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Recent findings of the tree fruit self-incompatibility studies

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Summary: This review endeavours to collect all recent and substantial contributions to the quickly deepening fields of tree fruit self-incompatibility studies and hence updating previously published reviews. Studies carried out to discover the molecular basis of gametophytic self-incompatibility are summarized and a newly described model for the solanaceous plants is also outlined. We describe recent findings in all economically important fruit tree crops involving apple, European pear, sweet and sour cherries, almond, Japanese plum, sloe, Japanese apricot, European apricot and peach. Additional DNA sequences are now available for both the pistil and pollen component genes in several species and their molecular, evolutionary or economic implications are discussed in the light of the fruit setting behaviour.

Key words: F-box, *Malus, Prunus, Rosaceae,* self-incompatibility, *S*-genotype, *S*-RNase, tree fruits

Studies on the molecular basis of gametophytic self-incompatibility

Studying self-incompatibility (SI) in various plant genera represents a continuously intensifying field within plant science. This is also reflected by the fact that more than 50 scientific reports are published yearly dealing with this type of research. This review intends to highlight the latest achievements regarding the molecular bases of SI and also the results arose from the most recent studies in each fruit tree species. We demostrate the most essential contributions in details that accumulated during the last two years in order to be able to provide updated description of the state of the art.

Self-incompatibility prevents the production of "self" seed and consequent inbreeding by providing a recognition and rejection system for "self," or genetically identical pollen. Studies of gametophytic SI (GSI) species at a molecular level have identified two completely different *S*-genes in the *Solanaceae*, *Rosaceae* and *Scrophulariaceae* with *S*-RNase as the pistil *S*-component and an F-box protein (SFB) as the pollen *S*-component. Based on studies with loss-of-function SFB mutant sweet cherry cultivars, an updated model for the self-incompatibility response was described by *Sonneveld* et al. (2005), supposing the presence of a general inactivation mechanism with SFB proteins to prevent self *S*-RNases from being degraded, and not recruit non-self *S*-RNases for degradation. However, this has also several unclear details regarding the precise role of SFB in protecting self *S*-RNases from degradation.

The intensive research on solanaceous species revealed that non-*S*-locus factors (the so-called modifier genes) could also be required for the SI response. *Juárez-Díaz* et al. (2006a) reported that a new thioredoxin *h* from *Nicotiana alata* (NaTrxh) was secreted similarly to animal. With fractionation studies as well as transiently expressing a NaTrxh-green fluorescent fusion protein in *Nicotiana benthamiana* and *Arabidopsis thaliana* leaves it was demonstrated that NaTrxh was a secretion protein such as *S*-RNases and NaTTS (*N. alata* transmitting tissue-specific protein). *In vitro* reduction assays identified that *S*-RNase was one of the several potential substrates of the NaTrxh in the extracellular matrix (ECM). Furthermore, NaTrxh was proved by affinity chromatography to specifically interact with *S*-RNase. Because NaTrxh is localized in the ECM of the stylar transmitting tract and it shows specific interaction with *S*-RNase to reduce it *in vitro*, *Juárez-Díaz* et al. (2006a) suggested that this thioredoxin *h* might be involved either in general pollen–pistil interaction processes or particularly in *S*-RNase-based self-incompatibility. It is confirmed by the observation that NaTrxh is six-fold higher expressed in SI *Nicotiana alata* than in SC *Nicotiana plumbaginifolia* (*Juárez-Díaz* et al., 2006b). Based on this information, *Halász* et al. (unpublished) could determine stylar RNase activity in extracts supplied with antioxidants in a *Prunus* fruit tree, apricot.

Goldraij et al. (2006) proposed an alternative model for the self-incompatibility reaction in *Nicotiana*. This model dictates that compatibility is due to *S*-RNase compartmentalization, rather than its degradation. *S*-RNase, HT-B, and 120 kDa glycoprotein (see review by *Cruz-Garcia* et al., 2003) are secreted into the transmitting tract. They are taken up by endocytosis and transported to the vacuole (*Figure 1*). An unidentified pollen protein (PP) is hypothesized to degrade HT-B in compatible pollen tubes. Regardless of the

degradation mechanism, in HT-B's absence, *S*-RNase remains compartmentalized, which results in compatibility. So *S*-RNases are present and stable but cytotoxicity does not occur because they are sequestered. However, the *S*-RNase/SLF or SFB interaction was suspected to determine *S*-specificity because these two genes reside in close proximity within the *S*-locus and both genes show high levels of interallelic variability (*Lai* et al., 2002; *Halász* et al., 2006). In *Figure 1*, SLF is shown with both self- and non-self *S*-RNase binding sites, just as in the model proposed by *Ushijima* et al. (2004) for *Prunus* species. It is not clear how *S*-RNase gains access to SLF but access must be indirect if *S*-RNase is sequestered in a vacuole. Perhaps, there is a mechanism for recruiting SLF to the membrane, or perhaps a small amount of *S*-RNase escapes to the cytoplasm. What is clear is that the self-interaction prevents the hypothetical pollen factor from degrading HT-B, while the non-self interaction facilitates its degradation. When it is released from the vacuole in the late stages of an incompatible pollination, *S*-RNase interferes with pollen homeostasis and this acts as a self-reinforcing mechanism for rejection.

