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Original scientific paper

EVALUATION OF PHENOTYPIC DIVERSITY OF APPLE *(Malus* sp.*)* GERMPLASM THROUGH THE PRINCIPLE COMPONENT ANALYSIS

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A wide range of apple cultivars representing *Malus* sp. germplasm were collected from southern part of Serbia, where this specie has been in cultivation for a long time. Data for twelve traits were collected for 18 cultivars for three consecutive years. Cultivars differed in phenology, fruit weight, yield and chemical composition were evaluated. Correlations were identified among phenological and compositional traits. Principal component analysis (PCA) was performed to study correlation among variables in set of apple cultivars evaluated and to interpret relationships among genotypes as a tool for germplasm characterization. Dulabija, intended for table consumption, and Stambolka, suitable for the processing, were identified as promising. Because of the large diversity identified, data should be collected for additional local apple cultivars.

Key words: autochthonous cultivars, genetic resources, phenotypic traits

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INTRODUCTION

Apple is economically the most important fruit tree crop due to its abundance in most of the temperate regions, fruit quality, and propagation ease (PEREIRA-LORENZO *et al*., 2009). Apples have been cultivated since 4000 BC. The primary centre of *Malus* is within the region from Asia Minor to the Western China. The Old Silk Road from central Asia to the Danube played an important role in the dispersal of the cultivated apple.

The genus *Malus* is characterized by a large diversity resulted by system of incompatibility, selection, accumulation of somatic mutations, fostering by human activities during the long history of its cultivation, artificial crossing and transportation to distant habitats. In temperate regions, each country has its own local cultivars, and since the apple is a long-lived tree and vegetatively propagated, cultivars known hundreds of years ago still exist. In spite of that, the diversity of local and well-adapted landraces has been replaced by a much narrower spectrum of bred cultivars that are often genetically rather similar. The use of novel diversity in apple breeding programs helps to develop new cultivars that will meet future market needs and opportunities, increase productivity, offer increased health benefits, reduce growing or handling costs, and reduce the risk of inbreeding depression (KUMAR *et al*., 2010). These new varieties may also have improved environmental adaptations or improved resistance to diseases and pests.

Large world collections and germplasm repositories represent a vast reservoir of desirable genes and numerous studies of apple genetic resources have been published (ZHI-QIN, 1999; PEREIRA-LORENZO *et al*., 2003; GARDINARIU *et al*., 2003; ERCISLI, 2004). Standardization and globalization in Serbian apple marketing have significantly reduced the numbers of local cultivars and most of them have nearly disappeared. While important quality parameters have been observed in the local apple cultivars, research and utilization of Serbian apple genetic resources has been recognized for some time (MILETIĆ *et al*., 2003; MRATINIĆ, 2005, MARIĆ *et al*., 2007).

This research was initiated to work toward preserving the genetic diversity of *Malus* sp. germplasm in southern Serbia. The purpose of this work was to quantify, characterize and describe the variability of 18 local cultivars using phenotypic characters, to promote conservation and management of genetic resources, plan breeding strategies in all directions and to give opportunities to the local farmers. Also, the objective was to evaluate the fruit parameters in the apple gene pool and identify and utilize significant correlations between some pomologic characters of interest in order to improve the choice of production objectives by using a limited number of characters. In addition, multivariate analysis was carried out to detect associations among genotypes and to identify the most useful variables for discrimination among genotypes.

MATERIALS AND METHODS

Eighteen apple (*Malus* sp.*)* cultivars from southern Serbia (municipalities Vranje, Bujanovac and Preševo) were analyzed in this study. Individual trees in villages of the region were selected according to economically valuable characters.

Trees were sampled from their planted locations and not placed in a common orchard. All accessions were examined for a set of 12 traits. Fruit characteristics were measured on fruit harvested at full maturity stage. Fifty fruits per tree were selected randomly for harvest. Data were collected for three consecutive years (2000-2002). The following traits were characterized for each tree:

Initial bloom (IB), full bloom (FB) and petal fall (PF) were determined when 10% of flowers were opened, 80% opened and 80% petal falls, respectively, and expressed as the number of days after April 15. Ripening time (RT) was the harvesting date and expressed as the number of days after September 15. Duration of flowering time (DFT) was the number of days between start of bloom and end of flowering time. Period from blooming time until the harvest time (FBHT) was represented as the number of days from full bloom until the ripening time. Fruit weight (FW) was measured for each fruit by scale in g and average was calculated. Yield (Y) is presented as kg tree^{-1}. A sub-sample was used to determine soluble solids content (SS) by refractometer. Titratable acidity (TA) was determined by neutralization to pH 7.0 with 0.1 N NaOH, whereas Luff - Schoorl method was used to measure total sugars content (TS). The mineral (MM) content was obtain by dry-ashing the samples at 550^{o}C in a muffle furnace.

