

***In vitro* pollen germination and pollen tube length as indicators of temperature effects on sweet cherry genotypes**

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Abstract. This study evaluated the effects of temperature on *in vitro* pollen germination and pollen tube growth in eight sweet cherry genotypes ('Burlat', 'Canetova', 'Dolga Šiška', 'G-2', 'Kordia', 'Lapins', 'Rita', and 'Sunburst') over two consecutive years (2022–2023). Experiments were conducted at 17°C and 20°C to assess genotypic variability and adaptability under changing climatic conditions. Results revealed considerable variability among genotypes: pollen germination ranged from 9.3% ('Kordia') to 53.9% ('Lapins'), while pollen tube length varied from 180.8 µm ('Dolga Šiška') to 669.3 µm ('Lapins'). Germination was generally higher at 17°C than at 20°C, contrary to expectations. 'Lapins', 'Canetova', and 'G-2' maintained stable pollen germination and tube growth across years, highlighting their potential as reliable pollenisers. In contrast, 'Dolga Šiška' and 'Sunburst' showed drastic reductions under less favourable conditions, confirming their sensitivity to environmental stress. Analysis of variance indicated that genotype was the dominant factor for pollen tube growth, while strong genotype × year interaction was the main determinant of pollen germination. These findings demonstrate that pollen performance is a valuable indicator of reproductive resilience, and they identify genotypes suitable for orchard planning to secure fruit set and yield stability under climate change.

Key words: *Prunus avium*, pollen viability, components of variability, climate change resilience

Introduction

Air temperature is an important climatic factor limiting the geographical distribution of plant species (Rosbakh & Poschlod, 2016). It is expected to increase globally by 1.4°C (in the 'low greenhouse gas emissions scenario') to 4.4°C (in the 'very high greenhouse gas

emissions scenario') by the end of this century, with frequent temperature extremes (IPCC, 2023). Considering these findings and the predicted climate change scenarios there is a risk that cherries will advance their phenological stages making them more exposed to increased risk of damage due to late frosts (Păltineanu & Chițu, 2020).

The apparent climate warming in Europe, including the Balkan region, leads to earlier flowering and an increased risk of frost (Drkenda *et al.*, 2018; Branchereau *et al.*, 2023). In Serbia, average annual temperatures have already risen by 1–1.5°C since the middle of the 20th century, with projections of 3–5°C by 2100 (Vuković *et al.*, 2018; Vujadinović Mandić *et al.*, 2022).

Rosaceae species, including sweet cherry (*Prunus avium* L.), have actinomorphic and hermaphrodite flowers that require pollination to produce fruit (Beltrán *et al.*, 2019). Fertilization occurs when the pollen grain adheres on the stigma, emits a pollen tube and reaches the ovule (Monselise, 2018). As the lifespan of the ovules is limited, fertilisation must take place within the effective pollination period (Sanzol & Herrero, 2001). Reproductive phases, such as pollen germination, pollen tube growth and fruit set, are among the most temperature-sensitive and vulnerable stages (Hedhly *et al.*, 2009; Zinn *et al.*, 2010; Bykova *et al.*, 2012; Hsu & Kim, 2025). Temperature dependence of pollen germination and tube growth shows species- and cultivar-specific responses, with optimal temperatures typically ranging from 20°C to 30°C for many plants, and minimum and maximum cardinal temperatures also varying. Higher temperatures accelerate the process of pollen tube growth, while extreme high or low temperatures inhibit it, impacting reproductive success and geographic distribution (Hsu & Kim, 2025). Higher temperatures also accelerate ovule degeneration, thereby shortening the effective pollination period and reducing fruit set (Hedhly *et al.*, 2005).

Appropriate orchard design, synchronisation of flowering and high pollen productivity of pollenisers are therefore crucial (Nyéki *et al.*, 2008; Nikolić *et al.*, 2012). There is considerable genotypic variation in cherry pollen germination rates, ranging from 6% to over 70% (Pirlak, 2002; Radičević *et al.*, 2008; Milatović & Nikolić, 2017). This trait is influenced by both genetic factors (Rakonjac *et al.*, 2024) and environmental stresses such as high temperatures and drought stress during microsporogenesis or anthesis (Popovska *et al.*, 2005). Understanding pollen performance under fluctuating thermal conditions is crucial as reproductive processes represent some of the most temperature-sensitive stages in sweet cherry ontogeny. Pollen germination and elongation of pollen tubes can therefore be considered as reliable indicators of climate resist-

ance, providing valuable criteria for the selection and breeding of genotypes capable of maintaining stable productivity under changing environmental conditions (Hedhly, 2011; Sharafi, 2011; Radičević *et al.*, 2025).

The aim of this study was therefore to determine the effects of temperature on pollen germination and pollen tube growth *in vitro* in eight sweet cherry cultivars and to identify possible differences in genotypic response that could help in the selection of cultivars for specific locations.

