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Analysis of Morphological Variability in Wild Cherry (*Prunus avium* L.) Genetic Resources from Central Serbia

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ABSTRACT

In thirty-three wild cherry (*Prunus avium* L.) accessions from Central Serbia, tree, leaf, fruit, and stone morphological characters were evaluated during three consecutive years. The goal was to detect relationships between the genotypes and to identify the most useful traits for discrimination among them. The study revealed a high variability in the set of the evaluated wild cherry accessions and considerable differences were found among them in all studied attributes. The majority of important correlations were determined among variables representing fruit and leaf size, and variables related to color. Cluster analysis distinguished wild cherry accessions into two distinct groups. In PCA, fruit and leaf traits such as leaf length and width, and fruit height, width, and weight, and skin flesh and juice color were predominant in the first two components, indicating that they were useful for the assessment of wild cherry germplasm characterization. These results indicate that these accessions must be conserved as valuable genetic resources to enrich the cherry gene pool and can be used for improving breeding efficiency of important horticultural traits worldwide.

Keywords: Cluster analysis, Fruit traits, Leaf traits, PCA.

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INTRODUCTION

Wild cherry (*Prunus avium* L.) is a diploid (2n= 16) member of the *Rosaceae,* typical out-crossing species with a monogenic and multiallelic gametophytic incompatibility system (Tehrani and Brown, 1992). It originated from the Caucasus region and is currently found across mainland Europe, Asia Minor, and North Africa (Webster, 1996). Sexual reproduction by the foraging behavior of bees may play a dominant role besides seed dispersal, while the seeds are mostly distributed by migratory birds, and often by humans (Webster, 1996). Since it occurs as an individual tree or small clusters, scattered throughout mixed forests, the potential for selecting it from natural populations is rather limited.

Natural population of Serbia meets a large number of economically important species, among others, wild cherry. It can be found mostly in mountainous areas up to the 1200 m altitude, where it often occurs as a forest edge species, in places where birds are nesting or as individual trees on the boundaries. The majority of these genotypes are potentially useful genetic sources of important pomological traits and available for cultivated sweet cherry improvement. Unfortunately, in our country, wild cherry belongs to the endangered species of forest trees (Banković *et al*., 2009).

Conservation of genetic resources has received increasing attention over the last

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decades. Therefore, the estimation of the diversity and its nature and magnitude are beneficial or even crucial to a breeding program. The availability and informative value of plant germplasm are becoming more and more important for the future preservation and sustainable use of genetic resources (Lacis *et al.*, 2010). This task is particularly challenging in forest trees whose life cycles are very long (Jolivet *et al.*, 2011). Since *P. avium* is widely distributed and has high level of genetic variability, which has not yet been well explored and exploited, ecotypes are expected to develop (Iezzoni, 2008). Prominent characteristics among cherry accessions such as tree habit and vigor, cropping efficiency, pomological traits, and resistance to pests and diseases should be considered as the traits important for the assessment. Thus, *in situ* methods are foreseen as the main component of conservation strategies and the first step in avoiding the disappearance of these genes from various growing and cultivation zones (Pérez-Sánchez *et al.*, 2008).

Since morphological characterization continues to be the first step for the description and classification of germplasm, numerous wild cherry genotypes have been evaluated and characterized for various traits, including diversity (Budan *et al.*, 2009; Gregorius *et al.*, 2011; Holtken and Gregorius, 2006; Ruisa, 1998; Stojecová and Kupka, 2009; Vaughan *et al.*, 2007). The most widely used multidimensional analysis methods in characterization of the plant genetic resources are principal component analysis (PCA) and cluster analysis. PCA enables visualization of differences among individuals, identification of possible groups and relationships among individuals and variables (Martínez-Calvo *et al.*, 2008). Principal components analysis is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences (Mattos *et al.*, 2010; Mratinić *et al.*, 2011). Cluster analysis allows analyzing simultaneously both quantitative and qualitative traits, and each entry is treated as an individual entity of equal weight. Cluster analysis has been employed to assess similarities among genotypes in plant breeding programs. When phenotypic repetition of several characters are found among a set of populations, lines or accessions' parents are then selected from among them for hybridization (Zeinali *et al.*, 2009)

On basis of these prerequisites, the main objectives of the current study were: (1) to quantify and characterize the variability of morphological traits within the set of 33 wild cherry accessions from the central Serbia, (2) to calculate the coefficients of correlation between studied characteristics, (3) to detect relationships between the genotypes, and (4) to identify the most useful traits for discrimination among the genotypes

MATERIALS AND METHODS

In this study, we included eight geographically separated *P. avium*

Table 1. Location of sampling sites, represented by the geographic longitudinal and latitudinal coordinates together with altitude of 33 *Prunus avium* L. accessions studied.

