A Molecular Description of Mutations Affecting the Pollen Component of the *Nicotiana alata S* locus

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ABSTRACT

Mutations affecting the self-incompatibility response of *Nicotiana alata* were generated by irradiation. Mutants in the M_1 generation were selected on the basis of pollen tube growth through an otherwise incompatible pistil. Twelve of the 18 M_1 plants obtained from the mutagenesis screen were self-compatible. Eleven self-compatible plants had mutations affecting only the pollen function of the *S* locus (pollen-part mutants). The remaining self-compatible plant had a mutation affecting only the style function of the *S* locus (style-part mutant). Cytological examination of the pollen-part mutant plants revealed that 8 had an extra chromosome ($2n + 1$) and 3 did not. The pollen-part mutation in 7 M_1 plants was followed in a series of crosses. DNA blot analysis using probes for S-RNase genes (encoding the style function of the *S* locus) indicated that the pollen-part mutation was associated with an extra *S* allele in 4 M_1 plants. In 3 of these plants, the extra *S* allele was located on the additional chromosome. There was no evidence of an extra *S* allele in the 3 remaining M_1 plants. The breakdown of self-incompatibility in plants with an extra *S* allele is discussed with reference to current models of the molecular basis of self-incompatibility.

FERTILIZATION in flowering plants begins when a pollen grain bearing the male gametes lands on a female stigma. Several mechanisms enable the stigma and style to discriminate between the different types of pollen it may receive, the best studied being self-incompatibility. If a pollen grain from a self-incompatible plant lands on its own stigma, or on the stigma of a genetically related plant, the pollen either will fail to germinate or will germinate to produce a pollen tube that grows poorly in the style and does not reach the ovary (de Nettancourt 1977). In many cases, this process is controlled by a single, multiallelic locus called the *S* locus. In solanaceous plants such as *Nicotiana alata* (ornamental tobacco), the *S* locus acts gametophytically and a haploid pollen grain is rejected by a diploid style when the same *S* allele is present in both. The only known product of the solanaceous *S* locus is an extracellular ribonuclease produced by the style (the S-RNase; McClure *et al.* 1989). S-RNases control the stylar phenotype of self-incompatible plants but do not control the pollen phenotype (Lee *et al.* 1994; Dodds *et al.* 1999). This suggests the *S* locus is bipartite, with different genes encoding the pollen component (pollen-*S*) and the style component (S-RNase) of the *S* locus. The product of the pollen-*S* gene is not known. As part of a strategy to define the nature of this product, we have generated a series of pollen-part mutations of the *S* locus (pollen-part mutant, PPM).

In previous studies, mutations affecting the pollen component of the *S* locus have been generated in *N. alata* (Pandey 1965, 1967; van Gastel and de Nettancourt 1975), *Petunia inflata* (Brewbaker and Natarajan 1960), and *Solanum tuberosum* (Olsder and Hermsen 1976; Hermsen 1978). Styles of PPM plants retain the ability to reject incompatible pollen.

Different types of lesions can cause mutations affecting the self-incompatibility response of pollen. The majority of pollen-part mutations in solanaceous plants are associated with duplications of an *S* allele (Brewbaker and Natarajan 1960; Pandey 1965, 1967; van Gastel and de Nettancourt 1975). The duplicated *S* allele is frequently on a short, additional chromosome known as a centric fragment, which segregates independently of the *S* locus. The self-incompatibility phenotype of pollen from these plants is only altered when the duplicated *S* allele and the allele present at the *S* locus are different (Brewbaker and Natarajan 1960). This phenomenon is called competitive interaction and requires two different *S* alleles to be present in the plant. In addition, competitive interaction results in progeny of backcross and selfed families having two different *S* alleles (Pandey 1967; van Gastel and de Nettancourt 1975; see de Nettancourt 1977).

A lesion in the pollen-*S* gene causes the other type of mutation affecting the self-incompatibility response of pollen. These are "true" pollen-part mutations and can be distinguished from plants carrying duplicated *S* al-

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TABLE 1

Pollination responses of plants from the M_1 generation

	Cross[a]						Phenotype	
Plant	Self	$S_3S_6 \times M_1$	$S_2S_2 \times M_1$	$M_1 \times S_2S_2$	$M_1 \times S_3S_3$	$M_1 \times S_6S_6$	Pollen	Pistil
M1-1	+	+	+	+	−	−	PPM	S_3S_6
M1-2	+	+	+	+	−	−	PPM	S_3S_6
M1-3[b]	−	−					INC	S_3S_6
M1-4[b]	−	−					INC	S_3S_6
M1-5	+	+	+	+	+	−	PPM	S_6S_6
M1-6	+	+	+	+	−	+	PPM	S_3S_3
M1-7	+	+	+	+	−	+	PPM	S_3S_3
M1-8	−	−	+	+	−	+	INC	S_3S_3
M1-9	+	+	+	+	−	−	PPM	S_3S_6
M1-10	+	+	+	+	−	−	PPM	S_3S_6
M1-11	+	+	+	+	−	−	PPM	S_3S_6
M1-12	+	+	+	+	−	−	PPM	S_3S_6
M1-13	−	−	−	+	−	−	—[c]	S_3S_6
M1-14	−	−	+	+	−	−	INC	S_3S_6
M1-15	+	+	+	+	−	−	PPM	S_3S_6
M1-16	+	+	+	+	−	−	PPM	S_3S_6
M1-17	−	−	+	+	−	+	INC	S_3S_3
M1-18	+	−	+	+	−	+	INC	S_3S_3 SPM
WT	−	−	+	+	−	−	INC	S_3S_6

+, compatible pollination; −, incompatible pollination; WT, an unmutated S_3S_6 plant; PPM, pollen-part mutant; SPM, style-part mutant; INC, pollen incompatibility response was the same as a WT plant.

[a] The female plant is listed first.

[b] These plants were self-sterile but were not characterized any further.

[c] The pollen phenotype of M1-13 could not be determined because of low pollen viability.

leles because they may be homozygous for *S* alleles and can produce homozygous progeny following backcross or self-pollinations.

Because none of the *N. alata* PPM plants generated in previous studies were available, we generated PPM plants using the same strategy applied in earlier studies (Pandey 1967; van Gastel and de Nettancourt 1975). Following irradiation of S_3S_6 *N. alata* plants, 18 M_1 individuals were isolated and characterized by pollination, cytological examination of root tip cells, DNA blot analysis with S-RNase gene probes, and protein blot analysis with S-RNase-specific antibodies. Eleven plants had mutations affecting the pollen component of the *S* locus and inheritance of the pollen-part mutation in seven of these PPM plants was followed through a series of crosses. The nature of the mutation in these plants is discussed with reference to current models of self-incompatibility.

MATERIALS AND METHODS

Screen for pollen-part mutants: Mature *N. alata* plants (genotype S_3S_6) received a total dose of either 8 or 10 Gy from a ^{60}Co source (1.4 Gy/min) housed at the CSIRO Division of Plant Industry, Canberra, Australia. The target tissue was floral buds containing pollen mother cells (see Dodds *et al.* 1993). Of 149 buds irradiated, 108 received 8 Gy and 41 received 10 Gy. Following irradiation, the floral buds were labeled and the pollen was collected at anthesis. This was either used directly in pollinations or stored at −70° until needed. Whenever practicable, each pollen sample was used to pollinate two flowers from an unmutated S_3S_6 plant (*i.e.*, an incompatible pollination). Following each pollination, 1% indole-3-acetic acid in lanolin was applied to the base of the flower. At maturity, the capsules were opened and the seeds collected. Before germination, seeds were surface sterilized for 1 hr with a hypochlorite solution (1% HClO in 0.1% Tween) and rinsed thoroughly in sterile water. Seeds were then placed on sterile MS agar containing 3% sucrose and incubated at 22°. When sufficiently strong, each seedling was transplanted into soil and grown as described by Anderson *et al.* (1989).

