ASSESSMENT OF SELF-(IN) COMPATIBILITY IN SOME SWEET CHERRY (*Prunus avium* L.) GENOTYPES

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The paper presents results of a three-year study of self-(in)compatibility in four economically important sweet cherry genotypes – 'Karina', 'Kordia', 'Regina' and 'Summit', under agro-environmental conditions of Western Serbia. Determination of *S-RNase* genotype, microscopic observation of the pollen tube growth rate and assaying of the fruit set level after self-pollination were used to assess the genotypes. 'Kordia' (S_3S_6), 'Regina' (S_1S_3) and 'Summit' (S_1S_2) are self-incompatible genotypes, with a considerable number of pollen tubes ending the growth in the middle third of the style and lack of fruit set. 'Karina', as S_3S_4 genotype, behaved as self-compatible, since its pollen tubes reached the base of the style and ovary, penetrating the nucellus. In addition, fruit set for 'Karina' was recorded in all three years of study (40.26%, 18.79% and 21.81%, respectively).

Key words: Prunus avium; S-genotype; self-pollination; pollen tube growth; fruit set

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is generally a self-incompatible, multipurpose fruit species used for its high quality fruits, timber and aesthetic value. Its self-incompatibility is controlled by a multi-allelic S-locus with gametophytic action, which prevents self-pollination. Interaction of an S-allele specific ribonuclease (S-RNases, BOŠKOVIĆ and TOBUTT, 2001) and a pollen S specific F-box protein (SFB, YAMANE *et al.*, 2003) determines the outcome of fertilization. Namely, only pollen tubes with an S-allele different from both stylar S-alleles can reach the ovary, otherwise the pollen tube growth will be arrested usually in the upper third of the style; another characteristic is the appearance of morphological deformity, especially on the tip of an incompatible pollen tube. The place of pollen tubes growth inhibition is characterized

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by deposition of callose that can be visually detected using aniline blue staining and ultraviolet (UV) light monitoring (KHO and BAËR, 1968). In addition to molecular genetics of incompatibility in sweet cherry, progress in discovery of new incompatibility alleles, understanding of their inheritance and interactions in heteroallelic pollen, folowed by monitoring of pollen tubes growth in the style of sour cherry were reported by TOBUTT *et al.* (2004) and BOŠKOVIĆ *et al.* (2006), as well as in myrobalan by SUTHERLAND *et al.* (2009).

Occasional sweet cherry cultivars are self-compatible; self-compatibility, as a desirable agronomic character, is a main objective of many breeding programmes worldwide. GRANGER (2004) reported the importance of pollenisers for both self-incompatible and self-compatible cultivars to ensure regular cropping in sweet cherry plantations. Knowledge of *S*-genotypes and blooming phenology of sweet cherry are very useful for breeders and growers to choose appropriate pollenisers. In order to achieve effective cross-pollination and fertilization, RADIČEVIĆ *et al.* (2011), based on average flowering period overlap and *S*-genotypes, reported recommendation for 21 economically important sweet cherry cultivars.

Self- and cross-(in)compatibility have traditionally been determined by the level of fruit set under field conditions. In the past self-incompatibility in sweet cherry was the rule, since obtaining fruits after selfing was exceptional. Recently, monitoring of pollen tubes growth in the pistils by fluorescence microscopy provides more reliable conclusions. CHOI and ANDERSEN (2005) showed that certain sweet cherry *S*-genotypes had a higher breakdown rate of self-incompatibility than others, as well as that the breakdown is related to air temperature during the flowering.

So far reported self-compatible cultivars have a narrow genetic base as they derive from JI2420 and JI2434 selections raised at the John Innes Institute, from crosses using X-ray irradiated pollen (SONNEVELD *et al.*, 2003). The self-compatibility of these selections is attributed to the loss of pollen-S function – namely, total deletion in the case of S_3 and the frameshift mutation in the case of S_4 (SONNEVELD *et al.*, 2005). Naturally self-compatible sweet cherry genotypes have been reported to date, i.e. Spanish cultivar 'Temprana de Sot' and its mutant 'Cristobalina' (WÜNSCH and HORMAZA, 2004a,b), and Italian cultivars 'Kronio' and 'Maiolina a Rappu' (MARCHESE *et al.*, 2007). The cause of self-compatibility of 'Kronio' and 'Maiolina a Rappu' is a natural pollen-part mutation of S_5 (MARCHESE *et al.*, 2007), whereas the mutation of a non S-locus EMPaS02, mapped on the LG3, leads to a loss of pollen function and self-compatibility in 'Cristobalina' (CACHI and WÜNSCH, 2011).

