S-RNase-mediated self-incompatibility

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Abstract

The Solanaceae, Rosaceae, and Scrophulariaceae families all possess an RNase-mediated self-incompatibility mechanism through which their pistils can recognize and reject self-pollen to prevent inbreeding. The highly polymorphic S-locus controls the self-incompatibility interaction, and the S-locus of the Solanaceae has been shown to be a multi-gene complex in excess of 1.3 Mb. To date, the function of only one of the S-locus genes, the S-RNase gene, has been determined. This article reviews the current status of the search for the pollen S-gene and the current models for how S-haplotype specific inhibition of pollen tubes can be accomplished by S-RNases.

Key words: Pollen–pistil interactions, self-incompatibility, self/non-self recognition, S-locus, Solanaceae, S-RNase.

Introduction

In order to assure genetic variability, many flowering plants have evolved strategies to prevent inbreeding and promote out-crosses. One effective means is self-incompatibility (SI), which allows the pistil of a flower to reject self (genetically related) pollen, but to accept non-self (genetically unrelated) pollen for fertilization. SI is widespread in flowering plants, but to date, genes controlling SI have been identified from only five families (for a recent review, see McCubbin and Kao, 2000). In all these families, a single highly polymorphic genetic locus, called the S-locus, determines the outcome of pollination. Haploid pollen carrying an S-haplotype different from the two S-haplotypes carried by the diploid pistil can grow down the style to effect fertilization. By contrast, pollen carrying an S-haplotype identical to one of the two carried by the pistil suffers growth arrest in the style.

Among the five families, the Solanaceae, Rosaceae and Scrophulariaceae all use a highly polymorphic gene at the S-locus, called the S-RNase gene, to control the pistil’s ability to recognize and reject self-pollen. This S-RNase-mediated mechanism is the focus of this review, and unless otherwise stated, the discussion concerns the results obtained from the studies of the Solanaceae.

The S-locus: a multi-gene complex

The S-RNase gene

The S-RNase gene is the first S-locus gene identified in the Solanaceae (Anderson et al., 1986). The allelic products of the S-RNase gene co-segregate with S-haplotypes in genetic crosses, and cDNA for any of its allelic variants invariably exhibits an S-haplotype specific RFLP (restriction fragment length polymorphism) when used as a probe in genomic blotting analysis. The S-RNase gene determines the S-haplotype specificity of the pistil in SI interactions, as has been demonstrated by both gain-of-function and loss-of-function experiments (Lee et al., 1994; Murfett et al., 1994). Furthermore, the RNase activity of S-RNases is essential for their function in rejecting self-pollen (Huang et al., 1994), but the carbohydrate moiety of S-RNases is not required for their function in self/non-self recognition (Karunananda et al., 1994). The coding sequences of different alleles of the S-RNase gene are highly divergent, with the amino acid sequence identity ranging from as low as 38% to as high as 92%. There are two hypervariable regions, named HVa...
and HVb, in which deletions and insertions of nucleotides are found when different alleles are compared. These two regions are necessary (Kao and McCubbin, 1996; Zurek et al., 1997) and in some cases appear to be sufficient (Matton et al., 1997) for determining the S-allele specificity of S-RNases. Recently, the crystal structure of an S-RNase of Nicotiana alata was determined at 1.55 Å resolution (Ida et al., 2001). The structure has revealed that both HVa and HVb regions lie next to each other on the surface of the molecule, and that they are connected via a hydrogen bond network.

The pollen S-gene

Several lines of evidence support the existence of a separate gene that controls S-haplotype specificity in pollen. (1) In Lycopersicon peruvianum, neither expression of the S1-RNase gene in pollen of transgenic S1S2 plants, nor expression of an antisense S3-RNase gene in pollen of transgenic S1S2 or S2S3 plants altered the SI behaviour of the pollen of the transgenic plants (Dodds et al., 1999). (2) SI breaks down in pollen grains that carry two different pollen S-alleles, as a result of duplication of part or the entire S-locus; however, in some cases this duplicated region does not contain the S-RNase gene (Golz et al., 1999, 2000). (3) A self-compatible cultivar of Pyrus serotina (Japanese pear, a species of the Rosaceae) has the Sx-RNase gene deleted from its S-locus, and this deletion only affects the pistil function, but not the pollen function (Sassa et al., 1997).

