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Influence of type of pollen-pistil interaction in pollen tube growth and fruit set

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Abstract. The aim of this work was to study possible differences between fully and half-compatible pollinations for pollen tube growth and fruit set in two self-incompatible almond cultivars. For this, flowers of the cultivars 'Masbovera' and 'Primorskyi' were emasculated and cross-pollinated with pollen of cultivars sharing one allele (half-compatible cross) or any allele (fully-compatible cross). Dynamics of pollen tube in the pistil at different times for each cultivar and pollination type were observed by fluorescence microscopy. In addition, fruit set and fruit drop were determined. Despite the results showed no differences between half- and fully-compatible pollinations for the germinated grains on the stigma, the number of pollen tubes reaching the ovary was significantly higher for the half-compatible pollinations. However, such differences do not seem to have an effect on fruit set and fruit drop.

Keywords. *Prunus dulcis* – Self-incompatibility – Pollen-pistil interaction – Fully-compatible – Half-compatible – Pollen tube growth – Fruit set.

Influence du type d'interaction pollen-pistil sur la croissance du tube pollinique et la nouaison

Résumé. *Le but de cette étude était de connaître les différences possibles entre des pollinisations compatibles et semi-compatibles pour deux variétés d'amandier auto-incompatibles en prenant en compte la croissance des tubes polliniques et la nouaison. Pour cela, les fleurs des variétés 'Masbovera' et 'Primorskyi' ont été émasculées et fécondées avec le pollen de variétés partageant un allèle semi-compatible ou un allèle quelconque de compatibilité. Pour chaque variété et chaque type de pollinisation, la dynamique de croissance des tubes polliniques dans le pistil à différents temps a été observée en microscopie à fluorescence. De plus, la nouaison et la chute des fruits ont été déterminées. Bien que les résultats obtenus ne montrent aucune différence pour le nombre de grains de pollen germés sur le stigmate entre pollinisations semi-compatibles et pleinement compatibles, le nombre de tubes polliniques atteignant l'ovaire était significativement plus élevé pour les pollinisations semi-compatibles. Cependant, de telles différences ne semblent pas avoir d'effet sur la nouaison et la chute des fruits.*

Mots-clés. Prunus dulcis – *Auto-incompatibilité – Interaction pollen-pistil – Pleinement compatibles – Semi-compatibles – Croissance des tubes polliniques – Nouaison.*

I – Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] exhibits self-incompatibility of the gametophytic type. This pre-zygotic barrier implies that those pollen tubes carrying an incompatibility allele expressed in the pistil are stopped in the style by the action of pistil-specific glycoproteins with ribonuclease activity named *S*-RNases (McClure *et al.*, 1989; Bošković *et al.*, 1997). Once pollen grains land on the stigma surface, undergo re-hydrated and germinate almost immediately. In the style, pollen-pistil interactions take place at the extracellular matrix (ECM) of the stylar transmitting tissue, which provides nutrients and chemical signals to guide pollen tubes towards the ovary (Herrero, 2001). Pollen tube growth is a tip-growth that occurs in a discontinuous way by deposition of callose plugs. The ECM is rich in glycoproteins, some with structural roles and others with a direct function in pollen tube growth (Cheung, 1996). However, during this period pollen tube growth seems to be mainly controlled by the incompatibility

interaction. Pollen tube dynamics through the pistil after self- and cross-pollination have been studied in self-compatible almonds by Ortega *et al.* (2002). Their results revealed the importance of mechanisms other than incompatibility in the control of pollen tube growth. However, studies comparing pollen tube growth and fruit set following half and fully-compatible interactions in self-incompatible almonds are scarce. The aim of this study was to determine the efficiency of fully and half-compatible pollinations for pollen tube growth and fruit set in two self-incompatible almond cultivars.

II – Materials and methods

1. Plant material

The self-incompatible almond cultivars 'Masbovera' (S_1S_9) and 'Primosrkyi' (S_5S_9) were cross-pollinated with pollen from cultivars sharing one *S* allele (half-compatible interaction, HC) or any *S* allele (fully-compatible interaction, FC) (Table 1).

