

Cryptic improvement for fertility by continuous selfing of diploid potatoes using *Sli* gene

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Summary

While a diploid potato was continuously selfed by a function of *Sli* gene, the level of heterozygosity reduced, and the fertility also reduced (inbreeding depression), which might be improved because continuous selfing could eliminate undesirable recessive alleles. To explore what was occurring as advancing self-generations in terms of fertility, 51 plants of an S₃ diploid potato family were evaluated for fertility-related traits and analyzed by RFLP markers. The level of heterozygosity was positively correlated with any of fertility-related traits ($r = 0.108 - 0.333$). At least six loci on six chromosome sections were associated with fertility-related quantitative trait loci (QTLs), of which three performed better in the heterozygotes and three in the homozygotes. By further continued selfing, QTLs performing better in heterozygotes were likely fixed to homozygotes with secondly better performance, and those performing better in homozygotes were fixed to the best genotypes. Thus, the selfed progenies were cryptically being improved for fertility by genetic fixation to the best or better genotypes in fertility-related QTLs.

Introduction

The inbreeding coefficient (F) measures the probable degree of heterozygosity and reduces at a rate of 50% in each self-fertilization (Allard, 1960). The derivation of F assumes no mutation, gene flow, or selection during inbreeding. However, gametic selection such as pollen competition, or zygotic selection such as full or partial lethality and sterility by recessive homozygotes, may occur, which can modify the genotypic frequencies, particularly in favor of the more heterozygous individuals (Brouwer & Osborn, 1997). Inbreeding often decreases vigor and fecundity (inbreeding depression). It is well known as classical examples that upon continuous selfing height of stalk, grain yields and plant height rapidly decreased in maize, and yields in alfalfa and onions (Allard, 1960; Jugenheimer, 1976).

Diploid potatoes and their related wild species (tuber-bearing *Solanum* species) are mostly outbreed-

ing by a gametophytic self-incompatibility system (Pushkarnath, 1942; Pandey, 1962; Cipar et al., 1964a). Controlled selfing had been impossible until recently, although self-compatible variants were occasionally found within self-incompatible species (Cipar et al., 1964b), and some of these variants were genetically investigated (Cipar, 1964; De Jong & Rowe, 1971; Olsder & Hermesen, 1976). Recently, we found a dominant gene, named *S*-locus inhibitor gene (*Sli*), which is sporophytically expressed to inhibit a function of *S*-allele in pollen, and alters self-incompatible plants to self-compatible ones (Hosaka & Hanneman, 1998a). Birhman & Hosaka (2000) introduced the *Sli* gene by crossing into cultivated diploid potatoes and confirmed its expression in the genetic background of cultivated diploid potatoes. This facilitates to obtain selfed progeny of diploid potatoes, and continuous selfing may produce highly homozygous lines, which are attractive in genetic analyses, genetic fixation of traits, production of TPS (true potato seed) varieties with

genetic uniformity, and heterosis breeding of the potato (Birhman & Hosaka, 2000).

Although diploid potatoes can be continuously selfed using the *Sli* gene, the actual approach to homozygosity during selfing is unknown and may differ from that predicted by the inbreeding coefficient (F). Phumichai et al. (2005) monitored the inbreeding process from a clone 97H32-6 to its S_4 progenies (inbred series A) and from a new hybrid clone (a cultivated diploid potato $\times$ 97H32-6) to its S_5 progenies (inbred series B) using restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers. The average heterozygosity levels reduced from 100% in S_0 to 10.7% in S_4 and to 8.6% in S_5 in the inbred series A and B, respectively, with slower reduction rates per generation (38.4 and 38.5%) than expected (50%). The slower decline of heterozygosity than expected has been reported by Brouwer & Osborn (1997) while inbreeding diploid alfalfa and by Scotti et al. (2000) while inbreeding tetraploid alfalfa. For the cause, unavoidable selection for vigor and fertility during selfing has been suggested (Brouwer & Osborn, 1997). Thus, we assumed that fertility-related traits were strongly correlated with heterozygosity and selection for fertility favored the more heterozygous plants (Phumichai et al., 2004). However, the S_4 progenies in the inbred series A were unintentionally derived from less heterozygous parents among the other S_3 plants. The other factors such as seed germination ability and vegetative vigor at an early growth stage might also had been involved in unintentional selection, resulting in more heterozygous plants that were actually used for measuring the heterozygosity (Phumichai et al., 2005). Since plants

used for evaluation of fertility-related traits and those for DNA analysis were always different in our past studies (Phumichai et al., 2004, 2005), the relationships between fertility or viability and heterozygosity were indirectly suggested.

