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Original scientific paper

ANALYSIS OF WILD SWEET CHERRY (*Prunus avium* L.) GERMPLASM DIVERSITY IN SOUTH-EAST SERBIA

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Ten wild growing sweet cherry (*Prunus avium* L.) genotypes from South-East Serbia with different fruit skin color were analyzed for its phenological, morphological and chemical traits. Agronomic evaluation of germplasm accessions revealed considerable diversity among different accessions for all the characters studied. The analysis of variance revealed significant differences among all genotypes for almost all examined properties. Cluster analysis showed adequate grouping of wild sweet cherry genotypes according to pomological characterization and distinguished them into two distinct groups. The first group had two subgroups and consisted of seven genotypes, while the second one included only three accessions. Despite of the significant differences among genotypes, the total concentration of phenols made a clear separation between the clusters. The

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level of genetic diversity in these wild sweet cherry genotypes is very high and therefore these trees are useful sources of variability for attributes studied and can be employed in further breeding programs or conservation.

Key words: analysis of variance, cluster analysis, pomology, variability

INTRODUCTION

This study concerns wild cherry (*Prunus avium* L.), which is a diploid member of the *Rosaceae* that occurs naturally in western Eurasia and northern Africa (RUSSELL, 2003). It is deciduous, vigorous, broadleaf tree with an erect pyramidal canopy shape that can reach heights of up to 25 m. Its growth is fast till 40 years and expected senescence is about 80 to 90 years (STOJECOVÁ and KUPKA, 2009). Wild sweet cherry is a pioneer species, capable of quickly colonizing clearings by seeds and suckers. It generally occurs as an individual tree or small clusters, often clonal, scattered throughout mixed forests, the potential for selecting it from natural populations is rather limited. It is essentially a lowland species with a maximum altitude of about 1900 m reported in France (RUSSELL, 2003). Because of its wide natural distribution and high levels of genetic variation, ecotypes are expected to develop. These local forms show high variation in tree and fruit size, productivity, ripening time, fruit quality and disease resistance (IEZZONI, 2008).

No matter the fact that the wild sweet cherry seeds are used for generative rootstock production, and its fruits are suitable for table consumption and as a local medicine, the majority of these wild sweet cherry genotypes are potentially useful genetic sources of resistance against diseases and pests, and available for cultivated sweet cherry improvement.

Sweet cherry, as compared to other fruit species, exhibit high genetic variability, that has not yet been well explored and exploited. A thorough assessment of wild sweet cherry germplasm could help to obtain archives of the existing genotypes, comprising most of the variability of important properties. Hence, prominent characteristics for fruit producers, such as morphology, chemical composition, host resistance to canker and cracking, self-fertility, dwarfing, seed germination, and tree size uniformity, should be considered by gene bank curators in their assessment of variation among sweet cherry accessions (HJALMARLSSON and ORTIZ, 2000).

Cluster analysis allows analyzing simultaneously both quantitative and qualitative traits, and each entry is treated as an individual entity of equal weight. Cluster analysis based on descriptive data on genetic resources can be useful in classifying accessions in a germplasm collection (LACIS *et al.,* 2009). This is the only possibility to classify accessions with unknown origin, as in case of the wild sweet genotypes.

Therefore, the aim of our paper were to quantify and characterize *in situ* and describe the variability of ten *Prunus avuim* L. accessions using phenotypic characters, because the knowledge of the genetic variation of wild sweet cherry could be important for their efficient use in further improvement and for

conservation purposes. Other objective was to obtain a dendogram of individuals and determine relation patterns by cluster analysis.

MATERIALS AND METHODS

Wild growing sweet cherry (*Prunus avium* L.) fruits were harvested from villages Kušići, Županjevica, Bukovica, Opaljenik and Rokci situated on mountain Golija, south-east Serbia, (latitude 20° 24' E, longitude 43° 45' N, and altitude 877-1200 m). The fruits were collected in three consequent years. The trees were approximately 40 years old, originating from seeds and were selected according to its phenotypic and organoleptic characteristics.

