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Morphological and biochemical evaluation of selected almond [*Prunus dulcis* (Mill.) D.A.Webb] genotypes in northern Serbia

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Abstract: In order to determine the overall degree of polymorphism and detect similarities among genotypes, 19 almond [*Prunus dulcis* (Mill.) D.A.Webb] genotypes were studied. Variation in traits related to phenology, morphology, and fruit quality was observed, and the results indicated a high morphological diversity of almond genotypes. The majority of important correlations were determined among the traits representing nut size (nut width, nut length, nut thickness, and nut weight) and leaf size (leaf length, leaf width, and leaf area). The lack of correlation between kernel size and chemical compounds enables the creation of a new almond cultivar with large kernels and improved quality. Principal component analysis showed considerable phenotypic diversity among the almond genotypes. Parameters with high discriminating values were those related to nut, kernel, and leaf size; ripening time; and tree habit. Genotypes B/04, 1/03, and 28/03 were singled out as the most promising for breeding and commercial growing.

Key words: Almond, correlations, principal component analysis, selection

Introduction

Almond [*Prunus dulcis* (Mill.) D.A.Webb syn. *Prunus amygdalus* (L.) Batsch] is a species of genus *Prunus* and subgenus *Amygdalus* (Rosaceae, subfamily Prunoideae). This species originated in Central Asia and dispersed through cold and xeric environments in the mountainous areas and deserts of western China and into Iran (Watkins 1976). Due to the high nutritive value of almond fruit and its favorable effects on human health (Kester et al. 1991; Sang et al. 2002a, 2002b; Amarovicz et al. 2005; Kodad et al. 2006a), the almond tree is of great importance throughout the world.

The Balkan Peninsula is a secondary center of genetic diversity of *P. amygdalus* (Korać et al. 1996). Natural populations of almond are very sparse in Serbia. They are sporadically encountered in the regions of *Quercus* sp. forests in the vicinity of Fruska Gora Mountain, Belgrade, and Negotin. In addition, almond genotypes of unknown origin are grown by households. Both are important as potential sources of variability; these genotypes can be used to introduce new genes or alleles in the cultivated almond.

Commercial almond production in Serbia is low considering the demand and economic potential. Almond cultivation is limited to a small number of locations, including Slankamen Hill (Čolić and Zec 2007; Čolić et al. 2009), where

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households grow seedlings of unknown origin that are characterized by large variability in traits. Studies of almond populations in the Slankamen Hill region were initiated to collect the basic material for breeding genotypes adapted to the agroecological conditions represented by hard winters and late frosts.

Traditional methods for cultivar characterization and identification of almonds are based on phenotypic observations. Morphological traits are useful for preliminary evaluation because they facilitate fast and simple evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions. Morphological characterization combined with multivariate statistical methods, such as principal component analysis (PCA), the most commonly applied, and cluster analysis, are useful tools for screening accessions (Lansari et al. 1994, 1998; Prats-Moya et al. 1997; Talhouk et al. 2000; Cordeiro et al. 2001; De Giorgio and Polignano 2001; Thakur et al. 2005; Chalak et al. 2007; Sorkheh et al. 2009, 2010). Multivariate techniques can help to evaluate large data sets and resolve several phenotypic and genotypic measurements into fewer more interpretable and more easily visualized groups.

PCA is a method of data reduction that transforms the original variables into a limited number of uncorrelated new variables. This method is therefore useful for representing a set of variables with a much smaller set of composite variables that account for much of the variance among the original set. It facilitates visualization of differences among individuals and the identification of possible groups and relationships among individuals and variables (Martínez-Calvo et al. 2008).

The objective of this study was to describe the variability in 19 selected almond genotypes, determine the correlation among traits, identify the most useful variables for discrimination among genotypes, and detect relationships among genotypes. Furthermore, an evaluation of economically valuable traits was performed to identify useful genotypes for almond producers and breeding programs.

