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REVIEW

Sexual incompatibility in *Rosaceae* fruit tree species: molecular interactions and evolutionary dynamics

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Abstract

Fruit crops have a growing economic importance worldwide and molecular genetics might be useful in solving many problems that arise during commercial production. One of the fields that have attracted intense attention is the molecular basis of self-incompatibility that may result in low fruit set. In tree fruits of the *Rosaceae* family, the incompatibility reactions take place between the pistil *S*-ribonuclease (*S*-RNase) and the pollen-expressed *S*-haplotype specific *F*-box (SFB) proteins. In most cases, the loss of self-incompatibility was associated with mutations in the *S-RNase* or *SFB* genes. A total of 27 non-functional *S*-haplotypes have been identified and characterized, most (24) of which emerged as a consequence of natural mutations. In the *Prunoideae*, most haplotypes are pollen-part mutants (50 %), while 8 are stylar-part mutants (36 %), one haplotype shows both pollen- and stylar-part mutations, and molecular changes for two haplotypes still have not been clarified. In contrast, non-functional natural haplotypes in the *Maloideae* are all stylar-part mutants. The analysis of such mutants may shed light on underlying molecular mechanisms as was the case with the establishment of the general inhibitor model that describes interactions between pollen and pistil *S*-proteins. However, several other molecules were supposed to contribute to the molecular interactions, at least in *Solanaceae*, a family with a similar self-incompatibility system. This review also endeavours to delineate the evolutionary implications of the *S*-locus mutations and collect limited data on non-*S*-locus molecular interactions and signaling events after self- and cross-pollination of fruit tree species.

Additional key words: crop evolution, *F*-box, *Maloideae*, *Prunoideae*, self-incompatibility, *S*-ribonuclease

Introduction

Rosaceous fruit trees belong to the subfamily *Maloideae* (apples, pears, and quinces) or *Prunoideae* (cherries, apricots, peaches, almonds and plums). All these species are grown for their fruits or nuts in vast regions of the temperate climate zone. Since parthenocarpy is rare (*Maloideae*) or does not occur at all (*Prunoideae*), fruit set depends on fertilization. All *Prunus* species have complete flowers containing both sexes, the female pistils and male anthers. The organs are present in close proximity; therefore, pollen grains can easily reach stigmata within the same flower. This would result in self-fertilization, which in a longer term may have deleterious effects on the fitness of individuals and the genetic structure of populations (Good-Avila *et al.* 2008). Self-fertilization would increase homozygosity and severely restrict variability, thus degrading the population's future adaptability. This phenomenon would be extremely harmful on an evolutionary scale, so plant species developed different barriers to self-fertilization that allow for the required levels of out-crossing. This mechanism is genetically controlled and is of the gametophytic type in the *Rosaceae* family, in contrast with the sporophytic control occurring in the *Brassicaceae* (De Nettancourt 2001, Panigrahi *et al.* 2011). In gametophytic self-incompatibility (GSI) the phenotype of the pollen is determined by its own haploid genotype. In *Rosaceae*, GSI is controlled by the single polymorphic *S*-locus (named after the term, sterility).

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Abbreviations: AA - amino acid; HV - hypervariable; PPM - pollen-part mutant; SC - self-compatibility; SFB - specific *F*-box; SI - self-incompatibility; SNP - single nucleotide polymorphism; SPM - stylar-part mutant; V - variable.

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Pollen-pistil interactions in gametophytic self-incompatibility

Fertilization is prevented when the *S*-allele expressed by the haploid pollen grain matches one of the *S*-alleles expressed in the pistil. Allelic series of the *S*-locus is labelled by different letters or numbers. Pollen grains from an S_1S_2 anther are incompatible with the S_1S_2 pistil. If two different cultivars have identical *S*-genotypes, they are mutually self-incompatible (SI), in other terms cross- or inter-incompatible. When pollen grains bear *S*-alleles different from the pistil they will be fully compatible. Growth of incompatible pollen tubes is arrested in the style, while one of the compatible pollen tubes reaches the ovary. The morphology of incompatible pollen tubes differs from that of compatible ones with an abnormally large deposit of callose along the incompatible pollen tubes (Halász *et al.* 2007a, Milatovic and Nikolic 2007). Fruitlets resulting from incompatible pollination are prone to dropping.

The past two decades have provided considerable gains in our understanding of the molecular basis of GSI. Incompatibility reactions take place between the pistil *S*-ribonuclease (*S*-RNase) (McClure *et al.* 1989) and the pollen-expressed *S*-haplotype specific *F*-box proteins (Entani *et al.* 2003, Lai *et al.* 2002, Ushijima *et al.* 2003). *S*-RNases are basic glycoproteins secreted into the style mucilage and show sequence motifs characteristic of the active site of the fungal T_2 RNases (Broothaerts *et al.* 1995, Kawata *et al.* 1988). RNase activity was shown to be of crucial importance for the incompatibility response (Huang *et al.* 1994) with pollen RNAs being the target molecules of the pistil RNase enzymes.

An *S*-haplotype-specific *F*-box (SFB or SLF) protein was proposed as the product of the pollen *S*-determinant gene in *Antirrhinum* and *Prunus* species since the gene is located within the highly divergent *S*-locus region, exhibits *S*-haplotype-specific sequence diversity, and is specifically expressed in pollen but not in styles or leaves (Entani *et al.* 2003, Lai *et al.* 2002, Ushijima *et al.* 2003). Sijacic *et al.* (2004) provided evidence of the function of SLF in transgenic *Petunia*. *F*-box proteins may be responsible for *S*-RNase degradation in pollen tubes since they are components of the ubiquitin-ligase complexes, which mediate protein degradation by the 26S proteasome (Hershko and Ciechanover 1998).

Putative models of interactions between pollen and pistil *S*-proteins

Several models have been proposed to describe molecular interactions behind the self-incompatibility reactions (Hua *et al.* 2008). When SFB was identified as the pollen-*S*, it seemed likely that non-self *S*-RNases were polyubiquitinated by SFB to be degraded by the 26S proteasome pathway, which would result in the inactivation of the non-self *S*-RNases (Ushijima *et al.* 2004). Concurrently, the activity of the cognate *S*-RNases is unaffected due to an *S*-haplotype-specific interaction in the pollen tube. The modified inhibitor model predicts that pollen *S*-allele products carry their *S*-allele-specificity domain, and a general inhibitor is responsible for the inhibition of *S*-RNases (Luu *et al.* 2001). This general inhibitor would bind and inhibit all *S*-RNases unless an *S*-RNase was bound to its cognate pollen *S*-allele product through their matching domains. Qiao *et al.* (2004) provided evidence that ubiquitin/26S proteasome activity was essential in compatible but not in incompatible interactions in *Antirrhinum*. In contrast to the previously described model (Ushijima *et al.* 2004), Sonneveld *et al.* (2005) proposed that SFB was not the inactivator of non-self *S*-RNases and hypothesized the presence of a general inactivation mechanism with SFB only conferring specificity to the reaction.

