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The importance of pollination type (self or cross) in two self-compatible almond cultivars: 'Francolí' and 'Guara'

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Abstract. Type of pollination (self or cross-pollination), and their influence on nuts, in two self-compatible almond cultivars 'Francolí' and 'Guara', growing in commercial orchards, was observed in 2006. One orchard with 'Glorieta' and 'Masbovera' cvs as pollinators of 'Francolí', and the second orchard with 'Ferraduel', 'Guara' and 'Masbovera' cvs. Four hundred fruits from 'Francolí' and 160 from 'Guara' were collected, characterized and put to germinate. Molecular markers (isozymes and SSRs) were applied to the seedlings to know the pollen source of each almond. 'Francolí' nuts showed that self-pollination was higher (77%) than cross-pollination (23%). Type of pollination in 'Guara' seeds seemed to be similar (61% self and 39% cross-pollination), although not all seedlings have been analyzed yet. So far, the results for both cultivars showed that the type of pollination has not affected the nut characters studied. Further observing years are planned.

Keywords. Almond – Isozymes – Molecular markers – Pollination – Self-compatible.

L'importance du type de pollinisation (auto ou croisée) chez deux cultivars d'amandier auto-compatibles, 'Francolí' et 'Guara'

Résumé. *Le type de pollinisation (auto ou croisée) et son influence sur les fruits, chez deux variétés d'amandier auto-compatibles, 'Francolí' et 'Guara', plantés en vergers commerciaux, a été étudié pendant l'année 2006. 'Glorieta' et 'Masbovera' se trouvaient comme pollinisateurs de 'Francolí', dans un verger, et 'Ferraduel', 'Guara' et 'Masbovera', étaient les trois variétés d'un deuxième verger. Quatre-cents fruits de 'Francolí' et 160 de 'Guara' ont été récoltés, caractérisés et mis à germer. On a utilisé des marqueurs moléculaires (isozymes et SSR) sur les semis germés pour connaître l'origine du pollen de chaque semis. Pour 'Francolí', les amandes provenant de l'autopollinisation étaient supérieures (77%) à celles provenant de la pollinisation croisée (23%). Pour 'Guara', malgré le fait que les fruits n'étaient pas tous analysés, la proportion était plus égalitaire : 61% provenaient d'autopollinisation et 39% de pollinisation croisée. Les premiers résultats montrent que le type de pollinisation (auto ou croisée), n'a pas d'influence sur les caractéristiques des fruits étudiés. Il est prévu de continuer ce travail dans les prochaines années.*

Mots-clés. *Amandier – Isoenzymes – Marqueurs moléculaires – Pollinisation – Autopollinisation.*

I – Introduction

Most of the almond cultivars are self-incompatible, but in the last years self-compatible cultivars, coming from different breeding programmes, are increasing in the orchards. Some questions about these self-compatible cultivars are still unknown. Do they need other cultivars in the orchard to have a good production? Is there any influence on fruit-set or fruit characteristics according the type of pollination (self or cross)?

Several approaches have been done trying to answer these questions, but the results obtained show inconsistencies. Studies carried out by Godini (1981) observed differences in 'Filippo Ceo' and 'Genco' cvs pollen tubes growth; Torre Grossa *et al.* (1994) showed differences in fruit set and kernel weight in 'Lauranne'; and Oukabli *et al.* (2000 and 2002) suggested that the type of

pollination seems to have a potential effect on ovule fertility, on fruit set and on fruit characters in 'Tuono' cv. In all these cases the results were favourable to cross-pollination in front of self-pollination. However, Socias i Company and Felipe (1992) observed similar rate of pollen tube growth, but different fruit set, due, probably to cage effects, in 'Guara' cv.; studies carried out by Dicenta *et al.* (2002) and Ortega *et al*. (2006) have not shown any difference between the two types of pollination (self and cross) in fruit characteristics, although a different behaviour in pollen tube growth, fruit set or fruit characteristics after self and cross-pollination of different self-compatible genotypes was reported. Socias *et al.* (2004) observed a different behaviour in fruit set when self and cross-pollination were compared in self-compatible selections, during 3 years, at this meaning, the authors suggest that these differences could be due not only to the genetic self-compatibility of the selection, but also to other genetic conditions of each genotype. Finally, a trial in self-compatible selections comparing fruits from bagged branches (self-pollinated flowers) and fruits from not bagged branches (self-pollinated and cross-pollinated flowers), showed that fruits from not bagged branches, presented higher nut and higher seed weight and volume (Vargas *et al.*, 2005).

