplasmic male sterility (CMS) has been given as the cause. This creates a partial sterility barrier against the interchange of genes between these groups. CMS has also hindered the utilization of Tuberosum as female parent in various interspecific crosses at both the 4x level and 2x levels (2, 4). This situation is important because many valuable Tuberosum clones have lower male fertility.

The presence of dominant restorer genes capable of reverting CMS in Group Tuberosum was reported by Peloquin and Iwanaga (10). Hanneman and Peloquin (7) elaborated more on this hypothesis to account for the CMS differences found among the progenies of different cultivars when pollinated by Phureja-haploid hybrids. This hypothesis might also explain the striking fertility differences found by Liberal (9) when comparing families involving USW-3 and USW-4 Tuberosum haploids pollinated by several diploid species.

Restorer genes have been found in other potato species. Two dominant epistatic restorer genes were reported by Grun and Aubertin (5) in *S. chacoense.* Rose (13) reported the occurrence of restorer genes in *S. stoloniferum.* F.L. Haynes (personal communication) has noticed a marked increase in male fertility in Tuberosum x Phureja/Stenotomum progenies if the Phureja/Stenotomum parents have been selected for early tuberization.

This investigation compared the intensity and frequency of the CMS phenomenon when the same Tuberosum females were crossed to Neo-Tuberosum clones from the Cornell Potato Breeding Program or the original Andigena introductions. 'Neo-Tuberosum' refers to Andigena potatoes selected through several cycles of recurrent selection for tuberization under long day conditions. In the process of adapting Andigena for use in the Cornell program, no selection for fertility in hybrid progenies was made. An improvement in the male fertility of Tuberosum x Neo-Tuberosum progenies as compared to Tuberosum x Andigena progenies could have practical applications as well as provide a better understanding of the evolutionary processes that may have taken place when Tuberosum evolved from Andigena.

Materials and Methods

Experimental Trials

In 1984, an observational study was performed of tuber progenies from 14 families, each represented by 25 flowering plants. These crosses were obtained from 7 Tuberosum cultivars pollinated by two different groups of male parents. One of the groups comprised two Tuberosum cultivars (NY 66 and T 73-29) and the other group consisted of bulk pollen of a Neo-Tuberosum population from the 5th cycle of selection (11). The majority of the Andigena introductions that gave rise to this Neo-Tuberosum population were obtained from N.W. Simmonds (14) in 1964 and 1966.

A field experiment conducted during 1985 compared the fertility of seedling progenies involving two Tuberosum females (Hudson and NY62) pollinated with bulk pullen from plants from each of five cycles of a Neo-Tuberosum population. These populations represented from zero to six cycles of recurrent selection for improved tuberization. Fifty seedlings per progeny per selection cycle were used. Data were taken only on plants that started to flower from the middle of July through the first week of August. Observations were made between day break and mid morning. No pollen samples were collected on hot, dry days. Berry formation was measured in late August.

During the winter of 1984/85 nine Andigena and seventeen Neo-Tuberosum clones derived by maternal line recurrent selection were used for controlled pollinations of the Tuberosum cultivars Hudson and Katahdin. These seventeen Neo-Tuberosum clones, each from a different maternal line family, were selected in the field in 1984 on the basis of being most tuberosum-like in vine and tuber appearance. These Neo-Tuberosum clones came from a broader base of Andigena accessions that included introductions from South America, the IR-1 collection at Sturgeon Bay, Wisconsin, the Commonwealth Potato Collection in the UK, as well as the Simmonds source. The countries of origin included Argentina, Bolivia, Chile, Ecuador, Mexico, and Peru.

The nine Andigena plants were obtained by sowing remnant seeds from 1971, the first cycle of selection and 1974, the second cycle of selection. Four plants per original Andigena accession were transplanted to 8 inch pots at the two leaf stage. When possible, bulk pollen was collected from the group of four plants to pollinate Hudson and Katahdin. Because of some failures in emergence and sterility problems with the Andigena plants, only nine of the intended crosses were successful. For this trial, three replications of twenty plants each were placed in the field in a completely randomized design. The data were analyzed as a 2x2 unbalanced factorial.