Code number	Location	Geographic coordinates		Altitude (m)	No. of individuals
		Longitude	Latitude		
K	Kotraža	20° 23' E	43° 71' N	428	9
G	Stanišići	20° 51' E	43° 30' N	813	6
X	Katići	20° 23' E	43° 58' N	632	8
Q	Kušići	20° 10' E	43° 50' N	1000	2
Z	Županjevica	20° 11' E	43° 53' N	1200	1
B	Bukovica	20° 15' E	43° 55' N	700	3
O	Opaljenik	20° 13' E	43° 51' N	900	1
R	Rokci	20° 21' E	43° 53' N	877	3

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populations, with the set of 33 accessions, located in the central Serbia (Table 1). All accessions were selected as phenotypically mature trees from a mixed deciduous forest. Within the study plots, wild cherry accessions were scattered as single trees or in small groups and its collection may be considered as a completely random sample. The trees were chosen as representative of the phenotypic variability observed in the field.

Accessions have been evaluated using a set of 19 traits: tree habit (TH), tree vigor (TV), cropping efficiency (CE), fruit (FW) and stone weight (SW), share of flesh (SF), fruit length (FL), width (FWD), fruit shape index (FSI), fruit stalk length (FSL), fruit shape (FS), fruit skin colour (FSC), fruit juice colour (FJC) and fruit flesh colour (FFC), leaf length (LL), leaf width (LW), leaf shape index (LSI), leaf area (LA) and incisions of leaf margin (ILM). The phenotypic traits were measured in three consecutive years (2006-2008), on a total of 50 randomly selected fruits and leaves from the same tree. For fruits, all observations were made on ripe fruit, ready for consumption, sampled randomly from the periphery of the trees. Leaves were sampled from the median section of one-year branches during harvesting time. FL, FWD, FSL, LL, and LW were measured by a caliper or by ruler (cm), LA was determined using Adobe PhotoShop CS 8.0, histogram level 254 and the data are given in cm^2. FW and SW were weighed using a scale (g). SF, which represents percentage of fruit flesh in total fruit weight, was obtained by calculation. FSI and LSI were calculated as a *FL/FWD* or *LL/LW* ratio, respectively. Tree, leaf, and fruit properties (TH, TV, CE, FS, FSC, FJC and FFC) were categorized according to IBPGR descriptor for cherry (Schmidt *et al.* 1985). Only trait ILM was described by modifying characteristics number 24 (Leaf blade: incisions of margin, where category 3 was `crenate`, 5 was `medium` and category 7 was `serrate`) from UPOV descriptor for plum (2002).

Statistical analysis was performed using average values from three-year measurements. To show the relationships among the traits, Spearman rank coefficients of correlation were performed. Cluster analysis applied to evaluate relationships among accessions performed by Ward's method using Euclidean distances. A principal component analysis (PCA) was performed to summarize the manifold data in the first principal component containing the highest possible variability of the data. The Eigen values of the 7 PCs were compared for each trait. Data analysis was conducted using the 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

The wild cherry genotypes from central part of Serbia were characterized by a set of 19 traits (Table 2). The wild cherry trees were characterized by accessions with a strong tree vigor (54.55%), mostly upright tree habit (60.61%) and with good yield effectiveness (51.52%). Only one accession (K10) had dropping tree habit.

Wild cherry genotypes had different LSI that varied from 1.63 (K1), which leaves could be considered as elliptic, up to 2.35 (K6) with lance-shaped leaves. Leaf area (LA) as an essential component to estimate plant growth ranged from 22.17 (R1) up to 43.46 cm^2 (G5).

Fruit characterization included several parameters of interest from the commercial growing viewpoint. The accessions had very small fruits ranging from 0.780 g (R3) to 2.376 g (K9), in accordance with the results of Karlidag *et al.* (2009) who studied wild-growing cherry genotypes in Coruh Valley, Turkey. Share of flesh showed a high range from 79.91% (K8) up to 90.65% (K6) that represented much wider interval of varying than found in grown cherry cultivars by Radičević *et al.* (2008). FSL varied from 3.1 (K1) up to 5.6 cm (K6), which was similar to the findings of Pérez-Sánchez *et al.* (2010). A uniform fruit shape for the wild

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Table 2. Description of wild cherry accessions [a].

