The Molecular Genetics of Self-incompatibility in *Petunia hybrida*

T. P. ROBBINS*, R. M. HARBORD, T. SONNEVELD and K. CLARKE

Plant Science Division, School of Biological Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

Received: 21 July 1999 Accepted: 20 October 1999

The cultivated petunia (*Petunia hybrida*) has been a popular system in which to study genetic, physiological and biochemical aspects of gametophytic self-incompatibility. As with other members of the Solanaceae a number of S-RNase genes have been isolated for functional S-alleles. We have identified S-RNase sequences for two additional functional S-alleles, S₁ and S₂. These alleles are more similar to alleles from other families of the Solanaceae (*Nicotiana* and *Solanum*) than to any petunia alleles reported previously. The total number of S-alleles in *P. hybrida* is at least ten in spite of its cultivated origin. However, most cultivars of *P. hybrida* are in fact self-compatible and this appears to arise from the prominence of a single previously described allele S₀. The implications of this observation for the origin of self-compatibility in *P. hybrida* are discussed. The S-locus of *P. hybrida* has recently been mapped using an indirect method involving T-DNA insertions. Seven T-DNA insertions that were previously shown to be closely linked to the S-locus were physically mapped on the long arm of chromosome III using fluorescent in-situ hybridization. The most tightly linked T-DNA insertions are in a sub-centromeric position. This is consistent with the centric fragments of *P. inflata* obtained by irradiation mutagenesis that carry additional S-loci and confer a pollen-part mutant phenotype. An S-linked restriction fragment length polymorphism (RFLP) marker, CP100 was used to confirm this chromosomal assignment and has provided evidence for S-locus synten in the Solanaceae.

© 2000 Annals of Botany Company

**Key words:** *Petunia hybrida*, petunia, self-incompatibility, self-compatibility, S-RNase, PCR, T-DNA.

**INTRODUCTION**

Over the past 30 years the cultivated petunia, *Petunia hybrida*, has contributed to many aspects of self-incompatibility research. The large flowers are amenable to controlled pollinations and provide sufficient anther and pistil material for biochemical analyses. The contributions of *P. hybrida* to both genetic and biochemical studies have been reviewed previously (Ascher, 1984). In this review, recent molecular genetic studies will be discussed in relation to the earlier findings. The contribution of *P. hybrida* will be placed in the context of the major advances in our understanding of S-ribonuclease (S-RNase) based incompatibility systems in other Solanaceae, and in particular the wild relative *P. inflata*.

*P. hybrida* is believed to have been derived in the early part of the last century from hybridization of two or more wild petunia species (Sink, 1984). Molecular fingerprinting (Peltier et al., 1994; Cerny et al., 1996) has supported the view that major contributions were from a purple flowered species (probably *P. integrifolia*) and a white flowered species (*P. axillaris* or *P. parodi*). The former species are invariably self-incompatible outbreeders whereas the latter species can be either self-incompatible or self-compatible (Tsukamoto et al., 1999). Although a number of self-incompatible cultivars of *P. hybrida* have been identified (Linskens and Straub, 1978; Ascher, 1984; this paper) the predominant condition is self-compatibility with abundant seed set on self-pollination.

As with all other solanaceous species studied to date, the self-incompatibility in *P. hybrida* is gametophytic with a single multiallelic S-locus. Polymorphic pistil proteins were found to be associated with the S-alleles originally described by Linskens and Straub (Rambo and Jackson, 1986). These were subsequently purified and N-terminal sequences were obtained for three alleles S₁, S₂ and S₃ (Broothaerts et al., 1989). These sequences showed homology with the C1 conserved domain of pistil S-proteins previously identified in *Nicotiana alata* and *Lycopersicon peruvianum* (Ioerger et al., 1991). These proteins therefore show all the properties anticipated of S-ribonucleases (S-RNases) that have now been extensively characterized in the Solanaceae (reviewed in Kao and McCubbin, 1996).

The first S-RNase cDNA sequences reported in *P. hybrida* were obtained by screening cDNA libraries with an oligonucleotide probe based on a conserved region (McClure et al., 1989). These S-proteins therefore show all the properties anticipated of S-ribonucleases (S-RNases) that have now been extensively characterized in the Solanaceae (reviewed in Kao and McCubbin, 1996).