Materials and methods

Plant material

The study included almond genotypes selected from a large autochthonous population in northern Serbia (Vojvodina) (230 m; 45°08′N-45°09′N, 20°06′E-20°13′E). Trees were selected after evaluation of over 100 trees on the basis of regular fruit production and observed phenotypic diversity. Selection of genotypes was mainly conducted according to relevant morphological traits of the tree and nut and phenology. The origin of the almond genotypes is unknown and the selected genotypes are self-incompatible. The trees from the examined genotypes are 15-20 years old. They are mostly individual trees from private gardens that have been grown without applying any agricultural practices. This study was conducted in situ.

Variables studied

To minimize the environmental effects, data were collected over 3 consecutive years (2004-2006), and 20 quantitative and 15 qualitative traits were analyzed. Genotypes were evaluated for tree, flower, leaf, nut, and kernel traits. The categories of tree habit, tree vigor, anthocyanin coloration on 1-year-old shoots, location of flower buds, color of petals, flower size, blooming density, nut shape, shell color intensity, marking of outer shell, softness of shell, pellicle color intensity, kernel shriveling, kernel pubescence, and kernel taste were evaluated according to International Board for Plant Genetic Resources (IBPGR) descriptors (Gülcan 1985). Nut length, width, and thickness, as well as kernel length, width, and thickness, were measured by caliper and expressed in millimeters. Nut weight and kernel weight were scale-weighed and expressed in grams. All observations were made on 30 ripe fruits sampled randomly from the periphery of the trees when the hull was fully desiccated and open along the suture. Kernel/nut ratio was expressed as a percentage. Leaves (30 per genotype) were sampled from the median section of 1-year-old branches during harvest time. Leaf length and leaf width were measured with a ruler and expressed in millimeters, while leaf area was determined using Adobe Photo Shop CS 8.0, histogram level 254; data are given in cm^2. Oil content was determined with a nuclear magnetic resonance (NMR) analyzer and crude protein content was

calculated using the Kjeldahl method. Dry matter was determined by drying to a constant weight at 105 °C, and mineral matter was determined by annealing at 600 °C. All chemical compounds are expressed as percentages. Blooming time was the date on which 90% of the flowers were open. Flowering duration was figured by calculating days from the onset to the end of flowering. Ripening time was the harvest date. For statistical analysis, blooming time was represented as the number of days from 1 March, while ripening time was represented as the number of days from 1 August. The fruit development period was expressed as the number of days from full bloom to ripening.

Data analysis

The mean values of 20 quantitative traits were calculated and the coefficients of variation were established as variability indicators. The frequency distribution of all 15 qualitative traits was represented in histograms. In order to unify the variation in 20 quantitative traits, the total interval of variation was divided into 5 categories into which the studied genotypes were placed.

Correlation coefficients were determined as Spearman's coefficient. Categories registered for each parameter were used to perform the PCA. This statistical procedure was applied to create a correlation matrix from which standardized principal component (PC) scores were extracted. Scatter plots of the first 2 PC scores were created. To determine which of the PC scores accounted for the greatest amount of variation for each trait, the eigenvalues of the 3 PC scores were compared for each trait. Data processing was performed using the statistical program Statistica (StatSoft, Inc., Tulsa, OK, USA).

Results

Scores for the 20 variables in 19 almond genotypes are shown in Table 1. All traits showed large differences, indicating a high level of morphological variation. This was confirmed by the relatively high coefficient of variation (CV) values established for the majority of the examined traits. In general, the highest levels of variation were found for leaf area (CV = 25.7%), kernel/nut ratio (CV = 23.3%), and nut weight (CV = 22.8%), whereas dry matter content (CV = 0.5%) and fruit development period (CV = 1.3%) showed the smallest differences among the genotypes.

