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FLOWERING TIME AND INCOMPATIBILITY GROUPS – CULTIVAR COMBINATION IN COMMERCIAL SWEET CHERRY (*Prunus avium* L.) ORCHARDS

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The paper presents results of eight-year study (1999–2006) of flowering phenophase in 21 introduced sweet cherry cultivars grown under the agro-environmental conditions of West Serbia. Flowering time, as well as progress and abundance of flowering were studied, and classification of the studied cultivars according to flowering time was derived. On the basis of mean several-year overlap in phenophase of full flowering and on the grounds of so far known data on classification of these cultivars among incompatibility groups, we have offered a recommendation for their cultivation in orchards whereby the most effective pollination and

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fertilization can be ensured as well as good fruit-set and satisfactory fruit yields.

Key words: cultivar, flowering, incompatibility groups, sweet cherry

INTRODUCTION

Sweet cherry, *Prunus avium* L., is economically important member of the Rosaceae family, which is mainly grown for fresh consumption. In addition, the fruits are suitable for processing, e.g. for different candy and milk products, canning, to produce juice, liqueur and jam.

Knowledge of flowering time of sweet cherry cultivars is essential for an adequate choice of cultivar combination that ensures the most effective pollination and fertilization for abundant fruit set. Thus accurate selection of cross-compatible cultivars and their arrangement in an orchard play the crucial role in obtaining high fruit yields.

Determination of possibilities of pollination and fertilization among cultivars belonging to different groups, defined according to this criterion – early, mid-early, mid-late and late flowering cultivars, is important issue in the study of sweet cherry flowering. Cropping of commercially important sweet cherry cultivars depends of the fact that full bloom of any cultivar varies from year to year and flower development varies within cultivars and trees (GRANGER, 2004). Short life of ovules and rapid collapse of stigma papillae in sweet cherry further impedes fertilization, particularly in cases of high air temperatures during flowering period.

Most sweet cherry cultivars are self-incompatible and certain pairs of cultivars are cross-incompatible. This incompatibility has been attributed to the gametophytic multi-allelic locus *S* with at least two genes, one encoding a stylar glycoprotein with ribonuclease activity (*S-RNase*; BOŠKOVIĆ and TOBUTT, 2001), and the other encoding a pollen-specific F-Box protein (*SFB*, YAMANE *et al.*, 2003).

Self and cros- (in)compatibility in sweet cherry have traditionally been determinated by monitoring the fruit set percentage under field conditions, and later on by observation of pollen-tube growth in the pistil using fluorescence microscopy. The first attempt to identify *S*-RNase alleles of sweet cherry cultivars using electrophoresis and activity staining was done by BOŠKOVIĆ and TOBUTT (2001). This method and methods based on polymerase chain reaction (PCR), which also have been developed, have enabled rapid determination of *S*-genotype and has made the pollination outcome foreseeable. A number of primers amplifying across the variable first or second introns of the *S*-RNase have been designed that allow the alleles to be distinguished on the basis of length polymorphism. Some 28 *S*-RNase alleles have been identified and characterized in sweet cherry in last decade (BOŠKOVIĆ and TOBUTT, 2001; SONNEVELD *et al.*, 2001; SONNEVELD *et al.*, 2003; DE CUYPER *et al.*, 2005; WUNSCH and HORMAZA, 2004; VAUGHAN *et al.*, 2008). Recent efforts have been focused on the identification and characterization of the sweet cherry gene which is expressed specifically in the pollen. Consensus primers for discrimination of *SFB* alleles that utilize the polymorphism of the intron present in the 5' untranslated region were developed by VAUGHAN *et al.* (2006). Some 17 *SFB* alleles have been reported in sweet cherry so far (IKEDA *et al.*, 2004; VAUGHAN *et al.*, 2006; VAUGHAN *et al.*, 2008).

By the mid-90's of the past century, based on 6 *S-RNaze* alleles identified by that time, sweet cherry cultivars were classified into 13 incompatibility groups, and O group ('universal donors'). The group of 'universal donors' was comprised of the genotypes which are compatible with members of the other groups and usually with each other, thus it is possibly that most members of O group are different genotypes.