Acession	TH[b]	TV	CE	LL	LW	LSI	LA	ILM	FS	FSC	FFC	FJC	FSL	FL	FWD	FSI	FW	SW	SF
K1	3	5	5	9.8	6.0	1.63	38.1	3	1	5	3	1	3.1	1.12	1.39	0.80	1.67	0.248	85.1
K2	7	7	7	10.2	5.4	1.89	32.9	5	4	4	1	0	3.2	1.14	1.10	1.04	1.13	0.186	83.5
K3	5	7	7	11.9	6.1	1.95	37.8	3	3	4	1	0	5.4	1.17	1.19	0.98	1.26	0.244	80.7
K4	3	5	5	10.4	5.8	1.79	34.8	3	4	5	3	2	4.5	1.41	1.38	1.04	1.93	0.223	88.3
K6	3	7	7	10.1	4.3	2.35	24.9	5	2	2	1	0	5.6	1.24	1.43	0.86	1.91	0.177	90.7
K7	3	5	7	10.7	6.0	1.78	34.9	5	2	4	2	0	4.5	1.07	1.15	0.93	1.14	0.210	81.5
K8	3	7	5	9.7	5.5	1.76	34.5	3	3	6	4	2	4.3	1.08	1.11	0.97	1.09	0.218	79.9
K9	3	9	7	10.4	6.1	1.70	32.2	3	1	7	5	3	4.5	1.27	1.56	0.81	2.38	0.251	89.4
K10	7	7	7	10.6	6.3	1.68	40.1	5	1	5	3	1	3.6	1.05	1.13	0.91	1.17	0.168	85.3
G1	3	5	5	9.7	4.7	2.06	33.2	5	2	6	4	2	4.4	1.04	1.12	0.93	1.11	0.201	81.6
G2	5	7	7	9.7	5.6	1.73	35.7	5	2	5	3	2	4.7	1.24	1.35	0.93	1.79	0.240	86.4
G3	5	5	5	10.4	5.2	2.00	33.0	5	1	7	4	4	3.8	0.98	1.11	0.88	0.97	0.129	86.7
G4	5	5	5	10.1	5.0	2.02	32.6	7	2	6	4	3	3.8	1.04	1.15	0.90	1.24	0.173	86.0
G5	5	5	3	11.4	5.5	2.07	43.5	7	2	4	4	1	4.7	1.13	1.22	0.93	1.49	0.201	86.5
G6	5	7	7	9.7	5.0	1.94	28.3	5	3	6	4	2	4.3	1.13	1.16	0.97	1.12	0.170	84.7
X1	5	5	5	9.8	5.0	1.96	29.3	5	2	4	4	1	4.8	0.97	1.08	0.91	1.00	0.198	80.1
X2	5	7	5	10.3	5.1	2.02	31.1	5	2	6	4	3	3.2	1.19	1.26	0.95	1.50	0.218	85.3
X3	3	7	7	11.0	5.8	1.90	38.4	3	3	6	4	2	4.4	1.05	1.05	1.00	1.05	0.152	85.1
X4	5	7	7	10.1	5.2	1.94	31.1	7	2	5	3	2	3.3	1.03	1.11	0.92	1.06	0.186	82.3
X5	3	7	7	9.9	4.7	2.11	27.1	5	2	6	4	3	4.0	1.03	1.11	0.93	1.12	0.169	84.9
X7	3	7	7	9.9	5.2	1.90	35.6	3	3	6	4	2	3.9	1.14	1.17	0.98	1.23	0.183	84.2
X8	3	7	7	11.0	6.0	1.83	37.1	3	4	7	5	4	4.0	1.41	1.31	1.08	1.94	0.201	89.6
X9	3	5	5	8.8	5.5	1.60	34.1	3	2	5	3	2	3.2	1.07	1.16	0.92	1.11	0.166	85.0
B1	5	7	5	8.2	4.4	1.87	25.9	3	4	1	2	0	3.4	1.10	1.03	1.07	1.19	0.175	85.7
B2	3	9	7	9.4	5.7	1.63	39.1	5	2	2	3	1	4.0	0.96	1.03	0.93	0.91	0.154	83.5
B3	3	5	5	9.8	4.8	2.12	31.6	7	4	1	2	1	3.9	1.17	1.08	1.08	1.39	0.200	85.6
O1	3	7	3	8.0	5.6	1.46	32.6	5	3	7	5	4	3.2	1.01	1.00	1.01	1.16	0.141	87.9
Q1	5	7	7	9.5	4.9	1.97	33.6	3	4	7	5	4	5.3	1.18	1.13	1.04	1.34	0.179	86.6
Q2	5	7	3	8.0	4.3	1.90	25.3	5	2	7	5	4	3.2	1.06	1.16	0.91	1.30	0.144	89.2
R1	3	9	7	7.4	4.2	1.78	22.2	3	4	7	5	4	4.4	1.05	0.89	1.18	0.91	0.168	81.3
R2	3	7	7	8.9	4.2	2.10	26.7	7	4	6	4	4	4.3	1.07	1.00	1.07	1.12	0.165	85.7
R3	3	3	5	8.1	4.3	1.87	24.2	3	3	7	5	4	3.6	0.95	0.99	0.96	0.78	0.127	84.6
Z1	3	5	7	9.4	4.8	2.00	34.0	5	4	5	3	2	4.0	1.08	1.02	1.06	1.05	0.163	84.8
Average	-	-	-	9.8	5.2	1.89	32.6	-	-	-	-	-	4.07	1.11	1.16	0.97	1.29	0.186	85.1
CV(%)	-	-	-	10.3	12.0	9.6	15.4	-	-	-	-	-	16.5	10.0	12.4	8.6	27.7	17.8	3.2

