UDC 575:630
DOI: 10.2298/GENSR1402411D
Original scientific paper

INCOMPATIBLE POLLEN TUBES IN THE PLUM STYLE AND THEIR IMPACT ON FERTILIZATION SUCCESS

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Đorđević M., R. Cerović, S. Radičević, D. Nikolić (2014): *Incompatible pollen tubes in the plum style and their impact on fertilization success-*. Genetika, Vol 46, No. 2, 411-418.

The pistils of plum (*Prunus domestica* L.) cv. 'Čačanska Lepotica' were self- and cross-pollinated in order to investigate occurrence of incompatible pollen tubes in the style, and their impact on fertilization success. The investigation also included open pollination variant. The highest percentage of these stopped the growth in the upper third of the style. Under cross-pollination variant, 15.4% and 12.1% of pollen tubes observed in the upper part of the style in the first and the second years of study, respectively, were found incompatible. In view of the above parameters, in the self-pollination variant, 15.0% and 17.0% of pollen tubes were found incompatible by years. As for the open pollination, percentages of incompatible pollen tubes in the upper part of the style by years were 14.0% and 14.4%, respectively. The occurrence of incompatible pollen tubes did not influence the fertilization success in these pollination variants.

Key words: fluorescence microscopy, incompatible pollen tubes, *Prunus domestica*

INTRODUCTION

Self-incompatibility (SI) is one of the most significant mechanisms in plants which prevents self-fertilization (and inbreeding accordingly), and hence promotes cross-pollination (FRANKLING-TONG and FRANKLIN, 2003). SI has been classified into heteromorphic and homomorphic types. The SI reaction in the heteromorphic type is associated with flower morphology. In the homomorphic SI type, an incompatible reaction occurs regardless of the flower morphology. The homomorphic SI is divided into the sporophytic (SSI) and gametophytic

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(GSI) types, depending on how the pollen *S* phenotype is controlled. In the sporophytic type, pollen *S* phenotype is determined by the *S* genotype of the plant that produces pollen, while in the gametophytic type, pollen *S* phenotype is determined by the *S* genotype of pollen itself (RYUTARO and IEZZONI, 2010).

In GSI, pollen tubes most commonly terminate their growth in particular parts part of the style, which varies among species and is governed by different internal or external factors; it also occurs at the stigma surface (in some grass species) and in the ovary (in *Theobroma cacao* (FRANKLING-TONG and FRANKLIN, 2003). The *Rosaceae*, *Solanacae*, and *Plantaginaceae* share the same gametophytic self-incompatibility (GSI) mechanism (MC CLURE, 2004). During the last two decades, genes for the two proteins controlling the allele specificity of GSI recognition in *Prunus* have been identified. It is now known that two separate genes at the *S* locus control male (pollen) and female (pistil) specificities. Ribonuclease (RNase) and F-box genes were identified as the pistil *S* and pollen *S* determinant genes (RYUTARO and IEZZONI, 2010). In plum (*Prunus domestica* L.), identical allelic formulae for two cultivars are very unlikely owing to the 3 component-genome (D_1D_2C), where each genome has a single gene control, and each of the three genes contains multiple alleles (BOTU *et al*., 2002).

The fluorescence microscopy method including staining with aniline blue clearly distinguishing compatible from incompatible pollen tubes (NIKOLIĆ and MILATOVIĆ, 2010). The walls of the pollen tubes contain callose (β-1,3- glucan) which binds with aniline blue during staining; when lighted with ultraviolet light, they become fluorescent. Using the fluorescence microscopy method, NIKOLIĆ and MILATOVIĆ (2010) examined self-compatibility in 18 cultivars of the European plum and classified them into two groups: self-compatible and self-incompatible.

The aim of this work was to examine occurrence and characteristics of incompatible pollen tubes in the style of plum (*Prunus domestica* L.) under three pollination variants: open pollination, cross-pollination and self-pollination, in order to examine its impact on fertilization success.