Thirty fruits from each wild sweet cherry genotype were picked in mature stage and used for performing analyses. Twenty-five variables were measured as described:

1-3. Start bloom (SB), full bloom (FB) and petal fall (PF) were expressed as the number of days from May 1st.

4-5. Beginning of ripening time (BRT) was determined when 5-10% ripe fruits can be observed and full maturity (FM) when almost all the fruits can be easily removed from the stalk, and were expressed as the number of days from July 1st.

6–9. Fruit length (FL), fruit width (FWD), stone length (SL) and stone width (SW) were measured by caliper in cm, respectively.

10–11. Fruit (FW) and stone weight (SW) were measured by scale in g, respectively.

12–13. Leaf length (LL) and leaf width (LW), in cm.

14. Leaf area (LA) was determined using Adobe PhotoShop CS 8.0, histogram level 254, data are given in cm^2.

15. Leaf stalk length (LSL), in cm.

16. The skin color (SC) was evaluated by three panelists according to UPOV (2006).

17. Soluble solids content (SS) was determined by refractometer (Atago, pocket PAL-1) in °Brix.

18-20. Total sugar (TS), invert sugar (IS) and sucrose content (SC) determined by Bertrand method, in %.

21. Titratable acidity (TA) was measured by neutralization to pH 7.0 with 0.1 N NaOH, expressed as percent of malic acid equivalent.

22. Pectin content (PC) was determined by Carbazole spectrophotometric determination method, in %.

23. The total concentration of phenols (TP) was estimated by Folin-Ciocalteu method, as mg/l.

24. The total anthocyanin content (AC) was investigated according to the procedure described in European Pharmacopoea 6.0. (2008), mg/l

25. The vitamin C (VC) was determined by an iodometric titration, mg $100g^{-1}$.

Differences between accessions and years of investigation were determined by analysis of variance (ANOVA). The least significant difference (LSD) when necessary was used to determine if the difference between two genotype is large enough to be considered real at a fixed level of confidence (LSD 0.05 = 95% confidence and LSD 0.01 = 99% confidence). Cluster analysis was done with all the selected variables (except for SC) using the UPGMA method. The tree-plot of clusters obtained by this procedure was used to decide the ultimate number of clusters by which the seedlings could be assessed. A statistical analysis was performed using software Statistica 6.0 for Windows (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Scores for the 25 variables in 10 wild sweet cherry genotypes are shown in Tables 1 and 2. SB, FB and PF of examined genotypes showed a narrow range (5, 2, 5 days, respectively). Late flowering genotypes are preferable since early flowering in wild cherry is often disadvantageous because late frosts are destroying open flowers and preventing fruit from forming (VAUGHAN *et al*., 2007). The differences for BRT and FM were slightly higher (9 and 7 days, respectively). Since accessions studied originate from a small geographic area, no different patterns for the phenological traits could be revealed.

Table 1. Phenology and morphological traits of fruit, stone and leaf in wild sweet cherry genotypes

	SB[a]	FB	PF	BRT	FM	FW	FL
1	11.05	19.05	29.05.	13.07.	19.07.	1.34	1.18
2	13.05	19.05	31.05.	14.07.	22.07.	1.05	1.08
3	08.05	18.05	26.05.	05.07.	15.07.	1.19	1.10
4	08.05	18.05	26.05.	05.07.	15.07.	0.91	0.96
5	08.05	18.05	26.05.	05.07.	15.07.	1.39	1.17
6	12.05	20.05	29.05.	05.07.	15.07.	1.30	1.06
7	11.05	20.05	27.05.	13.07.	22.07.	1.16	1.01
8	08.05	18.05	25.05.	11.07.	19.07.	0.91	1.05
9	08.05	18.05	25.05.	05.07.	18.07.	1.12	1.07
10	08.05	18.05	25.05.	05.07.	18.07.	0.78	0.95
LSD 0.05	1.62	0.96	1.25	1.46	3.13	0.28	0.11
LSD 0.01	2.50	1.47	3.15	2.26	4.82	0.43	0.17