[a] **Tree vigor (TV):** 1= Very weak; 2= Weak; 3 = Medium; 4= Strong, 5= Extremely strong; **Tree habit (TH)**: 3= Upright; 5= Spreading; 7= Dropping; **Cropping efficiency (CE):** 3= Poor; 5= Intermediate, 7= Good; **Incisions of leaf margin (ILM):** 3= Weak; 5= Medium, 7= Strong; **Fruit shape (FS):** 1= Kidney-shaped; 2= Flat-round; 3= Round, 4= Elongate, 5 = Cordate; **Fruit skin color (FSC)**: 1= Yellow; 2= Orange red; 3= Vermilion on yellow ground colour; 4= Vermilion; 5= Wine red; 6= Mahagony, 7 = Black; **Fruit flesh color (FFC)**: 1= Cream-white; Cream-yellow, 2= Pink; 3= Red; 4= Dark red, 5= Black red; **Fruit juice color (FJC):** 0= Colourless; 1= Pink; 2= Red; 3= Purple-dark red, 4= Black red.

[b] For explanation of character symbols, see ‘‘MATERIALS AND METHODS’’.

cherry genotypes was not observable, but rather a frequent tendency for 'flat around' to 'elongate' fruit shapes (39.40 and 27.27%, respectively) was observed, sometimes tending more towards 'round' or 'kidney-shaped' fruit shapes (21.21%, 12.12%, respectively). In this study, fruit skin color ranged from `yellow` to `black`. This is in accordance with Usenik *et al.* (2008), who stated that fruit skin color was a widely varying characteristic among cherry cultivars. The majority of the studied genotypes had wine red skin color (21.21%), dark red flesh color (36.36%), and red juice color (27.27%).

Among the qualitative characteristics, the largest variability corresponded to external and internal color of the fruit (including skin, flesh, and juice color). For quantitative traits, indicated by the value of coefficient of variation (Table 2), the highest variation was detected for fruit and stone weight (27.7%, 17.8%, respectively).

Important differences were found in the correlations among the studied traits (Table 3). Our results showed positive correlation between the tree vigor and crop efficiency (r= 0.45, P= 0.011), indicating that those traits can be considered as reliable indicators of yield. These results are not in agreement with previous reports of Rakonjac *et al.* (2010) for sour cherry, where more vigorous genotypes showed lower yield efficiency. These differences may well reflect different fruiting types of sour and sweet cherries. Absence of significant correlation between CE and FW (r= 0.04), determined in our study, is in agreement with Iezzoni and Mulinix (1992) who determined that the fruit size had little effect on the yield, but contrasted with the negative correlation reported by Whiting *et al.* (2005).

