Theor Appl Genet (2003) 106:904–911
DOI 10.1007/s00122-002-1159-y

E. Ortega · F. Dicenta

Inheritance of self-compatibility in almond: breeding strategies to assure self-compatibility in the progeny

Received: 18 June 2002 / Accepted: 20 August 2002 / Published online: 30 January 2003

Abstract To assure self-compatibility in the progenies, three different crosses were conducted for the first time in an almond breeding programme: self-pollination (266 descendants from 30 families), crosses between parents sharing an *S*-allele (108 descendants from five families) and crosses with homozygous self-compatible parents (62 descendants from five families). Depending on the cross, self-compatibility in the progenies was determined by observing pollen tube growth (by means of fluorescence microscopy), stylar *S*-RNases analysis or allele-specific PCR. The results obtained fit with the accepted hypothesis of inheritance of self-compatibility and the three crossing strategies used ensured 100% of self-compatible descendants. These strategies increase the efficiency of the breeding programme and avoid the laborious task of evaluating this characteristic. From the breeding point of view, self-fertilisation and crosses between relatives tend to produce inbreeding. Furthermore, these methods reduce the possibilities of choosing the parental combination. The use of homozygous self-compatible parents does not have any of these disadvantages. As far as we know, this is the first time that allele-specific PCR has been used for early selection of self-compatible seedlings. The advantages and disadvantages of the three methodologies used to determine self-compatibility are discussed.

Keywords Almond · Breeding methods · Inheritance · *Prunus dulcis* · Self-compatibility

Communicated by H.F. Linskens

E. Ortega · F. Dicenta (✉)
Departamento de Mejora y Patología Vegetal,
Centro de Edafología y Biología Aplicada del Segura,
Consejo Superior de Investigaciones Científicas. P.O. Box 4195,
E-30080, Murcia, Spain
e-mail: fdicenta@cebas.csic.es
Tel.: +34-968396309, Fax: +34-968396213

Introduction

Self-incompatibility is an evolutionary advantage in flowering plants due to its effectiveness for avoiding inbreeding and encouragement of outcrossing (Whitehouse 1951). However, when the commercial part of the crop is the seed, as in almond [*Prunus dulcis* (Mill.) D.A. Webb], self-incompatibility implies higher costs of management to obtain good yields (Dicenta et al. 2002a). For this reason, self-compatibility has become an important objective in almond breeding programmes (Socías i Company and Felipe 1992; Duval and Grasselly 1994; García et al. 1996; Godini and Palasciano 1997; Vargas et al. 1997; Gradziel and Kester 1998).

In *Prunus* species the self-incompatibility system is gametophytic and controlled by a single *S* locus with multiple alleles, the self-compatibility allele (S_f) being dominant over the others of the *S* series (Crane and Lawrence 1929). This monofactorial genetic control of self-compatibility has been demonstrated in cherry (*Prunus avium* L.) (Crane and Brown 1937), almond [*P. dulcis* (Mill.) D.A. Webb] (Socías i Company 1984; Dicenta and García 1993) and apricot (*Prunus armeniaca* L.) (Burgos et al. 1997, 1998).

The first studies on the transmission of self-compatibility in almond to offspring were conducted by Socías i Company and Felipe (1977), who found that the self-compatible parent was heterozygous and that self-compatibility was the dominant trait. In subsequent studies, Socías i Company and Felipe (1988) observed a higher than expected rate of self-compatible individuals resulting from crosses between the self-compatible cultivars Genco and Tuono, both originating from the Apulia region of Italy. This was explained by the presence of a lethal allele linked in repulsion to self-compatibility or by inbreeding effects.

Grasselly et al. (1981) found that almost all the descendants from the cross Ferragnès × Filippo Ceo were self-compatible and attributed this fact to the possibility that the self-compatible parents were homozygous. Grasselly and Olivier (1984), when crossing self-incom-

patible cultivars with Tuono, obtained the results that would be expected if this parent were heterozygous for self-compatibility. However, in some of the crosses with the female parent related to Cristomorto, the proportion of self-compatible individuals was higher than expected. Grasselly (1985) attributed this phenomenon to the presence of a common self-incompatibility allele of the *S* series.

Dicenta and García (1993) studied the inheritance of self-compatibility in 742 almond seedlings, from 25 families, by fluorescence microscopy. The observed frequencies of self-compatible descendants were in accordance with the accepted theory concerning the gametophytic system of *Prunus*. The results confirmed the presence of a common allele in the cultivars Genco, Tuono and Ferragnès. In addition, different crossing strategies that would assure self-compatibility were proposed.