Materials and Methods

Site and plant material. A two-year study (2022–2023) examined flowering phenology and pollen functionality in eight sweet cherry genotypes (*Prunus avium* L.), including two genotypes selected in the Republic of Serbia ('Canetova' and 'G-2'), five introduced cultivars ('Burlat', 'Kordia', 'Lapins', 'Rita', and 'Sunburst'), and one autochthonous cultivar from North Macedonia ('Dolga Šiška'). The trials were carried out at cherry gene-bank collection of the Faculty of Agriculture, University of Belgrade, at the experimental site 'Radmilovac' (44°45' N; 20°35' E; 130 m altitude) for 'G-2', 'Lapins', and 'Rita'; cherry orchard at experimental site 'Ljubić' of the Fruit Research Institute, Čačak (43°54' N; 20°18' E; 242 m altitude) for 'Canetova' and 'Burlat'; commercial cherry orchard in Ohrid, North Macedonia (41°07' N; 20°48' E; 695 m altitude) for 'Dolga Šiška', 'Kordia', and 'Sunburst'. The standard cultivation practices (pruning, fertilisation, pest and disease control) were applied in the orchards.

Air temperature monitoring. Air temperature in the orchard was recorded from January to April, and average mean, maximum and minimum daily temperatures were calculated for the flowering period. The flowering period was defined by the beginning, full flowering and end of flowering (growth stages 61, 65 and 69, respectively, according to the BBCH scale; Meier, 2018). Measurements were taken at a height of 1.5 m using an automatic MeteosCompact weather station (Pessl Instrument GmbH, Weiz, Austria) placed in the immediate vicinity of the experimental orchards.

Pollen performance in vitro at different temperature regimes. Branches with flower buds at the 'balloon' stage were collected and transported to the laboratory,

where the anthers were removed to Petri dishes and dried at room temperature (24–48 h) until the pollen grains were released. The pollen of each genotype was placed in three Petri dishes (three replicates) on a medium containing 0.7% agar and 15% sucrose. The dishes were then placed in FOC 225I incubators (Velp Scientifica, Usmate, Italy) at two different temperatures (17°C and 20°C). After an incubation period of 24 hours, pollen germination and tube growth were stopped by adding formaldehyde.

At least 300 pollen grains were counted in each replication to determine the germination percentage. The pollen was considered germinated if the length of the pollen tube was greater than the pollen grain diameter. For pollen tube length, 20 randomly selected pollen tubes were measured per replication. Observations were conducted using a Leica DM2000 light microscope equipped with a Leica DFC320 digital camera, and a computer system running Leica IM100 image processing software (Leica Microsystems, Wetzlar, Germany).

Statistical analysis. The experiment was designed as a three-factorial (genotype, temperature and year). For in vitro pollen germination, whose values are expressed as a percentage, a data transformation of the arcsine square root was performed. Data were statistically analysed by analysis of variance (ANOVA) and Tukey test for post-hoc comparisons using the statisti-

cal software package STATISTICA, version 8 (StatSoft Inc., Tulsa, OK, USA). The variance components were calculated based on the expected MS from the three-factorial ANOVA and expressed as percentages (Annicchiarico, 2002).

Results and Discussion

Monitored flowering phenology is summarized in Figure 1. The earliest onset of flowering was observed in ‘G-2’ (last week of March, Belgrade), while the latest occurred in ‘Dolga Šiška’ (last week of April, Ohrid). At the Čačak and Belgrade sites, flowering stages in 2023 occurred earlier than in 2024, influenced by air temperatures (Table 1). A lower number of frosty days and slightly higher temperatures in March advanced flowering onset by seven days in Čačak and five days in Belgrade, depending on genotype. Flowering duration was prolonged in 2023 at Belgrade by an average of 12 days, lasting from 23 days (‘G-2’) to 27 days (‘Lapins’), mainly due to lower daily temperatures in April (Table 1). At the Ohrid locality, no interannual differences in flowering phenology were observed. Although locality was the key factor influencing flowering, similar phenological patterns were observed across both years.

