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S Allele Discrimination in Styles of *Petunia hybrida* Bearing Stylar-conditioned Pseudo-self-compatibility*

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Summary. A cross between a 0% pseudo-self-compatible (PSC) plant ($S_{3.3}$) and a 100% PSC plant ($S_{1.1}$) yielded an F_1 population which, when selfed, produced a high mean seed set which was not significantly different than that produced when the F_1 was backcross pollinated by the 100% PSC parent. Backcross pollinating the F_1 with the 0% PSC parent yielded no seed. No $S_{3.3}$ plants were recovered in the F_2 populations, indicating that pollen tubes containing the S_3 allele were inhibited during pollen tube growth of the selfed F_1 plants. Apparently stylar-conditioned PSC does not remove all discriminatory power from these petunia styles. Crossing the F_1 ($S_{1.3}$) with an self-incompatible (SI) plant ($S_{2.2}$) produced plants which were used for computation of a standard linkage test. An approximate map distance of 28 units was found between the *S* specificity locus and the major gene(s) which influenced its expression. Other generalized PSC modifying genes apparantly were not linked with the *S* locus.

Key words: *Petunia hybrida* – Pseudo-self-compatibility – Self incompatibility – Crossovers – Stylar-part mutant

Introduction

Self incompatibility (SI), a genetically controlled physiological response, refers to a mating system in which individuals produce normal male and female gametophytes but fail to produce seed when self-pollinated (Bail 1971). The control of self incompatibility by a single *S* gene with multiple alleles determines whether fertilization will occur by inhibiting normal pollen tube growth in styles which match the *S* specificity carried by the pollen tube (Kojan 1950). Genetic PSC has been attributed to the action of non-allelic genes which affect normal *S*-gene activity and result in self seed (Mather 1943). The seed produced from self pollination can reach levels approaching those obtained from crosses with plants containing unmatched *S* alleles. Modifying genes which affect the pseudo-self-compatibility (PSC) expression in *Petunia* (Mather 1943; Takahashi 1973) appear to be freely segregating relative to the *S* locus.

The objective of this study was to determine whether the expected 1:2:1 segregation of *S* alleles occurred upon selfing a heterozygote which was obtained by crossing an SI plant with a highly PSC plant. A second objective was to determine whether there was linkage between the *S* locus and a gene or gene complex modifying it.

Materials and Methods

Petunia seeds were germinated on sphagnum moss under mist. Upon germination the seedlings were transferred and grown in a greenhouse with temperatures of 21/15°C (day/night) or as low as could be maintained by placing the plants approximately three feet from a fan and pad cooling system. Water on the pads was turned off nights throughout the experiment to reduce *Botrytis*. When the first true leaf had developed, seedlings were transplanted. Flowers were emasculated prior to anthesis when used for outcross pollinations. Self pollinations were made on or one day after anthesis. Five flowers were used for each pollination, but to obtain an average effect over time, not more than three flowers per plant were pollinated with any pollen source on any given day. Capsules were harvested prior to dehiscing and seeds were counted using an electronic seed counter with an error of ± 2-4%.

Mean seed set was calculated for each type of pollination on each plant as well as progeny means for the populations. The PSC level of each plant was calculated by dividing the mean self seed set by the mean number of seeds obtained by crossing the plant, as seed parent, to an unrelated pollen source which was SI and differing in *S* alleles. This value was multiplied by 100 to give a percent PSC.

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F_1 From a Cross Between a 0% PSC and a 100% PSC Plant

Originally, 20 plants from the F_1 cross between an I_7, 0% PSC plant ($S_{3.3}$), and an I_7, 100% PSC plant ($S_{1.1}$), were selfed (Fig. 1). Subsequently, 10 randomly chosen plants, designated 77-93-1 to 10, were selfed and backcross pollinated by the original parents, which were maintained vegetatively. These plants were also pollinated with related and unrelated individuals bearing the same *S* alleles as the parents. The F_1 population was selfed and backcross pollinated during two different periods. The first crosses were done in April and again in late October, when the F_2 populations were pollinated. Since the response was similar and data are more complete for the pollinations done in late October, only those data are presented.

