Theor Appl Genet (2007) 115:151–158
DOI 10.1007/s00122-007-0532-2

ORIGINAL PAPER

Negative inbreeding effects in tree fruit breeding: self-compatibility transmission in almond

J. M. Alonso Segura · R. Socias i Company

Received: 8 September 2005 / Accepted: 10 March 2007 / Published online: 30 May 2007

Abstract Inbreeding depression has been observed in most fruit trees, negatively affecting the offspring of related parents. This problem is steadily increasing due to the repeated utilization of parents in breeding programmes. In almond, self-compatibility transmission from 'Tuono' to its offspring remains partially unexplained due to deviations from the expected genotype ratios. In order to test if these deviations could be due to inbreeding, the *S*-genotypes of the seedlings of four almond families, 'Tuono' (S_1S_f) × 'Ferragnès' (S_1S_3), 'Tuono' (S_1S_f) × 'Ferralise' (S_1S_3) and reciprocal crosses were studied. The *S*-genotype determination of each seedling by separation of stylar *S*-RNases and by *S*-allele-specific PCR amplification gave identical results. The ratio of *S*-genotypes of the family 'Tuono' × 'Ferralise' was the one least adjusted to the expected 1:1 ratio, because the number of self-compatible seedlings (S_fS_3) was less than a half the number of self-incompatible ones (S_1S_3). A mechanism acting against inbreeding would favour cross-breeding in the following generation to increase heterozygosity. This fact stresses the need to avoid crosses between related parents in fruit breeding programmes.

Introduction

Inbreeding depression is a phenomenon frequently appearing in the offspring of related parents in most plant species, including fruit trees. Self-incompatibility has shown to be of evolutionary advantage to avoid inbreeding (de Nettancourt 1977), but presents many negative effects from the horticultural point of view. As a consequence, most fruit breeding programmes aim to obtain new self-compatible cultivars (Socias i Company 1990), thus raising the risk of inbreeding. This inbreeding increase is frequently aggravated by the utilization of a reduced number of parents in many fruit breeding programmes (Janick and Moore 1996).

Working with fruit trees implies more time, more space and, undoubtedly, more speculation than working with annual species (Socias i Company 1998). However, almond (*Prunus amygdalus* Batsch) may be a model species for the study of inbreeding in fruit trees because almond, with very few exceptions, is an obligate outcrosser, due to the presence of gametophytic self-incompatibility (Socias i Company et al. 1976) and inbreeding would be expected in an obligate outcrosser. Furthermore, the almond genetic pool has been gradually reduced, first by the empirical selection and clonal propagation of cultivars and, later on, by the utilization of a reduced number of genotypes as parents in the breeding programmes leading to crosses between related parents, including backcrosses, crosses between full sibs and half sibs, and from selfing self-compatible cultivars (Grasselly et al. 1981; Kester and Asay 1975; Kester et al. 1991; Socias i Company 1990; Socias i Company et al. 2004). As a consequence, inbreeding symptoms have appeared in several offsprings, implying a general reduction of the fitness in these genotypes, due to a slow vegetative growth, even dwarfing in some cases, a low differentiation of flower buds, a large amount of flower sterility, low fruit set, bark cracking of the trunk, accompanied sometimes by exudates of gummosis, and necrosis of 1-year-old branches and leaves, which sometimes may cause plant death. These symptoms have been confirmed as genetic and not pathogenic (Grasselly and Olivier 1988).

Communicated by H. Becker.

J. M. Alonso Segura · R. Socias i Company (✉)
Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Apartado 727, 50080 Zaragoza, Spain
e-mail: rsocias@aragon.es

In spite of the inbreeding risks, most almond breeding programmes have fostered the development of self-compatible cultivars to solve the management and pollination problems raised by self-incompatibility (Socias i Company 1990). As the seed is the commercial part of the fruit, efficient pollination and ovule fertilization are both required for acceptable crops, implying the joint planting of at least two inter-compatible cultivars, the presence of pollinating insects, and good weather conditions for insect activity. Although self-compatibility was discovered in almond in 1945 by Almeida (1945), no attention was paid to the issue until the 1970s and the establishment of its genetic basis is relatively recent and has been based on studies conducted concurrently with breeding programmes. After assessing the transmission of self-compatibility (Socias i Company and Felipe 1977), Socias i Company (1984) suggested that self-compatibility was dominant over self-incompatibility and that the self-compatible cultivars used in the breeding programmes were heterozygous.