Rovira et al. (1997) studied self-compatibility in 1,242 descendants from 22 different crosses between self-compatible and self-incompatible almond cultivars, by determining fruit set in bagged branches. In one of these progenies, self-compatibility was also studied by means of fluorescence microscopy. In most cases, the data fitted the 1:1 ratio of self-compatible: self-incompatible descendants expected from a cross between a self-compatible and a self-incompatible cultivar with no common alleles.

Bošković et al. (1997b, 1999) applied the analysis of stylar *S*-RNases by non-equilibrium pH gradient electrofocusing (NEPHGE), to detect self-compatible seedlings in almond progenies from crosses in which one parent was self-compatible. The results showed that, in general, the observed segregations of *S* alleles approximated to those expected, half of the progeny being self-compatible. These results were in agreement with data from fruit set and pollen tube growth, showing the reliability of this technique.

The objective of this work was to study the inheritance of self-compatibility in different almond progenies, from three different cross strategies designed to obtain 100% of self-compatible descendants, and to confirm whether the obtained results are in accordance with the accepted hypothesis of inheritance of this characteristic.

Materials and methods

Plant material

Four hundred and thirty six seedlings from 40 almond progenies, obtained from three types of crosses designed to assure self-compatibility, were studied, namely:

1. two hundred and sixty six descendants from self-fertilisation of 30 self-compatible selections were obtained by bagging branches having flower buds at stage 'D', in 1994 (Table 1),
2. one hundred and eight descendants from five crosses between the self-incompatible cultivar Ferragnès and a self-compatible selection with a common *S*-allele were obtained by hand pollination or open pollination in a plantation of Ferragnès and Guara cultivars, in 1996 (Table 3), and
3. sixty-two descendants from five crosses between self-incompatible cultivars and homozygous self-compatible selections were obtained by hand pollination in 2000 and 2001 (Table 5).

The ages of the trees, when they were studied, were 5 and 4 years for crosses 1 and 2 respectively, and 1 year or 1 month in the third type of cross.

Methods

Self-compatibility of the descendants was determined by three different methods:

1. observing pollen tube growth through the pistil, by means of fluorescence microscopy, following hand self-pollination in the laboratory (crosses 1 and 2),
2. *S*-RNases analysis by NEPHGE (crosses 1 and 2, except for Ferragnès × Guara), and
3. allele-specific PCR (cross 3).

Fluorescence microscopy

A sample of flower buds at stage 'D' (Felipe 1977) was collected and emasculated to dry the pollen. Ten pistils were placed in trays on moist foam and were maintained under controlled conditions (22 ± 1 °C and 70–80% relative humidity) to prevent dehydration of pistils. After 24 h, pistils were self-pollinated using a paintbrush. Seventy-two hours after self-pollination, each sample of pistils was kept in a small glass bottle containing 5 ml of FAA (a fixing solution made up of 40% v/v formaldehyde, glacial acetic acid and 70% ethanol, at a ratio of 1:1:18). The pistils were then washed several times and autoclaved for 30 min at a pressure of 1 kg cm^{-2}, in a solution of 5% sodium sulphite, to soften the tissue and to enhance staining. The stain used was 0.1% aniline blue in 0.1 N potassium phosphate. Finally, the pubescence was removed before squashing the pistils (Linskens and Esser 1957; Martin 1959).

The penetration level of pollen tubes along the pistil was determined in a sample of five pistils by fluorescence microscopy, 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. When the results from the five pistils were not conclusive, a set of five more pistils was observed. A descendant was considered as self-compatible when pollen tubes reached the ovary of two or more pistils.

S-RNase analysis

For the analysis of stylar *S*-RNases, the method described by Bošković et al. (1999) was followed. Specifically, gels contained 4% Pharmalyte pH 3–10 and 1.2% Pharmalyte pH 6.7–7.7, and the running conditions of NEPHGE were 1 h at 150 V, 2 h at 300 V and 2 h at 400 V.

The zymograms obtained showed a single band in heterozygous self-compatible descendants (S_iS_f), which corresponded to the S_i allele product in the style, or no band for the homozygous self-compatible descendants (S_fS_f), due to the absence of ribonuclease activity in the product of the S_f allele in almond styles (Bošković et al. 1999).

Allele-specific PCR

Genomic DNA was extracted from young leaves following the miniprep version of the CTAB extraction method of Doyle and Doyle (1987), with the modifications of Sonneveld et al. (2001).

For the PCR, approximately about 20 ng of DNA were used in a reaction volume of 25 µl, containing 2.5 µl of 10× PCR buffer, 0.12 mM of dNTP mix, 0.125 µM of each primer and 0.2 µl of *Taq* DNA polymerase (5 units/µl, Qiagen). Cycling parameters were those described by Tamura et al. (2000).

