Mutations conferring self-compatibility in *Prunus* species: From deletions and insertions to epigenetic alterations

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ABSTRACT

Self-incompatible *Prunus* species show a gametophytic self-incompatibility system, but self-compatibility is an interesting horticultural trait in these fruit tree species. Self-compatibility has recently attracted a particular attention since molecular approaches have been applied to the elucidation of the interacting pollen–pistil mechanism and to the identification of the genes involved in pistil–pollen recognition. Both mutations of the *S*-RNase gene expressed in the pistil and the *SFB* gene expressed in the pollen have been reported to explain breakdown of the incompatibility system in *Prunus*. Stylar-part mutations have revealed that ribonuclease activity of the *S*-RNases is required to inhibit pollen growth and have shown different activity levels for some *S*-RNases. The self-compatibility observed in some cultivars has been reported to be due to a defective pollen *S*-function, such as in sweet cherry, apricot, Japanese apricot and peach. Breakdown of self-incompatibility has also been associated with mutations affecting modifier genes unlinked to the *S*-locus, such as in sweet cherry, apricot, Japanese plum, and almond. Additionally, a double phenotypic expression of the same *S*-genotype has been observed in Japanese plum, sweet cherry and almond. The nature of these different mutations has only been identified in a few cases, including deletions, insertions, shift mutations and, more recently, epigenetic alterations.

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1. Introduction

Self-incompatibility (SI) is an important biological feature from the evolutionary point of view in order to maintain heterozygosis in a species and, as such, a higher variability prone to better adaptability (de Nettancourt, 1977). SI, however, is an inconvenient attribute in non-parthenocarpic crop species, such as in stone fruit trees, where the commercial part of the crop is obtained after an elaborate process of pollination, ovule fertilization and ovary growth. Thus, self-compatibility (SC) is an interesting trait from the horticultural point of view as it might solve many problems related to pollination and fertilization, especially in *Prunus* species where a gametophytic SI (GSI) system operates. Consequently, several breeding programes in the past aimed at obtaining SC cultivars in mainly self-incompatible (SI) species, such as sweet cherry, *Prunus avium* L. (Lewis and Crowe, 1954), and almond, *Prunus amygdalus* Batsch (Socias i Company, 1990). However, the breeding programmes for SC were undertaken when the genetic

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bases for SI/SC were unknown, with the founded expectation that SC would be transmitted to the offspring, as it was later confirmed.

Molecular approaches have been more recently applied to the elucidation of the interacting pollen–pistil mechanism and to the identification of the genes involved in pistil–pollen recognition. Genetically it has been established that SC/SI is controlled by a single polymorphic locus containing at least two linked genes, one specifically expressed in the pistil and the other in the pollen (Kao and Tsukamoto, 2004). The pistil component of SI in Rosaceae, Solanaceae and Plantaginaceae has been determined to be an *S*-RNase (McClure et al., 1989; Tao et al., 1997). *Prunus* species led behind the other Rosaceous species in the identification and cloning of *S*-RNases until Tao et al. (1997) reported the *N*-terminal sequences of almond *S*-RNases, leading to their identification in sweet cherry (Tao et al., 1999) and almond (Ushijima et al., 1998). An *S*-haplotype-specific F-box (*SFB*) was considered to be a good candidate gene for the pollen *S*-determinant in Japanese apricot, *Prunus mume* (Sieb.) Sieb. & Zucc. (Entani et al., 2003), almond (Ushijima et al., 2003), and sweet cherry (Yamane et al., 2003a), showing a tight linkage with the *S*-RNase gene (Ikeda et al., 2004, 2005). The examination of the different *S* haplotypes in Rosaceous fruit trees was extensively undertaken by Yamane and Tao (2009). The genetic structure of the *S* haplotype in these species, as well as the molecular basis for the reaction of pollen tube growth resulting in a compatible or incompatible pollination was reviewed by Tao and Iezzoni (2010), especially in comparison with other families with the *S*-RNase type of SI.

Although the dysfunction of either the pistil or the pollen *S* determinant leads to SC in *Prunus* (Tao and Matsumoto, 2012), other possible mutations may also have taken place (Hegedűs et al., 2012). Consequently, our objective was to review the different mutations suggested as possible bases for the change from SI to SC in the different *Prunus* species (Table 1) in order to understand better the possible genetic mechanisms acting in the SC/SI interaction in stone fruit trees.

2. Stylar-part mutations at the *S*-locus

Stylar-part mutations (SPM) in the *S*-locus have been reported in several families with GSI such as Solanaceae (Ioerger et al., 1991; Royo et al., 1994) and Rosaceae (Sassa et al., 1997; Ushijima et al., 1998, Sonneveld et al., 2005), revealing that ribonuclease activity of the *S*-RNases is required to inhibit pollen growth. A mutation or an alteration in the coding region of the *S*-RNase gene could be the reason of the change of SI activity in some cases of *Prunus* SC, as mentioned in other species. In *Lycopersicon peruvianum* (L.) Mill., some spontaneous SC accessions have an amino acid substitution at one of the essential histidines in the catalytic domain of the *S*-RNase that leads to a complete loss of enzymatic activity (Kowyama et al., 1994; Royo et al., 1994). In *Petunia inflata* R. Fries, the mutant S_3 allele mutagenised by replacing the codon for His-93 with a codon for asparagine, producing a mutant protein that does not exhibit any detectable ribonuclease activity (Huang et al., 1994).

