

A. M. Golmirzaie · K. Bretschneider · R. Ortiz

**Inbreeding and true seed in tetrasomic potato.****II. Selfing and sib-mating in heterogeneous hybrid populations of *Solanum tuberosum***

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**Abstract** Inbreeding depression may affect the performance of consecutive generations of potatoes propagated by true potato seed (TPS). The effect of inbreeding was established using selfed and sib-mated generations of five TPS families. Correlation coefficients were calculated between the level of inbreeding and different traits. Inbreeding depression was expressed mainly by pollen viability, as measured by its stainability ( $r = -0.912$ ,  $P < 0.01$ ), and tuber yield ( $r = -0.837$ ,  $0.01 < P < 0.05$ ). The results also indicated that without unavoidable selection inbreeding depression is expected to be more evident. Furthermore, the TPS families responded quite differently to inbreeding depression. They did not show the same amount of depression for yield as they did for the characters concerning fertility. The high tuber-yielding families displayed a greater inbreeding depression for tuber yield than the lower-yielding families.

**Key words** Hybrid vigor · True potato seed**Introduction**

The use of true potato seed (TPS) is an innovative and inexpensive method of potato production for the developing world. The propagation of potatoes by TPS, however, raises important questions that conventional potato breeders have not addressed (Ortiz 1997). For

example, inbreeding, a natural event in many crops, is an aspect one should consider when producing TPS.

Inbreeding depression as a consequence of homozygosity could influence the performance of the next generation in sexually propagated polysomic polyploids (Panella and Lorenzetti 1996; Levings et al. 1967; Dessureaux and Gallais 1969; Abdalla 1970; Hecker 1972). Moreover, the performance of open-pollinated TPS (produced mainly by selfing or sib-mating) will depend on the amount of outcrossing and the effect of inbreeding on each specific trait.

Although inbreeding studies in potato have been done by using elite breeding lines or cultivars as original parental materials, they did not continue beyond the  $S_2$  generation. We also lack extensive information about the effect of inbreeding on the reproductive characters of potato (Atlin 1985). Therefore, the present study was conducted to determine the effect of inbreeding in advanced selfed and sib-mated generations of TPS progenies.

**Materials and methods**

True potato seeds from five families of different genetic backgrounds (Table 1) were planted and their seedlings were selfed and sib-mated to obtain generations with different levels of inbreeding. Single plants from the non-inbred and highly heterozygous parental generation ( $S_0$ ) were selfed in order to obtain the  $S_1$  generation. The  $S_2$  resulted from selfing  $S_1$  plants, whereas the  $S_3$  was produced by selfing  $S_2$  plants. The  $sib_1$  generation was produced by pollinating  $S_0$  plants with bulked pollen of the same generation. Similarly,  $sib_2$  and  $sib_3$  generations were derived from bulk crosses within  $sib_1$  and  $sib_2$ , respectively.

An experiment including seven generations ( $S_0$ ,  $S_1$ ,  $S_2$ ,  $S_3$ ,  $sib_1$ ,  $sib_2$ , and  $sib_3$ ) of the five families, which were selected because of their adaptation to the mid-elevation tropics, was performed at the Andean highland station of the International Potato Center at Huancayo during 1985–1986 at 3200 miles above sea level. The experiment was carried out in this cool location under irrigated conditions in order to avoid losses and suppression of traits caused by high temperatures or drought.

