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Genetic and morphological diversity of introduced cultivars of almonds (*Prunus amygdalus* L.) in Bosnia and Herzegovina

Jasna Hasanbegović[1], Semina Hadžiabulić[1], Mirsad Kurtović[2], Fuad Gaši[2], Sezai Ercisli[3*], Boris Dorbić[4], Melekber Sulusoglu Durul[5 *]

[1] *Department of Agriculture, Agro Mediterranean Faculty, Džemal Bijedić University of Mostar, Bosnia and Herzegovina*
[2] *Department of Agriculture, Faculty of Agricultural and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[3] *Department of Horticulture, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Türkiye*
[4] *Marko Marulic, Polytechnic of Knin, Knin, Croatia*
[5] *Department of Horticulture, Faculty of Agriculture, Kocaeli University, 41380 Kocaeli, Türkiye*

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Abstract

The main morphological and genetic characterization of seven introduced almond cultivars in Bosnia & Herzegovina was conducted. The almond cultivars included three from Italy (Tuono, Genco, Supernova), two from France (Ferragnes and Ferraduel), and two from the USA (Texas and Nonpareil). Genetic characterization was utilized by using 10 microsatellite markers, with nine markers from Prunus persicae and one from Prunus armeniaca. The results of genetic characterization revealed an average of 5.40 alleles per primer per locus. The average number of effective alleles for the 10 SSR loci of introduced cultivars was 3.92. The Shannon Information Index averaged 1.41. The observed heterozygosity (Ho) and expected heterozygosity (He) averaged 0.53 and 0.69, respectively. Morphological analyses of the fruit of introduced almond cultivars in Bosnia & Herzegovina indicated favorable agroecological conditions for their cultivation and spread. The results suggest that these introduced almond cultivars could be utilized in breeding programs to enhance the genetic diversity of the local almond population in Bosnia & Herzegovina.

Keywords: Genetic characterization, Morphological characterization, Almond, Introduced cultivars, Microsatellites.

1. Introduction

Almond (*Prunus dulcis*) (Miller) D.A. Webb, syn. *Prunus amygdalus* Batch), is a fascinating fruit species that holds a unique position among other fruits [1]. The primary objectives of almond breeding globally include enhancing compatibility, late flowering, flowering dynamics, productivity, fruit quality improvement, and cost reduction [2]. Gizdić [3] stated that the selection of almonds is characterized by late flowering, self-fertilization, high yields, quality kernel, easy harvesting, resistance to diseases, and pests and favorable kernel properties (amount of oil, kernel yield, etc.).

Almond kernels rich for important nutritional elements for human health and nutrition including protein, unsaturated fatty acids, vitamin E, ash, riboflavin, phytosterols and polyphenols. It has been found that it reduces cholesterol absorption and lowers LDL-cholesterol level in the blood.

The demand for almond cultivation in the world is increasing and the reason for its increase in different almond producer countries is the high income obtained from unit area. It has been determined that consumption of almonds reduces the risk of heart diseases by 30% or even 45%.

Studies conducted on the origins of cultivated genotypes, as well as differentiating the genetic foundation and traits of the vast yet largely untapped genetic pool of peaches and almonds for breeding initiatives [4-10]. Cultivated almonds exhibit the highest level of polymorphism among all cultivated fruit species [10-15]. With this kind of species, the most important agronomic characteristics are quantitative. Quantitatively hereditary traits make up most of the variability, and by selecting during the breeding process, those that are superior are selected [2,16-17].

Morphological markers have been using in almond classification for a long time. The main morphological characteristics of almond nut and kernel were nut weight, nut length, nut width, nut thickness, kernel mass, kernel length, kernel width, kernel thickness, and kernel percentage.

Molecular markers have been increasingly utilized to

* Corresponding author.
E-mail address: sercisli@gmail.com (S. Ercisli).
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assess genetic diversity and identify genotypes of almonds and peaches in recent years [5-7,18-22-27]. Testolin et al. [20] developed the initial set of almond SSR markers, which have been effectively utilized for molecular characterization and cultivar identification [10,20]. In a study by Cipriani et al. [28], a set of microsatellites in the peach genus (*Prunus persica* L. Batsch), labeled UDP, was identified, with an investigation into their transferability to related *Prunus* species. The study revealed a high success rate of transferability in various species: 71% in sour cherries, 76% in sweet cherries, apricots, and Japanese plums, 82% in almonds and European plums, and 94% in the nectarine genome [29].

