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# Evaluation of almond selections for fruit set under field conditions

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**SUMMARY** – Fruit set was studied over three years in 11 almond (*Prunus amygdalus* Batsch) selections of the Zaragoza breeding programme, previously selected for their fruit and kernel traits and self-compatibility, following five different pollination treatments: open pollination (control), open pollination of a reduced number of flowers (control-D), artificial self- and cross-pollination after emasculation, and autogamy in bagged branches. Significant differences were observed in all cases: among selections, among years and among treatments. There was also a significant interaction in all cases (selection x year, selection x treatment, and year x treatment) as well as for the three variables together. In general, higher scored sets were observed for the control-D treatment, showing that the flowers left had a better ability to set fruit, probably due to their less competitive conditions. Also cross-pollinations as a whole produced better sets than self-pollinations, but not in all cases, showing differences in the ability to set self fruit among the selections, a trait to be considered in further evaluations. Sets in the bagged branches scored the lowest, revealing the differences in autogamy ability of each selection, as well as the wellknown set reduction produced by the bag effect. These sets could be explained by the genetic self-compatibility as deduced by the pollen tube growth, as well as to the flower morphology, allowing the stigma and anthers to be in contact. Year effects were observed, but they did not affect all the treatments and selections at the same level, thus stressing the need for autogamy evaluation during more than one year for such an important commercial trait as fruit set in almond.

**Key words:** *P. amygdalus*, fruit set, autogamy, breeding.

**RESUME** – "Evaluation des sélections d'amandier pour la nouaison du fruit en conditions de terrain". La nouaison a été étudiée pendant trois années chez 11 sélections d'amandier (*Prunus amygdalus* Batsch) du programme d'amélioration génétique de Saragosse, déjà repérées pour leurs caractères du fruit et de l'amandon ainsi que pour leur auto-compatibilité, suivant cinq différents traitements de pollinisation : pollinisation libre (témoin), pollinisation libre d'un nombre réduit de fleurs (témoin-D), auto-pollinisation et pollinisation croisée artificielle après émasculature, et autogamie de branches ensachées. Des différences significatives ont été trouvées dans tous les cas : entre sélections, entre années et entre traitements. Il y a eu aussi une interaction significative dans tous les cas (sélection x année, sélection x traitement, et année x traitement) ainsi que pour l'ensemble des trois variables. En général, les taux de nouaison les plus élevés ont été mesurés pour le traitement témoin-D, ce qui montre que les fleurs laissées en nombre réduit ont une plus grande possibilité de nouaison, probablement par leur condition moins compétitive. Les pollinisations croisées ont également donné des nouaisons plus élevées que les auto-pollinisations, mais pas dans tous les cas, ce qui montre qu'il y a des différences entre les sélections pour ce caractère et que l'on doit en tenir compte dans les pas suivants d'évaluation. La nouaison chez les branches ensachées a été la moindre et montre les différences entre les sélections pour l'autogamie et la réduction connue de la nouaison dû à l'effet du sac. Les nouaisons observées peuvent être mises en relation avec l'auto-compatibilité génétique déduite de la croissance des tubes polliniques ainsi qu'avec la morphologie de la fleur qui peut permettre le contact du stigmate et des anthères. On a aussi observé des effets de l'année, mais pas dans la même direction chez tous les traitements et sélections, ce qui accentue le besoin d'évaluer l'autogamie pendant plus d'une année pour assurer le niveau d'un caractère ainsi important que la nouaison chez l'amandier.

**Mots-clés :** *P. amygdalus*, nouaison, autogamie, amélioration.

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## Introduction

Most almond breeding programmes have fostered the development of self-compatible cultivars to overcome the problems related to cross-pollination of a mostly self-incompatible species as almond (Socias i Company, 2002). Consequently, self-compatibility is a primary trait to be considered during evaluation of seedlings of the breeding programme. Several approaches have been used to assess the level of self-compatibility, such as pollen tube growth and fruit setting following artificial

pollinations (Socias i Company and Felipe, 1987), bagging of branches (Grasselly and Olivier, 1984), or even enclosing whole trees in cages, with or without honey bees (Godini *et al.*, 1994; Socias i Company and Felipe, 1992). Self-compatible seedlings might also be detected by molecular markers (Bošković *et al.*, 1999; Channuntapipat *et al.*, 2001; Ma and Oliveira, 2001), although this information has only a genetic interest, not horticultural. Probably every method has advantages and limitations, but the final evaluation of self-compatibility of a cultivar or selection is its productivity under field conditions, i.e. with solid blocks of one clone isolated from any other almond clone and even in the absence of pollinating insects.