In recent times, the identification of new sweet cherry *S* alleles has induced rise in number of known incompatibility groups up to 27 (TOBUTT *et al.*, 2004), i.e. 36 (SCHUSTER *et al.*, 2007). Newly identified alleles have inevitably led to the reinterpretation of *S*-genotype of some sweet cherry cultivars and to their belonging to incompatibility groups accordingly. Numerous 'universal donors' have been fallen into some of above-mentioned incompatibility groups.

Developing self-compatible cultivars is a major accomplishment of sweet cherry breeding work. Self-compatible sweet cherries are being increasingly grown in commercial orchards and it was thought that the need for pollenisers could be avoided (GRANGER, 2004). In order to achieve effective cross-pollination in sweet cherry orchards two or three cross-compatible cultivars must be planted, so the pollenisers are very important for both self-incompatible and self-compatible cultivars (GRANGER, 2004; SCHUSTER *et al.*, 2007).

The above aspects of biology of fertilization, with particular focus on respective specificities in sweet cherry, necessitate seeking potentially effective pollenisers or cultivar combinations ensuring maximum long-term economic returns. Based on long-term study of flowering phenophase of introduced sweet cherry cultivars in West Serbia and knowledge of the belonging to incompatibility groups, the final purpose of this research is contribution to the choice of cultivars suitable for growing in commercial orchards to assure fruit set, as well as for designing crosses in breeding programmes.

MATERIALS AND METHODS

Plant material

Twenty-one sweet cherry cultivars – 'Bigarreau Hativ de Burlat' ('Burlat'), 'Bing', 'Compact Stella', 'Drogan's Yellow', 'Emperor Francis', 'Germersdorfer', 'Hedelfingen', 'Inge', 'Kordia', 'Lambert', 'Lapins', 'Lyons Early', 'Merchant', 'Napoleon', 'Souvenir des Charmes' ('Souvenir', 'Moreau'), 'Stark Hardy Giant', 'Summit', 'Sunburst', 'Van', 'Vega' and 'Vista' – were used. The assessed cultivars maintained at the Fruit Reseach Institute – Čačak (Ljubic site planting). The planting was established in 1988, at tree spacing 6 x 4 m. Cultivars were grafted on wild cherry (*Prunus avium* L.) seedling and planted nine trees each. Modified pyramidal crown was the applied training system.

Some of the above-mentioned cultivars are widely grown in Serbia ('Burlat', 'Lyons Early', 'Germersdorfer', 'Napoleon', 'Souvenir', 'Van'), while some others are currently gaining in popularity ('Kordia', 'Lapins', 'Summit', 'Sunburst'). In addition, some of analyzed cultivars in this paper have excellent pomo-technological and other traits, as mentioned by RADIČEVIĆ and CEROVIĆ (2003), and RADIČEVIĆ *et al.* (2009).

Flowering phenophase investigation

To investigate the flowering phenopase, we studied flowering time, progress and abundance of flowering by monitoring and taking notes on flowering onset (a date when 10 to 20% of flowers are open), full flowering (90–100% flowers are open) and end of flowering (90% of petals fallen off) and abundance of flowering (graded as follows: excellent (5), very good (4), good (3), poor (2), bad (1) and without flowers (0)). Given flowering phenogram involves average data obtained over the eight-year period (1999–2006). The studied sweet cherry cultivars are classified into four groups in terms of flowering time: early, mid-early, mid-late and late.Based on an average long-term overlap in full flowering phenophase (in the course of 5–8 days) (CEROVIĆ *et al.*, 2005) as well as on the basis of so far known data on classification of the studied cultivars according to incompatibility groups, we have offered a recommendation for their growing in orchards whereby the effective pollination and fertilization can be ensured.

