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Original scientific paper

EXAMINING SELF-COMPATIBILITY IN PLUM (*Prunus domestica* L.) BY FLUORESCENCE MICROSCOPY

Dragan NIKOLIĆ and Dragan MILATOVIĆ

Faculty of Agriculture, Belgrade, Serbia

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Self-compatibility in 18 European plum cultivars was examined using the method of fluorescence microscopy. According to self-compatibility, cultivars were divided into two groups: self-compatible and self-incompatible. In self-compatible cultivars the number of pistils, where pollen tubes reached the base of the style varied from 32.00% (Anna Späth) to 91.18% (Wangenheims Frühzwetsche). Mean number of pollen tubes at the base of style in these cultivars ranged from 0.52 to 3.97. Cultivars were considered self-incompatible if pollen tubes stopped their growth in the style along with forming characteristic swellings at their tips. Of the studied cultivars, 13 were found to be self-compatible: Wangenheims Frühzwetsche, Čačanska Lepotica, Valjevka, California Blue, Čačanska Rodna, Italian Prune, Stanley, Požegača, Herman, Bluefre, Jelica, Ruth

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Corresponding author: Dragan Nikolić, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia, e-mail: nikolicd@agrif.bg.ac.rs

Gerstetter and Anna Späth, while 5 were found to be self-incompatible: Čačanska Rana, Zimmers Frühzwetsche, Čačanska Najbolja, Pacific and President.

Key words: plum, pollen tube growth, self-compatibility

INTRODUCTION

Plum is the most important fruit species in Serbia. Plum trees are precocious and well cropping, have small requirements for ecological conditions and orchard management practices, and can be grown on higher altitudes. The fruits are used for table consumption, drying, freezing and processing. The largest amount of plum fruits produced in Serbia (more than 75%) is processed into brandy (MIŠIĆ, 2006).Important factor for successful plum growing is the knowledge of the cultivar's degree of self-fertility. Diploid plum species such as *Prunus salicina* Lindl. and *Prunus cerasifera* Ehrh. are mostly self-incompatible, while fertility relations of the hexaploid European plum (*Prunus domestica* L.) varieties vary between self-fertility, partial self-fertility and self-incompatibility (SZABÓ, 2003). There are also some male sterile plum varieties (e.g. Crvena Ranka, Tuleu Gras, Tuleu Timpuriu). From the aspect of production practice and breeding, self-fertile varieties are of the highest value, because when growing partially self-fertile, self-incompatible and self-sterile varieties it is necessary to provide adequate pollinators.Self-compatibility is usually determined by monitoring fruit set in pollinated and isolated flowers under field conditions. The disadvantage of this method is that fruit set varies from year-to-year depending on weather conditions. Another method used is the pollination of flowers and observation of pollen tube growth in the style under fluorescent microscope. It enables reliable conclusions regarding the self-compatibility (VITI *et al*., 1997). In our country, this method is applied in sour cherry (NENADOVIĆ-MRATINIĆ, 1984; CEROVIĆ, 1992, 1994), sweet cherry (CEROVIĆ *et al*., 2003, 2005), apricot (MILATOVIĆ and NIKOLIĆ, 2005, 2007) and plum (KUZMANOVIĆ *et al.*, 2007; ĐORĐEVIĆ *et al*., 2008). Besides two mentioned biological methods, two molecular methods have recently been used to determine self-compatibility: detection of stylar ribonucleases (*S*-RNases) and DNA amplification and identification by PCR analysis (HALÁSZ and HEGEDÜS, 2006).The aim of this study was to examine self-compatibility of the major plum varieties grown in our country, including some Serbian varieties.

MATERIALS AND METHODS

Plant material was taken from plum variety collection orchard at the experimental estate "Radmilovac" of the Faculty of Agriculture in Belgrade. Eighteen plum cultivars were examined, including seven Serbian cultivars. Research was conducted over a 2-year period (2004 - 2005).Shoots with flower buds in the "balloon" stage were taken for the analysis and placed in jars with 5% sucrose solution. Jars were kept in the laboratory at room temperature (20 ± 2ºC), and the water in them was changed every day. Flowers were emasculated immediately, and detached anthers were left in open Petri dishes to dry and release pollen.