In the world for all almond producer countries, the cultivars Tuono, Genco, Supernova, Ferragnes, Ferraduel, Texas and Nonpareil are widely cultivated due to their better agronomic nut characteristics.

The objective of this research is to assess and analyze the morphological and genetic traits of seven almond cultivars (Tuono, Genco, Supernova, Ferragnes, Ferraduel, Texas and Nonpareil) in Bosnia & Herzegovina (Mostar, Gnojnice) utilizing SSR markers.

2. Materials and methods

2.1. Plant material and experimental site

The almond cultivars analyzed were chosen at the Gnojnice site near Mostar, Bosnia & Herzegovina (latitude 43.645812N; longitude 18.969383E). The sample comprised seven introduced almond cultivars (Tuono, Texas, Ferraduel, Ferragnes, Genco, Nonpareil, and Supernova). The study encompassed the examination of both morphological and genetic traits of the introduced almond cultivars.

The morphological characteristics of almonds were studied by monitoring and measuring various traits such as fruit mass, fruit length, fruit width, fruit thickness, kernel mass, kernel length, kernel width, kernel thickness, and kernel percentage in two consecutive research years, 2018 and 2019. To enhance the comprehension of almond germplasm classification and description, descriptive descriptors for almonds were employed. These descriptors were formulated by the International Plant Genetic Resources Institute (IBPGR - International Plant Genetic Resources Institute for Almond 1981) and the International Union for the Protection of New Plant Varieties (UPOV-International Union for the Protection of New Plant Varieties, for almond 2006). The IBPGR descriptors are predominantly utilized in the preservation of plant genetic resources, whereas the UPOV descriptors are commonly applied for describing and safeguarding newly developed cultivars through breeding techniques. For genetic analysis, in 2018 the sampling process involved marking selected trees and collecting leaves in April from each marked tree. Approximately four young DNA extraction sheets were collected on average to maximize DNA yield and purity. The leaves were stored in the Gene Bank of the Faculty of Agriculture and Food in Sarajevo and kept in a deep freezer at -80°C until extraction. Lyophilization, a cold drying process of leaf tissue, was carried out under reduced pressure using a lyophilizer (Christ, model Alpha 1-2 LDplus). This method was employed to prevent DNA molecule degradation during the drying of plant material. Dried almond leaf samples were vacuum-sealed in PVC bags and stored at -80°C until DNA isolation. DNA isolation was carried out using 10-20 mg of powdered leaf tissue. The isolation and genetic characterization procedures were conducted at the Institute of Genetic Engineering and Biotechnology, University of Sarajevo INGEB. The DNA isolation process followed a modified CTAB protocol [30,31], commonly used for plant samples.

Following the successful isolation of DNA from almond samples, a PCR protocol was established. Ten genomic microsatellite primers were utilized for DNA amplification, with nine developed in *Prunus persica* by Testolini et al. [19], Cipriani et al. [28], and Dirlewanger et al. [32], subsequently applied in genetic analysis and cultivar identification of *Prunus dulcis* L. Additionally, one genetic microsatellite marker originated from *Prunus armeniaca* was used. From the total of 14 microsatellite markers employed by the aforementioned authors, the ten markers

Table 1. Characteristics of 10 microsatellite markers originating from *Prunus persica* and one from *Prunus armeniaca* used for the study of almond cultivars.

Marker	Primer sequence (5′ → 3′)	SSR motifs and number of repeats	The origin of the marker	Reference	The size of base pairs
UDP97-402	F:TCCCATAACCAAAAAAAACACG:C R:TGGAGAAGGGTGGGTACTTG	(AG)17	*Prunus persica*	Testolini *et al.* [19]	108-152
UDP98-411	F:AAGCCATCCACTCAGCACTC R:CCAAAAACCAAAACCAAAGG	CT and GT	*Prunus persica*	Testolini *et al.* [19]	154-180
UDP96-005	F:GTAACGCTCGCTACCACAAA R:CCTGCATATCACCACCCAG	(AC)16TG(CT) 2CA(CT)11	*Prunus persica*	Cipriani *et al.* [28] Testolini *et al.* [19]	155
UDP98-407	F:AGCGGCAGGCTAAATATCAA R:AATCGCCGATCAAAGCAAC	(GA)29	*Prunus persica*	Cipriani *et al.* [28]	212
PacA33	F:TCAGTCTCATCCTGCATACG R:CATGTGGCTCAAGGATCAAA	(GA)16	*Prunus armeniaca*	-	188-196
BPPCT039	F:ATTACGTACCCTAAAGCTTCTGC R:GATGTCATGAAGATTGGAGAGG	(GA)20	*Prunus persica*	Dirlewanger *et al.* [32]	154
BPPCT014	F:TTGTCTGCCTCTCATCTTAACC R:CATCGCAGAGAACTGAGAGC	(AG)23	*Prunus persica*	Dirlewanger *et al.* [32]	215
BPPCT026	F:ATACCTTTGCCACTTGCG R:TGAGTTGGAAGAAAACGTAACA	(AG)8GG(A)6	*Prunus persica*	Dirlewanger *et al.* [32]	134
BPPCT034	F:CTACCTGAAATAAGCAGAGCCAT R:CAATGGAGAATGGGGTGC	(GA)19	*Prunus persica*	Dirlewanger *et al.* [32]	228
BPPCT040	F:ATGAGGACGTGTCTGAATGG R:AGCCAAACCCCTCTTATACG	(GA)14	*Prunus persica*	Dirlewanger *et al.* [32]	135

