

**INITIAL AND FINAL FRUIT SET IN SOME PLUM (*Prunus domestica* L.)  
HYBRIDS UNDER DIFFERENT POLLINATION TYPES**

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The paper presents results of two-year study (2009–2010) of initial and final fruit set in promising plum (*Prunus domestica* L.) hybrids developed at Fruit Research Institute – Čačak, under different pollination conditions. The following hybrids were studied: 38/62/70 ('Hall' x 'California Blue'), 32/21/87 ('Stanley' x 'Scoldus'), IV/63/81 ('Large Sugar Prune' x 'Scoldus'), 22/17/87 ('Čačanska Najbolja' x 'Zh'lta Butilcovidna'), 29/29/87 ('Stanley' x 'Scoldus') and 34/41/87 ('Valjevka' x 'Čačanska Lepotica'). Each of the hybrids was studied both under self-pollination and open pollination. *In vitro* pollen germination was also performed as well as characteristics of flowering phenophase and flowering

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abundance. Generally, the results suggest lower flowering abundance in the second year of the study. Pollen germination ranged from averagely 25.31% (29/29/87) to 40.01% (38/62/70). With averagely 31.59% final fruit set under self-pollination and 29.38% under open pollination variants, respectively, hybrid 34/41/87 gave the best results. The lowest performance was observed in hybrid 32/21/87 with 1.61% and 7.69% final fruit set under self- and open pollination variants, respectively.

*Key words:* flowering, fruit set, plum, pollen, promising hybrids

## INTRODUCTION

Plum is an important fruit species, interesting for various forms of industrial and domestic processing, and for fresh consumption. Grown on the area of 135,632 ha and with an average annual production of 419,813 t (over 2000–2007, FAO Statistic Division, 2009), European plum (*Prunus domestica* L.) is a leading fruit species in Serbia (MRATINIĆ *et al.*, 2006), providing Serbia the fourth ranking in total production on global scale (MILOŠEVIĆ *et al.*, 2010).

Plum belongs to *Rosaceae* family, *Prunoideae* subfamily, and *Prunophora* subgenus which includes a number of different species of *Prunus* genus. The best-known and most widely grown species are *Prunus domestica* L., *Prunus salicina* Lindl., *Prunus subcordiata* Benth. and *Prunus instititia* L. (VOČA *et al.*, 2009). European plum (*Prunus domestica* L.) is a hexaploid fruit species ( $2n=6x=48$ ), originating from natural crossing of *Prunus spinosa* (4x) and *Prunus cerasifera* (2x) genera (DECROOQ *et al.*, 2004; ILGIN *et al.*, 2009).

Self-fertility, ability to set fruit following self-pollination, is an important trait in many *Prunus* species that contributes to higher, more consistent yields. Most *Prunus* fruit tree species exhibit a homomorphic gametophytic self-incompatibility (GSI) system, in which specificity of self/nonself-recognition is controlled by products encoded within the *S* locus; in the pollination event, a self-incompatibility (SI) reaction is triggered when the same *S* allele specificity is expressed in both the pollen and pistil (TAO and IEZZONI, 2010). During the last two decades, much progress has been made in understanding of the molecular basis of the gametophytic self-incompatibility system in *Prunus* species – sweet and sour cherry, almond, apricot, and plum. While diploid plum types are mainly self-incompatible, fertility level of European plum varies between self-fertility, partial self-fertility and self-incompatibility (SZABÓ, 2003). In this line, the important factor for successful plum growing is gaining knowledge on a degree of self-fertility of a cultivar (NIKOLIĆ and MILATOVIĆ, 2010). HEGEDUS and HALASZ (2006) report that almost half the total number of cultivars within this species are self-sterile.

On the other hand, knowledge of flowering time of plum cultivars is essential for an adequate choice of cultivar combination that ensures the most effective pollination and fertilization for abundant fruit set. Successful pollination and fertilization is one of the most important factors affecting the financial outcome in commercial fruit growing (KOSKELA *et al.*, 2010).