This hypothesis is consistent with the modified inhibitor model (Luu *et al.* 2001), and further supported by the argument that in the original inhibitor model a loss of function of the *SFB* gene should lead to universally incompatible pollen instead of universal compatibility, because pollen tubes would lack a mechanism to inhibit *S*-RNases. This new model suggests a role for SFB proteins to prevent self *S*-RNases from being degraded, and not recruit non-self *S*-RNases for degradation as is dictated by other models (Sonneveld *et al.* 2005). In this model, SI occurs when *S*-RNase and the cognate SFB proteins form a stable complex that prevents *S*-RNases from being degraded. Therefore, *S*-RNases remain intact and can degrade pollen RNA, which will inhibit protein synthesis and pollen tube growth. In the case of a compatible cross, the *S*-RNase and the F-box protein encoded by different alleles (or by the same allele but one of the genes experienced a deleterious mutation) are not able to form a stable complex, so the *S*-RNases will consequently be polyubiquitinated and degraded, which results in undamaged pollen RNA, unimpaired protein biosynthesis and fruit set. However, further biochemical investigations are required to clarify several details.

Goldraij *et al.* (2006) proposed an alternative model for the SI 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. An unidentified pollen protein (PP) is hypothesized to degrade HT-B in compatible pollen tubes. In the absence of HT-B, *S*-RNase remains compartmentalized, which results in compatibility. So *S*-RNases are present and stable but cytotoxicity does not occur because they are sequestered. The self-interaction prevents the hypothetical PP from degrading HT-B, and vacuoles will be disrupted. When released from the vacuole, *S*-RNase

interferes with pollen homeostasis, thus acting as a self-reinforcing mechanism for rejection. The initial interaction between *S*-RNases and SFBs is still unknown, although an interaction seems crucial to channel downstream reactions that alternatively lead to a compatible or incompatible reaction. Whether this model works in either or both of the subfamilies of the *Rosaceae* still awaits further clarification.

Molecular changes behind the transition from SI to SC phenotype in the *Rosaceae* and their crop evolutionary aspects

In general, the transition from SI to self-compatibility (SC) is one of the most frequently travelled roads in the evolution of plant mating systems (Good-Avila *et al.* 2008, Igic *et al.* 2008). However, a large fraction of angiosperms are SI suggesting that SI provides a macroevolutionary advantage (Igic *et al.* 2008). SC cultivars are known in many species including pears, almond, apricots, sweet and sour cherries, Japanese and European plums. The SC phenotype is known to be of natural origin in most cases except for sweet cherry and apple which were X-ray irradiated or genetically transformed, respectively, to break the incompatibility barrier (Lewis 1949, Van Nerum *et al.* 2000).

If self-incompatibility had been the original sexual reproduction strategy in *Rosaceae*, self-compatibility would have occurred naturally by accidental mutations with deleterious effects on one or both elements of the bipartite SI system. The identification and characterization of mutated non-functional *S*-haplotypes have significantly enriched our knowledge on the molecular basis of SI mechanisms and helped to determine genetic changes behind the transition from SI to SC.

A total of 27 non-functional *S*-haplotypes have been identified and characterized from tree fruits, most (24) of which emerged in consequence of natural mutations. In the *Prunoideae*, most haplotypes are pollen-part mutants (PPM; 50 %, 11 from 22), while 8 of them are stylar-part mutants (SPM; 36 %), one haplotype shows both pollen- and stylar-part mutations, while mutations in two haplotypes still have not been clarified. Non-functional *S*-haplotypes were more frequent (altogether 7) in the tetraploid species *Prunus cerasus*, while diploid species carried fewer (*P. avium* carries 4, 2 of which are artificial, *P. webbii* carries 4, one of which also occurs in *P. dulcis*, *P. persica* carries 3, *P. armeniaca* carries 3, *P. dulcis* carries 2, *P. mume* and *P. salicina* each carry only one non-functional haplotype). In contrast, non-functional haplotypes in the *Maloideae* are all SPMs including one each from *Pyrus serotina* and *P. communis*.

Cherries: Sweet cherry is renowned as a classical example of a SI fruit tree species. This situation fuelled the necessity for SC genotypes that would result in increased grower returns by facilitating production. Therefore, flower buds of a parental accession (Napoleon, S_3S_4) were X-ray irradiated and pollens were crossed on nominally incompatible egg plants (Lewis 1949). The resultant hybrids were selected and they formed the basis for further breeding activity. The S_3'- and S_4'-haplotypes originated with this treatment. Their subsequent molecular analysis revealed that four nucleotides were deleted in SFB_4' as compared with the wild-type SFB_4 (Sonneveld *et al.* 2005, Ushijima *et al.* 2004). DNA double-strand breaks induced by ionizing radiation such as X-ray are often repaired *via* a non-homologous end-joining pathway in plants, which frequently induces a deletion of short sequence repeats (Gorbunova and Levy 1997). The TTAT deletion is located just before the HVa (hypervariable) region and leads to a frame-shift, producing transcripts for a defective SFB lacking the two HVa regions and causing a premature termination of the protein due to a stop codon being out of position. In the case of the S_3'-haplotype the *SFB* gene was deleted (Sonneveld *et al.* 2005). The absence of SFB_3 results in a compatible phenotype indicating that pollen SFB is not included in *S*-RNase degradation and hence a general inactivation mechanism must operate in pollen tubes (Sonneveld *et al.* 2005).

Natural SC haplotypes are also known in sweet cherry. One of them (S_5') was proved to be a PPM with a premature stop codon induced by a single base pair substitution in the *SFB* upstream of the HVa region (Marchese *et al.* 2007), while two others (S_3 and S_6 of cv. Cristobalina) are also known to be PPM with mutations outside the *S*-locus (Wünsch and Hormaza 2004). Recently, Cachi and Wünsch (2011) have found that the locus associated with SC in Cristobalina is located in LG3, in contrast with the *S*-locus in LG6.