Additional S-locus linked genes

In addition to the S-RNase gene and the pollen S-gene, genetic and molecular analyses have revealed that the S-locus also contains a number of tightly linked genes. Bernacchi and Tanksley (1997) carried out linkage analysis and QTL (quantitative trait linkage) mapping of an interspecific backcross of self-incompatible L. hirsutum × self-compatible L. esculentum, and showed that the S-locus of tomato harbours most major QTL for several floral traits (e.g. flower size and flower number). They propose that the S-locus gene complex evolved early during the evolution of flowering plants, and the linkage has been maintained for a long period of time. To date, none of the S-linked genes that control floral traits have been identified.

mRNA differential display and subtractive hybridization have been used to identify genes that are linked to the S-locus, based on the prediction that S-linked genes show allelic sequence polymorphism (Dowd et al., 2000; Li et al., 2000; McCubbin et al., 2000a). Recombination analysis was then used to assess the degree of linkage of these genes to the S-locus. No recombination was found for nine pollen-expressed genes of P. inflata from the analysis of more than 1000 F2 plants (Y Wang, X Wang, A McCubbin, T-h Kao, unpublished results) and no recombination was found for a pollen-expressed gene of N. alata from the analysis of 215 F2 plants (Li et al., 2000). For the P. inflata genes, even though they are very tightly linked to the S-locus, those which have been studied show very low allelic diversity in their deduced amino acid sequences (unpublished results).

Sequencing and expression analyses of a 76 kb region of the S-locus of the Brassicaceae that contains both male and female S-haplotype determinants revealed the presence of a dozen additional genes (Suzuki et al., 1999). These genes are expressed in anther and/or pistil, and five of them are specifically or predominantly expressed in the anther or pistil. It is interesting that even though the SI systems of the Solanaceae and Brassicaceae are completely different, both S-loci, in addition to containing the genes involved in SI, also contain other genes that are expressed in reproductive organs. This raises the possibility that the S-locus also controls the developmental processes of reproductive organs and/or the process of sexual reproduction. Thus, the S-locus appears to contain a complex of tightly linked genes that control both genetic and morphological mechanisms of reproduction. This is reminiscent of heteromorphic SI systems in which multiple S-locus-linked genes controlling morphological traits and genetic identity act together to result in SI responses (de Nettancourt, 1977).

Structural organization of the S-locus

Suppression of recombination

Since two separate polymorphic genes, the S-RNase gene and the pollen S-gene, control pistil and pollen S-haplotype specificity respectively, these two genes must be tightly linked and co-evolve as a genetic unit in order to maintain SI. Recombination at the S-locus could destroy this tight linkage, causing breakdown of SI by generating different S-haplotype specificities for the pollen and pistil. Classical genetic studies have never uncovered any evidence of recombination between pollen and pistil S-alleles (de Nettancourt, 1977). Moreover, the immediate flanking regions of the S-RNase gene show a very high degree of allelic sequence diversity and contain highly repetitive sequences (Kaufmann et al., 1991; Coleman and Kao, 1992; Chung et al., 1995; Matton et al., 1995). These results are consistent with a lack of recombination in this region of the S-locus. Suppression of recombination would allow S-haplotypes, once generated, to retain their identity for a long period of time.

Recent mapping results have suggested that the chromosomal location of the S-locus may be a major factor in the suppression of recombination. Bernacchi and Tanksley (1997) found that the S-locus of L. peruvianum is most strongly associated with a marker near the centromere of chromosome 1. ten Hoopen et al. (1998) and Harbord et al. (2000) mapped some T-DNA insertions and RFLP markers
to regions near the S-locus on chromosome III of *Petunia hybrida*, and found that those most tightly linked are located in a sub-centromeric region. Consistent with the mapping results, Entani *et al.* (1999) showed by fluorescence in situ hybridization that the S-locus of *P. hybrida* is located very near the centromere. Thus, the S-locus is located near a chromosomal region in which the rate of recombination is greatly reduced (Copenhaver *et al.*, 1998; Mahtani and Willard, 1998).