Emasculated flowers were hand-pollinated after about 24h. Fixation of pistils was done four days (96h) after pollination. Fixation was carried out with FAA fixative consisting of ethanol (concentration 70%), glacial acetic acid and formaldehyde in the ratio 90:5:5 (BURGOS *et al*., 1997). Fixed material was kept at +4ºC in the refrigerator until staining. Before staining, pistils were rinsed in running water for 10-15 minutes. Thereafter, they were immersed in 8M NaOH solution and left to stand overnight (12-24h) to soften their tissues. Then they were rinsed again in running water for about 2h. Staining was done with 0.1% aniline blue dissolved in 0.1M K_3PO_4 for about 24h. To prepare pistils for microscopic examination, the style was separated from the ovary. The style was squashed, while the ovary was cut longitudinally with a razor blade so that penetration of pollen tubes into the ovules could be seen (CEROVIĆ, 1994).The examination of pistils was done in the Laboratory for microscopy at the Faculty of Agriculture in Belgrade. Observation was done under the fluorescent microscope "Leica DMLS", using the I3 blue filter (wavelength 450 - 490 nm). At least 20 pistils were analysed from each cultivar. In accordance with the recommendations given by LOPEZ *et al.* (2001) cultivars were considered self-compatible if pollen tubes reached the ovary in at least 25-30% of the pistils.

RESULTS

Testing of pollen tube growth in the pistil after self-pollination enables determination of self-compatibility in plum cultivars. Pollen grains placed on the stigma surface begin to germinate and elongate into pollen tubes that grow through the stylar tissue to the ovary (Figure 1 and 2). The walls of the pollen tubes content callose (β-1,3-glucane), which binds with aniline blue during staining. When lighted with blue or ultraviolet light they become fluorescent - show a golden yellow colour that creates a sharp contrast on a dark background. Thanks to that the growth of pollen tubes in the pistil can be observed.

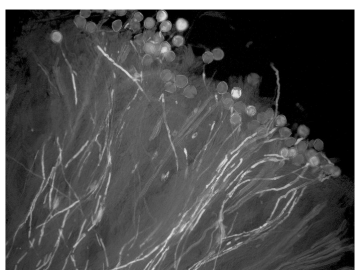
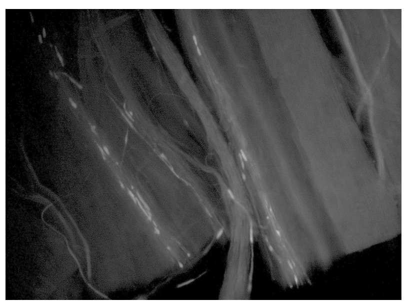


Figure 1. Germinated pollen grains on the stigma of the pistil, cultivar Italian Prune

Figure2. Entry of the pollen tube into the ovule, cultivar Wangenheims Frühzwetsche

Plum cultivars were considered self-compatible if at least one pollen tube reached the base of the style in the majority of pistils (Figure 3). Of the 18 cultivars studied, 13 were self-compatible. They were: Wangenheims Frühzwetsche, Čačanska Lepotica, Valjevka, California Blue, Čačanska Rodna, Italian Prune, Stanley, Požegača, Herman, Blufre, Jelica, Ruth Gerstetter and Anna Späth. In these cultivars, the percentage of pistils with at least one pollen tube reached the base of the style varied from 32.00% to 91.18% (Table 1). The average number of pollen tubes at the base of the style ranged from 0.52 in cultivar Anna Späth to 3.97 in cultivar Wangenheims Frühzwetsche.