exhibiting the highest allelic polymorphism were selected and utilized in this study (Table 1).

The genetic microsatellite markers utilized in this research are considered a highly reliable tool for the examination of genetic diversity due to their adaptively neutral nature. Amplification of the microsatellite sequences was conducted using the PCR system ABI GeneAmp® PCR System 9700. Fluorescently labeled primers were used for multiplexing and analysis of the PCR product on a DNA genetic analyzer. The amplification of the chosen loci was carried out in two separate PCR reactions (Mix 1 and Mix 2) consisting of five microsatellite loci each. The total volume of the PCR reaction was 15 µl (Table 2).

The amplification was carried out using Taq DNA polymerase with a protocol previously optimized [33, 34]. The temperature conditions for the PCR reactions were identical in both cases, as shown in Table 3.

This method allows for accurate and precise determination of allele sizes, as the internal standard helps correct for any variations in electrophoresis conditions. The GeneMapper ID 5 software also aids in the analysis of the PCR products, providing reliable and reproducible results. Overall, this technique provides a robust and efficient way to analyze allele sizes in genetic studies.

2.2. Biostatistical analysis of molecular and morphological data

The analysis of the tested microsatellite markers for informativeness included calculating various parameters such as the number of detected alleles (Na), effective number of alleles (Ne), ratio of effective to detected alleles (Ne/Na), Shannon's information index, observed (*Ho*), and expected (*He*) heterozygosity using the software Cervus. The evaluation of the morphological characteristics of almonds was analyzed using principal component analysis (PCA) based on the correlation matrix in the computer program Rv. 3.2.3. PCA was conducted using the mean values of the quantitative traits that were investigated. The graphic representation illustrates combinations of experimental factors based on the first two components, displaying the spatial distribution of analyzed almond cultivars. The principal components effectively summarize the data variability and highlight the relationships between variables in a succinct manner.

3. Results

3.1. Genetic analysis of introduced almond cultivars

The allele frequencies for seven introduced almond cultivars Tuono, Genco, Supernova, Ferragnes, Ferraduel, Texas, and Nonpareil using 10 microsatellite markers (UDP97-402, PacA33, BPPCT026, BPPCT034, BPPCT040, UDP96-005, BPPCT014, UDP98-411, UDP98-407 and BPPCT039) are depicted in Table 4.

The allele frequency for cultivars were between 112-154, 178-188, 140-156, 210-248, 132-160, 130-158, 178-194, 160-170, 180-200 and 134-154 for UDP97-402, PacA33, BPPCT026, BPPCT034, BPPCT040, UDP96-005, BPPCT014, UDP98-411, UDP98-407 and BPPCT039 microsatellite markers, respectively (Table 4).

Upon examination of Table 5, it is evident that the cumulative number of alleles identified across seven different introduced almond cultivars when using 10 microsatellite primer pairs was 54, correlating to an average of 5.40 alleles per locus.

The range of detected alleles varied from 2 (BPPTCT014) to 9 (BPPCT034) among the different SSR loci (Table 5). The average number of effective alleles for the 10 SSR loci in the introduced cultivars was 3.92. This suggests that, on average, there are 4 alleles per locus that contribute to the majority of the genetic diversity within

Table 2. The proportion of components used in PCR reaction Mix 1 and Mix 2.

