Study of Pollen-Pistil Interactions on Crosses between 'Rocha' Pear and Potential Pollinators

L. Silva
APAS
2550 - 458 Peral
Cadaval
Portugal

J. Sanzol
Departamento de Fruticultura
CITA-DGA
Zaragoza
Spain

M. Herrero
Departamento de Pomologia
EE Aula Dei-CSIC
Zaragoza
Spain

C.M. Oliveira
Instituto Superior de Agronomia
Technical University of Lisbon
Lisbon
Portugal

Keywords: gametophytic self-incompatibility, pollen tube, pollinators, 'Rocha' pear

Abstract

Currently, 'Rocha' pear production relies mainly on spraying with growth regulators, as this cultivar can set fruit parthenocarpically. The identification of suitable pollinators and cross pollination is of the most important for the development of alternatives, which are safer to consumers and to the environment. Observations of pollen-pistil interactions between the cv. Rocha and potential pollinators were carried out through fluorescence microscopy. Crosses with 'Aurora' and 'Limonera' and selected 'Rocha' clones, Clone 1, Clone 2, Clone 4c, Clone Z and Clone T behaved as incompatible. But, 'Beurré Precoce Morettini', 'Doyenné du Comice', 'Passe Crassane', 'Clapp's Favourite', 'General Leclerc', 'Beurré d'Avril', 'Coscia', 'Tosca', 'Carapinheira', 'Amêndoa' and 'Água' showed themselves to be compatible with 'Rocha'. The percentage of styles with pollen tubes at the base of the style varied between 0 and 4% in the incompatible crosses and between 61 and 96% in the compatible crosses. The number of pollen tubes registered per flower varied between 0–0.04, in the first case, and 4.1–8.9 in the latter. These results analysed in combination with the S genotypes for these cultivars showed that crosses between cultivars either sharing a common allele or with two different alleles were indistinguishable in terms of pollen tube growth in the style and presumably this can explain why other authors have not found differences in fruit set. These results also confirm previous work conducted by other groups on the molecular identification of the S-alleles coding for the S-RNases involved in the self-incompatibility mechanism.

INTRODUCTION

Most pear (*Pyrus communis*) cultivars have been classified as completely or almost completely self-incompatible (Crane and Lewis, 1942; Modlibowska, 1945). Others could be partially self-compatible, depending on the environment (Lewis and Modlibowska, 1942; Griggs and Iwakiri, 1954; Nyeki et al., 2000; Tassinari et al., 2004). Under suitable conditions of temperature and high pollen density on the stigma, Medeira and Avelar (1998) have observed pollen tubes in the ovary of 'Rocha' four days after self-pollination. The evidence suggests that, in these conditions, fertile flowers are able to yield seeded fruits in the present cultivar. Moreover, cv. Rocha can set fruit parthenocarpically either naturally or as a result of hormone spraying (Silva et al., 2005). Nevertheless, the existence of inter-compatible cultivars as pollinizers in orchards assures the production of stable economic crops, with production of seeded fruits.

Self-incompatibility is controlled in the family Rosaceae by a homomorphic, gametophytic, monofactorial, multiallelic incompatibility system and pollen rejection occurs in the style. The incompatibility locus controls self-incompatibility as well as

intra-specific cross-incompatibility (De Nettancourt, 2001). Knowledge of S-alleles has become critical for establishing cross-incompatibilities between cultivars. Only recently molecular techniques have started to be used for the clarification of S-genotype constitutions for example, through PCR-based methods (Tassinari et al., 2001). Recent work has established that the S-genotype is *SaSi* for the cultivar 'Rocha' (Mota and Oliveira, 2005). Also, the S-genotype of potential pollinators for the cv. Rocha and of different selected clones has also been established.

However, the assignment of S-genotypes to molecular patterns needs to be verified by an efficient *in vivo* test. The evaluation of pollen tube penetration into the ovule was established as a reliable method in pear to determine pollen-pistil incompatibility (Sanzol and Herrero, 2002).

In this work cross-compatibility in 'Rocha' pear with potential pollinators and some selected clones of 'Rocha' was studied by examining pollen tube growth, through fluorescence microscopy. The objective was to select the best pollinators to cv. Rocha.