MATERIALS AND METHODS

Plant material and experiment design

Plum cultivar 'Čačanska Lepotica' was the model plant for the investigation. A two years' trial was set up in the eight year old orchard of 'Čačanska Lepotica' planted at the site of Fruit Research Institute, Čačak. The orchard was establishied with one-year old virus-free plants, planting density being 4 × 1 m. The cultivar was grafted on *Prunus cerasifera* L. seedling rootstock. The experiment was set up as a randomized block design, in three replications with three trees each (nine trees in total).

Plum cv 'Čačanska Lepotica' was developed at Fruit Research Institute Čačak, from the cross of 'Wangenheims Frühzwetsche' × 'Požegača' (MILENKOVIĆ *et al*., 2006). Plum cv 'Čačanska Najbolja', used as polleniser in experiment, resulted from the same cross (MILENKOVIĆ *et al*., 2006).

Pollination and pistil fixation

Two-year old branches of 'Čačanska Lepotica' were chosen having a synchronized population of flowers at the late balloon stage, whereas all the other flowers were removed. All the selected branches had about 20-30 flowers. In this way, about 450 flowers were chosen, each replication involving 150 flowers from all sides of three plum trees. The single-pistil flowers were emasculated and protected with polythene bags to prevent uncontrolled pollination.

Pollination treatments in the period of full bloom included: (a) pollen from the same cultivar to test self-compatibility; (b) pollen from a different cultivar ('Čačanska Najbolja') to test cross-compatibility, each treatment including 70-80 flowers. Manual pollination was performed once for each flower by brushing dehiscing anthers against the receiving stigmas until pollen could be seen on the stigma surface. Upon the pollination, pollinated pistils were subjected to the triple successive fixation – 72, 144, 240 hours. Pistils were fixed in FPA solution (70% ethyl alcohol:40% formaldehyde:propionic acid in a 90:5:5 mixture) and stored at 4ºC until use. Concurrently with the flower fixation under cross- and self-pollination, randomly chosen flowers of cv 'Čačanska Lepotica' were subjected to fixation for the purpose of the study of pollen tubes growth in the style under the conditions of open pollination.

Staining and microscopic observation of pistils

Aniline blue staining was used for the study of pollen tube growth in the style (KHO and BAËR, 1971; PREIL 1970). To prepare pistils for microscopic examination, the style was separated from the ovary, divided longitudinally and squashed. Pollen tubes in the style and the ovary were observed under ultraviolet (UV) light, on the research microscope Olympus BX61, Japan. For the incompatible pollen tubes the percentage was based on the cumulative value identified in all fixation periods (terms). To examine quantitative parameters of pollen tubes growth per treatment and per each fixation term, fifteen samples were tested approximately. The following parameters were determined: the number of pollen tubes in the upper third of the style, the number of pollen tubes penetrating the ovary; the region of the termination of the longest pollen tube in the style – the upper, middle or lower third of the style, and penetration of pollen tubes into the ovary loculi, mycropila or nucellus. The percentage of incompatible pollen tubes was calculated in regard to the average number of pollen tubes in the upper third of the style.

Statistical analysis

Means and standard errors were calculated for the measured parameters. The data were statistically analyzed using two-factor analysis of variance (ANOVA). The significance of differences among mean values was determined by Duncan's multiple range test at $P \leq 0.05$. Data analysis was performed using the SPSS statistical software package, Version 8.0 for Windows (SPSS. Inc., Chicago, IL).

RESULTS

Quantitative parameters of pollen tubes growth efficacy.

Pollination treatment did not have a statistically significant effect on the number of pollen tubes in the style and ovary (Table 1). The impact of year as the variability factor, as well as interaction among the factors, significantly influenced the number of pollen tubes in the upper part of the style, while in the ovary this impact was not significant. However, the highest average number of pollen tubes in the style and ovary was registered in the cross-pollination variant (51.32 and 4.13, respectively), while the lowest was registered in the self-pollination variant (47.0 and 3.3, respectively). The influence of year was the strongest in the self-pollination variant (Tab. 1).

