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Pollen Tube Expression of Pseudo-self-compatibility (PSC) in *Petunia hybrida**

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Summary. An $S_{1,1}$ self-incompatible (SI) petunia plant which showed atypical seed set was found in an I_7 population. This plant showed a strong SI reaction when selfed but produced varying amounts of seed when used as the seed parent in crosses with unrelated individuals homozygous for the same *S* allele. Reciprocal crosses yielded no seed indicating that the reaction was a stylar response. Self seed obtained by high temperature treatments produced 18 plants, all of which exhibited the parental characteristics, the ability to reject self pollen but accept, to varying degrees, pollen bearing the same *S* allele from unrelated plants. Several petunias homozygous for S_1, and exhibiting various levels of PSC as determined by self seed set, progeny tests and temperature treatments, were used as pollen parents. The mean seed set of these crosses produced a ranking of the pollen parents which reflected the PSC levels obtained by other methods. The behavior of the F_1 and F_2 populations suggests that the pollen discriminating ability may be a simply inherited, dominant character in these plants. The styles of these unusual petunias illustrate the participation of the pollen tube in determining PSC.

Key words: *Petunia hybrida* – Pollen-mediated-pseudo-self-compatibility – Gametophytic Genes – Self Incompatibility

Introduction

The self-incompatibility reaction in *Petunia* is gametophytically controlled by one locus with a series of *S* alleles (Lewis 1944). This phenomenon normally functions by preventing fertilization by inhibiting growth of pollen tubes in a style which contains *S* alleles which match those carried by the pollen tubes. Plants which exhibit a functional SI system but set some self seed have been termed PSC. Henny and Ascher (1976) found PSC in *Nemesia* to be stylar-conditioned, since the pollen of the PSC plants produced no seed when tested on SI styles of plants bearing the same *S* alleles. They reported that stylar-conditioned PSC could produce seed sets which were equal to those obtained from crossing with a plant bearing a different *S* allele. Atwood (1942), working with *Trifolium repens*, attributed PSC to quantitative, additive genes which affected the expression of the *S* alleles. The PSC found in *P. hybrida* by Takahashi (1973) was found to be the result of a stylar reaction which resulted in faster pollen tube growth in styles of plants which expressed higher levels of PSC. He also attributed a portion of the PSC level observed to the increased vitality of some pollen.

SI *P. hybrida* plants bearing *S* alleles in common, as determined by diallel crosses, usually do not set seed when cross pollinated with related or unrelated sources bearing the same *S* alleles. We found an SI petunia plant which, after preliminary crosses with plants bearing the same *S* alleles, set varying amounts of seed. This plant showed the normal SI reaction when used as a pollinator with other SI plants. The abnormal reaction observed with this plant caused us to investigate its behavior further.

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 20/15°C (day/night) or as low as could be maintained by placing the plants approximately 3 feet from a fan and pad cooling system. Water on the pads was turned off at night throughout the experiment to reduce *Botrytis*. Three weeks after sowing, the seedlings were transplanted into 2 inch peat pots containing equal parts of peat moss, perlite and soil. Later the plants were individually potted into 4 inch, and eventually 5 inch, plastic pots. Each plant was given a numerical code

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with the first two digits indicating the year planted, the next digits indicating the row number and the last digit(s), the individual plant number. Flowers were pollinated on or one day after anthesis. All polinations were repeated on five flowers. Unused flowers were removed daily to prevent pollen contamination. To obtain an average effect over time, not more than three flowers per plant were pollinated with any pollen parent on any given day. The pollinations were made over a 3 week period. Seed capsules were harvested prior to dehiscing and the seeds counted using an electronic seed counter with a counting error of ±2-4%. Known tester plants, as determined by the original diallele crosses, were used as pollen parents for the crosses made. These testers were each homozygous for an *S* allele and exhibited differing PSC levels and varying degrees of environmentally induced SI breakdown (Flaschenriem 1978).

Mean self seed set was calculated for each seed parent. Pollen-mediated-pseudo-self-compatibility (PMPSC) is the term which will be used in this paper to refer to the PSC level due to the influence of the pollen tube. The level of PMPSC was calculated by pollinating an $S_{1.1}$ plant by another $S_{1.1}$ plant and dividing this seed yield by the seed obtained when the same plant was pollinated with a pollen source having a different *S* allele. This value was then multiplied by 100 to give a percentage of PMPSC. Frequency distribution histograms were constructed for percentage of PMPSC for each pollen source.