Plum breeding work at Fruit Research Institute was initiated in 1946 since when 14 plum cultivars have been developed within different breeding programs, with a large number of promising hybrids currently being evaluated (PAUNOVIĆ *et al.*, 2011). Self-fertility of the new genotype is one of the breeding objectives of plum breeding work (MILENKOVIĆ *et al.*, 2006).

The aim of this paper was to examine the degree of the initial and final fruit set in self- and open pollination variants in six promising plum hybrids developed at Fruit Research Institute – Čačak, in order to obtain data of interest for plum orchard establishing, further investigation of reproductive biology of European plum, and breeding work.

## MATERIALS AND METHODS

### *Plant material*

The experiment included six promising plum (*Prunus domestica* L.) hybrids, developed at Fruit Research Institute – Čačak: 38/62/70 ('Hall' x 'California Blue'), IV/63/81 ('Large Sugar Prune' x 'Scoldus'), 32/21/87 ('Stanley' x 'Scoldus'), 22/17/87 ('Čačanska Najbolja' x 'Zh'lta Butilcovidna'), 29/29/87 ('Stanley' x 'Scoldus') and 34/41/87 ('Valjevka' x 'Čačanska Lepotica'). Experiment conducted over 2009–2010 in the experimental orchard at the Ljubić site of Fruit Research Institute, Čačak (altitude of 260 m). The orchard was established in autumn 2002 with standard one-year-old plants grafted on Myrobalan (*Prunus cerasifera* L.) seedlings, planted at 6 x 5 m (333 trees per ha). The training system is open vase. Conventional cultural practices were applied in the planting.

### *Flowering time investigation*

To investigate the flowering phenophase, flowering time, progress and abundance of flowering were studied. Monitoring and taking notes on flowering onset (10–20% open flowers); full flowering (80–100% open flowers); end of flowering (fallen over 90% petals) were done. Abundance of flowering is expressed on scale 0–5: excellent (5), very good (4), good (3), poor (2), very poor (1) and no flowers (0).

### *Pollen germination test*

For examination of pollen viability, *in vitro* pollen germination test was used. The test involved collecting branches with buds in late balloon stage. Anthers of each hybrid were separated from flowers in laboratory, placed into paper boxes and dried at 20°C for 24–28 h until cracking and pollen dusting. Pollen of each hybrid was grown in the three Petri dishes, in the *in vitro* medium containing 12% sucrose and 1% agar (CEROVIĆ *et al.*, 2003) and maintained at 24°C for 24 hours. Flowers, anthers and pollen were carefully manipulated to avoid any contamination of samples by pollen. Three visual fields of each Petri dish were monitored under OLIMPUS BX61 microscope (light regime); the visual field included about 100

pollen grains. As germinated, pollen grains with pollen tubes length of twice of the grain diameter were counted.

#### *Initial and final fruit set investigation*

Emasculation of flowers was done at late balloon stage, by removing the perianths and anthers. Emasculated flowers were counted, and isolated with paper bags in order to prevent uncontrolled pollination. At the beginning of full flowering, they manually pollinated with their own pollen (prepared as described above), and isolated once more with the bags; the bags removed two weeks after pollination. 300 flowers of each hybrid were pollinated per year of the study. Also, branches were marked and flowers counted on the same day (late balloon stage) to examine fruit set in open-pollination variant (300 flowers of each hybrid). Initial fruit set was monitored by counting fruits 3–4 weeks after the pollination, while the final fruit set was checked at the beginning of fruit ripening phenophase.