All variables related to fruit size were correlated with each other (r= 0.72–0.92, P< 0.01), and this was also the case for the variables related to leaf size (r= 0.64–0.82, P< 0.01) and color traits (r= 0.80-0.86, P< 0.01). A correlation between the sweet cherry fruit weight and fruit diameter was detected by Brown (1988), Theiler-Hedricth (1990) and Demirsoy and Demirsoy (2004). A close relationship between traits could facilitate or hinder gene introgression since strong selection for a desirable trait could favor the presence of another desirable trait from this population (Dicenta and García, 1992).

According to the results of our study, the fruit shape index was correlated with the fruit width (r= −0.56, P= 0.001), while the fruit length had almost no influence (r = 0.16). Similar results were obtained also for the leaf shape index, which was highly correlated only with the leaf width (r= −0.57, P= 0.001). Very close results were obtained by Rakonjac *et al* (2010) for Oblačinska sour cherry clones.

Fruit color traits tended to correlate negatively with almost all leaf traits (with the exception of small positive correlation between FSC and LW), but only the negative correlations of FJC with LL (r= -0.35, P= 0.048) and FFC with LL (r= -0.42, P= 0.008) were statistically significant. Similar findings were determined between fruit color traits and fruit traits related to size, where the majority of correlations were small and negative, without any significance. Weak correlations between fruit color traits and other properties examined indicate that each of those is genetically independent.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among wild cherry accessions. Obtained dendrogram had two main clusters (Figure 1). The first cluster (I) included the majority (25) of the accessions studied and could be divided into five sub-groups (IA, IB, IC, ID and IE). Eight wild cherry genotypes were placed in the second cluster (II). No evidence of relationships for most of the accessions according to their geographic origin was found as the contrary to the results of Moreno and Trujillo (2005) for sweet cherry cultivars and Li *et al.* (2009) for Chinese sour cherry (*Prunus pseudocerasus* Lindl.).

The average accession distance (d) among

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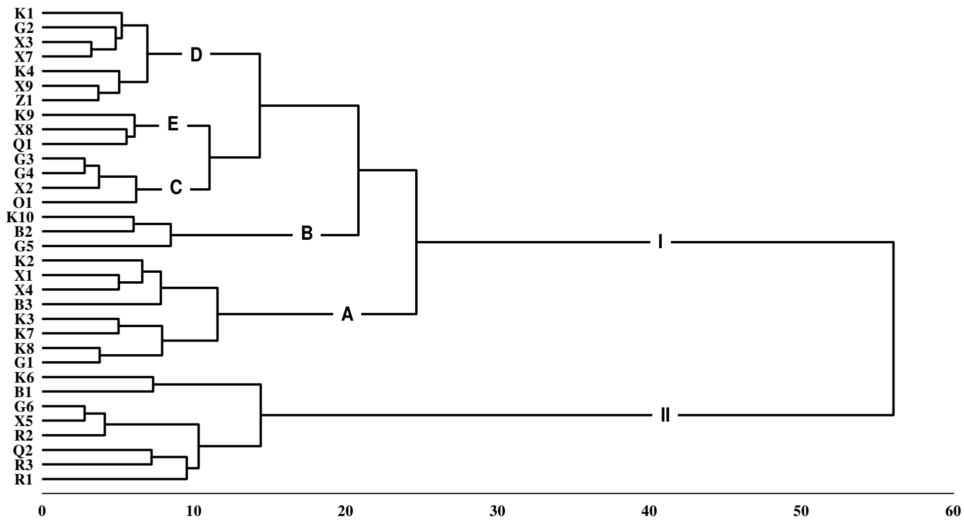
Table 3. Correlation matrix among characteristics studied in wild cherry accessions.