**Molecular cloning of the S-locus region**

A BAC library of the S<sub>2</sub>-S<sub>2</sub> genotype of *P. inflata* has been constructed and screened using cDNAs for the nine genes that are tightly linked to the S-locus and cDNA for the S<sub>2</sub>-RNase gene as probes (McCubbin *et al.*, 2000b). Interestingly, all except one of the BAC clones isolated contain only one of the S-linked genes. Collectively, the genomic DNA fragments contained in these BAC clones cover approximately 1.3 Mb. This large physical size is consistent with recombination suppression at the S-locus.

The approach of chromosome walking is being undertaken to isolate additional BAC clones in order to construct a BAC contig for the S-locus. This contig will allow the determination of the physical size of the S-locus, as defined by the chromosome region where recombination is suppressed. The BAC clones obtained can be used for sequencing and/or cDNA selection to identify additional genes located at the S-locus for subsequent study of their functions. The sequence information will also reveal the types and organization of repetitive sequence elements located at the S-locus and may shed light on the molecular basis for recombination suppression. Recently, a 63.7 kb region of the Antirrhinum S-locus containing the S<sub>2</sub>-RNase gene has been sequenced (Lai *et al.*, 2002). Of the six putative genes whose deduced amino acid sequences show homology with known proteins in the databases, four encode retrotransposons. The abundance of retrotransposons is also a characteristic of a 328 kb region of the *P. inflata* S-locus containing the S<sub>2</sub>-RNase gene (X Wang, T-h Kao, unpublished results).

**Approaches to identifying the pollen S-gene**

**Functional genomic approach**

Ultimate understanding of the molecular mechanism of the S-haplotype specific recognition and rejection of self-pollen tubes by S-RNases requires the identification of the pollen S-gene. One approach is to introduce flanking regions of the S-RNase gene of one S-haplotype into recipient plants and examine the SI behaviour of the pollen of the transgenic plants. This ‘functional genomic’ approach takes advantage of the competitive interaction phenomenon to determine whether a region contains the pollen S-gene. This phenomenon refers to the breakdown of SI in tetraploid plants that carry two different S-haplotypes (Lewis, 1947; Brewbaker, 1954; de Nettancourt, 1977). This is because pollen grains that carry two different pollen S-alleles fail to function in the SI interaction. This phenomenon has also been observed in pollen-part self-compatible mutants that contain a short additional chromosome (called a centric fragment) which bears at least part of the S-locus. Among the pollen grains produced by these self-compatible mutants, only those that inherit the centric fragment carrying a pollen S-allele different from the endogenous pollen S-allele fail to function in SI.

Because of the large physical size of the S-locus and because of suppression of intergenic recombination at the S-locus, the pollen S-gene could be at a large distance from the S-RNase gene. Thus, this functional genomic approach will be greatly facilitated by the use of BAC clones that contain large genomic DNA inserts (typically larger than 100 kb). The BIBAC2 vector used in the construction of the S<sub>2</sub>S<sub>2</sub> library of *P. inflata* is a binary Ti-plasmid based vector, so clones isolated from the library can be directly used for *Agrobacterium*-mediated transformation of plants (Hamilton *et al.*, 1996; Hamilton, 1997; Liu *et al.*, 1999).

The strategy for the functional genomic approach is outlined in Fig. 1. As stated above, pollen grains carrying two different pollen S-alleles fail to function in SI. So, if, for example, S<sub>1</sub>S<sub>2</sub> plants are transformed with BAC clones isolated from an S<sub>2</sub>S<sub>2</sub> library, the transgenic plants will be self-pollinated to see whether SI breaks down in any of them. If an S<sub>1</sub>S<sub>2</sub> transgenic plant carries a single copy of the pollen S<sub>2</sub>-transgene, it will produce four different genotypes of pollen grains. Among them, S<sub>1</sub>, S<sub>2</sub>, and S<sub>2</sub> plus the pollen S<sub>2</sub>-transgene should be incompatible with S<sub>1</sub>S<sub>2</sub> pistils. However, S<sub>1</sub> plus the pollen S<sub>2</sub>-transgene should be compatible with S<sub>1</sub>S<sub>2</sub> pistils because of competitive interaction. So, upon self-pollination of such a transgenic plant, only S<sub>1</sub> pollen with the pollen S<sub>2</sub>-transgene will be able to grow down the style to effect fertilization. This pollination will result in two different genotypes in the progeny, S<sub>1</sub>S<sub>1</sub>+pollen S<sub>2</sub>-transgene, and S<sub>1</sub>S<sub>2</sub>+pollen S<sub>2</sub>-transgene. That is, all the progeny should inherit the transgene. Also no S<sub>2</sub>S<sub>2</sub> genotype will be obtained in the progeny, and this will serve as a control for the breakdown of SI being due to competitive interaction.