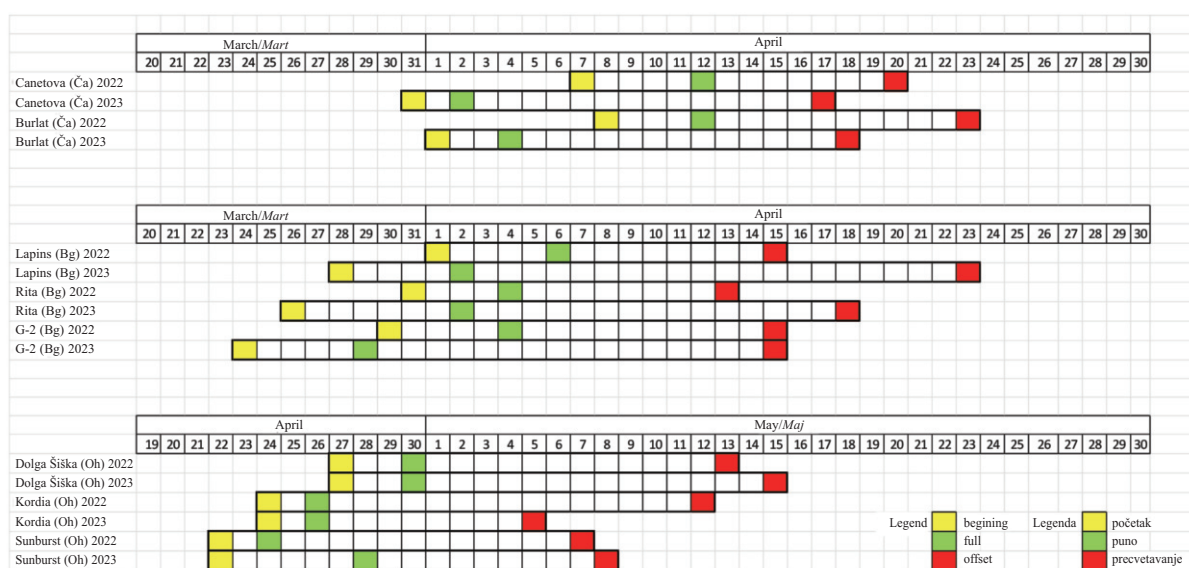


Figure 1. Flowering phenology of sweet cherry genotypes (2022–2023)

Slika 1. Fenologija cvetanja genotipova trešnje (2022–2023)

Table 1. Air temperatures from January to April at the experimental sites Belgrade, Čačak and Ohrid (2022–2023)

Tabela 1. Temperatura vazduha u periodu januar–april na eksperimentalnim lokalitetima u Beogradu, Čačku i Ohridu (2022–2023)

Climatic parameter <i>Klimatski parametar</i>	Year <i>Godina</i>	Belgrade/Beograd				Čačak/Čačak				Ohrid/Ohrid			
		January <i>Januar</i>	February <i>Februar</i>	March <i>Mart</i>	April <i>April</i>	January <i>Januar</i>	February <i>Februar</i>	March <i>Mart</i>	April <i>April</i>	January <i>Januar</i>	February <i>Februar</i>	March <i>Mart</i>	April <i>April</i>
Mean air temperature (°C) <i>Prosečna temperatura vazduha (°C)</i>	2022	2.8	7.6	7.2	12.5	0.5	4.1	4.4	10.9	1.9	4.9	3.6	10.5
	2023	4.9	3.9	9.0	10.3	3.4	3.1	7.8	9.5	3.89	2.8	7.4	8.9
Minimum air temperature (°C) <i>Minimalna temperatura vazduha (°C)</i>	2022	-8.8	-0.8	-5.4	0.8	-16.4	-6.8	-9.9	-3.8	-12.0	-4.7	-9.7	-1.9
	2023	-0.9	-9.2	-3.8	-0.6	-3.8	-10.8	-6.2	-1.6	-5.2	-11.2	-4.8	-2.9
Maximum air temperature (°C) <i>Maksimalna temperatura vazduha (°C)</i>	2022	6.1	18.5	23.0	25.5	15.6	18.5	22.3	28.1	13.5	19.5	22.4	28.9
	2023	16.9	19.0	24.1	23.0	16.9	21.9	26.4	25.5	15.2	24.9	22.3	23.3
Number of frosty days <i>Broj dana sa mrazom</i>	2022	17.0	1.0	9.0	0.0	26.0	10.0	26.0	10.0	23.0	23.0	27.0	5.0
	2023	5.0	12.0	6.0	4.0	13.0	21.0	11.0	5.0	25.0	25.0	11.0	4.0

In vitro pollen germination (Table 2) showed considerable variability among the sweet cherry genotypes, ranged from 9.28% for ‘Kordia’ at both 17°C and 20°C in 2023 to 53.85% for ‘Lapins’ at 17°C in 2023. Satisfactory germination rates (around 50%) were obtained for ‘Burlat’ at 17 °C in both years and for ‘Lapins’ at 17 °C in 2023. In 2022, the highest average values were recorded for ‘Burlat’ (49.01%), followed by ‘Sunburst’ (36.66%), while the ranking of genotypes shifted markedly in 2023. In that year, ‘Lapins’ (46.70%) and ‘Rita’ (42.11%) achieved the highest averages, performing better than in previous year, which suggest some resilience or adaptability to the prevailing conditions. In contrast, the pronounced decrease observed in ‘Dolga Šiška’ (10.08%) and ‘Sunburst’ (10.75%) indicates that these genotypes are more sensitive to unfavourable temperature regimes,

potentially limiting their suitability in scenarios of increasing climate variability.

