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Molecular background of self-incompatibility in apricot

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ABSTRACT *Prunus* species show gametophytic self-incompatibility, a trait encoded by a single multiallelic locus, termed *S*-locus. The *S*-gene products in styles are pistil ribonucleases, while the pollen component is an F-box protein suggesting that the ubiquitin-mediated protein degradation system has a pivotal role in self/non-self recognition. Using non-equilibrium pH gradient electrophocusing and PCR analysis with several *S*-gene specific degenerate primer pairs allowed to identify nine new alleles in apricot. Their schematic structure was determined and confirmed to be different from the previously described alleles. Monitoring of different self-(in)compatibility alleles were also successful using a combination of the two techniques. Preliminary results for the molecular background of fruit set behaviour in apricot are discussed.
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KEY WORDS

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non-equilibrium pH gradient electrofocusing
PCR
Prunus armeniaca L.
self-incompatibility
S-ribonuclease

Species of the *Rosaceae* family show gametophytic self-incompatibility (GSI) to prevent inbreeding depression. This intercellular reaction is controlled by a single multialleleic locus, the *S*-locus (de Nettancourt 1977). The *S*-gene product in styles is a ribonuclease enzyme (*S*-RNase; McClure et al. 1989), while the recently identified pollen product is an F-box protein (Entani et al. 2003; Romero et al. 2004). Successful fertilization may only occur when the *S*-allele carried by the haploid pollen grain does not match any of the two alleles reside in the pistil. Otherwise, pollen tube growth towards the ovary will be arrested.

Growth inhibition is provoked by stylar RNases, which can enter the pollen tubes and degrade rRNA to inhibit protein synthesis of the developing tubes. Self/non-self recognition assumes that non-self *S*-RNases are polyubiquitinated by the SCF_{SFB} complex to be degraded by the 26S proteasome pathway, which results in the inactivation of the non-self *S*-RNases (Ushijima et al. 2004). Concurrently, activity of the cognate *S*-RNases is unaffected in the pollen tubes.

Self-compatibility (SC) in many rosaceous species is presumed to be evolved due to an accidental genetic modification. In almond a pistil-part mutation resulted in an inactive *S*-RNase molecule, while in cherry and Japanese apricot self-compatible pollen-part mutants carry defective SFBs, that polyubiquitinate not only the non-self *S*-RNases but also cognate *S*-RNases for subsequent degradation by the 26 S proteasome pathway.

Almost all the European apricot cultivars have been traditionally considered self-compatible (Kostina 1977); nevertheless more and more exceptions were found (Nyujtó et al. 1985; Burgos et al. 1997). One *S*-allele for SC and several alleles for self-incompatibility were described in Mediterranean and American cultivars (S_1-S_7; Burgos et al. 1998) and in Hungarian and Central Asian genotypes (S_8-S_{16}) (Halász et al. 2005).

Our experiments were carried out to acquire preliminary knowledge concerning the operation of GSI system in Hungarian apricot cultivars.

Materials and Methods

Plant material

Apricot accessions were used in the experiments from the orchard of the Corvinus University of Budapest, Department of Genetics and Plant Breeding in Szigetcsép.

Non-equilibrium pH gradient electrofocusing (NEpHGE)

Forty styles with stigmas uncontaminated with pollen were harvested just before or soon after anthesis. The extraction procedure of stylar ribonucleases was carried out by Bošković and Tobutt (1996). The extracts were separated on vertical slab gels as described earlier (Halász et al. 2005). The catalyte and analyte used, as well as staining procedure were carried out according to Bošković and Tobutt (1996).

DNA extraction, *S*-PCR analyses and sequencing

Genomic DNA was extracted from fully expanded young leaves using the DNeasy Plant Mini Kit (Qiagen). PCR was conducted using EM-PC2consFD and EM-PC3consRD for the amplification of the second intron region according to Sutherland et al. (2004). PCR products were cloned into a pGEM-T Easy vector (Promega) and sequenced in an automated sequencer.

Results and Discussion

In a previous report, *S*-genotyping of a wide range of apricot

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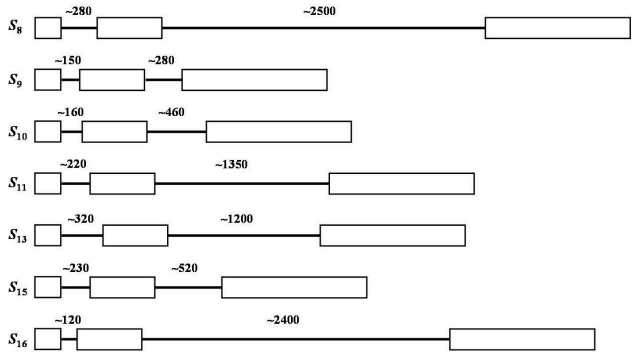

Figure 1. Schematic structures of apricot S_8-, S_9-, S_{10}-, S_{11}-, S_{13}-, S_{15}- and S_{16}-RNase genes deduced from the genomic PCR amplification products.

accessions revealed nine putative new self-incompatibility alleles (Halász et al. 2005). As both methods used (NEpHGE and *S*-PCR analysis with cherry consensus primers) had several advantages and shortcomings, the results provided by their combination were further clarified. Sweet cherry consensus primers amplifying the 1st and 2nd introns and the flanking exon regions worked with a nearly 70% efficiency, amplifying 11 from the total of 16 alleles. Due to an allele-specific length polymorphism, this enabled to determine the putative structure of 7 new alleles (Fig. 1), and could be clearly distinguished from S_1, S_2 and S_4 (Romero et al. 2004).

A more degenerate primer set designed from several available *Prunus S*-alleles (Sutherland et al. 2004), was applied to further improve the efficiency of *S*-allele detection. This could successfully amplify all alleles. Several amplification products were cloned and sequenced and found to be partial *S*-RNase sequences. To monitor allele flow from generation to generation, hybrids and their parents were obtained from apricot breeding programs. Our results show that the unique self-incompatibility alleles carried by the Armenian accessions were also inherited in the progeny.

S-allele constitution of commercial cultivars is of great interest. Cultivars of Hungarian Óriás group are mutually self-incompatible (Nyujtó et al. 1985), which was confirmed by their identical *S*-genotypes (S_8S_9). Recent cultivars were obtained from crosses between the Hungarian Óriás and several self-fruitful cvs. to combine favourable fruit characteristics with self-compatibility. Our analysis showed that self-compatibility was successfully introduced to several descendants.

Self-compatibility is a trait associated with the S_c-allele in apricot. Since this allele encodes a ribonuclease enzyme with great activity, it is presumed that a pollen-part mutation might contribute to the breakdown of SI reaction. Our results further corroborated this hypothesis and a comprehensive study is in progress to characterize the molecular background of self-compatibility in apricot.

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