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A. M. Golmirzaie · K. Bretschneider  
International Potato Center (CIP),  
Apartado Postal 1558, Lima 100, PerúR. Ortiz (✉)  
Royal Veterinary and Agricultural University, Thorvaldsensvej 40,  
DK 1871 Frederiksberg C, Copenhagen, Denmark

**Table 1** Parentage of TPS families. dms = *Solanum demissum*, tbr = *Solanum tuberosum* Gp. *Tuberosum*, adg = *S. tuberosum* Gp. *Andigena*, phu = *S. tuberosum* Gp. *Phureja*, chc = *Solanum chacoense*, neotbr = *neotuberosum*. (Gp. *Andigena* adapted to long days)

Family	Male parent	Female parent
375512.23 × 81M12.6	(dms × tbr) × adg	phu × (tbr × chc)
7.20PVX × DTO28	adg	tbr × (phu × tbr)
377895.12 × 377904.10	neotbr × tbr	neotbr × (tbr × phu)
103.12SRLL × 377904.10	unknown	neotbr × (tbr × phu)
377895.12 × 377904.10	neotbr × adg	neotbr × (tbr × phu)

Seeds of the crosses belonging to one generation of a family were mixed and sown in the greenhouse nursery. After 8 weeks the seedlings were transplanted to the field. The experimental field layout was a randomized complete block design with three replications. The treatments were arranged following a split-plot design. The main plot was the family and the subplot was the inbreeding generation. Each plot consisted of 30 plants. The distance between rows was 90 cm, with 30 cm between plants within the same row.

The following traits were evaluated: seed germination, survival 52 days after transplanting and at harvest, plant vigor, uniformity and shape of the vine, number of flowers, pollen germination and stainability, vine earliness, number and weight of berries, seeds per ten berries, number of marketable and unmarketable tubers, and marketable and unmarketable tuber weight. Pollen grains were stained with acetocarmine glycerol jelly (Marks 1954) to determine their viability.

A factorial analysis for split-plot designs was performed to evaluate this experiment. A least-significant difference test at the 0.05 probability level was used to compare family means and the family × inbreeding generation. Correlation and regression analyses were carried out to study the inbreeding effect by regressing the trait performance (dependent variable Y) on the level of inbreeding (independent variable X).

## Results

Significant differences between families ( $P < 0.05$ ) were detected in the analysis of variance (ANOVA) for plant vigor, vine shape, number of flowers, pollen germination and viability, vine earliness, number and weight of berries, survival at harvest, and number of marketable weight of tubers. Similarly, the generations of inbreeding were statistically different ( $P < 0.05$ ) according to the ANOVA for plant vigor, vine shape, number of flowers, pollen germination and viability, number and

weight of berries, number of seeds per ten berries, and the number and weight of tubers. The interaction of the family × inbreeding generation was significant ( $P < 0.05$ ) in the ANOVA for the number of flowers, pollen germination, the number and weight of berries, and the number and weight of tubers.

Table 2 indicates the inbreeding effect on a set of reproductive and agronomic traits, expressed as a percentage of the  $S_0$  generation. Table 3 shows the correlation coefficients between the level of inbreeding and trait performance. A significant inbreeding depression (negative coefficient) was detected for pollen viability and tuber yield across the seven generations. Inbreeding depression was detected only in the selfed generations for the number of tubers and plant survival, whereas this occurred for pollen viability in the sib-mated generations.

Inbreeding did not affect the traits in the same way in the different families (Table 4). The  $4x - 2x$  family, 375512.23 × 81M12.6, was affected by inbreeding depression only in respect of pollen viability, whereas the  $4x - 4x$  family, derived from a cross between two unrelated clones 103.12 SRLL × 377904.10, was not affected by inbreeding depression in any trait. Furthermore, the families did not show the same amount of inbreeding depression for tuber yield as they did for pollen viability. The higher tuber-yielding families also displayed a greater inbreeding depression in the first selfed generation ( $S_1$ ) for tuber yield than did the lower-yielding families (Table 5).

The amount of inbreeding depression also varied between the measured characters. For example, the  $S_3$  of the cross 375512.33 × 81M12.6 had only 82.6% as much tuber weight as the  $S_0$ , whereas the  $S_3$  of 377896.12 × 377904.10, 103.12SRLL × 377904.10, and 377895.12 × 377904.10 yielded 70.3%, 43.3%, and 50% of their respective  $S_0$ .