This study aimed to evaluate the self-(in)compatibility in four modern sweet cherry cultivars after self-pollination under West Serbia conditions, using a PCR amplification of *S*-*RNase*, by microscopic observation of the pollen tube growth rate and by assaying of the fruit set level.

MATERIALS AND METHODS

Plant material and experimental design

Four sweet cherry cultivars – 'Karina', 'Regina' (originating from Germany), 'Kordia' (Czech Republic) and 'Summit' (Canada), grafted on Gisela 5, were used in this study. The experiment was conducted over three years (2008–2010) in sweet cherry planting at the 'Preljina' facility of the Fruit Research Institute, near Čačak (43°53' N; 20°21' E; 350 m above the sea), Western Serbia, which is a typical area for fruit cultivation. The mean daily temperature

during the flowering phenophase was 12.1° C, 13.7° C and 12.3° C for the respective years of the experiment. The planting was established in 2005, in a randomized block design, with nine trees of each cultivar (three trees in three replications), at a 4.0×1.5 m distance.

DNA extraction and S-allele investigations

Leaves were harvested during the growing season, frozen in liquid nitrogen and stored at -80° C prior to DNA extraction. Frozen leaf sample was ground with four ball-bearings in Retsch Mixer Mill MM 400. Total genomic DNA was than isolated using the CTAB method described by DOYLE and DOYLE (1987), with addition of 1% β -mercaptoethanol and 2% polyvinylpyrrolidone (PVP 40) in the extraction buffer. *S*-genotyping of the assessed cultivars was performed according to the protocols described by SONNEVELD *et al.* (2001, 2003). In order to determine the *S*-alleles, polymerase chain reactions (PCR) were carried out by using the consensus primer pair specific for the second intron (PaConsII-F + -R) and the allele-specific primers for *S*₁, *S*₂, *S*₃ and *S*₄ (SONNEVELD *et al.*, 2001, 2003). Amplification products were separated by electrophoresis in 1.5% agarose gel (70 V for 3 h), visualised by ethidium bromide staining and sized by comparison with a 1 Kb plus DNA ladder (Invitrogen).

Pollination procedure

The branches with flowers at the late balloon stage were used for the testing. Some 600 flowers per each cultivar were chosen, each replication involving 200 flowers from all sides of three cherry trees. The single-pistil flowers were emasculated and bagged with paper bags to avoid contamination. Simultaneously, the anthers were collected from flowers at the late balloon stage and allowed to dehisce for 24–48 hours at 20°C. Pollen of each cultivar had a good *in vitro* germination ability (RADIČEVIĆ *et al.*, 2013). The collected pollen was used for self-pollination, at the begining of full flowering. Protective bags were removed ten days after the self-pollination.

Fixation and microscopic observation

A total of 30 flowers of each treatment (10 flowers per replication) was fixed 72, 144 and 240 hours after pollination in FPA (70% ethanol, propionic acid and formaldehyde, 90:5:5 percentage by volume). Aniline blue staining was used for the study of pollen tube growth in the pistil and carried out according to the protocol described by KHO and BAËR (1968), and PREIL (1970). In order to obtain squash preparation, the styles were separated from the ovaries, opened along the suture and covered with a husk. The ovaries were also opened along the suture. For better monitoring of the penetration of pollen tubes into the nucellus, the primary ovules were cut longitudinal-tangentially (CEROVIĆ, 1997). Pollen tubes were monitored under UV light, on the OLYMPUS BX61 microscope, by the AnalySIS software (using Multiple Image Analysis). The total number of pollen tubes was recorded in the style (upper, middle, lower third and the base of the style) and in the ovary, at magnification of 200X and 100X, respectively. The number of tubes was presented as average of three fixation samples. The penetration of the pollen tube into the nucellus was monitored 240 hours after pollination. The data were presented as percentage of pistils with penetration of pollen tubes into the nucellus.