The first S-RNase cDNA sequences reported in *P. hybrida* were obtained by screening cDNA libraries with an oligonucleotide probe based on a conserved region (Clark et al., 1990). They reported S-RNase sequences for three alleles also named S₁, S₂ and S₃, but obtained from an independent source (Ascher, 1984). A comparison of the putative mature S-RNase sequences encoded by these alleles confirms that they are distinct from the Linskens alleles previously characterized by Broothaerts (Fig. 1). In
expression for self-incompatibility (Dodds et al., 1994). These findings are similar to those in immature anthers for the S but not the transcriptase-polymerase chain reaction (RT-PCR) in very low levels of expression were detected using reverse transcriptase-polymerase chain reaction (RT-PCR) in immature pistils, before the S-RNase has accumulated to sufficient levels, and this allows the genera-

by pollinating immature pistils, before the S-RNase has

in ovaries. The accumulation of stylar mRNA is consistent with the onset of self-incompatibility as revealed previously (Lee, Singh and Kao, 1992). Additional allele specific primers were subsequently used to isolate full coding region sequences by 5′ rapid amplification of cDNA ends-polymerase chain reaction (5′RACE-PCR) as described by Xue et al. (1996).

The full amino acid sequences predicted for the S1 and S3 S-RNase precursors are aligned in Fig. 3. Both S-RNase sequences contain the conserved domains C1–C5 typical for S-RNase sequences from the Solanaceae (Ioerger et al., 1991). The S3 sequence is most closely related to S1 from Nicotiana alata (67% identity) and the most similar allele in P. hybrida is S3 (51% identity; Ai et al., 1992). The S3 sequence is most closely related to S1 from Solanum chacoense (66% identity) and the most similar allele in P. hybrida is again S3 (58% identity). The higher level of interspecific rather than intraspecific similarity is an established feature of many S-RNase sequences in the Solanaceae (Ioerger, Clark and Kao, 1990).

The predicted amino acid sequence of S1 can be compared with the N-terminal sequence for the purified S1 protein published previously (Broothaerts et al., 1989). The predicted molecular weight (22.1 kD) is in approximate agreement with that reported

addition they are all distinct from the three S-RNase sequences reported for the wild species P. inflata (Ai et al., 1990). Two additional S-RNase sequences have been reported in P. hybrida, S1 and S3, the former of which appears to be non-functional (Ai, Kron and Kao, 1991; Ai, Tsai and Kao, 1992). There may be as many as ten distinct S-alleles in P. hybrida cultivars suggesting a fairly complex breeding history involving more than two parent individuals.

Expression analysis in P. hybrida at the protein level showed that the Linskens S2 glycoprotein accumulates in the top half of the pistil during the latter stages of floral bud expansion (Broothaerts et al., 1989). The accumulation of corresponding mRNAs was studied using the Ascher S5–S3 alleles by Clark et al. (1990). They showed that all three alleles were highly expressed in styles only weakly in ovaries. The accumulation of stylar mRNA is consistent with the onset of self-incompatibility as revealed previously in Nicotiana alata (Cornish et al., 1987). As in other Solanaceae, it is possible to overcome self-incompatibility by pollinating immature pistils, before the S-RNase has accumulated to sufficient levels, and this allows the generation of homozygous S-allele stocks.

In addition to the anticipated expression in the pistil, very low levels of expression were detected using reverse transcriptase-polymerase chain reaction (RT-PCR) in immature anthers for the S3, but not the S2 RNase (Clark and Sims, 1994). These findings are similar to those in N. alata (Dodds et al., 1993), and recent studies in Lycopersicon peruvianum call into question the relevance of these low levels of expression for self-incompatibility (Dodds et al., 1999). The pollen-part mutations described in P. inflata (Brewbaker and Natarajan, 1960) and the inability to alter pollen phenotype using S-RNase transgenic approaches (Lee, Huang and Kao, 1994) is consistent with the view that the pollen determinant of self-incompatibility (pollen-S) is controlled by a separate, as yet unidentified, gene at the S-locus.