In 1986, a Tuberosum clone, NY 76, was used for a test-cross. At harvest of the factorial comparison, tubers from a random sample of approximately 100 F1 clones from six Tuberosum x Neo-Tuberosum hybrid progenies were selected. Those progenies represented four Neo-Tuberosum clones that had produced seedling families with the largest percentage of male fertile plants in the pollen stainability test in the previous factorial comparison. The hybrid progenies were: Hudson x 5M1913-7, Hudson x

5M1371-1, Katahdin x 5M1371-1, Hudson x 5M1168-2, Hudson x 5M1044-1 and Katahdin x 5M1044-1.

Emasculated flowers were pollinated in the greenhouse. No pollinations were made with hybrid progenies from the Neo-Tuberosum clone 5M1168-2 in 1986, because they flowered later than the tester clone. Fifty-seven progenies were obtained from the other three F1 hybrid families. Reciprocal crosses involving NY 76 as pollen parent onto various F1 clones were attempted but almost no crosses could be achieved due to the poor pollen quality of NY 76. Data were kept on the number of pollinations made by each of the F1 clones onto NY 76. These data were used for testing the accuracy of pollen stainability in predicting fertility.

Controls were produced by using bulk pollen from Andigena clones representing the original introductions on the same female tester. Other controls included four Neo-Tuberosum clones (5M619-1, 5M738-3, 5M1097-2, and 5M1140-3) that had produced a large proportion of sterile plants in the factorial experiment.

Experimental Observations

Observations were recorded from individual plants growing in the field from flowering through berry formation. Average progeny values were obtained for analysis of the data. Pollen quality for the observational study was assessed by staining pollen grains with acetocarmine stain solution. The differential pollen staining solution proposed by Alexander (1) was used in the 1985 and 1986 trials. This test allows an accurate and easy determination of viability. With this stain, aborted pollen grains are stained green, whereas non-aborted pollen are stained red. For all the stainability determinations, only pollen from freely dehiscent anthers was used. Approximately 200 pollen grains were counted per sample and 5% was used as the fertility threshold for both types of stain. Some red-staining pollen grains were shrivelled or otherwise not round in form. These were not considered viable.

Flower abnormalities followed Grun's (4) descriptions and included anthers reduced in size, greenish, or having various deformations in shape. These abnormalities are thought to be caused by disturbances during the pollen development process. Other morphological abnormalities recorded were anthers fused to styles, added vestigial or whole pistils, and ventral styled anthers. The latter abnormalities do not necessarily result in male sterility but provide evidence of gene-cytoplasm interactions (4). Other morphological observations were scored according to subjective scales and included the following: amount of pollen shed, berry set, flower abnormalities and extent of flowering. The first three traits were given a 1 to 3 scale and the latter a 1 to 5 scale (see footnotes to Tables 2 and 3). In addition, anther color was classed in three categories: green, yellow and orange.

Results and Discussion

Male Fertility Effects

Results of the 1984 experiment are presented in Tables 1 and 2. Male fertility levels of progenies from bulked pollen of clones from the fifth cycle of selection were studied. Bulk pollen from this population was used to pollinate Tuberosum clones and the percentage of male fertile plants (more than 5% stainable pollen) in the progenies was found to be about 40%. The percentage of plants from those progenies that shed some pollen was around 80% (Table 1). Flower abnormalities were quite infrequent in those progenies, none having any significant differences from those found in intra-Tuberosum progenies (Table 2).

Progenies of Andigena were not available as control comparisons. However, it has been reported that Andigena possesses several sterility inducing factors, mostly dominant genes, that interact with Tuberosum cytoplasm in hybrid progenies (3,8). Male fertility values observed in Tuberosum-Andigena hybrids most commonly are below 10%. The factorial experiment in 1985 gave further evidence of this. Thus the behavior of Neo-Tuberosum progenies was more like that of Tuberosum progenies than that expected of Andigena progenies.