Fruit set examination

Fruits were counted in each treatment at the beginning of ripening. Fruit set was recorded as the percentage of fruits per total number of self-pollinated flowers remaining after the last fixation.

Data analysis

Standard error was calculated for the measured parameters. The data were statistically analyzed using two-factor analysis of variance (ANOVA). In the results expressed in percentages, the arcsin data transformation was used. The significance of differences among mean values was determined by Duncan's multiple range test at $P \leq 0.05$. Data analysis was done using SPSS statistical software package, Version 8.0 for Windows (SPSS. Inc., Chicago, IL).

RESULTS

Genotyping of assessed sweet cherry cultivars. PCR analysis to determine S-genotypes of 'Karina', 'Kordia', 'Regina' and 'Summit' by using consensus and allele-specific primers for S-RNase gave patterns consistent with S_3S_4 , S_3S_6 , S_1S_3 , and S_1S_2 genotypes, respectively. Banding paterns from amplification with consensus primers for the second intron and specific amplification for S_1 , S_2 , S_3 and S_4 are given in Fig. 1 and 2, and scored in Tab. 1. The S-genotypes for the assessed cultivars were in agreement with results reported by SCHUSTER *et al.* (2007) and SCHUSTER (2012).



Figure 1. PCR amplification of genomic DNA with consensus primers for the S-RNase second intron of assessed sweet cherry cultivars analysed on a 1.5% agarose gel and stained with ethidium bromide: 'Karina' (lane 1), 'Kordia' (lane 2), 'Regina' (lane 3), 'Sumit' (lane 4); 1Kb plus DNA ladder (M)

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Figure 2. PCR amplification of genomic DNA with primers specific for S₁ (a), S₂ (b), S₃ (c) and S₄ (d) of assessed sweet cherry cultivars analysed on a 1.5% agarose gel and stained with ethidium bromide: 'Karina' (lane 1), 'Kordia' (lane 2), 'Regina' (lane 3), 'Sumit' (lane 4); 1Kb plus DNA ladder (M)

Cultivar	Results with consensus primer for the secon intron		Amplification with allele-specific primers			
	Allele 1	Allele 2	S_{I}	S_2	S_3	S_4
'Karina'	S_3	S_4			+	+
'Kordia'	S_3	S_6^*			+	
'Regina'	S_{I}	S_3	+		+	
'Summit'	S_I	S_2	+	+		

Table 1. S-genotypes of the assessed sweet cherry cultivars

*S6 allele was confimed with consensus primer



Figure 3. Pollen tubes growth in the pistil after self-pollination: a) pollen tubes arrested in the upper and in the middle third of style ('Regina', 144 h after self-pollination);b) incompatibility breakdown ('Karina', 144 h after self-pollination); c) penetration of the pollen tube into the nucellus ('Karina', 240 h after self-pollination)

