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Floral characterization of some self-compatible almond selections

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SUMMARY – Bud density, bloom phenology, flower morphology, and pollen tube growth were studied in seven almond (*Prunus amygdalus* Batsch) selections of the Zaragoza breeding programme. Bud density varied from 0.25 to 1.19 bud/cm and may be qualified from medium to high. Blooming time was late for all selections and the first flowers opened during the third week of February, which helped avoiding frost damage until that time. Self-compatibility was confirmed in all selections and was assessed by their own pollen growth, reaching the base of the style in all cases. Furthermore, pollen tube growth was similar both after self- and cross-pollination, confirming the effectiveness of their own pollen as it grows as fast as the foreign cross-compatible pollen. Genetic self-compatibility was also confirmed by the *S*-genotype of the selections, as determined by separation of the stylar *S*-RNases. The importance of the style is also shown by the effect of the style length on the pollen tube growth and the stigma/anther position inside the flower. The stigma position indicates the possibility of natural autogamy in two of the seven selections.

Key words: *Prunus amygdalus*, breeding, flower morphology, self-compatibility, autogamy.

RESUME *– "Caractérisation florale de quelques sélections d'amandier auto-compatibles". La densité florale, la phénologie de la floraison, la morphologie florale et la croissance des tubes polliniques ont été évaluées chez sept sélections d'amandier (*Prunus amygdalus *Batsch) issues du programme d'amélioration génétique de l'amandier de Saragosse. La densité florale varie de 0,25 à 1,19 bourgeons/cm et peut être qualifiée de moyenne à élevée. Ces sélections présentent une floraison tardive et les premières fleurs s'ouvrent pendant la troisième semaine du mois de février, permettant d'échapper aux dégâts causés par les gelées tardives. L'auto-compatibilité de ces sélections a été confirmée par l'étude de la croissance de leur propre pollen qui atteint la base du style chez toutes les sélections. La croissance des tubes polliniques après l'auto-pollinisation et la pollinisation croisée a été similaire, confirmant l'efficience du propre pollen qui croît à la même vitesse que le pollen étranger. Aussi, l'auto-compatibilité de ces sélections a été confirmée par la détermination des allèles *S *par la séparation des* S*-RNases stylaires. L'importance du style a été mise en évidence par l'effet de la longueur du style sur la croissance des tubes polliniques et par la position du stigmate par rapport aux anthères à l'intérieur de la fleur. La position du stigmate indique la possibilité d'autogamie naturelle chez deux des sept sélections.*

Mots-clés : Prunus amygdalus*, amélioration, morphologie florale, auto-compatibilité, autogamie.*

Introduction

Almond (*Prunus amygdalus*, Batsch) is a self-incompatible species with a gametophytic system controlled by the multiallelic locus *S* (Socias i Company *et al*., 1976) with pollination entrusted to insects, not to the wind (Kester and Griggs, 1959). Thus, insect-dependant cross-pollination among inter-compatible and simultaneously blooming cultivars is required in order to ensure that all the flowers could be efficiently pollinated to reach a 25-40% fruit set (Kester and Griggs, 1959), an economically acceptable threshold for a commercial crop. For this reason, self-compatible cultivars will be an alternative to self-incompatible ones to expand monovarietal orchards (Socias i Company, 1978).

Most commercial almond cultivars are early blooming. As a result, almond cultivation was restricted to regions with low risk of spring frosts. However, the expansion of the culture into inland Mediterranean areas, where the occurrence of spring frost is common and coincident with bloom of most almond cultivars, may reduce or even eliminate yields. Moreover, in these growing regions, unfavourable weather conditions (rain, wind) during bloom may also reduce yields by interfering with pollinating insects, mainly honeybees (Bernad and Socias i Company, 1995). Also, it is important to take into account the amount of flowers, because it is correlated with yield (Grasselly, 1972).

In order to overcome these problems, the breeding programme of Zaragoza was focused on obtaining self-compatible and late blooming cultivars (Felipe and Socias i Company, 1985). Six new cultivars have been released from it: 'Guara', 'Aylés', 'Moncayo' (Felipe and Socias i Company, 1987), 'Felisia', 'Cambra' and 'Blanquerna' (Socias i Company and Felipe, 2001). However, this program is continuing to obtain better performing cultivars (Socias i Company *et al.*, 1998). The aim of this work was to characterize seven selections from this programme in relation to some flower traits and to evaluate their self-fruitfulness based on the efficiency of their pollen tube growth and the stigma/anthers position.