The primers used were AS1II (forward, 5′-TATTTTCAATTTG-TGCAACAATGG-3′) and AmyC5R (reverse, 5′-CAAAATACCA-CTTCATGTAACAC-3′), synthesised by Tamura et al. (2000), which represent C1 and C5 conserved regions in *Rosaceae S*-RNases.

Statistical analysis of data

For statistical analysis, crosses were grouped by the parental genotypes. In each case, after determining the expected frequencies (according to the established hypothesis of inheritance of self-compatibility), the test of goodness-of-fit chi-square (χ^2) was applied. The null hypothesis (Ho) established in each case was: self-fertilisation (50% S_iS_f and 50% S_fS_f), crosses with a common allele (50% S_1S_f and 50% S_3S_f), and crosses with homozygous self-compatible genotypes (50% S_iS_f and 50% S_xS_f, where S_i and S_x are two different self-incompatibility alleles).

Results

Table 1 indicates the genetic origin of progenies from self-fertilisation, the total number of individuals studied in each case, the percentage of self-compatible (SC) and self-incompatible (SI) individuals determined by fluores-

Table 1 Genetic origin of progenies, number of descendants (N) and percentage of self-compatible (SC) and self-incompatible (SI) individuals, determined by observation of pollen tube growth. Percentage of individuals for each *S*-genotype was determined by stylar ribonuclease assay

Generation 1	Generation 2	Descendants						
		Pollen tube growth			*S*-RNases			
		N	SC	SI	S_1S_f	S_3S_f	S_fS_f	S_1S_3
Ferragnès (S_1S_3)[a] × Genco (S_1S_f)[a]								
C1122 (S_1S_f)[b]	C1122 × C1122	14	100.0	0.0	21.4	0.0	78.6	0.0
C1123 (S_1S_f)	C1123 × C1123	8	100.0	0.0	62.5	25.0	12.5	0.0
C1126 (S_3S_f)[b]	C1126 × C1126	14	100.0	0.0	0.0	28.6	71.4	0.0
C1127 (S_3S_f)[b]	C1127 × C1127	18	100.0	0.0	11.2	44.4	44.4	0.0
C1135 (S_3S_f)	C1135 × C1135	7	100.0	0.0	0.0	57.1	42.9	0.0
Ferragnès (S_1S_3) × Tuono (S_1S_f)[a]								
C1184 (S_3S_f)	C1184 × C1184	8	100.0	0.0	0.0	37.5	62.5	0.0
C1188 (S_1S_f)	C1188 × C1188	6	100.0	0.0	50.0	16.7	33.3	0.0
C1190 (S_3S_f)	C1190 × C1190	3	100.0	0.0	0.0	66.7	33.3	0.0
C1192 (S_1S_f)	C1192 × C1192	10	100.0	0.0	70.0	10.0	20.0	0.0
C1206 (S_3S_f)	C1206 × C1206	8	100.0	0.0	12.5	37.5	50.0	0.0
Genco (S_1S_f) × Ferragnès (S_1S_3)								
C2055 (S_3S_f)	C2055 × C2055	8	100.0	0.0	0.0	25.0	75.0	0.0
C2062 (S_3S_f)	C2062 × C2062	12	91.7	8.3	16.7	33.3	41.7	8.3
C2075 (S_3S_f)[b]	C2075 × C2075	6	100.0	0.0	0.0	16.7	83.3	0.0
C2078 (S_3S_f)[b]	C2078 × C2078	4	100.0	0.0	0.0	100.0	0.0	0.0
C2082 (S_3S_f)	C2082 × C2082	6	100.0	0.0	33.3	66.7	0.0	0.0
Genco (S_1S_f) × Tuono (S_1S_f)								
C3105 (S_fS_f)	C3105 × C3105	3	100.0	0.0	0.0	0.0	100.0	0.0
C3106 (S_1S_f)	C3106 × C3106	12	100.0	0.0	33.3	0.0	66.7	0.0
C3108 (S_1S_f)	C3108 × C3108	10	100.0	0.0	10.0	10.0	80.0	0.0
C3119 (S_1S_f)	C3119 × C3119	4	100.0	0.0	25.0	0.0	75.0	0.0
C3128 (S_1S_f)	C3128 × C3128	5	100.0	0.0	80.0	0.0	20.0	0.0
Tuono (S_1S_f) × Ferragnès (S_1S_3)								
C1010 (S_3S_f)[b]	C1010 × C1010	11	100.0	0.0	0.0	72.7	27.3	0.0
C1013 (S_3S_f)	C1013 × C1013	10	100.0	0.0	20.0	70.0	10.0	0.0
C1050 (S_3S_f)[b]	C1050 × C1050	10	100.0	0.0	20.0	60.0	20.0	0.0
C1054 (S_3S_f)[b]	C1054 × C1054	8	100.0	0.0	0.0	50.0	50.0	0.0
Tuono (S_1S_f) × Genco (S_1S_f)								
C1276 (S_1S_f)	C1276 × C1276	10	100.0	0.0	30.0	0.0	70.0	0.0
C1306 (S_1S_f)[b]	C1306 × C1306	12	100.0	0.0	50.0	8.3	41.7	0.0
C1312 (S_1S_f)	C1312 × C1312	12	100.0	0.0	25.0	0.0	75.0	0.0
C1319 (S_1S_f)[b]	C1319 × C1319	2	100.0	0.0	50.0	0.0	50.0	0.0
C1322 (S_1S_f)	C1322 × C1322	10	100.0	0.0	70.0	0.0	30.0	0.0
C1328 (S_1S_f)	C1328 × C1328	15	100.0	0.0	26.7	26.7	46.6	0.0
Total		266	99.7	0.3				