In the evolutionary study conducted in 1985, an increase was found in the fertility of progenies involving two Tuberosum females with bulk pollen

TABLE 1. — *Paired comparisons for percentage of plants shedding pollen*[1] *and percentage of pollen fertile plants*[2]. *Tuberosum (Tbr) pollinated by Tuberosum and by Neo-Tuberosum (NeoTbr). 1984.*

	Pollen Shedding Plants			Pollen Fertile Plants		
	Males			Males		
Tuberosum Female	Tbr %	NeoTbr %	Difference %	Tbr %	NeoTbr %	Difference %
NY 59	92	84	8	40	28	12
NY 66	100	70	30	72	30	42
NY 67	92	88	4	64	50	14
NY 68	83	70	13	54	35	19
NY 69	96	67	29	80	14	66
NY 70	92	79	13	88	79	9
T 73-29	100	86	14	95	43	52
X	93.6	77.7	15.9	70.4	39.9	29.3
t			2.59*			3.57**

[1]Proportion of pollen shedding plants to number of flowering plants × 100.
[2]Proportion of pollen fertile plants to number of flowering plants × 100.
Plants with over 5% round, stainable pollen considered pollen fertile.
Angular transformation performed for analysis.
*Significant at 5% level.
**Significant at 1% level.

TABLE 2. — *Paired comparisons for berry formation, pollen stainability, and flower abnormalities. Tuberosum pollinated by Tuberosum (Tbr) and by Neo-Tuberosum (NeoTbr), 1984.*

Tuberosum Female	Tbr	Berries[1] (1-3) NeoTbr	Diff.	Tbr	Pollen Stainability[2] (0-3) NeoTbr	Diff.	Tbr	Deformity[3] (1-3) NeoTbr	Diff.
NY59	1.36	1.40	−.04	.91	.57	.34	2.96	2.80	.16
NY66	2.24	1.60	.64	1.61	.94	.67	2.76	2.56	.20
NY67	1.68	1.38	.30	1.22	1.43	−.21	2.80	2.62	.18
NY68	1.76	1.56	.20	1.15	1.31	−.16	2.56	2.56	.00
NY69	2.00	1.20	.80	1.74	.40	1.34	2.88	2.87	.01
NY70	2.20	1.57	.63	2.35	1.91	.44	2.84	3.00	−.16
T 73-29	2.60	1.53	1.07	2.7	.82	1.88	3.00	3.00	.00
X	1.98	1.46	.51	1.67	1.05	.61	2.83	2.78	.06
t			3.6**			2.12			1.20
C1[4].			.35			.71			.12

[1]1-3 scale 1:no berries, 3:many berries formed
[2]0-3 scale 0:0-5%, 1:5-20%, 2:20-50%, 3:>50% pollen grains stained
[3]1-3 scale 1:deformed flowers, green anthers, 3:normal appearing
[4]Confidence interval at 5% significant level
**Significant at 1% level

from the Neo-Tuberosum population at different stages of selection (Figure 1). Similarly, a significant linear relationship ($r=0.87$, $P<0.05$) was found between the male fertility values of these progenies and the data of Rasco *et al.* (12) on early tuber yield from seedling plants of this Neo-Tuberosum population at the same stages of selection. Once again, the average fertility value over years and females was almost 40%. The highest male fertility values for the most advanced selection cycles crossed to NY 62 ranged around 65%. By contrast, the fertility value for the progenies from Neo-Tuberosum of earlier cycles crossed onto NY 62 was 20%.

Other related male fertility indicators such as pollen shedding, anther color, and freedom from flower abnormalities were also improved (Tables 1 and 2). In this experiment and in the observational study, the genetic constitution of the female parent had some importance in determining the actual fertility outcome of a particular cross.

