Expression of the Sfa-allele in homozygote genotypes (SfaSfi) indicates a mutation in the stylar part of the Sf haplotype as origin of self-compatibility in almond

Kodad O., Socias i Company R., Alonso J.M.

in

Kodad O. (ed.), López-Francos A. (ed.), Rovira M. (ed.), Socias i Company R. (ed.).
XVI GREMPA Meeting on Almonds and Pistachios

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 119

2016
pages 55-58

Kodad O., Socias i Company R., Alonso J.M. **Expression of the Sfa-allele in homozygote genotypes (SfaSfi) indicates a mutation in the stylar part of the Sf haplotype as origin of self-compatibility in almond.** In : Kodad O. (ed.), López-Francos A. (ed.), Rovira M. (ed.), Socias i Company R. (ed.). *XVI GREMPA Meeting on Almonds and Pistachios*. Zaragoza : CIHEAM, 2016. p. 55-58 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 119)

Expression of the S_{fa}-allele in homozygote genotypes ($S_{fa}S_{fi}$) indicates a mutation in the stylar part of the Sf haplotype as origin of self-compatibility in almond

O. Kodad[1,2,*], R. Socias i Company[1] and J.M. Alonso[1]

[1]Unidad de Hortofruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA),
Av. Montañana 930, 50059 Zaragoza (Spain)
[2]Département d'Arboriculture. Ecole Nationale d'Agriculture de Meknès, BP S/40, Meknès (Morocco)
*e-mail: osama.kodad@yahoo.es

Abstract. The S_f allele shows two different expressions: an active form (S_{fa}) inducing self-incompatibility and an inactive form (S_{fi}) inducing self-compatibility. Their interaction was studied in several hetero/homozygous genotypes ($S_{fi}S_{fa}$) in order to establish if self-compatibility was dominant as previously suggested. The seedling genotype was determined by PCR amplification of genomic DNA with universal and specific primers and the phenotype by pollen tube growth. The results showed full self-incompatibility of the $S_{fi}S_{fa}$ genotypes as a result of the recognition of any kind of S_f pollen (S_{fi} or S_{fa}) by the style, where S_f-RNase was produced due to the presence of the S_{fa} allele. These results confirm the allelism of the S_f allele with the series of *S* alleles of self-incompatibility and that a mutation in the stylar part of the S_{fa} haplotype has led to the self-compatibility of the S_{fi} form. The recognition of the S_{fi} pollen by the S_{fa} style confirms that the presence of the S_{fi} haplotype does not ensure self-compatibility, and that in these hetero/homozygous genotypes the expression of S_{fa} is dominant over that of S_{fi}.

Keywords. *Prunus amygdalus* Batsch – Self-compatibility – Breeding – S_f allele – Allele expression – Allele recognition.

L'expression de l'allèle S_{fa} dans les génotypes homozygotes $S_{fi}S_{fa}$ indique une mutation dans la partie stylaire du haplotype S_f comme l'origine de l'auto-compatibilité chez l'amandier

Résumé. *L'allèle S_f montre deux formes d'expressions différentes: une forme active (S_{fa}) qui confère l'auto-incompatibilité et une forme inactive (S_{fi}) qui induit l'auto-compatibilité florale. Leur interaction a été étudiée chez quelques génotypes hétéro/homozygotes ($S_{fi}S_{fa}$) pour établir si l'auto-compatibilité est dominante comme a été suggéré antérieurement. Le génotype des semis a été déterminé par l'amplification PCR de l'ADN génomique avec des amorces universelles et spécifiques et le phénotype a été évalué par l'étude de la croissance du tube pollinique dans le style. Les résultats ont montré que les génotypes $S_{fi}S_{fa}$ sont complètement auto-incompatibles due à la reconnaissance des deux formes de pollen S_f (S_{fi} ou S_{fa}) au niveau du style, où S_f-RNase est produite par la présence de l'allèle S_{fa}. Ces résultats confirment l'allelisme de l'allèle S_f avec la série d'allèles S de l'auto-incompatibilité florale chez l'amandier et indiquent qu'une mutation dans le style de l'haplotype S_{fa} a généré l'auto-compatibilité de la forme S_{fi}. La reconnaissance du pollen S_{fi} dans le style S_{fa} confirme que la présence du haplotype S_{fi} n'assure pas l'auto-compatibilité, et que l'expression du S_{fa} chez ces hétéro/homozygotes est dominante sur le S_{fi}.*