When comparing the years, a decrease in pollen germination was observed in four genotypes in 2023, which the largest decline recorded for ‘Sunburst’ (-25.91%). In contrast, four genotypes exhibited improvements, with ‘Rita’ showing the greatest increase (+30.45%). Genotypes such as ‘Burlat’ and ‘G-2’ maintained relatively stable performance, with only minor differences between years. ‘Burlat’ also displayed the lowest variation in pollen germination across temperature treatments (from 49.01% to 48.04%), whereas ‘Rita’ showed the highest variation (from 11.66% to 42.11%). The consistent performance of ‘Burlat’ and ‘G-2’ suggest their potential as cultivars capable of maintaining pollen viability under variable environmental conditions.

Table 2. Pollen germination (%) of eight sweet cherry genotypes at 17 °C and 20 °C over two years (2022–2023)

Tabela 2. Klijavost polena (%) kod osam genotipova trešnje na temperaturama od 17 °C i 20 °C tokom dve godine (2022–2023)

Genotype/Genotip	2022			2023		
	17°C	20°C	Average/Prosek	17°C	20°C	Average/Prosek
‘Burlat’	51.49	46.53	49.01	52.84	43.25	48.04
‘Canetova’	36.63	27.75	32.19	25.73	28.56	27.14
‘Dolga Šiška’	42.99	26.22	34.60	10.62	9.55	10.08
‘G-2’	36.54	24.93	30.74	34.74	31.85	33.29
‘Kordia’	9.28	9.28	9.28	14.13	9.93	12.03
‘Lapins’	28.31	17.33	22.82	53.85	39.55	46.70
‘Rita’	16.83	6.50	11.66	42.39	41.82	42.11
‘Sunburst’	39.53	33.79	36.66	13.22	8.28	10.75
Average/Prosek	32.70	24.04	28.37	30.94	26.60	28.77

Previous studies have reported considerable variation in *in vitro* pollen germination in sweet cherry, ranging from 13 to 63% (Bolat & Pirlak, 1999; Hormaza & Herrero, 1999; Pirlak, 2002; Tosun & Koyuncu, 2007; Radičević *et al.*, 2008; Beyhan & Karakas, 2009; Rakonjac *et al.*, 2024). Based on earlier findings by Nenadovic-Mratinic (1996), Pirlak (2002) and Milatović & Nikolić (2017), who investigated the effect of 15, 25 and 35°C on *in vitro* pollen germination in sweet and sour cherry cultivars, higher germination at 20°C was expected. In contrast, in our study, higher pollen germination was observed at 17°C in both years, with the exception of ‘Canetova’ in 2023. This result suggests that the evaluated genotypes may have developed adaptability to lower temperatures.

In vitro pollen tube growth (Table 3) exhibited considerable variability among sweet cherry genotypes and across years. Pollen tube lengths ranged from 180.8 µm for ‘Dolga Šiška’ at 20 °C in 2023 to 669.3 µm for ‘Lapins’ at 17°C in 2022. The highest average values in 2022 were recorded for ‘Lapins’ (621.5 µm), followed by ‘Sunburst’ (613.5 µm) and ‘Canetova’ (594.9 µm). In 2023, pollen tube lengths decreased in all eight genotypes, with the largest reductions observed in ‘Dolga Šiška’ (-57.6%) and ‘Sunburst’ (-46.5%), indicating their pronounced sensitivity to unfavourable conditions. In contrast, ‘Lapins’ (-2.2%) and ‘Burlat’ (-5.9%) remained highly stable across years.

Although expected, in our study, *in vitro* pollen tube length did not correspond with pollen germination, as previously reported by Milatović & Nikolić

(2017). Our observation reveals the shortest tubes (average 324.8 µm) in ‘Burlat’, which exhibited the highest pollen germination (48.5%). This indicated that even high pollen germination percentages do not necessarily guarantee rapid pollen tube elongation. Similar discrepancies have been reported in other fruit tree species (Miaja *et al.*, 2000; Sharafi, 2011), suggesting that these two traits are genetically independent.

Previous research has shown that temperature has a greater effect on pollen tube elongation than on pollen germination. In peach, Hedhly *et al.* (2004) reported that temperature slightly influenced the final germination percentage but had a pronounced effect on the rate of germination. Within the temperature range studied in the laboratory (10, 20 and 30°C), an accelerating effect of increasing temperature on both pollen germination and pollen tube growth kinetics was observed at 20°C. Significant increases in pollen tube length with higher temperatures were also reported by Cerović & Ružić (1992) and Pirlak (2002). In contrast, our study does not support these findings (Table 3), as pollen tubes were longer at 17°C (599.1 µm) compared with 20°C (552.5 µm).

As shown in Table 4 and Figure 2, the ANOVA results indicate a significant effect of genotype and temperature on pollen germination. Additionally, significant effects of the $G \times Y$ and $G \times Y \times T$ interactions on pollen germination were observed.