Mix 1		Mix 2	
Components	**Concentrations in the reaction**	**Components**	**Concentrations in the reaction**
UDP97-402	0,50 µM	UDP96-005	0,50 µM
BPPCT026	0,50 µM	UDP98-411	0,50 µM
BPPCT034	0,50 µM	BPPCT039	0,50 µM
PacA33	0,50 µM	UDP98-407	0,50 µM
BPPCT040	0,50 µM	BPPCT014	0,50 µM
dNTP	0,3 mM	dNTP	0,3 mM
PCR pufer	1 X	PCR pufer	1 X
MgCl2	2 mM	MgCl2	2 mM
Taq pol.	0,5 U	Taq pol.	0,5 U
DNK	25 ng	DNK	25 ng
ddH2O	do 15 µl	ddH2O	do 15 µl

Table 3. PCR protocol temperature regime for two separate PCR reactions (Mix 1 and Mix 2).

	Protocol		
	Temperature (°C)	**Duration (min:sec)**	**Number of cycles**
Enzyme activation	94	1:00	
Denaturation	94	0:45	
Annealing	57	0:45	35
Extension	72	2:00	
Final elongation	72	4:00	

Table 4. Allele frequency calculated for seven analysed international almond cultivars with 10 SSR loci.

	Tuono	Genco	Nonpareil	Texas	Supernova	Ferraduel	Ferragnes
UDP97-402	120	114	112	112	112	114	134
	120	114	142	112	112	114	154
PacA33	184	178	184	184	178	178	178
	188	178	184	184	178	178	178
BPPCT026	142	142	140	144	142	142	146
	148	148	140	156	146	148	146
BPPCT034	220	210	236	220	226	210	226
	248	234	260	226	250	234	242
BPPCT040	134	134	132	134	144	134	142
	134	134	132	140	160	134	142
UDP96-005	130	142	142	142	142	140	134
	142	156	150	158	154	154	154
BPPCT014	178	178	178	178	178	178	178
	194	178	178	178	178	178	194
UDP98-411	160	170	160	170	164	170	166
	164	170	170	170	164	170	166
UDP98-407	180	180	200	180	184	180	184
	184	186	200	180	184	186	200
BPPCT039	142	134	150	126	148	134	148
	154	150	150	150	154	150	148

Table 5. Number of detected alleles (Na), effective number of alleles (Ne), ratio between the effective and detected number of alleles (Ne/Na), Shannon information index (I), observed (*Ho*) and expected (*He*) heterozygosity for ten SSR markers on seven samples of the international almond group.

Locus	Na	Ne	Ne/Na	I	*Ho*	*He*
UDP97-402	6.00	4.08	0.68	1.57	0.29	0.75
PacA33	3.00	2.18	0.73	0.88	0.14	0.54
BPPCT026	6.00	4.90	0.82	1.67	0.71	0.80
BPPCT034	9.00	7.54	0.84	2.11	1.00	0.87
BPPCT040	6.00	3.27	0.54	1.47	0.29	0.69
UDP96-005	8.00	4.90	0.61	1.83	1.00	0.80
BPPCT014	2.00	1.32	0.66	0.41	0.29	0.24
UDP98-411	4.00	2.97	0.74	1.23	0.29	0.66
UDP98-407	4.00	3.63	0.91	1.33	0.57	0.72
BPPCT039	6.00	4.45	0.74	1.63	0.71	0.78
Average	5.40	3.92	0.73	1.41	0.53	0.69

the 7 cultivars. The ratio of effective alleles to the detected number of alleles (Ne/Na) varied from 0.54 (BPPCT040) to 0.91 (UDP98-407), with an average value of 0.73 indicating good allelic capacity (3/4 allele contributes to genetic diversity). The Shannon Information Index ranged from 0.41 (BPPCT014) to 2.11 (BPPCT034), with an average value of 1.41, for introduced cultivars, which is a lower value, relative to the diversity index values in the total sample examined (1.58). The observed heterozygosity (*Ho*) in 7 introduced cultivars at the analyzed 10 SSR loci ranged from 0.14 (PacA33) to 1.00 (BPPCT034 and UDP96-005), with an average value of 0.53 (Table 5).

3.2. Morphological analysis of introduced almond cultivars

After analyzing the morphological characteristics of the tested almond cultivars, it was determined that the Genco cultivar had the highest average nut weight at 4.84 g, whereas the Texas cultivar had the lowest average nut weight at 2.99 g (Table 6).

In terms of nut dimensions, the Supernova cultivar stood out with an average nut length of 36.85 mm, while the lowest average nut length was observed in the Genco cultivar at 26.82 mm. The width of the almond nuts varied among the tested cultivars, with the Texas cultivar measuring 19.88 mm and the Tuono cultivar measuring 24.65 mm. The nut thickness ranged from 15.08 mm in the Nonpareil cultivar to 16.85 mm in the Supernova cultivar (Table 6).