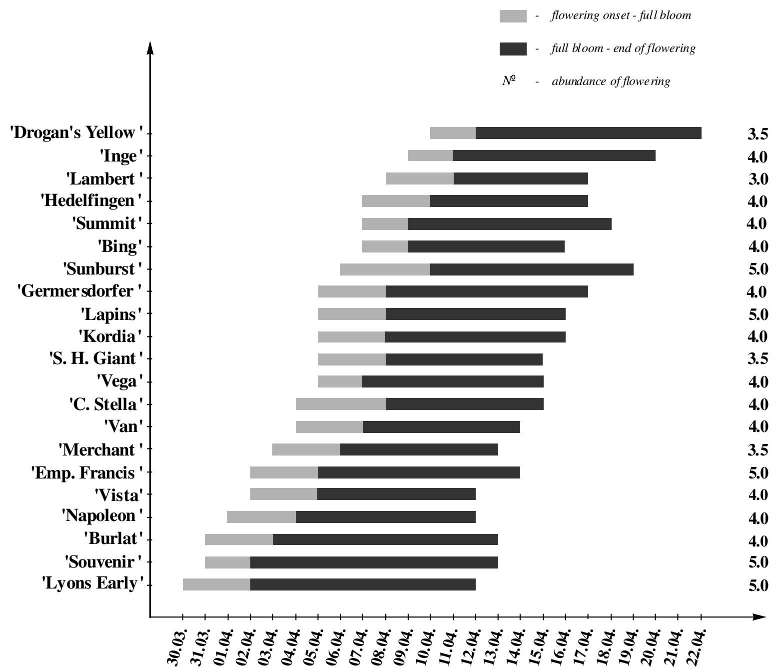
RESULTS AND DISCUSSION

The earliest average flowering onset was observed in 'Lyons Early', whereas the latest was in 'Drogan's Yellow' (Graph. 1). Flowering onset span in the assessed cultivars was 11 days. Reporting on the results of study of flowering time in 80 sweet cherry cultivars, HODUN and HODUN (2002) stated that the earliest and the latest flowering cultivars covered the span of three to nine days. Although flowering onset in sweet cherries depends on weather conditions, the sequence of flowering onset in cultivars grown under identical agro-environmental conditions depends on hereditary characteristics of cultivars, whereby this influence particularly dominates in years with earlier flowering onset.

In the studied sweet cherry cultivars, full flowering followed two to four days after flowering onset. In the majority of cultivars (the total of 13), full flowering began averagely three days after flowering onset.

In the studied sweet cherry cultivars flowering period took up between 9 ('Bing', 'Lambert') and 13 days ('Lyons Early', 'Souvenir', 'Burlat', 'Sunburst'). Sweet cherry belongs in the group of stone fruits that exhibit short period from the flowering onset to full flowering date (up to 3 days). MOGHADAM *et al.* (2009) reported that flowering period of 25 sweet cherry cultivars took approximately 11-18 days; keeping in view to weather conditions, the flowering time may change.

Fruit set in sweet cherries is largely governed by abundance of flowering. Five sweet cherry cultivars demonstrated excellent abundance of flowering, i.e. 'Lyons Early', 'Souvenir', 'Emperor Francis', 'Lapins' and 'Sunburst'; it was lowest (grade 3) in 'Lambert'.



Graphic 1. Phenophase of flowering in studied sweet cherry cultivars (1999–2006)

In terms of flowering onset, the studied cultivars were classified into four groups (Tab. 1). Cultivars which tend to belong to group that follows in sequence are marked with (+), whereas those inclined to the previous group are denoted with (-). The majority of the studied cultivars (the total of 9 cultivars, i.e. 42.86%) belong to the group of mid-late flowering cultivars. Our results concerning the sequence of flowering of some cultivars are mainly in agreement with those reported by HODUN and HODUN (2002), with the exception of 'Kordia'. However, 'Kordia' was classified as a late flowering cultivar, but according to our results it belongs to the group of mid-late flowering cultivars. Table 2 presents *S*-genotypes of the studied sweet cherry cultivars along with their classification into incompatibility groups. Seven *S*-alleles, within different combinations, are present in the assessed cultivars.On the basis of the average flowering period overlap in some sweet cherry cultivars during full flowering phenophase, *S*-genotype and classification of the studied cultivars among different incompatibility groups, we have offered recommendations for their effective cross-pollination and fertilization in orchards (Tab. 3). If cross-pollinated cultivars have identical *S*-alleles (incompatible), growth of pollen tubes is terminated at the upper third of the style; when cross-pollinated cultivars have either one common *S*-allele (semi-compatible) or none common *S*-alleles (fully compatible), pollen tubes penetrate the base section of the style and the ovary accordingly.