Materials and methods

Seven self-compatible and late blooming selections from the Zaragoza almond breeding programme were evaluated in this study. They originated from crosses between four self-compatible genotypes, 'Felisia', 'Guara', 'Moncayo' and selection A-10-6, with other self-incompatible cultivars, 'Marcona', 'Bertina' and 'Ferragnès'. These crosses are 'Felisia' × 'Bertina' (G-2-11 and G-3-24), A-10-6 × 'Marcona' (H-3-39), 'Felisia' × 'Moncayo' (I-3-65 and I-3-67) and 'Guara' × 'Ferragnès' (I-1-95 and I-2-12). They were grown as three contiguous trees at a trial plot at 6x6 m, grafted onto 'Garnem' almond × peach hybrid rootstock (Gómez Aparisi *et al.*, 2001).

To measure bud density, four branches were selected around the tree with homogeneous shape and position, about 1.5 m above ground and approximately 1 m long. During the winter of 2001, all the secondary branches were noted and measured, and all the flower buds, at stage B (Felipe, 1977), were counted (Socias i Company, 1988a).

To study pollen tube growth, flowers were collected from each selection at stage D (Felipe, 1977), and were emasculated and then placed in trays containing water, with the peduncle passing through the holes of a plastic mesh floating on the water. Two days after emasculation, the pistils were self- or cross-pollinated with 'Genco' pollen. Following pollination, samples of ten flowers were taken every 24 hr and fixed in FAA (formalin-acetic acid-ethanol 70%, 1:1:18), rinsed several times in water and autoclaved for 10 minutes at 1.2 kg/cm^2. For observation, pistils were prepared in squash slides (Socias i Company, 1979) stained with 0.1% aniline blue in 0,1N K_3PO_4 (Linskens and Esser, 1957). Pollen tube growth through the stigma and the style were observed using an ultraviolet microscope. Records were taken on the longer pollen tube in the style and expressed as the percentage of the length of the style.

Bloom observations were carried out in 2001. Full bloom was recorded as the number of days before or after 'Nonpareil' blooming time, which is considered as reference by GREMPA (Grasselly and Crossa-Raynaud, 1980). Flower morphology was also assessed by examining 20 flowers of each selection collected randomly around the canopy at the beginning of bloom. The distance from the stigma to the highest and the lowest anther were measured.

Results and discussion

Bud density

Significant differences were observed for bud density among the selections (Table 1). This confirms that bud density is a characteristic of each genotype and that selection for this trait can be made. Selection I-1-95 had the highest bud density (1.19 bud/cm), whereas selections G-3-24 and I-3-65 had the lowest values (0.32 and 0.25 bud/cm respectively). These results agree with those previously reported in other almond genotypes (Socias i Company, 1988a; Bernad and Socias i Company, 1998). Selections I-1-95, I-3-67 and G-2-11, having a high bud density, should ensure a good yield, because both traits are highly correlated (Grasselly, 1972). On the other hand, selections H-3-39, G-3-24 and I-3-65 may give a good yield if a maximum number (near 100%) of the flowers was efficiently pollinated (Kester and Griggs, 1959) and if the percentage of sterile flowers was low (Bernad and Socias i Company, 1995), even if they had a low bud density.

Table 1. Average bud density of seven almond selections (2002-2003)

Selection	Bud density (bud/cm)
I-1-95	1.19 a^y
I-3-67	0.78 ab
G-2-11	0.77 ab
I-2-12	0.67 bc
H-3-39	0.45 bc
G-3-34	0.32 c
I-3-65	0.25 c

y: mean separation by LSD test, P = 0.05.

Bloom evolution

Blooming of the seven selections was later than 'Desmayo Largueta' (a traditional Spanish cultivar), but none were later blooming than 'Ferragnès' (Fig. 1). Selections: H-3-39, I-1-95, G-3-24, G-2-11 and I-2-12 were rated as early-medium blooming, selection I-3-67 as medium-late blooming, and I-3-67 as the latest blooming (1 day before 'Ferragnès'). The late blooming time of selections I-3-65 and I-3-67 could be due to the presence of a major gene (*Lb)* (late bloom) in these selections, inherited from 'Felisia' (Socias i Company *et al.*, 1999). However, selections G-3-24 and G-2-11 were only early-medium blooming, despite having 'Felisia' as a parent. This could be explained by the fact that these two selections have not inherited the allele *Lb*.

