Self-(in)compatibility in 'AS-1', a Local Spanish Almond Cultivar

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Abstract

The finding of new self-compatible cultivars in local population is a breeder's objective in order to increase the sources of self-compatibility for the almond (*Prunus amygdalus* Batsch) breeding programmes. 'AS-1', a local Spanish selection, was considered to be self-compatible according to its self pollen tube growth and was incorporated in the almond breeding programme of Zaragoza as a source for this trait. However, both pollen tube growth and fruit set after self-pollination have shown that this selection is self-incompatible. The PCR analysis using specific and consensus primers revealed that its genotype is S_8S_{12}, where both alleles control self-incompatibility and do not confer self-compatibility in almond. The field crosses of 'AS-1' and 'Marcona Flota', of the same *S* genotype, were incompatible, confirming the *S* genotype of 'AS-1' determined by PCR analysis. The sequencing of the SFB *S* haplotype showed that 'AS-1' presents SFB^8 and SFB^{12}. Moreover, the absence of any notable deletion or insertion upstream from the HVa and HVb regions in the sequence of the SFB^8 and SFB^{12} *S*-haplotypes confirm their identity. All these results, including pollination tests, PCR analysis and cloning and sequencing of the *S* alleles of 'AS-1', indicate that this local selection is self-incompatible.

INTRODUCTION

The limited number of sources for self-compatibility utilised in almond breeding programmes (Socias i Company, 2002) recommended the search for new self-compatible forms among the different almond populations, mainly in local forms, such it happened with the local cultivars of the Puglia region in Italy (Grasselly and Olivier, 1976). As a result of an initial search, 'AS-1' was noticed in Tamarite de la Litera, Huesca province, Spain, as a single late-blooming tree, isolated from other simultaneously blooming almond trees and setting fruit every year, thus presumably self-compatible. Herrero and Felipe (1975) conducted controlled field pollinations and pollen tube growth observations in several cultivars and reported that 'AS-1' was self-compatible.

'AS-1' was included in the Zaragoza breeding programme and Socias i Company and Felipe (1988) reported a 3:1 (SC:SI) distribution in the progeny of the cross 'Tuono' × 'AS1', as expected in a cross involving two different heterozygous self-compatible cultivars. When 'AS-1' was open-pollinated, these authors found four self-compatible seedlings in a progeny of 15, thus presumably showing a self-compatible behaviour both in expression and transmission.

Thus the aim of this work was to confirm the molecular identity of the *S*-RNases and SFBs of 'AS-1', as well as the phenotypic expression of its self-(in)compatibility by pollen tube growth studies after controlled pollinations.

MATERIALS AND METHODS

Plant Material

Two Spanish cultivars were studied, 'AS-1' and 'Marcona'. Plant samples were obtained from the Spanish almond germplasm collection located at the CITA de Aragón in Zaragoza, maintained as living plants grafted on the almond × peach hybrid clonal rootstock INRA GF-677 using the standard management practices (Espiau et al., 2002). Pollen of 'Marcona Flota' was obtained from CEBAS (CSIC), Murcia (Spain).

Cloning and g DNA Sequencing of S-RNase and SFB S-haplotype

Genomic DNA was extracted from leaves following the method based on Doyle and Doyle (1987). For PCR amplification the forward primer PaConsI-F and the reverse primer EM_PCconsRD were used according to Ortega et al. (2006). A fragment of the SFB was PCR-amplified according to Yamane et al. (2003) with primers SFB-C1F and SFB-FB3 (Ushijima et al., 2003). Prior to cloning, the band sizes corresponding to the target alleles were purified and quantified on 1.5% agarose gel. The purified PCR products were cloned into the vector pCR2.1 using the TA Cloning Kit.

Pollination Tests

Physiological self-compatibility was assessed by pollen tube growth and fruit set after artificial hand pollination. Self- and cross-compatibility was assessed in the laboratory by observation of pollen tube growth in the style under a fluorescence microscope. Fruit set was evaluated in the field on several branches, where at least 100 flowers per branch were emasculated and self-pollinated. In other branches, the flowers were cross-pollinated with 'Marcona' and 'Marcona Flota' pollen. Fruit set was recorded in June for all treatments.

RESULTS AND DISCUSSION

PCR amplification of 'AS-1' genomic DNA using primers PaConsI-F and EM_PCconsRD produced only one fragment (Fig 1). The cloning and sequencing analysis revealed that this band corresponds to the S_{12} allele, confirming the results previously obtained by Kodad et al. (2008). For the other allele, we assume that it is identical to S_8 sequenced in 'Nonpareil' by Ushijima et al. (1998), since the specific primer designed for the S_8 allele from the second intron of 'Nonpareil' amplifies a similar band of 640 bp in 'AS-1' (Fig 2).

