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SADRŽAJ:

Evaluation of autochthonous apple varieties (*Malus domestica*) in the area of Tomislavgrad

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Abstract

The research included seven autochthonous apple varieties in the area of Tomislavgrad, Stipanjići locality, during 2018, which represent the natural wealth of our country. In seven autochthonous apple varieties: Petrovača bijela, Budimka, Ljutika-Divljaka, Ledarica, Bedrica, Ljepocvjetka-Cvjetača and Jonatanka, phenological characteristics were monitored and analyzed with morphological-pomological properties of fruits. The following phenological characteristics were monitored: flowering phenophases, germination of the first fruits, fruit growth, fruit ripening and full maturity. Five characteristics for the description of apple fruits were determined using the UPOV descriptor for apples (UPOV, 2003). The results of monitoring the morphological and pomological properties of fruits show that the lowest average fruit weight was in the variety of Cvjetača (58,38 g), while the highest average of fruit weight had variety Bedrica (140,59 g). In the result is evident that the lowest value of fruit width had variety Cvjetača (53,72 mm), and the highest had variety of Jonatanka (70,83 mm). The Cvjetača variety had the lowest average fruit lenght (47,24 mm), while the Bedrica variety had the highest (59,72 mm). The Budimka variety had the highest average stem length (2,73 cm), while the Divljak variety had the lowest average stem length (1,43 cm). The Jonatanka variety had the largest average cup concavity and was (1,28 mm), while the Petrovača bijela variety (0,41 mm) had the lowest average cup concavity.

Based on the this research, the examined autochthonous apple varieties can be used a valuable genetic material in breeding programs, and can be used to create new varieties, as well as for propagation in commercial plantations.

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[3] Hadžiabulić, S., Hasanbegović, J., Šupljeglav Jukić, A., Aliman, J., Skender, A., Hadžić, E. (2022). Evaluation of autochthonous apple varieties (*Malus domestica*) in the area of Tomislavgrad. *Glasilo Future*, *5*(3), 16–30.

Key words: autochthonous, apple, varieties, phenological, morphological-pomological properties.

Introduction

Cultivated apple (*Malus domestica* Borkh.) belongs to the family Rosaceae, in the genus *Malus* subfamily Maloideae. The origin of this culture is still disputable, but most morphological and molecular data indicate that *Malus sylverstris* (Ledeb.) roem from Central Asia is the main maternal ancestor of the cultivated apple (Robinson et al., 2001; Harris et al., 2002). The genetic diversity of *M. domestica* Borkh., in BiH, is considered endangered due to the spread of genetically uniform varietal composition, which represents the basis for commercial apple production, at the expense of old local (indigenous and introduced) varieties that disappear by eliminating extensive plantations (Kurtović et al., 2005). According to research (Kellerhals et al., 2004), the preservation of local apple varieties is reflected in the fact that heterogeneous genetic material is a source of genes for quality pomological characteristics, as well as resistance to abiotic and biotic factors.which are the basis for future breeding and sustainable agricultural production. Domestic and domesticated apple varieties that are present in the region of former Yugoslavia have been mentioned as early as the 19th century, in the Plant Directory printed in Zagreb (Šulek, 1879), which enlists more than a hundred domestic apple varieties, including eight varieties, named identical or similar to the genotypes included in this study ('Budimka', 'Zelenika', 'Kolačara', 'Petrovača', 'Rebrača', 'Ružmarinka', 'Samoniklica', 'Šarenika'). As can be seen, most of the enlisted names are descriptive, not toponymic, and therefore they cannot serve as reliable references for research on the actual origin. The great genetic diversity of fruit crops present in Bosnia and Herzegovina shows that fruit species represent a significant source of genetic variability that can serve as a starting material in fruit breeding programs. Research (Bubić, 1952 and Bubić, 1977) states that the inventory of the autochthonous apple gene pool was present in the literature published after the Second World War, where autochthonous apple varieties found on the territory of Bosnia and Herzegovina are described in detail. Many researchers from the territory of Bosnia and Herzegovina have been researching plant genetic resources of fruit crops, which has resulted in the publication of scientific papers using modern molecular methods of plant breeding that are widely used in apples and pears (Gaši et al., 2010, 2013a, 2013b, 2013c.), chestnut (Skender., 2010, 2013), almonds (Hasanbegović et al., 2021) and figs (Hadžiabulić et al., 2005). Literature sources citing research on indigenous populations and cultivated varieties of fruit trees, their wild relatives and free populations of fruit species have recently been a great challenge to a large number of researchers working in this field. Therefore, a group of authors (Hadziabulić et al., 2011, 2014, 2017; Hasanbegović et al., 2017, 2020; Skender et al., 2017a, 2017b, 2019; Šupljeglav Jukić et al., 2020), indicates the existence of a large gene pool of fruit trees in order to preserve fruit. in the territory of Bosnia and Herzegovina, which can serve as a good starting material in breeding programs. The aim of the research was to make an inventory of seven autochthonous apple varieties in the area of Tomislavgrad and to evaluate