Self-compatibility implies, first of all, a pollen tube growth similar both after self- and cross-pollination with a cross-compatible pollen. Secondly, this good pollen tube growth after self-pollination must be followed by similar sets, which probably is not always the case. And thirdly, these sets must reach the level of a commercial crop. From a horticultural point of view there is a fourth requirement, that these sets must be obtained by autogamy. All these aspects have been stressed during the evaluation process (Oukabli *et al.*, 2000; Socias i Company and Felipe, 1987; Torre Grossa *et al.*, 1994), but autogamy has received particular attention only later (Dicenta *et al.*, 2001; Godini *et al.*, 1992; Socias i Company and Felipe, 1992; Socias i Company *et al.*, 2002; Vargaa *et al.*, 1998).

Only natural autogamy, that is, the ability of a genetically self-compatible cultivar to pollinate itself in the absence of insects (Weinbaum, 1985), can allow solid plantings of one single cultivar. Flower morphology, in particular the relative positions of the stigma and anthers, has major importance for natural autogamy (Bernad and Socias i Company, 1995; Godini *et al.*, 1994).

Our objective was to evaluate fruit set under field conditions of 11 almond selections previously evaluated for their nut and kernel traits, and whose pollen tube growth and flower morphology were already known (Ben Njima and Socias i Company, 1995; Bernad and Socias i Company, 1995).

## Materials and methods

Eleven selections from the Zaragoza breeding programme (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 Verdrière' x 'Tuono' (B-4-2, B-5-2, B-5-7, 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). The sets were studied during three consecutive years on the same trees, an experimental plot of three contiguous trees of each selection grafted on the almond x peach hybrid clonal rootstock INRA GF-677, maintained at the Spanish almond germplasm collection of the SIA of Zaragoza, according to a usual growing management.

For set estimates, several branches all around the trees were selected and assigned randomly to the treatments. The treatments applied were: open pollination, open pollination of a reduced number of flowers, self-pollination, cross-pollination with a cross-compatible pollen, and bagging, although bagging was done only during two years. Control branches were left intact for open pollination. In another treatment (Control-D), only the flowers at stage D (Felipe, 1977), as if they were to be emasculated, were left for later natural open pollination, to evaluate the effects of deblossoming on set. On the branches used for artificial pollination treatments, open flowers and closed buds were removed, leaving only the flowers at stage D, being later emasculated and self- and cross-pollinated with 'Marcona' pollen. Bagged branches were covered before any flower opening, and the bags were removed just after petal fall. The total number of branches per treatment was four to six, but only one branch was bagged, due to the difficulties of bagging. The initial number of flowers, including closed flower buds, was counted on each branch, independently of the treatment applied. After deblossoming (control-D treatment) and emasculation (artificial pollinations) the remaining number of flowers or pistils were also counted. Fruits were counted in early June, after fruit fall, and sets were obtained for all selections as shown in Table 1 for selection A-10-2.

Fruit sets were statistically analysed, after angular transformation, by the General Linear Model procedure of SAS software (SAS Institute, 2000). Mean values were analysed by Duncan's multiple range test.

Table 1. Fruit sets in field treatments for selection A-10-2

Year	Treatment	Initial number of flowers	Flowers left	Fruits	Fruit set (% of flowers left)
1	Control	1111	1111	109	9.54
	Control-D	1062	422	51	12.08
	Selfed	1022	521	42	8.06
	Crossed	1168	782	66	8.44
2	Control	727	727	222	30.54
	Control-D	669	290	151	52.07
	Selfed	827	436	173	39.68
	Crossed	768	377	150	39.79
	Bag	222	222	12	5.41
3	Control	1029	1029	219	21.28
	Control-D	1014	423	131	30.97
	Selfed	1051	410	74	18.05
	Crossed	1027	427	96	22.48
	Bag	184	184	2	1.09

## Results

Fruit sets varied according to the clone, the year and the treatment (Table 2). The analysis of variance of the sets showed that there were significant differences for all the variables as well as for the interaction among the variables (Table 3).

As a whole, the effect of the year was significant and the average set for the second year (46.66%) was the highest, followed by that of the third year (29.47%) and finally that of the first year (16.55%). The same pattern of variation was observed in all the selections, showing the important year effect of fruit set (Socias i Company *et al.*, 2002). These differences cannot be attributed to frosts, the most important limiting factor for fruit set in almond, as frosts did not occur during blooming in any of the three years of study. Probably other factors, such as temperature, water status, or nutritional conditions, could be responsible for this variation, as it was the same for all selections.

The treatments also showed a significant effect as a whole. Higher sets were observed for the control-D treatment (44.14), followed by crossing (38.51), control (31.80), selfing (22.92), and bagging (9.16). However, the effect was different for each selection, showing their different levels of self-compatibility and autogamy. Thus, for some selections, such as A-10-2 and D-3-5, there are no differences between selfing and crossing, showing their full self-compatibility. However, sets after bagging of these two selections show that they possess very different levels of autogamy.