In some crosses deviations have been observed from the expected ratios of 1:1 (self-compatible × self-incompatible) or 3:1 (self-compatible × self-compatible). These deviations were explained by the presence of a common allele between the self-compatible pollen parent (i.e. S_1S_f) and the self-incompatible seed parent (i.e. S_1S_x). In this type of cross, only the pollen grains carrying the S_f allele would be able to grow through the pistil of the seed parent and achieve fertilization, thereby giving rise to an offspring of only self-compatible seedlings (Dicenta and García 1993; Grasselly et al. 1985). However, this explanation does not apply to all cases and cultivars with identical *S* alleles may give rise to different phenotypic ratios in their offspring (Socias i Company and Felipe 1988). Consequently, inbreeding or the presence of lethal or deleterious genes has been suggested to explain these deviations (Socias i Company 1990).

The deviations from the expected ratios in self-compatibility transmission have been mostly described in crosses involving two self-incompatible cultivars from the French breeding programme possessing identical *S*-alleles by descent (S_1S_3): 'Ferragnès' and 'Ferralise' (Grasselly and Crossa-Raynaud 1980). 'Ferralise' has an inbreeding coefficient of 0.25 (Lansari et al. 1994), as coming from the cross of two full sib cultivars, 'Ferraduel' and 'Ferragnès' (Crossa-Raynaud and Grasselly 1985), being the only known inbred almond cultivar used in breeding programmes. As a consequence of their identical *S*-genotypes, 'Ferragnès' and 'Ferralise' are cross-incompatible (Socias i Company and Felipe 1994; Socias i Company and Alonso 2004) and the offsprings obtained from the cross of these cultivars with another cross-compatible cultivar would be expected to show a similar allele transmission.

Both cultivars have been used in crosses with 'Tuono', a self-compatible cultivar with the genotype S_1S_f (Crossa-Raynaud and Grasselly 1985), thus sharing one allele with these two cultivars probably identical by descent because the S_1 allele of 'Ferragnès' and 'Ferralise' has been inherited from 'Cristomorto', a cultivar which originated in the same Italian region of Puglia as 'Tuono'. In progenies where 'Tuono' is the male parent, all the seedlings are expected to be self-compatible because in the cross $S_1S_3 \times S_1S_f$ only the S_1S_f and S_3S_f genotypes can be obtained. This was not confirmed in the first studies (Grasselly and Olivier 1984; Grasselly 1985), probably because phenotypes were established by the level of fruit set in bagged branches, resulting in a number of self-compatible phenotypes much higher in the offspring of 'Ferragnès' × 'Tuono' than in that of 'Ferralise' × 'Tuono'.

When the genotype was determined in some of these populations, results were different. With the *S*-RNase band determination, Ballester et al. (1998) in a cross 'Ferragnès' × 'Tuono' found that all seedlings were genotypically self-compatible, showing the only two possible genotypes, S_1S_f and S_3S_f. Duval et al. (2001) studied the population of 'Ferralise' × 'Tuono' of Grasselly (1985) and found the same genotype segregation as Ballester et al. (1998), but with a discrepancy between the genotypes and some of the previously determined self-incompatible phenotypes, although no explanation was given to this differential phenotype expression.

'Tuono' has also been used as seed parent in the cross 'Tuono' × 'Ferragnès' (Socias i Company and Felipe 1988; Dicenta and García 1993). In this case, a similar ratio of self-compatible (S_3S_f) and self-incompatible (S_1S_3) seedlings is expected, but only phenotype observations were made, with a higher percentage of self-compatible than self-incompatible seedlings. So far there are no references to any family coming from the cross 'Tuono' × 'Ferralise', although 'Ferralise' has been used in several breeding programmes mainly because of its very late blooming time (Grasselly and Olivier 1988; Socias i Company and Felipe 1994). Inbreeding symptoms have been observed in its offspring, although the whole range of fitness could be observed among its seedlings and some self-compatible ones have shown a good vigour and high density of good quality flowers (Alonso and Socias i Company 2005c).