The majority of almond genotypes (Figure 1) were characterized by a spreading tree habit (7 genotypes) and strong tree vigor (14 genotypes). Based on anthocyanin presence in the bark of 1-year-old shoots, almond genotypes were classified into 3 groups. In 16 genotypes, the flower buds were mainly on 1-year-old shoots, whereas distribution was mixed in 3 genotypes. The dominance of genotypes with light pink petal color was observed. Most of the genotypes had intermediate flower size. Oblong nut shape, intermediate shell color intensity, intermediate marking of the outer shell, and hard shell were determined in most genotypes (Figure 1). Pellicle color intensity varied from extremely light to dark, but intermediate (11 genotypes) was predominant. Slightly wrinkled and intermediate wrinkled kernel shriveling were dominant. Kernel pubescence was low in 3 genotypes, intermediate in 7 genotypes, high in 6 genotypes, and extremely high in 3 genotypes. Kernel taste was predominantly sweet (13 genotypes) compared to slightly bitter (6 genotypes). In terms of blooming density, the genotypes were classified into 3 categories; most of the genotypes had intermediate blooming density.

Table 2 shows only the significant correlations ($P < 0.05$) with an r-value over 0.45 between variables studied. The highest positive correlation coefficients were between leaf width and leaf area ($r = 0.93$) and nut thickness and nut weight ($r = 0.80$). The highest negative correlation coefficient was found between oil content and crude protein content ($r = -0.60$).

Results from the PCA presented in Table 3 indicate that the first 3 components explained 42.13% of the total variability observed; PC1, PC2, and PC3 accounted for 17.90%, 13.46%, and 10.78% of variance, respectively. PC1 showed 6 variables with higher scores (over 0.60 absolute value) related to nut size (nut length, width, thickness, and weight) and kernel size (kernel length and weight). The highest contribution of PC2 corresponded to tree habit, variables related to leaf size (leaf width and area), and ripening time. The separation along PC3 was primarily due to variations in blooming time.

The scatter plot (Figure 2) shows the distribution of almond genotypes on the PC1 and PC2 plots

Table 1. Mean values and coefficient of variations (CVs) of 20 quantitative traits observed in 19 almond genotypes.

