Self-incompatibility and S-allele identification in new apricot cultivars

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Abstract

In the last years, an important renewal of plant material is taking place in apricot, with the introduction of new cultivars from different breeding programs. The pollination requirements of many of these cultivars are unknown. In this work, the self-(in)compatibility and the S-alleles have been studied in 40 apricot cultivars. Self-(in)compatibility was analysed by self- and cross-pollination experiments followed by the observation of pollen tube growth through the pistil under the microscope. The S-alleles of the cultivars analysed were determined by PCR amplification of the *S-RNase* gene. The self-(in)compatibility and the S-RNase genotype of 31 cultivars are firstly reported here, identifying one new apricot S-RNase allele. These results, combined with information from previous studies, allow to allocate the cultivars in their corresponding incompatibility group.

Keywords: Incompatibility groups, pollen tubes, pollination, *Prunus armeniaca*, S-genotype, S-RNase.

INTRODUCTION

In the last years, an important renewal of plant material is taking place in apricot (*Prunus armeniaca*), with the introduction of new cultivars from different breeding programs (Zhebentyayeva et al., 2012). Most traditional Spanish apricot cultivars are self-compatible (Burgos et al., 1997a). Nevertheless, self-incompatible cultivars have been used as parental genotypes in some breeding programs, resulting in the recent release of new self-incompatible cultivars, which need to be grown together with cross-compatible cultivars to ensure a commercial yield. However, in many cases, the pollination requirements of these new cultivars are unknown.

The gametophytic self-incompatibility (GSI) reaction in *Prunus* is genetically determined by a polymorphic locus named *S*, encoding two linked genes that determine the pistil and pollen phenotypes (Charlesworth et al., 2005). A ribonuclease (S-RNase) determines the allele specificity of the style (Tao et al., 1997) and an F-box protein (SFB) determines pollen allele specificity (Ushijima, 2003).

Self-(in)compatibility in apricot can be evaluated accurately by the observation of pollen tube growth through the style in self-pollinated flowers. This approach allows the isolation of pollination failure from other possible factors affecting fruit set in orchard conditions (Burgos et al., 1993; Rodrigo and Herrero, 1996; Alburquerque et al., 2002). The identification of the S-RNase gene in apricot (Romero et al., 2004; Sutherland et al., 2004) allowed to develop an S-allele genotyping PCR strategy, similar to those developed for cherry and almond (Sutherland et al., 2004). In the first PCR study of S-allele genotyping in apricot reported, seven self-incompatibility alleles (*S*₁ to *S*₇) and one allele for self-compatibility (*S*_c) were identified (Vilanova et al., 2005). Afterwards nine additional S-alleles

(S_8 - S_{16}) were identified by Halász et al. (2005). These studies allowed the determination of different apricot *S*-genotypes from different countries (Halasz et al., 2010; Kodad et al., 2013).

In this work, self-(in)compatibility and *S*-alleles have been studied in a group of 40 apricot cultivars. Self-compatibility was determined by the observation of pollen tube growth under the microscope following self-pollination. The *S*-alleles present in each cultivar were identified by PCR analysis. The results obtained allow assigning each cultivar to its corresponding incompatibility group.

MATERIALS AND METHODS

Trees of 40 apricot cultivars from different collections and orchards of Aragón (Spain) were used, including traditional and new cultivars from different breeding programs (Table 1). Leaf samples were used for *S*-allele genotyping and flowers for pollination experiments.

Pollination experiments to establish self-(in)compatibility were carried out in the laboratory and pollen tube growth was observed under the microscope according to Rodrigo and Herrero (1996). For this purpose, pollen was obtained from flowers at the balloon stage by removing and drying the anthers at room temperature during 24 h. The pollen was sieved and stored at -20°C until further use. Flowers from each cultivar were collected at balloon stage 1 day before anthesis, emasculated and maintained on wet florist foam at room temperature. For each cultivar, 50 flowers were hand pollinated 24 h after emasculation, 25 of them self-pollinated and 25 cross-pollinated with compatible pollen from 'Canino', which is considered universal donor (Alburquerque et al., 2002; Vilanova et al., 2005) as control. Seventy-two hours later, the pistils were fixed in acetic acid: alcohol (1:3). For observation under the microscope, the pistils were stained with 0.1% (v/v) aniline blue in 0.1N K_3PO_4 . Pollen tube growth along the style until the base of the style was observed under an Olympus BH2 microscope with UV epifluorescence using a BP 355/425 exciter filter and a LP 470 barrier filter.

PCR analyses were carried out from genomic DNA isolated following the protocol described by Hormaza (2002) and using the DNeasy Plant Mini Kit (Qiagen). The primer pair SRC-F and SRC-R (Vilanova et al., 2005) was used to amplify the first intron of the apricot *S*-RNase in a PCR reaction of 15 µl, containing 10x NH₄ Reaction Buffer, 25 mM Cl_2Mg , 2.5 mM of each dNTP, 10 µM of each primer, 100 ng of genomic DNA and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK). The temperature profile used had an initial step of 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C and 3 min at 72°C, and a final step of 5 min at 72°C. On the other hand, the primers Pru-C2 and Pru-C4R were used to amplify the second intron as recommended in Vilanova et al. (2005), but with the addition of 10 cycles and 55°C of annealing temperature as indicated in Sonneveld et al., (2003).