Considering all these works, our study was focused on the behaviour of two self-compatible almond cultivars 'Francolí' and 'Guara' with natural autogamy (Socias i Company and Felipe, 1992; López *et al.*, 2005; Vargas, personal communication), in the orchards, under field conditions without any manipulation, trying to avoid any possible external effect (bagged branches, hand pollination, cage, etc.). The aim of this work was to study the percentage of self and cross-pollination that occurs in these two cultivars and investigate if the type of pollination (self or cross) has an influence on fruit characteristics.

II – Materials and methods

Two almond orchards in Vilallonga del Camp (Tarragona, Spain), both planted in 1997 (the first one of nearly 2 ha and the second one of 1 ha), having self-compatible cultivars, were chosen for this study: one orchard (A) with 'Francolí' cv. and 'Glorieta' and 'Masbovera' cvs, as pollinators, and the other one (B) with three cultivars: 'Guara', 'Ferraduel' and 'Masbovera'. The two orchards are irrigated and no bees are added at flowering time. Some isozyme genes are known for these cultivars (Arús *et al.*, 1992; Arús *et al.*, 1994) and for our study we chose those that could give us some information about the seedlings pollen source (Tables 1 and 2). Also, molecular markers, as microsatellites (SSRs), are known for 'Francolí', 'Glorieta' and 'Masbovera' (data not published), but not for 'Guara' and 'Ferraduel'. As occurred in isozymes, we chose two SSRs, that could give us some information about the 'Francolí' seedlings pollen source (Table 3). In August 2006, four hundred almonds from 'Francolí' (orchard A) and 160 from 'Guara' (orchard B), were collected. Fruits were characterized in the laboratory by its weight, length, wide and thickness, and then put to germinate (3 months at 4ºC). After this period of time seeds were planted in little pots and transferred to the greenhouse. When plantlets started growing, isozymes were used to know the pollen source of each almond. About 0.02 g of young developing leaves was crushed in 100 µl of extraction buffer (Arús *et al*., 1992). The extract was absorbed into 3 x 8 mm paper wicks (Whatman no. 3) and loaded into a horizontal starch gel (11.5% Connaught hydrolysed starch). One enzymatic system, phosphoclucomutase (PGM), was studied in 'Francolí' seedlings and four, aspartate amino transferase (AAT), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH) and phosphoclucomutase (PGM) in 'Guara' seedlings. Staining assays for these enzymes and the gel/electrode buffers used for each one are detailed in Arús *et al.* (1994). Observing isoenzymatic variability, it was not possible to know the male genitor for all the seedlings, and therefore a more specific molecular marker as SSRs, was used in these plants. Two SSR loci were used for 'Francolí' seedlings: "MS50" and "MS34". These two markers are specific polymorphic SSRs for *Prunus*. Young leaves were crushed with liquid nitrogen to be used later as departure material for the DNA extraction (Doyle and Doyle, 1990). The amplification of the SSRs was made by PCR with a PE 9700 termocicler (Applied Biosystems) and for its later

detection was used the system of hair electrophoresis ABI PRISM (Applied Biosystems). For each of the markers, the size of the detected alleles was considered, using the software of GeneScan® analysis (Applied Biosystems). SSRs markers for 'Guara' seedlings have not been used yet.