Genotype	NL	NWI	NTH	NW	KL	KWI	KTH	KW	K/N	LL	LWI	LA	DM	MM	OC	CP	BT	FD	RT	FDP
1/03	39.40	24.04	15.13	4.95	27.64	13.44	5.41	1.14	23.33	104.67	28.88	23.65	92.64	3.86	51.23	24.23	5 Apr	10	30 Aug	147
10/03	32.81	20.27	13.85	3.74	22.25	11.34	4.56	0.79	22.70	82.84	17.00	11.48	93.25	3.32	55.51	23.70	7 Apr	15	1 Sep	147
11/03	35.55	20.64	13.40	3.62	25.24	11.91	4.89	0.74	20.83	73.45	25.30	16.95	93.55	3.63	56.14	21.15	5 Apr	12	31 Aug	148
12/03	34.24	23.27	13.72	4.09	24.32	13.86	4.97	0.92	22.70	80.48	26.92	17.12	93.79	3.62	50.30	24.16	7 Apr	10	29 Aug	146
14/03	35.57	22.06	13.63	4.75	24.18	11.70	4.54	0.74	14.93	69.72	20.68	12.18	93.95	3.54	53.51	23.18	6 Apr	15	30 Aug	146
15/03	34.97	20.71	12.34	3.72	25.02	11.33	4.93	0.67	19.30	74.85	26.00	16.68	93.29	3.30	49.58	24.02	7 Apr	15	29 Aug	144
16/03	40.81	24.20	15.20	6.06	27.87	13.74	5.15	1.05	18.73	80.59	24.33	14.92	93.94	3.27	52.53	22.90	8 Apr	16	5 Sep	150
17/03	33.84	23.17	14.83	4.32	24.00	13.67	6.08	0.99	25.07	74.88	26.36	14.74	93.82	3.21	55.34	21.83	9 Apr	12	1 Sep	145
18/03	33.36	21.91	15.91	5.80	25.09	11.95	4.55	0.83	15.80	76.76	20.89	12.68	93.74	3.61	45.82	26.22	7 Apr	12	1 Sep	147
19/03	34.72	24.77	12.69	4.30	24.23	16.51	4.69	0.79	19.63	82.20	23.61	14.87	93.18	3.66	50.37	25.46	3 Apr	10	28 Aug	147
22/03	30.91	20.78	13.07	2.53	21.82	12.65	5.07	0.86	33.50	67.44	20.00	10.60	92.86	3.64	53.18	25.03	9 Apr	14	29 Aug	146
23/03	35.74	18.73	13.75	3.44	25.63	10.88	5.19	0.85	25.17	68.67	20.47	10.59	93.49	3.69	52.67	24.39	6 Apr	16	4 Sep	150
24/03	37.88	24.42	16.07	5.55	28.72	13.66	4.63	0.81	15.67	80.81	25.96	16.51	93.04	3.81	49.45	23.26	8 Apr	12	2 Sep	147
25/03	34.27	22.83	16.03	5.00	22.85	13.29	4.94	0.62	12.43	73.44	20.28	11.52	93.19	4.33	49.63	26.85	7 Apr	12	2 Sep	148
27/03	43.31	22.84	16.70	6.29	27.71	11.48	4.31	0.91	15.03	89.61	25.00	17.86	93.68	4.09	52.74	23.30	7 Apr	15	5 Sep	150
28/03	43.15	24.27	15.97	6.54	30.21	14.52	6.60	1.29	21.53	86.36	23.89	16.17	92.94	3.76	51.75	22.95	8 Apr	17	31 Aug	146
29/03	37.04	22.42	15.42	4.84	26.35	12.80	4.83	0.95	21.57	77.18	25.75	15.10	94.44	3.69	46.74	23.55	13 Apr	15	4 Sep	144
A/04	36.54	25.29	15.10	4.05	22.49	11.96	6.37	0.94	23.20	71.36	26.45	14.17	93.55	3.41	49.82	22.47	9 Apr	12	1 Sep	145
B/04	32.76	23.12	13.35	4.69	23.96	13.85	5.41	1.02	23.40	92.32	36.56	25.57	93.82	3.52	55.90	20.94	7 Apr	16	1 Sep	147
CV	9.5	7.8	9.0	22.8	9.2	10.8	12.3	18.4	23.3	11.6	17.3	25.7	0.5	7.6	6.5	5.6	5.4	16.5	7.5	1.3

Legend: Nut length (NL), nut width (NWI), nut thickness (NTH), nut weight (NW), kernel length (KL), kernel width (KWI), kernel thickness (KTH), kernel weight (KW), kernel/nut ratio (K/N), leaf length (LL), leaf width (LWI), leaf area (LA), dry matter (DM), mineral matter (MM), oil content (OC), crude protein content (CP), blooming time (BT), flowering duration (FD), ripening time (RT), and fruit development period (FDP).