In this study, 51 plants of the S_3 family were evaluated for both fertility and heterozygosity, and the prosperity or decline of segregating loci detected in the S_3 generation were investigated through the consecutive two selfing generations bearing on fertility and viability.

Materials and methods

Plant material

The initial diploid clone 97H32-6 was selfed by a function of the *Sli* gene originally derived from a self-compatible variant of *Solanum chacoense* Bitter (Phumichai et al., 2004). By three consecutive selfings, an S_3 family (2H22) was obtained, which were evaluated for fertility and heterozygosity. Each genotype was denoted by a hyphenated number with the family identity. Further two consecutive selfings generated seven S_5 families. Genetic fixation of segregating loci detected in the 2H22 family was analyzed for the most advanced progenies that contained more than ten plants (four S_5 families and one S_4 family) (Figure 1).

Evaluation for fertility

Fifty-one plants of the 2H22 family were grown through three seasons (once as seedlings and twice

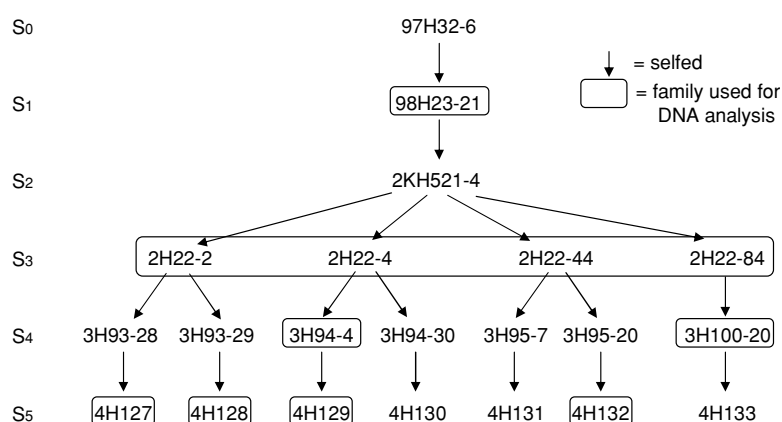


Figure 1. Pedigrees of the inbred families used in this study.

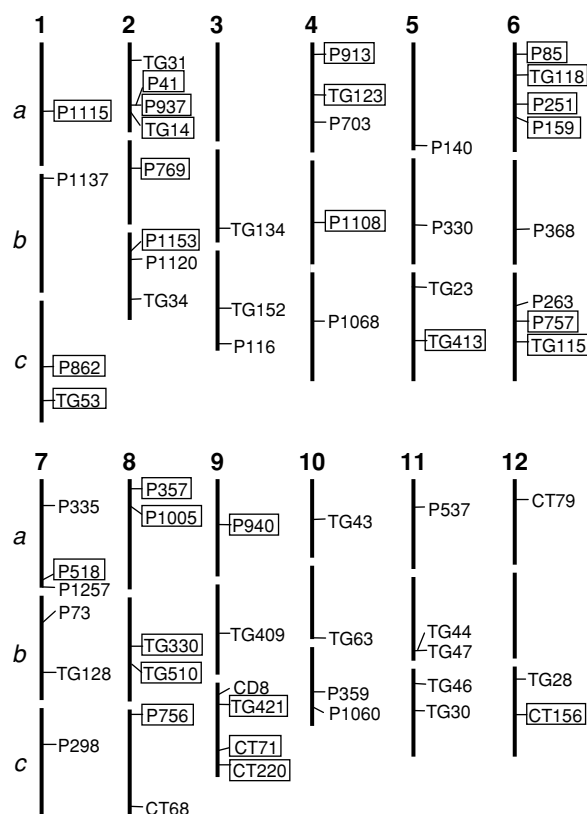


Figure 2. Each chromosome linkage map was trisected into *a*, *b* and *c*, which were monitored by RFLP loci shown in this map. Segregating loci in the S_3 family 2H22 were shown in boxes.