In the 1985 factorial experiment the fertility levels of progenies from Andigena introductions and a second population of Neo-Tuberosum were compared when crossed to two Tuberosum females. Large differences were found in all male fertility traits. No female and no interaction effects were found to be statistically significant for male fertility related traits. These differences favored the Neo-Tuberosum progenies over Andigena progenies (Table 3). Male fertility levels of progenies originating from Neo-Tuberosum averaged 20%, almost three times as much as progenies originating from

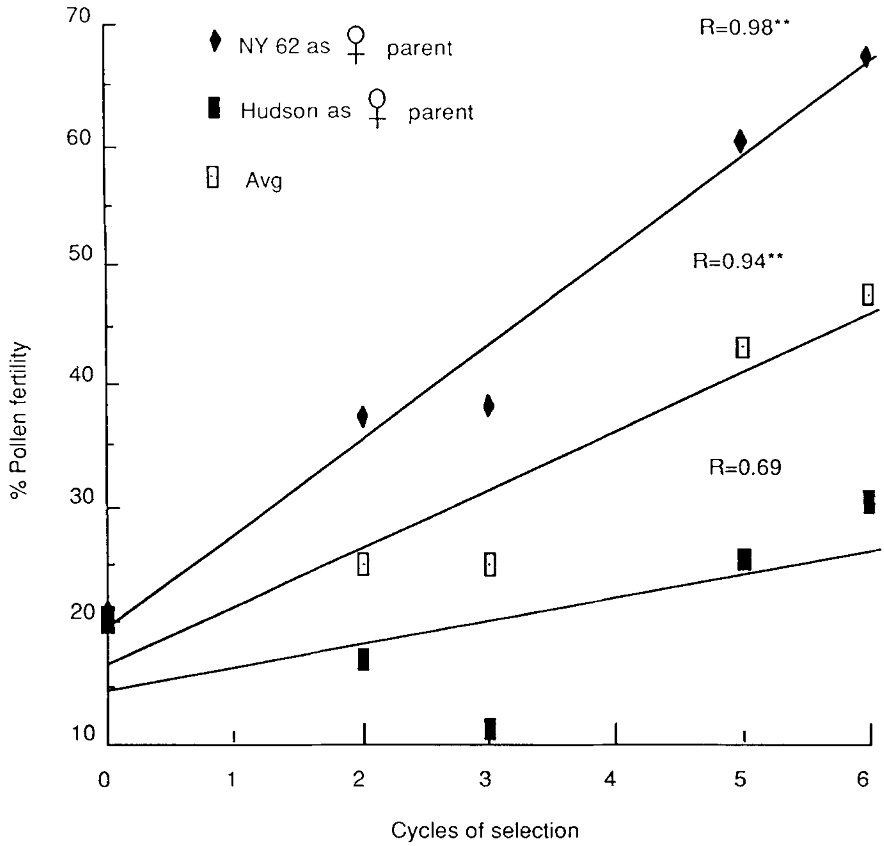

FIG. 1. Linear regression. Percentage of pollen fertile plants in progenies (Tbr × NeoTbr) at 5 stages of selection, 1985.

Andigena. This difference was largely produced by progenies from four of the Neo-Tuberosum clones (5M1044-1, 5M1168-2, 5M1371-1, and 5M1913-7). When averaged over the two Tuberosum females, the progenies of these four clones had more than 50% of pollen fertile plants, reaching in some instances 70-80% (Figure 2). These values are comparable to fertility levels among fertile Tuberosum clones. Morphological traits were very much in agreement with pollen stainability determinations. Controlled crosses in the greenhouse during 1986, of a randomly selected sample of F1 hybrid clones from this experiment rated as pollen fertile by the stainability test, onto one Tuberosum female, confirmed the stainability test results.