2. Pollen tube growth through the pistil

Flowers buds at 'D' stage from 'Masbovera' and 'Primorskyi' were collected from trees placed at Santomera (Murcia, Spain). The flowers were emasculated and, for each cultivar, a sample of 40 pistils for each cross was placed on trays with wet floral foam. Additionally, for each cultivar 40 more pistils were self-pollinated. One day later (coinciding with anthesis) the pistils were self or cross-pollinated by hand using a paintbrush. For each cross, a sample of 10 pistils was collected and fixed in FAA solution 24, 48, 72 and 96 hours after pollination, and later prepared for fluorescence microscopy as indicated in Ortega *et al.* (2002). For each pistil, the number of pollen grains germinated on the stigma, the number of pollen tubes in the ovary and the percentage of penetrated ovaries 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.

3. Fruit set

The indicated crosses were also made in the fields. For each cross 50 flower buds at 'D' stage were emasculated and pollinated by hand, and the percentage of fruit set was determined at 30 and 60 days following pollinations, which correspond to initial and final fruit set respectively.

4. Data analysis

For data analysis, pollen tubes in the ovary were expressed as a percentage of the number of germinated pollen grains in the stigma. Pistils with less than 10 germinated pollen grains were excluded from the analysis. To determine the differences between the two compatible interactions, data were analysed by the General Linear Model procedure using SAS software (SAS Institute, 1989). Percentage values were previously transformed by calculating the arc sin value of the square root. Mean values were analysed by Duncan's multiple range test.

III – Results and discussion

As expected, no pollen tubes were observed in the ovary following self-pollinations, thus confirming the self-incompatible status of the studied cultivars (Table 1). Likewise, fruit set was nil for self-pollinations (Table 2).

For 'Masbovera', in general, no differences between HC and FC interactions were observed for the percentage of germinated pollen grains in the stigma (Table 1). The differences between

both interactions at 48 h could be a consequence of the variability of the character. For the number and percentage of pollen tubes in the ovary differences were only observed at 96 h, the values being higher for the HC interaction. This seems to be due to the remarkably high number of pollen tubes reaching the ovary for the pollinators 'Cristomorto', 'Ferragnès' and 'Glorieta'. For the HC pollinations with 'Marta' and 'Primorskyi' the same number of pollen tubes than in the case of FC pollinations with 'IXL' and 'Ramillete' were observed.

Table 1. Number of pollen grains germinated on the stigma, number and percentage of pollen tubes in the ovary for each cultivar and type of interaction (half- and fully-compatible), and for self-pollination. Different letters indicate significant differences (shadowed in grey) between interactions at each time, according to Duncan's multiple range test (*P*=0.05)

♀	♂	Stigma				Ovary				Ovary (%)			
		24	48	72	96	24	48	72	96	24	48	72	96
'Masbovera' (S_1S_9)	'IXL' (S_7S_8)	43	76	60	49	0	3	6	8	0	3	9	17
	'Ramillete' (S_6S_{23})	70	92	93	77	0	1	6	5	0	1	7	7
	Full-compatible	54	84a	78	63	0	2	6	6b	0	2	8	12b
	'Cristomorto' (S_1S_2)	43	60	89	71	0	2	7	13	0	3	8	19
	'Ferragnès' (S_1S_3)	44	81	71	65	0	3	5	10	0	3	8	18
	'Glorieta' (S_1S_5)	80	78	66	54	0	2	10	12	0	3	18	23
	'Marta' (S_1S_f)	68	73	74	63	0	1	6	8	0	1	8	13
	'Primorskyi' (S_5S_9)	24	59	70	15	3	4	8	5	15	13	13	39
	Half-compatible	52	70b	74	59	1	3	7	10a	3	5	11	20a
'Primorskyi' (S_5S_9)	'Desmayo L.' (S_1S_{10})	65	69	66	56	0	0	1	3	0	0	2	5
	'IXL' (S_7S_8)	71	78	64	63	0	0	1	3	0	0	3	5
	'Ramillete' (S_6S_{23})	89	63	72	69	0	0	1	2	0	0	1	3
	Full-compatible	75	71b	67b	63b	0b	0b	1b	3b	0b	0b	2b	5b
	'Glorieta' (S_1S_5)	79	97	99	85	3	2	5	5	4	2	5	7
	'Masbovera' (S_1S_9)	90	78	72	71	5	4	5	6	5	7	7	9
	Half-compatible	85	87a	86a	78a	4a	3a	5a	5a	5a	5a	6a	8a
Total	Full-compatible	67	76	76	63	0b	1b	3b	4b	0b	1b	4b	8b
Total	Half-compatible	62	75	72	65	2a	3a	7a	9a	3a	5a	9a	16a
Self-pollination													
'Masbovera' (S_1S_9)	'Masbovera' (S_1S_9)	57	77	93	78	0	0	0	0	0	0	0	0
'Primorskyi' (S_5S_9)	'Primorskyi' (S_5S_9)	55	29	50	23	0	0	0	0	0	0	0	0

For 'Primorskyi', significantly higher values were observed for the germinated pollen grains in the stigma in the case of HC pollinations. Pollen tubes from HC pollinators reached the ovary shortly after pollination.