Pearson correlation coefficients were calculated using the individual means for the 12 traits. Means across the three year data were used to create a correlation matrix from which standardized principal component (PC) scores were extracted to determinate the relationships among the cultivars and among variables using statistic program 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA). Common components coefficients, eigenvalues, and relative and cumulative proportions of the total variance expressed by single traits were calculated. To determine which of the PCs accounted for the greatest amount of variation for each trait and each accession, the eigenvalue of the 3 PCS were used. The first two components having maximum variance were selected for creating scatter plots for all accessions studied.

RESULTS AND DISCUSSION

Scores for the 12 variables in 18 autochthonous apple accessions are shown in Fig. 1 and Table 1. Cultivars with different phenologic and pomologic composition were present as the accessions studied.

The earliest IB (Fig. 1) was recognized in cultivars Buzlija and Stambolka (22nd April) and the latest in Paša Šerbetka (6th May). There was an approximately 16 day of difference in full bloom between the earliest and latest cultivars. First PF was noticed in Stambolka (27th April), and the last in the cultivars Paša Šerbetka and Budimka (13th May). No matter the fact that all the cultivars flowered midseason or late midseason, later flowering apple accessions should be favour because of its possibility to avoid freezing injury. The RT of selected genotypes started from the last week of September till the first week of November. Duration of flowering time (DFT) showed a narrow range (7 days) while FBHT varied in 45.66 days.

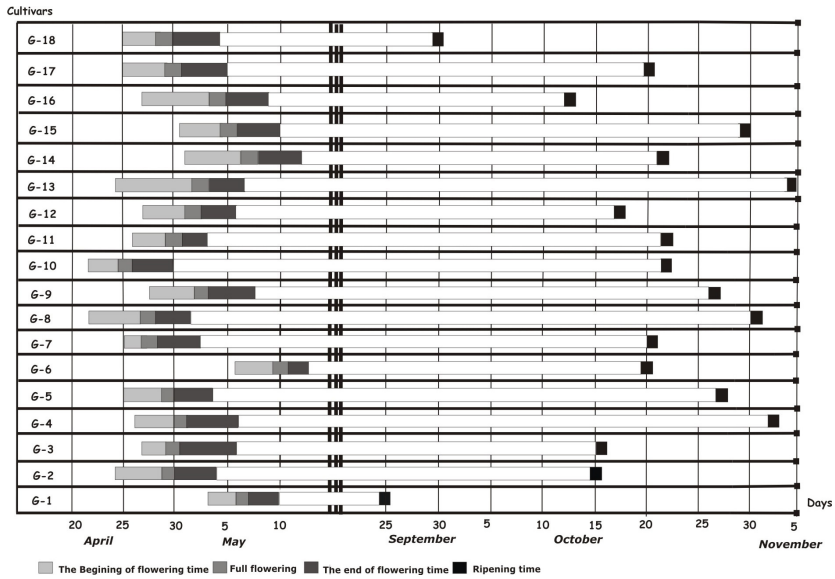

Figure 1. Flowering and harvest season of 18 apple accessions from south Serbia

FW (Table 1) is a variable character and ranged from 70.00 g (Stambolka) to 193.33 g (Pašinka). Similar findings have been reported by PIRLAK *et al*. (2003) for promising native summer apple cultivars grown in Turkey. It is well known that genetics, environment and cultural practices all interact to determine fruit weight. Since our accessions similar environmental conditions with nearly no agro-technical practice, it demonstrated that most native Serbian apple germplasm have more than a good genetic potential for this character. Exceptions to this rule are four genotypes (Laljče, Stambolka, Avajlija and Budimka) that are lacking the genes necessary for large fruit size.

Another character to be taken into consideration was the yield that varied from 81.33 (Pašinka) to 366.67 kg tree^{-1} (Stambolka) and showed big differences between autochthonous cultivars. From 18 apple genotypes grown in south part of Serbia (Table 1) the 33.33% of them had yields below 100 kg tree^{-1}, the 16.67% over 300 kg tree^{-1} and the most (50.00%) of genotypes analyzed in this paper were characterized by yields of 100-300 kg tree^{-1}.