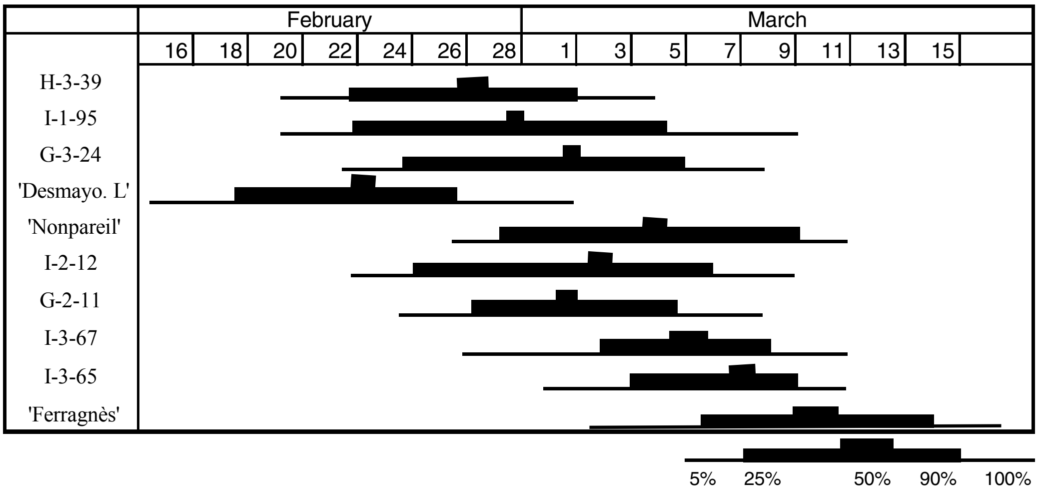


Fig. 1. Blooming time of studied selections as compared to the reference cultivars in 2002.

Pollen tube growth

The dynamics of the pollen tube growth confirmed the genetic self-compatibility of these selections. Pollen tubes had reached the base of the style four days after self-pollination. In general, no significant differences were observed between self- and cross-pollination regarding the dynamics of pollen tube growth, given that the growth of the own pollen tubes and the foreign ones was practically similar and in both cases the pollen tubes traversed the pistil and reached the base of the styles at the same time (Table 2), showing the efficiency of self-pollination. These results agree with those of Vasilakakis and Porlingis (1984), Ben-Njima and Socias i Company (1995) and Socias i Company (2001).

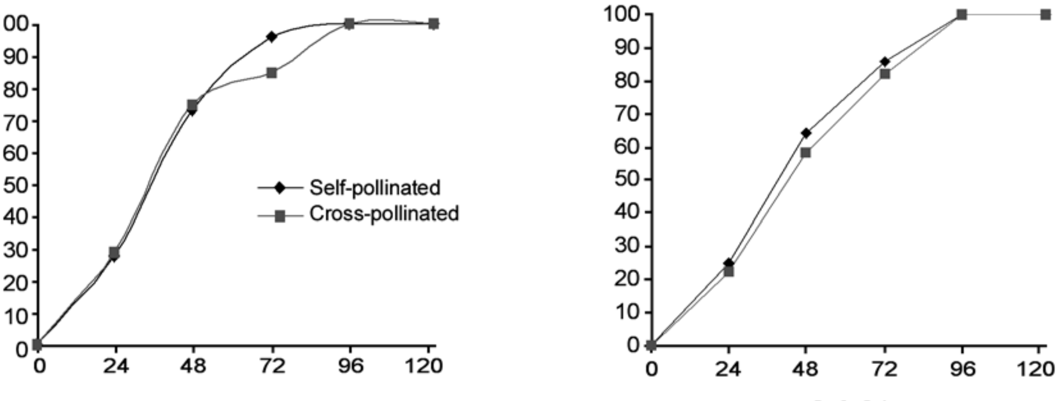
Table 2. Mean percentage of the style length traversed by the longest pollen tube at different time intervals after self-pollination and cross-pollination

Selection	Time after pollination (hours)									
	24		48		72		96		122	
	SP[†]	CP	SP	CP	SP	CP	SP	CP	SP	CP
G-2-11	32.32 ns	32.35	41.58 ns	49.54	81.61**	69.90	100**	93.53	100 ns	100
G-3-24	36.30*	32.46	57.66 ns	54.06	83.55 ns	83.24	95.12 ns	93.01	100 ns	100
H-3-39	27.89 ns	28.41	50.69 ns	54.27	87.35 ns	91.64	100 ns	100	100 ns	100
I-3-65	27.89 ns	28.88	73.05 ns	74.51	96.17**	85.02	100 ns	100	100 ns	100
I-3-67	24.87*	22.08	64.24 ns	58.14	85.97 ns	82.22	100 ns	100	100 ns	100
I-1-95	21.65 ns	22.57	52.09 ns	46.93	83.35**	90.69	100 ns	100	100 ns	100
I-2-12	18.01 ns	20.11	61.63 ns	66.14	84.82 ns	86.50	100 ns	100	100 ns	100

[†] SP = self-pollinated; CP = cross-pollinated with 'Genco' pollen.

ns,*,** Non-significant or significant at P = 0.05 or 0.01, respectively. Significance tested by ANOVA of arcsin-transformed pollen tube growth.