This model differs from the *S*-RNase degradation model in several respects. One important difference is the proposal that compatibility is due to *S*-RNase sequestration. The model has three main steps: (1) *S*-RNase uptake and sequestration, (2) an *S*-RNase/SLF interaction that affects HT-B stability, and (3) a self-reinforcing cytotoxic step that results in pollen rejection. While only the second step is *S*-specific, each step involves interactions between pollen and pistil factors. In this model, pollen plays a more active role than in the *S*-RNase degradation model. Pollen factors are responsible for uptake of stylar factors from the stylar ECM, sorting to the vacuole, maintenance of vacuole integrity, recognition of *S*-RNase, and degradation of HT-B. Since these processes are important for proper sequestration of *S*-RNase, the cytotoxic activity of *S*-RNase comes into sharper focus: by interfering with translation of proteins needed for these protective functions, release of *S*-RNase when the vacuole breaks down reinforces incompatibility. For more details, see reviews by *McClure* (2006) and *McClure & Franklin-Tong* (2006).

Untill now, there are only suggestions that modifier genes may act in the SI reactions of fruit tree species (*Zuccherelli* et al., 2002; *Wünsch & Hormaza*, 2004; *Vilanova* et al., 2006), which seems to be confirmed by the fact that RNase based GSI in various plant families have common evolutionary origin (*Igic & Kohn*, 2001). However, several differences were detected between solanaceous and rosaceous GSI (as discussed by *Tao* et al., 2007) and no similar molecular components encoded outside the *S*-locus have been identified or described in fruit trees. *Quiao* et al. (2004) found that proteasomal inhibitors blocked compatible pollinations but not incompatible ones in *Antirrhinum*, which could be reconciled with the compartmentalization model for example in a way that HT-B may be the target molecule of the 26S proteasomal degradation. This and other options must be checked in the future.

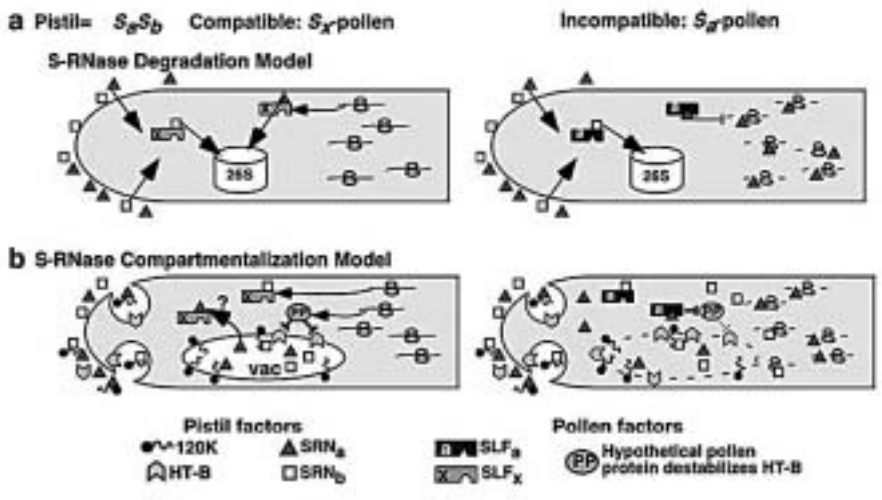

Figure 1 Alternative models for *S*-RNase-based SI. Compatible (S_x-, *left*) and incompatible (S_a-, *right*) pollinations are shown on an S_aS_b pistil. Symbols for pistil factors (*S*-RNase, HT-B, and 120 K) and pollen factors (SLF and PP) are shown below. (A) *S*-RNase degradation model. *S*-RNase enters the pollen tube cytoplasm from the ECM (*arrows*). SLF occurs as a complex with other proteins that are omitted for simplicity. A compatible non-self *S*-RNase/SLF interaction (*left*) results in ubiquitylation and degradation by the 26S proteasome, and pollen tube growth therefore continues normally. An incompatible self *S*-RNase/SLF interaction (*right*) does not result in *S*-RNase degradation; cytotoxicity results in RNA degradation and consequent pollen tube growth inhibition. (B) *S*-RNase compartmentalization model. *S*-RNase, 120 K, and HT-B are taken up by endocytosis and sorted to a vacuole. In a compatible interaction (*left*), *S*-RNase remains compartmentalized. *S*-RNase is present and stable but cytotoxicity does not occur because it is sequestered. A hypothetical pollen factor (PP) causes degradation of HT-B in compatible pollen tubes. It is not known how *S*-RNase gains access to SLF (*arrow*, *question mark*). In an incompatible interaction (*right*), the action of PP is inhibited, the vacuole breaks down, *S*-RNase is released into the cytoplasm, RNA is degraded as a consequence, and pollen tube growth is inhibited (After *McClure & Franklin-Tong*, 2006)

Apple (*Malus* × *domestica* Borkh.)