them by examining phenological and morphological characteristics and to select varieties that are characterized by agronomic characteristics.

Materials and methods

The research included the selection of appropriate autochthonous apple varieties, phenological monitoring and morphological-pomological analysis of fruits. The subject of this research are seven autochthonous apple varieties Petrovača bijela, Budimka, Ljutika-Divljaka, Ledarica, Bederica, Ljepocvjetka-Cvjetača and Jonatanka, which were located as individual trees in the above locality.. Samples were collected from the Stipanjići site, in the municipality of Tomislavgrad during 2018. The fruits of the mentioned autochthonous apple varieties, 30 fruits from each variety from 7 trees of each variety, were collected from Stipanjići, in the municipality of Tomislavgrad while in the phase of full maturity in specially marked bags and delivered to the laboratory of the Agromediterranean Faculty of Mostar where morphological-pomological analysis was performed. Monitoring of phenological characteristics implies phenophases of generative buds: swelling of generative buds, opening of generative buds, flowering, fruit formation, fruit growth and fruit ripening. When determining a particular variety of apple, the external characteristics of the fruit will be observed and measured. The measured morphological parameters are: fruit weight, fruit width, fruit height, stalk length and cup depression. Twelve phenotypic characteristics to be selected for the description of apple fruits were determined using the UPOV descriptor for apples (UPOV, 2003) (International Union for the Protection of New Varieties of Plants) - 'Apple'.

Table 1. *Overview of climatological data for Livno-Tomislavgrad*

Month	**Average monthly temperatures (°C)**	**Average monthly precipitation (mm)**	**Average monthly humidity (%)**
I	3,6	92,0	84
II	/,03	170,8	86
III	4,7	183,8	87
IV	12,8	78,7	76
V	15,7	139,9	81
VI	18,2	34,4	78
VII	20,5	50,0	76
VIII	20,6	94,7	78
IX	15,8	29,9	78
X	12,3	169,8	81
XI	7,4	11,9	84
XII	0,6	101,1	85
Average	**11,0**	**1258,0**	**80**

Data obtained by measuring the morphological and pomological characteristics of fruits of tested apple cultivars were processed in the statistical package XL STAT2017 analysis of variance (Fisher's

test) to determine the existence of the influence of factors on the observed properties. The direction in which this influence takes place is shown by the analysis of the mean values done using the Tukey test. The significance of the obtained differences was tested by the Tukey test. As a fine step, Pearson 's correlation coefficient for the examined statistical parameters was made. The analysis of the results of the assessment of morphological characteristics of 7 autochthonous apple cultivars was performed using the Principal Component Analysis (PCA) (Hotelling, 1936) based on the correlation matrix in the computer program R c. 3.2.3 (R core team, 2016). The principal components analysis (PCA) was performed using the mean values of 5 quantitative characteristics. Combinations of modalities of experimental factors based on the first two components are presented graphically in the form of spatial distribution of the analyzed autochthonous apple varieties. The main components explain the variability of the data in a concise way as well as the interrelationships of the variables. A hierarchical cluster analysis was also performed, which classified the examined autochthonous apple varieties into the corresponding clusters.

Results and discussion

After conducting the practical experimental work, the results were obtained by monitoring seven indigenous apple varieties at the locality of Stipanjići, in the municipality of Tomislavgrad in the vegetation year 2018.