Figure 3 illustrates the situation for those single clones that belonged to families that were represented both in the Andigena and in the second

TABLE 3. — *Neo-Tuberosum and Andigena main effects for anther color, pollen shedding, berry formation, and percentage of pollen fertile plants. Tuberosum × Andigena and Tuberosum × Neo-Tuberosum (NeoTbr) crosses. Reduced model, 1985.*

Male Population	Anther[1]	Pollen[2]	Berries[3]	Fertility[4] (%)[6]	Fertility[5] (%)[6]
NeoTbr	2.28	1.38	1.19	19.48	21.74
Andigena	1.90	1.15	1.03	7.36	10.39
Difference	.382	.226	.159	12.12	11.36
t	5.01**	3.3**	2.7**	3.21**	2.65**

[1]1-3 scale 1:green anthers, 2:yellow anthers, 3:orange anthers
[2]1-3 scale 1:no pollen shed, 3:much pollen shed
[3]1-3 scale 1:no berries, 3:many berries formed
[4]Proportion of pollen fertile plants over total number of plants
[5]Proportion of pollen fertile plants over number of flowering plants
[6]Angular transformation performed for analysis
** Significant at 1% level

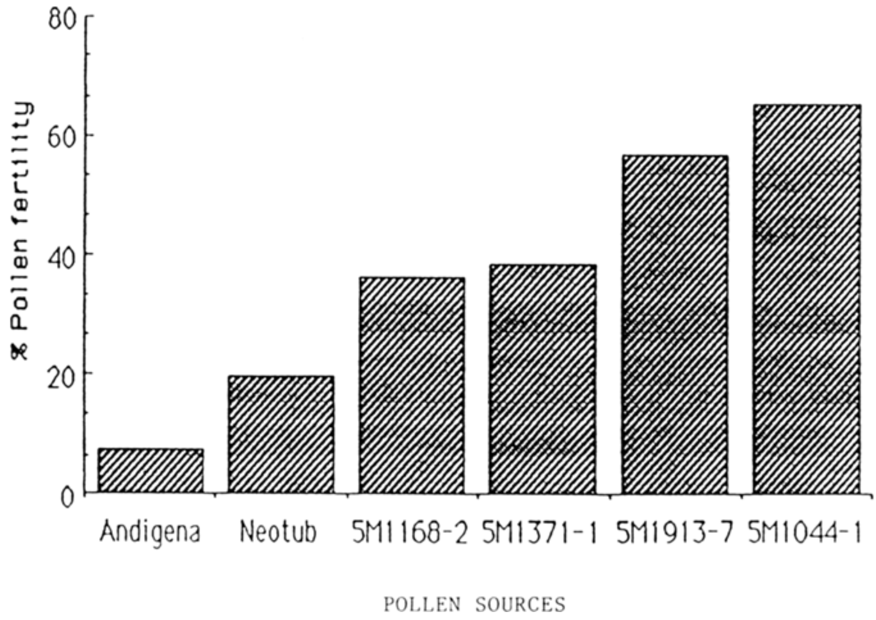

FIG. 2. Percentage of pollen fertile plants in progenies of 2 Tbr females, 9 Adg families, 17 NeoTbr clones and 4 selected NeoTbr clones, 1985.