CULTIVAR (A)		Number of pollen tubes					
		Stu	Stm	Stl	Bst	Ovr	
'Karina'		$51.66 \pm 3.99^{\circ}$	23.51 ± 7.88^{a}	16.20 ± 5.99^{a}	4.88 ± 1.27^{a}	2.01 ± 0.41^{a}	
'Kordia'		61.59 ± 8.08^{b}	$13.51 \pm 2.59^{\circ}$	0.57 ± 0.25^{b}	0.09 ± 0.03^{b}	$0.08\pm0.04^{\rm b}$	
'Regina'		96.83 ± 5.98^{a}	17.14 ± 1.60^{b}	0.30 ± 0.10^{b}	$0.08\pm0.05^{\rm b}$	0.02 ± 0.01^{b}	
'Summit'		59.92 ± 6.97^{b}	6.18 ± 1.69^{d}	0.24 ± 0.22^{b}	0.02 ± 0.01^{b}	$0.04\pm0.04^{\rm b}$	
YEAR (B)							
2008.		66.68 ±8 .66 ^b	10.92 ± 1.35^{b}	2.27 ± 1.12^{b}	1.43 ± 0.70^{b}	0.79 ± 0.39^{a}	
2009.		$49.66 \pm 4.70^{\circ}$	$6.81 \pm 1.59^{\circ}$	$0.18 \pm 0.09^{\circ}$	$0.15 \pm 0.08^{\circ}$	0.12 ± 0.06^{b}	
2010.		86.16 ± 3.83^{a}	27.53 ± 4.99^{a}	$10.54 \pm 5.07^{\rm a}$	2.22 ± 1.17^{a}	0.70 ± 0.34^{a}	
$A \times B$							
	2008.	$46.75 \pm 0.46^{\text{fg}}$	$14.78 \pm 0.15^{\circ}$	8.49 ± 1.31^{b}	5.43 ± 0.24^{b}	3.01 ± 0.11^{a}	
'Karina'	2009.	$41.50 \pm 1.00^{\text{gh}}$	1.92 ± 0.42^{g}	$0.64 \pm 0.19^{\circ}$	$0.49 \pm 0.26^{\circ}$	$0.41 \pm 0.11^{\circ}$	
	2010.	66.72 ± 3.59^{e}	53.84 ± 3.73^{a}	39.48 ± 2.77^{a}	8.72 ± 1.41^{a}	2.63 ± 0.10^{b}	
	2008.	$53.53 \pm 2.11^{\rm f}$	9.12 ± 0.32^{cde}	$0.13 \pm 0.06^{\circ}$	0.02 ± 0.02^{c}	$0.00\pm0.00^{\rm d}$	
'Kordia'	2009.	38.87 ± 3.55^{h}	8.25 ± 1.52^{def}	$0.07 \pm 0.03^{\circ}$	$0.10 \pm 0.00^{\circ}$	0.08 ± 0.02^{d}	
	2010.	92.36 ± 1.44^{bc}	23.17 ± 2.77^{b}	$1.53 \pm 0.25^{\circ}$	0.14 ± 0.07^{c}	0.17 ± 0.09^{d}	
'Regina'	2008.	115.80 ± 4.68^{a}	$15.17 \pm 0.66^{\circ}$	$0.41 \pm 0.19^{\circ}$	$0.24 \pm 0.08^{\circ}$	0.05 ± 0.01^{d}	
	2009.	76.03 ± 2.55^{d}	$14.34 \pm 1.81^{\circ}$	$0.00 \pm 0.00^{\circ}$	$0.00 \pm 0.00^{\circ}$	0.00 ± 0.00^{d}	
	2010.	98.67 ± 1.81^{b}	21.93 ± 3.10^{b}	$0.50 \pm 0.10^{\circ}$	$0.00 \pm 0.00^{\circ}$	$0.00\pm0.00^{\rm d}$	
'Summit'	2008.	$50.64 \pm 1.68^{\rm f}$	$4.60 \pm 1.30^{\rm efg}$	$0.04 \pm 0.04^{\circ}$	$0.05 \pm 0.02^{\circ}$	0.11 ± 0.11^{d}	
	2009.	$42.23 \pm 0.23^{\text{gh}}$	$2.75\pm0.38^{\rm fg}$	$0.00 \pm 0.00^{\circ}$	$0.00 \pm 0.00^{\circ}$	0.00 ± 0.00^{d}	
	2010.	$86.89 \pm 4.18^{\circ}$	11.20 ± 3.55^{cd}	$0.67 \pm 0.67^{\circ}$	$0.00 \pm 0.00^{\circ}$	$0.00\pm0.00^{\rm d}$	
ANOVA							
А		**	**	**	**	**	
В		**	**	**	**	**	
$A \times B$		**	**	**	**	**	

Table 2. The number of pollen tubes in certain parts of pistils in the sweet cherry genotypes after self-pollination, i.e. upper third of the style (Stu), middle third of the style (Stm), lower third of the style (Stl), base of the style (Bst), and ovary (Ovr)

within each column, the same letters indicate no significant difference at the $P \le 0.05$ according to Duncan's multiple range test

