Original scientific paper
10.7251/AGSY1505378M

INFLUENCE OF TEMPERATURE ON POLLEN GERMINATION AND POLLEN TUBE GROWTH OF PLUM CULTIVARS

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Abstract

The temperature is an important environmental factor that affects pollen performance during the reproductive phase of fruit tree species. Pollen germination is one of the main factors for successful fertilization and fruit set in plum (Prunus domestica L.). The study was carried out to determine the effect of three different temperatures (5, 15 and 25°C) on the pollen germination and pollen tube growth in vitro of six European plum cultivars: ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Jojo’, ‘Top’, ‘Toper’ and ‘Stanley’. Germination rate and pollen tube growth were determined on a culture medium containing 15% sucrose and 0.7% agar. Temperature significantly affected pollen germination of all studied cultivars. High germination rates (50–76%) were obtained at the temperatures of 15°C and 25°C. However, satisfactory germination rates (29–47%) were also obtained at the temperature of 5°C in some cultivars (‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’). The influence of temperature was more prominent on the pollen tube growth. The length of pollen tubes was six to twelve times higher at 15°C and 25°C in comparison with 5°C. This has led to the conclusion that the temperature of 5°C, although it could be enough for pollen germination, is not enough for optimal pollen tube growth.

Keywords: *Prunus domestica*, pollen germination *in vitro*, pollen tube length.

Introduction

Pollen viability and its germination capacity is one of the main factors for successful fertilization in fruit trees. It is important for both breeding work and choice of the varietal composition in orchards.

Parfitt and Ganeshan (1989) studied seven different tests to evaluate pollen viability in several species of the genus *Prunus.* They concluded that two *in vitro* germination tests (hanging-drop and agar-plate) were the most reliable.

Temperature is the most important environmental factor affecting the success of fertilization and fruit set of stone fruit species. It affects different stages of a reproductive process, such as a stigmatic receptivity (Hedhly et al., 2003; Hedhly et al., 2005), pollen germination (Keulemans, 1984; Egea et al., 1992; Pirlak, 2002; Hedhly et al., 2004), pollen tubes growth (Cerović and Ružić, 1992; Hedhly et al., 2005; Milatović and Nikolić, 2014), and ovule viability (Stöser and Anvari, 1982; Postweiler et al., 1985; Cerović et al., 2000).

Keulemans (1984) examined the effect of six different temperatures (4, 6, 9, 12, 15 and 18°C) on pollen germination and pollen tube growth of six plum cultivars. The examined cultivars had different reaction on temperature. Cultivars ‘Czar’, ‘Opal’ and ‘Victoria’ were characterized by good germination and fast growth of pollen tubes at low temperatures.

The aim of this study was to determine the effect of temperature on pollen germination and pollen tube growth in six cultivars of European plum.

Materials and methods

Six plum cultivars: ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Jojo’, ‘Top’, ‘Toper’ and ‘Stanley’ were used as a material for testing the pollen performance. Plant material was taken from a collection orchard of the Experimental farm “Radmilovac” of the Faculty of Agriculture in Belgrade. The orchard was planted in 2009. The rootstock was Myrobalan (*Prunus cerasifera* Ehrh.) seedlings, the crown form was Central Leader, and tree spacing was 4.5 × 3 m.

To collect pollen samples, twigs with flower buds in the “balloon” stage were taken from the experimental orchard. The twigs were transported to the laboratory where anthers were isolated from the flower buds. They were left to desiccate and release pollen in open Petri dishes for 24–48 h. Pollen was sowing using a fine brush in Petri dishes on previously prepared medium which consisted of 0.7% (w/v) agar-agar and 15% (w/v) sucrose. Petri dishes were then transferred to incubators ‘FOC 225I’ (Velp Scientifica, Usmate, Italy) at three different temperatures: 5, 15 and 25°C. After incubation of 24 hours, 40% (v/v) formaldehyde was added in Petri dishes to prevent further growth of pollen tubes.