Genetic analyses of sour cherry selections identified seven independent, non-functional *S*-haplotypes (S_1', S_{6m}, S_{6m2}, S_{13}', S_{13m}, S_a and S_d) with disrupted pistil and/or pollen function (Hauck *et al.* 2002, 2006a,b, Yamane *et al.* 2003b). For the majority of these, an equivalent wild-type *S*-haplotype has been described in sweet cherry, the diploid progenitor of sour cherry. Among these haplotypes, there are two PPMs (S_1' and S_{13}'), 3 SPMs (S_{6m}, S_{6m2} and S_{13m}) and 2 haplotypes (S_a and S_d) with unknown molecular modifications. The coding sequence of S_1' *SFB* contains a 615 bp *Ds*-like element (Hauck *et al.* 2006b), the S_{13}' has a 1 bp substitution that led to a premature stop codon before the V2 variable region (Tsukamoto *et al.* 2006). The S_{6m}-haplotype contains a 2.6 kb insertion upstream of *S-RNase* (Yamane *et al.* 2003b), while the S_{6m2} and S_{13m} suffered 1 and 23 bp deletions in their *S-RNase* gene, respectively, that led to premature stop codons (Tsukamoto *et al.* 2006).

Tsukamoto *et al.* (2006) put forward an intriguing speculation on why sour cherry had a relative high frequency of non-functional *S*-haplotypes. The *S*-haplotype mutations in sour cherry may be a conse-

quence of genomic instability associated with polyploidization (Zhao *et al.* 1998). The accelerated evolution exhibited by one of the paralogous gene pairs was possible since it was presumably free from selection pressure to maintain its original function. In contrast, sweet cherry has only one pair of *S*-haplotypes and is thus subject to functional constraints. Additionally, those non-functional *S*-haplotypes that emerged in sour cherry would likely be maintained hidden in the population since sour cherry is SI when only one non-functional *S*-haplotype is present (Hauck *et al.* 2006a). According to the so called one-allele-match model, SC selections must contain a minimum of two non-functional *S*-haplotypes.

Apricots: In Japanese apricot, the naturally occurring S_f-allele has also undergone a pollen-part mutation. Ushijima *et al.* (2004) designated SFB_f, of which the putative coding region was disrupted in the middle by an approx. 6.8 kb insertion. The insertion generated a stop codon, therefore, the SFB_f transcripts encode a putative SFB_f protein lacking 195 amino acids (AAs) residues at the C-terminal half including the HVa and HVb regions and contains 37 additional residues encoded by the inserted sequence.

In European apricot, Vilanova *et al.* (2006) 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 which results in the expression of a truncated protein. Halász *et al.* (2007a) isolated a new *S*-haplotype, S_8, which was clarified to be the non-mutated wild type version of the S_C-haplotype and hence S_C=S_8'. The S_C- and S_8-*RNases* had 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 pollen showed a typical incompatible phenotype in S_CS_9 styles. The isolated apricot SFB_8-allele was the first known progenitor allele of natural self-compatibility in *Prunus* species.

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 could not be detected. Very similarly, no *S*-locus linked mutations or pollen-*S* duplications were detected in cv. Katy, known to be SC (Wu *et al.* 2011). Results suggest that these cultivars have an additional mutation, presumably outside the *S*-locus, which causes a loss of pollen-*S* activity when present in pollen. These findings support a hypothesis that modifying factors other than the *S*-locus are required for GSI in apricot.

In China, the centre of origin for apricot, SI types predominate; however, most European cultivars are self-compatible (Halász *et al.* 2007a). This indicates that human selection might have had an immense contribution to the evolution and dissemination of the SC phenotype. The S_C-*RNases* differed more than their corresponding *SFB* sequences. The evolutionary model of Newbigin and Uyenoyama (2005) assumes that new specificities arise by consecutively accumulating point mutations in both the pollen and pistil genes through an intermediate state of SC, with natural selection favouring mutations in pollen and disfavouring mutations in the pistil specificity gene. However, when pollen function is destroyed, the absence of selection favouring the preservation of SI may permit increased mutations in the *S-RNase*. Consequently, single nucleotide polymorphisms (SNPs) occurring within the non-functional S_C-haplotype allowed monitoring allele-flow through the dissemination routes of apricot, confirming that Mediterranean cultivars are closer to each other than to Hungarian cultivars. The SNPs between the *S*-allele sequences from these cultivar groups must have accumulated during the last 2000 - 2500 years after the southern and northern European dissemination routes of apricot had diverged (Halász *et al.* 2007a). *S*-genotyping in apricot was also used to confirm genetic relationships between Turkish and Hungarian apricots (Halász *et al.* 2010).

Almonds: In almond, similarly to S_f, the allele S_{n5} in *P. dulcis*, which can be regarded as S_{2m} (*i.e.* a mutated version of the *P. dulcis* active S_2-allele), and *P. webbii* alleles S_{n2}, which can be regarded as S_{1m} (*i.e.* a mutated version of the *P. dulcis* active S_1-allele), and S_{n3} indeed appear to cause self-compatibility due to loss of pistil function (Bošković *et al.* 2007). The causes of inactivation most likely lie outside the coding sequence for all those alleles. Preliminary data indicated inactivation being caused by the methylation of the promoter and sequence regions, at least in the case of S_{n3}. Recently, another explanation was given for the inactivity of S_f-RNases, suggesting that a currently unidentified mutation in a non-*S* modifier locus has resulted in the breakdown of SI phenotype (Fernández i Martí *et al.* 2009).

The cv. Supernova was reported as a late flowering SC mutant, obtained by the irradiation of the early-flowering SI cv. Fascionello. Marchese *et al.* (2008) clarified that Supernova is actually a mutant of the SC cv. Tuono, so the self-compatibility of Supernova appears not to be derived from irradiation, inducing a new self-compatibility mutation, but from the presence of S_f in the cultivar from which it is derived. In contrast to the many non-functional haplotypes described from almond, all commercial cultivars carry the S_f-allele indicating their more or less common and recent origins from breeding programs.

Peaches: Hegedűs *et al.* (2006) were the first to identify two *S*-haplotypes in peach with ribonuclease activity. Tao *et al.* (2007) clarified that both peach *S*-haplotypes, S_1 and S_2 as well as S_{2m}, a newly detected haplotype, encode mutated pollen *SFB*, while only S_{2m} has a mutation that affects the function of the pistil *S-RNase*. A cysteine residue in the C5 domain of the S_{2m}-RNase is substituted by a tyrosine residue, and hence affects

correct folding or reduces RNase stability. The *SFB* gene in the S_1-haplotype is a mutant version of almond S_k-haplotype, encoding a truncated *SFB* due to a 155 bp insertion, which forms a direct repeat of the coding sequence resulting in a frame shift and premature stop codon. 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 transposon-like 5 bp insertion leading to a premature stop codon between the V1 and V2 regions.