Phenophases of flowering and ripening of fruits of tested apple varieties

Table 2. *Phenophases of flowering of examined apple varieties*

	Beginning	**Full**	**End**	**Duration (days)**
Petrovača bijela	28.02.2018.	15.03.2018.	25.03.2018.	26
Jonatanka	18.03.2018.	30.03.2018.	10.04.2018.	23
Budimka	10.03.2018.	23.03.2018.	03.04.2018.	24
Lederica	12.03.2018.	25.03.2018.	05.04.2018.	13
Bedrica	20.03.2018.	30.03.2018.	10.04.2018.	21
Cvjetača	15.03.2018.	27.03.2018.	07.04.2018.	20
Divljaka	16.03.2018.	29.03.2018.	09.04.2018.	24

By analyzing Table 2, it can be concluded that the earliest beginning of flowering was recorded in the variety Petrovača bijela (February 28), and the latest beginning of flowering was recorded in the variety Bedrica (March 20). The earliest end of flowering was recorded in the cultivar Petrovača bijela (March 25), and the earliest end of flowering was recorded in the cultivar Jonatanka and Bedrica (April 10). The longest flowering period was recorded in the variety Petrovača bijela 26 days, and the shortest flowering period was recorded in the variety Lederica 13 days.

Table 3. *Monitoring of the phenophase of growth and ripening of fruits of the examined apple varieties*

	Fruit set	**Fruit growth**	**The beginning of fruit ripening**	**Full maturity**
Petrovača bijela	15.04.2018.	01.05.2018.	30.05.2018.	28.06.2018.
Jonatanka	30.04.2018.	16.05.2018.	26.07.2018.	30.08.2018.
Budimka	27.04.2018.	17.06.2018.	24.07.2018.	12.08.2018.
Lederica	20.04.2018.	15.05.2018.	10.06.2018.	02.07.2018.
Bedrica	10.04.2018.	05.05.2018.	30.05.2018.	15.06.2018.
Cvjetača	20.05.2018.	28.05.2018.	25.06.2018.	02.07.2018.
Divljaka	24.05.2018.	16.06.2018.	04.08.2018.	06.09.2018.

The earliest fruit set was recorded in the Bedrica variety (April 10), while the latest beginning of fruit set was recorded in the Divljaka variety (May 24). The beginning of fruit growth started at the earliest with the variety of Petrovača bijela (May 1), and at the latest with the variety of Budimka (June 17). The varieties of Petrovača bijela and Bedrica had the earliest beginning of fruit ripening (May 30), and the latest in the varieties of Cvjetača (August 4). The phenophase of full maturity was first recorded in the cultivar Bedrica (June 15), and the latest in the cultivar Divljaka (September 6) (Table 3).

Results of morphological characteristics of examined autochthonous apple varieties

Analyzing Table 4. It can be concluded that the highest average fruit weight was in the Bedrica apple variety and it was (140,59 g), while the lowest average fruit weight was in the Cvjetača variety (58,38 g). The highest average fruit width was recorded in the Jonatanka variety (70,83 mm), while the Cvjetača variety had the lowest average fruit width (53,72 mm). The highest average fruit lenght was in the variety Bedrica (59,72 mm), and the lowest in the variety Cvjetača (47,24 mm). The Budimka variety had the lowest average fruit stalk length (2,73 cm), while the Divljaka variety (1,43 cm) had the lowest average stalk length. The highest average cup depression was in the Jonatanka variety, which was (1,28 mm), while the Petrovača bijela variety (0,41 mm) had the lowest average cup concavity (Table 4).

Table 4. *Morphological characteristics of autocthonous apple varieties*

	Fruit weight (g)	**Fruit width (mm)**	**Fruit length (mm)**	**Stalk length (cm)**	**Cup concavity (mm)**
Petrovača bijela	75,39	64,55	55,37	1,74	0,41
Jonatanka	135,23	70,83	57,76	1,76	1,28
Budimka	132,84	65,72	55,72	2,73	0,63
Cvjetača	58,38	53,72	47,24	2,21	0,53
Divljaka	97,11	69,21	53,32	1,43	0,6
Lederica	109,5	65,51	54,14	1,6	0,73
Bedrica	140,59	63,23	59,72	2,58	0,65

Based on the results of one-factor analysis of variance, it can be concluded that the cultivar factor had a statistically significant effect on the examined statistical characteristics of fruit weight, fruit length, fruit width, fruit stalk length and fruit cup concavity.