Pollination analysis: Plants were self-pollinated by spreading pollen from a dehiscent anther over the stigmas of four or more flowers. A pollination was compatible if a large capsule developed and incompatible if the flower abscised in the week following pollination. To determine the stylar self-incompatibility phenotype of a plant, immature floral buds were emasculated and pollinated with pollen from a plant of known *S* genotype soon after petal opening. Four such pollinations were usually done for each plant. Similar crosses were used to determine the self-incompatibility phenotype of pollen from the plant.

DNA blot analysis: Genomic DNA was extracted from the leaves of *N. alata* plants as described by Bernatzky and Tanksley (1986). Leaf DNA (5 μg) was digested to completion with *Hin*dIII or *Bam*HI (Promega, Madison, WI). The DNA was fractionated on a 0.8% agarose gel run in 1× TBE and transferred to a nylon membrane (Amersham, Buckinghamshire, UK) as described by Sambrook *et al.* (1989). S-RNase cDNA fragments were radiolabeled with random primers (Primagene, Promega). Hybridization of the radiolabeled S-RNase cDNAs to the DNA blots was done in 50% formamide, 5×

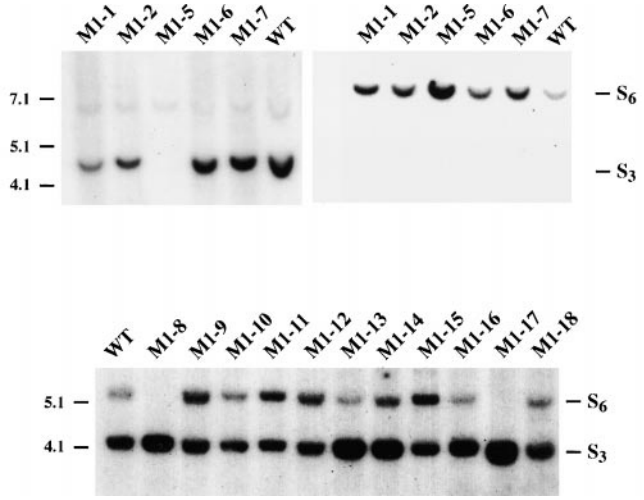

Figure 1.—DNA blot analysis of the M_1 plants. Each lane contains genomic DNA (5 μg) from the indicated M_1 plant or an unmutated S_3S_6 plant (WT). DNA was digested with *Bam*HI (top) or *Hin*dIII (bottom) and probed with the S_3-RNase cDNA and the S_6-RNase cDNA separately (top) or both the S_3- and S_6-RNase cDNAs (bottom). Molecular weight standards (in kilobases) are shown on the left of the figure and the identity of the S-RNase hybridizing bands is indicated at the right of the figure.

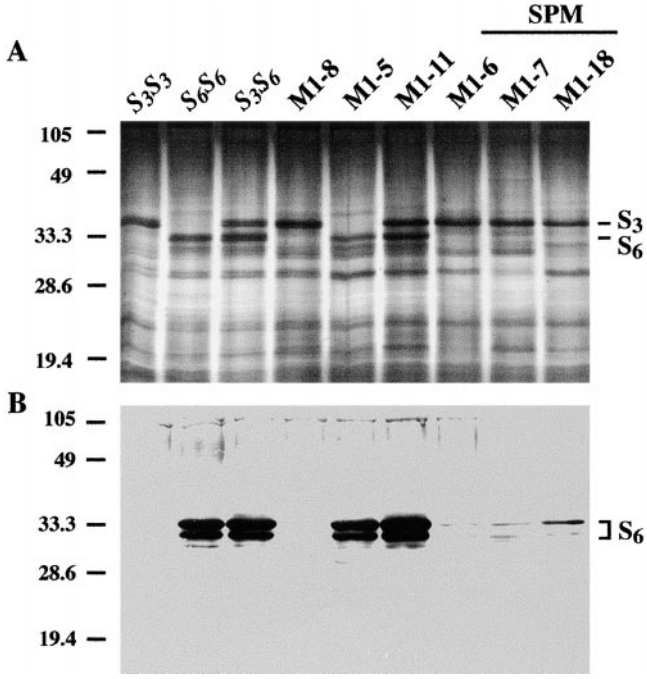

Figure 2.—S-RNase accumulation by the pistils of unmutated *N. alata* plants and selected M_1 plants. Buffer-soluble protein was extracted from pistils of the indicated M_1 plants and pistils of unmutated S_3S_3, S_6S_6, and S_3S_6 plants. M_1 plants that have an S_6-RNase gene but fail to reject S_6 pollen are indicated (SPM). Proteins were separated by SDS-PAGE and either stained with silver (A) or blotted onto nitrocellulose and incubated with an antiserum specific to the S_6-RNase (B). Protein bands corresponding to the S_3- and S_6-RNases (A) or the S_6-RNase (B) are indicated. Molecular weight standards (in kilodaltons) are shown at the left of the figure.

SSPE, 5× Denhardt's solution, 0.5% SDS, 50 μg/ml denatured herring sperm DNA at 42° for 12 hr. After hybridization, the membranes were washed twice (30 min each time) at 42° in 0.2× SSPE, 0.2% SDS and exposed to film.

Western blot analysis: Styles were collected and stored at −70°. Proteins were extracted from plant tissue in an extraction buffer (100 mm Tris-HCl, pH 8, 50 mm EDTA, 0.1% polyvinylpyrrolidone, 28 mm β-mercaptoethanol) to give a 25% solution. Protein concentrations were determined using a colorimetric assay (Bradford 1976) with BSA as a standard. Stylar proteins (15 μg) were fractionated on a 15% SDS-polyacrylamide gel according to the method of Laemmli (1970). Proteins were transferred to a nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) in transfer buffer (48 mm Tris-HCl; 39 mm glycine; 0.0374% SDS; 20% methanol) using a semidry electrophoretic transfer cell apparatus (Transblot, Bio-Rad, Richmond, CA). A replicate gel with 5 μg stylar proteins in each lane was stained with silver (Bio-Rad). The membrane was incubated with a protein G-purified sheep antiserum for the S_6-RNase (Dodds *et al.* 1993) as described by Harlow and Lane (1988). Bound antibodies were detected using biotinylated anti-sheep immunoglobulin and streptavidin-horseradish peroxidase (Amersham) according to the manufacturer's instructions.

Cytology: Root tips were collected from hydroponically grown plants. After harvest, the root tips were placed in a saturated solution of α-bromonaphthalene and incubated for 2 hr at room temperature with occasional agitation. Root tips were then washed with water and fixed in ethanol:acetic acid (3:1) for 12 hr at 4°. After fixation, the root tips were placed in a 70% ethanol solution and stored at 4° for up to 1 month before analysis. For cytology, fixed root tips were treated with 0.2 n HCl solution for 10 min at 55°. After acid hydrolysis, the root tips were washed with water and placed in a staining solution [2% synthetic orcein (Gurr) in 45% acetic acid] for 40 min. Root tips were destained in a solution of 45% acetic acid. Macerated root tips were spread and examined under phase contrast optics using a Zeiss Universal microscope. Images were captured with a Zeiss MC63 photographic unit using Tmax100 film (Kodak, Rochester, NY).

RESULTS

Production of the M_1 generation: Developing *N. alata* flower buds (genotype S_3S_6) were irradiated with either 8 or 10 Gy of γ-rays from a ^{60}Co source. Pollen was subsequently collected at anthesis and used to pollinate pistils of unmutated S_3S_6 plants. From 300 pollinations, only three capsules that contained seeds were recovered. Two capsules, containing 7 viable seeds, were produced following pollination with pollen irradiated with 8 Gy and one capsule, containing 11 seeds, was from pollen irradiated with 10 Gy. The seeds were germinated and the seedlings were grown to maturity. Plants were numbered M1-1 to M1-18. M1-1 to M1-4 came from one capsule, and M1-5 to M1-7 from the other capsule formed by pollen irradiated with 8 Gy. The remaining plants came from the capsule formed by pollen irradiated with 10 Gy. The plants grew normally and were not visibly different from nonmutagenized *N. alata* plants.