**Pollen-part mutant approach**

The pollen-part mutants identified by Golz *et al.* (2001) have been used to delimit the region of the S-locus that contains the pollen S-gene. By examining the presence or absence of several S-linked genes in different duplicated S-locus fragments, Golz *et al.* (2001) have determined the order of these marker genes for the S<sub>3</sub>-haplotype, and placed the pollen S-gene between marker 48A and the S-RNase gene. Ushijima *et al.* (2001) have used a different approach to delimit the pollen S-gene in their study of a...
self-compatible cultivar of *Prunus dulcis* (almond, a rosaceous species) that is defective in both pollen and pistil functions. One of the causes for the breakdown of SI in this cultivar is the deletion of the *S*-locus of the *S*-haplotype. Although the chromosomal deletion extends beyond a 200 kb region containing the *S*-RNase gene, genomic DNA blotting analysis revealed that only ~70 kb of this region immediately flanking the *S*-RNase gene contains sequences that exhibit *S*-haplotype specific diversity. If one assumes that recombination is suppressed at the *S*-locus, but not in regions outside the *S*-locus, the pollen *S*-gene of the *S*-haplotype is likely to reside within this 70 kb region. This finding also suggests that the *S*-locus of almond, and perhaps other rosaceous species as well, may be much smaller than that of the Solanaceae.

**Direct sequencing of flanking regions of the S-RNase gene**

Another approach to identifying the pollen *S*-gene is determining the sequences of BAC clones or cosmid clones that contain the flanking regions of the *S*-RNase gene. In *Antirrhinum* (of the Scrophulariaceae family), a gene, named *SLF* (*S*-locus F-box), encoding an F-box-containing protein was found approximately 9 kb downstream from the *S*-RNase gene of *S*-haplotype (Lai *et al*., 2002). This gene is expressed in pollen and tapetum. Interestingly, in *P. inflata*, a similar gene was found downstream of the *S*-RNase gene of *S*-haplotype (X Wang, A McCubbin, S Huang, T-h Kao, unpublished results). Many F-box-containing proteins are components of SCF ubiquitin–ligase complexes which, together with ubiquitin-activating enzymes (E1s) and ubiquitin-conjugating enzymes (E2s), mediate protein degradation by the 26S proteasome (Bai *et al*., 1996). It will be interesting to determine whether the *SLF* gene is involved in SI, and if so, whether it is the pollen *S*-gene.

**Identifying S-RNase-interacting pollen proteins**

A fourth approach to identifying the pollen *S*-gene is finding pollen proteins that interact with S-RNases. However, to date, no pollen proteins that possess the expected properties of the pollen *S*-allele products have been identified by this approach. Interestingly, a pollen protein potentially involved in ubiquitin-mediated protein degradation has been found to interact with S-RNases of *P. hybrida* by the yeast two-hybrid assay (Sims and Ordanic, 2001). This protein, named PhSBP1, contains a RING-HC domain found in many proteins that function as E3 ubiquitin ligases. However, PhSBP1 is not specific to pollen, its interaction with S-RNases is not *S*-haplotype-specific, and its gene does not show any allelic sequence diversity. Thus, Sims and Ordanic (2001) suggest that if PhSBP1 is indeed involved in SI, it is more likely to play a general role, rather than being the determinant of pollen *S*-specificity.

**Models for S-haplotype specific inhibition of pollen tubes**

**Receptor and inhibitor models**

Two different types of models have been proposed to explain how S-RNases specifically inhibit the growth of self-pollen tubes. One predicts that the pollen *S*-allele products are receptors for S-RNases and the other predicts that the pollen *S*-allele products are inhibitors of S-RNases (Haring *et al*., 1990; Thompson and Kirch, 1992; Kao and...
McCubbin, 1996). Since the RNase activity of S-RNases is essential for their function in pollen rejection, both ‘receptor’ and ‘inhibitor’ models assume that growth inhibition of self-pollen tubes results from specific degradation of pollen tube RNAs by ‘self’ S-RNases. However, these two models differ in how S-haplotype-specific rejection is determined. According to the receptor model, the membrane or cell wall-bound receptors of pollen tubes function as specific gatekeepers to allow the S-RNase of the same S-haplotype (i.e. self S-RNase), but not S-RNases of different S-haplotypes (i.e. non-self S-RNases) to enter, and thereby only the growth of self-pollen tubes is inhibited. The inhibitor model predicts that all S-RNases enter pollen tubes, but the inhibitors residing in the cytoplasm of the pollen tubes inhibit the activity of non-self S-RNases, but not that of the self S-RNase.