Due to the discrepancies on self-compatibility transmission in some of the progenies from 'Tuono' and to the presence of inbreeding in 'Ferralise' but not in 'Ferragnès', our objective was to assess how self-compatibility was transmitted from 'Tuono' when crossed with these two cultivars with the same *S* genotype but with a different inbreeding coefficient. This transmission was studied by ascertaining the genotype of each individual, avoiding the previous

Table 1 Origin of the seedlings studied and expected genotypic ratios

S-genotype cross	Progeny	No. of seedlings	Expected genotypic ratios
$S_1S_f \times S_1S_3$	'Tuono' × 'Ferragnès'	37	50% Self-incompatible, 50% S_1S_3
	'Tuono' × 'Ferralise'	22	50% Self-compatible, 50% S_3S_f
$S_1S_3 \times S_1S_f$	'Ferragnès' × 'Tuono'	39	100% Self-compatible, 50% S_1S_f 50% S_3S_f
	'Ferralise' × 'Tuono'	91	

phenotype assignment because some self-compatible genotypes are showing a self-incompatible behaviour (Alonso and Socias i Company 2005b).

Materials and methods

Plant material

Seedlings from four crosses of 'Tuono' (S_1S_f genotype) by 'Ferragnès' and 'Ferralise' (S_1S_3) and their reciprocals were studied (Table 1). The progenies belong to the almond-breeding programme of the Unidad de Fruticultura from the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) aiming to obtain new self-compatible and late blooming cultivars. The crosses were made in 1993 and at fall, fruits were collected and stratified for 2 months. In December, germinated kernels were placed in a nursery where they grew in 1994. In January 1995, seedlings were planted in a breeding plot and maintained according to usual growing management. Rates of seed germination and plant survival were similar for all families.

Stylar *S*-RNase analysis

S-genotype was predicted by separation of stylar RNases linked to self-incompatibility alleles. The stylar *S*-RNases were obtained from each seedling, during the 2001 and 2002 blooming seasons, from 30 flower buds collected at stage D (Felipe 1977). Two samples per seedling of 15 styles including stigmas from these buds were crushed in liquid nitrogen, and the frozen powder was suspended in 0.5 ml of extraction solution (Bošković and Tobutt 1996). The slurry was centrifuged at −4°C for 1 h at 14,500 rpm and the supernatant was stored at −80°C. About 60 µl of stylar extract by sample was separated electrophoretically on vertical slab gels, consisting of 7.5% polyacrylamide with 10% sucrose, 5.6% Pharmalyte pH 3–10 and 1.7% Pharmalyte pH 6.7–7.7 (Amersham Biosciences Europe, Freiburg, Germany) using NEPHGE II (Bošković et al. 1997, 2003) comprising 1 h at 150 V, 1 h at 300 V and 3 h at 400 V, obtaining a right separation of the S_1 and S_3 RNases, corresponding to the only two incompatibility alleles present in these populations.

Gels were stained for ribonuclease activity based on Wilson (1971), being extended the incubation period after RNA treatment to 20–30 min. After incubation, the gels were fixed for about 3 min with 7% acetic acid before the addition of the toluidine blue O solution (Bošković and Tobutt 1996).

S-allele identification by PCR

Affirmation of S_f presence is not possible by separation of the RNases because the self-compatibility allele (S_f) does not codify a product with RNase activity, thus its presence in a seedling is deduced by the observation of a single RNase band (Bošković et al. 1997; Tao et al. 1997). As a consequence, *S*-genotypes predicted by *S*-RNases must be confirmed by *S*-allele-specific PCR identification. For this analysis, genomic DNA was extracted from leaves of each seedling using a protocol based in Gepts and Clegg (1989).

For the amplification of the S_1, S_3 and S_f alleles present in the different progeny seedlings, several primers previously used in almond *S* allele identification were tested (Tamura et al. 2000; Ma and Oliveira 2001; Channuntapipat et al. 2001, 2003) because primers do not always allow a correct *S* identification in all analysis conditions. As a consequence, ConF/ConR primers (Channuntapipat et al. 2001) were used for S_1 identification, SfF/SfR primers (Channuntapipat et al. 2003) for specific S_f-allele identification, and S3F and S3R (Alonso and Socias i Company 2005a) for specific S_3-allele identification.

PCR reactions were made and the products separated according to Martínez-Gómez et al. (2003). The pattern of amplification products of each seedling was compared with the parent cultivars' bands and the *S*-genotypes were correlated with those obtained with the *S*-RNase analysis.

Statistical analysis

In each cross, the expected frequencies were determined according to the established hypothesis of inheritance of self-compatibility. The test of goodness-of-fit Chi-square (χ^2) was applied to each cross and to the pooled data of each type of progeny, utilizing Yates (1934) correction for one degree of freedom in small samples.