Cultivars were considered self-incompatible if pollen tubes growth stopped in the style with the formation of the characteristic swellings at their tips due to greater accumulation of callose (Figure 4). Self-incompatibility was found in five of the plum cultivars studied: Čačanska Rana, Zimmers Frühzwetsche, Čačanska Najbolja, Pacific and President. In these cultivars, pollen tubes did not reach the base of the style. Species of the genus *Prunus* have gametophytic incompatibility system, determined by a series of multiple alleles at a single locus. This type of incompatibility occurs most often in the upper third of the style, which was confirmed in our work. Most characteristic signs of incompatibility were the expansion and bifurcation of the pollen tube tips (Figure 5 and 6). STÖSSER (1982) found the cessation of pollen tubes growth in the style in seven self-incompatible plum cultivars using the method of fluorescence microscopy. SEMENAS and KOUKHARTCHIK (2000) in various plum species and hybrids found that incompatible barriers occurred just below the stigma.



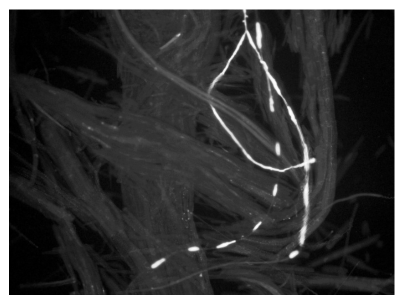


Figure 3. The base of the pistil with many pollen tubes, cultivar Čačanska Lepotica

Figure 4. Cessation of pollen tubes growth with forming characteristic swellings, cultivar Pacific

Table 1. Pollen tube growth of plum cultivars 96 h after self pollination

Cultivar	Origin	Number of pistils examined	Percentage of pistils with at least one pollen tube at the base of the style	Mean number of pollen tubes at the base of the style	Conclusion about cultivar
Wangenheims Frühzwetsche	Germany	34	91.18	3.97	self-compatible
Čačanska Lepotica	Serbia	22	86.36	2.18	- II -
Valjevka	Serbia	24	75.00	1.56	- II -
California Blue	USA	26	69.23	2.54	- II -
Čačanska Rodna	Serbia	33	66.67	1.94	- II -
Italian Prune	Italy	58	58.62	1.43	- II -
Stanley	USA	36	55.55	1.08	- II -
Požegača	Serbia	34	50.00	1.15	- II -
Herman	Sweden	20	50.00	1.70	- II -
Bluefre	USA	53	37.74	0.94	- II -
Jelica	Serbia	25	36.00	0.84	- II -
Ruth Gerstetter	Germany	21	33.33	0.62	- II -
Anna Späth	Germany	25	32.00	0.52	- II -
Čačanska Rana	Serbia	29	0.00	0.00	self-incompatible
Zimmers Frühzwetsche	Germany	26	0.00	0.00	- II -
Čačanska Najbolja	Serbia	20	0.00	0.00	- II -
Pacific	USA	22	0.00	0.00	- II -
President	England	31	0.00	0.00	- II -

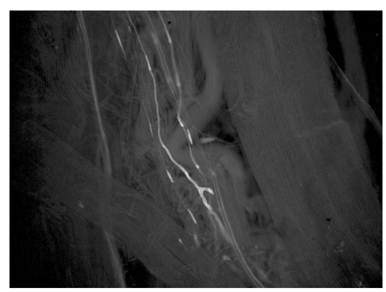


Figure 5. Incompatible pollen tube with a broadened tip in the upper third of the style, cultivar Čačanska Rana

Figure 6. Bifurcation of the pollen tube in the upper third of the style, cultivar President

BOTU *et al*. (2002) reported that gametophytic incompatibility genetic system is present in plum cultivars, and there are 3 component genomes (D_1D_2C), each genome with one gene control and probably with multiple alleles, so it is difficult to find the same alellic formula for two cultivars. Using molecular methods for testing self-compatibility of domestic plum SUTHERLAND *et al*. (2004) obtained the partial *S*-allele genotypes for 19 cultivars. HEGEDÜS and HALÁSZ (2006) explained that a variable extent of self-compatibility in European plum cultivars may be attributed to heteroallelic pollen. In the heteroallelic pollen *S*-alleles may interact competitively, so that the pollen is rejected in styles having any of the same *S*-alleles.