Twelve of the 18 M_1 plants formed large capsules after self-pollination and the remaining 6 plants were self-

TABLE 2

Summary of the pollination, DNA blot, stylar protein, and cytology analyses of plants from the M_1 generation

Plant	S phenotype		S-RNase genes	S-RNase proteins	Chromosome no.[a]	Type of mutation
	Pollen	Pistil				
M1-1	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-2	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-5	PPM	S_6S_6	S_6	S_6	$2n$	PPM
M1-6	PPM	S_3S_3	S_3 + S_6	S_3, S_6 (trace)	$2n$ + 1	PPM/SPM
M1-7	PPM	S_3S_3	S_3 + S_6	S_3, S_6 (trace)	$2n$	PPM/SPM
M1-8	INC	S_3S_3	S_3	S_3	$2n$ + 1	REV
M1-9	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-10	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$	PPM
M1-11	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-12	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-13	—[b]	S_3S_6	S_3 + S_6	S_3, S_6	$3n$ + 1	Polyploid
M1-14	INC	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	REV
M1-15	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1	PPM
M1-16	PPM	S_3S_6	S_3 + S_6	S_3, S_6	$2n$ + 1[c]	PPM
M1-17	INC	S_3S_3	S_3	S_3	$2n$ + 1	REV
M1-18	INC	S_3S_6 SPM	S_3 + S_6	S_3, S_6 (trace)	$2n$	SPM
WT	INC	S_3S_6	S_3 + S_6	S_3, S_6	$2n$	—

REV, revertant plant (see text); other abbreviations are defined in Table 1.
[a] $2n$ = 18 chromosomes; $2n$ + 1 = 18 chromosomes plus a centric fragment; $3n$ + 1 = 28 chromosomes.
[b] The pollen phenotype of M1-13 could not be determined because of low pollen viability (see Table 1).
[c] Cytology of a plant from the backcross family of M1-16 (see text).

sterile (see Table 1; data are incomplete for M1-3 and M1-4, which were self-sterile but were not characterized further). Crosses to *N. alata* plants of known *S* genotypes were used to characterize the pollen and pistil self-incompatibility phenotype of each M_1 plant (Table 1). Ten plants had pistils that rejected pollen from S_3S_3 and S_6S_6 plants, which indicated their pistil phenotype was S_3S_6; five plants had pistils that rejected S_3 pollen but accepted S_6 pollen and therefore had S_3S_3 as their pistil phenotype; and one plant rejected S_6 pollen but accepted S_3 pollen, which indicated its pistil phenotype was S_6S_6.

Capsules formed following the pollination of S_3S_6 pistils with pollen from 11 of the 12 self-fertile M_1 plants. This showed these plants carried mutations affecting the self-incompatibility phenotype of their pollen. Capsules did not form following similar pollinations using pollen from the self-fertile plant M1-18, indicating this plant carried a mutation affecting the self-incompatibility phenotype of its styles (a style-part mutant, SPM). M1-18 and 3 of the 4 self-sterile M_1 plants (M1-8, M1-14, and M1-17), produced viable pollen (capsules formed after pollination of a compatible S_2S_2 pistil). Interestingly, these plants did not have mutations affecting the self-incompatibility phenotype of their pollen, even though they were all grown from seed formed after an incompatible pollination. Presumably the normal self-incompatibility response of M1-8, M1-14, M1-17, and M1-18 pollen arose because the mutation that had allowed the pollen tubes to grow through an incompatible style "reverted" to an unmutated state after fertilization. The self-incompatible M_1 plants are therefore described as revertants (REVs). M1-13 did not produce viable pollen as no capsules formed after pollination of an S_2S_2 plant.

DNA blot analysis of M_1 plants: The *S* genotype of 16 M_1 plants was determined by DNA blot analysis using the S_3- and S_6-RNase cDNAs as probes (Figure 1). The S_3-RNase gene was present in all plants except M1-5 (S_6S_6). Similarly, the S_6-RNase gene was present in all plants except M1-8 and M1-17 (both S_3S_3). Thus, with the exception of M1-6 and M1-7 (both S_3S_3) and M1-18 (S_3S_3 SPM), the *S* genotype of the M_1 plants determined by DNA blot analysis matched the pistil phenotype.

Detection of S-RNases in the pistils of M_1 plants: To understand the discrepancy between the S phenotype and *S* genotype in plants M1-6, M1-7, and M1-18, the accumulation of S-RNases by the styles of these plants was assessed by SDS-PAGE and Western blot analysis. Buffer-soluble proteins were extracted from the styles (including stigmas) of unmutated plants (S_3S_3, S_6S_6, and S_3S_6), and from the indicated M_1 plants (Figure 2). Stylar protein was separated by SDS-PAGE and either stained with silver (Figure 2A) or transferred to a nitrocellulose membrane and incubated with an antiserum specific for the S_6-RNase (Figure 2B).

In Figure 2A, the S_3-RNase appeared as a band of M_r 35 kD and the S_6-RNase as a band of M_r 33 kD. In Figure 2B, the S_6-RNase appeared as a major band of 33 kD

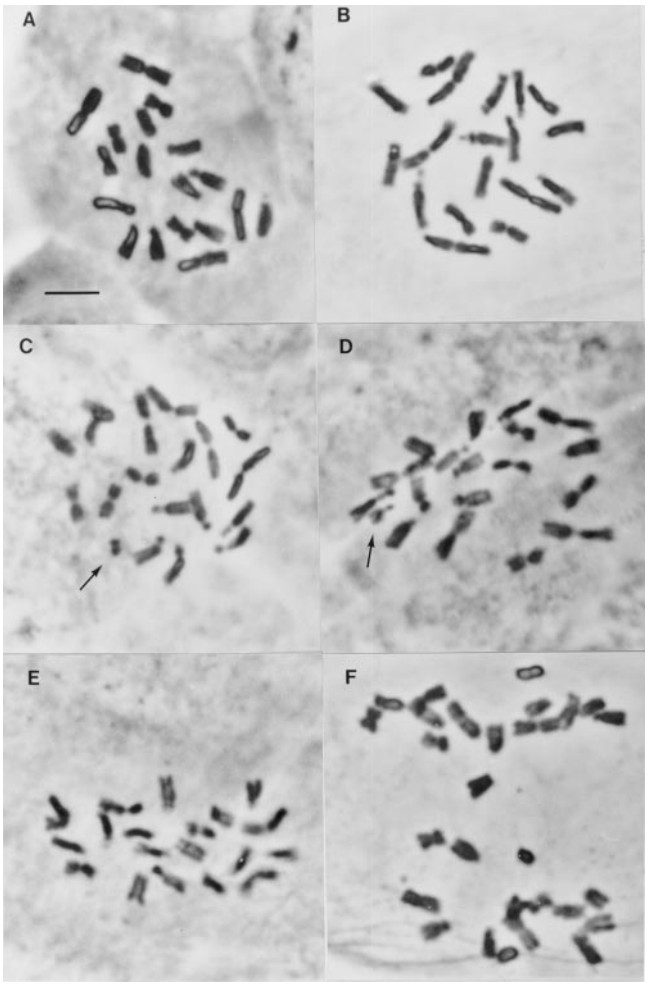

Figure 3.—Micrographs of metaphase chromosomes in the root tip cells of an unmutated S_3S_6 plant (A) and representative M_1 plants (B–F). The unmutated S_3S_6 plant (A) and plant M1-5(B) both contain 18 chromosomes. Nineteen chromosomes were seen in M1-1 (C), M1-6 (D), and M1-14 (E). The additional chromosome in M1-1 and M1-6 (C and D) was smaller than the rest (arrow). All chromosomes in M1-14 (E) were similar in size. Cells from M1-13 contained 28 chromosomes (F). Bar in A, 5 μm.

identical in size to the protein seen in Figure 2A. A second band of M_r 31.5 kD and a third, less abundant band of M_r 30 kD were also seen. The amount of S_3- and S_6-RNase extracted from styles of M1-11 and the other nine S_3S_6 M_1 plants (data not shown) was similar to that in the style of an unmutated S_3S_6 plant. Likewise, the amount of S_3-RNase in the style of M1-8 and M1-17 (both S_3S_3) and the amount of S_6-RNase in M1-5 styles (S_6S_6) was similar to that in unmutated S_3S_3 and S_6S_6 styles, respectively (Figure 2A and data not shown). However, although M1-6, M1-7, and M1-18 had the S_6-RNase gene, little or no S_6-RNase could be detected in their styles. It is likely that the level of S_6-RNase in these plants was less than that required to reject S_6 pollen (Table 1). All three plants are therefore SPMs with a lesion affecting the style-part of the S_6 allele. This allele will be referred to as S_6^{spm}. Plant M1-18 had this mutation alone; plants M1-6 and M1-7 had pollen-part mutations as well. The results of protein and Western analyses of all M_1 plants are summarized in Table 2.

