

# Inheritance of self-compatibility in almond

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The incidence of self-compatibility in 742 almond trees, resulting from 25 inter-cultivar crossings in which at least one of the parents is self-compatible (Genco and Tuono), was determined by means of microscopic observation of the pollen tube growth in the pistil. The data obtained are consistent with the assumption that self-incompatibility in the genus *Prunus* is controlled by a single, multi-allelic gene whose expression in the pollen is gametophytic. The 100 per cent occurrence of self-compatible specimens in the cross where the Ferragnès cultivar is the female parent is due to the existence of an allele shared with the self-compatible cultivars used. The high number of progeny examined and the method used to ascertain the self-compatibility make the results very reliable, as opposed to other less precise methodologies or tests with few seedlings.

**Keywords:** fluorescence microscopy, fruit breeding, intraspecific crossings, *Prunus dulcis* Miller, self-pollination.

## Introduction

Most cultivars of almond are self-incompatible. Its cultivation, therefore, requires the planting of at least two cross-compatible cultivars with coincident flowering time and the setting up of beehives to secure enough pollinating agents. Moreover, favourable environmental conditions must be obtained for bees to work effectively. The separate collection of the different cultivars is also necessary, either because of the different morphology of their fruits or their different maturing times.

Jointly these factors have a large impact on the profitability of the crop, hence the breeding of self-compatible cultivars is a priority in almond breeding schemes.

Only a limited number of the numerous almond cultivars grown worldwide are self-compatible. The majority come from the Italian region of Apulia (Godini, 1979; Reina *et al.*, 1986). Prominent among these are Tuono and Genco (Stazione Agraria Sperimentale di Bari, 1951, 1957), Filippo Ceo (Grasselly & Oliver, 1976), Mazzetto — synonymous for Tuono — (Jaouani, 1973), Exinograd (Cyprus Agricultural Research Institute, 1973), Falsa Barese, Ferrante and Palatina (Godini, 1977).

As these cultivars were shown to be capable of transmitting their self-compatibility to their offspring (Socías i Company & Felipe, 1977), using them has proved to be the most effective method for obtaining new self-compatible cultivars.

A knowledge of the inheritance of self-compatibility is an essential step in the attainment of such an objective. The incompatibility system in the genus *Prunus* appears to be controlled by a single, multi-allelic gene whose expression in the pollen is gametophytic (Crane & Brown, 1937; Crane & Lawrence, 1947; Lewis & Crowel, 1954).

This system seems to occur in the almond species, although there are few works to this effect (Grasselly, 1985; Jraidi & Nefzi, 1988; Socías i Company, 1989). In general, the plant material studied has not been very abundant and occasionally the interpretation of the results has not been accurate.

## Plant material and methodology

The presence or absence of self-compatibility has been ascertained in 742 seedlings belonging to 25 families produced by manual pollination, in which one or both parents are the self-compatible varieties Genco or Tuono (Table 1).

The self-compatibility of the progenies has been ascertained by means of microscopic observation of the pollen tube growth in the pistil, according to the following procedure.

**Table 1** Number and percentage (%) of self-compatible (SC) and self-incompatible (SiC) seedlings by families

Crosses	Total	SC	%	SiC	%	$\chi^2$	Significance
Peraleja × Genco	20	9	45.0	11	55.0	0.200	0.654
Garrigues × Genco	37	18	48.6	19	51.4	0.027	0.869
Atocha × Genco	59	27	45.8	32	54.2	0.424	0.515
Ramillete × Genco	48	13	27.1	35	72.9	10.083	0.001
Del Cid × Genco	47	11	23.4	36	76.6	13.294	0.000
Genco × Genco	2	2	100.0	0	0.0	—	—
Ferragnès × Genco	44	41	93.2	3	6.8	—	—
Mono × Genco	14	6	42.9	8	57.1	0.286	0.593
Titan × Genco	2	1	50.0	1	50.0	—	—
Wawona × Genco	1	0	0.0	1	100.0	—	—
Genco × Garrigues	17	8	47.1	9	52.9	0.059	1.808
Genco × Ramillete	26	10	38.5	16	61.5	1.384	0.239
Genco × Ferragnès	25	14	56.0	11	44.0	0.360	0.548
Genco × Mono	21	13	61.9	8	38.1	1.190	0.275
Genco × Tuono	20	19	95.0	1	5.0	—	—
Tuono × Genco	51	47	92.2	4	7.8	—	—
Peraleja × Tuono	54	32	59.3	22	40.7	1.851	0.173
Garrigues × Tuono	43	15	34.9	28	65.1	3.930	0.047
Atocha × Tuono	22	14	63.6	8	36.4	1.636	0.200
Ramillete × Tuono	53	32	60.4	21	39.6	2.283	0.131
Del Cid × Tuono	18	7	38.9	11	61.1	0.889	0.346
Tuono × Tuono	1	1	100.0	0	0.0	—	—
Ferragnès × Tuono	50	50	100.0	0	0.0	—	—
Mono × Tuono	14	11	78.6	3	21.4	4.571	0.032
Tuono × Ferragnès	53	36	67.9	17	32.1	6.811	0.009
Total	742	437	58.9	305	41.1		

