THE ALMOND s_f ALLELE: AN ALLELE IN QUESTION

INTRODUCTION

Once self-compatibility in almond was rediscovered in the 1970s, its importance in almond growing was clearly stressed and its relevance in almond breeding was fully understood (Socias i Company, 1978). The horticultural importance of almond self-compatibility is obtaining commercial yields after an acceptable fruit set (Socias i Company et al., 2009). Therefore, one of the major challenges in the breeding process has been the evaluation of self-compatibility (Socias i Company et al., 2010). The first approaches included fruit set (Almeida, 1945) and pollen tube growth (Socias i Company et al., 1976), but this evaluation was focused on the expression of self-compatibility, not in its genetic identification. These approaches involved both field and laboratory tests, usually laborious and time-consuming, subject to several external contingencies, such as climatic conditions handling procedures, because working with fruit trees implies more work, more space and more time than in annual species (Socias i Company, 1998).

The first results suggested that, as in other Rosaceous species, almond showed a mono-allelic gametophytic self-incompatibility system (Socias i Company et al., 1976). As a consequence, self-compatibility could be due to the presence of an S_f allele, as it happened in other close species. First of all, transmission to the offspring was quickly confirmed (Socias i Company and Felipe, 1977), and transmission data indicated that S_f was dominant over the *S* alleles of self-incompatibility (Socias i Company, 1984). The establishment of its heritability following a Mendelian pattern (Socias i Company and Felipe, 1988), allowed a better definition of crosses in the breeding process (Socias i Company, 1990). However, all these approaches were based on the phenotypic expression of self-compatibility or -incompatibility in the parents and the offspring of the almond crosses.

ALLELE IDENTIFICATION

Identification of *S* alleles was first attempted in order to establish cross-incompatibility groups by test pollination crosses (Kester et al., 1994). However, this approach also involved laborious field tests and could not allow the identification of the S_f allele. Only after Bošković et al. (1999) found no RNase activity for the S_f allele, an efficient identification of this allele could be initiated, although limited by the assumption that lack of RNase activity was due to the presence of the S_f allele. However, the presence of one band is not enough to assess the presence of the S_f allele. The absence of RNase activity may not only be due to the lack of transcription of the *S*-RNase in the pistil, but also to the very low level of this transcription, as reported in Japanese plum (*Prunus salicina* Lindl.) by Watari et al. (2007). Inbreeding may also produce an incompatible expression of self-compatible genotypes with a single RNase band (Alonso and Socias i Company, 2005a). Two different RNase bands may coincide after electrophoresis separation, thus giving a wrong "one band" result when a real superposition of two bands is occurring.

The more recent advances in genetic analysis at the gene level have allowed a closer approach to the S_f allele in almond. First, *S* alleles, including S_f, were identified by PCR analysis using conserved and allele-specific primers (Channuntapipat et al., 2001; Ma and Oliveira, 2001). Various consensus primer sets have been designed to determinate *S*-genotypes in almond. They were designed from conserved regions of *S*-genes to amplify across the second intron (Channuntapipat et al., 2003; Tamura et al., 2000), the first intron (Ortega et al., 2005), or both (Sutherland et al., 2004). However, PCR primers designed from conserved regions do not always distinguish between alleles with a similar number of nucleotides (López et al., 2004). In addition, the detection of some alleles is masked by the presence of another allele, thus giving a wrong single band. This confusion was first detected by Channuntapipat et al. (2003) when the presence of either S_1 or S_7 masked the amplification of S_8 by PCR when using conserved primers. The same masking has also been observed with other alleles (Alonso and Socias i Company, 2005b; Fernández i Martí et al., 2009).

As a consequence, other primer sets have been designed specifically to amplify S_f (Channuntapipat et al., 2001; Ma and Oliveira, 2001). Screening efficiency and flexibility have been also greatly increased with the development of successful multiplex PCR techniques by Sánchez Pérez et al. (2004). This technique avoids the problem of the masked presence of an allele by the expression of another. Once the S_f allele could be identified, the amino acid sequence of its RNase could be determined. However, since the beginning, several amino acid sequences for the S_f-RNase have been deposited in the databases by different authors.