Independently of the treatments, selections showed different ability to set fruit. Thus, B-5-7 with a mean set of 50.21 and D-3-5 with 46.40 showed consistently the highest sets for all treatments, mainly for the control, their mean sets being significantly higher than those of all other selections, whereas D-4-15, with a mean set of 11.31, showed the lowest sets, significantly lower than the other selections.

## Discussion

Sets followed different patterns of variation which may be attributed to the year, the treatment or the selection. The large differences observed between the three years, with a significant year effect, showed that the same treatments may act differently depending on unspecified conditions, some of them probably related to the biological entity of fruit trees. Trees are not mechanical objects reacting always in the same direction and grow, furthermore, in commercial orchards, under field conditions, with many environmental influences, which cannot all be taken into consideration. The different sets observed cannot be attributed to frosts, because there were no frosts during the blooming season or afterwards, but to other different conditions, as previously stated, which could not be measured.

Table 2. Summary of fruit sets (% of fruits over flowers left) in the field treatments

Selection	Year	Treatment				
		Control	Control-D	Selfed	Crossed	Bag
A-10-2	1	9.54 a	12.08 a	8.06 a	8.44 a	-
	2	30.54 b	52.07 d	39.68 c	39.79 c	5.41 a
	3	21.28 b	30.97 c	18.05 b	22.48 b	1.09 a
A-10-6	1	13.76 b	39.23 d	1.82 a	22.11 c	-
	2	63.29 b	68.69 bc	1.72 a	71.10 c	0.61 a
	3	27.10 b	34.39 c	3.80 a	58.07 d	0.32 a
A-10-8	1	15.92 a	25.98 b	20.91 ab	23.59 b	-
	2	20.87 b	28.44 c	26.53 bc	24.49 bc	5.33 a
	3	28.05 b	56.00 c	26.90 b	30.58 b	5.86 a
B-4-2	1	14.57 c	27.23 d	2.46 a	9.56 b	-
	2	50.14 c	50.63 c	9.44 a	14.46 b	11.18 a
	3	18.66 a	47.69 c	36.36 b	59.74 d	16.98 a
B-5-2	1	7.95 ab	11.34 b	3.37 a	25.92 c	-
	2	37.84 b	47.26 c	0.35 a	61.31 d	0.75 a
	3	27.43 c	25.36 c	2.64 bo	24.15 c	0 a
B-5-7	1	8.09 a	30.77 c	15.74 b	28.26 c	-
	2	59.97 b	88.80 e	65.71 c	80.14 d	18.28 a
	3	36.25 bc	60.82 d	42.56 c	34.05 b	27.60 a
B-5-9	1	7.45 a	18.35 c	11.11 b	11.41 b	-
	2	68.88 d	91.59 e	62.60 c	56.12 b	1.88 a
	3	30.59 b	49.24 d	39.91 c	52.80 d	1.11 a
C-11-1	1	2.78 b	1.07 ab	0.68 a	16.27 c	-
	2	55.03 b	55.73 b	0.44 a	69.58 c	0.59 a
	3	49.95 c	74.93 d	3.36 b	46.60 c	0.23 a
D-3-5	1	22.41 a	43.12 c	40.00 bc	35.71 b	-
	2	73.86 d	83.86 e	49.12 c	39.05 b	30.84 a
	3	23.58 a	72.84 d	28.77 b	50.10 c	20.52 a
D-4-15	1	2.42 a	6.41 b	2.11 a	9.56 b	-
	2	15.98 b	23.40 c	19.66 bc	44.74 d	1.54 a
	3	5.83 b	3.03 b	4.72 b	15.79 c	0 a
E-5-7	1	6.71 a	36.82 d	15.74 b	28.25 c	-
	2	62.33 c	77.06 d	38.16 a	45.58 b	38.69 a
	3	40.52 c	27.81 b	15.32 a	14.97 a	12.63 a

Mean fruit sets followed by different letters in the same line are significantly different at  $P \geq 0.05$  by the Duncan's test.

On the other hand, it is very difficult to establish a good set level for a commercial almond crop because of the large variation in bloom density among almond cultivars (Socias i Company, 1988). Kester and Griggs (1959) reported set averages for open pollination of 30.2 for 'Nonpareil' and 33.4 for 'Texas', the two leading commercial cultivars in California. The highest control fruit set was 73.86 for D-3-5 in year 2 (Table 2), with an average for the three years of 33.05 for the same selection, thus comparable to those reported in California. The sets can be considered high, except for selections A-10-2 and D-4-15, especially if related to bloom density, because all selections have high bud densities, ranging from 0.46 buds  $\text{cm}^{-1}$  for A-10-6 to 1.02 for C-11-1 (Bernad and Socias i Company, 1998), thus similar to or higher than that measured for 'Nonpareil', 0.46 (Socias i Company, 1988).