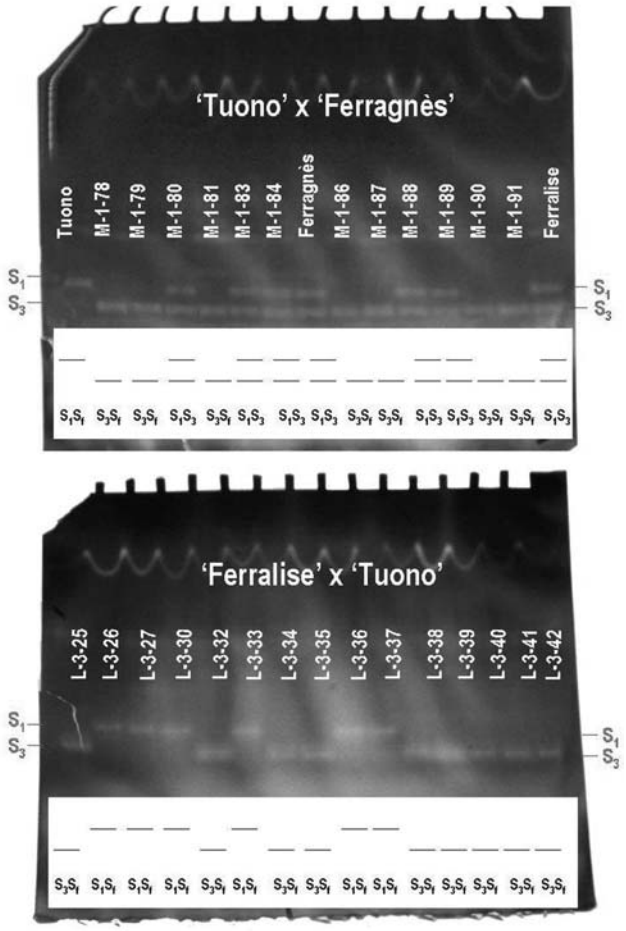

Fig. 1 Zymogram of the stylar *S*-Rnases for the *S* genotype identification in the families 'Tuono' × 'Ferragnès' and 'Ferralise' × 'Tuono'

Results

Genotype prediction by *S*-RNase expression

As expected, 'Tuono' (S_1S_f) only showed one band with RNase activity (Fig. 1), that of S_1-RNase, since the expression of the self-compatibility allele has no RNase activity. On the other side, the self-incompatible parents, 'Ferragnès' and 'Ferralise', showed the two same bands with RNase activity, corresponding to the S_1- and S_3−RNases.

In the families where 'Tuono' was the female parent, according to the gametophytic system, only two genotypes are possible, S_3S_f and S_1S_3 (Fig. 1a). Consequently, all seedlings showed the S_3−RNase inherited from the self-incompatible parent, because the pollen carrying the other allele, S_1, was unable to grow in 'Tuono' pistils and reach the ovule for its fertilization. If only S_3−RNase was identified in the stylar extract of a seedling, its genotype was considered to be S_3S_f due to the presence of the undetected S_f and therefore it was assumed to be self-compatible. The seedlings with two bands were considered to be S_1S_3, having inherited S_1 from 'Tuono' instead of S_f, and therefore were assumed to be self-incompatible.

In the families where 'Tuono' was the male parent (Fig. 1b), only the pollen carrying the S_f allele could grow in the 'Ferralise' and 'Ferragnès' pistils to reach and fertilize the ovules, giving rise to S_1S_f and S_3S_f genotypes. All the seedlings of these families showed stylar extracts with a single *S*-RNase band, either the S_1− or the S_3−RNase, and therefore the presence of the S_f allele was assumed, as well as their self-compatibility.

S-genotype determination by *S*-allele-specific PCR

Channuntapipat et al. (2001) designed the ConF and ConR primers (Table 2), consensus to the conserved regions of the published sequences of the *S*-RNase alleles S^b, S^c and S^d of almond (Ushijima et al. 1998). These primers allow the amplification of fragments from different *S* alleles and have allowed the identification of some of them, including the S_1 and S_f alleles involved in the four families studied. When these primers were used in our progenies, they were also found to amplify one fragment from the S_3 allele with a size of 1,196 bp (Fig. 2a, b). This fragment is only 9 bp smaller than the S_f amplified product, hindering their differentiation, which was not detected by Channuntapipat et al. (2001). In seedlings of the S_1S_f genotype, S_1 amplification frequently produces the inhibition or a weak amplification of the S_f allele (Fig. 2a, b), as already described by Channuntapipat et al. (2001), who concluded that the presence of either S_1 or S_7 in a seedling masked the amplification of the other allele by PCR.