[a] *S*-genotype determined by stylar ribonuclease assay by Bošković et al. (1997a)
[b] *S*-genotype determined by stylar ribonuclease assay in this study. The others were inferred from the progeny data

Table 2 Total number of descendants (N) for each group of crosses, according to the parental *S* genotype, and percentage of descendants for each genotype. Degrees of freedom (*df*), chi-square (χ^2) and *p*-value

Generation 2	Descendants						
	N	S_1S_f	S_3S_f	S_fS_f	*df*	χ^2	*p*-value
$S_1S_f \times S_1S_f$	120	43.3	0.0	56.7	1	2.13	0.14
$S_3S_f \times S_3S_f$	121	0.0	52.9	47.1	1	0.40	0.53
$S_fS_f \times S_fS_f$	3	0.0	0.0	100.0	–	–	–

Table 3 Genetic origin of progenies, number of descendants (N) and percentage of self-compatible (SC) and self-incompatible (SI) individuals, determined by observing pollen tube growth. Percentage of individuals for each *S*-genotype determined by stylar ribonuclease assay

Generation 1	Generation 2	Descendants				
		Pollen tube growth			*S*-RNases	
		N	SC	SI	S_1S_f	S_3S_f
Ferragnès (S_1S_3) × Tuono (S_1S_f)						
C1197 (S_1S_f)	Ferragnès × C1197	19	89.5	10.5	52.6	47.4
Genco (S_1S_f) × Ferragnès (S_1S_3)						
C2075 (S_3S_f)	Ferragnès × C2075	29	100.0	0.0	41.4	58.6
Tuono (S_1S_f) × Ferragnès (S_1S_3)						
C1050 (S_3S_f)	Ferragnès × C1050	21	100.0	0.0	61.9	38.1
C1054 (S_3S_f)	Ferragnès × C1054	19	100.0	0.0	68.4	31.6
Unknown origin						
Guara (S_1S_f)	Ferragnès × Guara	20	[a]	[a]	25.0	75.0
Total		108	97.4	2.6		

[a] Not determined

Table 4 Total number of descendants (N) for each group of crosses, according to the *S*-genotype of the self-compatible parent, and percentage of descendants of each genotype. Degrees of freedom (*df*), chi-square (χ^2) and *p*-value

Generation 2	Descendants					
	N	S_1S_f	S_3S_f	*df*	χ^2	*p*-value
$S_1S_3 \times S_1S_f$	37	40.5	59.5	1	1.32	0.25
$S_1S_3 \times S_3S_f$	69	55.1	44.9	1	0.71	0.40

cence microscopy and the *S*-genotypes determined by *S*-RNase analysis.

In general, the percentage of self-compatible individuals obtained in each progeny was the 100% expected, except for one case of self-incompatibility in the 266 individuals studied. This case was also confirmed by the stylar ribonuclease assay (S_1S_3 genotype). In the study of *S*-RNases, important deviations from the expected 1:1 frequency for each genotype were observed in some progenies.

Data from these progenies were divided into three groups according to the *S*-genotype of the parent (Table 2). The unexpected *S*-genotypes were not considered in this analysis. For each group, a percentage close to 50% for both heterozygous (S_fS_i) and homozygous (S_fS_f) individuals (all self-compatible) was observed. The null hypothesis proposed was accepted in both cases at a 5% significance level. In the case of homozygous parents the chi-square test could not be applied, since there was only one possible category (zero degrees of freedom).

