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Self-fertilization in homozygous and heterozygous self-compatible almonds

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Abstract

In homozygous self-compatible genotypes 100% of the pollen grains are potentially able to grow through their own pistil, and thus the rate of self-fertilization could be higher than in heterozygous self-compatible genotypes. To evaluate the advantages of growing homozygous self-compatible almonds, pollen tube growth along the pistil at different times following self-pollination, and fruit set were studied in four homozygous and four heterozygous self-compatible seedlings. The results showed important differences between homozygous and heterozygous individuals for the percentages of pollen tubes in the third section of the style at 24 and 48 h, the pollen tube growth rate being higher in the homozygous. Twenty-four hours following self-pollination only the homozygous individuals showed pollen tubes in the ovary. However, at 72 and 96 h those values were similar for both genotypes, suggesting that space and availability of nutrients become the main limiting factors, overcoming the genetic interactions between pollen and pistil. In general, fruit set was similar in homozygous and heterozygous individuals. Interestingly, one of the homozygous individuals showed problems of fruit development, which might be explained by its inbred origin.

Keywords: Almond; Self-compatibility; Self-fertilization; *Prunus dulcis*; Pollen tube growth; Fruit set

1. Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] is a self-incompatible species with a gametophytic system controlled by a multi-allelic *S* locus (Gagnard, 1954). In this type of system, the self-incompatibility alleles (S_i) are expressed in the style as ribonucleases (*S*-RNases), which specifically reject those pollen tubes with the same *S* genotype (Kao and McCubbin, 1996).

However, self-compatible almond cultivars have been described in the Italian region of Apulia (Stazione Agraria Sperimentale di Bari, 1957; Jaouani, 1973; Grasselly and Olivier, 1976; Godini, 1977; Godini et al., 1992; Palasciano and Godini, 2001), Portugal (Almeida, 1945), and India (Kumar and Kumar, 2000). To explain the origin of self-compatibility in almond cultivars from Apulia two different hypotheses have been proposed. Grasselly and Olivier (1976) suggested that some of those cultivars had a mutation, which was transmitted to other cultivars within the Apulian population during the selection process conducted by the growers. Other authors pointed out that self-compatibility was probably transmitted by natural hybridization of the cultivated almond species with the self-compatible wild species [*Prunus webbii* (Spach) Vierh.] (Godini, 1979; Socias i Company, 1984; Reina et al., 1986; Yamashita et al., 1987). More recently, Bošković et al. (1999) demonstrated that self-compatibility in almond is due to the absence of ribonuclease activity in the style, and suggested a mutation either in almond or in *P. webbii* as its origin.

Growing self-compatible almonds in single cultivar orchards would be desirable, as lower costs in crop management and higher yields are expected. For this reason, self-compatibility is one of the main objectives in almond breeding programmes (Socias i Company and Felipe, 1988; Duval and Grasselly, 1994; Godini and Palasciano, 1997; Vargas et al., 1997; Gradziel and Kester, 1998; Dicenta et al., 2002a). Experimentally, this characteristic has been introduced in almond by hybridization with other *Prunus* species such as peach [*P. persica* (L.) Batsch] (Anderson, 1972; Zaiger, 1978) and the wild *Prunus* species *P. webbii*, *P. mira* and *P. fenzliana* (Gradziel and Kester, 1998; Gradziel et al., 2001). However, most breeding programmes have achieved this aim by crossing a self-compatible cultivar and a self-incompatible cultivar of

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good agronomic characteristics (Felipe and Socias i Company, 1987; INRA, 1991; Socias i Company and Felipe, 1999; Duval, 1999; Egea et al., 2000). For this reason, the self-compatible individuals selected in such programmes are heterozygous for self-compatibility (S_iS_f).

Homozygous self-compatible almond cultivars (S_fS_f) are of great interest as in these genotypes 100% of the pollen grains are potentially able to grow down their own pistil following self-pollination, unlike the heterozygous in which 50% of self-pollen grains are rejected in the style. In addition, homozygous self-compatible genotypes could be used in breeding to assure self-compatibility in the progeny (Ortega and Dicenta, 2003).