**IDENTIFICATION OF TWO NOVEL S-RNAS IN SELF-INCOMPATIBLE P. HYBRIDA**

We have studied two self-incompatible petunia cultivars, one (T1U) homozygous for the S3 allele (Linskens and Straub, 1978), the other cultivar (V13) homozygous for a previously uncharacterized allele provisionally called S5 (Harbord, Napoli and Robbins, 2000). To further characterize these alleles a degenerate PCR cloning procedure was adopted to identify S-RNase sequences expressed in pistils. A degenerate primer based on the C2 conserved domain of S-RNases from the Solanaceae (Ioerger et al., 1991) was used to amplify partial S-RNase sequences by RT-PCR of pistil RNA (Xue et al., 1996). Several identical cloned PCR products with homology to other S-RNases were obtained for each allele.

Confirmation that these cDNA sequences corresponded to the S5 and S3 RNases was obtained by designing allele specific primers based on the hypervariable domain and the 3′ untranslated region. These were used for genomic PCR analysis of a small family that segregated for both alleles (Fig. 2). Allele specific PCR products were found to cosegregate perfectly with the S-genotypes determined by test pollination. In a more extensive analysis of 90 progeny from the same cross, the two allele specific PCR products segregated as alleles of the same locus (Clarke and Robbins, data not shown). These findings suggest that the cloned sequences represent functional alleles and not S-like RNases that are unlinked to the S-locus such as X2 in P. inflata (Lee, Singh and Kao, 1992). Additional allele specific primers were subsequently used to isolate full coding region sequences by 5′ rapid amplification of cDNA ends-polymerase chain reaction (5′RACE-PCR) as described by Xue et al. (1996).

**FIG. 1.** A comparison of N-terminal sequences reported for S-RNase genes of P. hybrida. The N-terminal sequences of the Linskens alleles reported by Broothaerts et al. (1989) are aligned with those of the Ascher alleles derived from cDNA sequences (Clark et al., 1990). The amino acids conserved between the C1 domain of all the Solanaceae S-RNases (Ioerger et al., 1991) are indicated with asterisks. Also included are the S1 and S3 alleles reported by Ai et al. (1992), the S5 allele reported by Broothaerts et al. (1991) and the S3 sequence reported here. Allele names in parentheses are those proposed to avoid future confusion.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Source</th>
<th>N-terminal sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Ascher</td>
<td>SFHDWQVLTVWPAGYCKVKG</td>
</tr>
<tr>
<td>S2</td>
<td>Ascher</td>
<td>NFDFYQQLTVWTPASCYRPR</td>
</tr>
<tr>
<td>S3</td>
<td>Ascher</td>
<td>NFDFYQQLTVWTPASCYRPR</td>
</tr>
<tr>
<td>S1 (S5L)</td>
<td>Linskens</td>
<td>DFDYQMVLTVWTPASFYXPRR</td>
</tr>
<tr>
<td>S2 (S3L)</td>
<td>Linskens</td>
<td>YFEYMQLVLTVWPPFAXKRX</td>
</tr>
<tr>
<td>S3 (S3L)</td>
<td>Linskens</td>
<td>EFELLQVLTVWTPASFYXKRG</td>
</tr>
<tr>
<td>Sx</td>
<td>Ai et al.</td>
<td>DFDYQMVLTVWTPASFYXPRR</td>
</tr>
<tr>
<td>So</td>
<td>Ai et al.</td>
<td>AFDHQLVLTVWTPASFYXKRG</td>
</tr>
<tr>
<td>Sb</td>
<td>Broothaerts</td>
<td>AFDHQLVLTVWTPASFYXKRG</td>
</tr>
<tr>
<td>Sv</td>
<td>this paper</td>
<td>YFEYMQLVLTVWPPFAXKRX</td>
</tr>
</tbody>
</table>

* *******
Robbins et al.—Self-incompatibility in Petunia

FIG. 2. S-RNase allele specific PCR products cosegregate with S-phenotypes in *P. hybrida*. A small family of plants segregating for the *S*<sub>y</sub> and *S*<sub>3</sub> alleles were generated by crossing an *S*<sub>y</sub>*S*<sub>y</sub> stock with pollen from an *S*<sub>y</sub>*S*<sub>3</sub> stock. The progeny were genotyped by test pollination with the *S*<sub>y</sub>*S*<sub>y</sub> stock and found to segregate 1:1 for *S*<sub>y</sub>*S*<sub>y</sub> and *S*<sub>y</sub>*S*<sub>3</sub> as anticipated. Progeny determined to carry the *S*<sub>y</sub> or *S*<sub>3</sub> allele are indicated together with the results obtained from two different allele specific genomic PCR reactions. A 500 bp product from *S*<sub>y</sub> allele specific PCR was found to cosegregate with the *S*<sub>y</sub> phenotype (upper panel) and a 450 bp product from the *S*<sub>3</sub> allele specific PCR with the *S*<sub>y</sub> phenotype (lower panel).