as vegetatively propagated plants by tubers or stem-cuttings) to evaluate fertility; (1) flowered or not flowered (scored 1 or 0, respectively), (2) self-fertility (berries containing seeds/self-pollinated flowers) (%), (3) number of seeds/berry, and (4) pollen stainability by aceto-carmin (%). Plants were grown in a green house in the fall and winter or an insect-proof screen house in the spring. To promote optimal growing conditions for flowering, light was supplemented to maintain 16 hours of daylight from October to March, while from April to July, plants were grown in natural daylight. Self-pollinations were made on as many flowers as possible.

Evaluation for heterozygosity

Total DNA was isolated by the method of Hosaka & Hanneman (1998b). Of 62 RFLP loci having been heterozygous in 97H32-6, 33 loci were already fixed as homozygotes in the 2H22 family (Phumichai et al.,

2004), thus the remaining 29 loci (Figure 2) were genotyped for each plant of those families shown in Figure 1. RFLP probes used were either single-copy tomato probes obtained from Dr. S. D. Tanksley, Cornell University (Tanksley et al., 1992) or potato probes (Hosaka & Spooner, 1992). RFLP detection procedures were described in Hosaka & Hanneman (1998b).

Locus-based observed percentage heterozygosity (LOPH) for each individual plant was calculated by $LOPH (\%) = 100 \times (\text{the number of heterozygous loci}/62)$. For the parental clone it was calculated by $LOPH (\%) = 100 \times (\text{the number of segregating loci in the selfed progeny}/62)$. Since some loci were localized together within a short map distance or co-segregated, each of 12 chromosomes was trisected into sections *a*, *b* and *c*, based on the map length (62 loci covered 32 chromosome sections) (Figure 2). Section-based observed percentage heterozygosity (SOPH) for each individual plant was calculated by $SOPH (\%) = 100 \times (\text{the number of sections containing at least one heterozygous$

locus/32). For the parental clone it was calculated by $\text{SOPH (\%)} = 100 \times (\text{the number of segregating sections in the selfed progeny}/32)$.

Data analysis

Allele A or B in each RFLP locus was determined by a linkage analysis of the segregation in the 2H22 family; that is, A alleles of the loci linked on the same chromosome and B alleles on the counterpart chromosome, respectively, in a coupling fashion. For the 2H22 family, Pearson's sample correlation coefficients between heterozygosity and fertility of the plants were calculated. Genetic effect of each locus on each fertility-related trait was analyzed by comparison of averages among AA, BB and AB genotypes by a single-factor analysis of variance (ANOVA) using Microsoft Excel 2004 for Mac version 11.1. If significant difference was found by ANOVA, genotypic differences were elucidated by the Bonferroni procedure, and the difference between homozygotes and heterozygotes was tested by *t*-test.

Results

Fertility in the 2H22 family

Of 51 plants of 2H22 family, 27 plants flowered and were self-pollinated onto 274 flowers. Most of the remaining 24 plants formed flower buds, but dropped at a premature stage of buds. The character of flowering or non-flowering, though scored 1 or 0, was considerably continuous among plants, that is, even for flowering plants, the degree of flowering varied from abundantly flowering to very rarely flowering, while for non-flowering plants the degree of bud formation and abortion was different between plants. Thus, fertility-related traits measured in this study were likely controlled by quantitative trait loci (QTLs). Twenty plants set 126 berries that contained a total of 8678 seeds. Pollen stainability was examined for only 15 plants out of 27 flowering plants due to a limited availability of flowers at the time. It varied from 10.5% to 75.4% with an average of 45.1% (Table 1).

Heterozygosity and fertility in the 2H22 family

Out of 62 loci spanned on 34 chromosome sections, 29 on 17 sections were segregating in 2H22 family (Figure 2), indicating 46.8% LOPH or 50.0% SOPH in 2KH521-4. Each of 2H22 plants was genotyped for 29 segregating RFLP loci. For example, 2H22-1 showed

Table 1. Pearson's sample correlation coefficients (*r*) between the degree of heterozygosity (LOPH or SOPH) and fertility-related traits in 2H22 family.

