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Syrian pear (*Pyrus syriaca*) as a pollinator for European pear (*Pyrus communis*) cultivars

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ABSTRACT

In Israel four European pear cultivars are grown: 'Spadona' is the main cultivar and 'Coscia', 'Gentile' and 'Spadochina' are its pollinators. However, molecular *S*-genotyping revealed that 'Spadona' is semi-compatible with its three pollinators. This explains, at least in part, the relatively low pear yield in Israel. The Syrian pear (*Pyrus syriaca*) grows wild in Israel and blooms intensively, overlapping the blooming of the cultivated European pears. Cross-fertilization between Syrian pear and 'Spadona' was shown to be efficient suggesting that Syrian pear might be a potent pollinator for 'Spadona'. Twenty-six Syrian pear seedlings, from different sites in north-east Israel were *S*-genotyped identifying 11 that are fully compatible with the four European pear varieties cultivated in Israel. By this screening, 24 different *S*-RNases were cloned; ten of them are new, whereas the other fourteen had been identified previously. In addition, seedlings of two wild pear species were also *S*-genotyped. Two seedlings from *Pyrus betulifolia* and one from *Pyrus korshinskii* were found to be genetically compatible with the four European pear cultivars. From these seedlings four *S*-RNases were cloned, two are new, one had been cloned previously and one was identical to an *S*-RNase allele cloned from Syrian pear in this work.

1. Introduction

The European pear belongs to the *Rosaceae* family, which carries the gametophytic self-incompatibility fertilization system (GSI). The GSI is controlled by a single multi-allelic locus (*S*-locus). The *S*-locus contains two haplotype-specific genes: the *S*-RNase gene, which is expressed in the pistil, and an F-Box gene named SFB (*S*-haplotype-specific F-Box), which is expressed in the pollen tube (McCubbin and Kao, 2000; Kao and Tsukamoto, 2004). Thus, pears depend on cross-pollination, and commercial orchards contain at least two cultivars that flower synchronically. The cultivar couples may be fully compatible, i.e., they differ in both of their *S*-loci, or semi-compatible, i.e., they share one of the two *S*-loci. Semi-compatibility between cultivars might lead to yield reduction, since half of the pollinator's pollen is rejected resulting in serious economical losses (Goldway et al., 1999; Zisovich et al., 2005; Sapir et al., 2008).

In a previous work (Zisovich et al., 2004a) it was found that 'Spadona', the main European pear cultivar grown in Israel, is semi-compatible with each of its three pollinators 'Gentile', 'Coscia' and 'Spadochina', i.e., the 'Spadonas' *S*-genotype is *S*101, *S*103 and its pollinators carry either *S*101 or *S*103, (Goldway et al., 2009) in addition to another *S*-haplotype. Thus, the full potential of cross-pollination is not being realized.

The *S*-haplotypes carried by 'Spadona' are very common: *S*-101 was found in 56% and *S*103 in 9% of 133 European pear cultivars that were analyzed (Goldway et al., 2009). Hence, among the cultivars that were evaluated for introduction into Israel, most were semi-compatible with 'Spadona' and the few that were fully compatible either did not flower synchronically with it, or produced low-quality fruit or were not accepted by the Israeli consumer.

The Syrian pear (*Pyrus syriaca*) grows wild in Israel and its intensive bloom overlaps the bloom of 'Spadona'. The objectives of the work were (1) to assess the suitability of Syrian pears as viable pollenizers of 'Spadona', and (2) to determine the *S*-genotypes of wild Syrian pear populations in north-eastern Israel so that they could serve as potent full compatible pollinators in the pear orchard.

Twenty-six Syrian pear seedlings from north-east Israel, the country's main pear orchard region, were analyzed, and 11 of them were found to be fully compatible with 'Spadona' and with the other three cultivars grown commercially in Israel. Similarly, two seedlings from *Pyrus betulifolia* and one seedling from *Pyrus korshinskii* were found to be compatible with the four European pear strains commercially cultivated in Israel.