In peach, a non-functional version of the *S*-haplotype could have prevailed over the almond functional *S*-haplotype due to selection pressure for SC in a humid area where pollinator insects with adequate activity might not have been reliably available (Tao *et al.* 2007). These genetic and environmental advantages of selfing are potentially counterbalanced by inbreeding depression (a reduction in the fitness of selfed progeny relative to outcrossed progeny as a result of increased homozygosity in selfed progeny that exposes deleterious recessive alleles to selection and decreases the contribution of overdominance to fitness) (Good-Avila *et al.* 2008). A weak selection pressure for SC could allow coexistence of both SI and SC individuals and might afford enough time for purging out all deleterious alleles from the populations. Consequently peach, a highly homozygous species, does not suffer from inbreeding depression. The selection pressure had to be mainly natural, since almond, one of the oldest domesticated plants, is SI and natural SC haplotypes are also rare in other fruit trees (Halász *et al.* 2007b, Marchese *et al.* 2007). Ornamental peaches, in which SI rather than SC is a character of preference, are also SC (Tao *et al.* 2007).

Plums: The S_e-haplotype of Japanese plum coding for self-compatibility was hypothesized to be a PPM (Beppu *et al.* 2005) and corresponds to S_5 in the numerical nomenclature (Halász *et al.* 2007b). Expression analyses of *S-RNase* showed that the S_e-*RNase* gene was transcribed in the style and hence the inhibition of transcription of the *S-RNase* gene is not responsible for self-compatibility in the S_e-haplotype of Japanese plum. Analysis of hexaploid European plum cultivars may provide additional non-functional haplotypes and confirmation for the one-allele-match model of the tetraploid sour cherry incompatibility (Hauck *et al.* 2006a).

Pears and apples: The first non-functional *S*-haplotype (S_4^{sm}) was identified in the Japanese pear (*Pyrus serotina*) cv. Osa-Nijisseiki. It is a natural SPM that resulted in the deletion of 236 kb including S_4-*RNase* (Okada *et al.* 2008). More recently, an SC haplotype has also been found in European pear (*Pyrus communis*). The S_{21}-haplotype carries natural SPMs including two consecutive indels of 2 and 30 nucleotides at the 3' UTR and a 561 bp retrotransposon insertion within the intron of S_{21}-*RNase* (Sanzol 2009).

Until now, naturally occurring self-compatible haplotype has not been identified in apple (*Malus* × *domestica*). By means of genetic transformation, sense and antisense S_3-*RNase* sequences were introduced into the cv. Elstar, which resulted in the absence of both S_3- and S_5-RNases in pistils of the transgenic lines conferring an SC phenotype (Van Nerum *et al.* 2000).

Summarizing all molecular changes behind the transition from SI to SC haplotypes, we can conclude that among the 13 characterized naturally occurring non-functional *S*-haplotypes, insertions represent the most frequent form of mutation (occurring in 8 haplotypes with inserted fragment sizes ranging from 5 to 6 800 bp). One bp substitution occurred in 3 haplotypes while deletions spanning 1 to 236 kb fragments were also detected in 3 haplotypes. These data illustrate that mobile element insertion is a considerable evolutionary force contributing to the breakdown of GSI, either by suppressing the expression or by interrupting the coding region of the relevant genes (Hauck *et al.* 2006b).

Concerning the unequal distribution between PPM and SPM in favour of the previous, it was proposed that under selection pressure for SC, PPMs might preferentially be selected compared to SPMs, not only because there are far more pollen grains, but also because pollen genotype directly determines the SI phenotype in GSI systems (Tao *et al.* 2007). Moreover, it is rather intriguing to consider that SC mutations in *Prunus* most frequently affect the pollen function, while pollen-part mutated SC haplotypes are completely unknown in the *Maloideae*. Sequence analyses have revealed that the *S*-loci of *Prunus, Petunia,* and *Antirrhinum* contain several *F-box* genes in addition to the pollen determinant *SFB*/*SLF* (Entani *et al.* 2003, Lai *et al.* 2002, Ushijima *et al.* 2003, Wang *et al.* 2004). In these species, however, each *F-box* gene is a single copy in a haplotype. In contrast, the *S*-haplotypes of apple and Japanese pear contain two or three copies of the *SFBB* (*S-locus F-box brothers*) genes (Sassa *et al.* 2007). Occurrence of multiple *SFBB* genes in a sole haplotype may appear inconsistent with the idea that they are the pollen determinant of GSI, since *S-locus F-box* genes are single copy genes in *Prunus* (Entani *et al.* 2003, Ushijima *et al.* 2003, Yamane *et al.* 2003b) and *Petunia* (Sijacic *et al.* 2004). However, apart from their multiplicity, *SFBB* genes are a good candidate for pollen-*S* in *Maloideae*, as they show linkage to the *S-RNase* gene, *S*-haplotype-specific sequence divergence, and pollen-specific expression. Genetic transformation might be a useful tool to declare the function of such multiple genes in the *Maloideae S*-haplotypes.

Several findings show that the *Maloideae S*-locus is larger than the *Prunus S*-locus. Analysis of the Japanese pear S_{4sm}-haplotype showed that the pollen-*S* gene must be located outside the deletion region by at least 110 kb (Okada *et al.* 2008). Additionally, the distances of $MdSFBB^{9\text{-}\alpha}$ and $MdSFBB^{9\text{-}\beta}$ from S_9-*RNase* gene are 42 and 93 kb, respectively. In contrast, the distances between *Prunus S-RNases* and *SFBs* are 380 to 36 kb

(Ushijima *et al.* 2004, Yamane *et al.* 2003b). It should be noted that *Maloideae* was confirmed to be of polyploid origin (Velasco *et al.* 2010), and polyploidization can activate retrotransposons (Madlung *et al.* 2005). The abundant retrotransposons found in the apple *S*-locus may help to prevent recombination at the chromosomal region and to maintain the tight linkage between *S-RNase* and pollen-*S* allele.