Evidence supporting both models has been obtained. For example, when a mutant S$_3$RNs lacking the RNase activity was produced in S$_2$S$_3$ transgenic plants of *P. inflata*, the pistils of the transgenic plants rejected S$_2$ pollen completely, but failed to reject S$_3$ pollen completely (McCubbin et al., 1997). This dominant-negative effect could result from competition of the mutant S$_3$-RNase with the endogenous S$_3$-RNase, but not with the endogenous S$_2$-RNase, for binding to the receptor for S$_3$-RNase.

On the other hand, the phenomenon of competitive interaction is consistent with the inhibitor model. According to the inhibitor model, when a pollen grain carries two different pollen alleles, for example, S$_1$- and S$_2$-alleles, the S$_1$-allele product would inhibit the RNase activity of all S-RNases except S$_1$-RNase and the S$_2$-allele product would inhibit the RNase activity of all except S$_2$-RNase. So, together the two pollen S-allele products would inhibit the RNase activity of all S-RNases, and thus, the growth of this pollen tube in the style cannot be inhibited by any S-RNases.

Several lines of recent evidence suggest that the specificity of the SI interaction more likely lies in the inhibition of S-RNases, as predicted by the inhibitor model, and not in the uptake of S-RNases into pollen tubes, as predicted by the receptor model. First, S-RNases have been shown by immunolocalization to be present in the cytoplasm of both self and non-self pollen tubes growing in the style, suggesting that uptake of S-RNases into pollen tubes is not S-haplotype specific (Luu et al., 2000). In this work, a monospecific polyclonal antibody against S$_{11}$-RNase of *Solanum chacoense* was used to examine the localization of this RNase in S$_{11}$S$_{13}$ styles that had been pollinated with S$_{11}$, S$_{12}$ or S$_{14}$ pollen. The S$_{11}$-RNase was found to accumulate in pollen tubes of all three S-haplotypes. Second, the identification of PhSBP1, a RING-HC domain containing protein that interacts with S-RNases, suggests that degradation of S-RNases (and thus inhibition of S-RNases) may be a mechanism in controlling SI interactions (Sims and Ordanic, 2001).

**Models for S-haplotype-specific inhibition of S-RNases**

Several mechanisms have been proposed to explain how pollen S-allele products could specifically inhibit non-self S-RNases (Fig. 2). One mechanism (shown in Fig. 2A) postulates that the pollen-S allele products, like S-RNases, contain two separate functional domains, an S-allele-specific domain and an inhibitor domain. The S-allele-specific domain of a pollen S-allele product interacts specifically with the corresponding S-allele-specific domain of its cognate S-RNase, and the inhibitor domain...
interacts with the catalytic domain of all S-RNases (Kao and McCubbin, 1997). The interaction between a pollen S-allele product and its cognate S-RNase would be through the S-allele-specific domains of these two proteins, and this interaction would prevent the interaction between the inhibitor domain of the pollen S-allele product and the catalytic domain of the S-RNase. That is, the specific interaction between matching S-allele-specific domains is thermodynamically favourable over the interaction between the RNase-inhibitor domain and the RNase-catalytic domain (Kao and McCubbin, 1997).

An interesting mechanism was recently proposed based on the assumption that the pollen S-allele products only contain the S-allele-specificity domain and that a general RNase inhibitor is responsible for the inhibition of S-RNases (Luu et al., 2001; see Fig. 2B). The authors’ laboratory had previously found that one of the hybrid S-RNases engineered from S11- and S13-RNases of S. chacoense possessed a dual S-allele specificity in that it was able to reject both S11 and S13 pollen (Matton et al., 1999). They subsequently found that, whereas pollen grains carrying both S11- and S13-alleles were accepted by pistils producing S11- and S13-RNases, as expected from competitive interaction, these pollen grains were rejected by pistils producing the dual specificity S11/S13-RNase. According to the mechanism proposed by Luu et al. (2001), the general RNase inhibitor produced by the pollen tube would bind and inactivate all non-self S-RNases. However, in the case of the self S-RNase, this binding would be prevented because the binding between the matching S-allele specific domains of a pollen S-allele product and its cognate S-RNase is more thermodynamically favourable.