Table 1. 'Francolí', 'Glorieta' and 'Masbovera' isoenzymatic genes

Genes	'Francolí'	'Glorieta'	'Masbovera'
Pgm-1	*bb*	*ab*	*ab*
Pgm-2	*ab*	*aa*	*aa*

Table 2. 'Guara', 'Masbovera' and 'Ferraduel' isoenzymatic genes

Genes	'Guara'	'Ferraduel'	'Masbovera'
Aat-1	*ab*	*aa*	*aa*
Aat-2	*bb*	*ab*	*bb*
Gpi-1	*fa*	*aa*	*aa*
Idh-1	*ab*	*aa*	*aa*
Pgm-1	*bb*	*bb*	*ab*

Table 3. 'Francolí', 'Glorieta' and 'Masbovera' SSRS markers

SSRS markers	'Francolí'	'Glorieta'	'Masbovera'
"MS50"	155/163	169/169	143/163
"MS34"	162/162	147/162	147/151

When pollen seeds source (self or cross) from 'Francolí' and from 'Guara' was known, results of fruit characteristics were compared between the two types of pollination. Analysis of variance was applied to the data and means separation was done by Duncan's multiple range test for $p \leq 0.05$.

III – Results and discussion

Characterisation of 396 fruits from 'Francolí' and 160 for 'Guara' are presented in Table 4. Fruits weight from 'Francolí' and 'Guara' were similar as described by Alegre *et al.* (2007): 3.9 g for 'Francolí' and 4.8 g for 'Guara'.

Table 4. Fruit characteristics in two almond cultivars 'Francolí' and 'Guara'

Cultivar	Number of samples	Weight	Length	Wide	Thickness
'Francolí'	396	3,83 (0,53)	36,10 (1,88)	21,10 (1,57)	15,33 (0,69)
'Guara'	139	4.94 (0.95)	40,28 (2,00)	27,11 (1,51)	17,90 (1,01)

Mean values (and SD).

Not all the seeds characterised in laboratory germinated, and only 139 from 'Francolí' and 93 from 'Guara' were possible to be studied by molecular markers when plantlets grew.

1. 'Francolí' seedlings

Seedlings from 'Francolí' were characterised by two isoenzymatic genes: *Pgm-1* and *Pgm-2*. Table 5 shows the different genotypes that could be found depending on the cross.

Table 5. *Pgm-1* and *Pgm-2* possible genotypes from 'Francolí' seedlings

Genes	'Francolí' x 'Francolí'	'Francolí' x 'Glorieta'	'Francolí' x 'Masbovera'
Pgm-1	*bb*	*ab bb*	*ab bb*
Pgm-2	*aa ab bb*	*aa ab*	*aa ab*

Individuals being *ab* for *Pgm-1* were cross-pollinated, and those being *bb* for *Pgm-2* were self-pollinated. Thus, 63 fruits could be classified: 33 were self-pollinated and 30 were cross-pollinated. Otherwise, the kind of pollination of 76 seedlings could not be known observing PGM banding patterns. For these individuals, two SSRs loci were used. Table 6 shows the different genotypes that could be found depending on the cross. Observing the genetic profile of the 76 seedling for these two SSRs loci ("MS50" and "MS34"), we concluded that 74 seedlings were self-pollinated and 2 cross-pollinated. From all seedlings analysed by isozymes and SSRs, 107 (77%) had been self-pollinated and 32 (23%) cross-pollinated.

Table 6. "MS50" and "MS34" possible genotypes from 'Francolí' seedlings

SSRs markers	'Francolí' x 'Francolí'	'Francolí' x 'Glorieta'	'Francolí' x 'Masbovera'
"MS50"	*155/155* 155/163 163/163	*155/169* *163/169*	*143/155* 155/163 *143/163* 163/163
"MS34"	162/162	*147/162* 162/162	*151/162* *151/162*

In italic: Specific SSRs to discriminate for self or cross-pollination.

2. 'Guara' seedlings

Seedlings from 'Guara' were characterised by five isoenzymatic genes: *Aat-1*, *Aat-2*, *Gpi-2, Idh-2* and *Pgm-1* (Table 7).