The *SFBB* genes might be paralogs all having the same or a derived function, or perhaps one or more of them have experienced a functional loss. Hence, it is possible that only one *SFBB* gene in a haplotype is the pollen determinant. However, this seems unlikely, since pear shows competitive interaction (Lewis and Modlibowska 1942) and multiple *SFBB* genes with *S*-specific polymorphisms are expressed in pollen with normal GSI function. Expressed non-*S SFBB* genes may competitively interact with the *SFBB* to break down GSI in pollen. Another possibility is that all the expressed *SFBB* genes act together as the pollen determinant. Analyses of pollen-part, self-compatible mutations of *Prunus* have found that all mutations, both natural and X-ray-induced, were loss-of-function type (Sonneveld *et al.* 2005, Ushijima *et al.* 2004). However, loss-of-function pollen mutations have not been reported in *Maloideae*, and pollen-part breakdown of GSI occurred only as a result of competitive interaction in tetraploid pears (Lewis and Modlibowska 1942). Moreover, the unequal distribution of pollen- and stylar-part SC mutants is evident between *Prunoideae* and *Maloideae* with the previous subfamily presenting mainly PPMs (although SPMs are also known) and *Maloideae* showing exclusively stylar-part SC mutants (except for competitive interaction in pollen). The occurrence of multiple pollen *S*-genes may explain the absence of the deletion-type of pollen SC mutation in *Maloideae*. A loss-of-function mutation in the pollen gene might be functionally restored or attenuated by another member of the multiple genes '*F-box brothers*'; however, this and similar scenarios require clarification.

The fact that SC haplotypes are more commonly found in *Prunus* than in the *Maloideae* might also be explained by the lack of orthology of the stylar- and pollen-*S* of *Prunus* with the *S*-locus genes of 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 (Ushijima *et al.* 2004). Second, *Prunus S-RNase* has an additional intron compared to maloideous and solanaceous *S-RNases* (Igic and Kohn 2001). Interestingly, *Prunus* has a pistil expressed non-*S*-RNase that has the same structure as maloideous and solanaceous *S-RNases* (Yamane *et al.* 2003a). It may be possible that *Prunus* recruited a different pistil-expressed *RNase* for GSI that may have been derived from the non-*S-RNase*. It was speculated that during evolution some of the ribonucleases involved in the defence of the plant against pathogens may have been converted to function in the defence of the plant against self-pollination (Lee *et al.* 1992).

Non-*S*-locus molecular interactions and signaling events after self- and cross-pollination

Since an increasing number of reports suggested the participation of non-*S* genes in the SI reaction (Fernández i Martí *et al.* 2009, Cachi and Wünsch 2011, Wu *et al.* 2011), the quest for putative candidate genes has been initiated. Modifiers known to be associated with SI in the *Solanaceae* SI system include 120 kDa glycoprotein (120 K), small asparagines-rich protein HT and pistil-specific extension-like proteins III (PELP III) (Cruz-Garcia *et al.* 2003, Cruz-Garcia *et al.* 2005), isoflavone reductase-like protein CP100 (Van Eldik *et al.* 1997) and proteins in the endomembrane system (Kumar and McClure 2010). Until recently, none of these proteins could have been isolated from tree fruit species. Proteomic differences after self- and cross-pollination may clarify additional loci in the fruit tree genome that have important roles in the molecular background of SI/SC phenotype.

Feng *et al.* (2006) compared the differences in the proteome of self- and cross-pollinated SI apricots by two-dimensional gel electrophoresis and liquid chromatography-electrospray ion trap tandem mass spectrometry (LC-ESI-MS/MS). Nine protein spots were exclusively expressed in a 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 unambiguously by *SEQUEST* in *NCBI* protein database: actin-12, enolase, MYB transcription-factor-like protein and heat-shock protein 70 were up-regulated in cross-pollinated pistils compared with self-pollinated pistils; whereas actin-7, actin-8 and fructose bisphosphate aldolase like protein were detected only in self-pollinated pistils.

Actins are highly conserved proteins that are involved in cell motility and are ubiquitously expressed in all eukaryotic cells (Hall 1998). As components of the cell cytoskeleton, actins play an important role in cytoplasmic streaming, organelle movement and extension growth. An intact actin cytoskeleton is essential for pollen germination and tip growth (Taylor and Hepler 1997). It has been shown that in *Papaver rhoeas*, SI results in actin depolymerization, and changes in actin filament levels or dynamics play a functional role in initiating programmed cell death (PCD) in pollen tubes (Thomas *et al.* 2006). Recently, PCD has also been implicated to specifically occur in incompatible *Pyrus pyrifolia* pollen tubes (Wang *et al.* 2009).

Heat-shock protein 70 is a stress-protein member of a family of molecular chaperones and is also developmentally regulated, independent of stress factors. The

MYB factors comprise one of the largest transcription-factor families and regulate the frequency of transcriptional initiation of specific genes (Ito 2005). The function of the MYB transcription-factor-like protein in compatibility interactions is not yet known. However, it has been shown that the MYB transcription-factor-like protein was expressed differently in the compatible and incompatible interactions between pollen and pistils of SI apricots (Feng *et al.* 2009).

In a subsequent study, the same authors extended the number of proteins successfully identified from apricot pistil after self- and cross-pollination of SI genotypes (Feng *et al.* 2009). A total of 30 protein spots from 2D-PAGE that were differentially expressed in compatible and incompatible pistils, were analyzed using LC-ESI-MS/MS and *SEQUEST* in the *NCBI* protein database, and 18 proteins were identified unambiguously. Nine of them, including receptor protein kinase-like protein, isoflavone reductase-like protein and ribose-phosphate pyro-phosphokinase were detected only in the SC pistils. Interestingly, the latter was shown to map on scaffold 3 of the peach genome (unpublished data), similar to the position of the unidentified gene responsible for cv. Cristobalina SC (Cachi and Wünsch 2011).

Six other proteins, including actin 7, a putative serine/threonine kinase, and *S*-RNase, were detected only in the SI pistils. A mitochondrial NAD-dependent malate dehydrogenase and a probable elongation factor G were up-regulated in SC, while heat shock cognate 70 was up-regulated in SI interaction. Interestingly, PCD was shown to induce massive protein degradation in tobacco, while some proteins (*e.g.* hsp70) remained unchanged (Chaves *et al.* 2011).

The results suggest that the processes upstream or downstream of self-compatibility and self-incompatibility reactions may include different proteins. The transcript abundance of *S-RNase* gene was higher in SI seedlings than that in SC seedlings (Feng *et al.* 2009). After self-pollination, an incompatibility *S-RNase* was induced only in SI and not in SC apricot, which may indicate that post-transcriptional regulation of *S-RNase* occurs in the SI apricot. The expression of isoflavone reductase-like protein was induced predominantly in the pistils of SC apricot. Isoflavone reductase (IFR) is involved in the production of isoflavonoid phytoalexins, which accumulate in response to pathogen attacks (Lers *et al.* 1998). Isoflavonoids are not synthesized in the *Solanaceae* and it was not expected that there would be an *IFR* gene in apricot. The predicted CP100 protein that was up-regulated in pistils upon pollen growth shows similarity to leguminous IFRs, and the IFR-like NAD(P)H oxidoreductase present in various plant species, such as maize and tobacco (Van Eldik *et al.* 1997). The IFR-like proteins do not have isoflavone reductase enzyme activity *in vitro*. However, the function of isoflavone reductase-like protein in the pistils of apricot in the compatible interactions is unknown.