75-579-1 and Its Progeny 77-80 Pollinated with $S_{1.1}$ Plants

An I_7 plant 75-579-1 ($S_{1.1}$) was selected because of its unusual ability to produce seed when pollinated with unrelated plants which were homozygous for the same *S* allele but produced no seed when reciprocally pollinated with SI $S_{1.1}$ pollen sources. This plant was derived from a diallel constructed to determine *S* allele classes of SI plants chosen from several inbred lines. Progeny 77-80 was obtained from selfing this plant after it had been given a temperature treatment of 30°C for 48 hours (Flaschenriem 1978). The seedlings of 77-80 were planted in April, 1977 and tested throughout the summer months for self seed set; all self pollinations yielded no seeds. Progeny 77-80 was selfed, backcross pollinated by its parent (75-579-1), and test cross pollinated using $S_{1.1}$ sources differing in PSC levels (Fig. 1).

Crosses of F_1 of 75-579-1 × 75-137-3, and F_2 Pollinated with $S_{1.1}$ Testers

In an attempt to better understand the behavior of 75-579-1, it was crossed with an $S_{1.1}$ plant 175-137-3) which was unrelated and showed a large degree of environmentally active PSC. This F_1 was backcross pollinated to determine whether the F_1 in the heterozygous condition would produce seed when pollinated with various S_1 pollen sources. Pollen sources for test cross pollinations onto the F_1 included the parents, two unrelated $S_{1.1}$ plants, one an SI (75-582-3) and the other a 100% PSC (75-582-4) plant as well as an unrelated plant bearing different *S* alleles ($S_{3.3}$).

Results and Discussion

Although strongly SI and homozygous for S_1, *P. hybrida* plant 75-579-1 produced seeds when pollinated by unre-

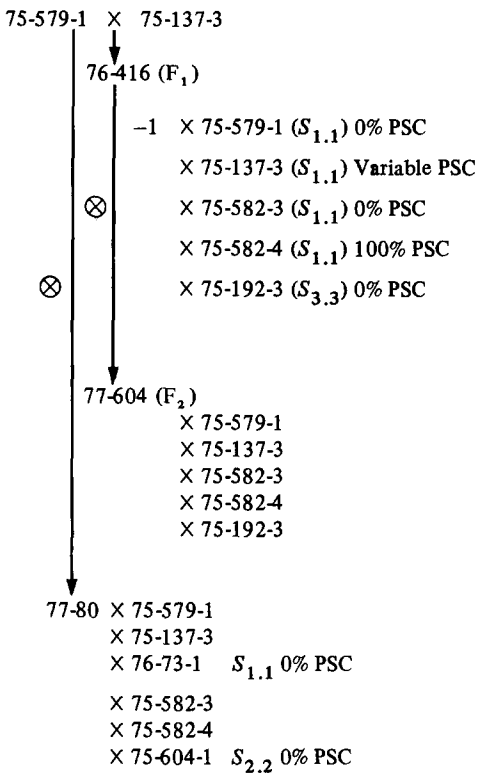

Fig. 1. The lineage of petunias used in studying the pollen tube expression of pseudo-self-compatibility in *Petunia hybrida*

Table 1. Seed set of preliminary crosses between 75-579-1 and other $S_{1.1}$ plants and an $S_{2.2}$ *Petunia* plant and pseudo-self-comptability (PSC) levels of pollen parents

Seed parent	Pollen parent	PSC level of pollen plant	Seed set
75-579-1 ($S_{1.1}$)	75-137-3 ($S_{1.1}$)	Unstable, range low to medium	186.2
75-579-1 ($S_{1.1}$)	75-582-3 ($S_{1.1}$)	Zero to low	249.7
75-579-1 ($S_{1.1}$)	75-582-5 ($S_{1.1}$)	Zero	143.0
75-579-1 ($S_{1.1}$)	75-652-4 ($S_{1.1}$)	Zero	101.7
75-579-1 ($S_{1.1}$)	75-604-1 ($S_{2.2}$)	Zero	401.7

Table 2. Frequency of *Petunia* plants 75-579-1 and 77-80 setting seed when parent and progeny were reciprocally pollinated

Crosses (parents)		Plants tested	Frequency of plants with various seed set				
Seed	Pollen		0.0	0.1-25	25.1-50	50.1-75	Mean
75-579-1	Selfed	1	1				0.0
75-579-1	77-80	18	18				0.0
77-80	Selfed	18	15	3			1.6
77-80	75-579-1	18	6	7	4	1	16.4

lated plants homozygous for S_1 (Table 1). The reciprocal pollinations failed. The pollen sources of S_1 produced seed set yields which were considerably lower than that obtained when an outcross (75-604-1) was used. Differences in seed yields from the various S_1 pollinators could not be attributed to differential fertility, since high seed yields were obtained when an $S_{3.3}$. plant was pollinated with each. This differential seed set suggests a unique PSC situation influenced by the S_1 pollen sources. Since the highest seed yield from an incompatible pollination of 75-579-1 was only about half that of a compatible pollination (Table 1), the style is apparently expressing a partial incompatibility reaction and the seed set must be designated PSC. Using plant 75-579-1 as the seed parent and its progeny 77-80 as the pollen source, failed to produce seeds (Table 2). The reciprocal pollinations resulted in a mean of 16.4 seeds per pollination for the 18 plants of 77-80. However, not all individuals in 77-80 set seeds from this backcross pollination; 6 plants were completely SI (Table 2).