Table 3 shows the results obtained in the case of crosses between Ferragnès (S_1S_3) and heterozygous self-compatible individuals, whose self-incompatibility allele is S_1 or S_3. In this case, the percentage of self-compatible individuals was also close to 100%. Two descendants were classified as self-incompatible by observing pollen tube growth; however, the results of the stylar ribonuclease assay indicated that the *S*-genotype of these individuals was self-compatible.

Although the number of individuals per family was not very high (19–29), most families showed around 50% of each genotype. This was not observed in the progeny from the cross Ferragnès × Guara, where the results deviated strongly from the expected.

When considering the results in two groups, regarding the genotype of the self-compatible parent, the null hy-

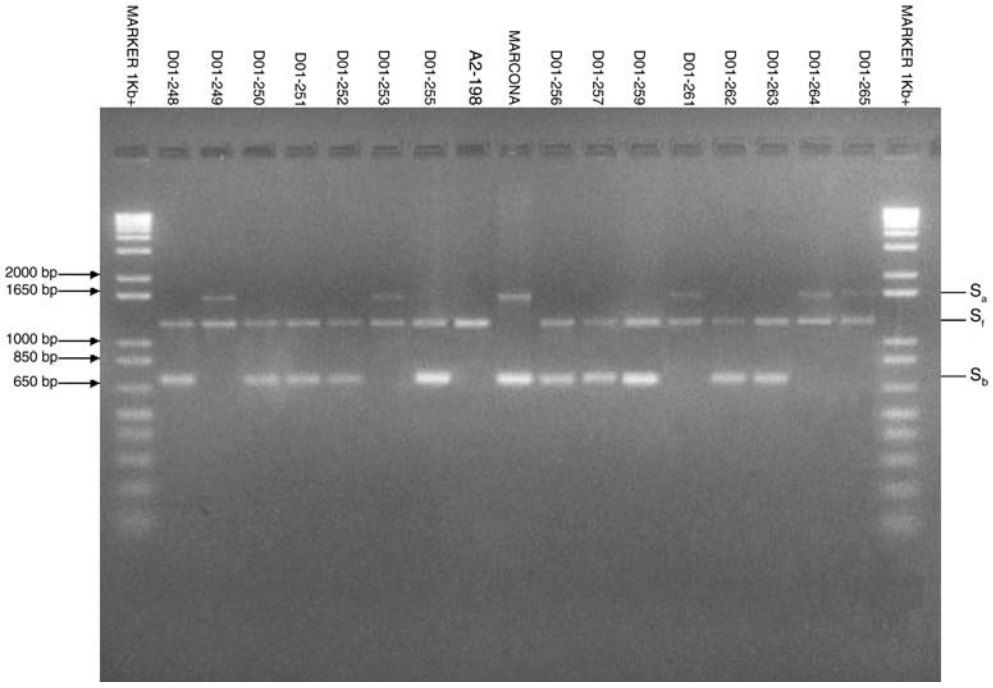

Fig. 1 PCR analysis of 15 individuals from the cross A2 198 (S_fS_f) × Marcona ($S_{11}S_{12}$). The bands corresponding to the S_{11} and the S_{12} allele of Marcona have been indicated as S_a (fragment of 1,650 bp) and S_b (fragment of 700 bp), since there is no reference to know which band corresponded to each allele. All seedlings were common to that of the female parent of 1,300 bp, corresponding to the S_f allele

Table 5 Genetic origin of progenies from crosses with homozygous self-compatible individuals (S_fS_f), number of descendants (N) and percentage of individuals for each *S*-genotype, determined by allele-specific PCR

Generation 1	Generation 2	Generation 3	Descendants		
			N	*S*-genotype	
Tuono (S_1S_f)[a] × Genco (S_1S_f)[a]					
C1312 (S_1S_f)[c]	C1312 × C1312			S_1S_f	S_5S_f
	A2146 (S_fS_f)[c]	A2146 × Desmayo Largueta (S_1S_5)[a]	15	66.7	33.3
Tuono (S_1S_f) × Genco (S_1S_f)					
C1328 (S_1S_f)[c]	C1328 × C1328			S^dS_f	S^eS_f
	A2198 (S_fS_f)[c]	A2198 × Marcona ($S_{11}S_{12}$)[b]	15	66.7	33.3
				S_5S_f	S^gS_f
	A2198 (S_fS_f)[c]	S5133 (S_5S_f)[c] × A2198	15	46.7	53.3
Tuono (S_1S_f) × Genco (S_1S_f)					
C1328 (S_1S_f)[c]	C1328 × C1328			S_5S_f	S^gS_f
	A2199 (S_fS_f)[c]	S5133 (S_5S_f)[c] × A2199	5	40.0	60.0
Genco (S_1S_f) × Tuono (S_1S_f)					
C3105 (S_fS_f)[c]	C3105 × C3105			S_1S_f	S_5S_f
	A2416 (S_fS_f)[c]	A2416 × Desmayo Largueta (S_1S_5)[a]	12	33.3	66.7
Total			62		