Differences among genotypes were highly significant, reflecting considerable genetic variability in pollen germination. Overall, genotype alone had a smaller effect, explaining approximately 7% of the

Table 3. Pollen tube length (µm) of eight sweet cherry genotypes at 17°C and 20°C over two years (2022–2023)

Tabela 3. Dužina polenove cevčice (µm) kod osam genotipova trešnje na temperaturama od 17°C i 20°C tokom dve godine (2022–2023)

Genotype/Genotip	2022			2023		
	17°C	20°C	Average/Prosek	17°C	20°C	Average/Prosek
‘Burlat’	367.4	302.3	334.8	251.2	378.4	314.8
‘Canetova’	580.8	609.0	594.9	500.5	499.6	500.0
‘Dolga Šiška’	485.2	494.9	490.0	234.3	180.8	207.6
‘G-2’	559.8	593.3	576.6	325.4	424.8	375.1
‘Kordia’	502.6	429.7	466.2	328.0	332.5	330.2
‘Lapins’	669.3	573.6	621.5	579.2	635.7	607.5
‘Rita’	402.7	374.0	388.3	288.4	290.8	289.6
‘Sunburst’	562.4	664.7	613.5	336.5	319.4	328.0
Average/Prosek	516.3	505.2	510.7	355.4	382.7	369.1

Table 4. Mean squares (MS) and variance components (%) from three-factor ANOVA for pollen germination and pollen tube length
Table 4. Sredine kvadrata (MS) i komponente varijanse (%) dobijene trofaktorijalnom ANOVA analizom za klijavost polena i dužinu polenovih cevčica

Source of variations Izvor varijacije	df	Pollen germination Klijavost polena		Pollen tube length Dužina polenove cevčice	
		MS	%	MS	%
Genotype (G)/Genotip (G)	7	1410.79**	7.3	233302.9**	52.6
Year (Y)/Godina (Y)	1	2.26 ns	0.0a	281413.1**	15.9
Temperature (T)/Temperatura (T)	1	986.11**	7.4	1576.0 ns	0.0
G × Y	7	1202.48**	75.5	33978.9**	13.2
G × T	7	36.72**	0.0	3882.6 ns	0.1
Y × T	1	102.34**	0.8	8841.0*	0.0
G × Y × T	7	51.81**	5.7	8435.9*	3.9
Error/Greška	64	8.18	3.2	4615.3	14.3

df – Degrees of freedom / Stepni slobode; a – Negative value is expressed as 0.0 / Negativna vrednost je izražena kao 0,0; *Significant difference for $p \leq 0.05$ / Značajna razlika za $P \leq 0,05$; **Very significant difference for $P \leq 0.01$ / Veoma značajna razlika za $P \leq 0,01$; ns – Not significant / Nije značajno.

total variation. Similarly, temperature exerted a significant influence, also accounting for around 7% of the variation, consistent with previous studies showing that temperature affects germination (Hedhly et al., 2004; Beltrán et al., 2019). The better adaptation of certain cultivars to lower temperatures during flowering may indicate their wider geographical adaptability (Milatović & Nikolić, 2017).

Interestingly, the year effect was not significant as a main factor, but the G × Y interaction was highly significant, accounting for the largest portion of the variance (75.5%). This interaction indicates that pollen germination capacity of individual genotypes var-

ied substantially between years, likely reflecting seasonal influences such as differences in flower development and pollen maturity at anthesis. Similar G × Y interaction effects have been reported in other multi-season studies of sweet cherry (Radičević et al., 2016). Smaller but significant contributions were also observed from the three-way interaction (G × Y × T), highlighting that temperature responses were not uniform across genotypes and seasons.

For pollen tube length, ANOVA revealed that genotype was the dominant factor, accounting for more than half of the explained variation (Table 4; Figure 3). This indicates strong genetic control over pollen

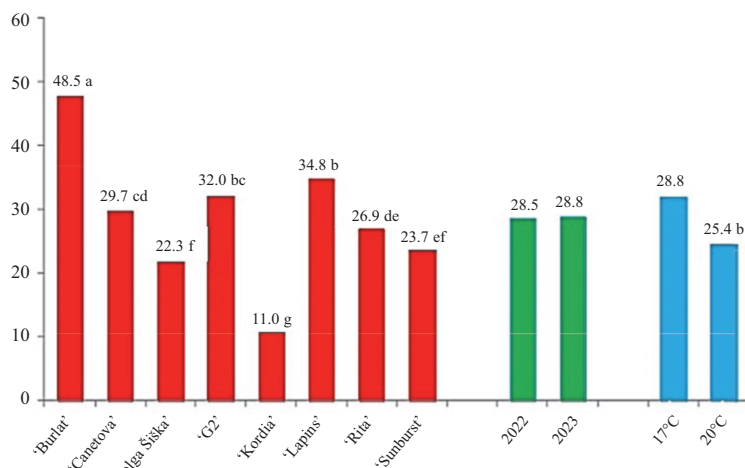


Figure 2. Mean values for main factors (genotype, year and temperature) for *in vitro* pollen germination; different letters indicate statistically significant differences ($p < 0.05$) according to Tukey test