	TV	CE	LL	LW	LSI	LA	ILM	FS	FSC	FJC	FFC	FSL	FL	FWD	FSI	FW	SW	SF
TH	0.06	-0.02	0.22	0.10	0.08	0.12	0.28	-0.16	-0.09	-0.22	-0.22	-0.13	-0.07	-0.03	-0.10	-0.11	-0.01	-0.06
TV		0.45**	-0.07	0.09	-0.20	-0.07	-0.16	0.06	0.05	0.08	0.07	0.12	0.16	0.06	0.16	0.18	0.09	0.08
CE			0.27	0.14	0.11	0.01	-0.16	0.16	-0.05	-0.26	-0.16	0.36*	0.19	0.06	0.16	0.04	0.13	-0.16
LL				0.64**	0.25	0.67**	0.10	-0.25	-0.15	-0.35*	-0.42*	0.38*	0.39*	0.49**	-0.30	0.36*	0.50**	-0.02
LW					-0.57**	0.82**	-0.24	-0.33	0.01	-0.16	-0.30	0.01	0.30	0.44*	-0.31	0.34	0.49**	-0.02
LSI						-0.32	0.44*	0.14	-0.20	-0.20	-0.08	0.40*	0.06	0.01	0.06	0.01	-0.08	0.07
LA							-0.02	-0.24	-0.10	-0.16	-0.33	0.07	0.19	0.27	-0.22	0.19	0.36*	-0.05
ILM								-0.17	-0.24	-0.14	-0.06	-0.09	-0.27	-0.17	-0.11	-0.16	-0.17	0.02
FS									-0.09	-0.05	0.10	0.11	0.28	-0.41*	0.92**	-0.14	-0.13	-0.04
FSC										0.80**	0.83**	-0.12	-0.04	-0.01	-0.03	-0.02	-0.17	0.12
FJC											0.86**	-0.12	-0.14	-0.13	0.04	-0.04	-0.28	0.18
FFC												-0.18	-0.07	-0.17	0.18	-0.05	-0.39*	0.31
FSL													0.31	0.24	0.04	0.26	0.31	-0.05
FL														0.72**	0.16	0.85**	0.60**	0.51**
FWD															-0.56**	0.92**	0.68**	0.51**
FSI																-0.26	-0.21	-0.13
FW																	0.65**	0.65**
SW																		-0.13

$^{*, **}$ Correlation significant at $P_{0.05}$ and $P_{0.01}$, respectively.

TH=Tree habit; TV=Tree vigor; CE=Cropping efficiency; LL= Leaf length; LW= Leaf width, LSI=Leaf shape index; LA= Leaf area; ILM=Incisions of leaf margin; FS=Fruit shape; FSC=Fruit skin color; FJC=Fruit juice color; FFC=Fruit flesh color; FSL=Fruit stalk length; FL=Fruit length; FSI=Fruit shape index; FW=Fruit; SW=Stone weight; SF= Share of flesh.



Figurer 1. Dendrogram of hierarchical analysis obtained by Ward's clustering method using 19 traits of wild cherry accessions.

wild cherry accessions based on the traits studied was *d*= 9.31, ranging from *d*= 2.8 (the most related accessions, both G3 and G4, and G6 and X5) to 24.0 (the most distantly related, G5 and R1). In fact, accession R1 was the most distant from any other particular accession examined (on average between any other accession, d= 13.4). In contrast, the accessions X2 and Z1 were the most similar to any wild cherry, possessing an average of *d*= 7.0 between them and any other accession.

Cluster analysis of wild cherry accession showed that the most important traits for grouping were leaf traits. The accessions represented in Cluster I were characterized by a larger leaf width ranging from 4.7 cm (G1) up to 6.3 cm (K10) and higher leaf area: 29.25 cm^2 (X1) up to 43.46 cm^2 (G5). This cluster was split off into five distinct sub groups, defined as cluster IA, IB, IC, ID, and IE, respectively. Sub-clustering was further done according to some fruit traits, such as FW and color properties.

The average fruit weight increased from the subgroup, IA to IE. Thus, sub-group IA had the lowest average FW (1.15 g) while the IE group had the highest (1.88 g). External and internal fruit colors also influenced the sub-grouping. Each sub-group had a different combination of FSC, FFC and FJC. Thus, IA (K2, X1, X4, B3, K3, K7, K8 and G1) group separated accessions with colorless to red color fruit juice. Both IB (K10, B2 and G5) and ID (K1, X3, X7, K4, X9, Z1 and G2) sub-groups had the same FFC, but the first one had accessions with pink juice while the majority of the accessions in ID showed red juice color. Sub-cluster IC (G3, G4, X2 and O1), that had the most related accessions, and IE (K9, Q1 and X8) were similar according to the purple-dark red to black-red juice color of the examined accessions. Distinction between them was determined by different FSC and FFC, where IE was pointed out as the sub-group of the accessions with the combination of the darkest skin – darkest flesh color.