The average weight of the kernel varied from 1.32 g in the Tuono cultivar to 1.59 g in the Genco cultivar. The Tuono had the smallest kernel length of 21.78 mm, while the Nonpareil cultivar had the largest average kernel length of 27.42 mm. The largest average kernel width was 15.57 mm in the cultivar Nonpareil, while the smallest was 14.35 mm in the cultivar Texas. The kernel thickness

Table 6. Morphological characteristics of seven almond cultivars.

Cultivars	Nut weight FW	Nut length FL	Nut width FWW	Nut thickness FT	Kernel weight KM	Kernel length KL	Kernel width KWW	Kernel thickness KT	Kernel yield KY
Ferraduel	4.16	33.89	22.13	15.64	1.41	25.38	14.67	0.79	33.92
Ferragnes	3.31	36.40	22.13	15.70	1.49	24.15	14.64	0.86	44.95
Teksas	2.99	28.88	19.88	15.68	1.38	22.42	14.35	1.16	46.10
Genco	4.84	26.82	22.74	15.53	1.59	26.92	14.98	0.91	32.97
Nonpareil	3.87	35.06	23.34	15.08	1.56	27.42	15.57	0.89	40.42
Tuono	4.21	27.57	24.65	15.77	1.32	21.78	15.48	0.89	31.39
Supernova	4.36	36.85	22.85	16.85	1.53	26.52	15.02	1.06	35.09

ranged from 0.79 mm in the Ferraduel cultivar to 1.16 mm in the Texas cultivar. Cultivar Texas had the highest average kernel percentage (46.10%), while the Tuono cultivar had the lowest average kernel percentage (31.39%) (Table 6, Figure 1).

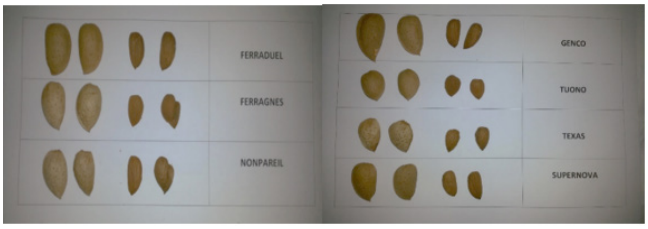

Fig. 1. Nut and kernel images of almond cultivars.

3.3. Multivariate data analysis

Upon analyzing Table 7, it is evident that the five main components of the PCA illustrate the contributions of the nine analyzed traits towards the total variability observed in the introduced almond cultivars. Each of the traits shows significant eigenvector values in one of the first five main components. The variables with the highest eigenvector values in these components are as follows:

PCA1 - Nut weight-FW, Nut width-FWW, Kernel weight-KM and Kernel yield-KY;

PCA2 - Nut thickness-FT;

PCA3 -;

PCA4 - Kernel width - KWW;

PCA5 - Nut length - FL, Kernel length – KL and Kernel thickness – KT.

The analysis of the results obtained indicates that the dominant eigenvectors in the first principal component (PCA) account for 43.17% of the total variance in the study. The key characteristics of the first component pertain to the morphometric attributes of almond cultivars, with nut weight, nut width, and kernel yield (21.79, 15.53, and 15.84) exhibiting the highest eigenvector values. Kernel weight, on the other hand, displayed lower eigenvector values (7.03). In the second principal component, which represents 66.26% of the overall variability in the experiment, the properties with the highest eigenvector values primarily relate to nut thickness (7.14) (Table 8).

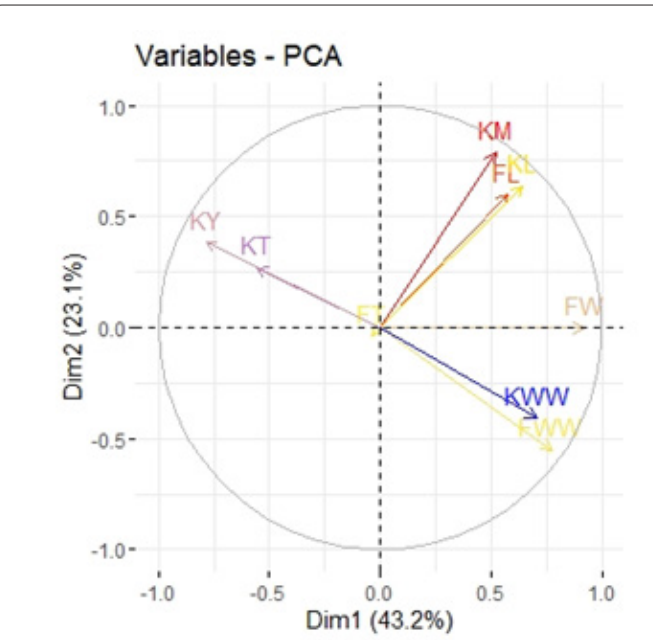

Fig. 2. The grouping and relationships between nine variables studied across seven almond cultivars, represented by the first two principal components (PC1 and PC2).