Table 1. Average number of pollen tubes in the upper third of the style and in the ovary

Treatment			Upper third of the style	Ovary
A	Open pollination		49.23 ± 4.26 a*	3.83 ± 0.30 a*
	'Čačanska Najbolja'		51.32 ± 4.66 a	4.13 ± 0.33 a
	'Čačanska Lepotica'		47.02 ± 5.77 a	3.26 ± 0.11 a
B	I year		47.37 ± 5.08 b	3.53 ± 0.29 a
	II year		53.02 ± 4.71 a	3.95 ± 0.20 a
A × B	Open pollination	I year	50.58 ± 4.73 a	4.40 ± 0.36 a
		II year	47.87 ± 3.79 a	3.27 ± 0.24 a
	'Čačanska Najbolja'	I year	49.57 ± 6.20 a	3.42 ± 0.39 a
		II year	53.10 ± 3.02 a	4.83 ± 0.26 a
	'Čačanska Lepotica'	I year	35.95 ± 4.21 ab	2.77 ± 0.11 a
		II year	58.03 ± 7.33 a	3.75 ± 0.10 a

**Mean values followed by the different lower-case letters in the column represent significant differences at P ≤ 0.05 according to Duncan's multiple range test*

In all the variants, an increasing number of pistils with ovaries penetrated by pollen tubes was observed, reaching its maximum on the tenth day after pollination (Fig. 1). It is noticeable that in both years of study, on the third and sixth days after pollination, the dynamics of pollen tubes growth is the most favourable in the open-pollination variant. In both years, on the tenth day after pollination, pollen tubes were observed to penetrate the nucellus in 70% of pistils. In the cross-pollination variant from days 6–10, dynamics of pollen tubes growth is more favourable in the second year of study where in 100% of pistils pollen tubes penetrate the nucellus. As for the self-pollination variant, on the tenth day after pollination in both years of the study, pollen tubes terminated their growth in the nucellus in 80% and 75% of pistils, respectively (Figure 1).

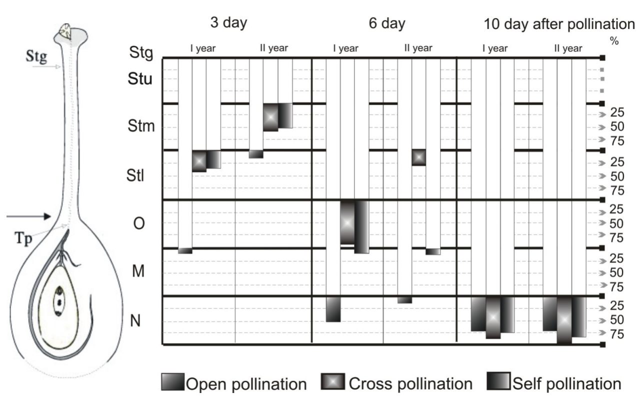

Figure 1.Dynamics of pollen tubes growth in certain parts of the pistils: Stg- stigma; Stu, Stm, Stl– upper, middle and lower part of the style; O – obturator; M – mycropile; N – nucellus

Incompatible pollen tubes. The fluorescence microscopy method enables relatively fast and efficient determination of incompatible pollen tubes in the style. In all the pollination variants, some pollen tubes in the style showed typical signs of incompatibility. The majority of pollen tubes with the cytological appearance of incompatibility were observed in the upper third of the style. These pollen tubes were with the formation of the characteristic swelling at the tip, with more or less fluorescence (Fig. 2a, b).