In a study by Skender et al. (2008), states that the average weight of the fruit of the indigenous apple variety Divljak in 2005 was (70,55 g), and in 2006 (70,33 g), and that the average weight of the fruit of the indigenous apple variety Petrovača was (82,51 g). Literature source Gaši et al., (2010), states that the average weight of autochthonous apple varieties Lederica, Budimka and Petrovača bijela was (150,70 g, 150,40 g and 101,60 g), respectively.The average fruit height of the varieties Lederica, Budimka and Petrovača white was (45,07 mm, 53,72 mm and 45,53 mm). The average fruit width of the autochthonous apple varieties Lederica, Budimka and Petrovača white was (71,42 mm, 73,75 mm and 62,86 mm). The average stalk compression of the autochthonous apple varieties of Lederica, Budimka and Petrovača white was (0,65 mm, 0,68 mm and 0,4 mm). The length of the stalk of the examined autochthonous apple varieties of Lederica, Budimka and Petrovača bijela was (1,45 cm, 0,99 cm and 1,75 cm).

The Tukey-Kramer test determined the lowest statistical significance that existed among the cultivars and thus singled out certain cultivars in relation to other examined cultivars (Table 5).

Table 5. *Tukey-Kramer test for the tested parameters*

Comparation		**Fruit weight**	**Fruit height**	**Fruit width**	**Stalk length**	**Cup concavity**
Bedrica	**Petrovača bijela**	*	*	ns	*	*
Bedrica	**Jonatanka**	*	*	*	*	*
Bedrica	**Budimka**	*	*	*	*	Ns
Bedrica	**Cvjetača**	*	*	*	*	*
Bedrica	**Divljaka**	*	*	*	*	*
Bedrica	**Lederice**	*	*	*	*	*
Jonatanka	**Petrovača bijela**	*	ns	*	Ns	*
Jonatanka	**Budimka**	ns	ns	*	*	*
Jonatanka	**Cvjetača**	*	*	*	*	*
Jonatanka	**Divljaka**	*	ns	ns	*	*
Jonatanka	**Lederice**	*	ns	*	*	*
Budimka	**Petrovača bijela**	*	ns	ns	*	*
Budimka	**Cvjetača**	*	*	*	*	*
Budimka	**Divljaka**	*	ns	ns	Ns	Ns
Budimka	**Lederice**	*	ns	ns	*	*
Lederice	**Petrovača bijela**	*	ns	ns	*	*
Lederice	**Cvjetača**	*	*	*	*	*
Lederice	**Divljaka**	*	ns	*	*	*
Divljaka	**Petrovača bijela**	*	ns	*	*	*
Divljaka	**Cvjetača**	*	*	*	*	*
Petrovača bijela	**Cvjetača**	*	*	*	*	*
P 0,05		4,92	2,66	2,07	0,11	0,03

*(statistically significant), ^{ns} (not statistically significant)

The results of the Tukey Kramer test indicate the existence of statistical significance visible in the table 5.

Pearson correlation for examined statistical parameters

The connection and the character of the connection between the individual examined parameters were determined by the regression-correlation analysis, and the strength of such connections was determined by the Pearson coefficients of correlations.

Analyzing the fruit weight, we can conclude that it is in a weak positive correlation and statistically significantly correlated with fruit width (r = 0,471), fruit height (r = 0,467), stalk length (r = 0,234), as well as with cup concavity (0,447). Fruit width is in medium strong statistically significant positive correlation with fruit height (r = 0,710), weak positive statistically significant correlation with cup concavity (r = 0,304), as well as weak negative statistically significant correlation with stalk length (r = -0,146). Fruit height was statistically significantly weakly positively correlated with stem length (r = 0,115), while in correlation with calyx indentation it did not show statistically significant correlations (r = 0,099). The length of the stalk is statistically in a significantly weak negative correlation with the cup depression (r = -0,116) (Table 6).