The latest review detailing information on apple self-incompatibility was reported by *Hegedűs* (2006). A recent research paper was published by *Cheng* et al. (2006), who designed degenerate primers from the conserved regions of the reported *Prunus*, *Anirrhinum* and *Petunia* SLF/SFB alleles, which allowed for isolation of more than a dozen cDNA clones from pollen of three different apple *S*-genotypes by reverse transcription polymerase chain reaction (RT)-PCR. With 5' and 3' rapid amplification of cDNA end (RACE), two full length cDNAs were synthesized, whose corresponding genes (SLF1 and SLF2) were specifically expressed in pollen in an *S*-haplotype-specific manner. PCR analysis of seven different cultivars further showed the linkage between SLF1 and S_1-RNase

and between SLF2 and S_2-RNase. The predicted ORFs of the two genes encode two F-box proteins of 393 amino acids in length with 70% amino acid identity. These features are consistent with the supposition that the isolated apple SLF1 and SLF2 are good candidates for pollen *S*-genes. Phylogenetic trees for the SLF/SFB and *S*-RNase proteins reported in the *Solanaceae*, *Scrophulariaceae* and *Rosaceae* (including apple) were constructed. It was found that the SLFs and *S*-RNases from apple were more closely related to those from *Petunia* (*Solanaceae*) and *Antirrhinum* (*Scrophulariaceae*) than to those from *Prunus* (*Rosaceae*), implying a potential co-evolution between SLF/SFB and *S*-RNase. Furthermore, six SLF-like genes that shared a high level of sequence similarity (amino acid identity from 68 to 72%) to SLFs were also isolated. Among them, three were confirmed to be expressed specifically in pollen in an *S*-haplotype-unspecific manner. The authors attributed the presence of multiple copies of SLF genes to a potential coevolution with the *S*-locus, which resulted in the polymorphism of these genes. Genome sequencing studies suggested that gene duplication was required for structuring eukaryotic genomes (*Bancroft*, 2001).

European pear (*Pyrus communis* L.)

The latest review summarizing available data on pear self-incompatibility was published by *Halász & Hegedűs* (2006). Until 2004, sequences for 13 pear *S*-RNase alleles were published and named following a letter–symbol nomenclature (S_a to S_e; S_h, S_i, and S_k to S_p) (*Zuccherelli* et al., 2002; *Zisovich* et al., 2004). However, the allele labelling system is burdened with inconsistencies. S_f and S_g were left out from the original nomenclature established by *Zuccherelli* et al. (2002) and S_j was assigned to an allele previously desribed as S_e (*Zisovich* et al., 2004). Therefore, some authors use labels S_a to S_d and S_h to S_p and neglect labels S_e to S_g.

Later, nine full-length cDNAs of *S*-ribonucleases were cloned from stylar RNA of European pear cultivars by RT-PCR and 3' and 5' RACE (*Takasaki* et al., 2006). Comparison of the nucleotide sequences between the nine *S*-RNases cloned and 13 putative *S*-alleles previously identified with genomic PCRs revealed that seven corresponded to S_a, S_b, S_d, S_e=$_j$, S_h, S_k and S_l alleles, and the other two were new *S*-alleles (designated as S_q and S_r). Genomic PCR with a set of 'FTQQYQ' and'EP-anti-IIWPNV' primers (*Ishimizu* et al., 1999) was used to amplify nine *S*-alleles; 1,414 bp (S_l), ca. 1.3 kb (S_k and S_q), 998 bp (S_e), 440 bp (S_b) and ca. 350 bp (S_a, S_d, S_h and S_r). Among these, *S*-alleles of similar size were discriminated by digestion with *Bae*I, *Bgl*II, *Bss*HII, *Hin*dIII, *Eco*O109I and *Sph*I. The PCR amplification of *S*-allele following digestion with the restriction enzymes provided a PCR-RFLP system for rapid *S*-genotyping of European pear cultivars harboring nine *S*-alleles. The PCR-RFLP system assigned a total of 63 European pear cultivars to 25 genotypes. Among these, 14 genotypes were shared by two or more cultivars, which were cross-incompatible.

Sanzol & Herrero (2002) were the first to describe *S*-alleles in European pear cultivars and labelled them with numbers (S_1 to S_4), which was not taken over by *Zuccherelli* et al. (2002), who established a completely different alphabetical nomenclature. Therefore, *Sanzol* et al. (2006) tried to establish the correspondence between the letter nomenclature used to name *S*-RNase sequences and the number-based nomenclature used to name *S*-alleles. They have amplified genomic DNA with newly designed consensus primers (MPyC1F, MPyC2F, MPyBC3F, MPyC5R and PycomS2R) from the cultivars 'Williams' (S_1S_2), 'Coscia' (S_3S_4), 'Butirra Precoce Morettini' (S_1S_3), 'Santa Maria Morettini' (S_2S_3) and 'Doyenne du Comice' (S_4S_5) and identified PCR products specifically associated with each *S*-allele. Cloning and sequencing of the amplification products and BLAST searches on the NCBI database allowed the authors to link *S*-RNase sequences with *S*-allele phenotypes and to determine a correspondence as S_1=S_j, S_2=S_l, S_3=S_k and S_4=S_b. Database searches using the pear *S*5-RNase protein sequence from 'Doyenne du Comice' found no exact matches. A genomic sequence for the S_a-RNase allele was available containing the first exon from the C1 region, the intron sequence and a small portion of the second exon (*Zuccherelli* et al., 2002). After comparing this sequence with the DNA sequence associated to the S_5-allele, both are identical for the first exon portion and the intron; however, the two deduced amino acid sequences differ in one residue located just downstream of the intron position. The functional identity or difference of the alleles S_5 and S_a should be checked in the future.

Sweet cherry (*Prunus avium* L.)