In order to clearly differentiate the S_3 and S_f alleles, a S_3 allele-specific identification was developed by the design of three new primers, the forward S3F and the reverse, S3R1 and S3R2 (Alonso and Socias i Company 2005a). The combinations of primers, S3F/S3R2, and that of S3F with ConR of Channuntapipat et al. (2001) (Fig. 2c), has allowed the amplification of S_3 fragments of 790 and 950 bp, respectively (Table 2), whose size differ from the fragment of 459 bp obtained by the SfF/SfR primer combination (Channuntapipat et al. 2003) for S_f-specific identification (Fig. 2d). The use of these primers has allowed identifying the two *S* alleles in all the seedlings of the four families studied and assigning them the correct genotype (S_1S_3, S_1S_f or S_3S_f).

S genotype proportions

The genotype distribution in the four families is shown in Table 3. The data available for the genotypes of families with the same origin (Ballester et al. 1998; Duval et al.

Table 2 Primer combinations and size of the *S* fragments amplified for the *S* allele identification in the seedlings studied

Reference	Primer name	5′ → 3′ Sequence
Alonso and Socias i Company (2005a)	S3F	CTTCTGCGCTTACGAGAGGTT
	S3R2	TGTGATTTCCACATGTCT
Channuntapipat et al. (2001)	ConF	GTGCAACAATGGCCACCGAC
	ConR	TACCACTTCATGTAACAACTGAG
Channuntapipat et al. (2003)	SfF	GTGCCCTATCTAATTTGTTGAC
	SfR	GACATTTTTTTAGAAAGAGTG

Primer combination	Size product (bp)	Allele objective
S3F/S3R2	790	S_3
ConF/S3R2	1,036	
S3F/ConR	950	
ConF/ConR	1,196	
	1,072	S_1
	1,205	S_f
SfF/SfR	449	

Table 3 Number of seedlings with each *S*-genotype obtained in each family

Cross	Progeny	S_fS_3	S_1S_3	Total	χ^2	α
$S_1S_f \times S_1S_3$	'Tuono' × 'Ferragnès'	21 (58.3%)	15 (41.7%)	36	0.69	0.40
	'Tuono' × 'Ferralise'	6 (28.6%)	15 (71.4%)	21	3.04	0.08
	'Tuono' × S1S3	27 (47.4%)	30 (52.6%)	57	0.07	0.79

Cross	Progeny	S_1S_f	S_3S_f	Total	χ^2	α
$S_1S_3 \times S_1S_f$	'Ferragnès' × 'Tuono'	22 (56.4%)	17 (43.6%)	39	0.41	0.52
	'Ferragnès' × 'Tuono' (Ballester et al. 1998)	28 (48.2%)	30 (51.7%)	58	0.01	0.89
	'Ferragnès' × 'Tuono' (addition)	50 (51.5%)	47 (48.5%)	97	0.04	0.84
	'Ferralise' × 'Tuono'	46 (52.3%)	42 (47.7%)	88	0.10	0.75
	'Ferralise' × 'Tuono' (Duval et al. 2001)	19 (63.3%)	11 (36.7%)	30	1.63	0.20
	'Ferralise' × 'Tuono' (addition)	65 (55.1%)	53 (44.9%)	118	1.02	0.31
	S_1S_3 × 'Tuono'	115 (52.0%)	106 (48.0%)	221	0.28	0.59

2001) have also been pooled in Table 3 in order to compare the results and consider how self-compatibility has been transmitted in each family.

In the progenies where 'Tuono' was the seed parent, thus where self-compatibility must segregate, a different distribution of the *S* genotypes was observed. While in the 'Tuono' × 'Ferragnès' progeny the percentage of self-incompatible seedlings (genotype S_1S_3) was 42%, in the 'Tuono' × 'Ferralise' progeny this percentage was 71% (Table 3). The χ^2 test confirmed the goodness of fit for the expected ratio of 1 S_3S_f : 1 S_1S_3 in the 'Tuono' × 'Ferragnès' cross, but in the 'Tuono' × 'Ferralise' cross the probability was five times lower and at the threshold of significance.