Table 1. Sweet cherries classified according to the time of flowering

C U L T I V A R S			
Early-flowering	***Mid-early flowering***	***Mid-late flowering***	***Late-flowering***
'Lyons Early'	'Vista'	'Vega'	'Lambert'
'Souvenir'	'Emperor Francis'	'Stark Hardy Giant'	'Inge'
'Burlat'	'Merchant'	'Kordia'	'Drogan'sYellow'
'Napoleon' (+)	'Van' (+)	'Lapins'	
	'Compact Stella' (+)	'Germersdorfer'	
		'Sunburst'	
		'Bing' (+)	
		'Summit' (+)	
		'Hedelfingen' (+)	
19.05%	**23.81%**	**42.86%**	**14.29%**

Table 2. S-genotypes of the studied sweet cherry cultivars

Cultivar	*S-allele constitution*	*Incompatibility group*	*Source*
'Lyons Early'	S_5S_6	XV	WIERSMA *et al.*, 2001
'Souvenir'	S_3S_9	XVI	SCHUSTER *et al.*, 2007
'Burlat'	S_3S_9	XVI	SONNEVELD *et al.*, 2003
'Napoleon'	S_3S_4	III	SONNEVELD *et al.*, 2003
'Vista'	S_2S_5	VIII	SONNEVELD et al., 2003
'Emperor Francis'	S_3S_4	III	WIERESMA *et al.*, 2001
'Merchant'	S_4S_9	XXI	SONNEVELD *et al.*, 2003
'Van'	S_1S_3	II	KAPPEL *et al.*, 2003
'Compact Stella'	$S_3S_{4'}$	*self-compatible*	KAPPEL, 2002
'Vega'	S_2S_3	IV	WIERSMA *et al.*, 2001
'S. H. Giant'	S_1S_2	I	SCHUSTER *et al.*, 2007
'Kordia'	S_3S_6	VI	ANDERSEN *et al.*, 2003
'Lapins'	$S_1S_{4'}$	*self-compatible*	WIERSMA *et al.*, 2001
'Germersdorfer'	S_3S_4	III	BARGIONI, 1996
'Sunburst'	$S_3S_{4'}$	*self-compatible*	WIERSMA *et al.*, 2001
'Bing'	S_3S_4	III	WIERSMA *et al.*, 2001
'Summit'	S_1S_2	I	WIERSMA *et al.*, 2001
'Hedelfingen'	S_3S_5	VII	BOŠKOVIĆ and TOBUTT, 2001
'Lambert'	S_3S_4	III	WIERSMA *et al.*, 2001
'Inge'	S_4S_9	XXI	SONNEVELD *et al.*, 2003
'Drogan'sYellow'	S_1S_5	XIV	SCHUSTER *et al.*, 2007

Table 3. Flowering time and (in)compatibility in the studied sweet cherry cultivars

♂ Cultivar ♀	'Lyons Early'	'Souvenir'	'Burlat'	'Napoleon'	'Vista'	'E. Francis'	'Merchant'	'Van'	'C. Stella'	'Vega'	'S. H. Giant'	'Kordia'	'Lapins'	'Germersdorfer'	'Sunburst'	'Bing'	'Summit'	'Hedelfingen'	'Lambert'	'Inge'	'Drogan's Yellow'
'Lyons Early'	⊗	‡	‡	‡	†	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	‡	‡	†	‡	‡	†
'Souvenir'	‡	⊗	⊗	†	‡	†	†	†	†	†	‡	†	‡	†	†	†	‡	†	†	†	‡
'Burlat'	‡	⊗	⊗	†	‡	†	†	†	†	†	‡	†	‡	†	†	†	‡	†	†	†	‡
'Napoleon'	‡	†	†	⊗	†	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Vista'	†	‡	‡	‡	⊗	‡	‡	‡	‡	†	†	‡	‡	‡	‡	‡	†	†	‡	‡	†
'E. Francis'	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Merchant'	‡	†	†	†	‡	†	⊗	‡	‡	‡	‡	‡	‡	†	‡	†	‡	‡	†	⊗	‡
'Van'	‡	†	†	†	‡	†	‡	⊗	†	†	†	†	†	†	†	†	†	†	†	‡	†
'C. Stella'	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Vega'	‡	†	†	†	†	†	‡	†	†	⊗	†	†	‡	†	†	†	†	†	†	‡	‡
'S. H. Giant'	‡	‡	‡	‡	†	‡	‡	†	‡	†	⊗	‡	†	‡	‡	‡	⊗	‡	‡	‡	†
'Kordia'	†	†	†	†	‡	†	‡	†	†	†	‡	⊗	‡	†	†	†	‡	†	†	‡	‡
'Lapins'	‡	‡	‡	†	‡	†	†	†	‡	‡	†	‡	†	†	‡	†	†	‡	†	†	†
Germersdorfer	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Sunburst'	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Bing'	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Summit'	‡	‡	‡	‡	†	‡	‡	†	‡	†	⊗	‡	†	‡	‡	‡	⊗	‡	‡	‡	†
'Hedelfingen'	†	†	†	†	†	†	‡	†	†	†	‡	†	‡	†	†	†	‡	⊗	†	‡	†
'Lambert'	‡	†	†	⊗	‡	⊗	†	†	†	†	‡	†	‡	⊗	†	⊗	‡	†	⊗	†	‡
'Inge'	‡	†	†	†	‡	†	⊗	‡	‡	‡	‡	‡	‡	†	‡	†	‡	‡	†	⊗	‡
'Drogan's Yellow'	†	‡	‡	‡	†	‡	‡	†	‡	‡	†	‡	†	‡	‡	‡	†	†	‡	‡	⊗