A novel PCR approach to determine and confirm the *S*-genotype of sweet cherries was reported by *Sonneveld* et al. (2006). The method involves PCR amplification with the fluorescently labelled PaConsI-F and the newly designed PaConsI-R2 consensus primer that immediately flank the first intron of cherry *S*-RNases. Fluorescent amplification products ranged from 234 to about 460 bp and could be sized accurately on an automated sequencer. Thirteen *S*-alleles reported in sweet cherry could be distinguished, except for S_2 and S_7, which have an amplification product of exactly the same size. S_{13}, which was also amplified, gave a microsatellite-like trace due to minor intra-allelic length variation. This method gives fast and accurate results and should be especially useful for medium/high-throughput genotyping of wild and cultivated cherries.

A similar method was elaborated by *Vaughan* et al. (2006) to exploit the putative allele-specific variations of the intron present in the 5' untranslated region (5'UTR) of the sweet cherry SFB gene. Primers were designed to amplify 12 SFB alleles, including the introns present in the 5'UTR; sequences representing the S_1, S_2, S_3, S_4, S_4', S_5, S_6, S_7, S_{10}, S_{12}, S_{13} and S_{16} alleles were cloned and characterized. Analysis of the introns confirmed sequence and length

polymorphism between the alleles. A novel, multiplex PCR-based method to genotype sweet cherry accessions was developed, which combines the simultaneous amplifications with primers for the intron of SFB and primers for the first intron of *S*-RNase alleles (both fluorescently labelled). Intron length polymorphisms can be then ascertained using a semi-automated sequencer. This approach helped to resolve all presently known *S*-alleles in sweet cherry in a single reaction. This method may extend the applicability of the previously detailed procedure described by *Sonneveld* et al. (2006).

Sour cherry (*Prunus cerasus* L.)

Three progenies of sour cherry were analysed to correlate self-(in)compatibility status with *S*-RNase phenotype (*Bošković* et al., 2006). The presence of S_4- and S_6-alleles at the same locus led to SI, whereas S_{13} and S_B at homoeologous loci led to self-compatibility. The failure of certain heteroallelic genotypes in the three crosses or in the SI seedlings was also detected. However, the success of $S_{13}S_B$ pollen on styles expressing corresponding *S*-RNases indicated competitive interaction or lack of pollen-*S* components. In general, the universal compatibility of $S_{13}S_B$ pollen may explain the frequent occurrence of S_{13} and S_B together in sour cherry cultivars.

The transition from self-incompatibility to self-compatibility (SC) is regarded as one of the most prevalent transitions in Angiosperm evolution, having profound impacts on the genetic structure of populations (*Hauck* et al., 2006b). Besides five fully functional (S_1, S_4, S_6, S_9 and S_{26}) *S*-haplotypes, genetic analyses of sour cherry (*Rosaceae*, *Prunus cerasus*) selections identified eight independent, non-functional *S*-haplotypes (S_1', S_{6m}, S_{6m2}, S_{13}', S_{13m}, S_a, S_d and S_{null}) with disrupted pistil component (stylar-*S*) and/or pollen component (pollen-*S*) function (*Hauck* et al., 2002; 2006a,b; *Tsukamoto* et al., 2006; *Yamane* et al., 2003) for which an equivalent wild-type *S*-haplotype was present in sweet cherry, the diploid progenitor of sour cherry.

The molecular bases of six of these non-functional *S*-haplotypes that confer unilateral incompatibility have been reported to date. Among these, there are two pollen-part mutants (S_1' and , S_{13}') 3 stylar-part mutants (S_{6m}, S_{6m2} and S_{13m}) and one has lost both pollen and stylar functions (S_{null}). The coding sequence of S_1' SFB contains a 615 bp *Ds*-like element (*Hauck* et al., 2006a), the S_{13}' has a 1 bp substitution that leads to premature stop codon (*Tsukamoto* et al., 2006). The S_{6m}-haplotype contains a 2.6 kbp insertion upstream of *S*-RNase (*Yamane* et al., 2003), while the S_{6m2} and S_{13m} suffered 1 or 23 bp deletions in their *S*-RNase gene, repectively, that lead to premature stop codons (*Tsukamoto* et al., 2006). S_1' along with 2 other previously characterized *Prunus* *S*-haplotype mutants, S_{6m} in sour cherry and S_f in Japanese apricot illustrate that mobile element insertion is an evolutionary force contributing to the breakdown of GSI (*Hauck* et al., 2006a). Since no RFLP fragment associated with S_{null} was visualized with either an *S*-RNase or an SFB probe (*Yamane* et al., 2001), the S_{null}-haplotype was hypothesized to contain a deletion of the *S*-locus (*Hauck* et al., 2006b).

The prevalence of non-functional *S*-haplotypes in sour cherry but not in sweet cherry (a diploid) suggests that polyploidization and gene duplication were indirectly responsible for the dysfunction of some *S*-haplotypes and the emergence of self-compatibility in sour cherry (*Tsukamoto* et al., 2006).