	FWD	SW	SL	SW	LL	LW	LA	LSL
1	1.13	0.18	0.83	0.55	9.48	4.85	33.62	2.73
2	1.02	0.16	0.81	0.54	9.42	4.75	33.95	3.07
3	1.03	0.17	0.81	0.52	8.24	4.37	25.87	2.61
4	1.03	0.15	0.71	0.53	9.41	5.72	39.10	2.57
5	1.08	0.20	0.89	0.55	9.77	4.80	31.63	3.52
6	1.16	0.14	0.72	0.53	8.04	4.25	25.29	2.45
7	1.00	0.14	0.70	0.55	8.01	5.55	32.64	2.08
8	0.89	0.17	0.79	0.58	7.42	4.22	22.17	2.14
9	1.00	0.16	0.75	0.52	8.94	4.21	26.74	2.69
10	0.99	0.12	0.65	0.50	8.11	4.34	24.24	2.64
LSD 0.05		0.026	0.068			0.40	6.62	0.44
LSD 0.01		0.040	0.105			0.61	10.18	0.67

[a] for explanation of character symbols, see ''Materials and Methods''

FW (Table 1) varied between 0.78 g (genotype 10) to 1.39 g (genotype 5). Similar findings have been reported by KARLIDAG *et al.*, (2009) for some wild sour cherry genotypes in Turkey with FW values between 0.76 and 2.11 g. The FL varied from 0.95 cm (genotype 10) to 1.18 cm (genotype 1) and fruit width from 0.89 (genotype 8) up to 1.13 cm (genotype 1). The lowest results regarding stone traits showed accession 10, and the highest accession 5. Leaves in sweet cherry are relatively large, elliptic with acute tips, petiole and strongly veined. LA ranged between 22.17 cm^2 to 39.10 cm^2 and showed big differences between genotypes.

Skin color (Table 2) is widely varying characteristic among sweet cherry cultivars and the most important indicator of quality and maturity of the fresh sweet cherries (USENIK *et al.*, 2006). Genotypes were arranged according to the fruit color (Table 2) from yellow (genotypes 3 and 5) to light red (genotype 4), brown red (genotype 2) and blackish (genotypes 1, 6, 7, 8, 9 and 10).

Another character to be taken into consideration was the SSC (Table 2), that ranged from 17.95 (genotype 6) to 28.65% (genotype 4). Accession 3, 4, 5 (with yellow and light red skin color) and genotype 10 with blackish skin color showed the highest soluble solid content (>25%). From our results can be concluded that wild growing sweet cherries had much higher SSC than commercially grown sweet cherry cultivars as reported by RADIČEVIĆ *et al.* (2008), KALYONCU (2009) and GARCIA-MONTIEL *et al.* (2010). The main sugars found in cherry cultivars have been glucose and fructose, followed by sorbitol and sucrose (USENIK *et al.*, 2008). Genotype 7 had the highest TS (18.21%) and the IS content (16.04%), while the SC was the highest in the genotype 1 (2.63%). Genotype 8 showed the lowest TS (10.335) and SC (0.71%). Our results are showing slightly higher values for reducing sugars than BERNALTE *et al.* (1999) reported for some sweet cherry cultivars, but much lower values for sucrose content found by USENIK *et al.* (2008). Important differences were

also found in TA. In our study TA was the highest in the yellow colored genotype 3 (2.00%) and the lowest in another yellow colored genotype 5 (1.34%). KARLIDAG *et al.*, (2009) reported acidity in wild growing sweet cherries from 0.98% to 1.53% which is in accordance with the results of this paper. Pectin content varied from 0.36 (genotype 4) up to 0.77% (genotype 8).

