

# Characterization of Some Self-compatible Almonds. I. Pollen Tube Growth

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**Abstract.** Pollen tube growth was studied in 10 almond [*Prunus amygdalus* Batsch, syn. *P. dulcis* (Mill.) D.A. Webb] selections of the Zaragoza breeding program, whose main objective is the development of self-compatible cultivars. Self-compatibility was evident in eight of the selections, as indicated by the fact that pollen tube growth was similar following self- and cross-pollination. In the other two selections, pollen tube growth differed following self- and cross-pollination, one showing self-incompatibility and the other an irregular progression of crossed pollen tubes. The importance of the style in sustaining pollen tube growth was evident, and pollen tube growth was influenced by style type.

Most commercial almond cultivars are self-incompatible (Socias i Company, 1990). Efficient, insect-mediated cross-pollination among cross-compatible and simultaneously blooming cultivars is required because any reduction in the number of cross-pollinated flowers can reduce yield (Kester and Griggs, 1959). Favorable weather at bloom is also necessary to maximize bee activity and pollen distribution. Fertilization must follow pollination because the seed is the commercial product. The presence of two or more cultivars in the same orchard, however, creates management problems, particularly at harvest, to provide for processing each cultivar separately. To overcome these problems, almond breeding programs have stressed the development of self-compatible cultivars, which would allow the establishment of single-cultivar orchards (Socias i Company, 1990).

Pollen tube growth has been used to assess self-compatibility in almond (Socias i Company et al., 1976). Selections of the Zaragoza breeding program are routinely examined for the presence of pollen tubes at the base of the style following self-pollination, but information on the location or the rate of pollen tube progression through the style is lacking. The importance of this growth rate is evident, because similar rates of pollen tube growth following self- or cross-pollinations have resulted in similar fruit sets in self-compatible cultivars (Socias i Company and Felipe, 1987, 1992a).

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The objective of this study was to compare self- and cross-pollen tube growth rates in some advanced selections from the almond breeding program at Zaragoza, as they could be related to self-compatibility and to the ability of the styles to regulate pollen tube growth.

## Materials and Methods

Ten selections from the Zaragoza almond breeding program (Felipe and Socias i Company, 1985) were evaluated in this study. They originated from crosses between two self-compatible cultivars, 'Tuono' and 'Genco', with other self-incompatible cultivars. These crosses are 'Tuono' x 'Ferragnès' (A-10-2, A-10-6, and A-10-8), 'Tardive de la Verdière' x 'Tuono' (B-4-2, B-5-2, and B-5-9), open-pollinated 'Tuono' (C-11-1), 'Titan' x 'Tuono' (D-3-5), self-pollinated 'Tuono' (D-4-15), and open-pollinated 'Genco' (E-5-7). They were grown in the department's almond germplasm collection (Socias i Company and Felipe, 1992b), grafted onto the 'GF677' almond x peach hybrid clonal rootstock, planted in 1982, and managed similarly. Virus status is periodically tested, and all selections are free from known viruses.

Flowers just ready to open, at stage D (Felipe, 1977) or popcorn stage, were collected from the field, emasculated, and placed in trays containing tap water. The peduncles extended through the holes of a plastic mesh floating on the water. Growth of pollen tubes on detached emasculated flowers have been shown to behave similarly to those on cut branches or on the tree (Socias i Company and Felipe, 1988).

Two days after emasculation, the pistils were either selfed or cross-pollinated with 'Marcona' pollen. Pollen was obtained from flowers at the same stage by scraping the anthers and drying the pollen until anther dehiscence at room temperature (Kester and Asay, 1975). After pollination, the trays were placed in constant-temperature chambers at

12C. Samples of 10 flowers from each selection and pollination treatment were collected every 24 h until six samples were completed. The pistils were fixed in FAA, rinsed several times in water, and autoclaved in a 5% solution of Na<sub>2</sub>SO<sub>3</sub> for 10 min at 1.2 kg·cm<sup>-2</sup>.

Pollen tube growth through the stigma and style was observed using an ultraviolet microscope. The pistils were prepared by the method of Socias i Company (1979). The central cylinder of transmitting tissue of each style, through which pollen tubes grow, was dissected and used for squash preparation after the callose deposits of the pollen tubes were stained with aniline blue (Linskens and Esser, 1957).