Cytology: Mitotic chromosomes in the root tips of an unmutated S_3S_6 plant and all M_1 plants were stained with orcein and examined by phase-contrast microscopy. Typically, four root tips were examined from each plant and the number of chromosomes in at least four cells from each root tip was counted. Figure 3 shows representative examples of these cells. The results for each M_1 plant are summarized in Table 2. The cells of an unmutated *N. alata* plant and four M_1 plants contained 18 chromosomes (Figure 3, A and B), which is the expected number of chromosomes in this species. The morphology of individual chromosomes also matched an earlier description of *N. alata* chromosomes made by Carluccio *et al.* (1974).

Eleven of the M_1 plants contained 19 chromosomes (Table 2) and one M_1 plant (M1-13) contained 28 chromosomes (Figure 3F). In plants with 19 chromosomes, the additional chromosome was generally smaller than the other chromosomes and varied in length from 1 μm (for example, M1-1; Figure 3C) to 1.7 μm (for example, M1-6; Figure 3D). In plant M1-14, none of the chromosomes was noticeably shorter than the others, making it difficult to say which was additional (Figure 3E). When identifiable, the additional chromosome had a constriction indicative of a centromere. In keeping with the nomenclature used by earlier researchers, the additional chromosome will be referred to as a centric fragment.

Breeding analysis of four PPM M_1 plants that had a centric fragment: One hypothesis to account for the pollen-part mutation in the four PPM M_1 plants with a centric fragment is that the centric fragment in these plants carries a duplicated *S* allele. This hypothesis was tested by correlating the presence of a centric fragment with the S phenotype and *S* genotype of plants produced by backcrossing an M_1 plant to an unmutated S_3S_6 plant or outcrossing it to an unmutated S_2S_2 plant. In one case, a family produced by self-pollinating an M_1 plant was used instead of a backcrossed family. *S* genotypes were determined by DNA blot analysis using S-RNase cDNAs as probes. Table 3 summarizes the results of this analysis for plants M1-1, M1-2, M1-6, and M1-11.

All plants in the backcross family of M1-1 had S_3S_6 as their pistil phenotype and, with one exception, all were PPMs. The *S* genotype of five PPM plants from the backcross family was determined by DNA gel blot analysis and, as expected, both the S_3- and S_6-RNase genes were present. Cytological analysis of root tip cells from two PPM plants showed both plants contained 19 chromosomes, with the additional chromosome apparently identical to the centric fragment in plant M1-1 (Figure 3C).

Two types of plants were present in the outcross family

TABLE 3

Pollination, DNA blot, and cytology data for progeny derived from four M_1 PPM plants carrying a centric fragment

Cross[a]	S phenotype of progeny		No. of progeny	S-RNase genes[b]	Centric fragment[c]
	Pollen	Pistil			
S_3S_6 × M1-1	INC	S_3S_6	1	ND	ND
	PPM	S_3S_6	12	$S_3 + S_6$ (5)	2 (2)
S_2S_2 × M1-1	INC	S_2S_6	5	$S_2 + S_6$ (5)	0 (2)
	PPM	$S_2S_3S_6$	6	$S_2 + S_3 + S_6$ (5)	2 (2)
S_3S_6 × M1-2	PPM	S_3S_6	8	$S_3 + S_6$ (5)	1 (1)
	PPM	S_6S_6	10	S_6 (5)	1 (1)
S_2S_2 × M1-2	INC	S_2S_3	5	$S_2 + S_3$ (5)	1 (2)
	INC	S_2S_6	3	$S_2 + S_6$ (3)	0 (1)
	PPM	S_2S_6	1	$S_2 + S_6$ (1)	1 (1)
S_3S_6 × M1-6	PPM	S_3S_3 SPM	11	$S_3 + S_6$ (5)	1 (1)
	PPM	S_3S_6	9	$S_3 + S_6$ (5)	1 (1)
S_2S_2 × M1-6	INC	S_2S_3	5	$S_2 + S_3$ (5)	0 (2)
	INC	S_2S_2 SPM	4	$S_2 + S_6$ (4)	0 (2)
	PPM	S_2S_3 SPM	1	$S_2 + S_3 + S_6$ (1)	1 (1)
M1-11 self	PPM	S_3S_3 SPM	5	$S_3 + S_6$ (5)	1 (1)
	PPM	S_3S_6	10	$S_3 + S_6$ (10)	1 (1)
S_2S_2 × M1-11	INC	S_2S_6	26	$S_2 + S_6$ (26)	0 (2)
	INC	S_2S_2 SPM	10	$S_2 + S_6$ (10)	0 (2)
	PPM	$S_2S_3S_6$	2	$S_2 + S_3 + S_6$ (2)	1 (1)
	PPM	S_2S_3 SPM	3	$S_2 + S_3 + S_6$ (3)	3 (3)

ND, not determined; other abbreviations are defined in Table 1.
[a] The female plant is listed first.
[b] The number of progeny examined by DNA blot hybridization is indicated in parentheses.
[c] The number of progeny with a centric fragment (number of plants examined) is indicated.

of M1-1; self-incompatible S_2S_6 plants and PPM plants with an $S_2S_3S_6$ (triallelic) pistil phenotype. DNA blot analysis of five plants from each class confirmed the presence of the S_2- and S_6-RNase genes in the self-incompatible plants and the S_2-, S_3-, and S_6-RNase genes in the triallelic PPM plants (Figure 4A). Cytological analysis found a centric fragment in the root tip cells of two triallelic PPM plants but not in two self-incompatible S_2S_6 plants. Similar classes of plants were also found in families produced by outcrossing M1-1 to S_1S_1 and S_7S_7 plants (data not shown).

PPM plants with either S_3S_6 or S_6S_6 as their pistil phenotype were found in the backcross family of M1-2 (Table 3). DNA gel blot analysis detected both the S_3- and S_6-RNase genes in representative S_3S_6 plants but only the S_6-RNase gene in representative S_6S_6 plants (Figure 4B). Both types of PPM plant inherited the centric fragment present in plant M1-2.

Three types of plants were found in the outcross family of M1-2: self-incompatible plants, either S_2S_3 or S_2S_6, and an S_2S_6 PPM plant. The expected S-RNase genes were detected by DNA blot analysis in representatives of each type of plant (Figure 4C). The S_2S_6 PPM plant and at least one of the S_2S_3 self-incompatible plants inherited the centric fragment from M1-2 (Table 3). Other self-incompatible plants lacked centric fragments. Similar types of plants were also found in a family produced by crossing M1-2 to an S_1S_1 plant (data not shown).

S_3S_3 or S_3S_6 PPM plants were found in the backcrossed family of M1-6 and the selfed family of M1-11 (Table 3). The S_3- and S_6-RNase genes were detected in representative S_3S_6 and S_3S_3 plants from both families (Figure 4, D and E). This indicated some plants inherited an S_6^{spm} allele. As mentioned above, plant M1-6 had a mutation affecting expression of the S_6-RNase gene and the S_3S_3 PPM plants in the backcross family presumably inherited this mutation. SDS-PAGE and Western analyses of plants from the selfed family of M1-11 found trace levels of S_6-RNase in the pistils of S_3S_3 PPM plants (data not shown). This showed M1-11 had the S_6^{spm} allele and was both a pollen and a style-part mutant (SPM/PPM). Both S_3S_3 SPM/PPM and S_3S_6 PPM plants inherited centric fragments from M1-6 and M1-11 (Table 3).

