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Cherry breeding work at Fruit Research Institute, Čačak – past, present and future

Sanja Radičević[1]*, Slađana Marić[1], Radosav Cerović[2]

[1]*Fruit Research Institute, Kralja Petra I 9, 32000 Čačak, Republic of Serbia*
[2]*University of Belgrade, Innovation Centre of the Faculty of Technology and Metallurgy in Belgrade Ltd., Karnegijeva 4, 11000 Belgrade, Republic of Serbia*
**E-mail: sradicevic@institut-cacak.org*

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Abstract. Cherry breeding work at Fruit Research Institute, Čačak has had a long tradition since its initiation in 1960, using planned hybridization within *Prunus avium* L. and *Prunus cerasus* L. as the main method. Two sweet cherry ('Asenova Rana' and 'Čarna') and two sour cherry cultivars ('Šumadinka' and 'Čačanski Rubin') have been released in the previous period, and three new sour cherry cultivars ('Sofija', 'Nevena' and 'Iskra') have been named and released in 2015. Current sour cherry breeding started in 2011, and is based on the use of domestic genotypes, well adapted to the environmental conditions of the area, and introduced genotypes – known sources of resistance, with a high cropping potential and good fruit quality. Special attention has been paid to sour cherry germplasm from the West Serbia region, whose variability is an abundant source of diversity – for clonal selection and using the autochthonous genotypes in commercial growing, or as parents in planned hybridization. Nowadays, one sweet cherry ('V/60') and two sour cherry ('GV-6' and 'GV-10') genotypes are under procedure of recognition at Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. The investigations are also focused on different aspects of cherry floral biology – flowering phenology, pollen quality, monitoring the pollen tubes growth in the pistil, and cytoembryology. Reproductive behaviour as pollen donors/recipients were considered from the context of genotypic specificities, and their expressions in relation to flowering temperature, especially warmer. Decades-long research work on self- and cross-(in)compatibility in cherries was started by monitoring fruit set percentage under field conditions, and later developed by observing pollen tubes growth in the pistil using fluorescence microscopy, to find solutions for the adequate choice of cultivars combinations that provide the best performance in terms of fruit set and yields. In addition, *S*-genotyping of autochthonous, domestic and foreign sweet cherry cultivars has also been begun by using consensus and specific primers for identification of *S-RNase* alleles.

Keywords: *P. avium, P. cerasus*, breeding, selection, new cultivars, floral biology, *S*-(in)compatibility

Introduction

Cherries are among the most popular temperate fruit crops, due to their attractiveness, nutritive value and suitability for fresh consumption, industrial and domestic processing. Cultivated cherries belong to the Rosaceae family, *Prunus* genus, *Cerasus* subgenus and comprise the diploid sweet cherry (*Prunus avium* L.) and the tetraploid sour cherry (*Prunus cerasus* L.). For the improvement of fruit growing in the Republic of Serbia (RS), cherries are of exceptional potential for both economic and traditional reasons. In the structure of fruit growing in the RS, cherry has important place, especially sour cherry which ranks third in the total fruit production and together with raspberry represents the country's most important exporting fruit species (Radičević et al., 2017). The average year production of sweet and sour cherry fruits for the period 2010–2019 was 21,234 and 108,666 tonnes, respectively (Statistical Office of the RS, 2020). The positive balance in foreign trade confirms that the RS is competitive in the production of fresh sweet cherries, as well as sour cherries which are mainly exported frozen.

The work on breeding and selection of new cherry genotypes at Fruit Research Institute, Čačak (FRI) resulted in realization of two sweet cherry and five sour cherry cultivars, as well as the numerous promising hybrids which are under the different phases of the evaluation process. Some of them are under procedure of recognition at Ministry of Agriculture, Forestry and Water Management of the RS. Using modern methods of fertilization biology and determination of *S*-genotype, as well as their complementary application in genotype identification, optimizing cultivars' composition and realization of productivity potential in commercial orchards are important parts of cherry breeding work at FRI.

Planned hybridization within *P. avium*

The work on breeding sweet cherries at FRI has been under way since 1960. Developing new genotypes with large, high-quality fruits resistant to cracking, with early, abundant and regular bearing, moderate vigour and compact growth habit were the main objectives of the programme. The cultivars was supposed to have crisp flesh and deep red or yellow ground colour, high flesh/stone ratio, improved resistance to pests and disesaes, and hardiness to winter and late spring frosts (Milenković et al., 2006). The breeding goal was also belonging to different weeks of sweet cherry ripening season, in order to provide the market with quality fruits in the widest possible time span and to rationally organize the harvest; the desirable were fruits of good storage ability and transportability. Self-compatibility was not of crucial importance as a breeding goal, but was also very desirable trait.