SS expressed in °Brix content was ranged from 12.55 (Hadži Sinan) to 19.24% (Pećka Šerbetka) although most of the apple cultivars showed values higher than 17.00%. Since SS is a very important quality attribute, influencing notably the fruit taste, the most valuable cultivars were Đulabija and Pećka Šerbetka, with soluble solid content over 19%. Furthermore, TS was between 8.65 and 12.18%, while TA was between 0.10 and 0.82% (Table 1). This range of values is in agreement with the results of PAGANINI *et al*. (2004) and BOSTAN (2009).

Table 1. Pomologic characters of 18 apple accessions from south Serbia

Cultivar	Code	FW[a]	Y	SS	TS	TA	MM
Đulabija	G-1	133.33	223.00	19.20	10.78	0.31	0.30
Pečka Šerbetka	G-2	136.67	276.67	19.24	9.98	0.16	0.26
Laljče	G-3	76.67	300.00	18.43	12.00	0.82	0.29
Vizajka	G-4	145.00	253.33	18.61	11.06	0.65	0.24
Kuljača	G-5	126.67	82.67	18.91	11.53	0.21	0.24
Paša Šerbetka	G-6	106.67	257.67	17.68	9.80	0.17	0.27
Krupna Šerbetka	G-7	167.33	91.00	17.26	8.65	0.12	0.28
Buzlija	G-8	145.67	240.00	17.06	10.93	0.43	0.27
Hadži Sinan	G-9	177.33	96.00	12.55	11.46	0.22	0.12
Stambolka	G-10	70.00	366.67	17.57	11.64	0.08	0.24
Sitna Šerbetka	G-11	111.67	200.67	16.74	11.01	0.12	0.18
Avajlija	G-12	84.00	268.33	17.23	12.03	0.16	0.24
Ulućanka	G-13	141.67	182.00	16.48	10.73	0.12	0.18
Budimka	G-14	86.67	132.67	17.99	12.01	0.16	0.26
Laknja	G-15	153.33	89.67	18.14	11.90	0.17	0.26
Demirka	G-16	142.67	83.00	18.81	11.50	0.27	0.28
Pašinka	G-17	193.33	81.33	18.27	12.18	0.17	0.27
Karapaša	G-18	123.33	353.33	17.99	10.59	0.10	0.30

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

Considering the fact that the balance between sugars and acids has an important role in consumer acceptance and that apple cultivars with sugar/acid ratios lower than 20 are sharp and appropriate for processing and cider production, only two cultivars (Laljče and Vizajka) can be used for this purpose, while the others are suitable for table consumption.

Correlations of interest both in determining how improvements in one trait may affect other traits and choosing candidates for indirect selection are presented in Table 2. Correlation coefficients between almost all phenology traits were found significant. However, IB was highly correlated to FB (r = 0.89), and to the PF (r = 0.95) and IB to PF (r = 0.88), therefore, those parameters can be used to predict each other. Also strong positive correlation was found between RT and FBHT (r = 0.96).

In this group of cultivars, Y had significant but negative correlation with FW (r = -0.63), which may be influenced by the methods of cultivar selection, since most of the cultivars included in this germplasm were selected by growers, who focused on selection of trees that produced either large fruits or high yields. Regarding the relationship between RT and chemical composition of the genotypes studied, the only significant but negative correlation was determined between RT and MM (r = - 0.52). Medium negative correlation coefficients was found between RT and SS (r = - 0.33), but relation between RT and TS as well as between RT and TA (r = 0.16, for both) did not reveal any connection. These relationships are not

very clear considering the fact that ŁATA and TRĄMPACZYŃSKA (2008) described a strong correlation between harvest date and chemical composition in apple. In terms of acid and soluble solid relatedness we found a positive but not significant correlation (r = 0.20). Also, according to VIEIRA *et al*. (2009) content of SS is not related to TA in apple, showing poor correlation. Furthermore, opposite to the results of VIEIRA *et al.* (2009), found in apple grown in Brazil, negative correlations between TS and SS (r = - 0.01) has been established in this study.

Table 2. Correlations among variables significant at the 5% (*) level.