Table 2. Skin color and chemical traits in wild sweet cherry genotypes

	SC[a]	SSC	TS	IS	SC	TA	PC	TP	AC	VC
1	blackish	18.53	11.44	8.67	2.63	1.57	0.43	1544	0.016	6.46
2	brown red	20.75	11.92	10.60	1.26	1.35	0.58	1551	0.026	7.82
3	yellow	26.63	12.98	11.74	1.18	2.00	0.50	1081	0.013	3.49
4	light red	28.65	13.21	12.06	1.10	1.44	0.36	1140	0.015	5.11
5	yellow	25.20	12.55	11.77	0.75	1.34	0.61	1063	0.045	3.32
6	blackish	17.95	11.70	10.76	0.90	1.64	0.52	1482	0.022	5.38
7	blackish	20.40	18.21	16.04	2.06	1.71	0.60	1405	0.017	5.43
8	blackish	21.35	10.33	9.59	0.71	1.59	0.77	1486	0.022	4.32
9	blackish	22.78	12.75	11.65	1.05	1.98	0.67	1388	0.014	4.32
10	blackish	26.15	11.55	9.70	1.77	1.81	0.61	1503	0.073	7.15
LSD 0.05	-	1.91	2.13	1.65	0.88					2.12
LSD 0.01	-	2.94	3.28	2.54	1.35					3.26

Phenolics (Table 2) are bioactive compounds concentrated in the skin of sweet cherries followed by flesh and pit, respectively. They contribute to sensory and organoleptic qualities of fruits, such as taste and astringency (FERRETTI *et al*., 2010), providing health-beneficial effects (TOMÁS-BARBERÁN and ESPÍN, 2001). Previously reported studies (ESTI *et al*., 2002; GONCALVES *et al*., 2004) indicate that plant genotype strongly affects total phenolic content in sweet cherries. The highest TP from our wild sweet cherry trees was recorded in genotype 2 (1544 mg/l) which had brown-red red skin color, while the lowest in genotype 5 (1063 mg/l) with yellow fruit skin color. This was quite expectable while knowing the facts that the major phenolics in sweet cherries are anthocyanins, especially in dark-colored fruits. Obtained values are in accordance with the results published by MOŽETIĆ *et al.* (2002) for different cultivars of sweet cherries from Nova Gorica region. Anthocyanins presence is universally associated with attractive, colorful and flavorful fruits (KARLIDAG *et al.,* 2009), while recently are connected with beneficial activities as food ingredients and as promoters of human health. According to the results obtained by GONZÁLEZ-GÓMEZ *et al*. (2009), the highest concentrations of anthocyanin pigments were found in the autochthonous sweet-cherry cultivars. Our data showed the highest amount of AC in the blackish fruits of the genotype 10 (0.073 mg/l), while in the yellow genotype 3 was measured the lowest level of AC (0.013 d mg/l). At 10 mg per 100 grams of flesh, fresh sweet cherries rank as a

moderate source of vitamin C. In our study VC in ten studied wild sweet cherry genotypes ranged from 3.32 (genotype 5) to 7.82 mg% (genotype 2), which was much lower that the results recorded by KARLIDAG *et al.* (2009) in wild sweet cherry genotypes originated from Turkey.

According to the analysis of variance, very significant differences between studied wild sweet cherry genotypes were determined for all the phenologic traits, FW, FL, stone properties, all leaf characteristics but LL, SSC, all the sugars and VC.

Since hierarchical cluster analysis is allowing the assessment of similarity or dissimilarity and clarifies some of the relationships among the accessions, it is widely used to study fruit germplasma. It was previously done for evaluation of sour cherry genepool (RAKONJAC *et al.,* 2010), raspberry seedlings (FOTIRIĆ AKŠIĆ *et al*., 2012) and apple genetic resources (MRATINIĆ and FOTIRIĆ AKŠIĆ, 2012).