Petri dishes with sowed pollen were observed under light microscope ‘Leica DM LS’ (Leica Microsystems, Wetzlar, Germany). The number of germinated and non-germinated pollen grains was counted and the percentage of germination was calculated. Petri dishes were divided into three parts, each part representing one repetition. In each repetition at least 300 pollen grains were analysed. Pollen was considered germinated if the length of the pollen tube was greater than the diameter of the pollen grain.

Pollen tube length was measured in pictures taken under the microscope ‘Leica DM LS’ using the ‘Leica IM 100’ programme. From all experiment variants (cultivars and temperatures) 60 pollen tubes were measured.

The experiment was conducted as two-factorial (cultivar, temperature), with three repetitions. The data were statistically analysed using the analysis of variance. Percentage data were subjected to arcsine square root transformation before the statistical analysis. Duncan’s multiple range test (5%) was performed for comparing means.

Results and discussion

Pollen germination ranged from 15.4% in the cultivar ‘Jojo’ at the temperature of 5°C to 76.3% in the cultivar ‘Cacanska Lepotica’ at the temperature of 25°C (Figure 1). In all studied cultivars pollen germination was significantly lower at the temperature of 5°C than at higher temperatures (15 and 25°C). Differences in pollen germination between temperatures of 15°C and 25°C were not statistically significant.

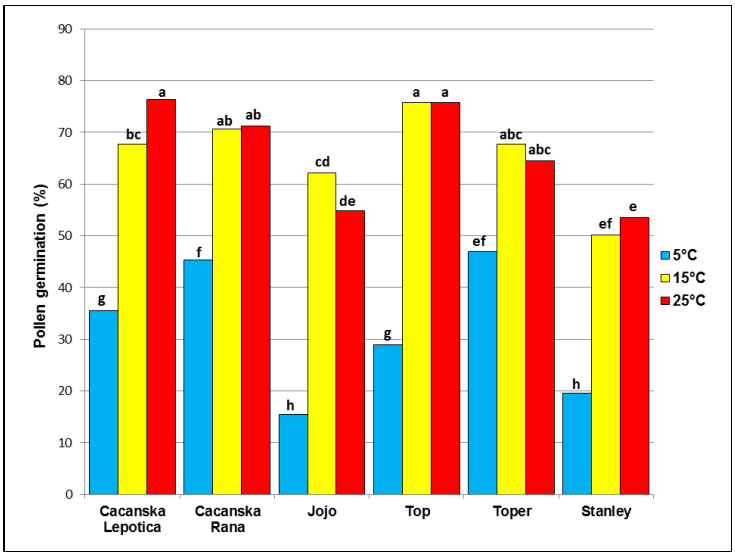

Figure 1. The influence of temperature on pollen germination of plum cultivars.

Differences between the cultivars were also significant (Table 1). Cultivars ‘Cacanska Lepotica’, ‘Cacanska Rana’, ‘Top’ and ‘Toper’ have higher germination rates (60-62% in average for all temperatures) comparing to cultivars ‘Jojo’ and ‘Stanley’ (41-44% in average).

Table 1. Analysis of variance for pollen germinatin and pollen tube length.

Source of variation	Pollen germination		Pollen tube length	
	df	Mean squares	df	Mean squares
Cultivar (G)	5	45.57**	5	2.12$^{n.s.}$
Temperature (T)	2	430.50**	2	437.91**
G x T	10	9.64**	10	1.55$^{n.s.}$
Error	36	17.07	36	125.477,4

**: statistically significant at 0.01 probability level; $^{n.s.}$ non-significant.

Cultivars ‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’ had significantly higher germination rates at the temperature of 5°C compared to ‘Jojo’ and ‘Stenley’. This could point to their better adaptation to colder weather conditions during flowering.