MATERIALS AND METHODS

Pear trees (*Pyrus communis* L. cv. Rocha) grafted on quince rootstocks and located in experimental orchards at APAS (Associação Produtores Agrícolas da Sobrena) and ENFVN (Estação Nacional de Fruticultura Vieira Natividade) located in the Oeste region were used. Buds with flowers at the balloon stage were tagged and emasculated by removing the petals and anthers with tweezers to avoid insect visits and self-pollination (Free, 1964). Flowers were hand pollinated two days after emasculation with self-pollen and pollen from cultivars 'Aurora', 'Dr. Jules Guyot' ('Limonera'), 'Beurré Precocoe Morettini', 'Doyenné du Comice', 'Passe Crassane', 'Clapp's Favourite', 'General Leclerc', 'Beurré d'Avril', 'Coscia', 'Tosca', 'Carapineira', Amêndoa and 'Água' and with selected 'Rocha' clones, Clone 1, Clone 2, Clone 4c, Clone Z and Clone T.

Pollen from each male parent cultivar and clone was obtained from flowers at the balloon stage by removing the anthers and placing them at room temperature on a petri dish during 48 hours. Controlled pollinations were then carried out using a small brush and using the corresponding pollen from each male parent.

Five days after pollination 20 flowers were collected and fixed in FAA (formalin: acetic acid: ethanol 70%) (1:1:18) (Johansen, 1940). Following fixation, pistils were washed with distilled water three times, for 1 h each and left overnight in 5% sodium sulfite. On the following day, they were autoclaved for 10 min at 1 kg/cm² (Jefferies and Belcher, 1974) and mounted in squash preparations with 0.1% aniline blue in 0.1 N K₂PO₄ (Linskens and Esser, 1957). This allows the detection of pollen tubes through the staining of their callose (Currier, 1957). Preparations were viewed under an Ortholux II microscope with UV epifluorescence using BP 355-425 exciter filter and LP 460 barrier filter. Pollen germination and pollen tube growth were monitored under the microscope. The distance travelled by the longest pollen tube was recorded and cross-compatibility was evaluated as the capacity of pollen tube to reach the base of the style.

RESULTS

Pollen tubes exhibited different performances depending on the cross (Tables 1 and 2). Self-pollination was performed and also cross-pollination of 'Rocha' with two cultivars with the same incompatibility alleles ('Aurora' and 'Limonera') (Mota and Oliveira, 2005; Takasaki et al., 2006). In these crosses pollen tube growth stopped at around 50% of the length of the style and practically no pollen tubes were observed at the base of the style.

In the other crosses performed with semi and totally compatible cultivars it was observed that pollen tubes grew the entire length of the style. The percentage of styles with pollen tubes in the base varied between 61 and 96% (Table 1). Considering that only one ovule of a flower needs to be fertilized to have fruit set (Sanzol, 2001), when we express this data as the percentage of flowers with at least one style with pollen tubes in the base it varied from 82 to 100%.

The number of pollen tubes in the base of the style was close to zero in incompatible crosses, showing homogeneity between crosses, while values for cross-compatible pollination were significantly higher presenting a large heterogeneity between pollinators (Table 1); this is similar to the results reported in other pear cultivars (Sanzol and Herrero, 2002).

All crosses performed between different selected clones of cultivar 'Rocha' proved to be inter-incompatible, although some pollen tubes were observed in the base of the style, which varied between 0.00 and 0.04 (Table 2). The percentage of styles with pollen tubes in the base varied between 0 and 4% (Table 2), and the percentage of flowers with at least one style with pollen tubes in the base varied from 0 to 20%.

DISCUSSION

The self-incompatibility of cv. Rocha and the inter-incompatibility between its selected clones has been confirmed in this work. The influence that many physiological and environmental factors, which operate modifying sterility and parthenocarpy, and the fact that self-incompatibility in *Pyrus* is not so sharply defined as in other genera of Rosaceae such as *Prunus* (Crane and Lewis, 1942; Zhang and Hiratsuka, 2000), may be a possible explanation for the results obtained in self-pollination and inter-pollination tests between selected clones of 'Rocha'.

Although in studies conducted in other pear cultivars, the observations of pollen tube growth in the style were not as sharply defined for the determination of pollen-pistil incompatibility as the observations of the presence of pollen tubes in the ovule (Sanzol and Herrero, 2002), in the present study the differences between compatible (61 to 96%) and incompatible (0 to 4%) crosses were clear, expressed as the percentage of styles with pollen tubes in the base.