In 254 crosses, 40 cultivars were used as female parents (acceptors) and 56 as male parents (donors). The best progenies were originated from the crosses 'Majova Rana' × 'Drogan's Yellow', 'Emperor Francis' × 'Cassin's Early', 'Majova Rana' × 'Schrecken Bigarreau', 'Stella' × 'Van', 'Van' × 'Stella', 'Stella' × 'Bing'. More than 5,000 hybrid seedlings were obtained, out of which 60 were designated as promising. Only 14 were singled out as elite, and certain number of them were tested in several localities in comparative cultivar trials. Two genotypes were released as cultivars – 'Asenova Rana' ('Drogan's Yellow' × 'Majova Rana') and 'Čarna' ('Majova' × 'Schrecken Bigarreau') (Stančević & Nikolić, 1994; Nikolić et al., 1996). The hybrid 'V/60' ('Majova Rana' × 'Primavera') of an very early ripening time (first week of sweet cherry ripening season) is under procedure of recognition at Ministry of Agriculture, Forestry and Water Management of the RS since 2017.

Planned hybridization within *P. cerasus*

Concurrently with sweet cherry breeding programme, the work on the improvement sour cherry genotypes at FRI was also started (1960 year). The breeding goals were to obtain self-fertile, highly cropping cultivars, with different ripening time, large and good quality fruits, suitable for freezing, industrial processing and fresh consumption, with favorable flesh/stone ratio, dark red or colourless juice that does not leak at separation from the stalk (Milenkovic et al., 2006). A special attention has paid to obtain genotypes tolerant/resistant to cherry leaf spot (*Blumeriella jaapii* (Rehm.) v. Arx.), brown rot (*Monilinia laxa* /Ader et Ruhl./ Honey ex Whetz.) and shot-hole (*Clasterosporium carpophilum* (Lév.) Aderh.) (Cerović et al., 1998; Lukić et al., 2012; Radičević et al., 2018a).

In the first phase, planned hybridization included 33 cultivars used as female parents and 52 as male parents, in 125 crosses. The best progenies were obtained from the crosses 'Köröser Weichsel' × 'Heimanns Konserven Weichsel', 'Köröser Weichsel' × 'Richmorency', 'May Sour Cherry' × 'Heimanns Konserven Weichsel', 'Köröser Weichsel' × 'Heimanns Rubin', 'Heimanns Konserven Weichsel' × 'May Sour Cherry' and 'Čačanski Rubin' × 'Heimanns Konserven Weichsel'. More than 20,000 hybrid seedlings have been obtained, 80 of which were singled out as promising; 25 were designated as elite, and evaluated in comparative cultivar trials. Two of them are registered as cultivars 'Čačanski Rubin' ('Shasse Morello' × 'Köröser Weichsel'), and 'Šumadinka' ('Köröser Weichsel' × 'Heimanns Konserven Weichsel').

In the second phase, 11 sour cherry genotypes were selected from the population of 3,000 hybrid seedlings (Cerović et al., 1998; Nikolić et al., 1999), and four of which have been singled out as elite, due to high quality fruits and a significant level of field resistance to economically most serious pests and causal agents of diseases (Radičević et al., 2010). Three of them were released as new sour cherry cultivars in 2015 (Radičević & Cerović, 2015):

'*Sofija*' ('Čačanski Rubin' × 'Heimanns Konserven Weichsel') – a mid-early ripening cultivar (the 2nd decade of June under the West Serbia condition), with large to very large roundish fruits (7.0 g) of rubired skin and an easily separating very long stalk; flesh and juice are red, sweet-subacid, of pleasant aroma and excellent quality; stone is medium large; regular and heavy cropper; shows high level of field resistance to *Blumeriella jaapii*;

'*Nevena*' ('Köröser Weichsel' × 'Heimanns Konserven Weichsel') – a mid-late ripening cultivar (the beginning of the 3rd of June under the West Serbia condition), with large to very large fruits (7.0 g) of dark red skin and short to medium long stalk; flesh is dark red, of pleasant aroma and high quality; juice is intensive coloured; stone is small to medium large; tolerant to *Blumeriella jaapii*; high-quality fruits are characterised by good transportability and suitability for both fresh consumption and different kinds of processing;

'*Iskra*' ('Köröser Weichsel' × 'Heimanns Rubin') – a mid-late ripening cultivar (the 3rd decade of June under the West Serbia condition), with large to very large flattened-spherical fruits (7.5 g) of medium long stalk, light red skin and juice, whitish mesocarp of pleasant aroma and excellent quality and small stone; regular and heavy cropper in the presence of adequate pollenisers; tolerant to *Blumeriella jaapii*; high quality fruits are suitable for both fresh consumption and different kinds of processing.