Slika 2. Srednje vrednosti glavnih faktora (genotip, godina i temperatura) za *in vitro* klijanje polena; različita slova označavaju statistički značajne razlike ($P < 0,05$) prema Tukey-testu

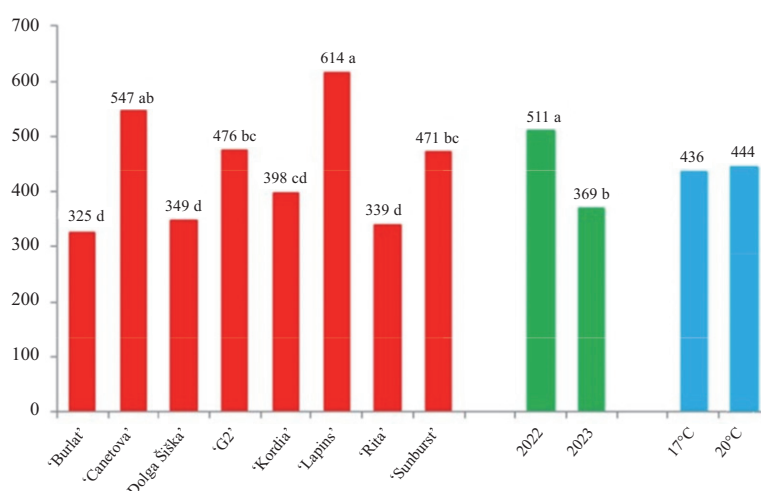


Figure 3. Mean values for main factors (genotype, year and temperature) for pollen tube length; different letters indicate statistically significant differences ($p < 0.05$) according to Tukey test

Slika 3. Srednje vrednosti glavnih faktora (genotip, godina i temperatura) za dužinu polenove tube; različita slova označavaju statistički značajne razlike ($P < 0,05$) prema Tukey-testu

tube elongation capacity, consistent with cultivar-specific physiological governing growth rate. Comparable genotype-driven differences in tube growth have been documented by Hedhly *et al.* (2004) and Acar & Kakani (2010). Unlike pollen germination, the year effect was significant, explaining nearly 16% of the variation, suggesting that seasonal factors directly influenced pollen tube growth performance.

This finding aligns with earlier reports that pollen tube elongation can vary considerably between years (Zhang *et al.*, 2018). In contrast, temperature, which played a major role in germination, did not contribute significantly as a main effect for tube length in the present dataset. However, significant $G \times Y$ interactions (13%) were observed, demonstrating that pollen tube growth responses were not stable across years for all genotypes. Radičević *et al.* (2016) also reported an interaction between temperature and cultivar, supporting this observation. A smaller but significant three-way interaction ($G \times Y \times T$; 3.9%), further supports the opinion that tube elongation kinetics are influenced by complex, genotype-specific, and environment-dependent dynamics. These findings are consistent with previous reports suggesting that temperature effects on pollen tube growth can be either masked or amplified depending on cultivar and environmental conditions (Hedhly *et al.*, 2004; Beltrán *et al.* 2019).

Conclusion

The results of this study underscore the strong genetic influence on pollen performance in sweet cherry, as evidenced by genotype-specific differences in both germination capacity and tube elongation. Among the analysed genotypes, 'Lapins' consistently exhibited high pollen germination rates and long pollen tubes across years, while 'Canetova' and 'G-2' also displayed relatively stable and moderate-to-high values for both traits. These genotypes demonstrated resilience to interannual variation in environmental conditions and maintained reproductive efficiency, making them promising candidates for reliable pollenisers in orchard design. In contrast, 'Dolga Šiška' and 'Sunburst' showed marked reductions in germination and tube growth under less favourable conditions, indicating greater sensitivity to environmental fluctuations.

In the context of ongoing climate change, characterised by increasingly frequent spring heat events and erratic weather patterns, identifying of genotypes that sustain pollen viability and tube growth under variable environmental conditions is of practical and strategic importance. Future research should aim to elucidate the specific pre-anthesis environmental factors driving the genotype $\times$ year ($G \times Y$) interaction and to further unravel the genetic mechanisms underlying the stable performance of resilient genotypes.