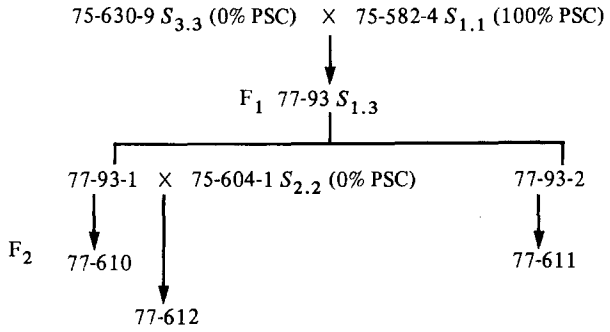

Fig. 1. The lineage of petunias used in studying the *S* allele discrimination in styles of *Petunia hybrida* and linkage of the *S* locus with the modifying gene(s)

F_1 ($S_{1.3}$) Crossed to an $S_{2.2}$ Plant

Plant 77-93-1 ($S_{1.3}$) was crossed with an SI (75-604-1, $S_{2.2}$) and this population was designated 77-612 (Fig. 1). Each of the 102 plants was selfed, backcross pollinated with 75-604-1, and the parents of the F_1. In all crosses and selfs, five flowers were used, as described for the previous populations. Mean self and cross seed set were calculated for each population cross.

F_2 Populations

Self seed of each of two plants of the F_1 population (77-93-1, 77-93-2) which showed the lowest self seed were planted and designated as populations 77-610 and 77-611 (Fig. 1). A total of 48 and 47 plants of these populations, respectively, were transplanted into 4 inch plastic pots and were grown and pollinated under the same environmental conditions as population 77-612 and the F_1 (which had been cut back and had resumed flowering).

Flowers of the F_2 populations were selfed, cross pollinated by the grandparents and tested for *S* alleles with known testers. In determining the *S* alleles of the individual F_2 plants, two additional testers, an SI sib ($S_{1.1}$) of the 100% PSC grandparent, 75-582-3, and an SI $S_{3.3}$, were used as seed parents to differentiate homozygous and heterozygous plants. Crosses using the $S_{3.3}$ plants as seed parents were used only in cases where further differentiation between $S_{1.3}$ and $S_{3.3}$ plants was required.

Results

F_1 From a Cross Between a 0% PSC and a 100% PSC Plant

Mean individual self seed set of the F_1 population was distributed over a relatively narrow range (Fig. 2). Mean self seed for the nine plants of progeny 77-93 was 228.0 seeds, with a range from 181.8 to 296.7 (Table 1). PSC level for seven of the nine plants was above 90% with only one individual with a mean PSC level below 60%. The mean PSC level for this population was 91.4%. Pollination of the F_1 with the high PSC parent or an SI sib of that parent (75-582-4, 75-582-3) both of which were $S_{1.1}$ or using an SI unrelated $S_{2.2}$ all produced progeny seed set means which were not significantly different from the progeny mean of the selfed F_1 population.

When the F_1 population was backcross pollinated with the 0% PSC plant ($S_{3.3}$), related to the parent (75-630-1) and an unrelated $S_{3.3}$ (75-250-2), both failed to produce

Table 1. Percent pseudo-self-compatibility and mean seed sets from self, compatible cross and test-cross pollinations of nine $S_{1.3}$ *Petunia* plants of population 77-93

				$S_{1.1}$ Pollen		$S_{3.3}$ Pollen		
Plant no.	self	Compatible cross	% PSC	75-582-4	75-582-3	75-630-9	75-630-1	75-250-2
1	184.8	342.2	53.9	210.8	156.0	0.0	0.0	0.0
2	181.8	240.4	75.6	234.4	140.0	0.0	0.0	0.0
4	206.6	137.0	100.0	146.8	174.5	0.0	0.0	0.0
5	201.0	192.2	100.0	97.8	277.0	0.0	0.0	0.0
6	237.4	255.6	92.9	160.6	299.2	0.0	0.0	0.0
7	220.4	165.0	100.0	126.4	167.0	0.0	0.0	0.0
8	296.7	253.0	100.0	346.6	315.8	0.0	0.0	0.0
9	269.4	265.8	100.0	230.2	199.2	0.0	0.0	0.0
10	253.6	185.6	100.0	170.2	216.0	0.0	0.0	0.0