Similar to the results observed in other studies on pear (Sanzol and Herrero, 2002), the homogeneity among crosses concerning the number of pollen tubes reaching the base of the style in incompatible crosses and the variability of this value observed for cross-compatible pollinations could be explained by the fact that the pollen tube numbers in the style base, in an incompatible mating, is restricted by the rejection system itself. On the other hand, since pollen tube growth in the style is heterotrophic at the expenses of the stylar reserves (Herrero and Dickinson, 1979), the pollen tube number in compatible crosses seems to be restricted by the potentiality of the female to support the growth of a certain population of pollen tubes and also by the pollen-pistil interaction. Differences between crosses in the number of pollen tubes reaching the base of the style have also been observed in other species, such as cherry (Hormaza and Herrero, 1996).

All the compatible crosses obtained can be used as pollinators of cv. Rocha. The cv. Passe Crassane hasn't been used lately due its high susceptibility to fire blight. 'Tosca' is a new introduction, and information about its flowering dates in comparison to 'Rocha' is still lacking. However, for the other cultivars and taking into account flowering dates (Sousa, pers. commun.; Silva, 2001), the cultivars 'Beurré Precoce Morettini', 'Água', 'Amêndoa', 'Coscia', 'Carapinheira', 'Beurré d'Avril' and 'Clapp's Favourite' cover the first part of flowering, whilst cvs. Doyenné du Comice and General Leclerc cover the final part of 'Rocha' flowering.

ACKNOWLEDGMENTS

The authors would like to thank the Director of ENFVN Rui de Sousa for facilities and to Reyes Lopez for technical support. This work was financially supported by CRUP - Acção Integrada Luso-Espanhola E-57/06 and project grants CICYT AGL2006-13529-C02-00/AGR) and by Grupo consolidado de Aragón A-43.

Literature Cited

- Crane, M.B. and Lewis, D. 1942. Genetical studies in pears III. Incompatibility and sterility. *J. Genet.* 43:31-44.
Currier, H.B. 1957. Callose substance in plant cells. *Amer. J. Botany* 44:478-488.