Considering data from both cultivars jointly, no differences were observed for the number of germinated pollen grains for HC and FC interactions. However, there were significant differences for the number and percentage of pollen tubes in the ovary, the values being higher for the HC interaction. Significant differences were not observed between both HC and FC interactions for fruit set and fruit drop, either when considering data from each cultivar

separately nor jointly. These results somehow contrast with those of Ortega *et al.* (2002) of a significantly higher number of pollen tubes in the upper part of the style for FC interaction, and no differences at the base of the style. This seems to indicate that there is an influence of the female genotype in the interaction, since the cultivars studied here are different from those studied by Ortega *et al.* (2002). On this regard, Hormaza and Herrero (1999) came to the same conclusion in sweet cherry, and indicated that these differences could be related to the nutritive support offered by the different female genotypes.

Table 2. Number of pollinated flowers, percentage of initial and final fruit set and percentage of fruit drop for each cultivar and type of interaction (half- and fully-compatible), and for self-pollination. Different letters indicate significant differences between interactions at each time, according to Duncan's multiple range test (*P*=0.05)

♀	♂	Number of flowers	Initial Fruit Set (%)	Final Fruit Set (%)	Fruit Drop (%)
'Masbovera' (S_1S_9)	'IXL' (S_7S_8)	50	42	40	5
	'Ramillete' (S_6S_{23})	46	54	54	0
	Full-compatible	48	48a	47a	3a
	'Cristomorto' (S_1S_2)	45	47	40	14
	'Ferragnès' (S_1S_3)	56	46	43	8
	'Glorieta' (S_1S_5)	70	51	47	8
	'Marta' (S_1S_f)	47	40	36	11
	'Primorskyi' (S_5S_9)	41	39	39	0
	Half-compatible	52	45a	41a	8a
'Primorskyi' (S_5S_9)	'Desmayo' (S_1S_{10})	53	30	26	13
	'IXL' (S_7S_8)	51	25	24	8
	'Ramillete' (S_6S_{23})	41	27	22	18
	Full-compatible	48	27a	24a	13a
	'Glorieta' (S_1S_5)	45	27	22	17
	'Masbovera' (S_1S_9)	50	36	36	0
	Half-compatible	48	32a	29a	9a
Total	Full-compatible	48	36a	33a	9a
Total	Half-compatible	51	41a	38a	8a
Self-pollination					
'Masbovera' (S_1S_9)	'Masbovera' (S_1S_9)	49	0	0	0
'Primorskyi' (S_5S_9)	'Primorskyi' (S_5S_9)	53	0	0	0

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References

Bošković R., Tobutt K.R., Batlle I. and Duval H., 1997. Correlation of ribonuclease zymograms and incompatibility genotypes in almond. In: *Euphytica*, 97, p. 167-176.

Cheung A., 1996. Pollen-pistil interactions during pollen-tube growth. In: *Trends Plant Sci*., 1, p. 45-51.

Herrero M., 2001. Ovary signals for directional pollen tube growth. In: *Sex. Plant Reprod.*, 14, p. 3-7.

Hormaza J.I. and Herrero M., 1999. Pollen performance as affected by the pistilar genotype in sweet cherry (*Prunus avium* L.). In: *Protoplasma*, 208, p. 129-135.

McClure B.A., Haring V., Ebert P.R., Anderson M.A., Simpson R.J., Sakiyama F. and Clarke A.E., 1989. Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases. In: *Nature*, 342, p. 955-957.

Ortega E., Egea J., Cánovas J.A. and Dicenta F., 2002. Pollen tube dynamics following half- and fully-compatible pollinations in self-compatible almond cultivars. In: *Sex. Plant Reprod.*, 15, p. 47-51.

SAS Institute, 1989. *SAS/STAT User's Guide*. Caey, N.C.: SAS Institute.