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Disruption of megagametophyte development caused by inbreeding in almond

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Abstract. A homozygous self-compatible almond with a high level of inbreeding producing a reasonable yield when cross-pollinated was experimentally found to drop fruits when self-pollinated. The aim of this work was to elucidate the causes of fruit abortion after selfing by studying pollen tube growth, megagametophyte development and fruit set following controlled self and cross-pollinations. Pollen tubes reached the ovary for both pollinations, however, differences were observed for embryo and endosperm development. Thus, while for cross-pollination a developing embryo and an incipient endosperm were observed, in self-fertilized ovules a degenerating embryo was observed in most cases. Thirty days after pollination, the percentage of fruit set was similar for both pollination types. In contrast, 60 days after self-pollination fruit set was very low or nil. These results indicate that the abnormal development of the megagametophyte in this genotype seems to be an expression of the high level of inbreeding.

Keywords. *Prunus dulcis* – Self-fertilization – Pollen tube growth – Megagametophyte development – Fruit set – Inbreeding.

Perturbation du développement du mégagamétophyte causée par consanguinité chez l'amandier

Résumé. Dans le cadre d'autofécondations contrôlées, des chutes de fruits ont été observées sur un amandier auto-compatible homozygote possédant un haut niveau de consanguinité et produisant un rendement raisonnable en pollinisation croisée. Le but de ce travail était d'élucider les causes d'avortement de fruits après autofécondation en étudiant la croissance des tubes polliniques, le développement du mégagamétophyte et les fruits issus de pollinisations croisées et d'autofécondations. Pour les deux types de pollinisations, les tubes polliniques ont atteint l'ovaire. Cependant, des différences ont été observées concernant le développement de l'embryon et de l'endosperme. Dans le cas de la pollinisation croisée, l'embryon et l'endosperme se développent, alors que dans la plupart des cas, un embryon dégénérant a été observé pour les ovules auto-pollinisés. Trente jours après la pollinisation, le pourcentage de fruits était semblable dans les deux cas. Au contraire, après 60 jours, le nombre de fruits issus d'autofécondation était très bas ou nul. Pour ce génotype, ces résultats indiquent que le développement incorrect du mégagamétophyte semble être une expression de son haut niveau de consanguinité.

Mots-clés. *Prunus dulcis* – Autofécondation – Croissance des tubes polliniques – Développement du mégagamétophyte – Nouaison des fruits – Consanguinité.

I – Introduction

Despite almond is a self-incompatible species, some self-compatible almond cultivars have been found mainly in Apulia (Italy) (Godini, 2002). In addition, homozygous self-compatible almonds have been experimentally obtained and their agronomic characteristics and self-fertility tested against heterozygous self-compatible ones (Dicenta *et al.*, 2002; Ortega and Dicenta, 2006). No differences between the two classes were observed for the studied characteristics and the very low productivity obtained in both cases was attributed to the inbred origin of the seedlings.

As in other *Prunus* species, almond megagametophyte (or embryo sac) is of the *Polygonum*-type and its developmental stages following compatible and incompatible pollinations have been

described by Pimienta and Polito (1982, 1983). Irregularities in the normal development of the embryo sac, finally resulting in embryo abortion and subsequent fruit drop, have been observed in several *Prunus* species, including almond (Bradbury, 1929; Harrold, 1935; Pimienta and Polito, 1982; Furukawa and Bukovac, 1989; Dittmann and Stösser, 1999). These irregularities include delayed differentiation of the mother cell, lack of polar nuclei fusion, lack of embryo sac elongation and lack of endosperm. The aim of this work was to elucidate the causes of massive fruit drop following selfing in a homozygous self-compatible almond selection by studying pollen tube growth, embryo sac development and fruit set after self- and cross-pollination.

II – Materials and methods

1. Plant material

The homozygous self-compatible selection 'A2-198' was obtained at CEBAS-CSIC almond breeding programme in 1998 by bagging the self-compatible selection 'C1328' (from a cross between the self-compatible cultivars 'Tuono' and 'Genco'). The S genotype of 'A2-198' was determined by IEF of stylar proteins and by PCR of S-RNases as indicated in Ortega and Dicenta (2003).

2. Pollination treatments and *in vitro* pollen germination

Self and cross-pollinations were carried out at flowering time in the fields at Santomera (Murcia, Spain). For each pollination treatment, flower buds at 'D' stage at four different branches were emasculated and hand-pollinated next day with own pollen or pollen from the cultivar 'Ramillete'. Viability of both pollen samples was *in vitro* tested by determining the percentage of germinated pollen grains according to Remy (1953).

3. Pollen tube growth through the pistil

Ninety-six hours following pollinations a sample of 10 pistils was collected and fixed in FAA solution. Later, pistil samples were prepared for fluorescence microscopy observation using the aniline blue staining protocol indicated in Ortega *et al.* (2002). For each pistil, the number of pollen grains germinated on the stigma, the percentage of pollen tubes on each section of the style, and the ovary were determined using an Olympus BH2 microscope under epifluorescence from a UV light-adapted system BH2-RFL-T2, with an Osram HBO 100 W/2 mercury lamp.