The first molecular approach to SI/SC in *Prunus* was undertaken by Bošković and Tobbutt (1996) when correlated the different SI alleles in sweet cherry with different bands of RNase activity after electrophoresis of the protein fraction of the styles. The same approach with similar results was applied to almond (Bošković et al., 1997), observing later that the SC allele did not show RNase activity (Bošković et al., 1999). As a result, heterozygous SC genotypes only showed a band corresponding to the stylar SI allele expression. Effectively, in these cases no RNase activity at all was observed linked to the SC allele expression (Bošković et al., 1999; Kodad et al., 2009; Fernández i Martí et al., 2010).

However, in some cases a low level of *S*-RNase transcription leading to low levels of *S*-RNase accumulation in the style has been suggested as conferring SC in sweet cherry, Japanese plum (*Prunus salicina* L.) and almond (Yamane et al., 2003b; Watari et al., 2007; Hanada et al., 2009). The level of *S*-RNase accumulation in the pistil is difficult to quantify. Consequently, the references to the absence of *S*-RNase activity may be due either to a complete absence or to a low transcriptional level.

The level of *S*-RNase accumulation has attracted an especial attention in a SC *Prunus* species such as peach, *Prunus persica* (L.) Batsch. Tao et al. (2007) reported an inactive S_{2m}-RNase in several peach cultivars, attributing the reduction of *S*-RNase stability to the replacement of a structurally important cysteine residue in the C5 region by tyrosine. The presence of a cysteine residue in the amino acid sequence of the almond S_f-RNase (Channuntapipat et al., 2001) could also be the reason of a reduced *S*-RNase stability. Bošković et al. (2007) wrongly suggested that a mutation in the C2 region of the S_f-RNase in 'Tuono' almond from histidine to arginine could be the origin of SC in this cultivar, although they had to correct this assertion in a note added in proof. It has already been reported that miss-sequencing of *S* alleles has led to some erroneous conclusions (Socias i Company et al., 2010).

The origin and the mechanism of almond SC are still unknown. Two hypotheses have been put forward to explain how SC appeared. First, a natural mutation in the SI system (Crossa-Raynaud and Grasselly, 1985), and second, a gene transfer through spontaneous inter-specific hybridisation between *P. amygdalus* and *Prunus webbii* (Vierh.) Spach (Socias i Company, 2004). Independently of its origin, it is accepted that the loss of activity of an *S*-RNase in the style is the possible reason for SC in almond (Bošković et al., 1999). Supporting this suggestion, Hanada et al. (2009) reported that the possible origin of SC in almond may be due to the lack or to the very low level of the transcription of the *S*-RNase in the pistil. The double expression of the S_f allele, as later explained in the section on this double expression, supports in almond the hypothesis of the stylar-part mutation (Socias i Company et al., 2012), although being outside of the *S*-RNase gene (Kodad et al., 2009). However, mutations in the *S*-RNase gene may also affect the pollen part in 'Jeffries' almond, which shows intraspecific unilateral compatibility (Kester et al., 1994). Ushijima et al. (2001) showed that at least two mutations had occurred to generate this reaction in 'Jeffries' almond: deletion of one of the haplotypes and duplication of the other, generating a dysfunctional *S*-haplotype affecting the pistil and pollen *S*-function.

The three-dimensional structure of the *S*-RNase gene has also been suggested as a possible reason for SC in almond. The modeled structures of the *S*-RNase gene consisted of mixed α and β folds, with six helices and six β-strands. However, the S_f-RNase gene contained an additional extended loop between the conserved domains RC4 and C5, which may be involved in the manifestation of SC (Fernández i Martí et al., 2012). Although it has not been definitely confirmed, monitoring of the 3D structure could shed some light on the alteration.

In Japanese plum, SC was attributed to the presence of the *Se* allele (Beppu et al., 2005), specifically to the pistil function of this allele, due to a low level of *S*-RNase accumulation (Watari et al., 2007). The presence of this allele, however, is not always associated to SC (Guerra et al., 2009), thus implying another type of mutation, although linked to the *Se* allele because the inheritance of SC has been correlated with the transmission of the *Se* allele (Beppu et al., 2010).

The study of SC/SI in sour cherry, *Prunus cerasus* L., is somehow intricate because of the polyploid origin of this species, as later commented. However, several mutations have been associated to a low expression or activity of the *S*–RNase protein (Tsukamoto et al., 2008). Thus, Yamane et al. (2003b) found an insertion of