Figures 1 and 2 display the distribution of seven almond cultivars based on the first two principal components, which were computed using a correlation matrix for nine morphological characteristics analyzed.

It can be argued that there was no clear segregation into distinct clusters observed in the study. By examining the first two principal components, which accounted for 66.3% of the overall variance, it is apparent that the different groups of almond cultivars did not cluster closely together at the center of the coordinate system. Additionally, Figure 2 provides a visual representation of the original properties that were analyzed. The graph indicates a strong positive correlation between kernel weight, kernel length, and nut length. Additionally, a positive correla-

Table 7. Eigenvalues, proportion of variance, and cumulative variance associated with the first five main components (PCA) from a nine-variable correlation matrix for seven almond cultivars.

Variables	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6
Eigenvalue	3.885	2.08	1.44	0.87	0.52	0.22
Proportion of variance (%)	43.17	23.09	15.96	9.62	5.75	2.41
Cumulative variance (%)	43.17	66.26	82.22	91.84	97.59	100.00

Table 8. Analysis of nine quantitative traits of introduced almond cultivars in total experimental variability.

	PCA1	PCA2	PCA3	PCA4	PCA5
Nut weight-FW	21.792	1.702	6.861	5.105	0.315
Nut length-FL	8.496	1.722	0.142	21.481	17.545
Nut width-FWW	15.534	1.461	0.126	5.656	0.197
Nut thickness-FT	0.042	7.142	6.087	3.583	15.211
Kernel weight-KM	7.033	2.995	0.378	9.242	0.479
Karnel length-KL	10.574	1.959	0.337	8.510	10.674
Karnel width-KWW	12.792	8.048	2.236	27.142	13.151
Karnel thickness-KT	7.892	3.442	21.426	9.492	42.418
Karnel yield-KY	15.844	7.062	8.406	9.788	0.0101

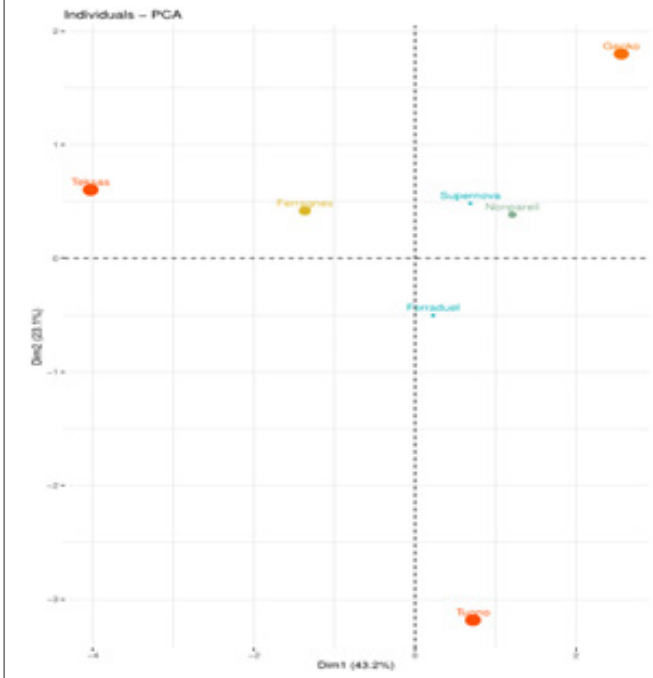

Fig. 3. Grouping of seven almond cultivars based on the first two principal components (PCA1 and PCA2), calculated using a correlation matrix for nine different characteristics.

tion is observed between kernel length and kernel yield. Conversely, a negative correlation is detected between nut width and kernel width.