Table 6. *Pearson correlation of morphological parameters of autochthonous apple varieties*

Variables	**Fruit weight**	**Fruit width**	**Fruit height**	**Stalk length**	**Cup concavity**
Fruit weight	**1**	0,471	0,467	0,234	0,447
Fruit width	0,471*	**1**	0,710	-0,146	0,304
Fruit length	0,467*	0,710*	**1**	0,115	0,099
Stalk length	0,234*	-0,146*	0,115*	**1**	-0,116
Cup concavity	0,447*	0,304*	$0,099^{ns}$	-0,116*	**1**

*(statistically significant), ^{ns} (not statistically significant)

Principal Component Analysis for autochthonous apple varieties

Analyzing the results of the five main components of the PCA analysis shown in Table 7, one can see the contribution of each of the 5 analyzed traits in the total variability present in the analyzed set of indigenous apple varieties. Each of the 5 observed traits is found with a high value of eigenvectors in one of the first five main components.

Table 7. *Eigenvalues, proportion of variance and cumulative variance associated with the first five main components (PCA), estimated from a correlation matrix with 6 variables in 7 autochthonous apple cultivars*

Variables	**PCA1**	**PCA2**	**PCA3**	**PCA4**	**PCA5**
Eigenvalue	2,846	1,385	0,583	0,136	0,050
Proportion of variance (%)	56,928	27,692	11,656	2,716	1,008
Cumulative variance (%)	56,928	84,620	96,277	98,992	100,000

The variables with the largest values of eigenvectors in the first five main components are presented:

PCA1– fruit weight, fruit width, fruit height;

PCA2 - stem length;

PCA3 - cup concavity.

Based on the Principal Component analysis (Table 7), it can be concluded that the eigenvalues of the vector of the first main component, which accounted for (56,928%) of the total variance, are morphological characteristics of the fruit that are small highest values in this component. The highest values of eigenvectors had the content of fruit mass and fruit width (29,500 and 27,250). Fruit widths had the lowest values of eigenvectors (24,548). From the graph it can be seen that there is a strong positive correlation between the characteristics of fruit weight and fruit height, as well as a strong negative correlation between fruit width and cup recess (Table 8).

Table 8. *Analysis of 5 quantitative properties of autochthonous apple varieties in the total variability of the experiment (significant sources of variability are in bold)*

	PCA1	**PCA2**	**PCA3**
Fruit weight - FW	**29,500**	8,747	0,209
Fruit length - FL	**27,250**	3,754	18,146
Fruit width - FWW	**24,548**	13,309	10,756
Stalk length - SL	0,012	**69,853**	2,132
Cup concavity - CR	18,690	4,336	**68,757**

As part of the second main component, which amounts to (84,620%) of the total variability of the experiment, the property with a high value for the eigenvector was related to the stalk length of indigenous apple varieties (69,853). Due to the visualization of the level of statistical significance of separation of individual genotypes and groups of dogwood genotypes, the following graphs present the examined autochthonous apple variety according to affiliation with 95% confidence.

From the figure 1, a strong positive correlation can be seen between the characteristics of fruit weight and fruit height, as well as a strong negative correlation between fruit width and cup concavity.

Figure 1 shows the distribution of 7 indigenous genotypes of apples obtained using the first two main components, which were calculated by a correlation matrix for 5 morphological characteristics of the fruit.