Data about self-fertility of plum varieties in the literature are usually obtained by examining fruit set in pollinated flowers under field conditions. Past research of fertility relations in various countries gave different, often contradictory results. This can be explained with using different limits for classification of a cultivar as self-incompatible, that vary in a wide range from <1% (SZABÓ, 2003) to 20% (ILIEV, 1985). In addition, fruit set under field conditions depends on many factors such as weather conditions, rootstocks, nutrition, pruning and health status of the tree (PEJKIĆ, 1978). The number of groups representing varieties of different degrees of self-fertility varied from 2 to 7, and most frequently a system of 3 groups (self-fertile, partially self-fertile and self-incompatible) is used (SZABÓ, 2003).

Based on the degree of fruit set obtained by self-pollination, ILIEV (1985) classified 14 plum varieties in three groups: self-incompatible (fruit set to 20%), partially self-fertile (fruit set from 20% to 70%) and self-fertile (fruit set above 70%). He found that self-fertile cultivars were Blufre and Požegača, and self-incompatible Pacific, which was confirmed by our results. Studying fruit set of 56 European and 10 Japanese plum varieties NYÉKI and SZABÓ (1996) established five groups of cultivars: entirely self-incompatible (fruit set 0%), self-incompatible (fruit set from 0.1% to 1%), partially self-fertile (fruit set 1, 1% to 10%), self-fertile (fruit set from 10.1% to 20%) and highly self-fertile (fruit set above 20%). Based on this criteria, they reported that cultivars Čačanska Najbolja, Pacific and President were entirely self-incompatible, Zimmers Frühzwetsche were self-incompatible, Italian Prune were self-fertile and Čačanska Rodna and Požegača were extremely self-fertile, which confirmed the results of our work. SZABÓ and NYÉKI (2000) as self-fertile classified two cultivars that we studied, Italian Prune as self-fertile and Čačanska Rodna as extremely self-fertile. Our findings confirm earlier conclusions about self-fertility of cultivars Wangenheims Frühzwetsche, Požegača, Italian Prune, Anna Späth, Stanley and the California Blue (PAUNOVIĆ, 1971), as well as cultivars Čačanska Lepotica and Čačanska Rodna (OGAŠANOVIĆ, 1985).

NYÉKI and SZABÓ (1996) for cultivars California Blue, Blufre, Čačanska Lepotica and Stanley, and PAUNOVIĆ (1971) for cultivar Ruth Gerstetter found that they are partially self-fertile. In our research, they proved to be self-compatible. It is possible that environmental factors affect the lower level of fertilisation in these cultivars under field conditions. Thus, THOMPSON and LIU (1973) found that chilly weather in the period after flowering caused poor fruit and consequently lower yield in cultivar Italian Prune. On the other hand, KEULEMANS (1994) states that high temperatures during the bloom period can be a limiting factor of fruit set in plum. The method of fluorescence microscopy, which we used for studying self-compatibility of plums in our work, enables to avoid the influence of environmental factors and thus the more accurate way to classify the cultivars only in the two groups (self-compatible and self-incompatible).

NYÉKI *et al*. (2000) state that many self-incompatible cultivars of stone fruits, including plums, are grown in commercial orchards, and that their yield depends on the presence of adequate pollinators. Such varieties should be provided with adequate pollinators that have approximately the same time of flowering. When choosing pollinators, phenomenon of inter-incompatibility that occurs between some varieties (TEHRANI, 1991; MIŠIĆ, 2006) should be taken into account. Considering the above mentioned, one of the goals of plum breeding should be a creation of self-compatible cultivars which can provide better pollination, and thus higher yields and eliminate the need for growing the additional varieties as pollinators.

CONCLUSION

The method of fluorescence microscopy provides a relatively fast and reliable determination of self-compatibility in plum cultivars. In self-compatible cultivars percentage of pistils with at least one pollen tube at the base of the style ranged from 32% to 91%. The average number of pollen tubes at the base of the style was relatively low and varied from 0.52 to 3.97. In self-incompatible cultivars pollen tubes stopped their growth in the style (usually in its upper third) with the formation of the characteristic thickenings at the tips. In these cultivars pollen tubes did not rich the ovary.