Pollen tube growth in a single style was recorded as the percentage of total length traversed by the longest tube (Lewis, 1942). Calculations were based on eight of the 10 samples, with the lowest and the highest measurements discarded; the percentages were statistically compared by means of a contingency table analysis and a *t* test.

## Results and Discussion

Patterns of pollen tube growth differed among the selections, especially concerning the progression of pollen tubes through the styles (Table 1). Although no pollen tubes had reached the base of the style 24 h after pollination, sharp differences were observed at 48 h. More differences were observed among than within selections for the progression of pollen tubes. Differences identified at 72 h largely had disappeared at 96 h, except for two selections. The main difference in pollen tube growth was determined by the style type and not by the pollen source.

Pollen tube growth analysis distinguished four distinct response patterns (Fig. 1). The fastest rate of pollen tube growth is represented by selections B-5-2, B-5-9, D-3-5, and D-4-15. Both self- and cross-pollen in this group had traversed the style and reached the base after 72 h. In some styles, pollen tubes had reached the base 48 h after pollination. This growth pattern is represented by D-3-5 in Fig. 1. In that group, pollen tube growth rates were practically identical following self- and cross-pollinations. When both pollinations were compared for all selections (data not shown), only one significant difference was observed for B-5-9 at 24 h with the *t* test. In all other cases, a similar rate of pollen tube growth was observed for the two pollen types in these four selections.

Selections A-10-2, A-10-6, A-10-8, and B-4-2 exhibited slower pollen tube growth and required at least 96 h to traverse the whole style. This growth pattern is exemplified by A-10-8 in Fig. 1. In this group, pollen tube growth did not differ following self- or cross-pollination (Table 1).

The similarity of growth of the two pollen types in the selections of these two groups was assessed by considering the combined number of styles with pollen tubes at their base, which revealed that there were no significant differences between self and cross treatments.

Table 1. Number of styles with pollen tubes at their base at various time intervals after pollination.

Times after pollination (h)	No. styles with pollen tubes at base for each selection																					
	A-10-2		A-10-6		A-10-8		B-4-2		B-5-2		B-5-9		C-11-1		D-3-5		D-4-15		E-5-7			
	S <sup>2</sup>	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C		
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
48	1	1	0	0	1	1	4	4	2	2	6	6	1	0	6	6	2	6	1	0	0	
72	7	6	2	4	5	4	6	7	7	8	8	8	1	7	8	8	8	8	7	1	0	
96	8	7	7	8	8	7	8	8	8	8	8	8	1	8	8	8	8	8	8	0	0	
120	8	8	8	8	8	8	8	8	8	8	8	8	2	8	8	8	8	8	8	0	0	
144	8	8	8	8	8	8	8	8	8	8	8	8	2	8	8	8	8	8	8	0	0	
Combined	32	30	25	28	30	28	36	37	33	34	38	38	7	31	38	38	34	38	32	1	1	
Significance	NS		NS		NS		NS		NS		NS		**		NS		NS		**			

<sup>2</sup>S = self-pollination; C = cross-pollination with 'Marcona' pollen.

NS, \*\* t values nonsignificant or significant at  $P \leq 0.01$ , respectively, for the comparison between self- and cross-pollination for each selection.

A third pattern, shown by selection C-11-1, demonstrates a bias against self-pollen. In that selection, the number of styles with pollen tubes at their base was  $\approx 4$  times greater for cross-pollen than for self-pollen (Table 1). Cross-pollen grew at a rate similar to that of the second group of selections, but self-pollen reached an upper limit of  $\approx 90\%$  (Fig. 1). The growth of the two pollens was similar at 24 h, but it differed significantly at 48 h and thereafter.

A fourth pattern is shown by selection E-5-7. Growth of the self-pollen was similar to that of the second group, but there was irregular growth of the cross-pollen. The progression of the two pollen types was not significantly different at 24 h, but cross-pollen growth stopped thereafter (Fig. 1). Only a single style was observed with cross-pollen tubes at its base (Table 1).