Three types of plants were found in the outcrossed family of plant M1-6: self-incompatible S_2S_3 plants, S_2S_2 SPM plants, and a S_2S_3 PPM plant (Table 3). DNA blot analysis found the S_2- and S_6-RNase genes in the S_2S_2 SPM plants and S_2-, S_3-, and S_6-RNase genes in the S_2S_3 PPM plant. Both types of plants had presumably inherited the S_6^{spm} allele from M1-6. Cytological examination found a centric fragment in the PPM plant.

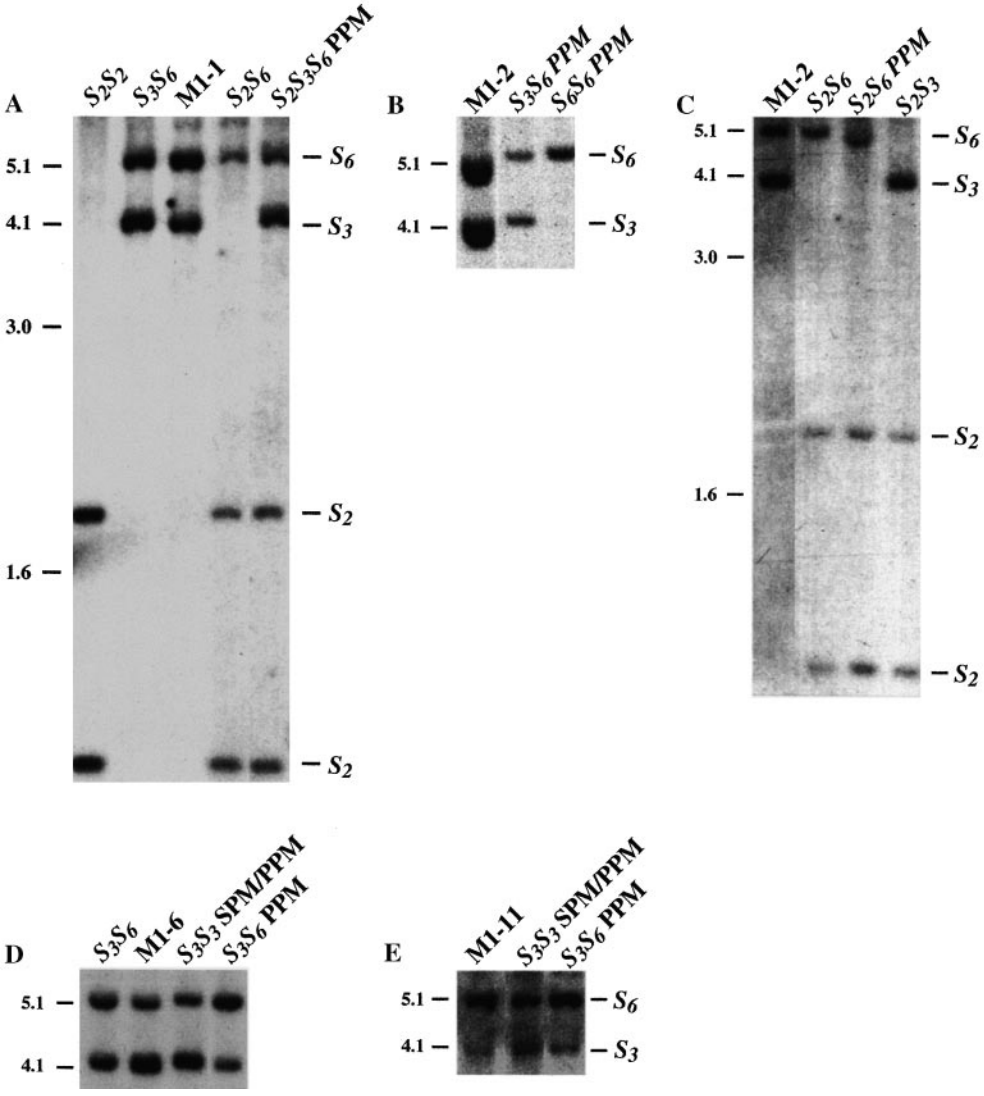

Figure 4.—DNA blot analysis of PPM plants with a centric fragment and representative progeny. Genomic DNA was isolated from the indicated plant, digested with *Hin*dIII and fractionated on an agarose gel. After transfer onto a nylon membrane, the blots were probed with ^{32}P-labeled S-RNase cDNA probes. (A) M1-1 and representatives of the phenotypic classes (self-incompatible S_2S_6 and $S_2S_3S_6$ PPMs) identified in the S_2S_2 outcross family (see Table 3). DNA from unmutated S_2S_2 and S_3S_6 plants is also shown. (B) M1-2 and representatives of the phenotypic classes identified in the S_3S_6 backcross family (S_3S_6 PPM and S_6S_6 PPM plants). (C) M1-2 and representatives of the phenotypic classes identified in the S_2S_2 outcross family (self-incompatible S_2S_3 and S_2S_6 plants and a S_2S_6 PPM plant). (D) M1-6 and representatives of the phenotypic classes identified in the S_3S_6 backcross family (S_3S_3 SPM/PPM and S_3S_6 PPM plants). (E) M1-11 and representatives of the phenotypic classes identified in the S_3S_6 backcross family (S_3S_3 SPM/PPM and S_3S_6 PPM plants). Molecular weight standards (in kb) are shown on the left of the figure and the S-RNase hybridizing bands are indicated at the right of the figure.

A similar range of pollination phenotypes was also present in the outcross family of plant M1-11, except that this family included self-incompatible S_2S_6 plants and $S_2S_3S_6$ PPM plants (Table 3). DNA blot analysis found the S_2- and S_6-RNase genes in the S_2S_6 self-incompatible and the S_2S_2 SPM plants and the S_2-, S_3-, and S_6-RNase genes in the S_2S_3 and $S_2S_3S_6$ PPM plants. The S_6^{spm} allele from plant M1-11 had therefore been inherited by the S_2S_2 SPM and S_2S_3 SPM/PPM plants. Cytological analysis found a centric fragment in the root tip cells of four PPM plants, but not in either the self-incompatible or SPM plants.

Breeding analysis of three PPM M_1 plants that lacked a centric fragment: One way to account for the pollen-part mutation in the three PPM plants that lack a centric fragment is to assume the mutation is caused by a lesion in the pollen-*S* gene. This hypothesis was tested by examining the S phenotype and *S* genotype of plants produced by backcrossing an M_1 plant to an unmutated S_3S_6 plant or outcrossing it to an unmutated S_2S_2 plant. *S* genotype was determined by DNA blot analysis using S-RNase cDNAs as probes. Table 4 summarizes breeding data for plants M1-5, M1-7, and M1-10.

PPM plants with S_3S_3, S_3S_6, and S_6S_6 pistil phenotypes were found in the backcross family of M1-5 (Table 4). DNA gel blot analysis detected the S_3- and S_6-RNase genes in S_3S_3 and S_3S_6 PPM plants, but only the S_6-RNase gene was present in S_6S_6 PPM plants (Figure 5A). SDS-PAGE and Western analyses found only trace amounts of the S_6-RNase in the pistils of S_3S_3 PPM plants, showing these plants had inherited an S_6^{spm} allele previously undetected in M1-5 (data not shown).

Self-incompatible S_2S_6 plants and PPM plants with either S_2S_2 or S_2S_6 as their pistil phenotype were found in the outcross family of M1-5 (Table 4). The S_2- and S_6-RNase genes were found in all plants tested (Figure 5B). The S_2S_2 PPM plant was presumably also an SPM and had inherited the S_6^{spm} allele from M1-5.