Based on the results obtained, the studied cultivars are divided into two groups. Self compatible were cultivars: Wangenheims Frühzwetsche, Čačanska Lepotica, Valjevka, California Blue, Čačanska Rodna, Italian Prune, Stanley, Požegača, Herman, Blufre, Jelica, Ruth Gerstetter and Anna Späth, while self-incompatible were cultivars: Čačanska Rana, Zimmers Frühzwetsche, Čačanska Najbolja, Pacific and President.

Since self-incompatibility occurs relatively frequently in plum cultivars, care should be taken in deciding cultivar composition of new orchard plantings.

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REFERENCES

BOTU, M., C. SARPE, S. COSMULESCU and I. BOTU (2002): The genetic control of pollen fertility, pollenizing and fruit set for the *Prunus domestica* L. plum cultivars. Acta Hort., *577*: 139-145.

BURGOS, L., J. EGEA, R. GUERRIERO, R. VITI, P. MONTELEONE and J.M. AUDERGON (1997): The self-compatibility trait of the main apricot cultivars and new selections from breeding programmes. J. Hort. Sci., *72*: 147-154.

CEROVIĆ, R. (1992): Pollen tube growth in sour cherry pistils in relation to fruit set. Adv. Hort. Sci., *6*: 107-111.

CEROVIĆ, R. (1994): Histocytological aspects of fertilization dynamics of sour cherry (*Prunus cerasus* L.). PhD thesis, Faculty of Biology, Belgrade.

CEROVIĆ, R., Đ. RUŽIĆ, S. RADIČEVIĆ and M. NIKOLIĆ (2003): Determination of specific cultivar composition in the pollinators for sweet cherry cv Asenova rana. Journal of Yugoslav Pomology, *37*(143-144): 85-94.

CEROVIĆ, R., S. RADIČEVIĆ, Đ. RUŽIĆ and M. KUZMANOVIĆ (2005): Determination of the cultivar composition of pollinators for sweet cherry cv Čarna. Journal of Pomology, *39*(152): 347-355.

ĐORĐEVIĆ, M., R. CEROVIĆ, D. NIKOLIĆ and S. RADIČEVIĆ (2008): Influence of pollination variants on the dynamic of pollen tubes growth and the fruits set in plum cv Čačanska lepotica. Journal of Pomology, *42*(163-164): 83-87.

HALÁSZ, J. and A. HEGEDŰS (2006): A critical evaluation of methods used for *S*-genotyping: from trees to DNA level. Int. J. Hort. Sci., *12*(2): 19–29.

HEGEDÛS, A. and J. HALÁSZ (2006): Self-incompatibility in plums (*Prunus salicina* Lindl., *Prunus cerasifera* Ehrh. and *Prunus domestica* L.). A minireview. Int. J. Hort. Sci., *12*(2): 137–140.

ILIEV, P. (1985): Degree of self-fertility in some *Prunus domestica* L. cultivars. Plant Science, *22*(7): 68-73.

KEULEMANS, J. (1994): Pollination and fruit set in self-incompatible plum cultivars. Acta Hort., *359*: 260-268.

KUZMANOVIĆ, M., S. RADIČEVIĆ and R. CEROVIĆ (2007): Study of the progamic phase of pollination in the plum cultivar Čačanska lepotica. Journal of Pomology, *41*(159): 89-93.

LOPEZ, M., J.M. ALONSO, P. MARTINEZ-GOMEZ, R. SOCIAS I COMPANY, T.M. GRADZIEL and I. BATLLE (2001): Biological and molecular assessment of self-incompatibility in almond. Nucis, *10*: 8-11.

MILATOVIĆ, D. and D. NIKOLIĆ (2005): The study of self-compatibility in apricot cultivars by means of fluorescence microscopy. Journal of Pomology, *39*(150): 171-178.

MILATOVIĆ, D. and D. NIKOLIĆ (2007): Analysis of self-(in)compatibility in apricot cultivars using fluorescence microscopy. J. Hort. Sci. Biotech., *82*(2): 170-174.