4. Embryo sac development

To study the post-fertilization events, a sample of 10 pistils was collected 20 days after pollination from branches of each treatment. The samples were fixed in FAA, and after removing the pericarp, the ovaries were dehydrated by immersion in increasing concentration of TBA. The samples were then embedded in paraffin wax, sectioned at 10 µm and stained according to Gerlach (1969). For each sample, the developmental stage of the ovule (determined by the shape of the embryo sac, presence or absence of a developed embryo, and presence of fused polar nuclei), and the endospermic nuclei were examined using a Leyca DMRB optical microscope.

5. Fruit set

For each pollination treatment the percentage of fruit set was determined on three different branches at 30 and 60 days following pollination, which correspond to initial and final fruit set respectively.

III – Results and discussion

1. Pollen tube growth through the pistil

A high pollen germination rate in the stigma and a continuous decrease of pollen tubes down the pistil was observed for both self and cross-pollination. Finally, a high number of pollen tubes reached the ovary in all cases (Table 1). These results indicate that both pollinations are compatible and suitable to ensure fertilization. In contrast, Alonso and Socias i Company (2005) observed an erratic pollen tube growth of self pollen in seedlings from self-pollination and attributed this to their inbred origin.

Table 1. Mean number of germinated pollen grains on the stigma and percentage of pollen tubes on each section of the style and in the ovary following self and cross-pollination of 'A2-198'

Pistil section	Self-pollination		Cross-pollination	
	Number of pollen grains	Pollen tubes (%)	Number of pollen grains	Pollen tubes (%)
Stigma	52	100	34	100
Style-1	29	56	19	56
Style-2	22	42	17	50
Style-3	17	33	13	38
Ovary	4	8	7	21

2. Embryo sac development

As shown in Fig. 1, morphology of the embryo sac for both pollinations was different from the typical elongated shape described by Pimienta and Polito (1983). This seems to be a consequence of the developmental stage of the ovules prior to pollination. For self-pollination an embryo was observed although unshaped and degenerated, and the spermatic nuclei were not apparent in many cases. For cross-pollination spermatic nuclei were present in most of the samples (Table 2).

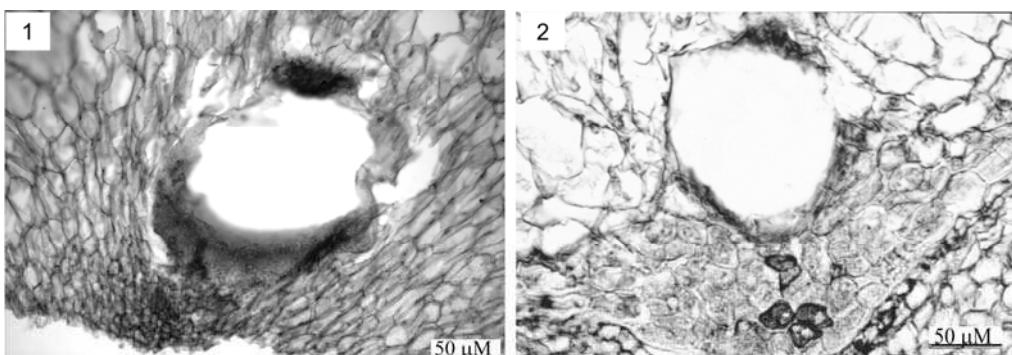


Fig. 1. Ovules of 'A2-198' following (1) cross-pollination and (2) self-pollination, both showing an abnormal structure with non-elongated embryo sac.

Table 2. Description of the embryo sac developmental stages observed by optical microscope in samples of 'A2-198' ovules following each pollination treatment

Sample	Self-pollination	Cross-pollination
1	Polar nuclei starting fusion	Fused polar nuclei
2	Fused polar nuclei	Embryo degenerating
3	Fused polar nuclei	Normal embryo starting divisions
4	Embryo sac not showing spermatic nuclei and degenerating embryo	Spermatic nuclei. No visible embryo
5	Embryo sac not showing spermatic nuclei and degenerating embryo	Normal embryo starting divisions
6	Fused polar nuclei. Degenerating embryo	Embryo degenerating and spermatic nuclei
7	Fused polar nuclei and egg cell	Embryo starting divisions and spermatic nuclei
8	Fused polar nuclei and no visible embryo	Fused polar nuclei
9	Embryo developing and spermatic nuclei	Embryo with small nuclei and spermatic nuclei
10	Embryo sac not showing spermatic nuclei and degenerating embryo	Embryo sac with spermatic nuclei and embryo starting divisions

3. Fruit set

Initial fruit set values were high for both pollinations (data not shown). On the contrary, final fruit set values while still suitable for cross-pollination, were very low or nil for self-pollination (data not shown). This indicates that for self-pollination the development of initially set fruits was disrupted, may be due to non-viable embryos. Oukabli *et al.* (2000) observed a delay in embryo sac development when the self-compatible almond cultivar 'Tuono' was self-pollinated. However, there were no differences for viability of embryos from self and cross-pollination.

Charlesworth and Charlesworth (1987) indicated that early-acting inbreeding depression, implies abortion of zygotes from self-fertilization due to homozygosis of deleterious recessive alleles.

In conclusion, these results indicate that the abnormal development of the megagametophyte in this genotype seems to be an expression of the high level of inbreeding. A more extensive study including additional genotypes and embryo developmental stages, which preliminary results support the findings of the present study, is in progress.

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