The number and characteristics of pollen tubes. A reduction in the number of pollen tubes growing down the style was recorded in all treatments (Tab. 2). In the pistils of 'Kordia', 'Regina' (Fig. 3a) and 'Summit', pollen tubes terminated their growth in the upper and the

middle part of the style. In 'Karina', pollen tubes reached the base of the style (Fig. 3b) and ovary, penetrating in some cases the nucellus, particularly 240 hours after pollination (Fig. 3c).

The differences in the number of tubes were progressively less significant in the lower parts of the pistils. In the lower third, the base of the style, and in the ovary, the number of pollen tubes was close to zero in 'Kordia', 'Regina' and 'Summit'. In 'Karina', the number of tubes in the lower pistilar parts was statistically significantly larger than in the other cultivars. The number of pollen tubes in all of the assessed cultivars, as well as in all of the pistil sections, was the lowest in 2009. Pollen tubes that stopped their growth in the upper and the middle part of the style were characterised by the formation of a broadened tip (Fig. 4a), or were thickened along the entire length exhibiting strong fluorescence (Fig. 4b).



Figure 4. Incompatible pollen tubes: a) a broadened tip; b) tubes thickened along the entire length

Pollen tubes growth efficacy

The efficacy of the growing pollen tubes was rated by the percentage of pistils with pollen tubes penetrating the nucellus 240 hours after self-pollination, and by fruit setting (Tab. 3). In 'Karina', pollen tubes in the nucellus were observed in 2008, while in the other two years they were not present in the nucellus, although they were observed in the ovary. However, the fruit set was obtained in 'Karina' in all three years, being the largest in the first year (40.26%) and the lowest in the second year (18.79%) of examination. In 'Kordia', 'Regina', and 'Summit', there were neither pollen tubes in the nucellus, nor fruit setting after self-pollination.

		Pollen tube growth			
CULTIVAR (A)		efficacy (%)			
		Ptn	Fs		
'Karina'		10.78 ± 5.78^{a}	26.95 ± 3.10^{a}		
'Kordia'		$0.00 \pm 0.00^{\rm b}$	0.37 ± 1.10^{b}		
'Regina'		$0.00 \pm 0.00^{\rm b}$	0.22 ± 0.84^{b}		
'Summit'		$0.00 \pm 0.00^{\rm b}$	0.14 ± 0.72^{b}		
YEAR (B)					
2008.		8.06 ± 4.53^{a}	10.07 ± 5.89^{a}		
2009.		$0.00 \pm 0.00^{\rm b}$	$5.25 \pm 3.01^{\circ}$		
2010.		$0.00 \pm 0.00^{\rm b}$	5.45 ± 3.61^{b}		
$A \times B$					
	2008.	32.25 ± 0.71^{a}	40.26 ± 0.15^{a}		
'Karina'	2009.	$0.00 \pm 0.00^{\rm b}$	18.79 ± 0.44^{b}		
	2010.	$0.00 \pm 0.00^{\rm b}$	21.81 ± 0.49^{b}		
	2008.	$0.00 \pm 0.00^{\rm b}$	0.00 ± 0.00^{e}		
'Kordia'	2009.	$0.00 \pm 0.00^{\rm b}$	$1.11 \pm 2.53^{\circ}$		
	2010.	$0.00 \pm 0.00^{\rm b}$	$0.00 \pm 0.00^{\rm e}$		
	2008.	$0.00 \pm 0.00^{\rm b}$	$0.00 \pm 0.00^{\rm e}$		
'Regina'	2009.	$0.00 \pm 0.00^{\rm b}$	0.67 ± 1.91^{cd}		
	2010.	$0.00 \pm 0.00^{\rm b}$	0.00 ± 0.00^{e}		
	2008.	$0.00 \pm 0.00^{\rm b}$	0.00 ± 0.00^{e}		
'Summit'	2009.	$0.00 \pm 0.00^{\rm b}$	0.42 ± 2.18^{cde}		
	2010.	$0.00 \pm 0.00^{\rm b}$	0.00 ± 0.00^{e}		
ANOVA					
А		**	**		
В		**	**		
$A \times B$		**	**		