The M locus is independent of and epistatic to the *S*-locus and encodes a membrane-anchored cytoplasmic serine/threonine protein kinase (*MLPK*) that is involved in SI signalling, acting downstream of *S receptor kinase* gene (*SRK*) in the *Brassicaceae* (Murase *et al.* 2004). The putative Ser/Thr kinase was strongly induced in SI apricot pistils 24 h after self-pollination (Feng *et al.* 2009), but further studies are needed to address its biochemical role in *Prunus* SI.

In summary, the very few available studies indicated that protein expression varied significantly between SC and SI apricot pistils. This could serve as a foundation in the search for unknown pistil proteins. Interestingly, proteomic studies indicated some commonalities in the SI systems with different molecular bases (*Brassicaceae*, *Papaveraceae* and *Rosaceae*). However, this is not surprising considering the end result of the incompatibility reaction will be the disorganization of the pollen tube. Identification and determination of the biological role of the pistil proteins that are expressed differently during SC and SI interaction could help clarify the molecular mechanism of pollen-pistil interactions in SI fruit trees.

Conclusions

Studies on the molecular background of sexual incompatibility in the rosaceous tree fruits span more than two decades. Although the pistil- and pollen component genes have been identified, their specific interactions still await clarification. More and more data suggest that other loci have crucial influence on the sexual (in)compatible phenotype, although the isolation of such a modifier locus from tree fruit species has not yet been achieved. In addition to the economical significance, this field of plant science may also have implications for declaring molecular networks and following plant evolution.

References

Beppu, K., Komatsu, N., Yamane, H., Yaegaki, H., Yamaguchi, M., Tao, R., Kataoka, I.: S_e-haplotype confers self-compatibility in Japanese plum (*Prunus salicina* Lindl.). - J. hort. Sci. Biotechnol. **80**: 760-764, 2005.

Bošković, R., Tobutt, K., Ortega, E., Sutherland, B., Godini, A.: Self-(in)compatibility of the almonds *P. dulcis* and *P. webbii*: detection and cloning of 'wild-type S_f' and new self-compatibility alleles encoding inactive *S*-RNases. - Mol. Genet. Genom. **278**: 665-676, 2007.

Broothaerts, W., Janssens, G.A., Proost, P., Broekaert, W.F.:

cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. - Plant mol. Biol. **27**: 499-511, 1995.

Cachi, A.M., Wünsch, A.: Characterization and mapping of non-*S* gametophytic self-compatibility in sweet cherry (*Prunus avium* L.). - J. exp. Bot.: in press, 2011.

Chaves, I., Alves, M., Carrilho, D., Duque-Magalhães, M., Ricardo, C., Regalado, A.: Protein changes during programmed cell death in tobacco. - Biol. Plant. **55**: 153-158, 2011.

Cruz-Garcia, F., Hancock, C.N., McClure, B.: *S*-RNase complexes and pollen rejection. - J. exp. Bot. **54**: 123-130, 2003.

Cruz-Garcia, F., Nathan Hancock, C., Kim, D., McClure, B.: Stylar glycoproteins bind to *S*-RNase *in vitro*. - Plant J. **42**: 295-304, 2005.

De Nettancourt, D.: Incompatibility and Incongruity in Wild and Cultivated Plants. - Springer-Verlag, New York 2001.

Entani, T., Iwano, M., Shiba, H., Che, F.-S., Isogai, A., Takayama, S.: Comparative analysis of the self-incompatibility (*S*-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. - Genes Cells **8**: 203-213, 2003.

Feng, J., Chen, X., Yuan, Z., Zhang, L., Ci, Z., Liu, X., Zhang, C.: Primary molecular features of self-incompatible and self-compatible F1 seedling from apricot (*Prunus armeniaca* L.) Katy × Xinshiji. - Mol. Biol. Rep. **36**: 263-272, 2009.

Feng, J., Chen, X., Yuan, Z., He, T., Zhang, L., Wu, Y., Liu, W., Liang, Q.: Proteome comparison following self- and across-pollination in self-incompatible apricot (*Prunus armeniaca* L.). - Protein J. **25**: 328-335, 2006.

Fernández i Martí, A., Hanada, T., Alonso, J., Yamane, H., Tao, R., Socias i Company, R.: A modifier locus affecting the expression of the *S*-RNase gene could be the cause of breakdown of self-incompatibility in almond. - Sex. Plant Reprod. **22**: 179-186, 2009.

Goldraij, A., Kondo, K., Lee, C.B., Hancock, C.N., Sivaguru, M., Vazquez-Santana, S., Kim, S., Phillips, T.E., Cruz-Garcia, F., McClure, B.: Compartmentalization of *S*-RNase and HT-B degradation in self-incompatible *Nicotiana*. - Nature **439**: 805-810, 2006.

Good-Avila, S.V., Mena-Alí, J.I., Stephenson, A.G.: Genetic and environmental causes and evolutionary consequences of variations in self-fertility in self incompatible species. - In: Franklin-Tong, V.E. (ed.): Self-Incompatibility in Flowering Plants. Pp. 33-51. Springer, Berlin - Heidelberg 2008.

Gorbunova, V., Levy, A.A.: Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions. - Nucl. Acids Res. **25**: 4650-4657, 1997.

Halász, J., Pedryc, A., Hegedus, A.: Origin and dissemination of the pollen-part mutated S_C-haplotype which confers self-compatibility in apricot (*Prunus armeniaca*). - New Phytol. **176**: 792-803, 2007a.

Halász, J., Hegedus, A., Szabo, Z., Nyeki, J., Pedryc, A.: DNA-based *S*-genotyping of Japanese plum and pluot cultivars to clarify incompatibility relationships. - HortScience **42**: 46-50, 2007b.

Halász, J., Pedryc, A., Ercisli, S., Yilmaz, K.U., Hegedus, A.: *S*-genotyping supports the genetic relationships between Turkish and Hungarian apricot germplasm. - J. amer. Soc. Hort. Sci. **135**: 410-417, 2010.

Hall, A.: Rho GTPases and the actin cytoskeleton. - Science **279**: 509-514, 1998.

Hauck, N., Yamane, H., Tao, R., Iezzoni, A.: Self-compatibility and incompatibility in tetraploid sour cherry (*Prunus cerasus* L.). - Sex. Plant Reprod. **15**: 39-46, 2002.