A genetic model demonstrating that the breakdown of SI in sour cherry is due to the accumulation of a minimum of two non-functional *S*-haplotypes within a single individual was developed and validated (*Hauck* et al., 2006b). The "one-allele-match" model dictates that a match between a functional pollen-*S* gene product in the pollen and its cognate functional *S*-RNase in the style would result in an incompatible reaction (*Figure 2*). A similar reaction would occur regardless of whether the pollen contained a single functional pollen-*S* gene or two different functional pollen-*S* genes. Consequently, heteroallelic sour cherry pollen is self-incompatible (as it was also experienced by *Bošković* et al., 2006), which is counter to the well-documented phenomenon in the *Solanaceae*. However, the absence of a functional match would result in a compatible reaction; thus, for successful self-fertilization, pollen must contain two non-functional *S*-haplotypes. The finding that sour cherry is SI when only one non-functional *S*-haplotype is present has significant evolutionary implications since non-functional *S*-haplotypes would be maintained in the population without causing an abrupt shift to SC.

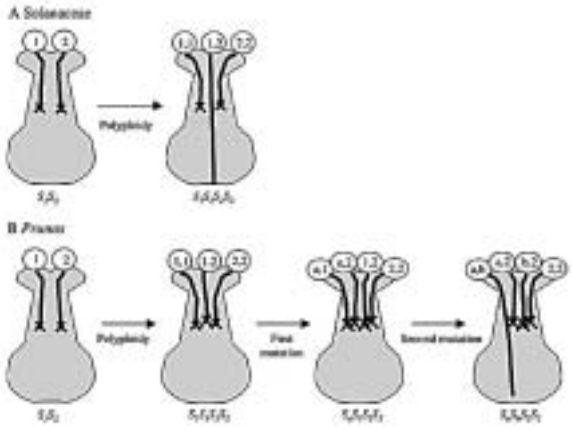

Figure 2 The consequences of polyploidy on GSI in (A) the *Solanaceae* and (B) *Prunus*. In the *Solanaceae*, polyploidy directly causes the conversion from SI to SC due to the compatibility of heteroallelic pollen. In *Prunus*, polyploidy does not directly result in a breakdown of SI. Rather, SC requires the loss-of-function for a minimum of two *S*-haplotype-specificity components (After *Hauck* et al., 2006b)

Almond [*Prunus dulcis* (Mill.) D.A. Webb]

Experiments aimed to solve fruit set problems arising from self-incompatibility of almond are quite intensive in Spain, where this species is of great economic importance. These studies involve both orchard experiments and

molecular investigations. From 2006 to the beginning of 2007 three reports were published to clarify several controversial points with fruit set and histological analyses.

At first, the putative advantage of homozygous self-compatible genotypes (where 100% of the pollen grains are potentially able to grow through their own pistil) over the heterozygous self-compatible genotypes (with only 50% of pollen grains that are capable of fertilization) was tested (*Ortega & Dicenta*, 2006). In general, pollen tube growth rates were different in the two genotypes but fruit set was similar in homozygous and heterozygous individuals. Interestingly, one of the homozygous individuals showed problems of fruit development, which might be explained by its inbred origin. The next study was commenced to clarify doubts that have arisen regarding the capability of the newly introduced self-compatible almond cultivars to produce good yields in monovarietal plantations, and the commercial quality of the fruits from self-pollination (*Ortega* et al., 2006b). Ten fruit traits were studied in 26 SC almonds following self-pollination and despite a few individuals showed differences between both pollination types for two of the traits, the results in general showed no influence of self-pollination in fruit quality. At last, the well-known negative effects of rainy weather on fertilization efficiency was investigated (*Ortega* et al., 2007). The results showed that adhesion of pollen grains to the stigma was very quick and strong and water sprays as simulated rain were not able to completely wash off pollen from the stigma surface and reduce subsequent fruit set, although it seems to affect adhesion in forthcoming pollinations.

To compile self-(in)compatibility almond genotypes, a review of 133 commercial cultivars of wide geographical origin was presented (*López* et al., 2006). The information summerized will be useful for both grower's cultivar choice when planting and for breeder's cross design when planning. The almond *S*-genotypes compiled were identified using five different methods: biological (pollination tests in the field and in the laboratory) and molecular (RNases, PCR and sequencing). In most cases, genotypes were assigned after combining more than one technique. Cultivars were classified into three categories: self-incompatible (99), self-compatible (16) and doubtful self-incompatible (18). A study of the 27 *S*-alleles already identified revealed that the geographical distribution of *S*-allele frequencies within the cultivated almond was uneven among the 133 cultivars. *S*-allele frequencies are related to geographical origin. Some alleles (S_1, S_5, S_7 and S_8) are more frequently observed than the others among cultivars. In the cultivated almond, the S_f-allele is only found in the Puglia region, Italy. The S_f frequency is three times higher in cultivars released from breeding programmes than in cultivars selected by growers. From the 351 resulting possible genotypes by combination of the 27 *S*-alleles identified [n(n-1)/2] only 20 cross-incompatibility groups (CIGs 0-XIX) have been established, which represents a small fraction of the whole genetic diversity of this polymorphic gene in almond.