Acknowledgments

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References

- Acar I., Kakani V.G. (2010): The effects of temperature on in vitro pollen germination and pollen tube growth of *Pistacia* spp. *Scientia Horticulturae*, 125(4): 569–572.
- Annicchiarico P. (2002): Genotype × environment interactions: challenges and opportunities for plant breeding and cultivar recommendations. *FAO Plant Production and Protection Paper No. 174*, FAO, Rome, Italy.
- Beltrán R., Valls A., Cebrián N., Zornoza C., García Breijo F., Reig Armiñana J., Garmendia A., Merle H. (2019): Effect of temperature on pollen germination for several *Rosaceae* species: influence of freezing conservation time on germination patterns. *PeerJ*, 7: e8195.
- Beyhan N., Karakas B. (2009): Investigation of the fertilization biology of some sweet cherry cultivars grown in the Central Northern Anatolian region of Turkey. *Scientia Horticulturae*, 121(3): 320–326.
- Bolat I., Pirlak L. (1999): An investigation on pollen viability, germination and tube growth in some stone fruits. *Turkish Journal of Agriculture and Forestry*, 23(4): 383–388.
- Branchereau C., Hardner C., Dirlewanger E., Wenden B., Le Dantec L., Alletru D., Parmentier J., Ivančič A., Giovannini D., Brandi F., Lopez-Ortega G., Garcia-Montiel F., Quilot-Turion B., Quero-García J. (2023): Genotype-by-environment and QTL-by-environment interactions in sweet cherry (*Prunus avium* L.) for flowering date. *Frontiers in Plant Science*, 14: 1142974.
- Bykova O., Chuine I., Morin X., Higgins S.I. (2012): Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography*, 39: 2191–2200.
- Cerović R., Ružić Đ. (1992): Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. *Journal of Horticultural Science*, 67: 333–340.
- Drkenda P., Musić O., Marić S., Jevremović D., Radičević S., Hudina M., Hodžić S., Kunz A., Blanke M.M. (2018): Comparison of climate change effects on pome and stone fruit phenology between Balkan countries and Bonn/Germany. *Erwerbs-Obstbau*, 60: 295–304.
- Hedhly A. (2011): Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany*, 74: 9–16.
- Hedhly A., Hormaza J.I., Herrero M. (2004): Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). *American Journal of Botany*, 91(4): 558–564.
- Hedhly A., Hormaza J.I., Herrero M. (2005): Influence of genotype-temperature interaction on pollen performance. *Journal of Evolutionary Biology*, 18(6): 1494–1502.
- Hedhly A., Hormaza J.I., Herrero M. (2009): Global warming and sexual plant reproduction. *Trends in Plant Science*, 14: 30–36.
- Hormaza J.I., Herrero M. (1999): Pollen performance as affected by the pistilar genotype in sweet cherry (*Prunus avium* L.). *Protoplasma*, 208(1/4): 129–135.
- Hsu H.W., Kim S.H. (2025): Temperature dependence of pollen germination and tube growth in conifers relates to their distribution along an elevational gradient in Washington State, USA. *Annals of Botany*, 135(1/2): 277–292.
- IPCC (2023): Summary for Policymakers. In: 'Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, Core Writing Team', Lee H., Romero J. (eds.), IPCC, Geneva, Switzerland, pp. 1–34.
- Meier U. (2018): Growth stages of mono- and dicotyledonous plants: BBCH monograph. Open Agrar Repository, Quedlinburg, Germany.
- Miaja M.L., Radicati L., Porporato M., Caramiello R., Fossa V., Vallania R. (2000): Morpho-physiological observations on pollen of sour cherry (*Prunus cerasus* L.) cultivars. *Acta Horticulturae*, 514: 311–320.
- Milatović D., Nikolić D. (2017): The effect of temperature on pollen germination and pollen tube growth *in vitro* of sweet cherry cultivars. *Acta Horticulturae*, 1161: 401–404.
- Monselise S.P. (2018): Handbook of fruit set and development. CRC press, Boca Raton, Florida, USA.
- Nenadovic-Mratinic E. (1996): The influence of temperature, sucrose concentration and time on pollen germination and pollen tube growth in sour and sweet cherry. *Acta Horticulturae*, 410: 443–448.
- Nikolić D., Rakonjac V., Fotirić-Akšić M. (2012): The effect of pollenizer on the fruit set of plum cultivar Čačanska najbolja. *Journal of Agricultural Sciences (Belgrade)*, 57(1): 9–18.
- Nyékí J., Soltesz M., Szabo T. (2008): Establishment and production techniques of sour cherry orchards (1st ed.). University of Debrecen, Debrecen, Hungary.
- Păltineanu C., Chițu E. (2020): Climate change impact on phenological stages of sweet and sour cherry trees in a continental climate environment. *Scientia Horticulturae*, 261: 109011.
- Pirlak L. (2002): The effects of temperature on pollen germination and pollen tube growth of apricot and sweet cherry. *Gartenbauwissenschaft*, 67: 61–64.
- Popovska M., Angelova E., Popovski B. (2005): Microsporogenesis of sour cherries in Skopje region. *Acta Horticulturae*, 667: 111–116.
- Radičević S., Cerović R., Đorđević M., Marić S. (2008): The study of the flowering phenophase and pollen germination in newly developed sweet cherry. *Journal of Pomology*, 42(163/164): 89–95.