Methods: Genomic DNA was prepared from 11 genotyped plants as described elsewhere (Harbord et al., 2000) and approx. 30–50 ng was used for genomic PCR. Genomic PCR was carried out in a manufacturer’s buffer with 3 mM MgCl<sub>2</sub>, 0.2 mM dNTPs and 0.5 units Taq polymerase (Bioline UK, Ltd) for 35 cycles of: 94°C 30 secs, specified annealing temperature 30 secs, 72°C 60 secs. The *S*<sub>y</sub> allele specific primers (V13-F1 5’ GGACGAAGCTGATTGTAAGGG and V13-R1 5’ CGATTTTCATATATTGGC) were used at 0.1 M and 45°C annealing temperature. The *S*<sub>3</sub> allele specific primers (PhS3-F1 5’ CACTACATTCGCGGGTAAGATGCTC and PhS3-R1 5’ CGCATGTATCACTTTGACGACAGG) were used at 0.2 μM and 60°C annealing temperature.

previously after deglycosylation (25-1 kD; Broothaerts et al., 1991). The *S*<sub>y</sub> allele was known to be functionally distinct from *S*<sub>y</sub> and *S*<sub>3</sub> in test pollinations but it was not clear how it was related to other previously described *P. hybrida* S-alleles. Based on the predicted sequence of the C1 region it is possible that *S*<sub>y</sub> is equivalent to the *S*<sub>2</sub> allele from Linskens allowing for some ambiguous amino acid sequences. Unfortunately we have not been able to obtain any *S*<sub>y</sub> stocks and we prefer to identify *S*<sub>y</sub> as a novel S-RNase until shown otherwise. It is worth pointing out that the *S*<sub>y</sub> and *S*<sub>3</sub> alleles of Ascher also appear identical in this region yet they are known to be functionally distinct (Dana and Ascher, 1986) and have different cDNA sequences (Clark et al., 1990).

To avoid future confusion between the *S*<sub>y</sub>*S*<sub>3</sub> alleles described by Linskens and Straub (1978) and the *S*<sub>y</sub>*S*<sub>3</sub> alleles from Ascher that were sequenced by Clark et al. (1990) it seems desirable to introduce a suffix, L, as shown in Fig. 1. Since this report contains the first full length sequence for one of Linskens’s alleles we propose that this allele should subsequently be called *S*<sub>y</sub>L. It is also important in any sequence comparisons to distinguish the *P. hybrida* alleles from the structurally distinct alleles of *P. inflata* that are also called *S*<sub>y</sub>*S*<sub>3</sub> (Ai et al., 1990). During the preparation of this manuscript two additional *P. hybrida* S-RNase sequences were submitted to the European Molecular Biology Laboratory (EMBL) database (accession numbers AB016522, AB016523). These amino acid sequences are identical to the *S*<sub>y</sub> and *S*<sub>3L</sub> alleles described in this report although they are presumably derived from distinct cultivars.

MOLECULAR BASIS OF SELF-COMPATIBILITY SUGGESTS A COMMON ORIGIN

In spite of its contribution to self-incompatibility research, most cultivars of *P. hybrida* are in fact self-compatible. Self-compatibility in *P. hybrida* has been studied by Ascher and colleagues and has been reviewed previously (Ascher, 1984). Ascher uses the term pseudo-self-compatibility (PSC) to describe both physiological and genetic causes for a breakdown in self-incompatibility. In some cases there is complex polygenic control and intermediate levels of seed set are observed. In at least one case it has been shown that PSC is not a result of reduced expression of an S-RNase (Clark et al., 1990). Ascher also describes lines with 100% PSC or rather full self-compatibility and in one case this arises from a loss of pollen function (Dana and Ascher, 1986). Interestingly, this line seems to lack a centric fragment which is characteristic of the pollen-part mutants arising from duplications described in *Petunia inflata* (Brewbaker and Natarajan, 1960). The mutation appears to be tightly, but not absolutely, linked to the S-locus.