Chi-squared and signification level.

From each seedling 10 buds were collected in state D (Felipe, 1977); they were emasculated in the laboratory, sparing only the pistil with a part of the calyx and the floral peduncle. These were carefully placed in trays with semi-stiff plastic mesh to hold the emasculated buds, thus allowing contact between the peduncle and the water on the tray. The pollen of the 10 emasculated buds were collected in small paper wells, to be used in subsequent self-pollination.

The trays containing the pistils and the wells carrying the pollen were placed in an atmosphere-controlled room, with a photoperiod of 12 h, at  $22 \pm 1^\circ\text{C}$  and a high relative humidity (70–80 per cent) to prevent the stigmas from drying up. After 24 h, the 10 pistils of each sample were self-pollinated using a small brush to apply the pollen.

Seventy-two hours after pollination the pistils were placed in small glass bottles with 5 ml of FAA, a fixing solution, made up of 5 per cent of formaldehyde (at 40 per cent), 5 per cent glacial acetic acid and alcohol 90 per cent (at 70 per cent v/v). At that point the peduncle

and the remains of the calyx were eliminated. The samples were thereafter kept in a refrigerator at  $4^\circ\text{C}$  pending observation.

Prior to microscopic examination, the pistils were washed and kept for 30 min in a sterilizer at  $1 \text{ kg cm}^{-2}$  in a solution of sodium sulphite at 5 per cent, which softens the tissue and enhances staining. The stain used was aniline blue at 0.1 per cent in 0.1 N potassium phosphate. Finally, the pistils were carefully stripped of their pubescence before being placed on slides. After crushing them, they were examined by fluorescence microscopy (Linskens & Esser, 1957; Martin, 1959).

Usually, five pistils per sample were examined and the level of penetration of the pollen tubes in each pistil ascertained. When the five pistils did not show homogeneous behaviour, the five remaining pistils were also examined. In general, the presence of at least two pistils in which the pollen tubes clearly reached the ovary was decisive in assigning self-compatibility to that sample.

The data from each family were analysed by goodness-of-fit  $\chi^2$ .

## Results and discussion

Table 1 shows the number and percentage of self-compatible (SC) and self-incompatible (SiC) seedlings for each family.

Note that the size of the sample analysed ranges from 14 to 59, except the (Wawona and Titan) × Genco progeny and those resulting from self-pollination. In the latter, although we started with a large number of seeds (García *et al.*, 1985), very few progeny were obtained, due presumably to the severe inbreeding also observed by other authors (Grasselly *et al.*, 1981; Grasselly & Oliver, 1988).

Note that in only six combinations of the 25 shown in Table 1 did all, or practically all, their progeny turn out to be self-compatible. These are the four combinations in which both parents are self-compatible and the two in which Ferragnès was used as female parent.

The results obtained from the progenies of the 19 remaining crosses gave either approximately 50 per cent self-compatible trees, such as (Peraleja, Garrigues and Atocha) × Genco, or showed marked deviations, either below or above this value. In the cases of Ramillete and Del Cid × Genco, however, flower sterility, pollinations were made outside the pollinating period or other uncontrolled factors, either genetic or atmospheric, may have contributed to the significance deviations from expected.

We can distinguish two groups according to their differential behaviour, as shown in Table 2. One can see that Group I includes the crosses between SC and SiC cultivars (except Ferragnès when it acted as female parent). These crosses produced approximately 50 per cent SC offspring. Note that Tuono behaved better than Genco as far as production of self-compatible offspring is concerned.

Group II shows the results obtained from crosses between SC parents, selfs and Ferragnès crosses (as female parent) with SC cultivars. The progenies belonging to this group display a percentage of SC trees which is very close to 100 per cent (95.2 per cent).