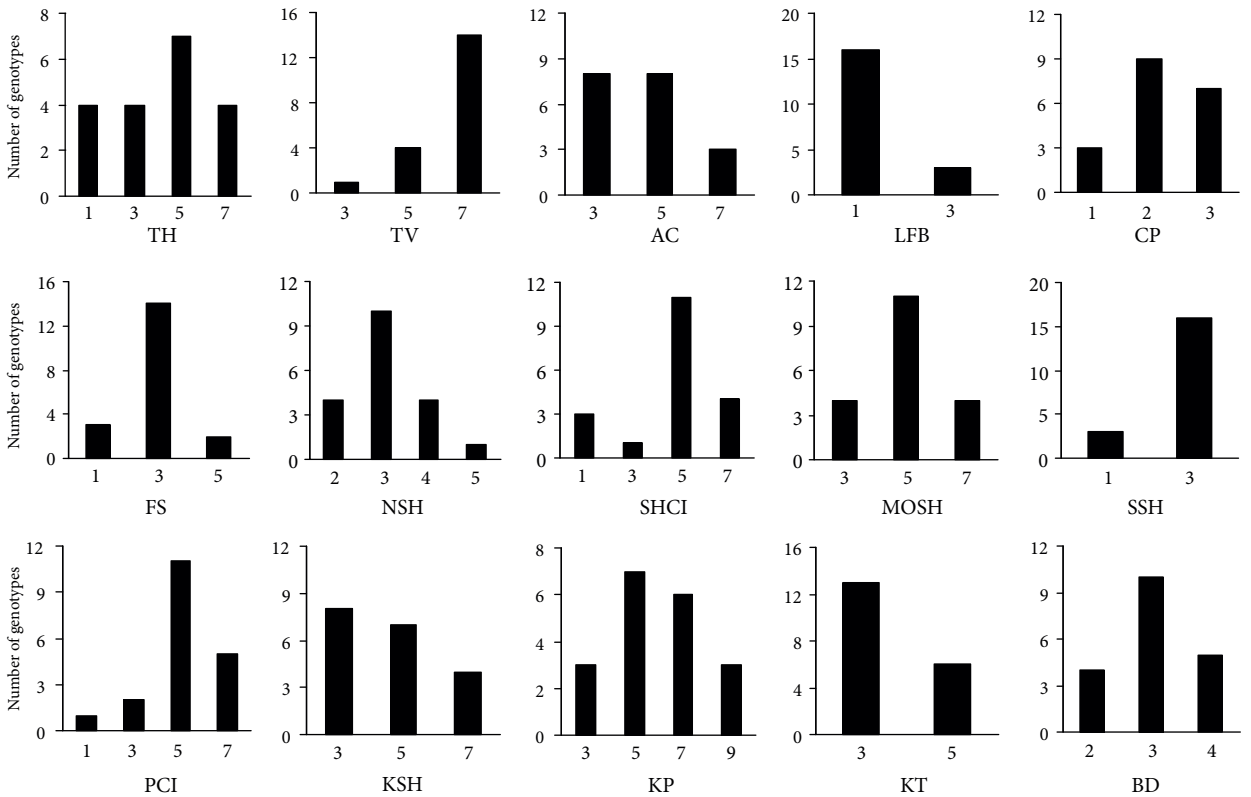

Figure 1. Frequency distribution of the 19 almond genotypes for 15 qualitative traits: tree habit (TH), tree vigor (TV), anthocyanin coloration on 1-year-old shoots (AC), location of flower buds (LFB), color of petals (CP), flower size (FS), nut shape (NSH), shell color intensity (SHC), marking of outer shell (MOSH), softness of shell (SSH), pellicle color intensity (PCI), kernel shriveling (KSH), kernel pubescence (KP), kernel taste (KT), and blooming density (BD).

Table 2. Correlation matrix among traits studied.

Traits	SHC	MOSH	NL	NWI	NTH	NW	KL	KWI	KW	LL	LWI	LA	MM	OC	CP	BT	FD	BD	RT	FDP
TH								−0.51			−0.54	−0.50					0.47			
AC	0.57	0.57																		0.56
FS															−0.54					
NSH			0.62				0.52										0.46			
SHC																		−0.56		
NL				0.57	0.69	0.70	0.78		0.57											
NWI					0.53	0.58		0.76	0.59											
NTH						0.80	0.50						0.53						0.58	
NW							0.62													
KSH															−0.48					
KP																0.47	0.49			
KL									0.48									0.63		
KWI									0.48											
KTH									0.57					−0.46						
KW										0.64	0.46									
LL											0.57	0.71								
LWI												0.93		−0.55						
OC															−0.60					
BT																				−0.47

Legend: Tree habit (TH), anthocyanin coloration on 1-year-old shoots (AC), flower size (FS), nut shape (NSH), shell color intensity (SHC), marking of outer shell (MOSH), nut length (NL), nut width (NWI), nut thickness (NTH), nut weight (NW), kernel shriveling (KSH), kernel pubescence (KP), kernel length (KL), kernel width (KWI), kernel thickness (KTH), kernel weight (KW), leaf length (LL), leaf width (LWI), leaf area (LA), oil content (OC), dry matter (DM), crude protein content (CP), blooming time (BT), flowering duration (FD), blooming density (BD), and ripening time (RT).