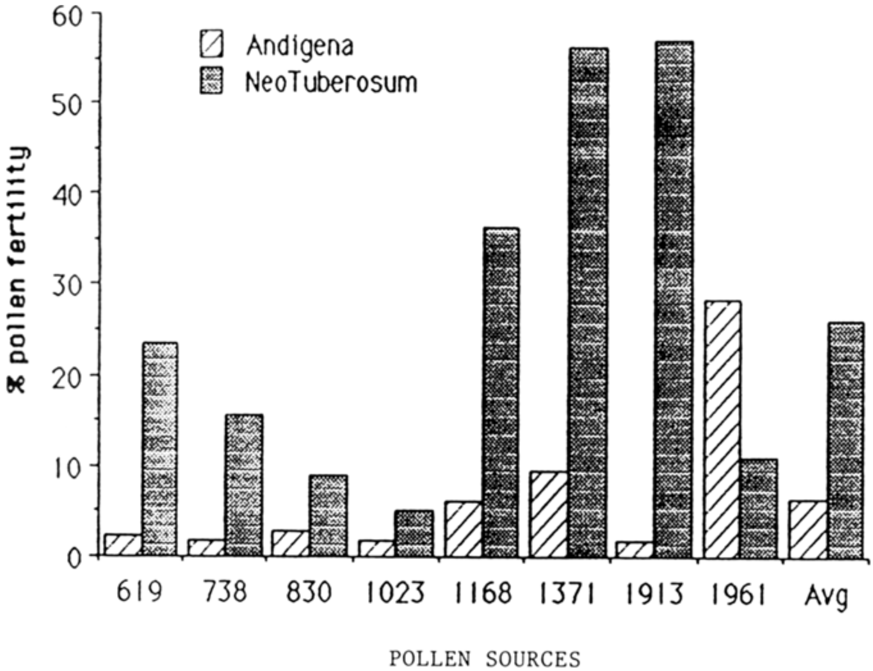

FIG. 3. Percentage of pollen fertile plants in hybrids between 8 Adg accessions on the fifth cycle selections of NeoTbr from them and Tbr used as the female, 1985.

Neo-Tuberosum populations. Seven out of eight of those clones experienced a quite remarkable increase in pollen fertility. For this particular set of clones, the Andigena progenies had an average fertility level of 6.8% and the Neo-Tuberosum progenies had an average level of 26.2%.

It can be concluded that the Cornell Neo-Tuberosum populations had experienced some changes in their genetic constitution affecting male fertility in hybrid progenies obtained with Tuberosum females. These changes had not been purposefully intended because the only applied selection pressure was for improved tuberization under long days and disease resistance. In addition, unconscious selection most likely favored genotypes with higher reproductive ability within the Andigena-Neo-Tuberosum populations.

Unexpectedly high values of male fertility were found in progenies from the test-cross in 1986. No clear segregation pattern could be shown for any of the traits observed. Most likely NY 76 was not susceptible to male sterility inducing factors from the male parent. This may support the hypothesis that minor genes in addition to major genes are involved in fertility restoration.

The question still remains why those restorer genes which supposedly were present in the Andigena population collected at Cornell were not expressed at the beginning of the selection program. Why only after some cycles of selection were those restorer genes more strongly expressed? One possible explanation for this is that during the adaptation process, essential modifier genes may have been increased in their frequency. These modifier genes were affected by pleiotropism or linkage with genes for adaptation to long days. These genes were supposedly affected by the recurrent selection program that developed the Neo-Tuberosum populations.

When efforts are made to widen the genetic base of Tuberosum potatoes, the Neo-Tuberosum population offers substantial possibilities for almost completely overcoming male sterilities due to CMS. These sterilities routinely occur when Andigena clones are used as pollinators onto Tuberosum. To alleviate undesirable CMS effects, the use of Neo-Tuberosum clones selected for producing male fertile progenies in hybrid crosses would be much faster and efficient than the cytoplasm substitution proposed by Grun and Staub (6). When breeding is performed at the 2x level this is further reinforced because of the much larger number of Tuberosum male sterile clones usually obtained. A Neo-Tuberosum population improved for fertility restoration would be a very valuable source for extracting haploids for breeding at that level.

Flowering Effects

Flowering ability changes expressed as percentage of flowering plants were shown in the evolutionary study. In this study, the degree of flowering was increased from 75% in the base population to roughly 100% in the 5th generation of selection cycle ($r=.82 P<0.05$). Moreover, progenies from the selection cycles and from clones with the highest male fertility levels showed the highest percentage of flowering plants and the largest scores for extent of flowering. In 1986, test-cross F1 hybrid progenies from clones with highest pollen fertility levels also showed the highest percentage of flowering plants and with the largest extent of flowering.

The genetic system controlling the photoperiod effect on tuberization and the system responsible for reproductive ability may be quite related. Some evidence in this direction was given by Turner (15). She proved that environmental conditions conducive to lower levels of assimilates in the plant reduced both the degree of flowering and the tuber dry weight.

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