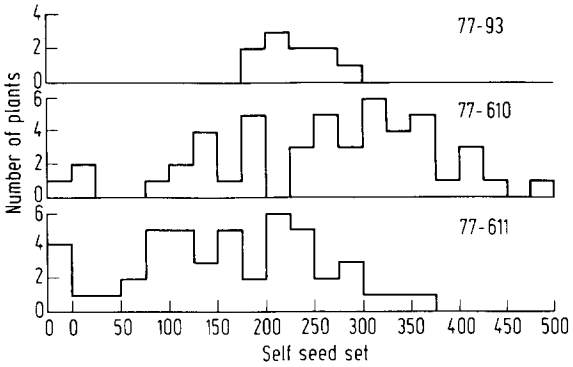

Fig. 2. Frequency distribution of self seed set of an F_1 and two F_2 populations of *Petunia hybrida* from crossing a 0% PSC plant, 75-630-9, with a 100% PSC plant, 75-582-4

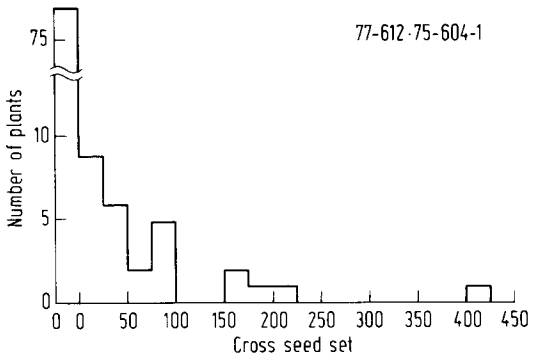

Fig. 4. Frequency distribution of cross seed set of population 77-612 backcross pollinated with 75-604-1 *Petunia hybrida* plants

seed even though all $S_{3.3}$ pollen sources produced abundant seed when tested with other *S* allele seed parents.

F_1 ($S_{1.3}$) Crossed to an $S_{2.2}$ Plant

Crossing 77-93-1 ($S_{1.3}$) with a 0% PSC $S_{2.2}$ (75-604-1) produced a population with a mean self seed level of 123.4 seeds (Fig. 3). A wide distribution of seed yields was obtained, ranging from 25 plants which produced zero seed to one plant which produced over 500 seeds. The mean of this population was considerably lower than the mean self seed set on the F_2 (77-610) of 259.4 (Fig. 2) and slightly lower than the F_1 self seed set of 184.8 seeds (Table 1).

Backcross pollination of progeny 75-612 by 75-604-1 ($S_{2.2}$) did not produce all zero seed set as would be expected (Fig. 4). About 25% of the plants produced mean seed set which was greater than zero.

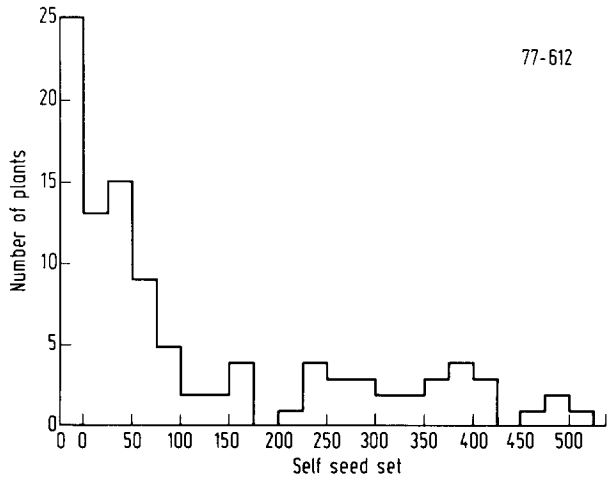

Fig. 3. Frequency distribution of self seed set of 77-612, a cross between 77-93-1 × 75-604-1 *Petunia hybrida* plants