Table 3. Pollen tubes growth efficacy, i.e. pistils with pollen tubes penetrating the nucellus 240 hours after self-pollination (Ptn), and fruit set (Fs)

within each column, the same letters indicate no significant difference at the $P \le 0.05$ according to Duncan's multiple range test

DISCUSSION

We have provided evidence for self-(in)compatibility of economically important sweet cherry cultivars based on the incompatibility genotypes, the number of pollen tubes in different parts of pistils and their growth efficacy.

The identity of 'Karina', 'Kordia', 'Regina' and 'Summit' was confirmed by *S-RNase* genotyping and S_3S_4 , S_3S_6 , S_1S_3 , S_1S_2 were obtained, recpectively. The PCR method with consensus and *S*-allele specific primers has already proved useful in genotyping cherry cultivars and progenies (SONNEVELD *et al.*, 2001; CACHI and WÜNSCH, 2011). In sweet cherry, *S-RNase* is the highly polymorphic locus, with 25 different alleles have been reported to date (VAUGHAN *et al.*, 2008; SCHUSTER, 2012). The most common *S*-allele in sweet cherry cultivars is the S_3 allele, followed by S_1 , S_4 and S_6 , as well as S_2 , S_5 and S_9 (SCHUSTER, 2012).

A dramatic reduction in the number of pollen tubes in certain parts of the pistils was the most pronounced in 'Kordia', 'Regina' and 'Summit', which were almost without pollen tubes in the lower third, the base of the style, and the ovary. This was followed by a lack of fruit set. Penetration of pollen tubes into the lower third, the base of the style and ovary was very rarely observed, usually on the tenth day after self-pollination. Our results for 'Kordia' and 'Regina' were in agreement with those of LECH *et al.* (2008), who reported that these cultivars practically did not set fruits after self-pollination. However, our data regarding the fruit set of 'Summit' were not consistent with the findings of above-mentioned authors, who stated that this cultivar reached 11% of fruit set after selfing.

Genetic determined pollen-pistil interaction plays a major role in regulation of pollen tube attrition. This interaction is superimposed over a physical and/or physiological pistilar restriction of pollen tubes growth along the style (HORMAZA and HERRERO, 1999). The fact that the genetic interaction seems to be more conspicuous in the upper than in lower half of the style is related to the self-incompatibility reaction (HORMAZA and HERRERO, 1999). *Prunus* species are characterised by gametophytic self-incompatibility, in which pollen tubes usually stopped their growth in the upper third or upper quarter of the style, or just behind the stigma – in sour cherry (CEROVIĆ, 1997), plum (NIKOLIĆ and MILATOVIĆ, 2010), and almond (ČOLIĆ *et al.*, 2010). Our study revealed that, in sweet cherry, a considerable number of tubes ended the growth in the middle third of the style. For apricot, MILATOVIĆ *et al.* (2010) reported that most incompatible pollen tubes stopped growth in the botom half of the style.

Altered morphology of pollen tubes is another important feature of this type of incompatibility, that has been reported in sour cherry, plum and almond (CEROVIĆ, 1997; NIKOLIĆ and MILATOVIĆ, 2010; ČOLIĆ *et al.*, 2010). Common characteristic of incompatible pollen tubes is their extension tip, with strong fluorescence, due to accumulation of callose. In sour cherry, extremely thickened incompatible pollen tubes, with a high deposition of callose in the walls, and a branched tip were also reported (CEROVIĆ, 1997). Although the pollen tubes in our study had similar characteristics to those described in other studies, the occurrence of branching was not observed. HEGEDÜS *et al.* (2012) stated that the consequence of incompatibility reaction is disorganization of the pollen tube, although the nature of the interaction of *S*-RNase and SFB proteins has not yet been resolved. Two current models attempt to explain this type of interaction: 'two-component inhibitor model' (SONNEVELD *et al.*, 2005) and 'sequestration model' (GOLDRAIJ *et al.*, 2006).