[a] *S*-genotype determined by stylar ribonuclease assay by Bošković et al. (1997a)
[b] *S*-genotype determined by stylar ribonuclease assay by Bošković et al. (1997b)
[c] *S*-genotype determined by stylar ribonuclease assay in this study. *S* corresponds to a new *S*-allele
[d] PCR fragment of 700 bp
[e] PCR fragment of 1,650 bp
[g] PCR fragment of 850 bp

pothesis-proposed 1:1 segregation for the S_1S_f:S_3S_f genotypes was accepted in both cases (Table 4).

Table 5 shows the results obtained by allele-specific PCR in progenies obtained from crosses with homozygous self-compatible individuals. In all cases, as expected, the *S*-genotype observed was self-compatible heterozygous. However, the percentages of each genotype deviated from the 50% expected in most cases.

Figure 1 shows the results obtained by PCR for the 15 descendants from the cross A2 198 (S_fS_f) × Marcona ($S_{11}S_{12}$). All seedlings showed a common band (around 1,300 bp) to that in the homozygous self-com-

Table 6 Total number of descendants (N) for each group of crosses with homozygous self-compatible individuals (S_fS_f), according to the *S*-genotype of the self-incompatible parent, and percentage of descendants for each genotype. Degrees of freedom (*df*), chi-square (χ^2) and *p*-value

Generation 3	Descendants								
	N	S_1S_f	S_5S_f	S_cS_f	S_aS_f	S_bS_f	*df*	χ^2	*p*-value
$S_fS_f \times S_1S_5$	27	51.9	48.1	–	–	–	1	0.04	0.84
$S_fS_f \times S_aS_b$	15	–	–	–	66.7	33.3	1	1.67	0.20
$S_5S_c \times S_fS_f$	20	–	45.0	55.0	–	–	1	0.20	0.66

patible parent, which presumably corresponded to the S_f allele.

When data were grouped according to the *S*-genotype of the self-incompatible parent (Table 6), the percentage of individuals of each genotype was closer to the 50% expected, with the exception of the progeny from the A2198 × Marcona cross. However, the segregation of *S*-alleles was the expected 1:1, with the *p*-value obtained in the three cases being >0.05.

Discussion

When isolated families were considered, the observed deviations from the expected rates could be explained by the low number of individuals studied in each progeny or by the presence of unexpected genotypes, because of the sporadic contamination of pistils with foreign pollen in the bagged branches. Once were eliminated the unexpected genotypes and families were properly grouped, frequencies of self-compatible:self-incompatible descendants fitted with the accepted hypothesis of inheritance of self-compatibility in *Prunus*.

The classification of two descendants as self-incompatible by observing pollen tube growth and as self-compatible by the stylar ribonuclease assay (Table 3), could be explained by problems related to the stigma receptivity or pollen viability of these descendants.

The coincidence observed between the results obtained by fluorescence microscopy and analysis of stylar *S*-RNases indicates the reliability of the latter technique, and also its ability to show unexpected results. This was observed also by Bošković et al. (1999) in different almond progenies.

In contrast to the other methodologies, allele-specific PCR does not require the first flowering of the tree (which takes 3 or 4 years) to identify the self-compatible seedlings. Only a small piece of a leaf from a seedling, shortly after germination of the seed, is needed for DNA extraction. For this reason, this method is an important tool for early marker-assisted selection of self-compatible seedlings and, as far as we know, it is the first time that this molecular marker has been used in an almond breeding programme. The use of molecular markers for self-compatibility was employed by Tao et al. (2000) in the Japanese apricot (*Prunus mume* Sieb. et Zucc.).

Regarding self-pollination, this is also the first time that inheritance of self-compatibility has been studied in a high number of seedlings following self-fertilisation by two different methods. In a previous study, Socías i Company and Felipe (1988) determined self-compatibility by fluorescence microscopy in a progeny of 65 individuals resulting from self-fertilisation of the almond cultivar Tuono. The results deviated from the proposed 3:1 ratio, with a higher than expected number of self-compatible seedlings. The authors attributed the low number of self-incompatible seedlings obtained to the presence of lethal genes linked in repulsion to the S_f allele. In addition to self-compatible and self-incompatible seedlings, an intermediate class (pseudocompatibility) was designated by Socías i Company and Felipe (1988). Pseudocompatible seedlings were considered as self-incompatible for statistical analysis. These results differed from ours, since we did not consider any intermediate class. In our case, the coincidence between the microscopy and ribonuclease assay results support the criteria adopted to assess self-compatibility. Dicenta and García (1993) observed that, although the progeny from self-pollination of the self-compatible cultivar they studied consisted of only three seedlings, all were self-compatible as expected.

