**Abstract**

Apricot (*Prunus armeniaca*) is a member of the Rosaceae originated in China. Most of the European apricot cultivars have been traditionally considered self-compatible (SC) although S-RNase-based-Gametophytic Self-Incompatibility (GSI), a common incompatibility mechanism found in the Rosaceae and genetically determined by a locus (*S*) with multiple alleles, is common in the species. This locus encodes an allele-specific *S-RNase*, expressed in the style, which inhibits the growth of pollen tubes with the same *S* alleles. In the last years, an important renewal of plant material is taking place worldwide, with the introduction of new cultivars from different breeding programs. The use of self-incompatible (SI) parental genotypes has resulted in an increasing number of SI new cultivars with unknown pollination requirements. In order to establish the incompatibility relationships among apricot cultivars, in this work we perform a *S-RNase* allele identification in a group of 48 cultivars from different breeding programs. The *S*-alleles of each cultivar were determined by PCR amplification of the *S-RNase* gene. The results allowed allocating the cultivars in their corresponding incompatibility groups, a highly valuable tool for fruit growers to design apricot orchards, and for breeders to choose parental genotypes in breeding programs.

**Keywords**: *Prunus armeniaca*, self-incompatibility, *S*-genotype, *S*-alleles

**INTRODUCTION**

Apricot (*Prunus armeniaca* L.) was originated in Central Asia where it has been cultivated for millennia and from where it spread worldwide. Apricot cultivars can be classified in six main ecogeographical groups (Layne et al., 1996). The majority of apricot cultivars originated in Middle-Asian and Iranian-Caucasian groups are self-incompatible (SI), however, most of the European apricot cultivars have been traditionally considered self-compatible (SC) (Hormaza et al., 2007).

In the last years, the introduction of new cultivars from different breeding programs has provided an important renewal of plant material (Zhebentyayeva et al., 2012). Although most traditional apricot cultivars are self-compatible (Burgos et al., 1997), the use of self-incompatible parental genotypes in breeding programs (Hormaza et al., 2007) has resulted in the recent release of new self-incompatible cultivars, which need to be grown together with cross-compatible cultivars to ensure an appropriate yield. However, the pollination requirements of many of these new cultivars are unknown.

Self-incompatibility in Rosaceae is based on cell-cell recognition that is determined genetically by a Gametophytic Self-Incompatibility System (GSI) that acts through the inhibition of the pollen tube growth in the style. This mechanism is controlled by a multiallelic locus named *S*, encoding two linked genes that determine the pistil and pollen genotype (Charlesworth et al., 2005). A ribonuclease (S-RNase) determines the allele specificity of the
The identification of the $S$-$RNase$ gene in apricot (Romero et al., 2004; Sutherland et al., 2004) allowed developing 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 (Halász et al., 2010; Kodad et al., 2013; Lora et al., 2017). Most of these $S$-alleles have been characterized using detection of $S$-locus genes by PCR fragment amplification and some of them have also been sequenced. The PCR-analysis can show little differences in the fragment size of some $S$-alleles, even if it was carried out on an automatic sequencer (Lora et al., 2017).

In this work, we determine the $S$-allele identification of 48 apricot cultivars. The genetic identification of some $S$-alleles that could not be distinguished in previous works has been solved by a sequencing approach. Each cultivar has been assigned to its corresponding incompatibility group.

**MATERIALS AND METHODS**

Adult trees of 48 apricot cultivars from different collections and orchards of Aragón (Spain) were used, including traditional and new cultivars from different breeding programs (Table 1).

Genomic DNA was extracted from young leaves and used for $S$-allele genotyping following the protocol described by Hormaza (2002) and using the DNeasy Plant Mini Kit (Qiagen). For the PCR analyses, 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 Cl2Mg, 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 94ºC, 35 cycles of 1 min at 94ºC, 1 min at 55ºC and 3 min at 72ºC, and a final step of 5 min at 72 ºC. For the amplification of the second intron, the primers Pru-C2 and Pru-C4R were used as recommended in Vilanova et al. (2005), but with the addition of 10 cycles and 55 ºC of annealing temperature as indicated in Sonneveld et al. (2003).

The PCR fragments were isolated using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel). Cloning was performed using CloneJET PCR Cloning Kit (Thermo) and by electroporation in E. coli Single-Use JM109 Competent Cells (Promega). The search for similarities was performed in NCBI blast (http://www.ncbi.nlm.nih.gov/BLAST, version 2.2.10).

**RESULTS AND DISCUSSION**

The $S$-alleles were characterized by using the primers SRc-F/SRc-R that allowed amplifying the first intron (Vilanova et al., 2005) and identifying eight alleles. Two of them, $S_1$ and $S_7$, showed a similar fragment size of 400 bp and, in those cases, the primers Pru-C2/Pru-C4R could distinguish both alleles, showing a fragment size of 2260 bp for the $S_1$ allele or 900 bp for $S_7$ according to Vilanova et al. (2005). Primers Pru-C2/Pru-C4R were initially designed to amplify the second intron in Prunus avium (Tao et al., 1999).