Character	Flowering	Self-fertility	Seed number	Pollen stainability
Number	51	27	20	15
Mean	(27 vs. 24) ¹	43.0%	68.2	45.1%
SD	—	35.13	30.26	18.25
<i>r</i> (LOPH)	0.229	0.108	0.195	0.228
<i>r</i> (SOPH)	0.187	0.128	0.173	0.333

¹ Flowering vs. non-flowering plants.

AB heterozygote genotypes in 16 loci on 10 chromosome sections being 25.8% LOPH or 29.4% SOPH. In the 2H22 family, the LOPH ranged from 6.5% to 38.7% with an average of 24.2%, while the SOPH ranged from 8.8% to 41.2% with an average of 27.3%. Correlation coefficients between LOPH or SOPH and fertility-related traits were always positive (Table 1), indicating that plants with higher heterozygosity were more fertile, although their correlations were all very weak.

Association between locus and fertility

Seven associations ($P < 0.05$) between RFLP genotypes and fertility-related traits were found using ANOVA (Table 2). As P41 and P937 were cosegregating loci, at least six loci on six chromosome sections were presumably associated with fertility-related QTLs. For the trait of flowering vs. non-flowering, four loci on three chromosome sections showed significant associations. The cosegregating loci P41 and P937 (both on chromosome section 2a) showed that BB homozygotes did not flower at all, and AB heterozygotes were better in flowering than AA homozygotes (the average score of AA:BB:AB was 0.41:0:0.67). Better flowering in the heterozygote was also supported by the *t*-test (Table 2). However, in TG115 (6c) and TG421 (9c) AA homozygotes showed the best flowering and the differences between heterozygotes and homozygotes were not significant for both loci. For the seed setting by self-pollinations, only P159 (6a) showed significant association, where both AA and BB homozygote genotypes were better than the AB heterozygote. ANOVA did disclose two significant loci P769 (2b) and CT156 (12c) associating with the pollen stainability, both of which showed better performance in the heterozygotes. However, the Bonferroni 95% confidence intervals did not disclose significant difference between

Table 2. RFLP loci significantly affecting fertility-related traits in 2H22 family ($P < 0.05$). Genotypic means were compared by ANOVA and sub-tested by t -test.

Trait	Chr. sect.	Locus	ANOVA for AA:BB:AB ¹	t -test for (AA + BB):AB ²
Flowering	2a	P41	0.41 ^{ab} :0 ^a :0.67 ^b $F = 4.276 (P = 0.020)$	0.33:0.67 $t = -2.472 (P < 0.05)$
	2a	P937	0.41 ^{ab} :0 ^a :0.67 ^b $F = 4.276 (P = 0.020)$	0.33:0.67 $t = -2.472 (P < 0.05)$
	6c	TG115	0.71 ^a :0.20 ^b :0.54 ^{ab} $F = 3.503 (P = 0.038)$	0.52:0.54 $t = -0.075 (ns)$
	9c	TG421	0.85 ^a :0.46 ^{ab} :0.40 ^b $F = 3.917 (P = 0.027)$	0.65:0.40 $t = 0.817 (ns)$
	6a	P159	68.5 ^a :54.2 ^{ab} :30.5 ^b $F = 3.714 (P = 0.039)$	64.2:30.5 $t = 2.647 (P < 0.05)$
Self-fertility	6a	P159	68.5 ^a :54.2 ^{ab} :30.5 ^b $F = 3.714 (P = 0.039)$	64.2:30.5 $t = 2.647 (P < 0.05)$
	6a	P159	68.5 ^a :54.2 ^{ab} :30.5 ^b $F = 3.714 (P = 0.039)$	64.2:30.5 $t = 2.647 (P < 0.05)$
Pollen stainability	2b	P769	29.1 ^a :57.0 ^a :52.6 ^a $F = 4.186 (P = 0.042)$	33.8:52.6 $t = -2.355 (P < 0.05)$
	12c	CT156	10.5 ^a :26.8 ^a :51.0 ^a $F = 5.742 (P = 0.018)$	21.4:51.0 $t = -3.255 (P < 0.05)$
	12c	CT156	10.5 ^a :26.8 ^a :51.0 ^a $F = 5.742 (P = 0.018)$	21.4:51.0 $t = -3.255 (P < 0.05)$

¹Superscripts ^a and ^b indicate significant differences between genotypic means using the Bonferroni 95% confidence intervals.