Hauck, N.R., Yamane, H., Tao, R., Iezzoni, A.F.: Accumulation of nonfunctional *S*-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid *Prunus*. - Genetics **172**: 1191-1198, 2006a.

Hauck, N.R., Ikeda, K., Tao, R., Iezzoni, A.F.: The mutated S_1-haplotype in sour cherry has an altered *S*-haplotype-specific F-box protein gene. - J. Hered. **97**: 514-520, 2006b.

Hegedus, A., Szabo, Z., Nyeki, J., Halasz, J., Pedryc, A.: Molecular analysis of *S*-haplotypes in peach, a self-compatible *Prunus* species. - J. amer. Soc. hort. Sci. **131**: 738-743, 2006.

Hershko, A., Ciechanover, A.: The ubiquitin system. - Annu. Rev. Biochem. **67**: 425-479, 1998.

Hua, Z.-H., Fields, A., Kao, T.-h.: Biochemical models for *S*-RNase-based self-incompatibility. - Mol. Plant **1**: 575-585, 2008.

Huang, S., Lee, H.S., Karunanandaa, B., Kao, T.: Ribonuclease activity of *Petunia inflata S* proteins is essential for rejection of self-pollen. - Plant Cell **6**: 1021-1028, 1994.

Igic, B., Kohn, J.R.: Evolutionary relationships among self-incompatibility RNases. - Proc. nat. Acad. Sci. USA **98**: 13167-13171, 2001.

Igic, B., Lande, R., Kohn, J.R.: Loss of self-incompatibility and its evolutionary consequences. - Int. J. Plant Sci. **169**: 93-104, 2008.

Ito, M.: Conservation and diversification of three-repeat Myb transcription factors in plants. - J. Plant Res. **118**: 61-69, 2005.

Kawata, Y., Sakiyama, F., Tamaoki, H.: Amino-acid sequence of ribonuclease T2 from *Aspergillus oryzae*. - Eur. J. Biochem. **176**: 683-697, 1988.

Kumar, A., McClure, B.: Pollen-pistil interactions and the endomembrane system. - J. exp. Bot. **61**: 2001-2013, 2010.

Lai, Z., Ma, W., Han, B., Liang, L., Zhang, Y., Hong, G., Xue, Y.: An F-box gene linked to the self-incompatibility (*S*) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. - Plant mol. Biol. **50**: 29-41, 2002.

Lee, H.-S., Singh, A., Kao, T.-H.: RNase X2, a pistil-specific ribonuclease from *Petunia inflata*, shares sequence similarity with solanaceous *S* proteins. - Plant mol. Biol. **20**: 1131-1141, 1992.

Lers, A., Burd, S., Lomaniec, E., Droby, S., Chalutz, E.: The expression of a grapefruit gene encoding an isoflavone reductase-like protein is induced in response to UV irradiation. - Plant mol. Biol. **36**: 847-856, 1998.

Lewis, D.: Structure of the incompatibility gene. II. Induced mutation rate. - Heredity **3**: 339-355, 1949.

Lewis, D., Modlibowska, I.: Genetical studies in pears. - J. Genet. **43**: 211-222, 1942.

Luu, D.-T., Qin, X., Laublin, G., Yang, Q., Morse, D., Cappadocia, M.: Rejection of *S*-heteroallelic pollen by a dual-specific *S*-RNase in *Solanum chacoense* predicts a multimeric SI pollen component. - Genetics **159**: 329-335, 2001.

Madlung, A., Tyagi, A.P., Watson, B., Jiang, H., Kagochi, T., Doerge, R.W., Martienssen, R., Comai, L.: Genomic changes in synthetic *Arabidopsis* polyploids. - Plant J. **41**: 221-230, 2005.

Marchese, A., Bošković, R.I., Martínez-García, P.J., Tobutt, K.R.: The origin of the self-compatible almond 'Supernova'. - Plant Breed. **127**: 105-107, 2008.

Marchese, A., Boskovic, R.I., Caruso, T., Raimondo, A., Cutuli, M., Tobutt, K.R.: A new self-compatibility haplotype in the sweet cherry 'Kronio', S_5', attributable to a pollen-part mutation in the *SFB* gene. - J. exp. Bot. **58**: 4347-4356, 2007.

McClure, B.A., Haring, V., Ebert, P.R., Anderson, M.A., Simpson, R.J., Sakiyama, F., Clarke, A.E.: Style self-incompatibility gene products of *Nicotlana alata* are ribonucleases. - Nature **342**: 955-957, 1989.

Milatovic, D., Nikolic, D.: Analysis of self-(in)compatibility in apricot cultivars using fluorescence microscopy. - J. hort. Sci. Biotechnol. **82**: 170-174, 2007.

Murase, K., Shiba, H., Iwano, M., Che, F.-S., Watanabe, M., Isogai, A., Takayama, S.: A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. - Science **303**: 1516-1519, 2004.

Newbigin, E., Uyenoyama, M.K.: The evolutionary dynamics of self-incompatibility systems. - Trends Genet. **21**: 500-505, 2005.

Okada, K., Tonaka, N., Moriya, Y., Norioka, N., Sawamura, Y., Matsumoto, T., Nakanishi, T., Takasaki-Yasuda, T.: Deletion of a 236 kb region around S_4-RNase in a stylar-part mutant S_4^{sm}-haplotype of Japanese pear. - Plant mol. Biol. **66**: 389-400, 2008.

Panigrahi, J., Kole, P., Kole, C.: RFLP mapping of loci controlling self-incompatibility in *Brassica campestris* and their comparative mapping with *B. napus* and *B. oleracea*. - Biol. Plant. **55**: 54-60, 2011.

Qiao, H., Wang, F., Zhao, L., Zhou, J., Lai, Z., Zhang, Y., Robbins, T.P., Xue, Y.: The F-Box protein AhSLF-S_2 controls the pollen function of *S*-RNase-based self-incompatibility. - Plant Cell **16**: 2307-2322, 2004.

Sanzol, J.: Pistil-function breakdown in a new *S*-allele of European pear, S_{21}°, confers self-compatibility. - Plant Cell Rep. **28**: 457-467, 2009.

Sassa, H., Kakui, H., Miyamoto, M., Suzuki, Y., Hanada, T., Ushijima, K., Kusaba, M., Hirano, H., Koba, T.: *S* locus F-Box brothers: Multiple and pollen-specific F-box genes with *S* haplotype-specific polymorphisms in apple and Japanese pear. - Genetics **175**: 1869-1881, 2007.

Sijacic, P., Wang, X., Skirpan, A.L., Wang, Y., Dowd, P.E., McCubbin, A.G., Huang, S., Kao, T.-H.: Identification of the pollen determinant of *S*-RNase-mediated self-incompatibility. - Nature **429**: 302-305, 2004.