Accessions in Cluster II (K6, B1, G6, X5, R2, Q2, R3 and R1) showed the lowest values of leaf traits among all the accessions studied. No other trait could be used to separate those accessions from the others since genotypes had different tree vigor, all kind of cropping efficiency, from yellow to black fruit skin color and from cream-white to black red fruit flesh color. The lowest similarities between the genotypes placed in this cluster were proved by the highest distance (7.68) in regard to the other clusters and sub-groups.

The overall organization of wild cherry accessions on dendrogram suggests that there is considerable phenotypic variability in natural populations in central Serbia. High level of diversity can be explained by the fact that the diversity of wild cherry genotypes was high due to the outcrossing or by seed dispersal from other populations. No evidence of relationships was found for most of the accessions according to their geographic location. Our results are not unexpected considering the fact that wild cherry as an open-pollinated species is characterized by a high degree of heterozygosity; therefore, in one locality, different genotypes can be found. Also, the locations of the accessions were relatively closely placed, so that the entire area can be considered as a wider population. Two previous studies (Frascaria *et al*., 1993; Mariette *et al*., 1997) based on isozymes also indicated very low level of differentiation among populations, and a comparatively high level of genetic diversity.

Principal component analysis (PCA) that was used to identify the most significant variables in the data set produced seven principal components with eigenvalues greater than one (Table 4). These components are enough to explain 86.59% of the total observed variability. Value of the extracted information is adequate considering the number of involved variables and the study’s purpose. The percentage of variation explained by the first four components was only 66%. According to Reim *et al*. (2012), this result has revealed a great morphological variation indicating a high genetic diversity within the wild cherry population and suggests that evaluation of different morphological characteristics remains necessary for a meaningful characterization.

The first component presented 25.78% of the variation and variables with higher scores on PC1 (over 0.70, absolute value) related to leaf (length and width), fruit size

Table 4. Eigenvalues, proportion of total variability and correlation between the original variables and the first seven principal components for wild cherry accessions.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Tree vigor	-0.10	0.32	0.23	-0.11	0.14	-0.14	0.68
Tree habit	-0.09	-0.20	-0.29	0.33	0.29	-0.65	0.28
Yield efficiency	-0.22	0.15	-0.44	0.34	0.54	-0.30	0.03
Life length	-0.77	0.23	0.07	-0.01	0.35	0.36	0.16
Life width	-0.71	-0.06	0.38	0.51	-0.02	0.12	0.15
Life length/width	0.05	0.32	-0.42	-0.69	0.35	0.21	-0.01
Leaf area	-0.60	0.11	0.37	0.37	0.01	0.40	0.22
Incisions of leaf margin	0.13	0.39	0.17	-0.56	0.14	0.09	0.37
Fruit shape	0.33	0.05	-0.78	0.27	-0.25	0.29	0.18
Fruit skin colour	0.27	-0.74	0.21	0.13	0.40	0.20	0.06
Fruit juice colour	0.42	-0.76	0.19	0.04	0.26	0.19	0.01
Fruit flesh colour	0.51	-0.77	0.02	-0.06	0.22	0.15	0.13
Fruit stalk length	-0.35	0.10	-0.49	-0.07	0.50	0.17	-0.30
Fruit height	-0.67	-0.37	-0.54	-0.05	-0.19	0.12	0.16
Fruit width	-0.85	-0.38	0.00	-0.29	-0.05	-0.12	-0.10
Fruit height/width	0.43	0.06	-0.69	0.37	-0.17	0.25	0.28
Fruit weight	-0.76	-0.49	-0.24	-0.26	-0.16	-0.09	0.03
Stone weight	-0.79	-0.02	-0.12	0.12	-0.04	0.04	-0.20
Share of flesh	-0.22	-0.58	-0.14	-0.49	-0.26	-0.10	0.31
Eigenvalue	4.89	3.08	2.60	2.08	1.40	1.21	1.17
% Variance	25.78	16.23	13.72	10.93	7.40	6.37	6.15
% Cumulative	25.78	42.02	55.74	66.67	74.07	80.44	86.59

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(height, width and weight), and stone weight. The second component explained 16.23% of the total variation and featured fruit skin color, flesh color, and juice color. The third component accounted for 13.72% of the variation in which fruit shape was dominant. Finally, PC4 explained 10.93% of the variation had high loading for leaf length/width ratio (Table 4.). The rest of the components varied to a less and extent about 35% of total variance.