## Discussion

Inbreeding depression is a common response to selfing in many naturally outbreeding species, mainly because of increased homozygosity. The results obtained for pollen viability and yield in the present experiment

**Table 2** Inbreeding effect, as expressed in terms of the relative percentage of the hybrid generation compared to the  $S_0$ , on reproductive and agronomic traits

Generation	Pollen viability (%)	No. of seeds (%)	No. of seeds berry <sup>-1</sup> (%)	No. of tubers (%)	Plant survival (%)	Yield (%)
$S_0$	100.0	100.0	100.0	100.0	100.0	100.0
$S_1$	66.8	49.0	86.5	90.5	98.6	80.2
$S_2$	52.4	35.5	47.6	83.6	98.3	55.3
$S_3$	55.7	29.9	82.0	82.2	97.6	63.4
Sib <sub>1</sub>	87.5	75.6	113.7	85.1	97.7	74.4
Sib <sub>2</sub>	70.5	81.4	88.5	88.7	97.1	69.5
Sib <sub>3</sub>	66.2	104.6	95.9	90.5	98.0	61.8

**Table 3** Coefficients of correlation ( $r$ ) and determination ( $R^2$ ) of the inbreeding coefficient for the reproductive and agronomic traits

Trait	Seven generations		$S_0$ to $S_3$		Sib <sub>1</sub> to Sib <sub>3</sub>	
	$r^a$	$R^{2b}$	$r$	$R^2$	$r$	$R^2$
Pollen viability	-0.912**	0.832	-0.911	0.829	-0.969	0.939
No. of berries	-0.554	0.307	-0.935	0.874	-0.309	0.290
No. of seeds berry <sup>-1</sup>	0.554	0.307	-0.583	0.339	-0.423	0.179
No. of tubers	-0.695	0.483	-0.977*	0.955	-0.418	0.175
Yield	-0.837*	0.701	-0.908	0.825	-0.888	0.789
Plant survival	-0.514	0.264	-0.976*	0.953	-0.567	0.322

\* and \*\* indicate significance at the 0.05 and 0.01 levels of probability

<sup>a</sup> $r$  = correlation coefficient

<sup>b</sup> $R^2$  = coefficient of determination

**Table 4** Inbreeding depression for five traits in five families as indicated by the significant regression coefficient

	Flowers plant <sup>-1</sup>	Pollen Germin-ability	Pollen viability	Berries plant <sup>-1</sup>	Seeds berry <sup>-1</sup>	Berry weight	Tubers plant <sup>-1</sup>	Plant survival	Yield
377512.23 × 81M12.6			*						
7.20PVX × DTO-28			*	*					*
377895.12 × 377904.10		*	**	*	*	*		**	
103.12SRLL × 377904.10									
377896.12 × 377904.10			*						
Effect of inbreeding (%)	0	20	80	40	20	20	0	20	20

\* and \*\* indicate significance at the 0.05 and 0.01 levels of probability

**Table 5** Inbreeding depression for tuber yield (kg per row of 30 plants) in the first selfed and sib-mated generations

Family	$S_0$	$S_1$	Sib <sub>1</sub>
103.12SRLL × 377904.10	22.827 (100%)	13.867 (61%)	14.370 (69%)
377895.12 × 377904.10	22.947 (100%)	10.750 (47%)	15.437 (69%)
377512.23 × 81M12.6	17.820 (100%)	16.957 (95%)	10.403 (58%)
377896.12 × 377904.10	15.490 (100%)	16.237 (105%)	12.633 (82%)
7.20PVX × DTO-28	14.677 (100%)	10.407 (71%)	9.083 (62%)

agree with this statement. Krantz and Hutchins (1929) and Mullin and Lauer (1966) also reported inbreeding depression for tuber yield in tetraploid potatoes, while Atlin (1985) and Iwanaga and Atlin (1986) reported a strong inbreeding effect on male fertility in the  $S_1$  and  $S_2$  generations. Therefore, both tuber yield and pollen viability are significantly affected by an increased level of homozygosity or the loss of heterozygosity.