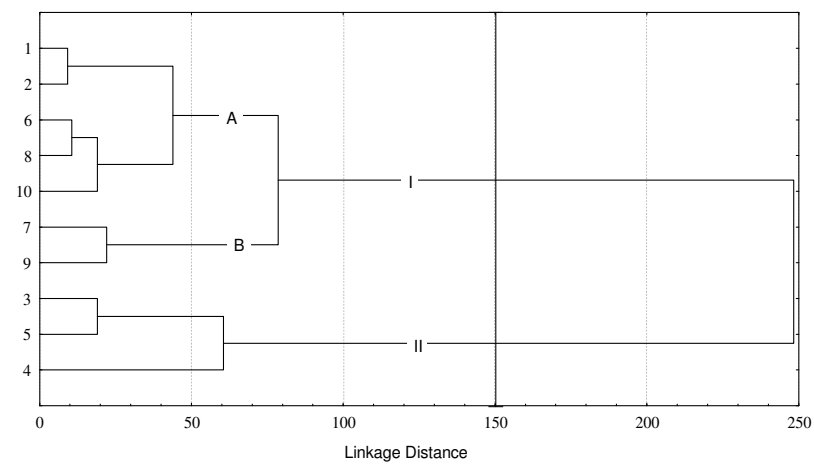

Figure 1 - Dendrogram of the 10 wild sweet cherry genotypes

Dendogram obtained by the cluster analysis showed adequate grouping of wild sweet cherry genotypes according to phenological and pomological characterization and distinguished them into two distinct groups (Figure 1). The average genotype distance (GD) among wild sweet cherry trees based on phenological, physical and chemical traits was GD = 219.2, ranging from GD = 9.2 (the most relate accessions, 1 and 2) to 488.2 (the most distantly related, 2 and 5). Cluster I included seven accessions (1, 2, 6, 7, 8, 9 and 10) which had brown red to blackish skin color. Those genotypes are mainly characterized by later full maturity, lower SSC and the sugars and very high TP (1405 – 1551 mg/l). This cluster was split off, into two distinct sub groups, defined as cluster IA and IB, respectively.

Sub-group (IA) consisted of five accessions (1, 2 6, 8 and 10) with higher TP than accessions 7 and 9 that formed sub-group (IB). Group II, where yellow and light red fruit skin colored genotypes were placed, was characterized by early full maturity (July 15th), high SSC content (25.20 – 28.65%), TS (12.55 – 13.21%), IS (11.74 – 12.06%) and the low TP (1063 – 1140 mg/l). Cluster analysis showed a considerable diversity in the wild sweet cherry genotypes where the total concentration of phenols was a determinant criterion for genotypes clustering.

CONCLUSION

Our results show large variations in phenological and pomological properties of wild growing sweet cherries, showing wide diversity among genotypes originating from south-east Serbia. Statistically significant differences in numerous characteristics are offering reliable data for the selection of genotypes with later flowering time or improved physical and chemical traits.

Present study confirmed the necessity of preserving these unique genetic resources and continuing its study no matter the fact that in practice, however, it is difficult to determine whether a specific genetic variant will be of future value. From the economic point of view detailed description of such germplasm through plant genetic resource that comprises collection, maintenance, characterization and evaluation of the genetic diversity within wild sweet cherry genotypes is also a guarantee of obtaining better results in the selection and genetic improvement programs.

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PROUČAVANJE DIVERZITETA DIVLJE TREŠNJE (*PRUNUS AVIUM* L.) NA PODRUČJU JUGO-ISTOČNE SRBIJE

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U ovom radu ispitivane su fenološke, morfološke i hemijske osobine deset genotipova divlje trešanje (*Prunus avium* L.) različite boje pokožice, poreklom iz jugoistočne Srbije. Izučavanjem ove germplazme otkriven je značajan biodiverzitet između ispitivanih biljaka. Analiza varijanse otktrila je značajne razlike između genotipova za skoro sve proučavane osobine. Klaster analizom genotipovi divlje trešnje su grupisani i na osnovu pomološke kategorizacije su izdvojene dve grupe. Prva grupa je sadržala dve podgrupe i obuhvatala je sedam genotipova, dok je drugoj pripadalo samo tri genotipa. Bez obzira na značajne razlike ovaka podela između proučavanih biljaka je izvršena na osnovu koncentracije ukupnih fenola. Nivo genetičkog diverziteta kod proučavanih genotipova divlje trešnje je veoma viosk i zbog toga ova stabla su koristan izvor varijabilnosti za pročavane osobine i mogu se koristiti u budućim oplemenjiačkim programima pri ukršanju između sorti i divljih genotipova.

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