Twenty four hours after pollination, significant differences at the 1% level were observed only between two selections (I-3-67 and G-3-24) for the two types of pollination (Table 2), with the own pollen tubes growing faster than the crossed ones (Fig. 2). This fact could be due to a weaker germination rate, penetration and growth of pollen tubes at the stigmatic surface (Certal *et al.*, 2002). In other tree selections (I-3-65, I-1-95 and G-2-11), significant differences for the pollen tube growth were observed at 48 and 78 hours after pollination (Table 2; Fig. 2). This could be due to the changes from autotrophous to heterotrophous feeding of the pollen tubes (Ben-Njima and Socias i Company, 1995) and to the availability of the nutritional reserves and the space required for the pollen tubes growth, which decreases along the style in the genus *Prunus*. The same results were reported in other species such as *Prunus persica* (Herrero and Arbeloa, 1989) and *Prunus avium* L. (Hormaza and Herrero, 1996).



I-3-65

G-3-24

Fig 2. Dynamics of pollen tube growth at different time intervals after self-pollination and cross-pollination of two selections.

Furthermore, a significant negative correlation (-0.95 at 1% level) was found between the length of the pistil and the pollen tube growth rate. Selections I-1-95 and I-2-12, with the longer pistils (13.22 and 14.31 mm, respectively), showed the slowest growth rates (0.097 and 0.086 mm/h). However, selection I-3-65 had the shortest pistils (10.98 mm) and showed the highest growth rate (0.164 mm). These results agree with those of Vasilakakis and Porlingis (1984) and Socias i Company (1988b).

Reciprocal anther/stigma position

The stigma level relative to the upper and lower anthers is shown in Fig 3. In three selections, G-2-11, G-3-24 and H-3-39, the upper anther was well below the stigma, making natural autogamy morphologically difficult. However, selections I-1-95 and I-2-12, had the upper anther just below the stigma, making autogamy morphologically possible, through the contact between the stigma and the anthers. In the two other selections, I-3-65 and I-3-67, the upper anther was above the stigma, making the contact between stigma and anthers possible, increasing the possibility for natural self-pollination.

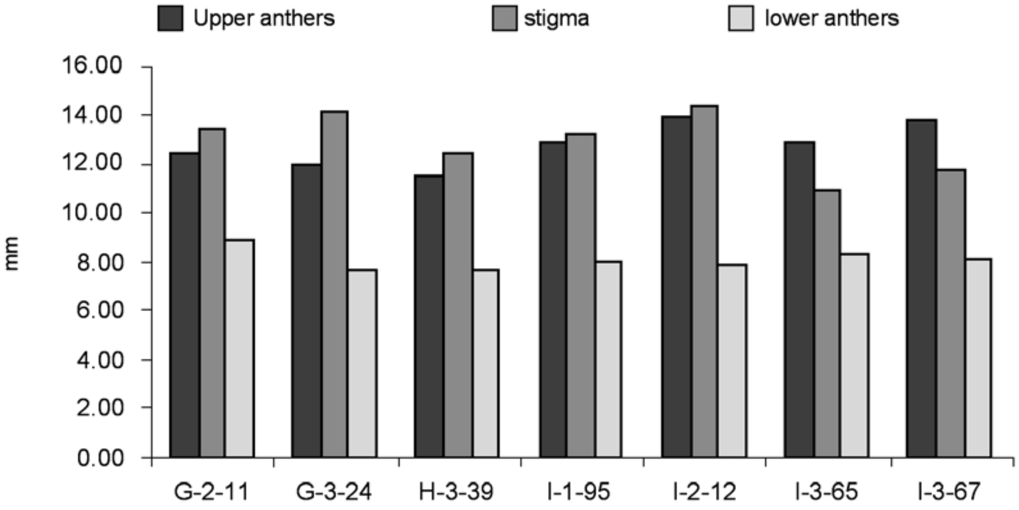


Fig. 3. Reciprocal stigma/anther position in the flowers of the almond selections.

The effective self-pollination observed in some self-compatible cultivars such as 'Truoito' (Vasilakakis and Porlingis, 1984) and 'Guara' (Socias i Company and Felipe, 1992) have been attributed to the close stigma/anther position, which facilitates the natural transfer of pollen to the stigma within the flower. Moreover, the intimate contact of the pollen grains and the stigmatic surface appears to be critical to reach an adequate fruit set. As a consequence, natural autogamy may be achievable with two selections, I-3-65 and I-3-67, because of their flower morphology. However, this trait needs to be studied and evaluated in the field to ensure a good fruit set without the intervention of pollinating insects.

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