Table 3. Eigenvalues, proportion of total variability, and correlations among the original variables and the first 3 principal components (PCs).

Variable	PC1	PC2	PC3
TH	0.026	0.794	0.163
TV	0.234	−0.369	−0.097
AC	−0.101	0.423	−0.573
LFB	0.168	0.065	0.366
CP	−0.479	0.144	−0.155
FS	0.111	0.218	0.328
NSH	0.482	0.438	0.382
SHC	−0.327	0.053	−0.365
MOSH	−0.158	0.028	−0.467
SSH	0.044	0.004	0.498
NL	0.869	0.253	−0.047
NWI	0.687	−0.217	−0.282
NTH	0.689	0.526	−0.038
NW	0.713	0.435	−0.344
PCI	0.278	−0.237	−0.231
KSH	0.215	0.322	0.562
KP	0.292	0.037	0.179
KT	−0.137	0.082	−0.240
KL	0.825	0.132	−0.093
KWI	0.356	−0.356	−0.289
KTH	0.258	−0.434	0.407
KW	0.788	−0.298	0.197
K/N	−0.169	−0.520	0.507
LL	0.584	−0.388	−0.298
LWI	0.507	−0.616	−0.070
LA	0.518	−0.601	−0.277
DM	−0.006	0.176	0.354
MM	0.359	0.348	−0.328
OC	−0.285	0.487	−0.290
CP	−0.161	−0.366	−0.151
BT	0.117	0.057	0.619
FD	0.159	0.265	0.335
BD	0.573	−0.046	0.040
RT	0.344	0.630	0.065
FDP	0.117	0.423	−0.416
Eigenvalue	6.264	4.710	3.773
% Var.	17.90	13.46	10.78
% Cum.	17.90	13.36	42.13

Legend: Tree habit (TH), tree vigor (TV), anthocyanin coloration on 1-year-old shoots (AC), location of flower buds (LFB), color of petals (CP), flower size (FS), nut shape (NSH), shell color intensity (SHC), marking of outer shell (MOSH), softness of shell (SSH), nut length (NL), nut width (NWI), nut thickness (NTH), nut weight (NW), pellicle color intensity (PCI), kernel shriveling (KSH), kernel pubescence (KP), kernel length (KL), kernel width (KWI), kernel thickness (KTH), kernel weight (KW), kernel/nut ratio (K/N), leaf length (LL), leaf width (LWI), leaf area (LA), dry matter (DM), mineral matter (MM), oil content (OC), crude protein content (CP), blooming time (BT), flowering duration (FD), blooming density (BD), ripening time (RT), and fruit development period (FDP).