Mots-clés. Prunus amygdalus *Batsch – Auto-compatibilité – Amélioration – S_f allèle – Expression d'allèle – Reconnaissance d'allèle.*

I – Introduction

Self-compatibility (SC) has been considered a priority objective in almond (*Prunus amygdalus* Batsch) breeding (Socias i Company, 1990). After confirming that SC was a transmissible trait (Socias i Company and Felipe 1977) it was attributed to the presence of the S_f allele, allelic to the series of *S* alleles of self-incompatibility (SI) (Socias i Company 1984), being inherited as a Mendelian trait (Socias i Company and Felipe 1988). In almond, it was firstly established that SC is due to the lack of RNase activity of the S_f allele (Bošković *et al*., 1999). However, Kodad *et al.* (2009; 2010) reported that three local Spanish cultivars with the S_f allele were self-incompatible (SI), denominating as S_{fa} the active form of the S_f allele, showing a SI expression, whereas the denomination S_{fi} has been suggested for the inactive S_f allele showing a SC expression (Fernández i Martí *et al*., 2009). The two forms of the S_f allele are not only identical for the coding region sequence (C1 to C5) (Kodad *et al*., 2009; Fernández i Martí *et al*., 2009), but also at the alignment of their 5'-flanking regions, as shown by the construction of a fosmid library (Fernández i Martí 2010). Later Kodad *et al*. (2010) reported that some Spanish almond cultivars sharing similar *S*-genotype, including the S_{fa}-allele, are cross-incompatible. In almond, some cases of cross-incompatibility have been reported in combinations sharing identical *S*-genotypes (Bošković *et al*., 2007; Fernández i Martí *et al*., 2009; Socias i Company *et al*., 2012). Thus, in this situation a question has arisen: what will be the expression of homozygote genotypes sharing the two forms of the *Sf*-allele? Consequently, our objective was to study the possible interaction between the two forms of the S_f allele when present in the same genotype.

II – Materials and methods

Three almond cultivars with identical *S*-genotype were included as parents to obtain seedlings for analysis. 'Belona' and 'Soleta' (SC, $S_{fi}S_{23}$) were used as female parents and crossed with 'Vivot' (SI, $S_{fa}S_{23}$) pollen to obtain $S_{fi}S_{fa}$ heterozygotes. The crosses were made in the spring of 2009, nuts were collected in the following fall, seeds were stratified and the germinated seedlings were placed in growing plots and later transferred to the open field for flowering.

Genomic DNA was extracted from leaves following the CTAB extraction method based on Doyle and Doyle (1987). The consensus primers AS1II (forward) and AmyC5R (reverse), designed from conserved coding regions flanking the second intron of almond S-RNases, were used, as well as specific primers for the identification of the S_{23}- and S_f-alleles. PCR products were separated in 1.5% (w/v) agarose gels. Band scoring was carried out using a standard 1 kbp DNA ladder (Invitrogen).

The phenotype of the seedlings was determined by pollen tube growth as described by Socias i Company *et al*. (2013), since this method has been shown to be an efficient method for SC evaluation (Socias i Company *et al*., 2014). The pistils were rated according to the level where pollen tubes were observed as defined by Socias i Company *et al*. (2013). Finally, each genotype was classified according to the average rate of all the pistils observed, pooling the data of the two years of observation in order to obtain the SC classification for the genotype.

III – Results and discussion

PCR amplification of genomic DNA of the progeny resulting from the two crosses studied was performed with specific primers to detect the presence of the S_{23} and S_f alleles as shown in Fig. 1. As expected, only two genotypes were observed in the offspring of the two crosses (Table 1). Only the 'Vivot' pollen carrying the S_{fa} allele could grow trough the pistils of the 'Soleta and Belona' cultivars, despite the genetic identity of this S_{fa} allele with the S_{fi} allele of the pistils, reaching the ovule and accomplishing its fertilisation giving progeny with two possible combinations: $S_{fi}S_{fa}$ and $S_{23}S_{fa}$.