The nearly identical growth rates observed in self- and cross-pollinations is consistent with the hypothesis of self-compatibility of the first two groups of selections (A-10-2, A-10-6, A-10-8, B-4-2, B-5-2, B-5-9, D-3-5, and D-4-15), as reported for other almond cultivars (Socias i Company and Felipe, 1987, 1992a). This finding counters Godini's (1981) suggestion that, in self-compatible almond cultivars, foreign cross-compatible pollen grows faster than the cultivar's own tubes. The growth rate at the temperature we used (12C) was also in agreement with previous results in almond (Socias i Company et al., 1976).

Self-pollen tube growth rate of C-11-1 was slower than that of cross-pollen tubes, with only a few styles having self-pollen tubes at their base. These results agree with previous tests with this selection, where only 17% of the styles observed during 5 years of study had pollen tubes at their base, while higher proportions were observed in the other selections. Consequently, we consider C-11-1 to be only partially self-compatible. In almond, partial self-compatibility has been indicated by very low levels of fruit set and by the growth of a single pollen tube in only some styles following self-pollination (Socias i Company and Felipe, 1988). As partial self-compatibility is considered insufficient to obtain a commercial crop in single-cultivar plantings (Socias i Company, 1990), this selection would be eliminated in the screening for new cultivars.

The opposite result is observed in E-5-7, where the foreign and presumably cross-compatible pollen does not grow well but behaves

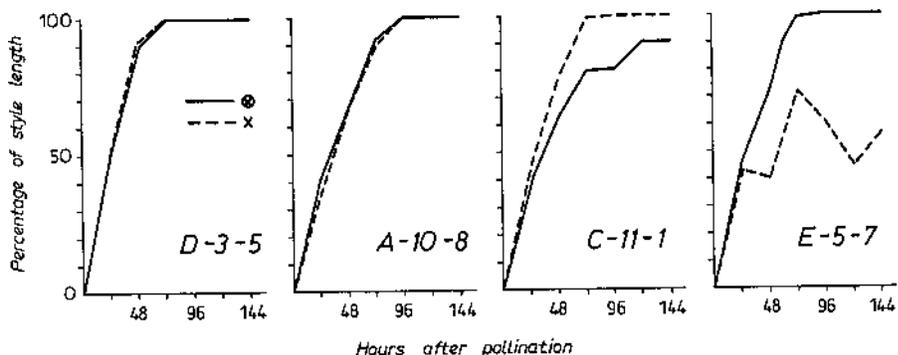


Fig. 1. Mean percentage of style length traversed by the longest pollen tube at different time intervals after (— ⊗) self- and (--- x) cross-pollinations of four selections.

as an incompatible pollen. Cases of cross-incompatibility are rare in almond (Socias i Company, 1990) and have been reported only for genetically related cultivars. This relationship is improbable here because E-5-7, an open-pollinated seedling of 'Genco', does not appear to have 'Marcona' as the pollen parent, as concluded from comparing the isozyme profiles of the seedling (Bernad and Socias i Company, 1994) with those of 'Genco' and 'Marcona' (Cerezo et al., 1989). Furthermore, we observed a similar behavior in this selection when pollinated by 'Vivot', another unrelated cultivar (Socias i Company and Rallo i Garcia, 1989), suggesting a possible rejection of foreign pollen by E-5-7 pistils. The irregular growth rate of foreign pollen tubes in E-5-7 pistils, which shows a decrease with time (Fig. 1), might be due to the retrogression of incompatible pollen tubes, as reported in almond (Socias i Company et al., 1976) and earlier in *Lycopersicon peruvianum* Mill. (de Nettancourt et al., 1973). This anomaly requires further study and could be related to the different stages of pollen tube progression through the styles, including changes from autotrophous to heterotrophous in the feeding pattern of the pollen tubes. Pistils of E-5-7 may be unable to sustain foreign pollen tube growth when their nutritional reserves are depleted.