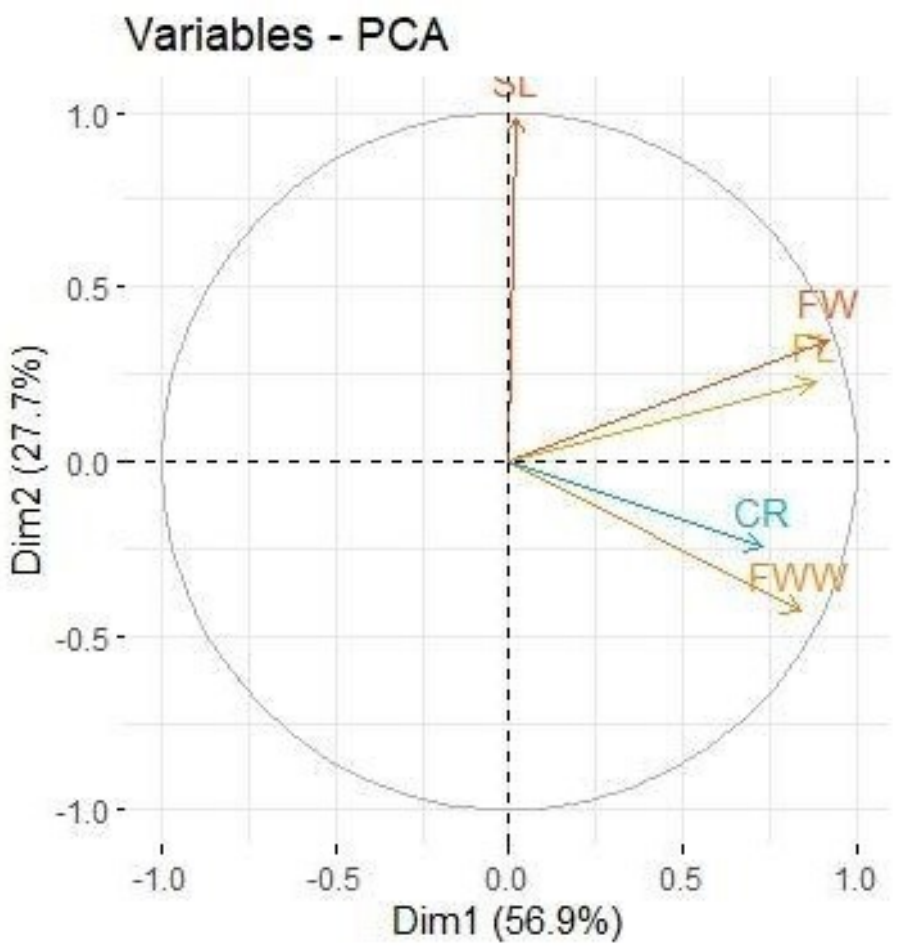

Figure 1. *Representation of grouping and interrelationship of 5 variables of examined 7 autochthonous apple varieties using the first two main components (PC1 and PC2)*

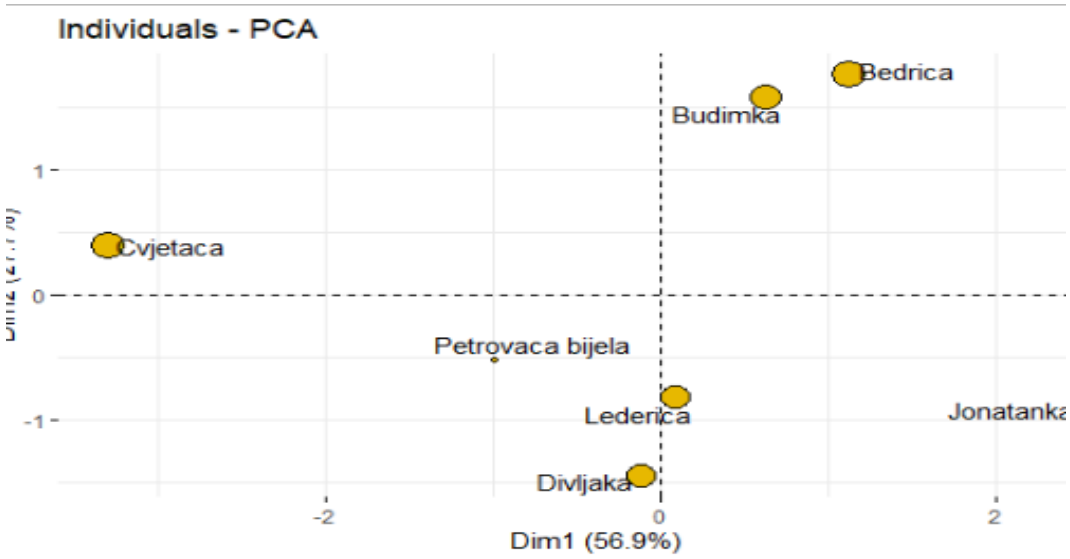

Figure 2. *Demonstration of grouping of examined 7 autochthonous apple varieties using the first two main components (PC1 and PC2)*

Based on the previous graphs, it is possible to notice that the first two components containing 84,620% of the total variability of the experiment, the examined apple cultivars occupied a certain position in the coordinate system.