ALLELE SEQUENCING

When the different sequences of the S_f-RNases deposited in the databases were compared, several differences could be observed between them. This diversity was closely examined by Hanada et al. (2009) in order to solve previous confusions on their identity. As a result of this examination, the sequences could be contrasted because most of them had been determined in 'Tuono' and genotypes derived from it, consequently for the same S_f-RNase. This identity allowed different sources of self-compatibility for the genotypes studied to be discarded. The first sequences by Channuntapipat et al. (2001) and Ma and Oliveira (2001) were already different. Further sequencings suggest that the sequence by Channuntapipat et al. (2001) was the correct and must be taken as the consensus sequence.

Figure 1. Multiple alignment of the deduced amino acid sequence of different *S* almond alleles. Accession numbers are referred in Table 1.

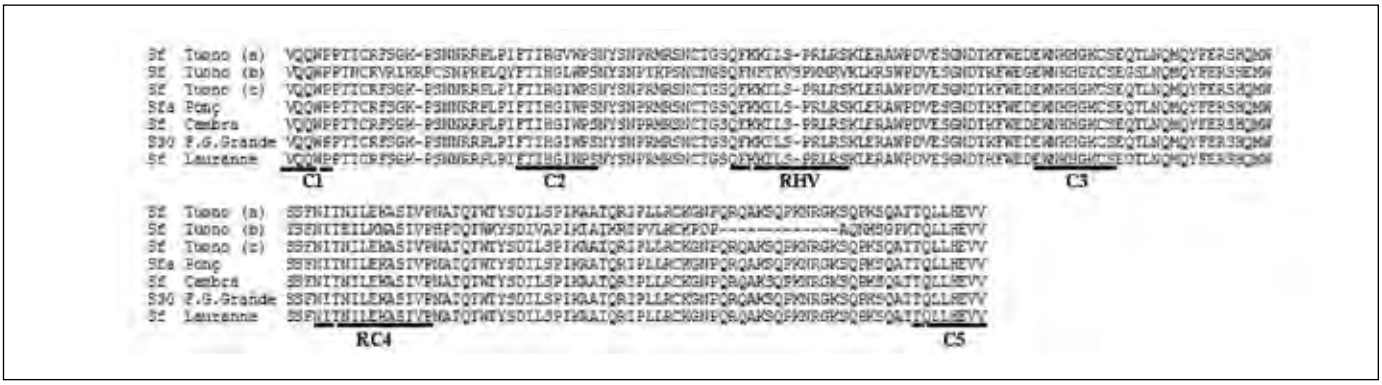

Table 1. Similarity of different almond *S*-RNases with the consensus S_f -RNase.

Allele	Genotype	Database code	Coincidence with the S_f consensus allele (%)	Reference
S_f consensus	'Lauranne' selection IRTA12-2	AY291117	100	Channuntapipat et al. (2001)
S_f	'Tuono'	AF157009	98	Ma and Oliveira (2001)
S_f	'Tuono'	DQ156217	64	Barckley et al. (2006)
S_f	'Tuono'	AM690356	99.3	Bošković et al. (2007)
S_f	'Cambra'	EU684318	100	Kodad et al. (2009a)
S_{fa}	'Ponç'	EU293146	100	Kodad et al. (2009a)
S_{fa}	'Alzina'	FJ887784	100	Kodad et al. (2010)
S_{fa}	'Garondès'	FJ887783	100	Kodad et al. (2010)
S_{fa}	'Vivot'	AB467370.1	100	Fernández i Martí et al. (2010a)
S_{30}	'Fra Giulio Grande'	AM690361	100	Bošković et al. (2007)

Ma and Oliveira (2001) showed valine instead of isoleucine and histidine instead of arginine in the C2 region, probably as a result of a mistake in sequencing (Fig. 1). Fig. 1 shows the alignment of the published sequences for the S_f-RNase as well as some other *S*-RNases for comparison and mostly agrees with the results of Hanada et al. (2009) and Fernández i Martí et al. (2010). The consensus sequence of Channuntapipat et al. (2001) was amplified in 'Lauranne' and selection IRTA12-2, two self-compatible genotypes deriving from 'Tuono', the cultivar utilized in several determinations as shown in Table 1, although not always correctly.