The new breeding programme within *P. cerasus* at FRI started in 2011 (with the beginning of project *TR31064 – Development and preservation of genetic potential of temperate zone fruits*, supported by the Ministry of Education, Science and Technological Development of the RS), and is in accordance with the requirements of the modern sour cherry production, regarding the breeding objectives, and the proper choice of parental genotypes to achieve the setted goals.

First of all, attention has paid to sour cherry germplasm from the West Serbia region, whose variability is an abundant source of diversity – for clonal selection and using the autochthonous genotypes as a 'final products' in commercial growing, or in conventional breeding (as parents in planned hybridization). Many of the autochthonous sour cherry genotypes have been evaluated, and some of them are selected as elite. Two of them ('GV-6' and 'GV-10') with large fruits, early ripening time and resistance to causal agents of cherry diseases (Radičević et al., 2018a; 2019) are under procedure of recognition at Ministry of Agriculture, Forestry and Water Management of the RS since 2019.

New planned hybridization programme aims to unify the desired characteristics of the genotypes obtained from the planned hybrid populations (domestic and introduced cultivars, promising hybrids) and from the natural populations (indigenous genotypes). The concept is influenced by the fact that the pedigree of the commercially important cultivars is dominated by a relatively small number of genotypes (Lukić et al., 2016). It is continued with strategies of earlier stages, by using genetic potential of German cultivars 'Heimanns Konserven Weichsel' and 'Heimanns Rubin', well adapted to Serbian agroecological conditions or their progenies - i.e. 'Šumadinka', 'Sofija' and 'Nevena'. On the other hand, the usage of autochthonous genotypes in breeding work is increased. Beside the earlier used 'Köröser Weichsel' ('Pandy Meggy') that significantly influenced the fruit quality of obtained sour cherry progenies, we also include some of the high-quality-fruited genotypes of Hungarian origin ('Erdi Botermo' and 'Ujfehertoi Furtos') and genotypes selected in West Serbia region ('GV-6' and 'GV-10').

High cropping potential, fruit quality to suit the needs of industrial processing and fresh consumption (fruit size, low share of stone in the total fruit weight and easily detachable stone, as well as a high soluble solids content with a good balance of sugars and acids), as well as the tolerance to *Blumeriella jaapii* are among the major breeding goals. In addition, obtaining earlier-ripening sour cherries is main goal too, since commercially important cultivars are characterised by the relatively short span in the ripening time. Self-fertility and suitability for mechanical harvesting (medium vigour and upright growth, uniformly ripening time, fruit firmness and resistance to bruises, easy detachment of stone and absence of juice leakage at stalk separation of fruit) are also desirable traits within FRI current breeding programme.

Floral biology investigation

The knowledge of possibilities for cross-pollination and fertilization in cherries is essential for an adequate choice of cultivar combination that ensures abundant fruit set.

Among genetically compatible cultivars, the possibility for effective pollination are related to their flowering time (early, mid-early, mid-late and late flowering cultivars), which is an important part of floral biology investigation at FRI (Radičević et al., 2008; 2012). Perennial overlap during the phenophase of full flowering lasting 5 to 8 days is the crucial for a successful cross-pollination in commercial orchards (Cerović et al., 2005); the overlap should be accompanied by a short span in the beginning of full flowering (the most 2–3 days).

Pollen quality is one the key aspects of reproduction process which to a great extent influences its efficacy and consequently realization of the cropping potential in sweet and sour cherries. Pollen germination *in vitro* is directly related to the regularity of the microsporogenesis process (Cerović, 1991a), and can be the first indicator of genotype adaptability to agro-environmental conditions (Radičević et al., 2012; 2013b). On the other hand, pollen morphology analysis in clarifying the classification of many plants has been recognized from taxonomists, paleobotanists and breeders. Application of scanning electron microscope (SEM) in characterization of sweet cherry pollen grains revealed differences in pollen size, coarseness, depth of ridges, striation of exine among the cultivars, and therefore this method could be used for identification and gene flow studies (Radičević et al., 2013b).