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Fig. 1. Locations of sampled Syrian pears in north-east Israel.

2. Materials and methods

2.1. Assessment of the suitability of Syrian pears as viable pollenizers of 'Spadona'

2.1.1. Hand pollination experiments

Hand pollination experiments were conducted in 2007and 2008 in the Yiftah pear orchards in north-east Israel. Ten representative 'Spadona' trees were chosen for the trials. At the first white stage (mid-March), four branches per tree, with at least 5 inflorescences on each branch, were caged with screen nets (15 mesh, 30% shade) to prevent bees and other insects from reaching the flowers. At the peak of the bloom, open flowers on the caged 'Spadona' branches were hand pollinated, each with a fresh flower of the examined pollinator. Fruit-set percentage was determined for each branch 3 weeks later, before the "June drop" (when many fruitlets drop from the tree). Seed number per fruit was determined at harvest.

The fruit-set of 'Spadona', hand-pollinated by its commercial pollinators, 'Coscia' and 'Spadochina', was the positive control and that of self-hand-pollinated 'Spadona' was the negative control.

2.1.2. Statistical analysis

All data were analyzed for statistical significance by the general linear model (GLM) procedure. Percentage data were subjected to Arcsine transformation before analysis to provide a normal distribution.

2.2. Determination of the S-genotypes of wild Syrian pears

2.2.1. Plant materials

Twenty-six Syrian pear seedlings from north-east Israel were *S*-genotyped. The trees were listed according to village or the geographical site in which they were located (Fig. 1). In addition, two *Pyrus betulifolia* seedling from the Hula experimental station and one *P. korshinskii* seedling from the botanical garden of the Hebrew University of Jerusalem were analyzed. Fresh leaves were picked from each of those trees and stored at −80 °C pending use.

2.2.2. DNA extraction

Extraction of DNA from the leaves was based on the method of Doyle and Doyle (1987). Briefly, 700 μl of extraction buffer (2% hexadecyltrimethylammonium bromide [CTAB], 100 mM of tris pH 8, 20 mM of ethylenediamine tetraacetic acid [EDTA] pH 8, 1.4 M of NaCl, 1% polyvinylpyrolidone [PVP] MW 40,000, and 1% β-mercaptoethanol) was added to 100–200 mg of leaves, which had been powdered under liquid nitrogen with a pestle and mortar. This mixture was incubated for 30 min at 65 °C, with occasional mixing. After cooling to room temperature, two extractions were performed with chloroform:octanol (24:1). The DNA was precipitated with ethanol and dissolved in double-distilled water (DDW). The DNA extract was kept at −20 °C pending use.

2.2.3. PCR amplification

The universal primers used for the PCR amplification of *S*-RNase alleles were based on the conserved peptide sequences "FTQQYQ" from C1 and "FI(D/N)CP(H/R)" from C5 (Ishimizu et al., 1998). The PCR was performed in the MyCycler apparatus (Bio-Rad, USA). The basic program was as follows: 1 cycle of 2 min at 94 °C; 10 cycles of 15 s at 94 °C, 30 s at 48 °C and 2 min at 70 °C; and 20 cycles of 15 s at 94 °C, 30 s at 48 °C and 2.5 min at 70 °C. The final cycle was 7 min at 70 °C.

Each PCR tube contained about 20 ng of DNA, 5 μl of 10× Ex Taq PCR buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of dNTP mixture, 20 pmol of each primer and 0.25 μl of Ex Taq at 5 unit/μl (Takara Bio. Inc. Japan), in 50 μl of reaction mixture.

2.2.4. Cloning and DNA sequence of PCR products

Each PCR product was cloned into pGEMT (Promega, Madison, WI, USA) and sequenced in both directions. For each *S*-RNase, at least two different colonies were sequenced. The *S*7 and *S*7a RNases,