#### Inbreeding effect on fertility and seed production

Dessureaux and Gallais (1969) found that inbreeding depression also affected fertility in alfalfa, a tetrasomic

polyploid-like potato. They also suggested that unfavorable interactions between identical alleles should be considered to explain the decline in fertility.

Strong inbreeding often leads to the complete loss of fertility. This makes it difficult to maintain advanced inbred lines (Krantz 1946). However, as the results of the present experiment indicated, the highly inbred plants contribute little to the pool of male gametes in a mixed population. Moreover, Iwanaga and Atlin (1986) reported that progenies resulting from two generations of selfing produced only 22% as much viable pollen per flower as did the  $S_0$  plants. They tested the poor quality of this inbred-derived pollen with a controlled experiment. This may explain why advanced open-pollinated generations derived from TPS showed less yield reduction than expected because of the small contribution of the inbred plants to the next generation. This was confirmed by Arndt and Peloquin (1990) who used a marker gene (yellow tuber flesh) to demonstrate that the highly heterozygous plants had a higher fertility and were more attractive to bumblebees (natural pollinators of potato) than highly inbred plants. Furthermore, male gametophyte viability, positively correlated with open-pollinated berry set, may be enhanced by sporophytic heterozygosity in tetrasomic potato (Ortiz and Peloquin 1994).

The production of berries decreased drastically with subsequent inbreeding but not with sib-mating (Table 2). The reduction in berry set was probably not

caused by loss of pollen fertility alone, but also by a reduction in female fertility. However, seed set was not affected by inbreeding. This indicated that pollen quality was not important for seed set within the berry. This also explained why the correlation coefficient between pollen germination and seed set was low ( $r = 0.263$ ). Nevertheless, pollen fertility may affect seed set in plants with low pollen viability, such as in the  $S_2$ .

### TPS production

The performance of the  $S_3$  for pollen viability and tuber yield (Table 2) could be the result of unavoidable selection for fertility during the process of self- and sib-pollination. Because of their improved flowering and fertility, the most heterozygous plants in the  $S_2$  were probably used as parents of the next generation. Moreover, the sib-mated progenies showed less yield reduction than the selfed progenies, which indicates that sib-mating between relatives could be less harmful than selfing. This is very important for a TPS production strategy using open-pollinated (OP) progenies. OP TPS derived from male-fertile tetraploid clones are mainly the result of selfing, especially in isolated fields, whereas OP plants derived from TPS progenies are the result of selfing, sib-mating, and outcrossing. The proportions will depend on the activity of bumblebees and the rate of outcrossing. Therefore, OP seeds derived from TPS progenies (a kind of synthetic) should have higher yields than those derived from single cultivars. Therefore, we need to identify those OP progenies whose performance does not decline over successive generations of open pollination.

### Different response to inbreeding depression and the selection of improved parental lines

The TPS families responded quite differently to inbreeding. High tuber-yielding families displayed a greater inbreeding depression for tuber yield than the lower-yielding families (Table 5). This sharp yield reduction in the  $S_1$  could result from the loss of high-order interactions in tetra- and tri-allelic loci (Busbice and Wilsie 1966) in the high-yielding families.

The different response of the families may also be attributed to genetic differences among the original parental populations. Thus, the possibility of selecting for tetraploid TPS progenies that are more or less tolerant to inbreeding will exist. This type of variation has been observed and exploited in other tetrasomic species (Pfeiffer and Bingham 1983). This was possible because selection during the inbreeding process was based on an additive type of gene action. However, in potato a non-additive type of gene action seems to be

more important for tuber yield (Mendoza and Haynes 1974; Mendiburu and Peloquin 1977; Ortiz et al. 1997). This means that high-yielding progenies in potato are the result of maximizing heterozygosity (Mendiburu et al. 1974; Peloquin and Ortiz 1992). Therefore, the use of inbreeding and selection to obtain improved parental lines seems to be limited in potato breeding.

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