²The t -test between homozygotes and heterozygotes was performed using the standard error given by $[MSE (1/n_1 + 1/n_2)]^{1/2}$, where MSE was the mean square in the ANOVA table and n_1 and n_2 were, respectively, the number of plants in AA + BB and AB, with the degree of freedom of $n_1 + n_2 - 2$.

Table 3. Viability in S_5 families

Pedigree			Seeds sown	Germinated plants	Matured plants
S_3 parent	S_4 parent	S_5 family			
2H22-2	3H93-28	4H127	77	75 (97.4%)	45 (60.0%)
	3H93-29	4H128	62	44 (71.0%)	28 (63.6%)
2H22-4	3H94-4	4H129	44	30 (68.2%)	11 (36.7%)
	3H94-30	4H130	71	46 (64.8%)	8 (17.4%)
2H22-44	3H95-7	4H131	62	24 (38.7%)	5 (20.8%)
	3H95-20	4H132	107	41 (38.3%)	10 (24.4%)
2H22-84	3H100-20	4H133	40	9 (22.5%)	1 (11.1%)

any genotypes likely due to the small sample size. No significant association was found for the seed number per berry.

Generation of S_4 and S_5 progenies

Out of 27 S_3 plants that produced selfed seeds, ten were used for generating S_4 plants. From a total of 424 S_4 plants grown, only 30 plants could be self-pollinated and eight of them set S_5 seeds. All except one that produced only a few seeds were grown as S_5 families (Table 3). The S_5 seeds germinated with a frequency from 22.5% to 97.4% depending on the family. Many of the young seedlings (59.9%), however, died or stopped growing, thus, the frequency of matured plants per germinated plants for each family ranged from 11.1% to

63.6%, which was highly correlated with the germination frequency ($r = 0.807$). Viability expressed by these two measurements differed by the S_3 parents, $2H22-2 > 2H22-4 > 2H22-44 > 2H22-84$ (Table 3). Although the 4H133 family with the lowest viability was descended from 2H22-84 with the lowest SOPH, the relationship between viability in S_5 and heterozygosity levels of their S_4 and S_3 parents was inconsistent (data not shown).

Reduction of heterozygosity from S_3 to S_5 progenies

The reduction rates of SOPH from parents to the selfed progenies were not very different in any selfing generations, ranging from 35.0% to 51.5% with an average of 44.9% (Table 4). These reductions occurred in the process from self-fertile parents (or selfed seeds) to matured progenies, thus, relating mostly to viability. The reduction rates of SOPH from an S_2 parent to S_3 parents and to S_4 parents that actually resulted in S_5 progenies were on average 48.6% and 23.1%, respectively (Table 5). These reductions occurred through the process of self-fertilization and growing.

Genetic fixation

Genetic fixation in the most advanced progenies (four S_5 families and one S_4 family) was analyzed for the segregating loci in 2H22 family (Table 6). All but

Table 4. Reduction rates of heterozygosity (SOPH) from a parent to the progeny population.

Parent			Progeny				Reduction rate (%)
Generation	Clone	SOPH (%)	Generation	Family	No.	Mean SOPH (%)	
S ₀	97H32-6	100	S ₁	98H23	15	57.4	42.6
S ₂	2KH521-4	50.0	S ₃	2H22	51	27.3	45.4
S ₃	2H22-4	23.5	S ₄	3H94	15	12.0	48.9
S ₃	2H22-84	17.6	S ₄	3H100	15	9.4	46.6
S ₄	3H93-28	23.5	S ₅	4H127	15	11.4	51.5
S ₄	3H93-29	23.5	S ₅	4H128	15	13.7	41.7
S ₄	3H94-4	17.7	S ₅	4H129	11	11.5	35.0
S ₄	3H95-20	23.5	S ₅	4H132	10	12.4	47.2
Mean							44.9