In the progenies where 'Tuono' was the pollen parent no self-compatibility segregation was expected, but that of the S_1 and S_3 alleles. The S_1S_f genotype was slightly more common than the S_3S_f one, although in both progenies the percentages were as expected (Table 3). The 1:1 ratio was also found by Ballester et al. (1998) and Duval et al. (2001) with the *S*-RNase analysis, and when the results of all these families are pooled the distribution is adjusted to the expected 1:1 ratio (Table 3).

Discussion

S-allele identification

Identical *S*-genotypes were obtained by the *S*-RNase identification and by the *S*-allele PCR fragment amplification, which allowed the unequivocal identification of the two alleles of each seedling. Unquestionably, the NEpHGE technique has been very useful in the advancement of the *S*-genotype knowledge, mainly in almond and cherry, but as the number of *S*-alleles to be identified increases (Bošković et al. 2003; Ortega 2002), its application

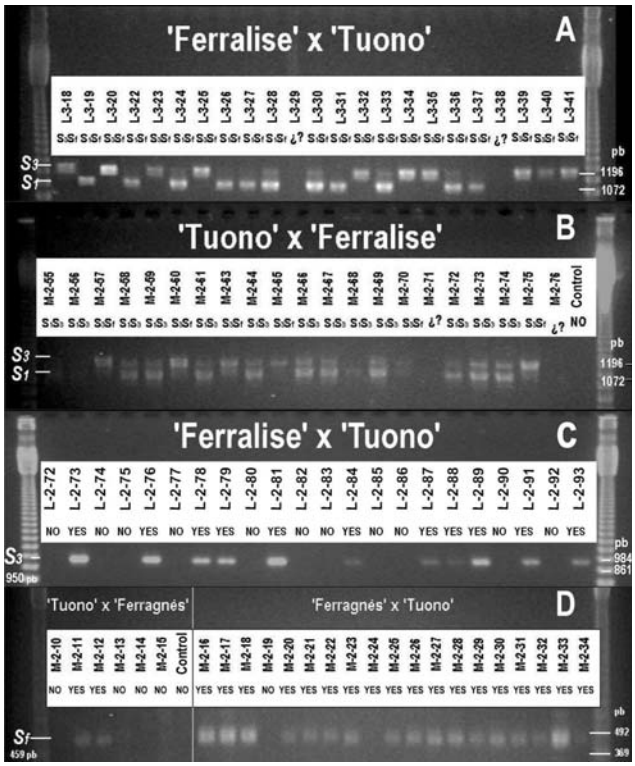

Fig. 2 *S*-allele identification by PCR by using different primers: ConF/ConR (**a**, **b**), S3F/ConR (**c**) and SfF/SfR (**d**)

becomes difficult to identify new alleles due to the narrow pH range in which many *S*-RNases have their isoelectric point.

When the conserved primers ConF/ConR (Channuntapipat et al. 2001) were assayed, one fragment from the S_3 allele was also amplified, but its size was similar to that of the S_f amplified fragment, making their differentiation difficult. Another problem appeared because the S_f fragment amplification with these primers was silenced in most cases by the amplification of the S_1 and S_3 fragments, and one of these two alleles is always present whenever a seedling of these families has the S_f allele.

The number of alleles amplified by the conserved primers ConF/ConR or AS1II/AmyC5R (Tamura et al. 2000) is so far unknown because these primers have only been tested in a few genotypes and, therefore, in a few *S*-allele combinations. Thus, identification by specific primers becomes especially interesting and the specific primers S3F and S3R (Alonso and Socias i Company 2005a) supplement those described by Channuntapipat et al. (2003) for S_3 allele identification. These new primers may be very useful in different European and Australian breeding programmes, because the S_3 allele is present in many progenies due to the utilization of cultivars deriving from 'Aï' such as 'Ferragnès' and 'Ferralise'.

The coincidence of *S* genotype assignment by the two methods shows that the self-compatibility allele product in almond does not have RNase activity probably due to either a deletion of the corresponding genomic fragment or to the production of a defective protein (Bošković et al. 1999). The appropriate work of the S_f-specific primers (Channuntapipat et al. 2003) in our families also confirms the possibility of their routine application as self-compatibility marker in segregant breeding progenies allowing the early detection of seedlings with a self-compatible genotype.