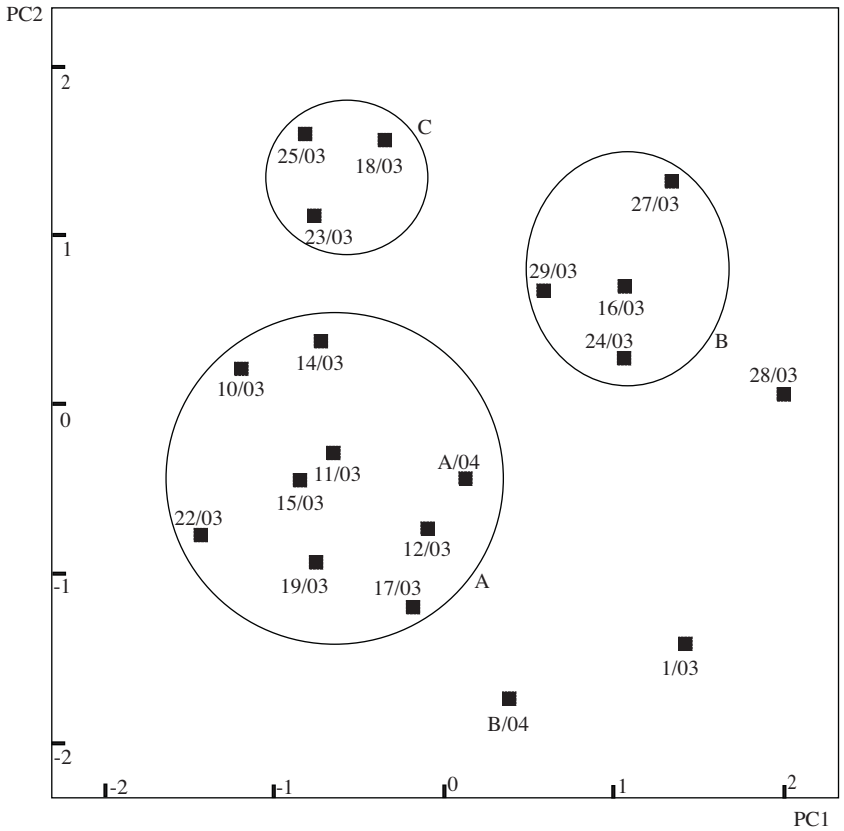

Figure 2. Factor scores for the first 2 principle components (PCs) for 19 almond genotypes.

and the geometrical distances among genotypes that reflect the similarity among them in terms of variables measured. Based on the position of almond genotypes, 3 groups of related genotypes were separated. Most genotypes (9) belong to group A. Group B consists of 4 genotypes that correspond with intermediate to high positive PC1 and PC2 values. The 3 genotypes that have a low to intermediate negative PC1 value and high positive PC2 value are in group C. Genotypes 28/03, B/04, and 1/03 can be considered unique.

Proceeding from negative to positive values of PC1, almond genotypes showed a general increase in nut and kernel size. Genotype 28/03 had the most positive value on PC1 due to its large nuts and kernels. The nut and kernel weights for this genotype were 6.54 g and 1.29 g, respectively, which represent the maximum values for these 2 characters. Due to their large nuts and kernels, genotypes 27/03 and 1/03 also had high positive values on PC1. On the diagram, these 2 genotypes are diametrically opposed as a result of differences in tree habit and ripening time, the characters highly loaded on PC2.

Proceeding from negative to positive values of PC2, genotypes were characterized by smaller leaf width and leaf area, wider spreading tree habit, and later ripening time. Genotypes 25/03 and 18/03, with a high positive value on PC2, had narrow leaves (20.28 mm and 20.89 mm, respectively) and small leaf area (11.52 cm^2 and 12.68 cm^2, respectively). However, genotype 27/03, which also had a high positive value on PC2, was characterized by late ripening time (5 September). On the other hand, genotypes B/04 and 1/03 had high negative values on PC2 due to their large leaves and extremely upright tree habits. The leaf area for these genotypes was 25.57 cm^2 and 23.65 cm^2, respectively.

Discussion

All examined genotypes are highly adapted to the environmental conditions in northern Serbia and could be a very interesting source of genetic diversity. The results of this study indicated a high morphological diversity of almond genotypes. Regarding qualitative traits, the highest variability was established for tree habit, nut shape, shell and pellicle color intensity, and kernel pubescence (Figure 1). With respect to CV values for quantitative values, high levels of variation were found in fruit, kernel, and leaf size, whereas kernel taste and shell hardness showed little difference.