Table 5. Reduction rates of heterozygosity (SOPH) from a self-compatible parent to the self-compatible progeny clone

S ₂ clone	SOPH (%)	S ₃ clone	SOPH (%)	S ₄ clone	SOPH (%)
2KH521-4	50.0	2H22-2	29.4	3H93-28	23.5
"	"	"	"	3H93-29	23.5
"	"	2H22-4	23.5	3H94-4	17.7
"	"	2H22-44	32.4	3H95-20	23.5
"	"	2H22-84	17.6	—	—
Reduction rate (%)		From S ₂ to S ₃		From S ₃ to S ₄	
Mean		48.6		23.1	
Range		35.2–64.8		20.1–27.5	

two loci were fixed as homozygotes in at least one of the progenies. The two loci still segregating in all the progenies were tightly linked P357 and P1005 on chromosome section 8a, which were not associated with any fertility-related traits. In the loci associated with fertility-related QTLs, fixation occurred towards its better performance. For example, P41 and P937 (both on 2a) (for flowering, the best in AB and better in AA, see Table 2) were fixed to AA homozygotes in all progenies.

Discussion

Heterozygosity continued to reduce by selfing at rates of 48.6% from S₂ to S₃, 23.1% from S₃ to S₄ and 43.9% from S₄ to S₅, which were almost equivalent to the previously obtained reduction rate (the average of 38.4% per selfing generation from S₀ to S₄) (Phumichai et al., 2005). The analysis of 2H22 family showed the positive, though very weak, correlations between heterozygosity and any of fertility-related traits. Thus, the lower reduction rate of heterozygosity than the expected 50% was possibly caused by an unintentional or unavoidable selection for plants of higher heterozy-

gosity with better fertility, supporting our previous suggestion (Phumichai et al., 2004) and agreeing with numerous inbreeding studies (Krantz, 1946; Wilsie, 1958; Jugenheimer, 1976; Ray & Bingham, 1992).

It is implied that, as the heterozygosity continues to reduce by selfing, the fertility also continues to reduce and the plants may become complete sterile. Interestingly, however, not all fertility-related QTLs expressed better performance in their heterozygote states (Table 2). For flowering, three QTLs were found, among which one QTL performed better in the heterozygotes and two in the homozygotes. By consecutive selfing, the QTL performing better in heterozygotes was likely to be fixed to the second better homozygotes, and those performing better in homozygotes were fixed to the best genotypes (Table 6). One QTL was found for the trait of self-fertility, which performed better in the homozygotes. For the pollen stainability two QTLs were found, both of which performed better in the heterozygotes (Table 2). However, the S₄ plants of the family 3H100 were all homozygotes for these two QTLs (Table 6), but successfully produced the S₅ progeny (see Figure 1). It has been hypothesized that continuous selfing may eliminate undesirable recessive alleles as they become homozygous, and

Table 6. Segregating RFLP loci in 2H22 family and their segregation or fixation in later selfing generations.