#### *Statistical analysis*

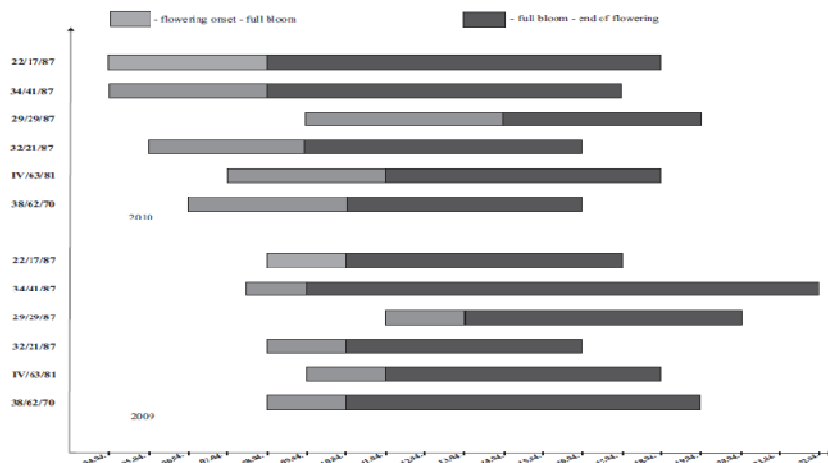
A completely randomized block system with five trees in three replications was used. The data were statistically analyzed using the analysis of variance model (ANOVA). The significance of differences among mean values was determined using LSD test at  $P \leq 0.05$  and  $P \leq 0.01$ .

### RESULTS AND DISCUSSION

The earliest beginning of flowering was observed in hybrid 34/41/87 (07 April) in the first, and in hybrids 34/41/87 and 22/17/87 (04 April) in the second year of study (Graph. 1). The latest beginning of flowering was recorded in hybrid 29/29/87 in both years. In all hybrids beginning of flowering was later in 2009, about 3 days on average. Similarly, the first experimental year was characterized by somewhat more explosive flowering, as phenophases of full flowering and end of flowering occurred for averagely 2.2 and 7.8 days, respectively. In 2010, full flowering phenophase occurred in 4.2 days after beginning of flowering, regardless of hybrid, whereas the average duration of flowering phenophase was 11.5 days. The shortest flowering phenophase was recorded most in hybrid 32/21/87 (averagely 9 days), while the longest was recorded in hybrid 34/41/87 (14 days).

Early flowering is the most critical phase in the production of plum because of the possible occurrence of late spring frosts. For this reason it is desirable that cultivars have late, longer and more abundant flowering. Besides the genetic background, flowering is determined by many environmental factors, of which the influence of climatic factors is considered as major (ROMANOVSKAJA and BAKŠIENE, 2009). Flowering time of some hybrids coincides with flowering time of 'Čačanska Lepotica' and 'Čačanska Rodna' (GLIŠIĆ *et al.*, 2011), which are classified as mid-early flowering cultivars (MILENKOVIĆ *et al.*, 2006). The data on flowering time of some hybrids correspond to the results of DINKOVA *et al.* (2005) for cultivar 'Stanley', classified as a late-flowering cultivar (NEUMÜLER, 2011). Concerning the duration of flowering phenophase, hybrids studied do not lag behind the cultivated

plum cultivar such as 'Čačanska Lepotica', 'Stanley', 'Mildora' and 'Ana Spat' (BOTU *et al.*, 2002; OGAŠANOVIĆ *et al.*, 2005; DRAGOYSKI *et al.*, 2005). Most of European plum genotypes flower at the approximately same time, providing an adequate flowering overlap for effective cross-pollination among them. In a warm spring, the total flowering period will be shortened, and many genotypes may overlap, resulting in effective cross-pollination, while in a cold spring, the whole flowering period is lengthened, and less overlapping will occur. However, in order to perform the classification of promising hybrids, it is necessary to perform phenological observations over a longer period.