Regarding crosses with a shared allele, Grasselly et al. (1981), when studying self-compatibility in seedlings from crosses between self-incompatible cultivars and the self-compatible Filippo Ceo, found that almost all the descendants were self-compatible. The authors suggested the possibility that the male parent was homozygous for self-compatibility. In later studies (Grasselly and Olivier 1984), the results from similar crosses with the self-compatible cultivar Tuono were those expected if this cultivar were heterozygous for self-compatibility. In addition, in the crosses with the female parent related to Cristomorto (Ferragnès and a selection from Cristomorto × Tardy Nonpareil), the proportion of self-compatible individuals was higher than expected. Grasselly (1985) explained this phenomenon by the presence of a common self-incompatible allele of the *S* series. These results were also observed in progenies from crosses between the cultivar Ferragnès and the self-compatible Tuono and Genco by Dicenta and García (1993), who accepted the hypothesis of Grasselly (1995). All these results are in agreement with our results from the second type of cross (Ferragnès × self-compatible genotypes sharing an *S*-allele).

As far as we know, this is the first time that homozygous self-compatible individuals have been used as parents in breeding crosses for fruit trees. Thus, the results obtained cannot be compared to earlier findings.

A general analysis of our data indicates that the three types of crosses studied assure self-compatibility in the offspring. So, they could be used in almond breeding programmes with this aim, thus avoiding the laborious task of determining this characteristic in the offspring and consequently increasing the efficiency of the breeding programme. However, one must take into account that crosses between genetically related individuals, and even more so self-fertilisation, could generate inbreeding effects in the progeny (Grasselly and Olivier 1981, 1988; Socías i Company and Felipe 1988; Lansari et al. 1994), which manifest themselves as high mortality, low productivity or necrosis and bark split.

In the current study, these effects were shown in most of the descendants from self-fertilisation as a low flower intensity and even lower productivity, which seems to be a consequence of an important delay to fruit-bearing (Dicenta et al. 2002b). These effects have been shown to a lesser degree in seedlings from parents sharing an *S*-allele (non-published data). However, for parents sharing an allele, but genetically distant (which do exist), this inbreeding could be avoided. An additional inconvenience of self-fertilisation and crosses sharing an allele strongly restrict the choice of parents in the cross-breeding programme.

On the contrary, inbreeding effects in seedlings resulting from crosses with homozygous self-compatible individuals are not expected, since they can be obtained from crosses between heterozygous self-compatible seedlings with genetically distant self-incompatible ancestors (Dicenta et al. 2002b). In addition, these homozygous genotypes can be crossed with any other genotype, ensuring self-compatibility in the offspring. Thus, we consider that this third type of cross is the most efficient way to obtain 100% of self-compatible descendants in an almond breeding programme.

Indeed, we have already selected some homozygous self-compatible selections with another important characteristic (for example early or late flowering time) for each breeding objective. Currently, these selections are being used as "joker self-compatible genitors" and the obtained seedlings are being evaluated in the field, as part of our breeding programme.

In conclusion, the proposed hypotheses of inheritance of self-compatibility have been contrasted, and the three strategies of crosses studied (self-fertilisation, crosses between parents sharing an *S*-allele and crosses with homozygous self-compatible parents) ensured self-compatibility in the offspring, thus increasing the efficiency of the breeding programme and eliminating the laborious task of evaluating this characteristic.

Acknowledgements This work has been financed by the project "Mejora Genética del Almendro" (AGF98-0211-C03-02) from the "Plan Nacional de I+D" of the Spanish Ministry of Education and Culture. We thank Dr. David Walker for revision of the English in the manuscript.

References

Bošković R, Tobutt KR, Batlle I, Duval H, Dicenta F (1997a) Correlation of ribonuclease zymograms and incompatibility genotypes in almond. Euphytica 97:167–176

Bošković R, Tobutt KR, Rovira M, Romero M, Batlle I, Duval H, Dicenta F (1997b) Inheritance of stylar ribonucleases in two almond progenies and their correlation with self-compatibility. Acta Hort 420:118–122

Bošković R, Tobutt KR, Duval H, Batlle I, Dicenta F, Vargas JF (1999) A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. Theor Appl Genet 99:800–810

Burgos L, Ledbetter C, Pérez-Tornero O, Ortín-Párraga F, Egea J (1997) Inheritance of sexual incompatibility in apricot. Plant Breed 116:383–386