Among the cultivars assessed in this study, the results obtained for 'Karina' showed an obvious difference in its behavior after self-pollination. Based on monitoring of pollen tube

growth and assaying the fruit set, incompatibility breakdown in 'Karina' was manifested as stable during the three years of investigation. In sweet cherry, incompatibile crosses have been recorded to produce fruit set les than 3% (CHOI *et al.*, 2002). Also, genotypes of the other *Prunus* species showing up to 5% fruit set after selfing are considered as self-incompatible (VILANOVA *et al.*, 2006). In addition, BEKEFI (2004) reported that fruit set ranging from 20.1% to 30% was referred to as high in sweet cherries. Considering all these facts, 'Karina' behaved as a self-compatible cultivar.

CHOI and ANDERSEN (2005) reported that the breakdown of self-incompatibility in sweet cherry, determined by the fruit set, showed significant differences in frequency among genotypes with different *S*-allelic constitutions. The highest frequency occured for S_3S_4 genotypes (self- and cross-incompatible combinations). The percentage of breakdown, expressed as pollen tube growth, had the highest breakdown rate at a higher temperature during the flowering. Based on the current data for 'Karina', the higher rate of incompatibility breakdown in the year with the highest temperature during the flowering was not observed, although S_3S_4 genotype of this cultivar was confirmed. In contrast, the number of pollen tubes and fruit set were the lowest in the year with the highest temperature. Our results are in agreement with the findings of HEDHLY *et al.* (2005) for compatible crosses in sweet cherry, indicated reducing of pollen tubes number at the base of the style on higher temperatures. The maximum daily temperature increased by 5– 7°C, resulting in a moderate increase in the average temperature of 1–3°C, was sufficient to drastically reduce fruit set in sweet cherry (HEDHLY *et al.*, 2007), which is consistent with the results obtained in this study.

Genotype-year interaction was significant for all the measured parametars, indicating that the effect of environmental conditions was genotype-dependent. The most important environmental factor that could affect pollen tube growth rate and fruit set is the temperature during the progamic phase. HEDHLY *et al.* (2004) reported that the response to temperature during the reproductive phase is genotype-dependent, reflecting the temperature adaptation of the genotype.

In addition to high quality fruit, extension of the season of maturity and resistance to economically important diseases, self-compatibility is also a useful characteristic in modern sweet cherry breeding programmes. The findings of this study suggest that 'Karina' shows self-compatible behaviour. Our current work is based on molecular and genetic analysis, which is undertaken primarily to discover the nature of self-compatible behaviour in 'Karina', using different combinations of classic crossing to obtain progenies for further study of the segregation of *S-RNase* and *SFB* alleles.

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UTVRÐIVANJE SELF-(IN)KOMPATIBILNOSTI KOD NEKIH GENOTIPOVA TREŠNJE (Prunus avium L.)

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Izvod

U radu su predstavljeni rezultati trogodišnjeg ispitivanja self-(in)kompatibilnosti kod četiri ekonomski značajna genotipa trešnje - 'Karina', 'Kordia', 'Regina' i 'Summit', u agroekološkim uslovima Srbije. Određena je alelna konstitucija *S-RNaze*, utvrđen stepen rasta polenovih cevčica metodom fluorescentne mikroskopije, kao i stepen zametanja plodova pri samooprašivanju navedenih genotipova. 'Kordia' (S_3S_6), 'Regina' (S_1S_3) i 'Summit' (S_1S_2) su self-inkompatibilne, sa značajnim brojem polenovih cevčica koje završavaju rast u srednjoj trećini stubića, uz odsustvo zametanja plodova. 'Karina' (S_3S_4 genotip) je ispoljila self-kompatibilnost, imajući u vidu da su polenove cevčice pri samooprašivanju dostizale bazu stubića i plodnik, prodirući u nucelus semenog zametka. Pored toga, zametanje plodova kod sorte 'Karina' utvrđeno je u svim godinama ispitivanja (40,26%, 18,79% i 21,81%, resp.).

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