Experiments by Takahashi (1972) with progeny of SI *P. hybrida* plants gave evidence for a unilateral PSC between progenies and their parents when *S* alleles matched. He found that SI plants heterozygous for *S* alleles could produce progeny which were SI when selfed and cross-incompatible with their parents when used as pollen parents. However, when crossed reciprocally as seed parents, several plants showed high fertility. The unilateral difference was attributed to the activity of the stylar conditioned genes weakening the SI reaction in the progeny, while also slowing the growth of the pollen tubes after self-pollinations, resulting in no self seed set. These changes, occurring in both stylar and pollen behavior, were attributed to inbreeding depression. Since 75-579-1 is an I_7 inbred, the unilateral differences should not be due to inbreeding depression. The unilateral differences we observed were low and occurred with two-thirds of the plants. Segregation of modifying genes might produce these results.

Pollinating the 18 $S_{1.1}$ plants of progeny 77-80 with several unrelated $S_{1.1}$. plants produced varying amounts of seeds. Figure 2 illustrates the results of these pollinations expressed as percentage of PMPSC. 75-137-3 was classified as an unstable SI because it produced zero to low seed, depending on seasonal and environmental conditions (Flaschenriem 1978). It also produced high self seed when given a temperature treatment (Flaschenriem 1978). When used as the pollen source on 77-80 (Fig. 2) the PMPSC level was 63.9%. A selection of the selfed progeny of 75-137-3 produced an SI (76-73-1) which showed no response to 30°C temperature treatment for 48 hours. The level of PMPSC expressed by this plant as tested by 77-80 was 36.8% (Fig. 2). Pollen source 75-582-3 (Fig. 2), an SI (0% PSC) plant which produced few seeds when given a temperature treatment, showed a PMPSC level of 57.6% (Fig. 2) on 77-80. Using 75-582-4 (100% PSC) produced a high PMPSC level of 71.8% (Fig. 2).

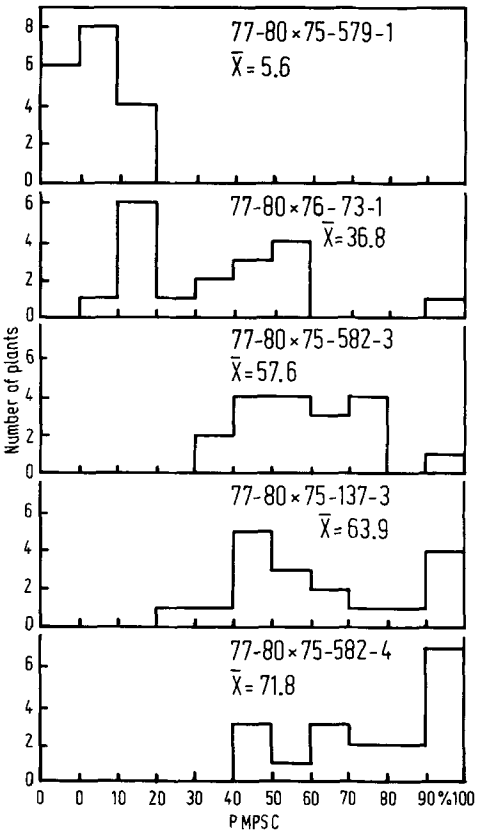

Fig. 2. Frequency distribution of percent pollen-mediated-pseudo-self-compatibility (% PMPSC) obtained by pollinating progeny 77-80 ($S_{1.1}$) with different $S_{1.1}$ pollen sources

The results of pollinating progeny 77-80 with $S_{1.1}$ pollen from plants which show different PSC levels demonstrated the effect pollen can have on the expression of PSC in these styles. The styles of 77-80 as well as 75-579-1 have the unique ability of permitting low seed set when incompatibly pollinated with pollen from a plant which is strongly SI and high seed set when pollinated with pollen from a high PSC plant. The ranking of these different crosses indicated that the PSC level of these $S_{1.1}$ plants, relative to high or low PSC can be determined by the seed set obtained when 77-80 is used as the seed parent.