‡	*Compatible*
†	*Semi-compatible*
⊗	*Incompatible*
(white)	*Flowering time overlap*
(light grey)	*Insufficient overlap in some seasons*
(dark grey)	*Insufficient overlap*

Self-compatibility of cultivars 'Compact Stella' ($S_3S_{4'}$), 'Lapins' ($S_1S_{4'}$), and 'Sunburst' ($S_3S_{4'}$) is attributed to a mutant S_4 allele, denoted $S_{4'}$, where the prime symbol indicates the loss of pollen-*S* function. These cultivars carry pollen-part mutations - a 4 bp deletion in a variable region of the S_4-haplotype-specific *SFB* gene leads to a shift the translation reading frame and premature termination of the pollen (SONNEVELD *et al.*, 2005), but retain normal stylar function. The outcome of the crosses of these cultivars depends on whether they are used as pollenisers or receptor cultivars. So, the cross of self-compatible cultivar as polleniser with cultivar with S_4 allele is compatible or semi-compatible, depending on the other alleles (Tab. 3).

Given recommendations referring to potentially good combinations of sweet cherry cultivars should be considered within the context of other pollination factors that have not been discussed in this paper, such as production, quality and transfer of pollen. Similarly, we find it necessary to suggest exceptional potential of combining mid-late flowering cultivars whose flowering period overlaps with that of the majority of other cultivars. Some earlier investigations point to high *in vitro* pollen germination in some of these cultivars – 'Van', 'Compact Stella' (CEROVIĆ *et al.*, 2003), and in self-compatible 'Sunburst' and 'Lapins' (RADIČEVIĆ *et al.*, 2008).

On the other hand, cultivars classified as those with extreme flowering period cannot be recommended as suitable pollenisers among these groups, even when their classification according to incompatibility groups allows cross-pollination and fertilization.

Cultivar combinations categorized as 'insufficient overlap in some seasons' can be suitable in some seasons and localities, provided that *S*-genotype requirements are satisfied, though the risk of insufficient overlap in full flowering remains. This can particularly be emphasized when relatively low temperatures occurring over the period of flowering onset in sweet cherry markedly postpone flowering onset in late flowering cultivars.

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VREME CVETANJA I GRUPE INKOMPATIBILNOSTI – SORTNA KOMPOZICIJA U KOMERCIJALNIM ZASADIMA TREŠNJE (*Prunus avium* L.)

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I z v o d

U radu su prikazani osmogodišnji rezultati (1999-2006. godine) ispitivanja karakteristika fenofaze cvetanja 21 introdukovane sorte trešnje u agroekološkim uslovima Čačka. Ispitivani su tok, trajanje i obilnost cvetanja, i izvršena klasifikacija ispitivanih sorti prema vremenu cvetanja. Na bazi prosečnog višegodišnjeg preklapanja u fenofazi punog cvetanja, kao i na osnovu do sada poznatih podataka o pripadnosti ovih sorti pojedinim grupama inkompatibilnosti, iznete su mogućnosti za njihovo gajenje u višesortnim zasadima koje mogu obezbediti efikasno oprašivanje i oplođenje, a time i realizaciju potencijala rodnosti.

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