The numerous self-compatible cultivars of *P. hybrida* developed for commercial and research purposes offer a diverse source of self-compatible stocks. In a previous study a commercial *F*<sub>1</sub> hybrid was crossed to self-incompatible
P. inflata to test the genetic basis of the self-compatibility (Ai et al., 1991). Two distinct S-alleles derived from the P. hybrida parent, Sv and S, were identified in progeny by SDS-PAGE analysis. All progeny carrying the Sv allele were self-compatible whereas progeny carrying the S allele were either self-compatible or self-incompatible. This suggested that the Sv allele is non-functional and the Sx allele is conditionally functional perhaps due to the segregation of modifier genes. Subsequently cDNA clones were obtained for both S-RNases (Fig. 1) and both alleles were self-compatible and the remainder were either fully self-compatible (27.7%) or partially self-incompatible (20.0%) setting intermediate levels of seed (Harbord et al., 2000). The latter class may be equivalent to the pseudo-self-compatible types described previously by Ascher (1984). Intermediate levels of seed set were also observed in the progeny of P. hybrida x P. inflata crosses that carry the Sx allele (Ai et al., 1990).

To analyse the segregation of S-alleles in this backcross two approaches were utilized. The first approach was indirect, using an S-linked RFLP marker CP100 which suggested that all self-incompatible progeny were homoygous for the Sv-linked allele of CP100 (Harbord et al., 2000). This suggested that the Sv allele may be non-functional and this was tested further by the cloning of an S-RNase from the V26 line (Robbins, unpubl. res.). Using the same approach described above for the functional alleles Sv and S, 3’RACE-PCR was used to amplify sequences that contain the conserved C2 domain from pistil RNA of the self-compatible V26 cultivar. Three independent clones with S-RNase homology were identical in size bands in full length published S sequence and found to amplify identical size bands in Sv and S (data not shown). These
primers were used to monitor the segregation of the $S_0$ allele in the backcross progeny described above. All the fully self-incompatible progeny were $S_0/S_0$ whereas the fully self-compatible progeny were $S_0/S_v$ (Fig. 4). The partially self-incompatible progeny were $S_0/S_v$ (data not shown) which was consistent with the genotyping using the CP100 marker (Harbord et al., 2000). This confirms that the $S_0$ allele is non-functional as reported by Ai et al. (1990), and suggests that unlinked modifiers are also segregating that weaken the self-incompatibility in the $S_0/S_v$ homozygotes.

The finding that the same $S_0$ allele is present in two apparently unrelated commercial stocks prompted us to conduct a survey of commercial cultivars and research stocks. Using the allele specific primers designed for $S_0$, we obtained genomic PCR products of the expected size for 12 out of 15 commercial $F_1$ hybrids tested and 13 out of 16 research stocks obtained from the Free University of Amsterdam (data not shown). This suggests that the $S_0$ allele is present in approx. 80% of existing research stocks and commercial cultivars and is probably a major contributor to the self-compatibility observed in many $P. hybrida$ varieties propagated today. However, it cannot be excluded that modifier loci are also present and preliminary data suggest that at least two inbred lines other than V26 (W80 and W115) show backcross segregations consistent with modifiers and polygenic control (Robbins and Harbord, unpubl. res.). The self-compatibility of V26 and other cultivars may therefore represent a compound effect of the $S_0$ allele and other unlinked compatibility factors. It is also possible that self-compatible cultivars carry functional $S$-alleles that are rendered inactive due to such modifiers as proposed for the original source of the $S_0$ allele (Ai et al., 1991).

It is interesting to speculate on why the $S_0$ allele should predominate in self-compatible varieties (Fig. 5). One possibility is that the self-compatible allele was present in the original crosses between wild species that gave rise to $P. hybrida$. Indeed, $P. axillaris$, which is believed to be one of the parental species, is frequently self-compatible in the wild (Tsukamoto et al., 1999). This is in contrast to $P. integrifolia$ which has been proposed as the other major species contributing to $P. hybrida$ (Peltier et al., 1994; Cerny et al., 1996), which is an obligate outbreeder. It will be interesting to see whether the $S_0$ allele is present in any of these natural populations of $P. axillaris$. An alternative possibility is that the self-compatibility arose as a result of hybridization. Some evidence to support this view is that the $S_0$ allele is expressed at high levels, has all the structural features of an S-RNase, and most significantly has been shown to have ribonuclease activity (Ai et al., 1992). These findings are consistent with the view that the S-RNase of the $S_0$ allele is fully functional, and whilst RNase function is not the sole prerequisite for stylar expressed S-gene function in the Solanaceae, the self-compatibility is most likely to result from a mutation affecting pollen-S function.