Burgos L, Pérez-Tornero O, Ballester J, Olmos E (1998) Detection and inheritance of stylar ribonucleases associated with incompatibility alleles in apricot. Sex Plant Reprod 11:153–158

Crane MB, Brown AJ (1937) Incompatibility and sterility in the sweet cherry (Prunus avium L.). J Pomol Hort Sci 15:86–116

Crane MB, Lawrence WJC (1929) Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. J Pomol Hort Sci 7:276–301

Dicenta F, García JE (1993) Inheritance of self-compatibility in almond. Heredity 70:313–317

Dicenta F, Ortega E, Canovas JA, Egea J (2002a) Self-pollination vs cross-pollination in almond: pollen tube growth, fruit set and fruit characteristics. Plant Breed 121:163–167

Dicenta F, Ortega E, Martínez-Gómez P, Bošković R, Tobutt KR (2002b) Comparison of homozygous and heterozygous self-compatible seedlings in an almond breeding programme. Euphytica 124:23–27

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Duval H, Grasselly Ch (1994) Behaviour of some self-fertile almond selections in the south–east of France. Acta Hort 373: 69–74

García JE, Dicenta F, Berenguer T, Egea J (1996) Programa de Mejora del Almendro del CEBAS (CSIC-Murcia). Fruticultura Profesional 81:64–70

Godini A, Palasciano M (1997) Growth and yield of four self-unfruitful and four self-fruitful almonds onto three rootstocks: a 13-year study. Acta Hort 470:200–207

Gradziel TM, Kester DE (1998) Breeding for self-fertility in California almond cultivars. Acta Hort 470:109–117

Grasselly Ch (1985) Avancement du programme "autocompatibilité" chez l'amandier. Options Méditerr CIHEAM/IAMZ 85/I:39–41

Grasselly Ch, Olivier G (1981) Difficulté de survie de jeunes semis d'amandiers dans certains descendances. Options Méditerr CIHEAM/IAMZ 81/I:65–67

Grasselly Ch, Olivier G (1984) Avancement du programme de création de varietés d'amandier autocompatibles. Options Méditerr CIHEAM/IAMZ 84/II:129–131

Grasselly Ch, Olivier G (1988) Phenomènes d'inbreeding dans les descendances issues d'autofécondation chez l'amandier. 7 Colloque GREMPA. Trapport EUR 11557, 73–78

Grasselly Ch, Crossa-Raynaud P, Olivier G, Gall H (1981) Transmission du caractère d'auto-compatibilité chez l'amandier (Amygdalus communis). Options Méditerr 71–75

Lansari A, Kester DE, Lezzoni AF (1994) Inbreeding, coancestry and founding clones of almonds of California, Mediterranean shores and Russia. J Am Soc Hort Sci 119:1279–1285

Linskens HF, Esser K (1957) Über eine spezifische Anfärbung der Pollenschläuche im Griffel und die Zahl der Kallosepfropfen nach Selbstung und Fremdung. Naturwissenschaften 44:16

Martin FW (1959) Staining and observing pollen tubes in the style by means of fluorescence. Stain Tech 34:125–128

Rovira M, Clavé J, Romero M, Santos J, Vargas FJ (1997) Self-compatibility in almond progenies. Acta Hort 470:66–71

Socías i Company R (1984) A genetic approach to the transmission of compatibility in almond. Options Méditerr Serie Études 2:123–128

Socías i Company R, Felipe A (1977) Heredibility of self-compatibility in almond. 3e Colloque du GREMPA, Bari, pp 181–183

Socías i Company R, Felipe AJ (1988) Self-compatibility in almond: transmission and recent advances in breeding. Acta Hort 224:307–317

Socías i Company R, Felipe AJ (1992) Self-compatibility and autogamy in "Guara" almond. J Hort Sci 67:313–317

Sonneveld T, Robbins TP, Bošković R, Tobutt KR (2001) Cloning of six cherry self-incompatibility alleles and development of allele-specific PCR detection. Theor Appl Genet 102:1046–1055

Tamura M, Ushijima K, Sassa H, Hirano H, Tao R, Gradziel TM, Dandekar AM (2000) Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. Theor Appl Genet 101:344–349

Tao R, Habu T, Yamane H, Sigiura A, Iwamoto K (2000) Molecular markers for self-compatibility in Japanese apricot (Prunus mume). Hort Science 35:1121–1123

Vargas FJ, Clavé J, Romero M, Batlle I, Rovira M (1997) Autogamy studies on almond progenies. Acta Hort 470:74–81

Whitehouse HLK (1951) Multiple-allelomorph incompatibility of pollen and style in the evolution of the angiosperms. Ann Bot NS 14:198–216