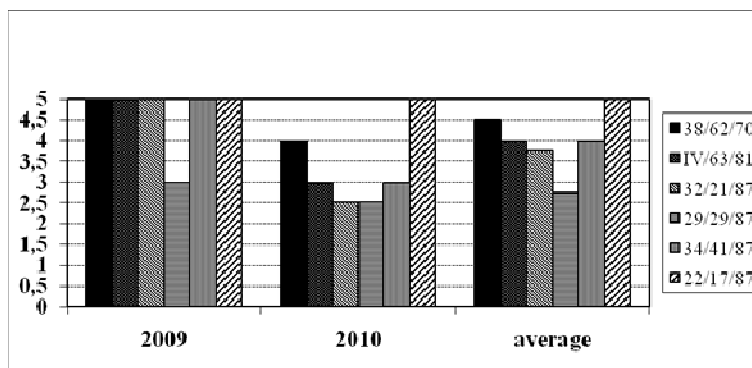
Graph.1. Phenophase of flowering in studied plum hybrids

Abundance of flowering (Graph. 2) ranged from an average of 2.75 (hybrid 29/29/87) to 5 (hybrid 22/17/87) and was more uniform in the first experimental year, when, with the exception of hybrid 29/29/87, all hybrids showed maximum yield potential. In the second year of study, slightly lower abundance of flowering was observed in hybrids, with the exception of 22/17/87, which maintained the abundance level gained in the previous year.

Also, in terms of abundance of flowering, the hybrids do not lag behind commercial plum cultivars characterized by high productivity (SOSNA, 2010; GLIŠIĆ *et al.*, 2011). High abundance of flowering of studied hybrids can be partially explained by good donor ability for high yield of parental cultivars 'Stanley' and 'Čačanska Lepotica' (NEUMÜLER, 2011).

Average pollen germination (Tab. 1.) ranged between 25.54% (hybrid 29/29/87) and 40.59% (hybrid 34/41/87). With an average of 35.09% of pollen grains germinated, the values were lower in the first year of study, although this does

not go for all hybrids. ANOVA indicated highly significant influence of basic factors of variability (hybrid specificity and year), and their interaction.



Graph. 2. Abundance of flowering in studied plum hybrids

Not only from the aspect of genetics and breeding but also from the aspect of plum production, is pollen viability a very important property. Additionally, it is of major importance that a cultivar has good pollen resource (SURANYI, 2006). According to this author, pollen germination in some cultivars of European plum ranged from 24 to 64.4%, while BOTU *et al.* (2002) and DJORDJEVIC *et al.* (2011) reported on somewhat lower values. Results of the study of plum hybrids are within the range of the results of authors cited above, while the variation of pollen germination per year can be explained by the regularity of microsporogenesis process (RADIČEVIĆ *et al.*, 2011).

In self-pollination variant, the largest percentage of initial and final fruit set was observed in hybrid 34/41/87 (46.12% and 31.56%, respectively), and the lowest in hybrid 32/21/87 (1.99% and 1.61%, respectively). The percentage of initial fruit set was higher in the first year of study, while in terms of final fruit set, the opposite tendency was recorded; the phenomenon was not evidenced in all hybrids examined (Tab. 1). ANOVA showed that hybrid specificity, year, and their interaction had a highly significant influence on the experimental results above.

The percentage of initial fruit set in open-pollination ranged from 29.46% (hybrid 29/29/87) to 49.04% (hybrid 22/17/87). All studied hybrids had a slightly higher initial fruit set in the first year of study. ANOVA showed that hybrid specificity and environmental factors, as well as their interaction are very significant source of variability. Disharmony in the results obtained for initial and final fruit set by year and by different type of pollination may be due to the impact of different factors that cause unfertilized flowers drop, and then the initial fruit set with different defects.