Sonneveld, T., Tobutt, K.R., Vaughan, S.P., Robbins, T.P.: Loss of pollen-*S* function in two self-compatible selections of *Prunus avium* is associated with deletion/mutation of an *S* haplotype-specific F-box gene. - Plant Cell **17**: 37-51, 2005.

Tao, R., Watari, A., Hanada, T., Habu, T., Yaegaki, H., Yamaguchi, M., Yamane, H.: Self-compatible peach (*Prunus persica*) has mutant versions of the *S* haplotypes found in self-incompatible *Prunus* species. - Plant mol. Biol. **63**: 109-123, 2007.

Taylor, L.P., Hepler, P.K.: Pollen germination and tube growth. - Annu. Rev. Plant Physiol. Plant mol. Biol. **48**: 461-491, 1997.

Thomas, S.G., Huang, S., Li, S., Staiger, C.J., Franklin-Tong, V.E.: Actin depolymerization is sufficient to induce programmed cell death in self-incompatible pollen. - J. cell. Biol. **174**: 221-229, 2006.

Tsukamoto, T., Hauck, N.R., Tao, R., Jiang, N., Iezzoni, A.F.: Molecular characterization of three non-functional S-haplotypes in sour cherry (*Prunus cerasus*). - Plant mol. Biol. **62**: 371-383, 2006.

Ushijima, K., Sassa, H., Dandekar, A.M., Gradziel, T.M., Tao, R., Hirano, H.: Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. - Plant Cell **15**: 771-781, 2003.

Ushijima, K., Yamane, H., Watari, A., Kakehi, E., Ikeda, K., Hauck, N.R., Iezzoni, A.F., Tao, R.T.: The *S* haplotype-specific F-box protein gene, *SFB*, is defective in self-compatible haplotypes of *Prunus avium* and *P. mume*. - Plant J. **39**: 573-586, 2004.

Van Eldik, G.J., Ruiter, R.K., Colla, P.H.W.N., Van Herpen, M.M.A., Schrauwen, J.A.M., Wullems, G.J.: Expression of an isoflavone reductase-like gene enhanced by pollen tube growth in pistils of *Solanum tuberosum*. - Plant mol. Biol. **33**: 923-929, 1997.

Van Nerum, I., Incerti, F., Keulemans, J., Broothaerts, W.: Analysis of self-fertility in transgenic apple lines transformed with an *S*-allele either in sense or antisense direction. - Acta Hort. **538**: 625-629, 2000.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S.K., Troggio, M., Pruss, D., Salvi, S., Pindo, M., Baldi, P., Castelletti, S., Cavaiuolo, M., Coppola, G., Costa, F., Cova, V., Dal Ri, A., Goremykin, V., Komjanc, M., Longhi, S., Magnago, P., Malacarne, G., Malnoy, M., Micheletti, D., Moretto, M., Perazzolli, M., Si-Ammour, A., Vezzulli, S., Zini, E., Eldredge, G., Fitzgerald, L.M., Gutin, N., Lanchbury, J., Macalma, T., Mitchell, J.T., Reid, J., Wardell, B., Kodira, C., Chen, Z., Desany, B., Niazi, F., Palmer, M., Koepke, T., Jiwan, D., Schaeffer, S., Krishnan, V., Wu, C., Chu, V. T., King, S.T., Vick, J., Tao, Q., Mraz, A., Stormo, A., Stormo, K., Bogden, R., Ederle, D., Stella, A., Vecchietti, A., Kater, M. M., Masiero, S., Lasserre, P., Lespinasse, Y., Allan, A.C., Bus, V., Chagne, D., Crowhurst, R.N., Gleave, A.P., Lavezzo, E., Fawcett, J. A., Proost, S., Rouze, P., Sterck, L., Toppo, S., Lazzari, B., Hellens, R.P., Durel, C.-E., Gutin, A., Bumgarner, R.E., Gardiner, S.E., Skolnick, M., Egholm, M., Van de Peer, Y., Salamini, F., Viola, R.: The genome of the domesticated apple (*Malus* × *domestica* Borkh.). - Nat. Genet. **42**: 833-839, 2010.

Vilanova, S., Badenes, M.L., Burgos, L., Martinez-Calvo, J., Llacer, G., Romero, C.: Self-compatibility of two apricot selections is associated with two pollen-part mutations of different nature. - Plant Physiol. **142**: 629-641, 2006.

Wang, C.-L., Xu, G.-H., Jiang, X.-T., Chen, G., Wu, J., Wu, H.-Q., Zhang, S.-L.: *S*-RNase triggers mitochondrial alteration and DNA degradation in the incompatible pollen tube of *Pyrus pyrifolia in vitro*. - Plant J. **57**: 220-229, 2009.

Wang, Y., Tsukamoto, T., Yi, K.-W., Wang, X., Huang, S., McCubbin, A.G., Kao, T.-H.: Chromosome walking in the *Petunia Inflata* self-incompatibility (*S*-) locus and gene identification in an 881-kb contig containing S_2-RNase. - Plant mol. Biol. **54**: 727-742, 2004.

Wu, J., Gu, C., Du, Y.-H., Wu, H.-Q., Liu, W.-S., Liu, N., Lu, J., Zhang, S.-L.: Self-compatibility of 'Katy' apricot (*Prunus armeniaca* L.) is associated with pollen-part mutations. - Sex. Plant Reprod.: in press, 2011.

Wünsch, A., Hormaza, J.I.: Genetic and molecular analysis in Cristobalina sweet cherry, a spontaneous self-compatible mutant. - Sex. Plant Reprod. **17**: 203-210, 2004.

Yamane, H., Tao, R., Mori, H., Sugiura, A.: Identification of a non-*S* RNase, a possible ancestral form of *S*-RNases, in *Prunus*. - Mol. Genet. Genom. **269**: 90-100, 2003a.

Yamane, H., Ikeda, K., Hauck, N.R., Iezzoni, A.F., Tao, R.: Self-incompatibility (*S*) locus region of the mutated S_6-haplotype of sour cherry (*Prunus cerasus*) contains a functional pollen *S* allele and a non-functional pistil *S* allele. - J. exp. Bot. **54**: 2431-2437, 2003b.

Zhao, X.-P., Si, Y., Hanson, R.E., Crane, C.F., Price, H.J., Stelly, D.M., Wendel, J.F., Paterson, A.H.: Dispersed repetitive DNA has spread to new genomes since polyploid formation in cotton. - Genome Res. **8**: 479-492, 1998.