The most recent advances in the molecular research of SI in almond are attributed to *Ortega* et al. (2006a). One year earlier, this group extended the number of known *S*-RNase alleles to 29 (*Ortega* et al., 2005). The new study aimed to resolve possible synonyms and to provide data for phylogenetic analysis, 21 almond *S*-RNase alleles were cloned and sequenced from the signal peptide region (SP) or first conserved region (C1) to C5, except for the S_{29}-allele, which could be cloned only from SP to C1. Nineteen sequences (S_4, S_6, S_{11}–S_{22}, S_{25}–S_{29}) were potentially new whereas S_{10} and S_{24} had previously been published but with different labels. The sequences for S_{16} and S_{17} were identical to that for S_1, published previously; likewise, S_{15} was identical to S_5. In addition, S_4 and S_{20} were identical, as were S_{13} and S_{19}. A revised version of the standard table of almond incompatibility genotypes was presented. Sliding window analysis identified regions where positive selection may operate; in contrast to the *Maloideae*, most of the region from the beginning of C3 to the beginning of RC4 appeared not to be under positive selection. Phylogenetic analysis indicated separate clades for four pairs of alleles with a 'bootstrap' support higher than 80%: S_5/S_{10}, S_4/S_8, S_{11}/S_{24}, and S_3/S_6. Various motifs up to 19 residues long occurred in at least two alleles, and their distributions were consistent with intragenic recombination, as were separate phylogenetic analyses of the 5' and 3' sections. Sequence comparison of phylogenetically related alleles indicated a putatively higher significance of the region between RC4 and C5 in defining specificity than that of the RHV region.

López et al. (2006) proposed 20 and *Ortega* et al. (2006) reported on 21 CIGs in almond. However, through reconciling the differences between the two studies in the allele labelling system and genotypes of the proposed CIGs, at least 23 CIGs might be established.

Japanese plum (*Prunus salicina* Lindl.)

Diploid Japanese plum cultivars are commonly self-incompatible. To date, 14 incompatibility alleles have been identified and labelled with alphabetical (S_a–S_n) and 5 with numeric codes (S_1, S_3–S_6). *Halász* et al. (2007) applied PCR amplification of the *S*-RNase alleles with degenerate and allele-specific primers in 10 Japanese plum cultivars and two pluots of unknown incompatibility alleles. Besides DNA sequencing, an additional method for the exact length determination of the first intron region was used for the first time for *S*-genotyping Japanese plums. The S_3-allele was shown to correspond to S_k in the alphabetic nomenclature, S_4 to S_c, S_5 to S_e, and S_6 to S_f. The S_5-allele-specific primer can be used as a reliable marker for SC in Japanese plum. 'Black Amber', 'October Sun', 'TC Sun', and 'Super Giant' share the S_bS_c genotype, which was confirmed by test crosses. These cultivars belong to the widest incompatibility group currently known in Japanese plum. An additional incompatibility group (S_cS_h) was established, including 'Green Sun' and 'Queen Rosa', a cultivar formerly known as a universal donor. By incorporating all previous and recent results, a table was assembled including 49 cultivars assigned

to I–VII CIGs, to the self-compatible group and to the group 0 of unique incompatible genotypes.

The SFB gene in the *S*-locus of Japanese plum was first time identified by *Zhang* et al. (2007). They determined eight novel sequences (S_a, S_b, S_c, S_e, S_f, S_h, S_7 and S_{10}) homologous to the SFB genes of other *Prunus* species and named these sequences PsSFB. The gene structure of the SFB genes and the characteristic domains in deduced amino acid sequences were conserved. Three sequences from 410 to 2,800 bp of the intergenic region between the PsSFB sequences and the *S*-RNase alleles were obtained. The eight identified PsSFB sequences showed *S*-haplotype-specific polymorphism, with 74–83% amino acid identity. These alleles were exclusively expressed in the pollen. These results suggest that the PsSFB alleles are the pollen *S*-determinants of GSI in Japanese plum.

Sloe (*Prunus spinosa* L.)

Prunus spinosa is an allotetraploid self-incompatible species. First molecular study on this species was carried out by *Nunes* et al. (2006) to test models for the generation of new GSI specificities, which require that neutral variability segregates within specificity classes. Furthermore, one of the models predicts greater ratios of non-synonymous to synonymous substitutions in pollen than in pistil specificity genes. All models assume that new specificities arise by mutation only. Twenty one SFB alleles from a wild *P. spinosa* population were sequenced. For seven of these, the corresponding *S*-haplotype was also characterized. The SFB data set was also used to identify positively selected sites. Those sites are likely to be the ones responsible for defining pollen specificities. Of the 23 sites identified as being positively selected, 21 are located in the variable (including a new region, V_n, described here) and hypervariable regions. Little variability is found within specificity classes. There is no evidence for selective sweeps being more frequent in pollen than in pistil specificity genes. The *S*-RNase and the SFB genes showed only partially correlated evolutionary histories. None of the models were found to be compatible with the variability patterns found in the sloe SFB and the *S*-haplotype data.

Japanese apricot (*Prunus mume* Sieb. et Zucc.)

All SC cultivars carry the S_f-haplotype with the pollen component SFB_f containing a 6.8 kbp insertion, which presents a loss-of-function mutation (*Ushijima* et al., 2004). Based on this information, *Habu* et al. (2006) elaborated a simple and rapid procedure for the detection of SC cultivars, the so-called loop-mediated isothermal amplification (LAMP) method. A set of 4 primers were specifically designed from 6 regions of the exon and the putative inserted sequence of SFB_f. Optimal reaction time at 63 °C was determined to be 90 minutes. This method uses isothermal conditions compared with thermal cycling executed by PCR, thus requires simple equipment. The amplification specificity is high since 6 different regions of the target allele are involved in the reaction. Finally, because the LAMP method is designed to synthesize a large amount of amplification byproduct from the target sequence, pyrophosphate ion is used to yield a white precipitation of Mg pyrophosphate. The result can be easily judged from turbidity of the reaction mixture by the naked eye sparing the time and cost of electrophoresis. It was concluded that the LAMP method combined with an ultrasimple DNA extraction protocol efficiently detected SC in Japanese apricot, and hence can be a useful tool for the marker-assisted selection.