F_2 Populations

The self seed sets of the F_2 progenies 77-610 and 77-611 are given in Fig. 2. The progeny mean self seed set for 77-610 was 259.4 seeds and ranged from zero to over 450 seeds. This population contained 26 $S_{1.1}$, 21 $S_{1.3}$ and 0 $S_{3.3}$ plants, with one plant not classified.

Populations 77-611 had a progeny mean self seed set which was lower (163.9 seeds) than population 77-610 (Fig. 2). The range for this population was zero to 350 seeds. The segregation ratio of this population was 21 $S_{1.1}$, 26 $S_{1.3}$ and 0 $S_{3.3}$ plants.

Discussion

Crossing an SI petunia with a 100% PSC plant produced a PSC progeny with high self seed set (Table 1). The F_2 segregated, producing plants exhibiting varying amounts of self seed, including some SI plants (Fig. 2). In addition, crossing the F_1 with an unrelated strongly SI plant further reduced the population mean self seed yield and produced more SI individuals than the F_2 (Fig. 3). This rapid gain from selection for SI resembles the effect reported by Henny and Ascher (1976) in selection for high PSC in *Nemesia strumosa*. They were able to change the PSC mean from 2.0 to 70.1% in three generations of selection. The response of PSC to selection in both *Petunia* and *Nemesia* suggest that few genes exert a major effect on this trait.

The similar mean self seed set and backcross pollination seed set when the $S_{1.1}$ (100% PSC) parent was used as the pollen source and the lack of seed when the $S_{3.3}$ (0% PSC) parent is used on the F_1, indicated that the styles of this highly PSC F_1 were able to discriminate between *S* alleles in pollen tubes. Since there was no seed set difference in the self or outcross to a plant with different *S* alleles, it is apparent that pollen tubes bearing the

S_1 allele are not inhibited in the styles of these plants. Also, since there was no difference in seed set when the pollen of a 100% PSC $S_{1.1}$ or that of a related plant with 0% PSC was used, this reaction was a stylar response. The inhibition of pollen tubes bearing S_3 regardless of the source indicated that the rejection phenomenon of S_3 pollen was also stylar conditioned.

The two classes, $S_{1.1}$ and $S_{1.3}$, observed in the F_2 populations are to be expected based on the behavior of backcross pollinations of the F_1. The recovery of two classes in an approximate 1:1 ratio would be expected if all of the F_2 seed was due to growth of only S_1 pollen tubes. Abnormal homozygous class ratios resulted when *P. violacea* plants heterozygous for *S* alleles were self pollinated by Harland and Atteck (1933). The F_2 of an $S_{1.4}$ produced 4 $S_{1.1}$, 15 $S_{1.4}$ and 10 $S_{4.4}$ plants. They attributed this deficiency to the weakness of the $S_{1.1}$ homozygote. East and Magelsdorf (1926) selfed *Nicotiana* plants heterozygous for *S* alleles and recovered no plants of one homozygous class. They concluded, based on the ratio of the two classes recovered, that the missing class was the result of a lethal homozygous condition. Since our $S_{3.3}$ parent was itself an I_7 and the ratio of the classes recovered was 1:1, the missing $S_{3.3}$ class could not be attributed to a lethal gene.

The reaction observed agrees with the classical explanation of a stylarpart mutation. Theoretically, a stylar-part mutation causes the loss of activity of the part of the *S* locus which delineates the specificity of the style for a particular *S* allele (Lewis 1949; de Nettancourt and Ecochard 1971; Pandey 1970). Pollen tubes bearing the specificity of the mutated *S* allele are not inhibited and produce seed. If the style is heterozygous for a normal *S* allele, pollen tubes bearing that allele are inhibited. The pollen, however, retains its capacity for an SI reaction. According to this theory, both S_1 alleles in the 100% PSC parents would be stylar-part mutants. The F_1, which was $S_{1.3}$, would appear self compatible because of the inactivity of the mutant S_1 in the style. However, pollen tubes bearing the non-mutant S_3 would be inhibited.