PPM plants with either S_3S_3 or S_3S_6 as their pistil phenotype were found in the backcross family of plant

TABLE 4

Pollination and DNA blot data for progeny derived from three PPM plants lacking a centric fragment

Cross[a]	S phenotype of progeny: Pollen	S phenotype of progeny: Pistil	No. of progeny	S-RNase genes[b]
S_3S_6 × M1-5	PPM	S_3S_3 SPM	3	$S_3 + S_6$ (3)
	PPM	S_3S_6	4	$S_3 + S_6$ (4)
	PPM	S_6S_6	13	S_6 (5)
S_2S_2 × M1-5	INC	S_2S_6	10	$S_2 + S_6$ (5)
	PPM	S_2S_2 SPM	1	$S_2 + S_6$ (1)
	PPM	S_2S_6	11	$S_2 + S_6$ (5)
S_3S_6 × M1-7	PPM	S_3S_3 SPM	8	$S_3 + S_6$ (5)
	PPM	S_3S_6	12	$S_3 + S_6$ (7)
S_2S_2 × M1-7	INC	S_2S_3	19	$S_2 + S_3$ (5)
	INC	S_2S_2 SPM	2	$S_2 + S_6$ (2)
S_3S_6 × M1-10	PPM	S_3S_6	8	$S_3 + S_6$ (8)
	PPM	S_6S_6	4	S_6 (2)
S_2S_2 × M1-10	INC	S_2S_3	9	$S_2 + S_3$ (5)
	PPM	S_2S_6	7	$S_2 + S_6$ (5)

[a] The female plant is listed first.
[b] The number of progeny examined by DNA blot hybridization is indicated in parentheses.

M1-7. As plants from both classes contained the S_3- and S_6-RNase genes, the S_3S_3 PPM plants presumably inherited the S_6^{spm} allele present in plant M1-7 (see above). Two types of plants were found in the outcrossed family of plant M1-7: self-incompatible S_2S_3 plants and S_2S_2 SPM plants (Table 3). No PPM plants were among the 21 plants examined. The S_2S_2 SPM plants in this family presumably inherited the S_6^{spm} allele and, consistent with this, DNA blot analysis found the S_2- and S_6-RNase genes in these plants.

S_3S_6 and S_6S_6 PPM plants were found in the backcross family of M1-10 (Table 4). DNA gel blot analysis detected the S_3- and S_6-RNase genes in the S_3S_6 PPM plants, but only the S_6-RNase gene in the S_6S_6 PPM plants (data not shown). Self-incompatible S_2S_3 and S_2S_6 plants and PPM plants with an S_2S_6 pistil phenotype were found in the outcross family of M1-10 (Table 4).

Breeding analysis of two revertant M_1 plants with a centric fragment: The revertant plants, M1-8 and M1-17, were crossed as the male parent to self-incompatible S_2S_2 plants. All progeny of these crosses were self-incompatible with an S_2S_3 pistil phenotype (Table 5). DNA gel blot analysis detected the S_2- and S_3-RNase genes in all the plants. The centric fragment was found in two of four plants examined from each family.

DISCUSSION

Our study of pollen-part mutations is part of a broader study aimed at identifying the pollen-*S* gene. Earlier examinations of pollen-part mutations relied on pollination behavior to identify *S* alleles in individual plants. A significant advance of this study was the availability of cDNA probes to identify a particular *S* allele and specific antibodies to identify S-RNase products of the *S* locus. This gives us an independent means of identifying *S* alleles and an opportunity to get a more precise description of the nature of the mutations.

Ionizing radiation causes chromosomal alterations such as inversions and deletions and has frequently been used to induce pollen-part mutations (de Nettancourt 1977). The classes of plants produced by mutagenesis in this study were similar to those described earlier (Pandey 1967; van Gastel and de Nettancourt 1975). Self-compatibility was mainly due to mutations affecting pollen phenotype (PPMs). The majority of PPMs expressed two *S* alleles (S_3 and S_6) in their pistil but a few expressed a single *S* allele (either S_3 or S_6). The proportion of homozygous to heterozygous PPMs seen in this study was also similar to that reported earlier. As observed in earlier studies, many plants had a centric fragment.

Style and pollen-part mutations are independent: We identified style-part mutations (SPMs) in three M_1 plants (M1-6, M1-7, and M1-18). Each plant had an S_6^{spm} allele and two had a pollen-part mutation as well (Table 2). Molecular analyses of plants in the M_2 generation also identified S_6^{spm} alleles in plants M1-5 and M1-11. Functional S_6 alleles masked the style-part mutations in these M_1 plants, which indicates the S_6^{spm} allele is recessive. In plants with an S_6^{spm} allele and no functional S_6 allele, low levels of stylar S_6-RNase were detected by Western blotting. The lesion in the S_6^{spm} allele is unknown but appears to act *in cis* and affects the level of expression of the S_6-RNase gene. However, our analysis of the M_2 families indicates the S_6^{spm} allele may not be completely penetrant and can in some instances become a functional S_6 allele. Breeding experiments showed that the pollen function

of the S_6^{spm} allele is normal and the mutation is independent of the pollen-part mutation. For example, 10 of the outcross progeny of M1-11 inherited the S_6^{spm} allele but not the pollen-part mutation (Table 3). Style-part mutations affecting the expression of the S_6 allele have also been noted in *N. alata* plants recovered from tissue culture (H. Du, A. E. Clarke and T. Bacic, personal communication). The S_6^{spm} allele in these plants arose in the absence of irradiation. It may well be that the S_6^{spm} allele described here also arose spontaneously in our stock lines and was not produced by irradiation. This mutation is not discussed further.

Evidence of a duplicated *S* allele in four M_1 plants: The inheritance of the pollen-part mutation in seven M_1 plants was studied. Four plants (M1-1, M1-6, M1-7, and M1-11) had a duplicated S_3 allele as judged by DNA blot analysis with S-RNase probes (Table 6) and the three remaining plants (M1-2, M1-5, and M1-10) did not (Table 7). We will discuss the pollen-part mutations in these two groups separately.

The outcross families of M1-1, M1-6, and M1-11 contained triallelic progeny, which indicates some of the pollen produced by these plants contained both the S_3 and S_6 alleles. For M1-1 and M1-11, the lack of S_2S_3 progeny in the outcross families showed that the S_3 allele was not at the *S* locus. The duplicated S_3 allele (dS_3) is therefore probably on the centric fragment as centric fragments were found in all triallelic progeny. On this basis, the *S* genotypes of M1-1 and M1-11 are S_6S_6 duplicated S_3 (S_6S_6dS_3) and $S_6S_6^{spm}$dS_3, respectively. The outcross progeny of M1-6 indicate that this plant has both the S_3 and S_6^{spm} alleles at its *S* locus. The duplicated allele in M1-6 is therefore either S_3 or S_6^{spm} and is associated with a centric fragment. The relative intensities of the S_3- and S_6-RNase hybridizing bands on blots of M1-6 DNA indicate $S_3S_6^{spm}$dS_3 as the most likely *S* genotype of M1-6 (Figure 4D).

The lack of PPM plants in the outcross family of M1-7 limited interpretation of the nature of the mutation in this plant. Like M1-6, DNA blot analysis indicated that all backcross progeny had both S_3 and S_6 alleles. Lack of segregation in the backcross family is indicative of a duplicated *S* allele (see below). Presumably the duplicated *S* allele is poorly transmitted through pollen unless selection is applied for the pollen-part mutation. The *S* genotype of M1-7 is the same as that of M1-6 as judged by S-RNase band intensities (data not shown). As M1-7 does not have a centric fragment, the duplicated S_3 allele must have been translocated to another chromosome.