Certain plants of progeny 77-80 tended to produce high PMPSC levels. These plants could be useful as seed parents for F_1 production involving crosses between $S_{1.1}$. parents. As a predictive tool, they would cause problems since they would not provide the differential needed to make selection effective. However, using plants which are close to the mean of the population 77-80 should be useful in predicting the relative strengths of $S_{1.1}$ plants. One plant showed 100% PMPSC regardless of the unrelated pollen source. This high level was attributed to the low outcross value obtained when crossed with 75-604-1 ($S_{2.2}$). This low seed set was probably due to sampling error.

The F_1 produced by crossing 75-579-1 (I_7) x 75-137-3 (I_4) was progeny 76-416 (Table 3). One of the three plants tested showed zero seed set when selfed and the other two plants showed low seed set. Backcross pollinating these plants with 75-579-1 resulted in zero seed set with all three plants, while two plants produced low seed set when pollinated with 75-137-3. Using a 100% PSC plant (75-582-4) as the pollinator produced higher seed set as compared with the 0% PSC sib (75-582-3). The overall average means of the seed produced when using the different pollinators showed that 75-582-4 produced three times as much seed as the SI pollen source 75-582-3, but the outcross to a different *S* allele produced about three times as much as the 100% PSC pollen source. These data indicated that the F_1 styles, like the parent, were able to demonstrate PMPSC among matched *S* allele pollinators.

An F_2 population was produced by bud pollinating the F_1 plant 75-416-1. Fifteen of the 20 plants tested were SI, while the remaining plants were distributed widely, with one plant averaging over 200 seeds per self pollination. The overall mean seed set was 23.8 seeds (Table 3). The occurrence in the F_2 of plants which produced low to moderate seed set levels would be expected based on the progeny tests of 75-137-3 (Flaschenriem 1978). It could also result from segregation of genes which would allow the *S* alleles of 75-137-3 pollen tubes to accomplish fertilization. Backcross pollinating the F_2 with grandparent 75-579-1 resulted in zero seed set from 19 of the plants with the other plant producing a mean seed set of less than 25 seeds (Fig. 3). The overall mean seed yield was 0.8 seeds (Table 3). This lack of seed set in all but one plant suggests that the F_2 plants could discriminate 75-579-1 as a pollen source which was not active in PMPSC. Backcross pollinating to the other grandparent (75-137-3) resulted in an overall mean seed set of 49.2 seeds with only four plants producing no seeds (Fig. 4). Using 75-582-3 as the pollinator resulted in no seeds from half of the plants and between 0 and 125 seeds per pollination from the remainder. The overall mean seed set was 28.0 seeds (Table 3). Crosses using 75-582-4 resulted in three plants which produced over 300 seeds when pollinated with this pollen source. However, six plants produced zero seed. The comparison of means of seed obtained from the F_2 population when pollinated with 75-582-3 was less than half the seed obtained when pollinated with 75-582-4. The F_2 population showed a discriminating ability of PMPSC similar to that of 75-579-1, 77-80 and the F_1. From the behavior of the F_1 and F_2 it appears

Table 3. The mean seed sets from self, compatible-cross and testcross pollinations of *Petunia* F_1 76-416 and F_2 population 77-604

		Pollen testers				
		$S_{1.1}$				$S_{3.3}$
Plant no.	Self	579-1	137-3	582-3	582-4	192-6
76-416-1	0.0	0.0	7.2	0.0	23.4	325.4
2	19.8	0.0	15.5	87.8	265.2	365.5
3	5.8	0.0	0.0	4.2	34.0	342.0
Means	8.5	0.0	7.6	30.7	107.5	344.3
77-604-1	0.0	0.0	31.4	0.0	33.2	173.0
2	0.0	0.0	0.0	0.0	0.0	227.0
3	0.0	0.0	62.0	16.6	45.2	261.8
4	0.0	0.0	37.6	0.0	95.0	234.8
5	0.0	0.0	2.4	0.0	0.0	264.8
6	0.0	0.0	43.6	0.0	0.0	289.0
7	0.0	0.0	27.0	17.4	219.8	318.0
8	68.4	15.2	143.6	67.4	129.8	304.0
9	0.0	0.0	28.8	59.8	10.0	274.0
10	0.0	0.0	0.0	0.0	30.2	228.5
11	0.0	0.0	0.0	0.0	0.0	179.0
12	140.6	0.0	158.2	41.8	236.4	314.5
13	218.8	0.0	195.2	102.2	106.4	347.8
14	0.0	0.0	20.8	41.8	165.8	354.8
15	22.6	0.0	127.2	102.0	223.4	316.6
16	0.0	0.0	57.4	0.0	0.0	305.6
17	25.0	0.0	9.6	22.2	21.8	305.0
18	0.0	0.0	6.0	0.0	37.4	386.2
19	0.0	0.0	34.0	89.0	45.0	384.8
20	0.0	0.0	0.0	0.0	0.0	198.8
Means	23.8	0.8	49.2	28.0	70.0	283.4