Assuming that the inheritance of self-compatibility in almond is the same as that in other *Prunus* species, we summarize in Fig. 1 the theoretical possibilities of crossing among SC and SiC cultivars, considering two assumptions: with and without a shared allele.

The crosses belonging to Group I, assuming 50 per cent SC:50 per cent SiC, could be assigned to hypothetical cases A, B or E in Fig. 1. As there is no reason to believe that the SiC varieties are related to either Genco or Tuono, it is unlikely that they share an allele with either of the latter, thus the hypothetical case E can be discarded, and they must correspond to case A where the SC cultivar was the female and case B where it was the male; both yielding an identical result.

As a consequence, our SiC parents (except Ferragnès) do not appear to share any alleles with either Genco or Tuono and, given the fact that they are intercompatible (García, 1978; García *et al.*, 1985; Egea *et al.*, 1988), it is expected that they will, at least, differ in one of their S alleles.

The crosses in Group II could conform to hypothetical cases F, G or H because they are the only cases which produce 100 per cent self-compatible seedlings in the offspring. In cases of self-pollination ( $SC_1 = SC_2$ ) and crosses between SC cultivars, the only theoretical possibilities are G or H, both of which give an identical result, which suggests that our two self-compatible parents have the same genotype for self-compatibility. The Ferragnès × SCs case, assigned to Group II, has to match necessarily hypothetical case F, this being the

**Table 2** Number and percentage of self-compatible (SC) and self-incompatible (SiC) seedlings by groups

	NF	Total	SC	%	SiC	%	$\chi^2$	Significance
<b>Group I</b>								
SiC × Genco	8	228	85	37.3	143	62.7	14.754	0.000
SiC × Tuono	6	204	111	54.4	93	45.6	1.588	0.207
Genco × SiC	4	89	45	50.6	44	49.4	0.011	0.915
Tuono × SiC	1	53	36	67.9	17	32.1	6.811	0.009
Total Group I	19	574	277	48.3	297	51.7	0.696	0.403
<b>Group II</b>								
SC × SC	2	71	66	93.0	5	7.0	—	—
Self-pollination	2	3	3	100.0	0	0.0	—	—
Ferragnès × SC	2	94	91	96.8	3	3.2	—	—
Total Group II	6	168	160	95.2	8	4.3	—	—

NF = Number of families. Chi-squared and significance level.

(a)

<p><b>A</b></p> <p>SC × SiC</p> <p><math>S_f S_f</math>      <math>S_2 S_3</math></p> <p><math>\frac{S_f S_2}{50\% SC}</math>    <math>\frac{S_f S_3}{50\% SiC}</math></p>	<p><b>B</b></p> <p>SiC × SC</p> <p><math>S_2 S_3</math>      <math>S_f S_f</math></p> <p><math>\frac{S_2 S_f}{50\% SC}</math>    <math>\frac{S_3 S_f}{50\% SiC}</math></p>
<p><b>C</b></p> <p>SC<sub>1</sub> × SC<sub>2</sub></p> <p><math>S_f S_f</math>      <math>S_f S_2</math></p> <p><math>\frac{S_f S_f}{75\% SC}</math>    <math>\frac{S_1 S_2}{25\% SiC}</math></p>	<p><b>D</b></p> <p>SC<sub>2</sub> × SC<sub>1</sub></p> <p><math>S_f S_2</math>      <math>S_f S_f</math></p> <p><math>\frac{S_f S_f}{75\% SC}</math>    <math>\frac{S_1 S_2}{25\% SiC}</math></p>

  

(b)

<p><b>E</b></p> <p>SC × SiC</p> <p><math>S_f S_f</math>      <math>S_1 S_2</math></p> <p><math>\frac{S_f S_2}{50\% SC}</math>    <math>\frac{S_1 S_1}{50\% SiC}</math>    Do not occur</p>	<p><b>F</b></p> <p>SiC × SC</p> <p><math>S_1 S_2</math>      <math>S_f S_f</math></p> <p><math>\frac{S_1 S_f}{100\% SC}</math>    <math>\frac{S_2 S_f}{Do not occur}</math></p>
<p><b>G</b></p> <p>SC<sub>1</sub> × SC<sub>2</sub></p> <p><math>S_f S_f</math>      <math>S_f S_f</math></p> <p><math>\frac{S_f S_f}{100\% SC}</math>    Do not occur</p>	<p><b>H</b></p> <p>SC<sub>2</sub> × SC<sub>1</sub></p> <p><math>S_f S_f</math>      <math>S_f S_f</math></p> <p><math>\frac{S_f S_f}{100\% SC}</math>    Do not occur</p>