Figure 3 demonstrates the distinct separation of the almond cultivars under examination, with no evident overlap observed between them. This absence of overlap suggests a lack of genetic exchange over time or highlights the anthropogenic influence on the establishment and dissemination of almond cultivars in Bosnia & Herzegovina. Furthermore, a hierarchical cluster analysis was performed (Figure 4). The results of the analysis clearly indicate divergence among the almond cultivars under study. Specifically, the samples were grouped into four distinct clusters. In the first and third clusters, one sample each from a total of seven almond cultivars was classified. The second cluster consisted of two out of the seven identified cultivars. The fourth-largest cluster included three almond cultivars. Specifically, the almond cultivars Texas and Tuono were classified in the first and third clusters respectively. The second cluster contained samples from the cultivars Supernova and Genco. The largest cluster, which included three cultivars, identified the samples as Nonpareil, Ferragnes, and Ferraduel.

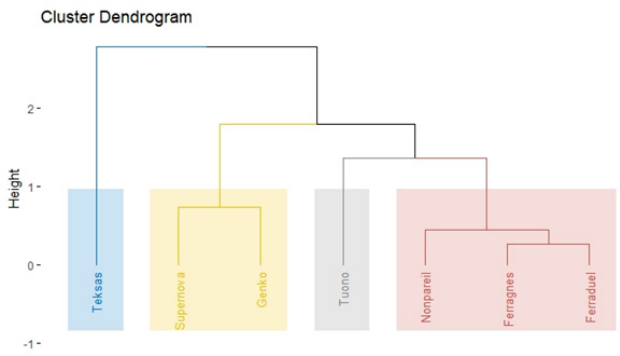

Fig. 4. Dendrogram showing the results of the Hierarchical Clustering on Principal Components (HCPC) analysis for the clustering of nine quantitative traits in seven almond cultivars. The clusters are represented by different colors: cluster 1 (blue), cluster 2 (yellow), cluster 3 (gray), and cluster 4 (red).

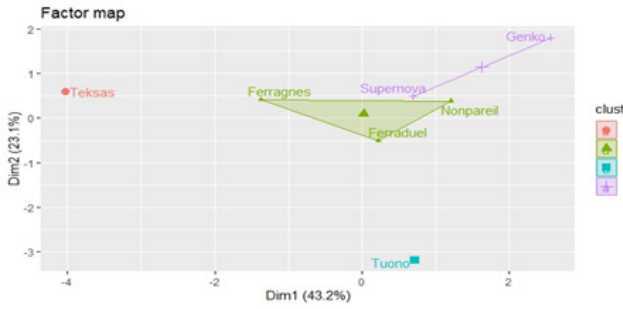

Fig. 4. The organization of examined almond cultivars into distinct clusters through hierarchical cluster analysis based on factor data analysis.

In order to determine the most promising almond cultivars with desirable traits, a thorough analysis was conducted on the average values of all quantitative traits. The findings from the factor analysis align perfectly with those of the hierarchical cluster analysis, which divides the almond cultivars into 4 distinct clusters (Figure 5). The almond cultivars in the right part of the coordinate system were clustered together, exhibiting the highest average values for the characteristics under examination. On the other hand, the almond cultivars in the left part of the coordinate system were grouped together, displaying lower values for the characteristics being studied.