European apricot (*Prunus armeniaca* L.)

The last two years supplied valuable information regarding the molecular bases of SC in apricot. *Vilanova* et al. (2006) have studied two SC cultivars of apricot, 'Currot' (S_CS_C) and 'Canino' (S_2S_C), sharing the naturally occurring S_C-haplotype. Sequence analysis showed that, whereas the S_C-RNase is unaltered, a 358 bp insertion is found in the SFB_C gene resulting in the expression of a truncated protein. The alteration of this gene is associated with SI breakdown.

Halász et al. (unpublished) could isolate a new *S*-haplotype, S_8, which was clarified to be the non-mutated wild type version of the S_C-allele. The relationship of the S_8- and S_C-alleles was validated in a multi-approach analysis using RNase activity assays, pollen tube growth and fruit set evaluation besides sequence analysis. S_C- and S_8-ribonucleases showed equally sized introns, identical 1st intron and cDNA sequences and equal levels of RNase activity. A controlled cross (♀ S_CS_9 × ♂ S_8S_9) did not result in fruit set and S_8 pollens showed typical incompatible phenotype in S_CS_9 styles. A newly designed consensus primer pair could resolve previously unidentified *S*-genotypes. The isolated apricot SFB_8-allele is the first known progenitor allele of self-compatibility in *Prunus* species. This allele occurs frequently in the Hungarian apricot gene pool and the evolvement of the mutation might be traced back to the Central Asian region. These results can be evaluated in the light of other molecular diversity studies in apricot (*Maghuly* et al., 2005; *Sánchez-Pérez* et al., 2006).

On the other hand, PCR-analysis of progenies derived from 'Canino' showed that pollen grains carrying the S_2-haplotype were also able to overcome the incompatibility barrier (*Vilanova* et al., 2006). However, alterations in the SFB_2 gene or evidence of pollen-*S* duplications were not detected. These results suggest that the 'Canino' cultivar has an additional mutation, not linked to the *S*-locus that causes a loss of pollen-*S* activity when present in pollen.

Research on apricot SI has been recently also started in China. Using real-time fluorescence quantification RT-PCR technology, spatio-temporal expression patterns of *S*-RNase gene between 'Katy' (SC) and 'Xinshiji' (SI) were compared (*Feng* et al., 2006a). The transcript abundance was distinctly diverse at the keystage (i.e., at 24 h after self-pollination) in both genotypes, and was greater in 'Xinshiji' than 'Katy'. In

the SI cultivar 'Xinshiji', the expression of *S*-RNase remained at a relatively high level after cross-pollination, but it dropped continuously after self-pollination and un-pollination. This study was continued with protein analyses involving two-dimensional gel electrophoresis and liquid chromatography-electrospray ion trap tandem mass spectrometry (*Feng* et al., 2006b). Nine protein spots were expressed in self-pollinated pistil and only one was expressed in cross-pollinated pistils. Sixteen and three protein spots were up- and down-regulated in cross-pollinated pistils, respectively, compared with self-pollinated pistils. Seven protein spots were identified: Actin-12, enolase, MYB transcription-factor-like protein, heat-shock protein 70 were upregulated in cross-pollinated pistils compared with self-pollinated pistils; and actin-7, actin-8 and fructose bisphosphate aldolase like protein were detected only in self-pollinated pistils.

Halász et al. (unpublished) extended the number of identified and characterized apricot *S*-alleles to a total of twenty.

Peach [*Prunus persica* (L.) Batsch]

Yamane et al. (2005) described the use of the yeast-based signal sequence trap (YSST), together with transient expression of identified proteins as fusions with the reporter green fluorescent protein (GFP), focusing on the complex extracellular interactions between peach pollen and pistil tissues. The assay was also used to confirm the extracellular localization of a recently identified pistil-specific basic RNase protein (PA1), as has been observed with *S*-RNases that are involved in self-incompatibility.

The most commercially grown peach cultivars do not require cross-pollination for reasonable fruit set. *Hegedűs* et al. (2006) carried out isoelectric focusing and native polyacrylamide gel electrophoresis of *S*-ribonucleases; PCR analyses of *S*-RNase and *S*-haplotype-specific F-box genes as well as DNA sequencing to survey the self-(in)compatibility allele pool and to uncover the nature of self-compatibility in peach. From 25 cultivars and hybrids with considerable diversity in phenotype and origin, only two *S*-haplotypes were detected. Allele identity could be checked by exact length determination of the PCR-amplified fragments and/or partial sequencing of the peach S_1-, S_2- and *P. davidiana* (Carr.) Franch. S_1-RNases (*Figure 3*). *S*-RNases of peach were detected to possess ribonuclease activity, and a single nucleotide polymorhism in the S_1-RNase was shown, which represents a synonymous substitution and does not change the amino acid present at the position in the protein. A 700-bp fragment of the peach SFB gene was PCR-amplified, which is similar to the fragment size of functional *Prunus* SFBs.