- Free, J.B. 1964. Comparison of the importance of insect and wind pollination of apple trees. *Nature* 201:726–727.
- Griggs, W.H. and Iwakiri, B.T. 1954. Pollination and parthenocarp in the production of Bartlett pears in California. *Hilgardia* 22:643–678.
- Jefferies, C.J. and Belcher, A.R. 1974. A fluorescent brightener for pollen tube identification in vivo. *Stain Technology* 49:199–202.
- Johansen, D.A. 1940. *Plant Microtechnique*. In: Ed. McGraw-Hill, New York.
- Herrero, M. and Dickinson, H.G. 1979. Pollen-pistil incompatibility in *Petunia hybrida*: changes in the pistil following compatible and incompatible intraspecific crosses. *J. Cell Sci.* 36:1–18.
- Hormaza, J.I. and Herrero, M. 1996. Dynamics of pollen tube growth under different competition regimes. *Sex Plant Reprod.* 9:153–160.
- Lewis, D. and Modlibowska, I. 1942. Genetical studies in pears. IV. Pollen-tube growth and incompatibility. *J. Genet.* 43:211–222.
- Linskens, H.F. and Esser, K. 1957. Über eine spezifische Anfärbung der Pollen-schläuche und die Zähl Kallosaproten nach selbstung und fremdung. *Naturwiss* 44:16.
- De Nettancourt, D. 2001. Incompatibility and incongruity in wild and cultivated plants. Springer, Berlin, Heidelberg, New York.
- Medeira, M.C. and Avelar, J.S.H. 1998. Polinização cruzada na pereira 'Rocha'. *Agronomia Lusitana* 43:99–106.
- Modlibowska, I. 1945. Pollen tube growth and embryo-sac development in apples and pears. *J. Pomol.* 21:57–89.
- Mota, M. and Oliveira, C.M. 2005. Identificação de alelos S na pereira 'Rocha' e determinação da compatibilidade entre cultivares. *Actas de Horticultura do V Congresso Ibérico de Ciências Horticolas, IV Congresso Iberoamericano de Ciências Horticolas*, Porto, Maio de 2005. 2:232–238.
- Nyeki, J., Soltesz, M. and Ivanciscs, J. 2000. Self-fertility of pear varieties conditioned by natural self-pollination (autogamy). *Int. J. Hort. Sci.* 6:110–113.
- Rodrigo, J. and Herrero, M. 1996. Evaluation of pollination as the cause of erratic fruit set in apricot 'Monique'. *J. Hort. Sci.* 71:801–805.
- Sanzol, J. 2001. Mecanismos reproductivos que regulan la fructificación en peral (*Pyrus communis* L.) cv. agua de Aranjuez. Tesis Doctoral Universidad Publica de Navarra. Departamento de Producción Agraria.
- Sanzol, J. and Herrero, M. 2002. Identification of self-incompatibility alleles in pear cultivars (*Pyrus communis* L.). *Euphytica* 128:325–331.
- Silva, A. 2001. Capítulo VII. Polinização. p.137–166. In: O livro da pera Rocha. Volume Primeiro. ANP.
- Silva, J.M., Barba, N.G., Barros, M.T. and Torres-Paulo, A. 2005. 'Rocha', The pear from Portugal. *Acta Hort.* 671:219–221.
- Takasaki, T., Moriya, Y., Okada, K., Yamamoto, K., Iwanami, H., Bessho, H. and Nakanishi T. 2006. cDNA cloning of nine S alleles and establishment of a PCR-RFLP system for genotyping European pear cultivars. *Theor. Appl. Genet.* 112:1543–1552.
- Tassinari, P., Zucchereli, S. and Sansavini, S. 2001. Contributo allo studio delle basi biologico-molecolari dell'incompatibilità gametofitica del pero (*Pyrus communis* L.). *Riv. Frutt. Ortoflora* 6:81–86.
- Tassinari, P., Zuccherelli, S. and Sansavini, S. 2004. Self-pollination and fertility in european pear (*Pyrus communis* L.) cultivars. *Acta Hort.* 663:677–680.
- Zhang, S. and Hiratsuka, S. 2000. Cultivar and development differences in S-protein concentration and self-incompatibility in the Japanese pear. *HortSci.* 35:917–920.

Tables

Table 1. Pollen tube performance. Pollen tube growth in cross-pollinations and self-pollination.

Pollinated cultivar	Pollinator cultivar	Styles (%) ^a	Flowers (%) ^b	Longest pollen tube ^c	Number pollen tube ^d
Rocha	Carapinheira	87	100	100	7.2
	Morettini	90	100	100	7.1
	Passe Crassane	88	100	100	6.8
	Doyenné du Comice	87	100	100	7.1
	Clapp's Favorite	93	100	100	7.4
	Coscia	84	90	100	5.7
	General Leclerc	96	100	100	8.9
	Beurré d'Avril	86	100	100	6.5
	Tosca	83	100	100	6.9
	Amêndoa	83	100	100	5.9
	Água	61	82	100	4.1
	Dr. Jules Guyot	0	0	50	0.0
	Aurora	0	0	50	0.0
	Rocha	1	4	100	0.0

^aPercentage of styles with pollen tubes reaching the base of the style.

^bPercentage of flowers with at least one style with pollen tubes reaching the base of the style.

^cMean length of the longest tube in the styles expressed as percentage of the style length.

^dMean number of pollen tubes at the base of the style.

Table 2. Pollen tube performance. Pollen tube growth in cross-pollinations between different clones of 'Rocha'.

Pollinated clone	Pollinator clone	Styles (%) ^a	Flowers (%) ^b	Longest pollen tube ^c	Number pollen tube ^d
Rocha	Clone 1	2	10	100	0.04
	Clone 2	4	18	100	0.04
	Clone 4c	0	0	75	0.00
	Clone Z	2	10	100	0.02
	Clone T	2	10	100	0.02
Clone 1	Rocha	0	0	75	0.00
Clone 2	Rocha	2	9	100	0.04
Clone 4c	Rocha	4	20	100	0.04
Clone 2	Clone 4c	0	0	50	0.00

^aPercentage of styles with pollen tubes reaching the base of the style.

^bPercentage of flowers with at least one style with pollen tubes reaching the base of the style.

^cMean length of the longest tube in the styles expressed as percentage of the style length.

^dMean number of pollen tubes at the base of the style.