Tab. 1. *In vitro* pollen germination and initial and final fruit set in studied plum hybrids

	Pollen germination	Self pollination		Open pollination	
		Initial fruit set (%)	Final fruit set (%)	Initial fruit set (%)	Final fruit set (%)
Hybrids (A)					
38/62/70	39.25 <sup>ab</sup>	26.47 <sup>b</sup>	9.80 <sup>c</sup>	34.62 <sup>c</sup>	17.73 <sup>bc</sup>
IV/63/81	37.54 <sup>b</sup>	41.30 <sup>a</sup>	15.92 <sup>c</sup>	33.83 <sup>c</sup>	14.06 <sup>c</sup>
32/21/87	38.09 <sup>b</sup>	1.99 <sup>c</sup>	1.61 <sup>e</sup>	40.37 <sup>b</sup>	7.63 <sup>d</sup>
29/29/87	25.54 <sup>c</sup>	25.71 <sup>b</sup>	8.85 <sup>d</sup>	29.46 <sup>d</sup>	22.23 <sup>b</sup>
34/41/87	40.59 <sup>a</sup>	46.52 <sup>a</sup>	31.56 <sup>a</sup>	46.35 <sup>a</sup>	29.39 <sup>a</sup>
22/17/87	37.98 <sup>b</sup>	32.46 <sup>b</sup>	22.80 <sup>b</sup>	49.04 <sup>a</sup>	30.63 <sup>a</sup>
Year (B)					
2009	35.09 <sup>b</sup>	37.18 <sup>a</sup>	13.19 <sup>b</sup>	55.80 <sup>a</sup>	20.70 <sup>a</sup>
2010	37.91 <sup>a</sup>	24.45 <sup>b</sup>	16.99 <sup>a</sup>	22.08 <sup>b</sup>	19.84 <sup>a</sup>
A x B					
38/62/70	26.96 <sup>g</sup>	45.34 <sup>b</sup>	17.04 <sup>de</sup>	39.79 <sup>e</sup>	10.85 <sup>ef</sup>
	51.53 <sup>a</sup>	7.6 <sup>de</sup>	2.56 <sup>hi</sup>	29.43 <sup>g</sup>	24.60 <sup>bc</sup>
IV/63/81	35.93 <sup>def</sup>	46.17 <sup>b</sup>	11.71 <sup>efgh</sup>	51.71 <sup>c</sup>	17.63 <sup>d</sup>
	39.16 <sup>cd</sup>	36.43 <sup>c</sup>	20.01 <sup>cd</sup>	15.95 <sup>h</sup>	10.50 <sup>ef</sup>
32/21/87	42.34 <sup>b</sup>	3.55 <sup>e</sup>	3.21 <sup>ghi</sup>	72.24 <sup>a</sup>	9.09 <sup>fg</sup>
	33.85 <sup>ef</sup>	0.33 <sup>e</sup>	0	8.50 <sup>i</sup>	6.16 <sup>g</sup>
29/29/87	25.07 <sup>g</sup>	35.10 <sup>c</sup>	5.21 <sup>fghi</sup>	44.42 <sup>d</sup>	30.98 <sup>a</sup>
	25.99 <sup>g</sup>	16.33 <sup>d</sup>	12.48 <sup>efg</sup>	14.51 <sup>h</sup>	13.47 <sup>e</sup>
34/41/87	38.06 <sup>de</sup>	41.40 <sup>c</sup>	27.81 <sup>bc</sup>	58.52 <sup>b</sup>	30.15 <sup>a</sup>
	43.11 <sup>b</sup>	51.63 <sup>a</sup>	35.31 <sup>a</sup>	34.19 <sup>f</sup>	28.62 <sup>ab</sup>
22/17/87	42.18 <sup>c</sup>	30.53 <sup>c</sup>	14.14 <sup>def</sup>	68.15 <sup>a</sup>	23.54 <sup>c</sup>
	33.78 <sup>f</sup>	34.38 <sup>c</sup>	31.46 <sup>ab</sup>	29.90 <sup>g</sup>	25.72 <sup>bc</sup>
ANOVA					
A	**	**	**	**	**
B	**	**	**	**	ns
A x B	**	**	**	**	**

The lowest percentage of final fruit set in open-pollination was recorded in hybrid 32/21/87 (7.63%) and the highest in hybrid 22/17/87 (30.63%). Slightly higher degree of final fruit set was observed in 2009, but the deviations from this trend were noticed in some hybrids. Statistical analysis inferred that hybrid specificity as well as the interaction between hybrid specificity and year had a highly significant influence on the experimental results, while the influence of year was not statistically significant.