Previous research has found that pollen germination depends on the genotype. Different authors have found great variation in the degree of pollen germination *in vitro* in European plum cultivars: Botu et al. (2002) 12-61%, Surany (2006) 25-64%, Koskela et al. (2010) 3-70%, Sharafi (2011) 46-72%, Nikolić et al. (2012) 30-67%. The results obtained in this study are within the specified values.

According to the states of Wertheim (1996) that pollen germination of 25% is considered as a threshold for satisfactory germination in plum, it can be said that the studied cultivars are characterized by good pollen germination rates. Therefore, they can be recommended as potential pollenizers for other cultivars.

The minimum length of pollen tubes was obtained in cultivar ‘Jojo’ at the temperature of 5°C and it was 127 µm, while the maximum length was obtained in ‘Stanley’ at 25°C and it was 1727 µm (Figure 2).

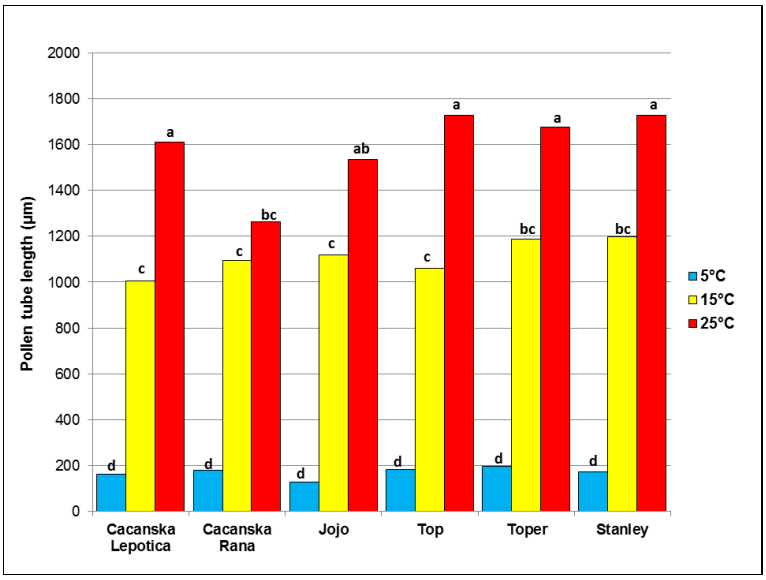

Figure 2. The influence of temperature on pollen tube length of plum cultivars.

The average values of pollen tubes length were the highest at 25°C (1589 µm), then at 15°C (1111 µm), while they were the lowest at 5°C (170 µm). The length of pollen tubes at temperature of 15°C was significantly higher than at the temperature of 5°C in all studied cultivars. Also, the length of pollen tubes at temperature of 25°C was significantly higher than at the temperature of 15°C in all cultivars, except ‘Cacanska Rana’. Differences between cultivars, as well as interaction Genotype × Temperature were not statistically significant (Table 1).

The average values of pollen tube length obtained in this study were higher than the values obtained by Sharafi (2011). Increasing of the temperature resulted in a significant increase in the length of pollen tubes, which confirms the results of previous studies (Cerović and Ružić, 1992; Pirlak, 2002; Hedhly et al., 2004; Milatović and Nikolić, 2014).

Conclusion

Temperatures of 15 and 25°C are optimal for pollen germination and pollen tube growth of European plums. However, the rates of pollen germination at 5°C were higher in some cultivars (‘Toper’, ‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Top’) than in others (‘Jojo’ and ‘Stanley’). Therefore, these cultivars could be better adapted to the low temperatures during flowering.

The influence of temperature was more prominent on the pollen tube growth. The length of pollen tubes was six to nine times higher at 15°C and seven to twelve times higher at 25°C in comparison with 5°C. This leads to the conclusion that the temperature of 5°C, although it could be enough for pollen germination, is not enough for optimal pollen tube growth.

Acknowledgements

This study was supported by the Ministry of Education and Science of the Republic of Serbia through the project TR 31063, and also by the EU FP7 project 316004 (project acronym AREA).

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