High absolute values of the correlations between variables related to fruit and leaf size, and PC1 or PC2 were also established by Krahl *et al*. (1991) and Rakonjac *et al*. (2010) in sour cherry, Hjalmarsson and Ortiz (2000) and Lacis *et al*. (2009, 2010) in sweet cherry and Khadivi-Khub *et al*. (2012) in several *Prunus* subgen. *Cerasus* species. This indicates that these traits could be sufficient for reliable germplasm characterization. These characteristics are also important in agricultural practice and breeding.

In this paper, in PC1, which explained the largest proportion of variability, as many as 6 traits showed a high loading. The summarization of these traits in one component reflected the strong correlation between leaf length and leaf width as well as the correlation between fruit height, fruit width, fruit weight and stone weight reciprocally. Similarly, in PC2, three traits (skin color, flesh color, and juice color) that had the most considerable loading were significantly correlated with each other. This result suggested a reduction of these nine traits to three main characters, namely, leaf size, fruit size, and fruit color, could be sufficient.

CONCLUSIONS

High variability has been found in the set of the evaluated wild cherry genotypes with regard to the characteristics studied. Although these accessions does not represent the whole wild cherry germplasm in Serbia, considerable genetic diversity observed in both quantitative and qualitative characteristics indicate rich and valuable plant material for cherry improvement. Characterization provided data for multivariate statistical analyses (cluster analysis and PCA), which allowed to obtain a general view on plant material variability in the collection, based on the complete range of described traits.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among wild cherry accessions. The obtained dendrogram had two main clusters. Cluster analysis distinguished wild cherry accessions into two distinct groups, where the first one was split off into five sub-groups.

In principal component analysis, fruit and leaf traits such as length and width, fruit height, width, and weight, and skin, flesh, and juice color are predominant in the first two components indicating that they are not only useful for the assessment of genetic diversity but also for wild cherry germplasm characterization. Description of the wild cherry genetic resources in Serbia makes a valuable contribution to establishing a targeted management and evaluation program. The evaluation can serve also as a base for further utilization of the available genetic resources in breeding, since a plant material description makes it easier to identify interesting properties and to ensure that the whole spectrum of variation is preserved.

In order to keep a valuable wild cherry natural germplasm resource, the following measures are proposed. Firstly, different occurrences of the *P. avium,* should be protected *in situ* as gene conservation stands. Secondly, *ex situ* conservation should be a good complement to the *in situ* measures, where material should be tested to verify disease infection status. Thirdly, an appropriate number of seeds can be sampled from all these populations, and stored in a gene bank. Finally, research about intraspecific variation and genetic structure by molecular markers must be intensified.

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تحلیل گوناگونی ریخت شناسی در منابع ژنتیکی آلبالو وحشی (*Prunus avium* L.) صربستان مرکزی

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چکیده

به منظور یافتن رابطه بین ژنوتیپ ها و شناسایی مفید ترین صفات برای تمایز بین سی وسه نمونه آلبالو وحشی(Prunus avium L.) از صربستان مرکزی، طی سه سال مطالعه، ارزیابی تنه درخت، برگ، میوه و هسته انجام شد.نتایج ارزیابی مزبور گوناگونی زیادی در میان نمونه های مطالعه شده نشان داد و تفاوت های متعددی در مورد صفات مورد مطالعه پیدا شد. روابط اصلی مهم بین متغیر های اندازه میوه و برگ و متغیر های مربوط به رنگ تعیین شد. با تحلیل خوشه ای، نمونه های آلبالو وحشی در دودسته متمایز گروه بندی شدند. در تجزیه به مولفه های اصلی(PCA) صفاتی از برگ و میوه مانند طول و عرض برگ، قد و عرض و وزن میوه، و رنگ پوست، گوشت وآب میوه در دو مولفه نخست چیره بودند و این نتیجه حاکی از آن بود که این صفات برای ارزیابی و مشخص کردن مواد ژنتیکی آلبالو وحشی مفید بودند. نیز، نتایج چنین اشاره داشت که نمونه های مطالعه شده می بایست به عنوان منابع ژنتیکی ارزشمندی حفاظت شوند تا بتوان ذخایر مشترک ژنتیکی را غنی ساخت و برای بهبود کارآیی بهنژادی صفات باغبانی در سراسر گیتی از آن بهره جست.

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