In a previous study we identified the $S$-alleles of 40 apricot cultivars, some of them showing a 420 bp or 430 bp fragment that was assigned as $S_x$ and $S_y$ respectively (Lora et al., 2017). These band sizes are close to the $S_x$ allele, which has been reported as 424 bp (Kodad et al., 2013) or 423 bp (Halasz et al., 2010). To elucidate if $S_x$ (420 bp) and $S_y$ (430 bp) correspond to $S_x$ or are different alleles, both fragments were cloned and sequenced, resulting
in a sequence of 414 bp for \(S_x\) and 421 bp for \(S_y\). These results showed differences of several bp from the PCR fragment analysed by an automatic sequencer and the real sequence. Differences in fragment size were also found in the \(S_x\) (334 bp herein, 327 bp in Vilanova et al., 2005; 332 bp in Kodad et al., 2013) and \(S_y\) (358 bp herein; 353 bp in Vilanova et al., 2005; 355 bp in Kodad et al., 2013) alleles.

The alignment of the \(S_x\) allele in the sequence database showed a 99% identity (100% cover) with an unpublished allele, \(S_{52}\) (KF951503.2). Since the \(S_x\) allele has not been previously sequenced, allele \(S_{52}\) could indeed correspond to the \(S_x\) allele. The \(S_x\) allele could also be identified with the primers Pru-C2/Pru-C4 showing a PCR-fragment of around 1400 bp that includes the second intron (Vilanova et al., 2005). Thus, we amplified it using these primers. The PCR results showed a fragment of 1400 bp strongly suggesting that \(S_y\), \(S_6\) and \(S_{52}\) are the same allele. Moreover, the primers Pru-C2/Pru-C4 enclose a sequence of 1386 bp in the allele \(S_{52}\). Thus, in this work, allele \(S_6\) was assigned to \(S_y\).

The sequence of the \(S_x\) allele showed sequence similarity to \(S\)-alleles, but not \(S\)-alleles of \textit{Prunus armeniaca}. The second intron of this allele was amplified with the primers Pru-C2/Pru-C4, showing a PCR-fragment of around 700 bp. Its cloning, sequencing and alignment revealed a 99% identity (100% cover) with the allele \(S_9\) (AY853594, Feng et al., 2006) and, consequently, the allele \(S_x\) was assigned to the allele \(S_9\). These results have enabled the identification of 12 cultivars that were previously identified with the allele \(S_x\) or \(S_y\) (Lora et al., 2017).

In this work, the \(S\text{-RNase}\) genotype has been analysed in a group of 48 apricot cultivars (Table 1). The \(S_c\) allele was identified in 13 cultivars, which were considered self-compatible (Vilanova et al., 2006). The cultivars 'Katy', 'Lorna' and 'Palsteyn' were also assigned to the self-compatible group, because they have been described as self-compatible although they did not exhibit the \(S_c\) allele (Zuriaga et al., 2013; Herrera et al., 2017; Lora et al., 2017). Although two alleles were identified in most cultivars, a unique allele was identified in 13 cultivars. Nineteen cultivars were assigned to seven incompatibility groups. Results herein showed differences in the \(S\text{-RNase}\) genotype of 'Palsteyn' \((S_1S_2)\) and 'Lorna' \((S_1S_2)\) reported in previous studies as \(S_1S_c\) (Raz et al., 2009) and \(S_1\) (Donoso et al., 2009).

These results are valuable for the selection of parental genotypes in apricot breeding programs and for an appropriate distribution of pollenizer cultivars in commercial orchards. Moreover, these results underline the need for a sequencing approach for accurate \(S\)-allele identification that unifies PCR fragment size reports.

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Table 1. Incompatibility groups and S-genotype of 48 apricot cultivars.

| I.G. | S-genotype | Cultivars analysed in this study |
|------|------------|---------------------------------|
| I    | S_1S_2     | Goldrich, Hargrand              |
| III  | S_2S_6     | ASF0401, Bergarouge, Moniqui     |
| VIII | S_2S_9     | ASF0402, ASF0405, Orangered¹, Stark Early Orange, Wonder Cot |
| XVIII | S_2S_3   | Mayacot, Sun Glo                |
| XIX  | S_2S_9     | Goldstrike, Magic Cot           |
| XX   | S_2S_9     | Durobar, Flodea, Henderson, Tsunami |
| XXI  | S_2S_9     | Goldbar                         |

Self-compatible cultivars

ASFO0404 (S_2), Bergecot (S_2S_2), Canino (S_2S_2), Charisma (S_2S_2), Faralia (S_2S_2), Flopria (S_2S_2), Kiotto (S_2), Mitger (S_2), Paviot (S_2S_2), Pricia (S_2S_2), Soledane (S_2), Tadeo (S_2), Tom Cot (S_2S_2), Katy (S_2S_2), Lorna¹ (S_2S_2), Palsteyn¹ (S_2S_2)

Unclassified

Aurora (S_2), Big Red (S_2), Early Queen (S_2), Golden Sweet (S_2), Harcot (S_2), JNP (S_2), Lilly Cot (S_2), Muñoz (S_2), Pandora (S_2), Perle Cot (S_2), Pinkcot (S_2), Veecot (S_2), Westley (S_2)

¹Cultivars in which the S-RNase genotype reported herein differs from that reported in other studies.