The cross of the $S_{1.3}$ and the strongly SI $S_{2.2}$ provided plants for the computation of a standard linkage test cross (Table 2). Counting the $S_{2.3}$ individuals in the next generation, which had high self seed set, and the $S_{1.2}$ plants with SI as recombinational (nonparental) types, 25 of the 88 plants classified were products of a crossover (Table 3). These data provide evidence of an approximate map distance of 28 units between the *S* specificity locus and the modifying gene or genes which influence its expression. Assignment of individuals to one of the four classes of parental and recombinational types was hindered by what are apparently the effect of environmental influences and generalized PSC which is freely segregating with respect to the *S* locus. Fourteen plants could not be assigned to parental or nonparental classes. The map distance indicated should be viewed as an approximate value because of the variability in this trait as measured by self seed set. Also, the arbitrary choice of 50 seeds or less as criteria for an SI individual may have reduced the accuracy of classification.

A second estimation of the map distance between the two loci can be calculated from the F_2. Table 4 illustrates

Table 2. The mean seed set from self and test-cross pollinations of plants showing crossovers between the *S* specificity locus and the PSC modifying gene(s) in *Petunia* cross 77-612

Plant no.	*S* Genotype	Pollen testers			
		Self	$S_{1.1}$	$S_{3.3}$	$S_{2.2}$
2	2.3 PSC	319.2	378.2	307.2	0.0
8	2.3 PSC	63.2	350.6	0.0	87.4
12	1.2 SI	32.8	182.0	565.2	0.0
17	1.2 SI	0.0	94.6	154.4	59.2
18	1.2 SI	4.8	190.0	255.4	0.0
27	1.2 SI	0.0	152.2	327.6	0.0
31	2.3 PSC	74.4	351.0	202.0	0.0
40	1.2 SI	26.6	224.4	377.6	168.0
42	1.2 SI	41.2	168.0	186.8	0.0
43	2.3 PSC	87.6	273.6	0.0	0.0
46	1.2 SI	15.2	0.0	681.0	0.0
48	1.2 SI	0.0	41.8	483.0	0.0
54	2.3 PSC	148.8	352.6	261.8	0.0
55	2.3 PSC	76.2	440.8	187.0	0.0
56	1.2 SI	41.0	431.8	541.0	0.0
57	2.3 PSC	299.0	474.8	441.7	12.4
60	1.2 SI	0.0	295.8	390.4	0.0
66	2.3 PSC	260.6	449.6	380.0	7.2
68	2.3 PSC	56.0	356.4	0.0	0.0
75	2.3 PSC	253.6	203.6	101.4	0.0
80	2.3 PSC	454.6	511.0	154.5	80.8
83	2.3 PSC	231.6	482.0	155.2	156.6
93	1.2 SI	0.0	61.6	319.8	0.0
95	2.3 PSC	68.0	322.6	231.0	0.0
99	1.2 SI	18.0	64.8	442.8	0.0

Table 3. Crossover frequency between *S* specificity gene and PSC gene(s) observed in *Petunia* plants from a cross between an $S_{1.3}$ PSC plant (77-93-1) and an SI $S_{2.2}$ (75-604-1)

Class	Type	Number
$S_{1.2}$ SI	Recombination	12
$S_{1.2}$ PSC	Parental	31
$S_{2.3}$ SI	Parental	32
$S_{2.3}$ PSC	Recombination	13
Total[a]		88

[a] 14 Plants could not be classified. Plants were classified as SI if mean self seed was less than 50 seeds

Table 4. Expected recoverable genotypes, origin of recombinant chromosome, phenotypic pattern from self and test cross pollinations, and number of F_2 plants obtained from self pollination of an F_1 $\frac{S1\ P}{S3\ +}$ fitting the expected phenotypes