The differences observed among the selections must be attributed to the style rather than to the pollen because the same foreign pollen was used in all the crosses. Differences in style behavior and length can be expected. The different behavior of these selections may have a genetic basis since the four groups basically correspond to the different crosses that gave rise to these selections, with the

exception of B-4-2. Selection B-4-2 was assigned to the second group, while its sibs were placed in the first group. Selections of the second and third group have longer pistils (Bernad and Socias i Company, 1995) and reduced pollen tube growth rates. Differences in style behavior may be explained by differences in pistil secretion after the pollen tubes cross the transmitting tissue of the style, as reported for peach (Herrero and Arbeloa, 1989).

The similar growth of cross- and self-pollen is consistent with the self-compatibility of eight of the selections studied. Selection E-5-7 also showed self-compatibility despite the lack of growth of the cross-pollen. Only C-11-1 must be considered not fully self-compatible among the selections tested.

#### Literature Cited

- Bernad, D. and R. Socias i Company. 1994. Caracterización morfológica y bioquímica de algunas selecciones autocompatibles de almendro. Info. Técnica Econ. Agraria 90V(2):103-110.
- Bernad, D. and R. Socias i Company. 1995. Characterization of some self-compatible almonds. II. Flower phenology and morphology. HortScience 30:321-324.
- Cerezo, M., R. Socias i Company, and P. Arús. 1989. Identification of almond cultivars by pollen isozymes. J. Amer. Soc. Hort. Sci. 114:164-169.
- Felipe, A.J. 1977. Almendro. Estados fenológicos. Info. Técnica Econ. Agraria 27:8-9.
- Felipe, A.J. and R. Socias i Company. 1985. L'amélioration génétique de l'amandier à Saragosse. Options Méditerran. CIHEAM/IAEMZ 85/1:9-14.
- Godini, A. 1981. Observing pollen tube growth in self compatible almond cultivars by means of

- fluorescence. Options Méditerran. CIHEAM/IAMZ 81/I:77–82.
- Herrero, M. and A. Arbeloa. 1989. Influence of the pistil on pollen tube kinetics in peach (*P. persica*). Amer. J. Bot. 76:1441–1447.
- Kester, D.E. and R. Asay. 1975. Almonds, p. 387–419. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.
- Kester, D.E. and W. H. Griggs. 1959. Fruit setting in the almond: The effect of cross-pollinating various percentages of flowers. Proc. Amer. Soc. Hort. Sci. 74:206–213.
- Lewis, D. 1942. The physiology of incompatibility in plants. I. The effect of temperature. Proc. Royal Soc., Ser. B 131:13–26.
- Linskens, M.F. and K. Esser. 1957. Über eine spezifische Anfärbung der Pollenschläuche im Griffel und die Zahl der Kallosepfropfen nach Selbstung und Fremdung. Naturwissenschaften 44:16.
- de Nettancourt, D., M. Devreux, A. Bozzini, M. Cresti, E. Pacini, and G. Serfatti. 1973. Ultrastructural aspects of the self-incompatibility mechanism in *Lycopersicon peruvianum* Mill. J. Cell. Sci. 12:403–419.
- Socias i Company, R. 1979. Aportación a las técnicas de observación de los tubos polínicos. Caso del almendro. Ann. Inst. Nacional Investigaciones Agraria, Ser. Prod. Veg. 10:233–236.
- Socias i Company, R. 1990. Breeding self-compatible almonds. Plant Breeding Rev. 8:313–338.
- Socias i Company, R. and A.J. Felipe. 1987. Pollen tube growth and fruit set in a self-compatible almond selection. HortScience 22:113–116.
- Socias i Company, R. and A.J. Felipe. 1988. Self-compatibility in almond: Transmission and recent advances in breeding. Acta Hort. 224:307–317.
- Socias i Company, R. and A.J. Felipe. 1992a. Self-compatibility and autogamy in 'Guara' almond. J. Hort. Sci. 67:313–317.
- Socias i Company, R. and A.J. Felipe. 1992b. Almond: A diverse germplasm. HortScience 27:717–718, 863.
- Socias i Company, R., D.E. Kester, and M.V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci. 101:490–493.
- Socias i Company, R. and J. Rallo i García. 1989. La pollinització de l'ametller 'Vivot'. Rev. Ciència (Inst. d'Estudis Balearics) 5:67–74.