**Fig. 1** Theoretical possibilities of crosses between SC × SiC and SC<sub>1</sub> × SC<sub>2</sub> and expected genetic frequency in a monogenic and gametophytic scheme of self-incompatibility. S<sub>f</sub> = self-compatible allele. S<sub>i</sub> = self-incompatible. (a) Without shared S<sub>f</sub>, (b) with shared S<sub>f</sub>.

only possibility for 100 per cent of the offspring to be SC with a SiC parent taking part. This is backed up by the possibility of Ferragnès sharing a self-incompatible allele with Genco and Tuono, transmitted from its Cristomorto parents (Ferragnès = Cristomorto × Ai), which belongs to the same population as Genco and Tuono. Moreover, if we observe their reciprocal crosses (Genco and Tuono) × Ferragnès, included in Group I, we can observe that these crosses conform to hypothetical case E, thus Ferragnès should have the same S<sub>1</sub> as Genco and Tuono.

The Chi-squared test (Table 1) discloses that the hypothesis 50 per cent SC: 50 per cent SiC, if we cross a SC cultivar with a SiC one (always barring Ferragnès × SC), must be accepted in practically all the crosses. Only in (Ramillete and Del Cid) × Genco did the results depart significantly from those expected and in Tuono × Ferragnès they hardly conformed at all. In the joint study of this group of crosses (Group I in

**Table 3** New planned crosses which will supply 100 per cent of the self-compatible issue. (See hypothetical cases in Fig. 1.)

<p>Cases A and B</p> <p>Seedling SiC × Parent SC</p> <p>Parent SiC × Seedling SC</p>	<p>Case E</p> <p>Seedling SiC × Parent SC</p> <p>Parent SiC × Seedling SC</p> <p>Seedling SiC × Seedling SC</p>
<p>Case F</p> <p>Parent SiC × Seedling SC</p>	<p>Cases G and H</p> <p>Parent SC × Seedling SC</p> <p>Seedling SC × Seedling SC</p> <p>Ferragnès × Seedling SC</p>

Table 2) we clearly perceive that our results do not significantly disagree with the established hypothesis.

Taken as a whole, these data suggest that self-compatibility in this material is determined by an allele of the self-incompatibility gene which is dominant over all the other alleles at this locus, a conclusion which agrees with those of other authors (Sociás i Company, 1984; Grasselly *et al.*, 1985; Sociás i Company & Felipe, 1988).

Only in the crosses in which Ferragnès was the female parent were the genotypic frequencies completely unexpected. This anomalous behaviour of Ferragnès has been studied by Grasselly *et al.*, (1981), and by Sociás i Company & Felipe (1988). Grasselly (1985) accounted for this phenomenon by arguing for the presence of a shared self-incompatibility allele, offering as evidence the possible kinship of Ferragnès with Genco and Tuono, although his theoretical development does not explain satisfactorily the results he arrived at. Sociás i Company & Felipe (1988), on the other hand, relate this high rate of SC seedlings to a decrease in SiC genotypes due to depression phenomena caused by inbreeding, or due to the presence of a lethal gene linked in repulsion to self-compatibility. The results we have arrived at in this work do not tally with this latter hypothesis and reaffirm that of Grasselly.

Now the inheritance of this characteristic has been demonstrated, we plan to carry out new crosses (Table 3) that will give 100 per cent self-compatible seedlings (F<sub>2</sub>), by using the most outstanding offspring (F<sub>1</sub>).

### Conclusions

The results obtained are consistent with the assumption of monofactorial control of self-compatibility, the gene concerned, S<sub>f</sub>, being dominant over all self-incompatible alleles of the S-gene. They also suggest that Genco and Tuono are heterozygous for S<sub>f</sub>.



The seemingly anomalous behaviour of Ferragnès, giving 100 per cent of SC seedlings when used as female parent in crosses with heterozygous self-compatible cultivars, suggests the presence of a shared self-incompatible allele in both parents.

The obligatory presence of a shared allele of  $S_i$ , when a heterozygous self-compatible cultivar self-pollinates, also gives rise to families with 100 per cent self-compatible seedlings. The confirmation of this assumption bears out the shared allele theory in the cases mentioned above.

That Genco and Tuono carry the same compatibility alleles ( $S_i S_i$ ) is confirmed by the fact that when they pollinate each other, the results are identical to those obtained when they are self-pollinated.

Finally, the possibility of using the most outstanding offspring to carry out new crosses which will secure 100 per cent of self-compatible seedlings, has been proposed.

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