Barckley et al. (2006) gave an amino acid sequence for 'Tuono' S_f identical to S_1, probably due to missampling. Consequently, they incorrectly suggested that the 'Tuono' genotype present in California, although self-compatible, showed a different S_f allele and could be a different clone than the 'Tuono' genotype studied in the other reports. As a consequence of this missampling, all the conclusions of this paper must be discarded.

A similar confusion was produced by the paper of Bošković et al. (2007), who had to recognize a missequencing in a note added in proof, thus invalidating most of the reasoning of their conclusions. Their 'Tuono' S_f did not really show the supposed histidine substitution instead of arginine in its sequence, thus confirming that the consensus sequence of Channuntapipat et al. (2001) must be maintained for all the S_f alleles so far sequenced. This consensus sequence is identical to the S_f sequence of 'Cambra', another cultivar derived from 'Tuono', to the S_{fi} sequence of 'Blanquerna', a cultivar derived from 'Genco', not from 'Tuono', and to five *S* alleles reportedly conferring self-incompatibility in almond (Table 1).

The identity of the S_f sequences from both self-compatible and self-incompatible genotypes gave rise to another question: the double expression of this allele (Kodad et al., 2009; Socias i Company et al., 2011). These two forms of the S_f allele are equally identified by specific primers and show an identical allele sequence (Fernández i Martí et al., 2009; Kodad et al, 2009).This double expression suggests that the coding region of the S_f gene may not be the exclusive origin of self-compatibility in almond (Kodad et al., 2009a) and that some genetic modification outside this coding region must be affecting that expression (Fernández i Martí et al., 2009), taking into account that this identity is not only restricted to the coding region (C1 to C5), as deduced from their sequences (Fig. 1), but also to the alignment of their 5'-flanking regions as shown by the construction of a fosmid library (Fernández i Martí et al., 2010).

All the almond self-compatible genotypes so far identified have shown the same S_f allele (Table 1), thus suggesting a monophyletic origin of self-compatibility in almond. Work in process (Fernández i Martí et al., in preparation) is proposing a point change in the S_f allele producing this expression change from self-incompatibility to self-compatibility.

ALLELE TERMINOLOGY

The mistakes in allele sequences observed by Bošković et al. (2007) led them to incorrectly name a new allele, S_{30}, which they wrongly considered different from S_f, although it is identical to S_f, but showing a different activity (Kodad et al., 2009a). This new name may create new confusions in almond *S* allele research because the identity of any allele must be preserved, once correctly defined by its sequence, in spite of showing a different phenotypic expression. As a consequence, the denomination S_{fa} was suggested for the active S_f allele showing a self-incompatible expression (Kodad et al, 2009a), whereas the denomination S_{fi} was suggested for the inactive S_f allele showing a self-compatible expression (Fernández i Martí et al., 2009). As already mentioned, these two forms of the S_f allele are equally identified by specific primers and show an identical allele sequence (Fernández i Martí et al., 2009; Kodad et al, 2009). Thus, the only difference between them is their expression, not their genetic identity.

As the priority sequence was the sequence published by Channuntapipat et al. (2001), and being considered the consensus sequence, any change in allele terminology must take into account this priority and cannot be based in erroneous results.

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R. Socias i Company, O. Kodad,
À. Fernández i Martí and J.M. Alonso
Unidad de Fruticultura, CITA de Aragón,
Av. Montañana 930, 50059 Zaragoza, Spain
E-mail: rsocias@aragon.es