Important parts of floral biology investigation at FRI are pollination experiments, followed by monitoring the pollen tubes growth in the pistil by fluorescent microscopy, and fruit set monitoring. So far it has been shown that pollen tube growth *in vivo* depended on pollen germination *in vitro* in both sour cherry (Cerović & Ružić, 1992a) and sweet cherry (Radičević et al., 2016). Although pollen-pistil interactions in cherries are manifested primarily through the *S-RNase*-based gametophytic self-incompatibility (GSI) system (Cerović, 1997; Bošković et al., 2006; Radičević et al., 2013a), they occur in crossing of compatible cultivars too, therefore the pollen performance and final outcome of the fertilization represent overlapping effect of the following factors – polleniser genotype, behaviour of pollenisers at different temperatures, the influence of pollinated cultivar on pollen performance, the influence of temperature on male-female relations (Radičević et al., 2016).

The reproductive phase is one of the most sensitive plant developmental stages to temperature stress that at blooming time has a detrimental effect on fruit set. Sweet cherry is considered as a species better adapted to the conditions of somewhat colder climates (Hedhly et al., 2005) and this also applies to sour cherry, in an even more pronounced way. A strong genotype-temperature interaction that could reflect the geographic origin of the pollen donor and its adaptation to the prevailing environmental conditions has been shown (Hedhly et al., 2005; Radičević et al., 2016). On the other hand, warm temperature stress accelerates ovule senescence which as a consequence leads to female gametophyte degeneration and ovule abortion (Hedhly, 2011). Assessment of female flower elements functionality based on fluorescent-microscopy and histological methods revealed its dependence on genotypic factors, and the temperature during the full flowering phenophase (Cerović, 1991b; Cerović & Ružić, 1992b; Cerović et al., 1999). Nowadays, the above-mentioned factors were considered in the context of temperature conditions (Cerović et al., 2014; Radičević et al., 2018b), bearing in mind the effects of global warming – warmer winter and incidence of seasons with higher temperatures during the flowering that change tree phenology in main cherry regions in Europe (Chmielevski et al., 2004; Wenden et al., 2016; Herrero et al., 2017), as well as in the Balkan region (Drkenda et al., 2018), including future projections 'stabilization' or 'constant-increase' scenarios (Đurđević et al., 2018; Vuković et al., 2018).

Species or genotypes that showed tolerance are very interesting genetic reservoirs of potential temperature-tolerance genes (Hedhly, 2011), so our work in the present time and future projections lead to the developing of programmes for obtaining cherry genotypes better adapted to the conditions of higher temperatures during flowering, which can respond to a challenge of global warming. Practical aspects are related to regionalization of cultivars (better/lower adapted to environmental conditions in terms of the flower female elements functioning), and choice of the well flowering-synchronized polleniser with potential to reach the ovule in time of its short vitality, and of adequate genetic compatibility.

The study of *S*-allelic constitution in cherry genotypes

Sweet and sour cherry exhibit a GSI system, which prevent self-fertilization and facilitate fertilization with pollen of other genotypes within the species. GSI is controlled by the multiallelic two linked genes of *S*-locus – *S-RNase* and SFB genes, expressed in the style and the pollen, respectively. Sweet cherries are generally self-incompatible and certain pairs of genotypes are cross-incompatible, requiring the availability of compatible pollen to ensure fruit set. Sour cherry includes both self-incompatible and self-compatible genotypes, which are characterised by more complex genetics. Thus the identification of *S*-genotype represents crucial information for cherry breeders and growers. Over the last decade, identification of *S*-genotype in autochthonous, domestic and foreign sweet cherry cultivars using consensus/allele-specific PCR methods has been applied in the laboratory of Department of Pomology and Fruit Breeding at FRI (Radičević et al., 2013a; 2015; Marić & Radičević, 2014; Marić et al., 2015; 2017; 2018; 2019a; 2019b; 2020). *S*-genotyping has become a useful tool for cultivar identification and discrimination (Radičević et al., 2015; Marić et al., 2015); determination of *S*-allelic constitution and incompatibility group in some old cultivars [i.e. 'Majova Rana' (S_1S_4; incompatibility group IX), 'Junska Rana' (S_3S_9; incompatibility group XVI); Marić & Radičević, 2014; Marić et al., 2015]; and released cultivars within breeding programmes of three institutions in the Balkan region [i.e. 'Alexus' (S_1S_2; incompatibility group I), 'Čarna' (S_1S_4; incompatibility group IX), 'Kossara' and 'Rosita' (S_2S_9; incompatibility group XLIII), 'Bucium' (S_3S_6; incompatibility group VI), 'Asenova Rana' and 'Rosalina' (S_3S_9; incompatibility group XVI), 'Margonia' (S_5S_6; incompatibility group XV); Marić et al., 2017; 2018] which have not been published earlier; assessment of self-(in)compatibility in some sweet cherry cultivars (Radičević et al., 2013a); diversity evaluation of local sweet cherry germplasm originated in the Balkan countries (Marić et al., 2019a; 2019b; 2020). Additionally, multi-disciplinary approach, based on S-genotyping and the average full flowering overlap, has been used for recommendation of cultivars in sweet cherry orchards, in order to obtain effective cross-pollination and fertilization ensuring maximum long-term economic returns (Radičević et al., 2011; 2015; 2018c). Future work will be focused on the analysis of segregating progenies in order to elucidate the nature of self-compatible behaviour in some sweet cherry genotypes, as well as on determination of *S*-allelic constitution in autochthonous genotypes and newly released sweet and sour cherry cultivars and hybrids within breeding programmes in the Balkan region.