Later, *Tao* et al. (2007) clarified that all three peach *S*-haplotypes, S_1, S_2, and S_{2m}, they have isolated encode mutated pollen determinants, SFB, while only S_{2m} has a mutation that affects the function of the pistil determinant *S*-RNase. A cysteine residue in the C5 domain of the S_{2m}-RNase is substituted by a tyrosine residue, thereby reducing RNase stability. The peach SFB mutations are similar the SFB mutations found in SC haplotypes of sweet cherry and Japanese apricot. It was proposed that under selection pressure for SC, pollen-part mutants might preferentially be selected compared to pistil-part mutants not only because there are many pollen grains but also because pollen genotype determines the SI phenotype in GSI system.

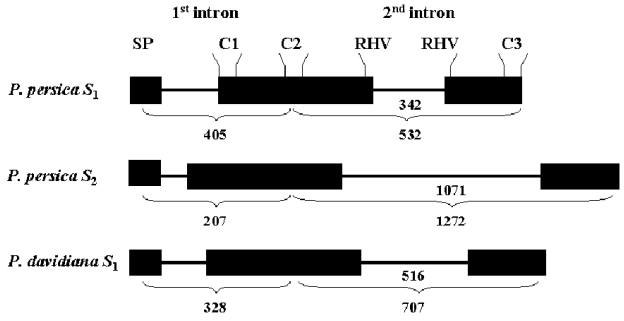

Figure 3 Structure of the *Prunus persica* S_1-, S_2- and *P. davidiana* S_1-RNase alleles. Boxes and lines are exons and introns, respectively (not to scale). Sizes of the fluorescently labelled first intron regions were obtained on automated sequencer; second intron region was sequenced, and the deduced intron lengths (above the brackets) are also marked. SP: signal peptide, C_1-C_3: conserved regions, RHV: rosaceous hypervariable region (After Hegedűs et al., 2006)

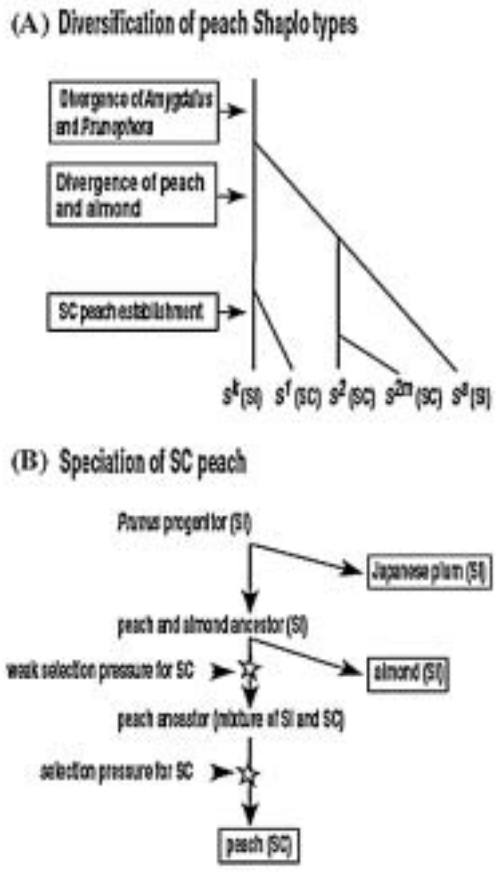

Figure 4 Schematic diagram for evolution of peach *S*-haplotypes (A) and SC peach speciation (B) (After *Tao* et al., 2007)

Tao et al. (2007) use different labels than those introduced by *Hegedűs* et al. (2006): S_1 and S_2 in Tao's study coincide with S_2 and S_1 of Hegedűs, respectively. SFB_1 of the S_1-haplotype, a mutant version of almond S_k-haplotype, encodes truncated SFB due to a 155 bp insertion. SFB_2 of the S_2 and S_{2m} haplotypes, both of which are mutant versions of the S_a haplotype in Japanese plum, encodes a truncated SFB due to a 5 bp insertion. Thus, regardless of the functionality of the pistil determinant, all three peach *S*-haplotypes are SC haplotypes. The finding that peach has mutant versions of *S*-haplotypes that function in almond and Japanese plum, which are phylogenetically close and remote species, respectively, to peach in the subfamily *Prunoideae* of the *Roasaceae*, provides insight into the SC/SI evolution in *Prunus* (*Figure 4*).

According to *Tao* et al. (2007) the *Prunus* SI recognition mechanism may have some differences or modifications compared to the mechanism(s) in the *Solanaceae* and *Maloideae*. The following observations support this view. First, phylogenetic analysis suggested that *Prunus* SFB and the solanaceous SLF may not be orthologous. Second, *Prunus S*-RNase has an additional intron compared to maloid and solanaceous *S*-RNases. Interestingly, *Prunus* has a pistil expressed non-*S*-RNase that has the same structure as maloid and solanaceous *S*-RNases. It may be possible that *Prunus* recruited a different pistil RNase for GSI that may have been derived from the non-*S*-RNase. A lack of orthology of the stylar-*S* and/or pollen-*S* of *Prunus* with the *S*-locus genes of the *Solanaceae* and the subfamily *Maloideae* of the *Rosaceae* (*Cheng* et al., 2006), may be one reason why pollen part mutant SC haplotypes are found more commonly in *Prunus* contributing to the transition from SI to SC.

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