Table 3. Analysis of variance of fruit sets

Source	DF	Type III SS	Mean square	F value	Probability
Clone	10	4.34770527	0.43477053	52.87	<.001
Year	2	6.76392653	3.38196326	411.29	<.001
Treatment	4	7.22935764	1.80733941	219.79	<.001
Clone x year	20	2.00279599	0.10013980	12.18	<.001
Clone x treatment	40	4.01363172	0.10034079	12.20	<.001
Year x treatment	7	0.54670659	0.07810094	9.50	<.001
Clone x year x treatment	70	2.40006007	0.03428657	4.17	<.001
Error	228	1.87481184	0.00822286		

The treatment of control-D was adopted because in artificial pollinations there is always a reduction in the number of flowers, when open flowers and retarded buds are eliminated at emasculation. Although Kester and Griggs (1959) reported that any reduction in the number of flowers is a reduction in the number of fruits, this is not always the case (Socias i Company and Felipe, 1987, 1992), and our results showed that in most cases (Table 2) fruit sets for control-D were significantly higher than for the control treatment. This set increase is probably due to a better ability to set fruit of the flowers left, as they are in a less competitive condition.

Sets after cross-pollination are similar to those reported for artificial pollination of fruit trees (Layne, 1983), showing large differences among the selections. However, most important are the differences between self- and cross-pollination of each selection. These were initially selected because of their pollen tube growth after self-pollination and their good nut and kernel traits. However, it was later shown that selection C-11-1 did not have a good pollen tube growth after self-pollination (Ben Njima and Socias i Company, 1995) and this is reflected in the very low sets after selfing. Other selections, such as A-10-6, B-5-2, and D-4-15, although they have a very similar pollen tube growth after selfing and crossing (Ben Njima and Socias i Company, 1995), showed a very important decrease of fruit set after selfing as compared to after crossing, thus indicating that a genetic self-compatibility is not an enough condition to ensure the horticultural self-compatibility required for a commercial cultivar. The genetical self-compatibility of these selections has also been confirmed by their RNases (J.M. Alonso and O. Kodad, unpublished).

For some other selections (B-4-2, B-5-7, and E-5-7) there is a significant decrease of fruit set after selfing as compared to after crossing, but the self sets were in most cases enough to ensure a commercial crop. Only for the remaining selections (A-10-2, A-10-8, B-5-9, and D-3-5) were sets after selfing and crossing comparable, ensuring their horticultural self-compatibility. However, only field autogamy can ensure a good behaviour of a cultivar in a commercial orchard, and this trait was examined by the bag treatment.

All selections showing a self-incompatible setting pattern (A-10-6, B-5-2, C-11-1, and D-4-15) confirmed these low sets in the bag, but other selections, even with good sets after selfing, such as A-10-2 and B-5-9, showed very low levels of autogamy. Both selections are characterized by possessing stigmas placed over the longest stamens (Bernad and Socias i Company, 1995), thus with a very low possibility of self-pollination in autogamy conditions, a trait explaining the low sets inside the bags. The remaining selections showed acceptable sets in the bag, mainly D-3-5 and E-5-7, and only A-10-8 was just under 6%, the level considered for self-compatibility in bagged branches by Grasselly *et al.* (1981).

This 6% level is low for a commercial crop, but in the bag there is a set decrease due a bag effect (Socias i Company *et al.*, 2002). Branch protection to avoid unwanted pollination creates a special environment inside the protection, mainly temperature changes (Larsen *et al.*, 1960). Warm temperatures adversely affect fruit set (Layne, 1983), and a temperature increase is likely whenever a branch is enclosed, especially on the sunny days, which often occur during the almond blooming period. Some ventilation took place through the plastic mesh of the bags, but its level could depend on the wind intensity, thus creating different environments inside the protection, more favourable or not to set fruit. Shading reduced fruit set in apple (Byers *et al.*, 1990) and this might also have

occurred in the bag environments due to the important light reduction produced inside the bag (Socias i Company *et al.*, 2002).

The results observed in this study show that set levels after self-pollination are not only related to the genetical self-compatibility of the selection under trial, but also to other genetic conditions of each genotype. Almond is a self-incompatible species with a possible genetic background of pseudo-self-compatibility (Socias i Company, 1990) resulting in the set differences observed in these selections. Furthermore, differences among the results of different years point to unspecified environmental conditions affecting fruit set of biological entities as fruit trees are. In spite of these variations, some selections, such as A-10-8, B-5-7, D-3-5, and E-5-7 show high levels of self-compatibility and autogamy, but these results stress the need for autogamy evaluation throughout more than one year.

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