In this work we have studied pollen tube growth and fruit set following self-pollination of homozygous and heterozygous self-compatible almond cultivars to evaluate the possible advantages of homozygous self-compatible cultivars regarding self-fertilization, and thus the convenience of growing single cultivar orchards.

2. Materials and methods

2.1. Plant material

Pollen tube growth and fruit set were studied in four homozygous ('A1473', 'A2198', 'A2206', 'A2416') and four heterozygous ('A1177', 'A1194', 'A1775', 'A2321') self-compatible almond seedlings following self-pollination by hand. The seedlings were obtained in 1993 by bagging branches of self-compatible almond selections from CEBAS-CSIC breeding programme (Murcia, Spain). The *S* genotype of these individuals was determined by non-equilibrium pH gradient electrofocusing (NEPHGE) of stylar ribonucleases (Dicenta et al., 2002b). Table 1 summarises information on the parentage and the *S* genotype of these seedlings.

2.2. Fluorescence microscopy

A sample of 40 flowers at stage 'D' (Felipe, 1977) was collected for each individual. The same day the flowers were emasculated, the pistils were placed in trays with the calyx inserted on wet floral foam and kept under controlled conditions (22 ± 2 °C, 12 h photoperiod and 75–80% relative humidity), and the anthers were placed on Petri dishes at room temperature for dehiscence. After 24 h, pollen viability was determined for each individual by observing the percentage of germinated pollen grains in vitro, following the procedure indicated by Remy (1953). In all cases the percentage of germination was higher than 65% (data not shown). The pistils were then self-pollinated using a paintbrush and kept again under the same controlled conditions.

A sample of 10 pistils per individual was collected from the trays at 24, 48, 72 and 96 h after pollination, then fixed in FAA solution and prepared for fluorescence microscopy observation as indicated in Ortega et al. (2002).

For each pistil the number of germinated pollen grains in the stigma, the number of pollen tubes in the first, second and third section of the style and the number of pollen tubes in the ovary were determined using an Olympus BH2 microscope with a UV light-adapted system BH2-RFL-T2, with illumination from an Osram HBO 100 W/2 mercury lamp. Pollen tubes at each section of the style were expressed as a percentage of the number of germinated pollen grains in the stigma for data analysis.

2.3. Fruit set determination

For each individual 60–100 flower buds at 'D' stage were emasculated on branches of these trees and then self-pollinated by hand. Self-pollinated branches on each tree were labelled and the percentages of initial and final fruit set were determined 30 and 60 days after pollination, respectively.

2.4. Statistical analysis

Differences between self-compatible genotypes (homozygous and heterozygous) and between individuals within each genotype were analysed following a nested general linear model procedure using SAS software (SAS Institute, 1989). In order to homogenize variances, the percentages of pollen tubes in each section of the style and in the ovary were previously transformed by calculating the angular transformation (arc sin value of the square root), and mean values were analysed by Duncan's multiple range test.

3. Results and discussion

3.1. Pollen tube growth

The analysis of variance (data not shown) indicated the presence or absence of differences for the number of pollen

Table 1
Parentage and *S* genotype (determined by NEPHGE) of the studied self-compatible almond seedlings

F1 parentage	F2 parentage	Seedling (F2)	*S* genotype (NEPHGE)
'Ferragnès' × 'Genco'	C1123 × C1123	A1473	S_fS_f
'Tuono' × 'Genco'	C1328 × C1328	A2198	S_fS_f
'Tuono' × 'Genco'	C1328 × C1328	A2206	S_fS_f
'Genco' × 'Tuono'	C3105 × C3105	A2416	S_fS_f
'Tuono' × 'Ferragnès'	C1010 × C1010	A1177	S_3S_f
'Tuono' × 'Ferragnès'	C1011 × C1011	A1194	S_3S_f
'Ferragnès' × 'Tuono'	C1192 × C1192	A1775	S_1S_f
'Genco' × 'Ferragnès'	C2062 × C2062	A2321	S_1S_f