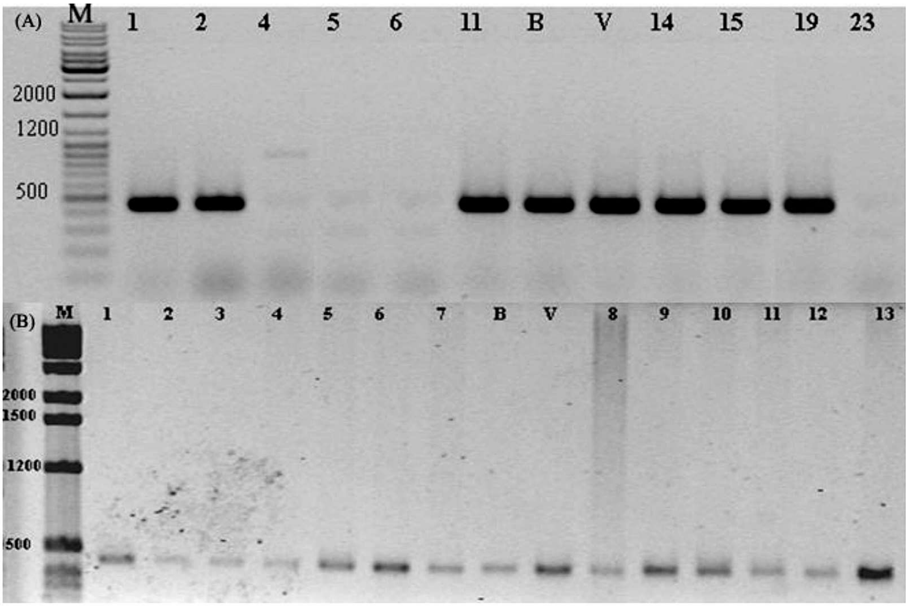

Fig. 1. Agarose gel showing *S*-allele fragments amplified with the *Sf*-specific primers (a) or with the S_{23}-specific primers (b) of some genotypes from the family 'Belona' × 'Vivot', compared to 'Vivot' (V) and 'Belona' (B). Lanes M, 1 kbp marker ladder.

Table 1. Distribution of the *S* genotypes in the offspring of the two almond crosses studied, 'Belona' × 'Vivot' and 'Soleta' × 'Vivot'

Cross	Genotype		χ^2	P
	$S_{fi}S_{fa}$	$S_{23}S_{fa}$		
'Belona' ($S_{fi}S_{23}$) × 'Vivot' ($S_{fa}S_{23}$)	15	10	0.64	0.01
'Soleta' ($S_{fi}S_{23}$) × 'Vivot' ($S_{fa}S_{23}$)	8	11	0.05	0.01
Total	23	21		

The results of pollen tube growth allowed the phenotypic characterisation of the seedlings. The seedlings of genotype $S_{fi}S_{fa}$ were fully SI and a single one was rated as only SI. These results show that the presence of the S_{fi} allele in these cases cannot be related to SC, but that the mechanisms of SI are fully active in this genotype. The homogeneity of results among all seedlings of $S_{fi}S_{fa}$ genotype may explain the interaction between both forms of the S_f allele. In previous studies it has been reported that the presence of the S_{fa} allele in some local almond cultivars showed a self-incompatible phenotype (Kodad *et al*., 2009; 2010; Fernández i Martí 2009) and cross-incompatibility when the two cultivars share the S_{fa}-allele (Kodad *et al*., 2010).

The full SI of the seedlings of $S_{fi}S_{fa}$ genotype indicates that a complete recognition of both alleles takes place in the pistils of this genotype. Taking into account that the S_{fi} allele does not code for any *S*-RNase and that the S_{fa} allele codes for the S_f-RNase (Kodad *et al*., 2009), only a *S*-RNase may be present in the pistils controlling the compatibility of the incoming pollen. The incompatibility of the self-pollination of these seedlings is explained by the recognition by the S_f-RNase of both kinds of pollen, those of S_{fi} and of S_{fa} genotypes. S_{fi} pollen, characterized by SC, was able to grow in S_{fi} pistils, but not in S_{fa} pistils, where the RNase produced by the S_{fa} pistils is able to recognize the S_{fi} pollen, thus stopping its growth and resulting in an incompatible pollination (Fernández i Martí *et al*., 2009). These observations, however, were in heterozygous S_fS_x genotypes, not in homozygous $S_{fi}S_{fa}$ genotypes. Our results show that the inactivation induced by the S_{fi} genotype only takes places in the pistil part, avoiding the production of the S_f-RNase, whereas the pollen part remains completely active, as shown by the recognition of the S_{fi} pollen by the S_f-RNase, probably due to the full genetic identity of both forms. Consequently, in the $S_{fi}S_{fa}$ genotypes, the presence of the S_{fi} allele is not a clue for SC, as generally accepted. These results confirm the hypothesis that S_f is allelic to the *S* alleles of SI (Socias i Company 1984) and suggest that S_{fa} is probably the original allele, being another allele of the *S* locus in a predominantly SI species such as almond. Consequently, S_{fi} expression may have resulted from a mutation, as first suggested by Grasselly and Olivier (1976). This mutation could have been an epigenetic change taking place in the upstream region of the S_f-RNase (Fernández i Martí *et al*., 2014).