To explain the phenomenon of competitive interaction, Luu et al. (2001) further hypothesized that the active forms of the pollen S-allele products are homotetramers. In the pollen grain carrying two different pollen S-alleles (e.g. S11 and S13), the pollen S-allele products would mainly form heterotetramers, which could not efficiently block the binding of the general inhibitor to the catalytic domain of their respective cognate S-RNases (e.g. S11- and S13- RNases). As a result, pollen grains carrying S11- and S13-alleles would not be rejected by pistils producing S11- and S13-RNases, or by pistils producing any other S-RNases. However, the dual-specificity S-RNase, S11/S13-RNase, would still interact efficiently with the heterotetramer of the pollen S11- and S13-allele products, and as a result, the general inhibitor could not bind to the catalytic domain of this S-RNase, so the pollen grains would be rejected by the dual-specific S11/S13-RNase.

The simple and the modified inhibitor models predict completely contrasting SI phenotypes of pollen, if the pollen S-gene is defective. Based on the simple inhibitor model (Kao and McCubbin, 1997), mutations in the pollen S-gene would be ‘lethal’, because absence of a functional S-RNase inhibitor would render the pollen tube unable to inhibit the RNase activity of any S-RNases and thus be rejected by the pistil of any S-genotype. On the contrary, the modified inhibitor model predicts that mutations in the pollen S-gene would render its product unable to prevent the binding of the general inhibitor to the catalytic domain of any S-RNase, and thus pollen tubes would be accepted by the pistil of any S-genotype. There were early reports, based on genetic studies, of self-compatible mutants that were caused by mutations in pollen S-alleles (Pandey, 1965; Olsder and Hermansen, 1976). However, the validity of these findings has recently been called into question by the results of Golz et al. (1999, 2001). They used gamma ray irradiation to mutagenize immature pollen of N. alata and identified pollen-part mutants from a large number of progeny derived from pollination using the irradiated pollen. No pollen-part mutants with deletions of pollen S-alleles were found, and instead, all the mutations identified resulted from competitive interaction between two different pollen S-alleles. That is, these mutants contained duplication of part or the entire S-locus, with the duplicated region (either in a centric fragment or in a translocated fragment) containing an additional pollen S-allele. The authors estimated that the frequency of deletions of pollen S-alleles, if they did occur, was less than 0.000002%, which is many orders lower than that observed at several other loci of tomato when immature pollen grains were subjected to similar radiation treatment.

**Models for S-haplotype-specific degradation of S-RNases**

Recent identification of SLF and PhSBP1, two proteins with potential functions in ubiquitin-mediated protein degradation, as possible SI components has raised an intriguing possibility that it is not the RNase activity of S-RNases that is inhibited, but rather the stability of S-RNases that is controlled by SI interactions. At present, it is difficult to envisage how allele-specific rejection of S-RNases would require two proteins, one a potential E3 ligase and the other a potential component of an E3 ligase complex. Because the SLF gene is located at the S-locus and specifically expressed in pollen and tapetum, it is likely to be involved in SI. SLF could be a general inhibitor of S-RNases, and the interaction between the matching S-allele-specific domains of a pollen S-allele product and its cognate S-RNase would prevent the binding of SLF to the self S-RNase. SLFs could be the product of the pollen S-alleles. In this case, an SLF could interact with self and non-self S-RNases differently, so that only non-self S-RNases would be degraded by the 26S proteasome.

**Conclusion**

Since the first report of the cloning of an allele of the S-RNase gene in 1986 by Anderson et al., a vast amount of
information has been obtained on the structure and function of S-RNases. Recent progress in genetic and physical mapping of the S-locus and cloning and sequencing of large regions of the S-locus has yielded valuable information about the structural organization of the S-locus and revealed a pollen-expressed gene that could potentially be involved in SI. BAC clones containing various S-locus regions could be used in transformation experiments to identify any additional genes that may be involved in SI or other reproductive processes.

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