These results were expected since the almond is self-incompatible. This high phenotypic variability corresponds with previous reports on molecular characterization using different markers as nuclear and chloroplast simple sequence repeats (Martínez-Gómez et al. 2003; Fathi et al. 2008; Zeinalabedini et al. 2008) or amplified fragment length polymorphisms (Sorkheh et al. 2007).

Established relationships between some traits can help breeders in setting goals for parental partner selection and breeding. Significant correlation coefficients between anthocyanin content in 1-year-old shoots and both shell color intensity (r = 0.57) and the marking of the outer shell (r = 0.57) (Table 2) indicate that this trait can be utilized as a morphological marker for early selection. In addition, the correlation between anthocyanin content in 1-year-old shoots and fruit development period (r = 0.56) can be used to select almonds for growth in severe climates. In our research, a positive correlation existed among most variables related to nut and kernel size (r = 0.50-0.80), which is in accordance with the findings of Thakur et al. (2005) and Tavassolian (2008). Therefore, these parameters can be used to predict each other. Talhouk et al. (2000), Ledbetter (2008), Tavassolian (2008), and Sorkheh et al. (2010) established significant correlations between nut weight and kernel weight; our results revealed a lack of correlation between these 2 traits. Sánchez-Pérez et al. (2007) concluded that shell hardness does not affect the weight of a kernel, which was also confirmed by our results. Significant correlation coefficients were determined between leaf length and leaf width (r = 0.57) and between leaf width and leaf area (r = 0.93), which corresponds with results obtained by Talhouk et al. (2000) and Sorkheh et al. (2009). In accordance with the results reported by the above authors, our work also confirmed the absence of a correlation between leaf size and nut size. Negative correlations were determined between crude protein content and flower size (r = −0.54) and between crude protein content and kernel shriveling (r = −0.48). If the goal is high protein content, then genotypes with small flowers and slightly wrinkled kernels should be the target of selection. In our research, oil content was negatively correlated with crude protein content (r = −0.60), as Kodad et al. (2006b) reported earlier. This makes it difficult to obtain genotypes with both high oil and high protein contents. In contrast, the absence of a correlation between kernel size and crude protein indicates that genotypes with both high quality and large-sized kernels can be obtained.

Using the PCA (Table 3), a high correlation was found between some traits and PCs that could reduce the number of traits studied in almond germplasm. Those traits were related to nut, kernel, and leaf size; ripening time; and tree habit. Our results correspond with those of Lansari et al. (1994), Talhouk et al. (2000), and Sorkheh et al. (2009), who used a similar analysis to compare kernel, nut, and leaf characters in different almond collections and found that the variables contributing to nut and kernel size were more important than leaf traits. High absolute values of correlations between variables related to fruit, nut, and leaf size; phenology; and PC1 or PC2 were also established in other species of the genus *Prunus*, such as apricots (Badenes et al. 1998; Ruiz and Egea 2008), peaches (Nikolić et al. 2010), sour cherries (Krahl et al. 1991), and sweet cherries (Hjalmarsson and Ortiz 2000). This indicates that these traits could be sufficient for reliable germplasm characterization. At the same time, these are the most important traits in agricultural practice and breeding. Moreover, PCA results indicated that the observed variability in the studied almond genotypes was more influenced by quantitative than qualitative traits. As quantitative traits, apart from genotype, are influenced by environmental factors, a combination of molecular markers and morphological data is the best choice for genetic variability analysis.

According to their position on the scatter plot (Figure 2), those almond genotypes with high PC1 scores could be good genitors for increasing nut and

kernel size. On the other hand, later ripening time could be attained using those genotypes with higher PC2 scores as genitors. According to their positions in Figure 1, genotypes B/04, 1/03, and 28/03 can be considered unique and the most promising for breeding or commercial growing. Genotypes B/04 and 1/03 have a kernel weight of over 1 g, while genotype 28/03, apart from the large kernel, has high oil content and a long flowering duration.

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