Acknowledgments

This research was supported by the Spanish grant AGL2010-22197-C02-01 and Research Group A12 of Aragón. Technical assistance by J. Búbal and O. Frontera is highly appreciated.

References

Bošković R., Tobutt K.R., Ortega E., Sutherland B.C. and Godini A., 2007. Self-(in)compatibility of the almond *P. dulcis* and *P. webbii*: detection and cloning of 'wild-type S_f' and new self-compatibility alleles encoding inactive *S*-RNases. In: *Molecular Genetics and Genomics*, 278, p. 665-676.

Bošković R., Tobutt K.R., Duval H., Batlle I., Dicenta F. and Vargas F.J., 1999. A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. In: *Theoretical and Applied Genetics*, 99, p. 800-810.

Doyle J.J. and Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. In: *Phytochemistry Bulletin*, 19, p. 11-15.

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

Fernández i Martí À., Hanada T., Alonso J.M., Yamane H., Tao R. and Socias i Company R., 2010. The almond S_f haplotype shows a double expression despite its comprehensive genetic identity. In: *Scientia Horticulturae*, 125, p. 685-691.

Fernández i Martí A., Gradziel T.M. and Socias i Company R., 2014. Methylation of the S_f locus in almond is associated with *S*-RNase loss of function. In: *Plant Molecular Biology*, 86, p. 681-689.

Grasselly C. and Olivier G., 1976. Mise en evidence de quelques types autocompatibles parmi les cultivars d'amandier (*P. amygdalus* Batsch) de la population des Pouilles. In: *Annales de l'Amélioration des Plantes*, 26, p. 71-75.

Kodad O., Socias i Company R., Sánchez A. and Oliveira M.M., 2009. The expression of self-compatibility in almond may not only be due to the presence of the S_f allele. In: *Journal of the American Society for Horticutural Science*, 134, p. 221-227.

Kodad O., Alonso J.M., Fernández i Martí À., Oliveira M.M. and Socias i Company R., 2010. Molecular and physiological identification of new *S*-alleles associated with self-(in)compatibility in local Spanish almond cultivars. In: *Scientia Horticulturae*, 123, p. 308-311.

Socias i Company R., 1984. Genetic approach to the transmission of self-compatibility in almond (*Prunus amygdalus* Batsch). In: *Options Méditerranéennes CIHEAM/IAMZ*, I/84, p. 123-127.

Socias i Company R., 1990. Breeding self-compatible almonds. In: *Plant Breeding Reviews*, 8, p. 313-318.

Socias i Company R. and Felipe A.J., 1977. Heritability of self-compatibility in almond. In: *III Coll. GREMPA*, Bari, 3-7 October, p. 181-183.

Socias i Company R. and Felipe A.J., 1988. Self-compatibility in almond: transmission and recent advances in breeding. In: *Acta Horticulturae,* 224, p. 307-317.

Socias i Company R., Kodad O., Fernández i Martí À. and Alonso J.M., 2012. The double expression of S_f in almond. In: *Acta Horticulturae*, 967, p. 121-125.

Socias i Company R., Kodad O., Fernández i Martí À. and Alonso J.M., 2013. Pollen tube growth and self-compatibility in almond. In: *Plants,* 2, p. 50-56.

Socias i Company R., Kodad O., Fernández i Martí À. and Alonso J.M., 2014. Self-compatibility expression in almond by pollen tube growth. In: *Acta Horticulturae,* 1028, p. 111-116.