According to NEUMÜLER (2011), in dependence with the level of fruit set at self-pollination, European plum cultivars can be classified into four groups: low ( $\leq 10\%$ ), intermediate (10-20%), high (20.1 to 40%) and very high ( $> 40\%$ ). The results showed that hybrids 38/62/70, 32/21/87 and 29/29/87 belong to the group of low self-compatible cultivars, hybrid IV/63/81 to the intermediate group, while hybrids 34/41/87 and 22/17/87 belong to the high self-compatible group. Studying the fruit set of some European plum cultivars BOTU *et al.* (2002) found that the fruit set at self-pollination ranged from 0.4% to 30%. These percentages vary from one year to another.

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.

Insufficient fruit set in plums may be due to a genetic predisposition for abnormal embryo sac development, and low temperature conditions during the flowering time, resulting in poor pollen tube growth. Effective pollination period shortened than required for the pollen tube reach the egg cell, also resulted in poor fruit set (JIA *et al.*, 2008).

#### CONCLUSION

Commercial plum production is based on high and regular yielding varieties. The main preconditions for high and regular yields are later and longer flowering, high abundance of flowering, very good resource of pollen and good fruit set. Priority is given to self-fertile cultivars because self-incompatible cultivars need adequate pollinizers that have approximately the same time of flowering. Also, phenomena of inter-incompatibility that exist between some cultivars should be taken into account.

The results obtained in this work can be helpful in determining which of promising hybrids can find place in plum orchards in Serbia in the future period.

Further research will be based on utilization of new pollinizers, studying fruit set in different combinations of cross-pollination, and the application of fluorescent-microscopy to study dynamics of pollen tube growth in some regions of pistil.

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**INICIJALNO I FINALNO ZAMETANJE PLODOVA HIBRIDA ŠLJIVE  
(*Prunus domestica* L.) U ZAVISNOSTI OD TIPA OPRAŠIVANJA**

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U radu su prikazani rezultati dvogodišnjih ispitivanja (2009-2010. godina) inicijalnog i finalnog zemetanja plodova perspektivnih hibrida šljive (*Prunus domestica* L.), stvorenih u Institutu za voćarstvo u Čačku, u zavisnosti od tipa oprašivanja. Ispitivani su hibridi: 38/62/70 ('Hall' x 'California Blue'), IV/63/81 ('Large Sugar Prune' x 'Scoldus'), 32/21/87 ('Stanley' x 'Scoldus'), 22/17/87 ('Čačanska najbolja' x 'Zh'lta Butilcovidna'), 29/29/87 ('Stanley' x 'Scoldus') i 34/41/87 ('Valjevka' x 'Čačanska lepatica'). Inicijalno i finalno zemetanje plodova ispitivani su u varijantama samooprašivanja i slobodnog oprašivanja. Istovremeno je obavljeno ispitivanje klijavosti polena *in vitro*, karakteristika fenofaze cvetanja i obilnosti cvetanja. Generalno se uočava niža obilnost cvetanja u toku druge godine ispitivanja. Klijavost polena je varirala od prosečno 25,31% (hibrid 29/29/87) do 40,01% (hibrid 38/62/70). Sa prosečno 31,59% finalno zemetnutih plodova u varijanti samooprašivanja i 29,38% u varijanti slobodnog oprašivanja hibrid 34/41/87 je dao najbolje rezultate, dok su najslabiji rezultati sa prosečno 1,61% finalno zemetnutih plodova u varijanti samooprašivanja i 7,69% u varijanti slobodnog oprašivanja dobijeni kod hibrida 32/21/87.

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