4. Discussion

In a study by El Hamzaoui et al. [35] conducted in Mo-

rocco, 68 almond cultivars were analyzed using 16 microsatellite markers. The study found an average of 13.3 alleles, with 6.7 alleles being detected on average. The *Ho* and *He* were found to be 0.64 and 0.80, respectively. These values were higher compared to the results obtained in this study, possibly due to the use of a greater number of microsatellite markers. Other studies have reported a lower number of alleles per locus, ranging from 6.3 to 8.4 [6,7,20,34], which is similar to the findings of this study. According to a group of authors [35-37], a smaller set of SSR markers (9) were utilized in their research, revealing an average of 17, 15.9, 14.6, and 14.6 alleles per locus. This limited number of markers led to a higher diversity within the Italian almond germplasm. Additionally, the average heterozygosity value established in their study was lower than the one reported by Distefano et al. [38] at 0.71. Additionally, Kadkhodaei et al. [22] conducted a study in Iran on 53 almond genotypes/cultivars using nine microsatellite markers. The study found that the UDP9841 locus was highly polymorphic, with 19 alleles and an *Ho* of 0.94. The average number of alleles per locus ranged from 8 to 17, with an overall average of 12.86. The study also observed a higher average number of effective alleles, with a value of 5.59. The study recorded higher values of the average Shannon information index (I) at 1.97, *He* at 0.80, and average PIC at 0.89. These values may be attributed to the large geographical distance from which the genotypes were originally from, including Spain, Iran, and the USA. In a study by Halász et al. [25], 86 almond genotypes from Central Asia to the USA were analyzed using 15 SSR markers for genetic characterization, revealing an average of 18.86 alleles per locus. The same findings were reported by Fernández i Martí et al. [36], who found an average of 18.66 alleles per locus. Hasanbegović et al. [39] also observed high genetic diversity in almond genotypes from Šibenik (Croatia) and Bar (Montenegro), attributing it to the significant geographical distance between the two locations. The expected heterozygosity (*He*) ranged from 0.24 (BPPCT014) to 0.87 (BPPCT034), averaging 0.69. Previous studies revealed the average number of alleles per locus in almonds was 6.3 to 8.6 [6,7,20,34]. Characterization of germplasm collections is a crucial aspect for plant breeders looking to utilize germplasm in breeding programs. The study of morphological characteristics is necessary to document and analyze genetic diversity within collections. This process aids in creating core collections that accurately represent the diversity present within the overall collection [40,41]. In the research of Sabate and Hook [42], it is stated that the quality of almond fruits is related exclusively to the morphological characteristics of the kernel: size, shape, double kernel, etc. In their research on almond genotypes from the Dalmatian region, Strikić et al. [43] observed that certain genotypes had an average nut weight of 3.82 g. The taste of the kernel varied from sweet to medium bitter depending on the genotype. Also, the shape of the nut and kernel ranged from elongated, medium wide, and wide to very wide depending on the genotype. This group of authors states that the almond population in Croatia is heterogeneous and that research will continue in the direction of identifying superior genotypes, in order to then create adequate breeding programs. According to Fathi et al. [44], a study was conducted on 51 almond genotypes from various provinces of Iran, as well as cultivars from Spain and the USA. It was found that the average nut weight among the genotypes was 9.33 g, with an average kernel weight of 3.05 g. The percentage of the kernel in the fruit was reported to be 28.72%. Additionally, the average kernel length was measured at 3.05 cm, nut width at 1.99 cm, and kernel thickness at 1.14 cm. Sepahvand et al. [45] also found similar results who investigated the gene pool of almonds in a sample of 155 genotypes from Iran. After the morphological analysis of the fruits, they concluded that the average nut weight was 4.48 g, the average weight of the kernel was 1.88 g, and the index of the shape of the nut was 0.42. A very important observation is that they did not find the presence of double kernel in the examined samples. The shell hardness detected in the tested sample was soft, semi-hard and hard. In a sample of 155 almond genotypes, some genotypes were singled out with regard to late flowering, which is a very important feature considering the sensitivity of almonds to early spring frosts. Ardjmand et al. [46] in the research of the Iranian almond gene pool stated that the average length of the nut ranged from 19.32 mm to 37.61 mm, the width of the nut from 16.44 mm to 24.55 mm, nut thickness from 12.37 mm to 16.4 mm. The weight of the kernel was within the limits of 0.78 g to 1.58 g, the other characteristics of the kernel were within the limits: length of the kernel (16.09-26.77 mm), width (9.94 -15.51 mm), kernel thickness (5.93-11.90 mm). The weight of the nut was from 1.46 g to 5.99 g, and the kernel ratio ranged from 22.37% to 57.89%. Previous studies also indicated high morphological diversity among horticultural plants [47-53].

5. Conclusions

Using 10 SSR primer pairs, all extremely polymorphic PCR products were successfully amplified, and gave positive, coherent, and reproducible results in all seven introduced almond cultivars, indicating a good selection of molecular markers. The findings of this study will serve as an addition to the existing genetic information on almond (*Prunus dulcis* L.) profiles in Bosnia & Herzegovina. The importance of genetic characterization of modern almond cultivars in Bosnia & Herzegovina lies in the creation of a database of SSR profiles that enables the identification of foreign germplasm infiltration into domestic varieties. Based on this research, certain almond cultivars have been identified as a substantial source of genetic variation in breeding programs. This can lead to the development of genotypes with desirable traits for commercial cultivation and expansion of almond culture in Mediterranean regions, particularly in Bosnia & Herzegovina where almond cultivation is currently overlooked.

Author contributions

Jasna Hasanbegović, Semina Hadžiabulić, Mirsad Kurtović, Fuad Gaši and Boris Dorbić contributed to the concept and design of the research, conducted the experiment, analyzed the data and wrote the draft manuscript. Sezai Ercisli and Melekber Sulusoglu Durul contributed the original draft and edited the last version. The authors confirm the sole responsibility for study conception, design, data collection, analysis of results and manuscript preparation.

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Conflict of interest

All authors declare no conflict of interest.

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