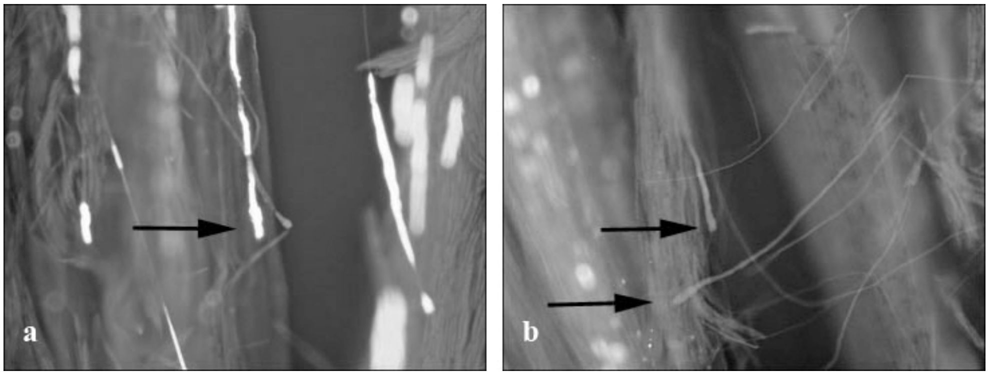

Figure 2a, b. Incompatible pollen tubes in the upper third of the style (magnified 200x). Typically swollen tips of pollen tubes (black arrows) that have ended incompatibly in the style

In some cases, fluorescence was not observed at the tips of incompatible pollen tubes, and they additionally had different morphological forms (Fig.3a, b).

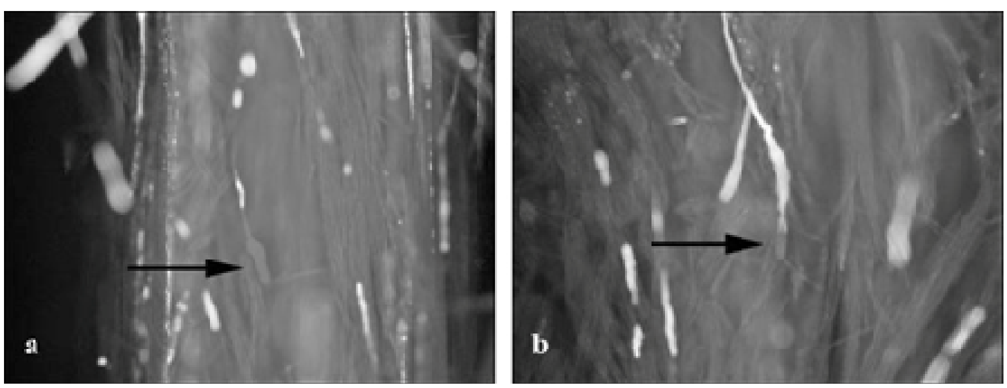

Figure 3a, b: Different morphological forms of incompatible pollen tubes (magnified 200x)

In several cases, the short pollen tubes, rather thick and wider at the tip were observed; they also showed intense fluorescence. The growth of these pollen tubes ended immediatelly after penetrating into style tissue (Fig. 4).

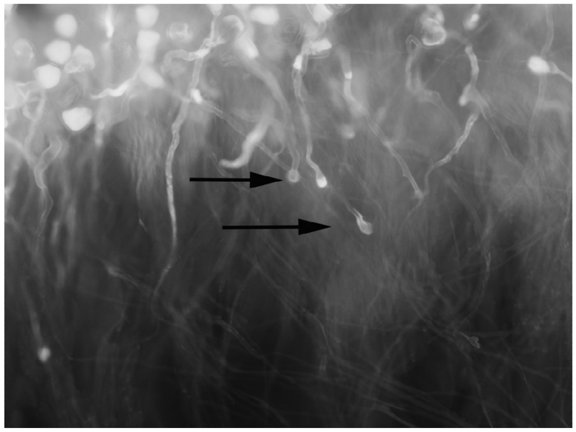

Figure 4. Incompatible pollen tubes just below the stigma (magnified 200x)

As for the cross-pollination variant, for all fixation periods in the first and the second years of the study, 15.4% and 12.1% of pollen tubes observed in the upper part of the style, respectively, were found incompatible. In the self-pollination variant, 15.0% and 17.0% of pollen tubes in the first and second years of study were found incompatible. Under open pollination, a number of pollen tubes in the upper part of the style was found incompatible in both years of the study - 14.0% and 14.4%, respectively.