	IB[a]	FB	PF	RT	DFT	FBHT	FW	Y	SS	TS	TA	MM
IB		0.89*	0.88*	-0.27	-0.35	-0.45	-0.07	-0.23	0.02	-0.11	-0.04	0.17
FB			0.95*	-0.14	0.00	-0.37	-0.01	-0.26	0.01	0.04	-0.05	0.07
PF				-0.15	0.05	-0.36	-0.08	-0.23	0.09	0.14	0.14	0.18
RT					0.43	0.96*	0.32	-0.42	-0.33	0.16	0.16	-0.52*
DFT						0.36	0.31	-0.29	-0.01	0.24	0.04	-0.09
FBHT							0.30	-0.31	-0.32	0.06	0.14	-0.49*
FW								-0.63*	-0.23	-0.23	-0.11	-0.17
Y									0.17	-0.10	0.20	0.21
SS										-0.01	0.20	0.81*
TS											0.22	-0.11
TA												0.20
MM												

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

Principal component analysis (PCA) has been used previously to evaluate germplasm of apple (CURRIE *et al*., 2000; ECHEVERRÍLA *et al*., 2005; PEREIRA-LORENZO *et al*., 2007). PCA, one of the multivariate statistical procedures, has been used to study correlations among fruit traits and to establish genetic relationships among cultivars within sets of apple cultivars. Associations between traits emphasized by this method may correspond to genetic linkage between loci controlling traits or a pleiotropic effect (ORAGUZIE *et al*., 2001).

The first three components with eigenvalues were able to explain more than 65% of the total trait variation. PC1, PC2 and PC3 accounted for 30.825%, 22.562% and 13.709%, respectively, of the trait variability (Table 3). In particular, the first component was negatively and strongly associated with RT and FBHT, but positively and strongly associated with flowering phases and MM. The second component was positively and strongly associated with flowering phases, but negatively with Y. The third component explains chemical properties such as TA, TS and SS. Character such as FW, an important yield component, was not useful to distinguish among accessions.

Table 3. Correlation between original variables and the first three principal components (PC) and contributions to the total variation (%) in apple germplasm

Variable	Component loadings			Cultivars	Component loadings		
	PC 1	PC 2	PC 3		PC 1	PC 2	PC 3
IB	0.717	0.632	-0.122	G-1	2.222	0.030	-0.366
FB	0.610	0.748	0.050	G-2	-0.040	-0.975	-0.325
PF	0.638	0.700	0.241	G-3	0.674	-0.840	1.859
RT	-0.788	0.383	0.274	G-4	-0.770	-0.104	1.500
DFT	-0.429	0.262	0.491	G-5	-0.677	-0.147	0.242
FBHT	-0.867	0.170	0.200	G-6	1.946	1.065	-0.903
FW	-0.415	0.432	-0.141	G-7	-0.432	-0.152	-1.459
Y	0.279	-0.728	-0.014	G-8	-1.178	-0.603	0.696
SS	0.466	-0.374	0.523	G-9	-1.250	1.701	-1.601
TS	-0.094	0.116	0.541	G-10	-0.337	-2.063	-0.580
TA	0.027	-0.102	0.690	G-11	-0.457	-0.389	-1.280
MM	0.610	-0.366	0.389	G-12	0.292	-0.287	-0.154
Eigenvalue	3.6992	2.707	1.6445	G-13	-1.315	0.848	0.071
% Var.	30.825	22.562	13.707	G-14	0.773	1.174	0.863
% Cum.	30.825	53.387	67.096	G-15	0.096	1.297	0.512
				G-16	0.275	0.509	1.059
				G-17	-0.540	0.416	0.589
				G-18	0.718	-1.481	-0.724

The first three principal factor loadings and contributions to the total variation (%) in apple germplasm are shown in Table 3. Autochthonous cultivars (Đulabija, Paša Šerbetka and Budimka) with the highest positive values on PC1 tend to have the latest flowering periods, while the cultivars with the lowest negative scores (Vizajka, Buzlija, Hadži Sinan and Uloćanka) have the latest RT and FBHT. Cultivars with the highest PC2 scores have the latest FB (Paša Šerbetka, Hadži Sinan, Budimka and Laknja), while the cultivars the lowest negative scores have the highest Y (Stambolka and Karapaša). PC3 represents mostly traits related to chemical composition such as TA (0.690), TS (0.541) and SS (0.523). High PC3 values were found for Laljče, Vizajka and Demirka.

The most desirable cultivars according to the grower's preferences were, in dependence of flowering season, those either with low PC1 and PC2 scores and high PC3 (early FB, later RT and the most quality one) or with high PC1 and PC3 (late flowering and the most quality one). While PC1 and PC2 were highly related to phenology, those cultivars with high PC1 and PC2 scores could be good genitors for lateness in flowering, while those with low PC1 scores could be parents of the accessions with the late maturity. This situation confirms the suitability of using phenology as a basis for selecting parental sources; nevertheless, studies through several years must be conducted before parental selection for a possible plant

breeding. On the other hand, accessions with low PC2 scores could be used to increase productivity. Better fruit quality (TA, TS and SS content) could be reached using as genitors those cultivars with higher PC3 scores.