A similar situation may apply in $Nicotiana sylvestris$ which is exceptional amongst self-compatible $Nicotiana$ species in having an apparently functional S-RNase (Golz...
Phylogenetic analysis places the *N. sylvestris* S-RNase with other functional S-RNases and the authors proposed the term 'relic S-RNase'. The self-compatibility of *N. sylvestris* may also result from a mutation in pollen-S. In contrast to this, a rare self-compatible accession in *Lycopersicon peruvianum* revealed an active site mutation that abolishes ribonuclease activity (Royo et al., 1994). This is consistent with the observation that S-RNases with a modified active site are also inactive in transgenic *Petunia inflata* (Huang et al., 1994). It will be interesting to examine whether pollen-part mutations are a frequent cause of self-compatibility in other cultivated species such as *L. esculentum.*

**CYTOGENETIC AND GENETIC MAPPING OF THE S-LOCUS IN P. HYBRIDA**

Until relatively recently the cytological position of the S-locus in *P. hybrid* was uncertain. There was a single report of linkage between the *Grandiflora/Undulata* locus on chromosome V and the S-locus based on segregation distortion (de Nettancourt, 1977). The seven chromosomes of *P. hybrid* can be divided into three classes based on centromere position (Smith, Oud and de Jong, 1973). The chromosomes are amenable to direct gene localisation by fluorescent *in-situ* hybridization (FISH) as demonstrated by ten Hoopen et al. (1996). However, the ability to map S-RNase genes directly by FISH has been hampered by the small target size (ten Hoopen and Robbins, unpubl. res.). A much larger target is provided by a T-DNA integrated into the genome and several transgenes have been localized previously on chromosome II (ten Hoopen et al., 1996). Recently, several T-DNA insertions have been mapped close to the S-locus of *P. hybrid* using a segregation distortion assay (Harbord et al., 2000). These T-DNA insertions offered an opportunity to map the S-locus by an indirect method.

In collaboration with colleagues at the University of Amsterdam we have localized seven of these T-DNA insertions by fluorescent *in-situ* hybridization (ten Hoopen et al., 1998). All seven S-linked T-DNA insertions were localized to the long arm of chromosome III. The two T-DNA insertions most tightly linked to the S-locus (within 2 cM) were shown to be within 20% of total chromosome length of the centromere whereas more loosely linked T-DNAs had a more distal location. This suggests that the S-locus is located in the vicinity of the centromere, a finding consistent with a class of self-compatible mutants generated by irradiation in *P. inflata* (Brewbaker and Natarajan, 1960). These mutants carry 'centric fragments', small centromere-bearing chromosome segments that confer additional S-specificity to otherwise normal diploid plants (see Golz et al., 2000).

An independent confirmation that the S-locus of *P. hybrid* maps to chromosome III was obtained using an anonymous potato RFLP marker, CP100. This RFLP marker had previously been shown to map genetically to the same position as a potato S-RNase gene (Gebhardt et al., 1991). Similarly, in *P. hybrid* this RFLP marker was shown to cosegregate with S-phenotypes in a testcross family of 48 individuals (Harbord et al., 2000). In a different mapping population the CP100 marker was found to cosegregate with a peroxidase isozyme locus (PrxA) previously mapped to chromosome III (ten Hoopen et al., 1998). Consistent with this localization was the observation that S-linked T-DNAs were loosely linked to a leaf visual marker, yg3 previously assigned to chromosome III. These observations are not in agreement with the reported linkage between the S-locus and the *Undulata* mutation on chromosome V. However, this was based on segregation distortions that were subsequently called into question by Reimann-Phillip (de Nettancourt, 1977).