MIŠIĆ, P. (2006): Plum. Partenon, Belgrade.

NENADOVIĆ-MRATINIĆ, E. (1984): Sexual incompatibility in sour cherry determined by fast method *in vivo*. Journal for Scientific Agricultural Research, *45*(160): 463-469.

NYÉKI, J. and Z. SZABÓ (1996): Fruit set of plum cultivars under Hungarian ecological conditions. Acta Hort., *423*: 185-192.

NYÉKI, J., Z. SZABÓ and M. SOLTÉSZ (2000): Association of varieties in stone fruit plantations. Int. J. Hort. Sci., *6*(3): 29-33.

OGAŠANOVIĆ, D. (1985): The most suitable pollinators for new plum cultivars. Journal of Yugoslav Pomology, *19*(71-72): 109-115.

PAUNOVIĆ, S.A. (1971): The investigation of self-fruitfulness and recommendation of pollinizers for some more important plum and prune cultivars. Journal of Yugoslav Pomology, *5*(17-18): 109-122.

PEJKIĆ, B. (1978): Productivity and non-productivity of fruit tree species. Nolit, Beograd.

SEMENAS, S. and N.V. KOUKHARTCHIK (2000): Investigations in pollination and fertilization in *Prunus* L. breeding via fluorescence microscopy. Acta Hort., *538*: 371-374.

STÖSSER, R. (1982): The pollen tube growth *in vitro* and *in vivo* in *Prunus domestica* L. Z. Pflanzenzüchtung, *88*: 261-264.

SUTHERLAND, B.G., K.R. TOBUTT and T.P. ROBBINS (2004): Molecular genetics of self-incompatibility in plums. Acta Hort., *663*: 557–562.

SZABÓ, Z. (2003): Plum (*Prunus domestica* L.). In: „Floral biology, pollination and fertilisation in temperate zone fruit species and grape". Akadémiai Kiado, Budapest, pp. 383-410.

SZABÓ, Z. and J. NYÉKI (2000): Floral biology of plum. Int. J. Hort. Sci., *6*(3): 11-27.

TEHRANI, G. (1991): Seventy-five years of plum breeding and pollen compatibility studies in Ontario. Acta Hort., *283*: 95–103.

THOMPSON, M.M. and L.J. LIU (1973): Temperature, fruit set, and embryo sac development in 'Italian' prune. J. Amer. Soc. Hort. Sci. *98*: 193-197.

VITI, R., P. MONTELEONE and R. GUERRIERO (1997): Incompatibility in apricot (*Prunus armeniaca* L.): Methodological considerations. J. Hort. Sci. *72*(6): 961-970.

ISPITIVANJE SAMOOPLODNOSTI KOD ŠLJIVE (*Prunus domestica* L.) METODOM FLUORESCENTNE MIKROSKOPIJE

Dragan NIKOLIĆ i Dragan MILATOVIĆ

Poljoprivredni fakultet, Beograd, Srbija

I z v o d

Kod 18 sorti domaće šljive ispitivana je samooplodnost metodom fluorescentne mikroskopije. Na osnovu samooplodnosti sorte su podeljene u dve grupe: autokompatibilne i autoinkompatibilne. Kod autokompatibilnih sorti broj tučkova kod kojih su polenove cevčice stigle do osnove stubića varirao je od 32,00% (Ana Špet) do 91,18% (Vangenhajmova). Prosečan broj polenovih cevčica u osnovi stubića kod ovih sorti je bio od 0,52 do 3,97. Sorte su smatrane autoinkompatibilnim ako su polenove cevčice zaustavljale rast u stubiću tučka uz formiranje karakterističnih zadebljanja na njihovim krajevima. Među proučavanim sortama 13 su se pokazale kao autokompatibilne: Vangenhajmova, Čačanska lepotica, Valjevka, Kalifornijska plava, Čačanska rodna, Italijanka, Stenli, Požegača, Herman, Blufri, Jelica, Rut geršteter i Ana Špet, a 5 kao autoinkompatibilne: Čačanska rana, Cimerova rana, Čačanska najbolja, Pacifik i Prezident.

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