Self-compatibility observed in some genotypes of several *Prunus* species has been attributed to a defective function of the pollen, which could be due to a mutation, deletion or insertion in the coding region of the SFB *S*-haplotype, such as in *P. avium* and *P. mume* (Ushijima et al., 2004). The sequence analysis of the amplified fragment from 'AS-1' showed that it is identical to the SFB^8 and SFB^{12} *S*-haplotypes, excluding the presence of the SFB^f *S*-haplotype. Moreover, the results obtained in the present work indicate the absence of any notable deletion or insertion upstream from the HVa and HVb regions in the sequence of the SFB^8 and SFB^{12} *S*-haplotypes.

No pollen tubes were observed at the style base after self-pollination in the laboratory and no fruit set was obtained after self-pollination in the field, indicating that

'AS-1' is self-incompatible. The high percentage of pistils with pollen tubes at the style base after pollination with 'Marcona' pollen shows that 'AS-1' pistils are able to sustain pollen tube growth if this pollen is cross-compatible. Cross-incompatibility of 'AS-1' and 'Marcona Flota', with the same *S* genotype (S_8S_{12}), agrees with the molecular data.

Herrero and Felipe (1975) incorrectly reported that 'AS-1' was self-compatible after controlled pollinations and pollen tube growth tests, with more than 74% of flowers with pollen tubes in the ovary, in disagreement with our results, but they did not obtain any fruit set when 'AS-1' was selfed, corroborating our results.

As a conclusion, based on the study of pollen tube growth, fruit set after self-pollination, PCR analysis and sequencing of the *S*-RNases and the SFB *S*-haplotypes, 'AS-1' must be reclassified as self-incompatible. The cross-incompatibility between 'AS-1' and 'Marcona Flota' corroborates the new Cross Incompatible Group XXI proposed by Kodad et al. (2007).

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Figures

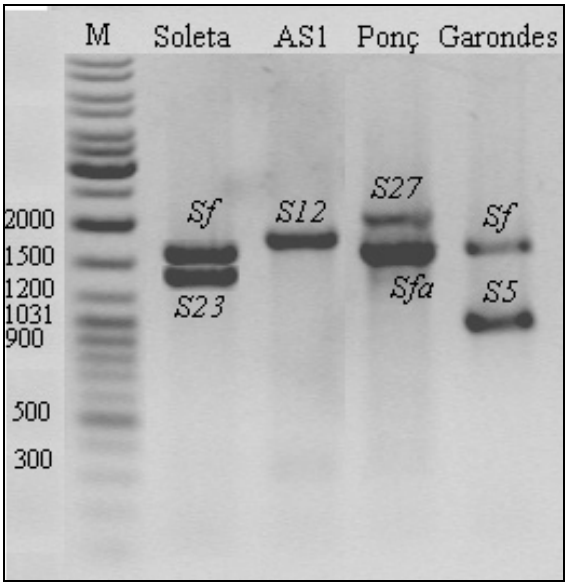

Fig. 1. *S*-genotype identification in several almond cultivars amplified with the primer pair PaConsI-F and EM_PCconsRD. M: 1kb Ladder (Fermentas).

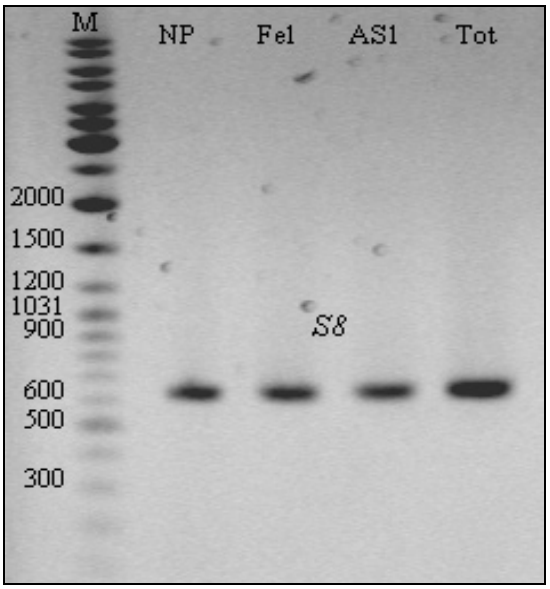

Fig. 2. Agarose (1.5%) gel showing *S*-allele fragments amplified with the specific S_8 primers in several almond cultivars. NP: Nonpareil (S_7S_8). Fel: Felisia (S_fS_8). AS-1 (S_8S_{12}). Tot: Totsol (S_8S_{31}). M: 1kb Ladder (Fermentas)