Chr. seg.	Locus	2H22 (S ₃)			3H100 (S ₄)			4H127 (S ₅)			4H128 (S ₅)			4H129 (S ₅)			4H132 (S ₅)			Associated trait ¹
		AA	BB	AB	AA	BB	AB	AA	BB	AB	AA	BB	AB	AA	BB	AB	AA	BB	AB	
1a	P1115	14	13	24	0	15	0	15	0	0	2	2	11	11	0	0	1	2	7	
1c	P862	13	14	24	2	3	10	7	1	7	4	3	8	1	5	5	0	10	0	
	TG53	13	13	25	2	3	10	7	1	7	4	3	8	1	4	6	0	10	0	
2a	P41	17	4	30	15	0	0	15	0	0	15	0	0	11	0	0	10	0	0	F (AB > AA > BB)
	P937	17	4	30	15	0	0	15	0	0	15	0	0	11	0	0	10	0	0	F (AB > AA > BB)
	TG14	17	4	30	15	0	0	15	0	0	15	0	0	11	0	0	10	0	0	
2b	P769	16	7	28	15	0	0	15	0	0	15	0	0	11	0	0	10	0	0	P (AB > AA or BB)
2c	P1153	14	16	21	15	0	0	2	2	11	2	4	9	2	2	7	10	0	0	
4a	P913	16	12	23	0	15	0	15	0	0	15	0	0	0	11	0	0	10	0	
	TG123	14	12	25	0	15	0	15	0	0	15	0	0	0	11	0	0	10	0	
4b	P1108	15	11	25	15	0	0	6	6	3	0	15	0	5	2	4	0	10	0	
5c	TG413	12	12	27	8	0	7	2	5	3	15	0	0	11	0	0	2	5	3	
6a	P85	15	6	30	15	0	0	15	0	0	7	3	5	11	0	0	10	0	0	
	TG118	15	7	29	15	0	0	15	0	0	8	1	6	11	0	0	0	6	4	
	P251	15	7	29	15	0	0	15	0	0	8	1	6	11	0	0	6	0	4	
	P159	14	7	30	15	0	0	15	0	0	15	0	0	11	0	0	0	5	5	S (AA > BB > AB)
6c	P757	14	14	23	5	2	8	2	5	8	1	8	6	11	0	0	4	2	4	
	TG115	17	10	24	15	0	0	4	5	6	2	5	8	11	0	0	2	3	5	F (AA > AB > BB)
7a	P518	20	8	23	0	15	0	15	0	0	15	0	0	0	11	0	1	3	6	
8a	P357	16	6	29	3	2	10	7	3	5	9	1	5	1	3	7	2	3	5	
	P1005	16	6	29	3	2	10	7	3	5	9	1	5	1	4	6	2	3	5	
8b	TG330	19	4	28	6	0	9	1	6	8	5	0	10	11	0	0	10	0	0	
	TG510	4	17	30	7	0	8	15	0	0	3	0	12	11	0	0	10	0	0	
8c	P756	8	13	30	15	0	0	0	15	0	0	15	0	0	11	0	3	2	5	
9a	P940	11	18	22	5	6	4	15	0	0	15	0	0	0	11	0	1	3	6	
9c	TG421	13	13	25	15	0	0	15	0	0	15	0	0	1	1	9	10	0	0	F (AA > BB > AB)
	CT71	15	14	22	15	0	0	15	0	0	15	0	0	5	3	3	10	0	0	
	CT220	18	13	20	15	0	0	15	0	0	15	0	0	11	0	0	10	0	0	
12c	CT156	10	11	30	0	15	0	5	2	8	2	4	9	2	0	9	0	10	0	P (AB > AA or BB)

¹ Genotypic effects on fertility-related traits shown in Table 2. F = flowering, S = self-fertility, P = pollen stainability.

may recover fertility with advancing generations (Busbice, 1968; Pfeiffer & Bingham, 1983; Ray & Bingham, 1992). This study, however, suggests that not only by eliminating undesirable recessive alleles but also by changing QTLs expressing best in the heterozygotes (overdominance QTLs) to the better homozygotes and by changing those expressing best in one of the homozygotes (dominance QTLs) to the best homozygotes the selfed progenies were cryptically being improved for fertility, even though there was no actual recovery of fertility.

Strong selection pressure obviously functioned through consecutive selfing generations both for fertility (20 out of 51 S₃ and 8 out of 424 S₄ plants were self-fertile) and for viability (2.5–58.4% of S₅ plants matured). However, under the selection pressure for viability, heterozygosity reduced at an average rate of 44.9%, which was only slightly lower than the expected

50%. The difference in viability among S₅ progenies was possibly due to the parental genotypic effects and not directly related to the heterozygosity levels. Only 23.1% of heterozygosity was reduced from self-fertile S₃ to self-fertile S₄ plants (selection pressure for viability and fertility). These results suggest that fertility rather than viability primarily affected the slower decline of heterozygosity.

The most homozygous S₃ parent (2H22-84, SOPH = 17.6%) became extinct in the subsequent S₅ generation because it produced only one matured, but non-flowering S₅ plant (Table 3). This was probably because the approach to homozygosity under selfing was too rapid (Genter, 1967). As suggested by Kinman (1952), the use of sib-mating or the composite sibbing of inbreds can slow the approach to homozygosity and permit smoother transition from overdominance QTLs to dominance QTLs before complete fixation occurs.

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