In four M_1 plants pollen-part mutations arise through competitive interaction of *S* alleles: According to their *S* genotypes, M1-1 and M1-11 can produce either true haploid pollen containing the S_6 allele or S_6dS_3-containing pollen. An S_3S_6 pistil will reject S_6 pollen. If competitive interaction occurs, S_6dS_3-containing pollen will be compatible on an S_3S_6 pistil and all the progeny of

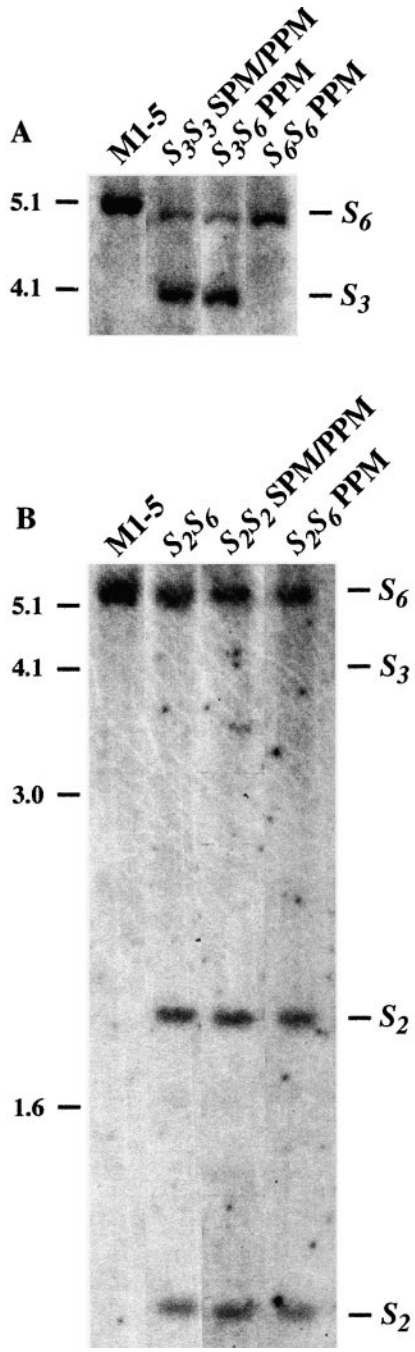

Figure 5.—DNA blot analysis of M1-5 and representative progeny. Genomic DNA was isolated from the indicated plant, digested with *Hin*dIII, and fractionated on an agarose gel. After transfer onto a nylon membrane, the blots were probed with ^{32}P-labeled S-RNase cDNA probes. (A) M1-5 and representatives of the phenotypic classes identified in the S_3S_6 backcross family (S_3S_3 SPM/PPM, S_3S_6 PPM, and S_6S_6 PPM plants). (B) M1-5 and representatives of the phenotypic classes identified in the S_2S_2 outcross family (self-incompatible S_2S_6 plants, S_2S_2 SPM/PPM, and S_2S_6 PPM plants). Molecular weight standards (in kb) are shown on the left of the figure and the S-RNase hybridizing bands are indicated to the right of the figure.

TABLE 5

Pollination, DNA blot, and cytology data for progeny derived from two REV plants

Cross[a]	S phenotype of progeny: Pollen	S phenotype of progeny: Pistil	No. of progeny	S-RNase genes[b]	Centric fragment[c]
S_2S_2 × M1-8	INC	S_2S_3	13	S_2 + S_3 (13)	2 (4)
S_2S_2 × M1-17	INC	S_2S_3	10	S_2 + S_3 (10)	2 (4)

[a] The female plant is listed first.
[b] The number of progeny examined by DNA blot hybridization is indicated in parentheses.
[c] The number of progeny with a centric fragment (number of plants examined) is indicated.

a backcross or self-pollination will be heterozygous. This was found experimentally.

M1-6 and M1-7 can produce true haploid pollen containing either S_3 or S_6^{spm} or pollen containing two *S* alleles, either S_3dS_3 or $S_6^{spm}dS_3$. An S_3S_6 pistil will reject all haploid pollen from M1-6 and M1-7. If competitive interaction occurs, only $S_6^{spm}dS_3$ pollen will be compatible on an S_3S_6 pistil and all the backcross progeny will have both S_3- and S_6-RNase genes. As this was observed, dS_3 must be able to interact with S_6^{spm} and not S_3 to produce a PPM phenotype. It is formally possible, as suggested by Pandey (1967), that the S_6^{spm} allele has a true pollen-part mutation and a second mutation linked to the S_6^{spm} allele that is lethal in pollen. According to this model, the role of the dS_3 allele is to complement the lethal mutation. However the $S_2S_6^{spm}$ progeny in the outcross family of M1-6 and M1-7 makes the presence of a lethal mutation near the S_6^{spm} allele unlikely.

Self-incompatibility models and competitive interaction: As our analysis of four M_1 plants led us to conclude that self-incompatibility breaks down in pollen grains containing dS_3 and either S_6 or S_6^{spm}, we sought to explain competitive interaction using current models of the molecular basis of self-incompatibility in the Solanaceae.

The two current models of self-incompatibility are the receptor model and the inhibitor model (Figure 6; McClure *et al.* 1989; Thompson and Kirch 1992). The receptor model proposes that the pollen product of the

TABLE 6

Summary of the genetics of four PPM plants carrying a duplicated S_3 allele

Plant	S phenotype	*S* genotype[a]	Cross[b]	Expected progeny[c]: S phenotype	Expected progeny[c]: *S* genotype	Observed progeny: S phenotype
M1-1	S_3S_6 PPM	$S_6S_6dS_3$	S_3S_6	S_3S_6 PPM	$S_3S_6dS_3$, $S_6S_6dS_3$	S_3S_6 PPM
			S_2S_2	S_2S_6	S_2S_6	S_2S_6
				$S_2S_3S_6$ PPM	$S_2S_6dS_3$	$S_2S_3S_6$ PPM
M1-6	S_3S_3 SPM/PPM	$S_3S_6^{spm}dS_3$	S_3S_6	S_3S_3 SPM/PPM	$S_3S_6^{spm}dS_3$	S_3S_3 SPM/PPM
				S_3S_6 PPM	$S_6S_6^{spm}dS_3$	S_3S_6 PPM
			S_2S_3	S_2S_3	S_2S_3	S_2S_3
				S_2S_2 SPM	$S_2S_6^{spm}$	S_2S_2 SPM
				S_2S_3 PPM	$S_2S_3dS_3$	Not found
				S_2S_3 SPM/PPM	$S_2S_6^{spm}dS_3$	S_2S_3 SPM/PPM
M1-7	S_3S_3 SPM/PPM	$S_3S_6^{spm}dS_3$	S_3S_6	S_3S_3 SPM/PPM	$S_3S_6^{spm}dS_3$	S_3S_3 SPM/PPM
				S_3S_6 PPM	$S_6S_6^{spm}dS_3$	S_3S_6 PPM
			S_2S_2	S_2S_3	S_2S_3	S_2S_3
				S_2S_2 SPM	$S_2S_6^{spm}$	S_2S_2 SPM
				S_2S_3 PPM	$S_2S_3dS_3$	Not found
				S_2S_3 SPM/PPM	$S_2S_6^{spm}$ dS_3	Not found
M1-11	S_3S_6 PPM	$S_6S_6^{spm}dS_3$	Self	S_3S_3 SPM/PPM	$S_6^{spm}S_6^{spm}dS_3$	S_3S_3 SPM/PPM
				S_3S_6 PPM	$S_6S_6dS_3$, $S_6S_6^{spm}dS_3$	S_3S_6 PPM
			S_2S_2	S_2S_6	S_2S_6	S_2S_6
				S_2S_2 SPM	$S_2S_6^{spm}$	S_2S_2 SPM
				S_2S_3 SPM/PPM	$S_2S_6^{spm}dS_3$	S_2S_3 SPM/PPM
				$S_2S_3S_6$ PPM	$S_2S_6dS_3$	$S_2S_3S_6$ PPM

[a] dS_3 denotes an additional S_3 allele. In M1-1, M1-6, and M1-11, dS_3 is on a centric fragment.
[b] In each case the M_1 plant was the staminate parent in a cross to the indicated pistillate parent.
[c] Expectations are based on the competitive interaction model (see text).