RESULTS AND DISCUSSION

Self-pollination of the 40 cultivars included in this study showed that 13 behaved as self-compatible and the other 27 were self-incompatible (Table 1). These results agree with previous reports for 'Canino', 'Katy', 'Palstein', 'Pavot' and 'Tadeo' as self-compatible (Rodrigo and Herrero, 1996; Burgos et al., 1997a; Jie et al., 2005; Wu et al., 2011), and also for 'Aurora', 'Bergarouge', 'Goldrich', 'Goldstrike', 'Hargrand', 'Harcot', 'Moniquí', 'Stark Early Orange', 'Stella', 'Sunglo' and 'Veecot' as self-incompatible (Egea and Burgos, 1996; Rodrigo and Herrero, 1996; Burgos et al., 1997b; Milatović et al., 2010).

On the other hand, PCR analyses with primers SRC-F/SRC-R and Pru-C2/Pru-C4R allowed the identification of eight different *S*-alleles (Table 1). Two of them showed band sizes for the first intron of 420 bp and 430 bp, very close but different to the size attributed to S_6 (424 bp) by Kodad et al., (2013). To verify if these bands correspond to new alleles the PCR products should be cloned and sequenced. In the meantime the bands were assigned to S_x (420 bp) and S_y (430 bp) alleles. The *S*-genotypes reported in this work include 32 cultivars of previously unknown *S*-genotype and 8 cultivars whose *S*-alleles had been reported previously (Table 1). In three of these ('Katy', 'Moniquí' and 'Palstein'), the *S*-alleles

identified herein differs from that reported in other studies (Jie et al., 2005; Vilanova et al., 2005; Wu et al., 2011; Zuriaga et al., 2013). The cultivars 'Perlecot', 'Pinkcot' and 'Robada' showed the S_c allele, but pollen tubes did not reach the base of the style and, therefore, will be further studied. The results also allowed clustering the 21 self-incompatible cultivars into 10 incompatibility groups, two of which had been described previously (Vilanova et al., 2005; Halasz et al., 2010) and the other eight are described for the first time in this work.

In apricot, self-compatibility has been associated with the presence of the S_c haplotype (Vilanova et al., 2005), which has recently been related to the loss of function of pollen S gene by other factors outside the S -locus (Wang et al., 2013; Zuriaga et al., 2013). This agrees with the results obtained in the present work, since the seven cultivars analyzed that presented allele S_c behaved as self-compatible. However, other S -haplotypes appear related to self-compatibility, since six self-compatible cultivars did not show the allele S_c but S_3 , S_2S_7 or S_7S_{X1} .

In this work, the self-(in)compatibility and the S genotype of 40 apricot cultivars has been established. The results allowed to identify 13 cultivars as self-compatible and 27 as self-incompatible. Eight new incompatibility groups have been identified (S_7S_X , S_2S_Y , S_2S_X , S_cS_X , S_3S_c , S_3S_X , S_YS_c and S_XS_Y). This information will be valuable for the selection of parental genotypes in breeding programs and for the appropriate combination of cultivars in commercial orchards.

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Table 1. Self(in)compatibility and S-genotype of 40 apricot cultivars

Cultivar	Self(in)compatibility SI/SC ¹	S-genotype
Goldrich	SI	S_7S_2
Hargrand	SI	S_7S_2
Harcot	SI	S_7S_4
Aurora	SI	$S_7S_X^3$
Goldstrike	SI	$S_7S_X^3$
Tomcot	SI ²	$S_7S_X^3$
Sunglo	SI	S_2S_3
Veecot	SI	$S_2S_Y^3$
Pandora	SI ²	$S_2S_Y^3$
ASF0401	SI ²	$S_2S_Y^3$
Bergarouge	SI	$S_2S_Y^3$
Moniqui	SI	$S_2S_Y^4$
Muñoz	SI ²	$S_2S_Y^3$
Magic Cot	SI ²	$S_2S_X^3$
Pinkcot	SI ²	$S_6S_X^3$
PerleCot	SI ²	$S_3S_C^3$
Henderson	SI ²	$S_3S_X^3$
Durobar	SI ²	$S_3S_X^3$
Robada	SI ²	$S_YS_C^3$
ASF0402	SI ²	$S_XS_Y^3$
ASF0405	SI ²	$S_XS_Y^3$
Gold Bar	SI ²	$S_XS_Y^3$
JNP	SI ²	$S_XS_Y^3$
Lilly Cot	SI ²	$S_XS_Y^3$
Stark Early Orange	SI?	$S_XS_Y^3$
Wondercot	SI ²	$S_XS_Y^3$
Stella	SI	$S_XS_Y^3$
Canino	SC	S_2S_C
Paviot	SC	$S_2S_C^3$
Mitger	SC ²	S_C^3
Soledane	SC ²	S_C^3
Tadeo	SC	S_C^3
Corbato	SC ²	S_C^3
ASF0404	SC ²	S_C^3
Lorna	SC ²	$S_7S_2^3$
Katy	SC	$S_7S_2^4$
Early Queen	SC ²	$S_7S_2^3$
Palstein	SC	$S_7S_2^4$
Westley	SC ²	$S_7S_2^3$
Golden Sweet	SC ²	S_3^3

¹SI: Self-incompatible, SC: Self-compatible²Self(in)compatibility first reported in this study³S-RNase genotypes first reported in this study⁴S-RNase genotype reported herein differs from that reported in other studies