DISCUSSION

Dynamics of pollen tubes growth was observed as the percentage of pistils with particular regions of the style and the ovary reached by the longest pollen tubes. According to MILATOVIĆ and NIKOLIĆ (2005) and MILATOVIĆ *et al*. (2010), in self-compatible apricot (*Prunus armeniaca* L.) cultivars at least one pollen tube was found in the ovary, whereas in self-incompatible cultivars pollen tubes growth was terminated at a ¾ of the style and was accompanied by the characteristic swelling at the tips of pollen tubes. Using the fluorescence microscopy method, ALONSO and SOCIAS and COMPANY (2005) categorized almond (*Prunus amygdalus* Batsch.) cultivars as self-compatible and self-incompatible. Thus, cultivars with more than 50% of pistils with their own pollen tubes in the style base are considered self-compatible, whereas those with less than 25% of pistils with their own pollen tubes in the base of the style are considered self-incompatible.

Cultivars were considered self-compatible if at least one pollen tube reached the base of the style, while cultivars were considered self-incompatible if pollen tubes stopped in the style with the formation of the characteristic swellings at the tips (NIKOLIĆ and MILATOVIĆ, 2010). In view of this, 'Čačanska Lepotica' behaved as self-compatible.

Prunus species are characterised by gametophytic self-incompatibility, in which pollen tubes with intense fluorescence usually stop their growth in the upper third or upper quarter of the style, or just behind the stigma – in sour cherry (TOBUTT *et al*. 2004), almond (ČOLIĆ *et al*., 2010), apricot (MILATOVIĆ *et al*., 2010) and sweet cherry (RADIČEVIĆ *et al*., 2013). The occurrence of self-incompatibility is a problem in fruits, since it excludes establishment of single-cultivar plantings and requires a presence of two or three compatible cultivars, in order to obtain high yields (DICENTA *et al*. 2002). In commercial orchards of GSI fruit species, cross-compatible cultivars that belong to different pollen incompatibility groups and same flowering time must be interplanted to ensure fruit set. The majority of incompatible pollen tubes in plum cv 'Čačanska Lepotica' were observed in the self-pollination variant. They were with morphological changes - wider at the tip, with more or less pronounced fluorescence under UV light. Additionally, only few pollen tubes were short, thick and wide at the tip, exhibiting intense fluorescence along the entire length. The occurrence of incompatible pollen tubes did not have an influence on the pollen tubes number and growth rate through the different regions of the style, because a number of pollen tubes which continued their growth were sufficient to reach the ovary and perform fertilization.

ACKNOWLEDGEMENT

This work was conducted under Research Project *TR31064-Development and preservation of genetic potential of temperate zone fruits*, supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Received January 29th 2014
Accepted May 28th, 2014

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INKOMPATIBILNE POLENOVE CEVČICE U STUBIĆU ŠLJIVE I NJIHOV UTICAJ NA STEPEN OPLOĐENJA

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Izvod

U radu su prikazani rezultati dvogodišnji ispitivanja prisustva polenovih cevčica sa znakovima inkompatibilnosti u stubiću sorte ‘Čačanska lepotica’ u tri varijante oprašivanja (slobodno-, strano- (‘Čačanska najbolja’) i samooprašivanje). Primenjena je fluorescentna metoda bojenja sa anilin plavim, kojom se može uočiti jasna razlika između kompatibilnih i inkompatibilnih polenovih cevčica. Mesto zaustavljanja rasta polenovih cevčica karakterisalo se njihovim izmenjenim morfološkim izgledom, sa proširenim vrhom koji je pod UV svetlom jače ili slabije fluorescirao. U najvećem broju slučajeva inkompatibilne polenove cevčice zaustavljale su se u svom rastu u gornjoj trećini stubića. Manji broj inkompatibilnih polenovih cevčica je konstatovan u regionu neposredno ispod stigme. Takve polenove cevčice u najvećem procentu su se završavale proširenim vrhom, koji je jače ili slabije fluorescirao. U obe godine ispitivanja procenat inkompatibilnih polenovih cevčica kretao se od 12,1% do 17,0%, s tim da je najveći procenat bio u varijanti samooprašivanja. Konstatovan broj inkompatibilnih polenovih cevčica, u svim ispitivanim varijantama oprašivanja, nije imao uticaja na stepen oplođenja.

Primljeno29.I.2014.
Odobreno 28. V. 2014.