Table 7. *Aat-1*, *Aat-2*, *Gpi-2, Idh-2* and *Pgm-1* possible genotypes from 'Guara' seedlings

Genes	'Guara' x 'Guara'	'Guara' x 'Ferraduel'	'Guara' x 'Masbovera'
Aat-1	*aa ab bb*	*aa ab*	*aa ab*
Aat-2	*bb*	*ab bb*	*bb*
Gpi-2	*ff fa aa*	*fa aa*	*fa aa*
Idh-2	*aa ab bb*	*aa ab*	*aa ab*
Pgm-1	*bb*	*bb*	*ab bb*

Individuals being *bb* for *Aat-1*, *bb* for *Idh-2* or *ff* for *Gpi-2* were self-pollinated, and those being *ab* for Aa*t-2* or *ab* for *Pgm-1* were cross-pollinated. Forty-four fruits could be classified: 27 self-pollinated and 17 cross-pollinated. The male genitor of 29 seedlings could not be known observing isoenzymatic banding pattern, therefore, SSRs will be used for these seedlings in the future.

Comparing fruit characters as weight, length, wide and thickness (Tables 8 and 9), from 'Francolí' and 'Guara' seeds coming from self and cross-pollination, no significative differences were found in any of the fruit characters studied. Our results agree with those carried out by Dicenta *et al.* (2002) and Ortega *et al.* (2006), who worked with different self-compatible genotypes; and differ from those reported by Oukabli *et al.* (2002) working with 'Tuono' cv. and Vargas *et al.* (2005) working with several self-compatible selections. It is important to be pointed out that most of the results of these works have been obtained using cages to isolate the trees, bagged branches, or hand pollinated flowers. Thus, may be an effect of manipulation should have an influence on these reported results. In the present assay we tried to avoid all these possible external effects. Considering all the inconsistent results obtained from researchers working with different genetic material (all self-compatible), we could consider that fruit characters depend not only on the type of pollination (self or cross), but also on the genetics of the selection studied.

Table 8. Weight, length, wide and thickness of self and cross 'Francolí' seedlings

'Francolí' seedlings	Number of seedlings	Weight	Length	Wide	Thickness
Self-compatible	107	3.89 a	36.21 a	21.17 a	15.43 a
Cross-compatible	32	3.83 a	36.22 a	21.14 a	15.39 a

Means separation in columns by Duncan's multiple range test at $p \leq 0.05$. Means within columns followed by the same letter are not significantly different.

Table 9. Weight, length, wide and thickness from self and cross 'Guara' seedlings

'Guara' seedlings	Number of seedlings	Weight	Length	Wide	Thickness
Self-compatible	27	4.88 a	39.91 a	26.71 a	17.67 a
Cross-compatible	17	4.86 a	39.73 a	26.65 a	17.56 a

Means separation in columns by Duncan's multiple range test at $p \leq 0.05$. Means within columns followed by the same letter are not significantly different.

IV – Conclusions

After this first year of studying the fruits from two self-compatible almond cvs. 'Francolí' and 'Guara', we can conclude that the molecular markers (isozymes and SSRs) are useful to investigate the male genitor of the seedlings and allowed us to know the type of pollination that had occurred (self or cross) in the orchard.

Results obtained in the orchard A show that most of the 'Francolí' flowers had been in advantage self-pollinated (77%), whereas 23% had been cross-pollinated. The type of pollination for 'Guara' flowers, in the orchard B seems to be more similar: 61% self and 39% cross-pollinated although not all the seedlings of this last cultivar have been analysed yet. The fact that there are no bees added in the orchards could favour the presence of self-pollinated seeds.

In both cultivars 'Francolí' and 'Guara' the type of pollination has not influenced the fruit characters studied: weight, length, wide and thickness in both of the cultivars. To verify these first results, further years of observation related to the type of pollination and its possible influence on almond fruit characteristics, are planned.

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