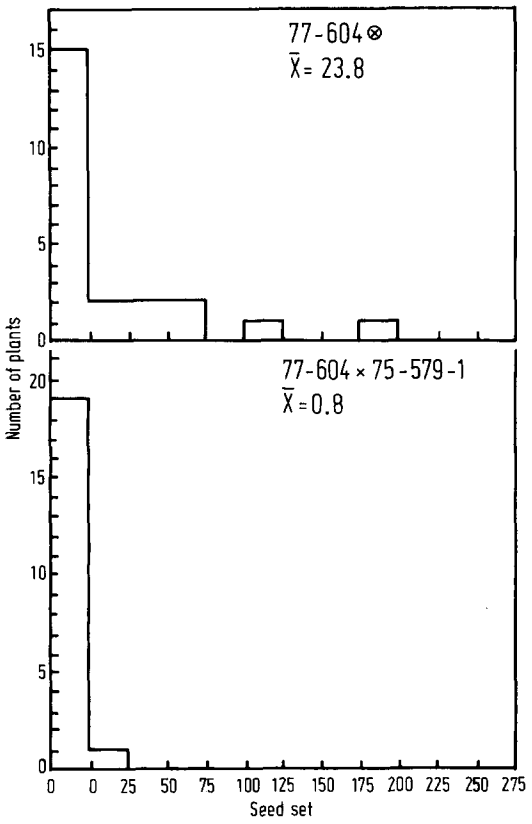

Fig. 3. Frequency distribution of self seed set of F_2 population 77-604 obtained from selfing an F_1 *Petunia* plant 76-416-1 and seed set from backcross pollinating the F_2 population by the grandparent 75-579-1

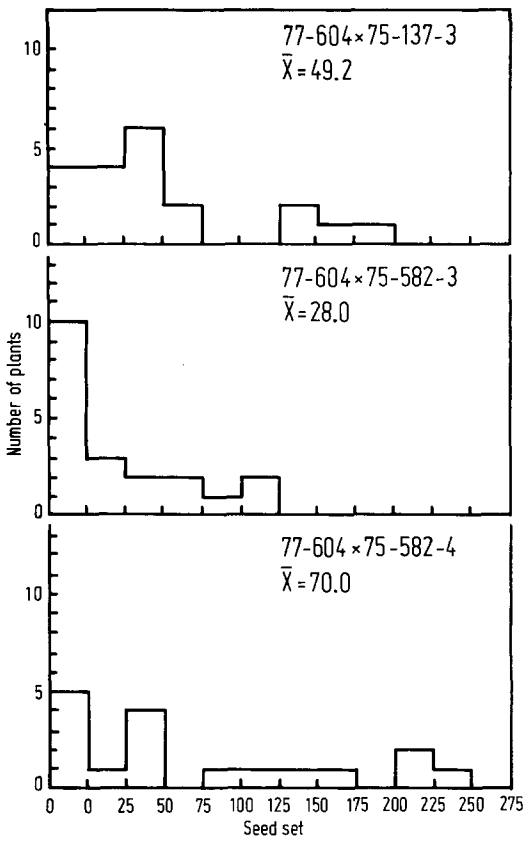

Fig. 4. Frequency distribution of seed set from backcross pollinating an F_2 *Petunia* population 77-604 by the pollen grandparent 75-137-3 and with plants 75-582-3 and 75-582-4

that the characteristic of discriminating PMPSC is a simply inherited trait which is dominant to non-discriminating.

The PSC levels expressed in *P. hybrida* show a stylar-conditioned response as measured using conventional plant materials. However, the SI reaction of 75-579-1, 77-80 and the F_1 and F_2 data indicated that these plants could measure a unique form of PSC, a form which is expressed by pollen. Since these plants show styles which discriminate among incompatible pollen tubes based on the pollen source, this difference in pollen tube growth must be due to gametophytically expressed modifiers in the pollen tubes. Discrimination among incompatible pollen tubes by these styles would increase progeny PSC levels because of gamete selection at the stylar level. The behavior of the pollen tubes of different S_1 sources on these styles provides evidence for non-S-allele, pollen tube-stylar interaction.

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