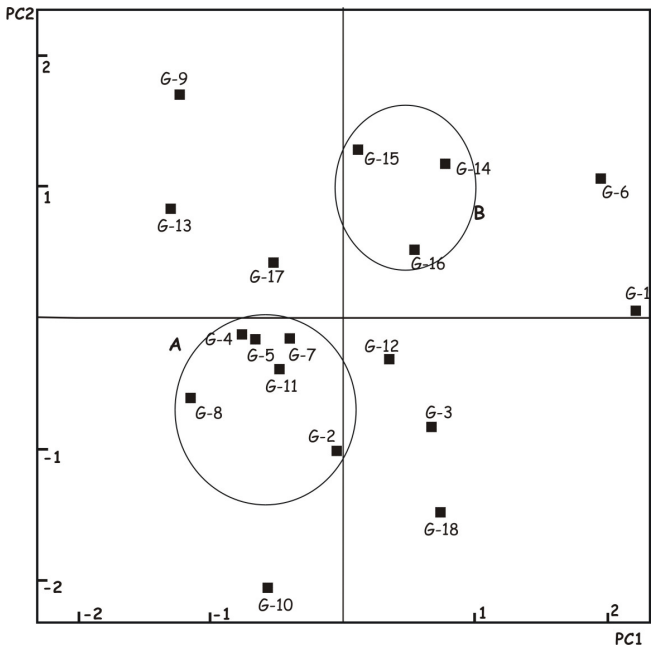

Figure 2. The scatter plot of the first two principal components (number of accessions corresponded to those of Table 1).

The PC analysis provided a simplified classification of the apple accessions for collecting and breeding. The scatter plot (Fig. 2) also shows geometrical distances among cultivars that reflect similarity among them in terms of variables measured. Two groups of related accessions were separated. Group A includes those accessions with low negative values of both PC1 and PC2. Second, B group, is consists of three genotypes that corresponded with positive both PC1 and PC2 value. For further collecting it is quite enough to take just one cultivar for each group, while the rest of the accessions can be considered as unique item. Especially interesting accessions are Đulabija and Stambolka that were disposed in gaps and are the most promising ones. Cultivar Đulabija is characterized by late flowering but early maturing (the shortest FBHT), very high soluble solid content (>19%) and satisfactory fruit weight and yield, so it can be intended for the table consumption. Cultivar Stambolka has the highest yields among all studied accessions and satisfactory soluble solid content and can be recommended for processing.

CONCLUSION

Accessions different in phenology, fruit weight, yield and chemical composition that represents apple germplasm of a small geographic area, where this specie has been cultivated for a long time were presented in this study. The results are certainly representative and valuable, and will provide some guidance for screening breeding resources for improving fruit quality and serve as a base for economically valuable phenotypes. A high correlation was found among some apple attributes that could reduce the number of pomological traits which need to be studied as additional information for breeding and orchard management. Principal component analysis (PCA) was used to establish similar groups of accessions, according to their characters, as well as to study relationships among pomological traits. Intensive production and conventional breeding could be improved in terms of later maturity and high yields if using cultivars with low PC1 and PC2 scores. Later flowering time could be reached using as genitors those genotypes with high PC1 and PC2 scores, while better fruit quality could achieve using as genitors those cultivars with higher PC3 scores. Dulabija, intended for table consumption, and Stambolka, suitable for the processing, were identified as promising. Because of the large diversity identified, further management and data collecting for additional autochthonous genotypes need to be implemented in order to avoid the genetic erosion and extinction of many local apple cultivars.

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PROUČAVANJE GENETIČKIH RESURSA JABUKE *(Malus sp.)* MULTIVARIJACIONOM ANALIZOM

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I z v o d

Veliki broj autohtonih sorti jabuke (*Malus* sp.) sakupljen je u regionu južne Srbije, sa teritorije gde se ova vrsta godinama gaji. Kod 18 autohtonih sorti koje su se međusobno razlikovale u fenologiji, masi ploda, prinosu i hemijskom sastavu proučavano je 12 osobina tokom tri godine. Korelacije su utvrđene između fenoloških i hemijskih osobina. Multivarijaciona analiza je primenjena kako bi se izučile korelacije između osobina kod svih sorti jabuke i objasnili odnosi između genotipova kao metod za karakterizaciju germplazme. Između svih proučavanih sorti, najperspektivnije su Đulabija, koja je namenjena za stonu upotrebu, i Stambolka, pogodna za preradu. Zbog velikog diverziteta, preporučuje se nastavak proučavanja ostalih autohtonih sorti.

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