In other members of the Solanaceae, the S-locus has been assigned to a chromosome genetically. Thus the S-locus of *Lycopersicon peruvianum* was assigned to chromosome I by segregation distortion (Tanksley and Loaiza-Figueroa, 1985) and the S-RNase of *Solanum tuberosum* was placed on the homeologous chromosome by RFLP analysis (Gebhardt et al., 1991). There is evidence of synteny around the S-locus in the Solanaceae, the CP100 marker is S-linked in petunia (Harbord et al., 2000) and potato (Gebhardt et al., 1991), and an anodal peroxidase is linked to the S-locus in both petunia (ten Hoopen et al., 1998) and potato (Bennatzy and Tanksley, 1986). Evidence is thus gathering in support of the view that no major rearrangement of the S-locus region has occurred during speciation of the Solanaceae although local rearrangements sufficient to inhibit recombination between alleles cannot be excluded.

During the preparation of this manuscript it was reported that a *P. hybrid* S-RNase gene has been localized directly by FISH at, or very near to, the centromere of chromosome III (Entani et al., 1999). Genomic clones of the S-RNase revealed that the gene was embedded in repetitive sequences present at the centromere of all petunia chromosomes. A centromeric localization for the S-locus opens up the possibility that recombination around the S-locus may be suppressed by centromeric heterochromatin. Recombination is likely to be suppressed at the S-locus to maintain linkage between a coevolved gene complex including the pollen recognition gene and the S-RNase gene. Comparison of S-RNase sequences in the Solanaceae lends support to this view (Clark and Kao, 1991).

If it is a selective advantage for the S-locus to be located within a centromere then this should be observed in other members of the Solanaceae. There is some support for this in tomato based on S-linked RFLP markers (Bennatzy and Miller, 1994) showing linkage to centromeric repeats (Broun and Tanksley, 1996). However, a centromeric location for the S-locus in other members of the Solanaceae may only be a reflection of synteny (ten Hoopen et al., 1998). For this reason it will be particularly interesting to establish the cytological position of S-RNase loci in other more distantly related families such as the Scrophulariaceae and Rosaceae (Richman, Brookharts and Kohn, 1997).

**FUTURE PERSPECTIVES**

The total number of S-alleles in *P. hybrid* currently stands at ten, and since the same alleles are now being recovered...
independently in unrelated cultivars it may not rise significantly in future surveys. This remains a surprisingly high level of diversity considering that plant breeders probably selected for self-compatibility at an early stage. This has almost certainly contributed to the prevalence of the non-functional \( S_0 \) allele in current stocks of \( P. \) *hybrida*. The molecular basis of self-compatibility of the \( S_0 \) allele is uncertain, but the available data are consistent with a functional S-RNAs. One possibility is that it is a pollen-part mutation of a previously functional allele, perhaps as a result of a duplication. It will be interesting to compare the situation in *P. hybrida* with other cultivated Solanaceae that were derived from self-incompatible species. Understanding the mechanisms that have led to self-compatibility during cultivation may facilitate the genetic engineering of self-compatibility into self-incompatible species of both the Solanaceae and the Rosaceae which share the same S-RNase mechanisms (Richman et al., 1997).

Self-compatibility can also arise from the presence of modifier genes as demonstrated in *P. hybrida*. The identity of such genes is difficult to predict in view of the simple models of self-incompatibility currently proposed (reviewed by Kao and McCubbin, 1996). However, *P. hybrida* offers several examples of such modifier genes (Ascher, 1984; Ai et al., 1991; Harbord et al., 2000) that could be genetically characterised. Such modifier genes may not show allele specificity and therefore offer a more general method of inactivating self-incompatibility systems.

Finally, as in all of the self-incompatibility systems discussed in this volume, a major goal remains the identification of the pollen determinant of S-locus specificity. The pollen-S gene must reside at the S-locus and high resolution genetic maps, including tightly linked flanking markers such as CP100, should allow the limits of the S-locus to be defined. The centromeric location and the suppression of recombination anticipated would predict that this may correspond to a physically large region. The application of S-linked transgenes to preselect for rare recombination events around the S-locus (Harbord et al., 2000) should facilitate this approach. In addition, these S-linked T-DNAs carry maize transposable elements which opens up the possibility of genetic screens to identify pollen-S.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Wim Broothaerts for supplying seed carrying the \( S_0 \) allele originally characterized by Prof. H. F. Linskens. Also Dr Ronald Koese (Free University of Amsterdam) for providing V13, V26 and other inbred lines of *P. hybrida* and Dr Christiane Gebhardt (Max Planck Institute, Cologne) for the potato RFLP marker, CP100. This work was supported by the UK Biotechnology and Biological Sciences Research Council.

LITERATURE CITED