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OPLEMENJIVANJE TREŠNJE I VIŠNJE U INSTITUTU ZA VOĆARSTVO, ČAČAK – PROŠLOST, SADAŠNJOST I BUDUĆNOST

Sanja Radičević[1*], Slađana Marić[1], Radosav Cerović[2]

[1]*Institut za voćarstvo, Kralja Petra I br. 9, 32000 Čačak, Republika Srbija*
[2]*Univerzitet u Beogradu, Inovacioni centar Tehnološko-metalurškog fakulteta u Beogradu d.o.o, Karnegijeva br. 4, 11000 Beograd, Republika Srbija*
[*]*E-mail: sradicevic@institut-cacak.org*

Rezime

Oplemenjivanje trešnje i višnje u Institutu za voćarstvo, Čačak ima višedecenijsku tradiciju (od 1960. godine), pri čemu je kao osnovna metoda korišćena planska hibridizacija u okviru trešnje (*Prunus avium* L.) i obične višnje (*Prunus cerasus* L.). U ranijem periodu su priznate dve sorte trešnje (Asenova rana i Čarna) i dve sorte višnje (Šumadinka i Čačanski rubin). Tri nove sorte višnje (Sofija, Nevena i Iskra) su priznate 2015. godine. Novi oplemenjivački program višnje je započeo 2011. godine, i baziran je na korišćenju domaćih genotipova dobro prilagođenih agroekološkim uslovima Republike Srbije i introdukovanih genotipova – poznatih izvora otpornosti, izraženog potencijala rodnosti i kvaliteta ploda. Posebna pažnja je posvećena selekciji autohtonih genotipova višnje krupnog ploda na području zapadne Srbije; varijabilnost ovih genotipova je bogat izvor diverziteta – za komercijalno gajenje, ili u roditeljskim kombinacijama za stvaranje novih sorti u okviru planske hibridizacije. Jedan genotip trešnje (V/60) i dva genotipa višnje (GV-6 i GV-10) su u postupku priznavanja kod Komisije za priznavanje sorti i podloga koštičavih vrsta voćaka pri Ministarstvu poljoprivrede, šumarstva i vodoprivrede Republike Srbije. Istraživanja su takođe usmerena na različite aspekte biologije cvetanja trešnje i višnje - karakteristike fenofaze cvetanja, kvalitet polena, monitoring rasta polenovih cevčica u stubiću, kao i citoembriologiju. Reproduktivno ponašanje kao donora/recipijenta polena razmatrano je u kontekstu genotipskih specifičnosti, i njihove ekspresije u odnosu na temperature tokom fenofaze cvetanja, a naročito u odnosu na više temperature. Istraživački rad na ispitivanju samo- i unakrsne (in)kompatibilnosti kod trešnje i višnje započelo je ispitivanjima inicijalnog i finalnog zametanja plodova pri kontrolisanom oprašivanju u poljskim uslovima, a kasnije nastavljeno praćenjem rasta polenovih cevčica u stubiću i plodniku korišćenjem fluorescentne mikroskopije, u cilju iznalaženja najboljih rešenja za izbor adekvatnih sortnih kompozicija koje daju najbolje rezultate u pogledu zametanja plodova i prinosa. Tokom poslednje decenije, uvedena su molekularna istraživanja u proučavanju samo-inkompatibilnog lokusa *S* kod trešnje, koja su dovela do suštinskog pomaka u sagledavanju i rešavamju problema sortne kompozicije oprašivača u proizvodnim i eksperimentalnim zasadima ove izrazito samobesplodne vrste voćaka. primena PCR-metode sa konsenzus prajmerima za prvi i drugi intron *S-RNaze*, kao i alel-specifičnim prajmerima omogućila je *S*-genotipizaciju autohtonih, domaćih stvorenih oplemenjivačkim radom i introdukovanih sorti i hibrida trešnje.

Ključne reči: *P. avium, P. cerasus*, oplemenjivanje, selekcija, nove sorte, biologija cvetanja, *S*-(in)kompatibilnost