- Radičević S., Cerović R., Nikolić D., Đorđević M. (2016): The effect of genotype and temperature on pollen tube growth and fertilization in sweet cherry (*Prunus avium* L.). *Euphytica*, 209: 121–136.
- Radičević S., Marić S., Glišić I., Cerović R., Đorđević M., Milošević N., Rakonjac V., Čolić S., Popovska M., Gjamovski V., Banović Đeri B. (2025): Pollen–pistil interactions in autochthonous Balkan sweet cherry cultivars – the impact of genotype and flowering temperature. *Agronomy*, 15(3): 646.
- Rakonjac V., Nikolić D., Čolić S., Glišić I., Đorđević M., Popovska M., Radičević S. (2024): Investigation of pollen morphology and viability of sweet and sour cherry genotypes by multivariate analysis. *Microscopy Research and Technique*, 88(1): 42–52.
- Rosbakh S., Poschlod P. (2016): Minimal temperature of pollen germination controls species distribution along a temperature gradient. *Annals of Botany*, 117: 1111–1120.
- Sanzol J., Herrero M. (2001): The ‘effective pollination period’ in fruit trees. *Scientia Horticulturae*, 90: 1–17.
- Sharafi Y. (2011): *In vitro* pollen germination in stone fruit tree of Rosaceae family. *African Journal of Agricultural Research*, 6(28): 6021–6026.
- Tosun F., Koyuncu F. (2007): Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: Pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. *Horticultural Science*, 34: 47–53.
- Vujadinović Mandić M., Vuković Vimić A., Ranković-Vasić Z., Đurović D., Čosić M., Sotonica D., Nikolić D., Đurđević V. (2022): Observed changes in climate conditions and weather-related risks in fruit and grape production in Serbia. *Atmosphere*, 13(6): 948.
- Vuković A.J., Vujadinović M.P., Rendulić S.M., Đurđević V.S., Ruml M.M., Babić V.P., Popović D.P. (2018): Global warming impact on climate change in Serbia for the period 1961–2100. *Thermal Science*, 22(6A): 2267–2280.
- Zhang L., Pappas C., Whiting M.D., Shen Y. (2018): Temperature effects on pistil viability and fruit set in sweet cherry (*Prunus avium* L.). *Scientia Horticulturae*, 242: 106–113.
- Zinn K.E., Tunc-Ozdemir M., Harper J.F. (2010): Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, 61(7): 1959–1968.

KLIJAVOST POLENA I DUŽINA POLENOVIH CEVI U *IN VITRO* USLOVIMA KAO INDIKATORI UTICAJA TEMPERATURE NA GENOTIPOVE TREŠNJE**Slavica Čolić^{1*}, Sladana Marić², Milena Đorđević², Ivana Glišić², Sanja Radičević², Milan Lukić³, Vera Rakonjac⁴**¹*Institut za primenu nauke u poljoprivredi, Bulevar despota Stefana 68b, 11000 Beograd, Republika Srbija*^{*}*E-mail: scolic@ipn.co.rs*²*Institut za voćarstvo, Kralja Petra I 9, 32000 Čačak, Republika Srbija*³*Institut za proučavanje lekovitog bilja 'Dr Josif Pančić', 11000 Beograd, Republika Srbija*⁴*Univerzitet u Beogradu, Poljoprivredni fakultet, Nemanjina 6, 11080 Zemun – Beograd, Republika Srbija***Rezime**

U ovom istraživanju ispitivan je uticaj temperature na klijanje polena i rast polenovih cevčica u *in vitro* uslovima kod osam genotipova trešnje (Burlat, Canetova, Dolga Šiška, G-2, Kordia, Lapins, Rita i Sunburst) tokom dve godine (2022–2023). Eksperiment je sproveden u uslovima konstantnih temperatura (17°C i 20°C) radi procene varijabilnosti genotipova i njihove adaptabilnosti na različite temperaturne uslove. Rezultati su pokazali značajne razlike među genotipovima: klijavost polena se kretala od 9,3% (Kordia) do 53,9% (Lapins), dok je dužina polenovih cevi varirala od 180,8 µm (Dolga Šiška) do 669,3 µm (Lapins). Klijavost polena je generalno bila bolja na 17°C nego na 20°C. Genotipovi Lapins, Canetova i G-2 pokazali su stabilne rezultate za klijavost polena *in vitro* i dužinu polenovih cevčica tokom obe godine ispitivanja,

što ukazuje na njihov potencijal kao pouzdanih opravišača. Nasuprot tome, Dolga Šiška i Sunburst su imale drastično smanjenje oba parametra u godini sa nepovoljnijim uslovima, što potvrđuje njihovu osetljivost na temperaturni stres. Analiza varijanse je pokazala da je genotip bio dominantan faktor za porast polenove cevčice, dok je na klijavost polena najveći uticaj imala interakcija genotip × godina. Ovi nalazi potvrđuju da je performans polena *in vitro* vredan indikator reproduktivne otpornosti i ukazuju na genotipove trešnje pogodne za planiranje zasada radi obezbeđivanja oplodnje i stabilnosti prinosa u uslovima klimatskih promena.

Ključne reči: *Prunus avium*, vijabilnost polena, komponente varijabilnosti, otpornost na klimatske promene