Parental effect on self-compatibility transmission

The self-compatibility transmission from 'Tuono' to its offspring showed a different pattern depending on the self-incompatible parent, 'Ferragnès' or 'Ferralise', in spite of their identical *S*-genotype, S_1S_3. Although the number of plants obtained and of surviving trees in this type of study is normally low (Gillen and Bliss 2005), the change from the expected ratios in the number of self-incompatible seedlings in the 'Ferralise' offsprings may suggest a mechanism acting against inbreeding.

As expected, in progenies where 'Tuono' was the male parent, therefore not segregant for self-compatibility, all plants showed a self-compatible genotype. However, in progenies where 'Tuono' was the female parent, therefore segregant for self-compatibility, opposite genotypic proportions were obtained. In the 'Tuono' × 'Ferragnès' family, the number of self-compatible seedlings was higher than that of self-incompatible seedlings, as Socias i Company and Felipe (1988) and Dicenta and García (1993) had observed in two different 'Tuono' × 'Ferragnès' families. On the contrary, in the 'Tuono' × 'Ferralise' family, only about a quarter of the progeny is of self-compatible genotype.

All the studied progenies come from half-compatible pollinations, thus implying that the genotypic proportions obtained only depend on the initial distribution of the *S* alleles in the haploid ovules of the seed parent because only one pollen genotype can reach the ovary to accomplish fertilization of these ovules. The subsequent survival of the new genotypes from the embryo stage to the seedling evaluation stage may also affect the observed proportion of each genotype.

Thus, 'Ferragnès' and 'Ferralise' ovules were either of S_1 or S_3 haploid genotype and could only be fertilized by S_f pollen from 'Tuono' because S_1 pollen is unable to reach these ovules. In all the families obtained with 'Tuono' as pollen parent, including those of Ballester et al. (1998) and Duval et al. (2001), S_1S_f genotypes are slightly more frequent than the S_3S_f ones, but show a good fit to the expected ratio of 1 S_1S_f:1 S_3S_f, as put forward by Crossa-Raynaud and Grasselly (1985) for half-compatible crosses.

On the contrary, 'Tuono' ovules were either of S_1 or S_f haploid genotype and could only be fertilized by S_3 pollen

from 'Ferragnès' and 'Ferralise' because S_1 pollen is unable to reach 'Tuono' ovules. In their progenies, the *S*-genotype distribution seems to depend on the self-incompatible pollen parent. In all 'Tuono' × 'Ferragnès' progenies, the self-compatible S_3S_f genotype is slightly more frequent, although not significantly, than the self-incompatible S_1S_3 genotype, whereas in the 'Tuono' × 'Ferralise' progeny, the S_1S_3 self-incompatible genotype is over 70%. The increase in the number of self-incompatible seedlings in the 'Ferralise' offspring may suggest a mechanism acting against inbreeding, favouring the survival of self-incompatible seedlings, those requiring cross-pollination to produce the new generation. In the crosses between 'Ferralise' and 'Tuono', there were always a lower number of S_3S_f plants than that of the other *S*-genotype. It may be possible that combinations of the genes present in the linked regions of S_f in 'Tuono' and of S_3 in 'Ferralise', adversely affect the performance of the S_3S_f plants, probably due to an inbreeding consequence (Socias i Company and Felipe 1994).

Inbreeding symptoms in almond have already been described as genetic and resulting in a general reduction of fitness. This vigour reduction may also include other aspects not described so far, such as a survival reduction of inbred genotypes or even of genotypes contributing to an increase of inbreeding. Inbreeding affects plant vigour, mainly reducing the efficiency in plant reserve accumulation, and this reduction may also affect the accumulation of reserves in the seed for embryo nourishment and also in the pistil to sustain pollen tube growth (Alonso and Socias i Company 2005b).

The utilization of inbred genotypes in almond breeding programmes aiming to obtain new self-compatible cultivars happens more frequently due to the utilization of a reduced number of parents. This reduces the almond genetic pool and, as a consequence, may decrease the efficiency of these programmes by the distortion of the Mendelian transmission of self-compatibility.

Acknowledgments Research conducted under the Spanish project CICYT AGL2004-06674-C02-01. JMA acknowledges a scholarship grant from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Training by Dr. I. Batlle (IRTA-Mas Bové), Dr. P. Martínez Gómez (CSIC-Murcia) and Dr. T.M. Gradziel (UC-Davis) as well as technical assistance by A. Escota, J. Búbal and J.M. Ansón are highly appreciated.

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