TABLE 7

Summary of the genetics of three PPM plants assuming the pollen-part of the S_3 allele has been duplicated

Plant	S phenotype	*S* genotype[a]	Cross[b]	Expected progeny[c]		Observed progeny: S phenotype
				S phenotype	*S* genotype	
M1-2	S_3S_6 PPM	$S_3S_6dS_3^p$	S_3S_6	S_3S_6 PPM	$S_3S_6dS_3^p$	S_3S_6 PPM
				S_6S_6 PPM	$S_6S_6dS_3^p$	S_6S_6 PPM
			S_2S_2	S_2S_3	S_2S_3	S_2S_3
				S_2S_6	S_2S_6	S_2S_6
				S_2S_3 PPM	$S_2S_3dS_3^p$	S_2S_3
				S_2S_6 PPM	$S_2S_6dS_3^p$	S_2S_6 PPM
M1-5	S_6S_6 PPM	$S_6S_6^{spm}dS_3^p$	S_3S_6	S_3S_3 SPM/PPM	$S_3S_6^{spm}dS_3^p$	S_3S_3 SPM/PPM
				S_3S_6 PPM	$S_3S_6dS_3^p$	S_3S_6 PPM
				S_6S_6 PPM	$S_6S_6dS_3^p$, $S_6S_6^{spm}dS_3^p$	S_6S_6 PPM
			S_2S_2	S_2S_6	S_2S_6	S_2S_6
				S_2S_2 SPM	$S_2S_6^{spm}$	Not found
				S_2S_2 SPM/PPM	$S_2S_6^{spm}dS_3^p$	S_2S_2 SPM/PPM
				S_2S_6 PPM	$S_2S_6dS_3^p$	S_2S_6 SPM
M1-10	S_3S_6 PPM	$S_3S_6dS_3^p$	S_3S_6	S_3S_6 PPM	$S_3S_6dS_3^p$	S_3S_6 PPM
				S_6S_6 PPM	$S_6S_6dS_3^p$	S_6S_6 PPM
			S_2S_2	S_2S_3	S_2S_3	S_2S_3
				S_2S_6	S_2S_6	Not found
				S_2S_3 PPM	$S_2S_3dS_3^p$	Not found
				S_2S_6 PPM	$S_2S_6dS_3^p$	S_2S_6 PPM

[a] dS_3^p denotes an additional pollen-part of the S_3 allele. In M1-2, dS_3^p is on a centric fragment.
[b] In each case the M_1 plant was the staminate parent in a cross to the indicated pistillate parent.
[c] Expectations are based on the competitive interaction (see text).

S locus (pollen-S) is a receptor that allows extracellular S-RNases to enter the pollen tube in an allele-specific manner (Figure 6A). Specific uptake of an active ribonuclease by the pollen tube causes an increased rate of RNA breakdown and an inability to synthesize protein. This leads to the dramatically slowed growth rate characteristic of incompatible pollen tubes (Lush and Clarke 1997).

The behavior of pollen-part mutations resulting from a duplicated *S* allele can be accommodated by the receptor model if it is assumed that the self-incompatibility response of pollen is critically dependent on the number of functional receptors. A pollen tube with two different *S* alleles will have fewer functional receptors than a pollen tube with a single *S* allele if the receptor (pollen-S) is a multimer and only homomeric forms of pollen-S are functional. However, heteromeric forms of pollen-S will occur only if the monomers encoded by different *S* alleles assort randomly.

The second model, the inhibitor model, proposes

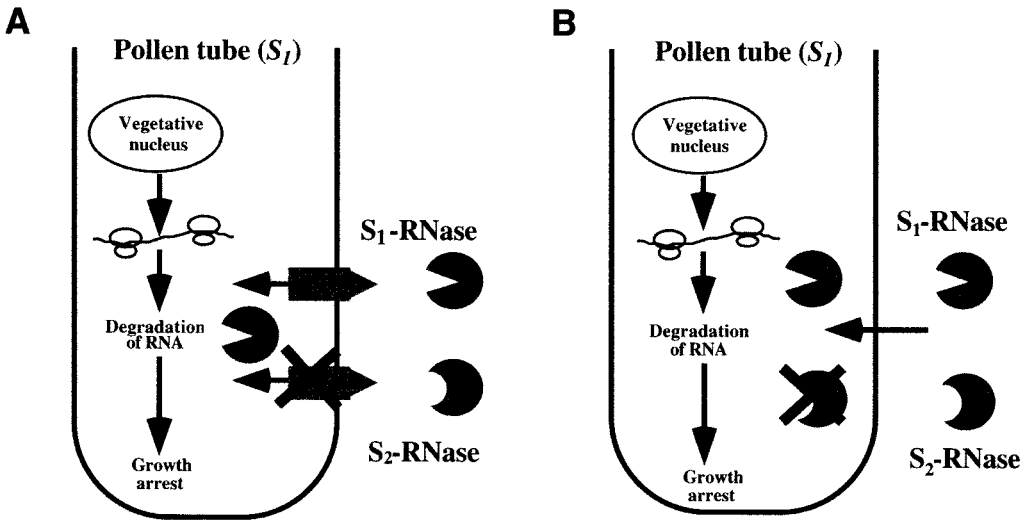

Figure 6.—Two models of events involved in inhibiting the growth of an S_1 pollen tube in an S_1S_2 style. (A) The receptor model; (B) the inhibitor model. See text for details.

that S-RNases enter pollen tubes nonspecifically (Figure 6B). Once inside, S-RNases encounter pollen-S, which is an inhibitor that can inactivate any S-RNases except those encoded by a matching *S* allele. According to this model, the inability of a pollen tube to detoxify matching S-RNases leads to increased rates of RNA degradation and consequently slowed growth. To explain why a pollen tube expressing two different *S* alleles can grow through an incompatible pistil, it is necessary to assume that the presence of two types of pollen-S inhibitor can inactivate all S-RNases, regardless of their allelic origin.

Although either model can explain competitive interaction, they make different predictions about the mutability of the pollen-*S* gene. According to the receptor model, pollen-part mutations could arise from deletions as well as duplications of the pollen-*S* gene. A pollen tube lacking the pollen-*S* gene would be unable to allow S-RNases to enter and thus would be able to grow through an incompatible style.

The inhibitor model, on the other hand, predicts that PPMs can arise only by duplication of an *S* allele as a pollen tube lacking the pollen-*S* gene is unable to detoxify any S-RNase. This makes mutations of the pollen-*S* gene lethal, as pollen tubes carrying these mutations are rejected by styles expressing S-RNases.

Using PPM plants to test the models of self-incompatibility: Identifying PPM plants that appear to lack a duplicated *S* allele is one way to test the two self-incompatibility models. In our study, three plants fall into this category: M1-2, M1-5, and M1-10. M1-5 is homozygous at the *S* locus, and the backcross families of M1-2 and M1-10 include both homozygous and heterozygous PPMs. There are no triallelic progeny in the outcross families of M1-2, M1-5, and M1-10. We might conclude that these plants have true pollen-part mutations and therefore support the receptor model. Certainly the presence of S_3S_6 and S_6S_6 plants in the backcross family of M1-2 suggests a true pollen-part mutation in the S_6 allele. However, there are some uncertainties, in particular the association between a centric fragment and the PPM phenotype.

M1-5 and M1-10 have no additional chromosome or other evidence of a duplication. The inheritance of the pollen-part mutation can be explained by assuming the mutation in these plants is linked to an S_6 allele. It is possible M1-5 and M1-10 carry true mutations in the pollen-part of the S_6 allele.

To date, the only attempt to understand PPMs at a molecular level has been by Thompson *et al.* (1991). Working with self-compatible dihaploid lines of *S. tuberosum*, bearing what appeared to be a translocation of an *S* allele, Thompson *et al.* (1991) used S-RNase gene probes and gels of stylar proteins to search for evidence of a duplicated *S* locus, but found none. On the basis of this lack of evidence, Thompson *et al.* (1991) concluded that the pollen-part mutation was caused either by the duplication of only a part of the *S* locus, or by a mutation in an allele-specific modifier locus unlinked to the *S* locus. Accordingly, it is possible that a duplicated pollen-part of the S_3 allele (dS_3^p), but not the style-part of the S_3 allele (the S_3-RNase gene), can account for the pollen-part mutations in M1-2, M1-5, and M1-10 (see Table 7). In M1-2, dS_3^p is presumably on the centric fragment. In M1-5 and M1-10, dS_3^p may be linked to an S_6 allele. Whether the lesion in M1-2, M1-5, and M1-10 is caused by a true mutation in the pollen-part of the S_6 allele or a duplication of the pollen-part of the S_3 allele is currently being investigated by DNA blot analysis using molecular markers linked to the *S* locus.

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