Expected recoverable genotypes and source of chromosome		Expected pattern from self and test crosses				Number of F_2 plants fitting phenotypic pattern
		Self	$S_{3.3}$ ♂	$S_{1.1}$ ♂	$S_{1.1}$ ♀	
Parental	$\frac{S1\ P}{S3\ +}$	+	–	+	+	26
Parental	$\frac{S1\ P}{S1\ P}$					
Nonparental, recombination in either male or female meiosis	$\frac{S1\ +}{S1\ P}$	+	+	+	–	44
Nonparental, two events, one male and one female	$\frac{S1\ +}{S1\ +}$	–	+	–	–	3
Nonparental, female meiosis	$\frac{S1\ P}{S3\ P}$	+	+	+	+	12
Nonparental, male meiosis	$\frac{S1\ +}{S3\ +}$	–	–	–	+	7
Nonparental, two events, one male and one female	$\frac{S1\ +}{S3\ P}$	+	+	–	+	2

the expected recoverable genotypes, the expected results from self pollination and tester crosses, and the number of F_2 plants fitting the expected phenotypic patterns. Upon self pollination, only pollen tubes bearing the S_1 allele will penetrate the $S_{1.3}$ F_1 style. Therefore, no crossovers occurring during microspore meiosis and involving the chromosome carrying S_3 can be recovered. In addition, the $S_{1.1}$ PSC phenotype includes parental and nonparental genotypes. Crossovers replacing the PSC factor (P) with wild type (+) in either male or female meiosis will be phenotypically the same as the parental $\frac{S1\ P}{S1\ P}$ genotype. The nonparental genotypes in this phenotypic class could be distinguished by progeny testing since self progeny of a plant heterozygous for PSC will produce three PSC to one SI. However, an estimation of the number of non-parental genotypes in the $S_{1.1}$ PSC phenotypic class can be gained without the progeny test by assuming that the two recoverable parental genotypes occur with equal frequency and subtracting the number of parental $S_{1.3}$ plants from the number of $S_{1.1}$ PSC plants. According to this assumption, 18 of the $S_{1.1}$ PSC plants should be non-parental genotypes.

Since none of the crossover events in microspore meiosis involving the chromosome bearing S_3 can be recovered, and since the origin of all other recombination events can either be determined or calculated (Table 4), it is possible to estimate the map distance using only crossover events occuring during female meiosis in the F_1. Assuming that one-half of the 18 $S_{1.1}$ PSC plants estimated to be nonparental by subtraction recombination during female meiosis and disregarding the seven $\frac{S1\ +}{S3\ +}$ self-incompatible plants as being nonparental because of crossovers during microspore meiosis, 26 nonparental plants resulted from crossovers during female meiosis. A total of 94 F_2 plants were characterized, giving a second estimate of 28 map units between the *S* locus and the PSC factor.

These data suggest that a major PSC gene or group of genes linked to the *S* locus interferes with the production of the *S* specificity by the style for the *S* allele in the cis position. The modifying gene or genes in the transposition apparently do not affect the expression of the *S* allele. Brieger (1927) reported that $S_{1.3}$ *Nicotiana sanderae* plants exhibited differential pollen acceptance in that the styles allowed S_3 pollen tubes to grow but inhibited S_1 pollen tubes. He hypothesized that there was a PSC factor 'P' which was linked to the *S* locus and removed the ac-

tivity of the allele with which it was linked. However, since the population tested contained only 15 plants, no map distance was given.

Theoretical treatments which produced the hypothesis of a multipartite *S* locus include tight linkage between the so-called activity and specificity subunits (Lewis 1949). Our data suggest that this linkage need not be as close as theorized. In a petunia breeding program aimed at producing F_1 hybrids using self incompatibility to reduce hand labor, this PSC factor would be most useful. The relatively loose linkage of the factor to the *S* locus will permit the recovery of SI individuals from small seedling populations.

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