The partial grouping of individual varieties is also visible, which indicates the fact that their average values of the examined characteristics are in a positive correlation with each other (Fig 2.).

Hierarchical cluster analysis

After the hierarchical cluster analysis, the existence of divergence of the examined autochthonous apple varieties is visible, which resulted in the separation of all analyzed samples into four different clusters (Figs. 3, 4.).

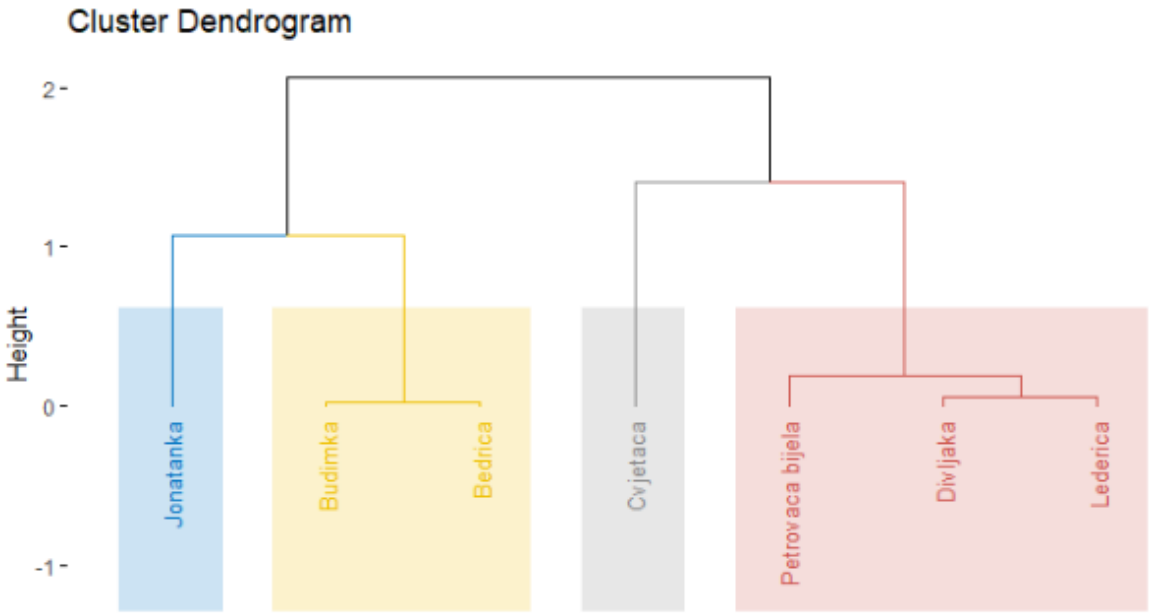

Figure 3. *Dendrogram created on the basis of the Euclidean matrix - Euclidean distance between average values for 5 quantitative traits in 7 indigenous apple varieties*

In the first cluster, 1 autochthonous variety of Cvjetača apple is classified. The second largest cluster includes 3 autochthonous apple varieties of Petrovača bijela, Lederica and Divljaka. The third cluster includes 2 autochthonous apple varieties of Bedrica and Budimka, and the fourth cluster includes 1 autochthonous apple variety of Jonatanka.

In order to identify autochthonous apple varieties, average values of quantitative characteristics were analyzed. Figure 4 and 5. they clearly show that the results of the factor analysis are in complete agreement with the hierarchical enema analysis, which classifies all examined apple varieties into 4 separate clusters. In the right part of the graph (coordinate system), apple varieties are grouped, which show the highest average values of the examined morphological characteristics (Jonatanka, Bedrica and Budimka). In the left part of the graph (coordinate system) are grouped varieties that have the

lowest average values of the examined characteristics, namely Cvjetača, Lederica, Divljaka and Petrovača bijela.

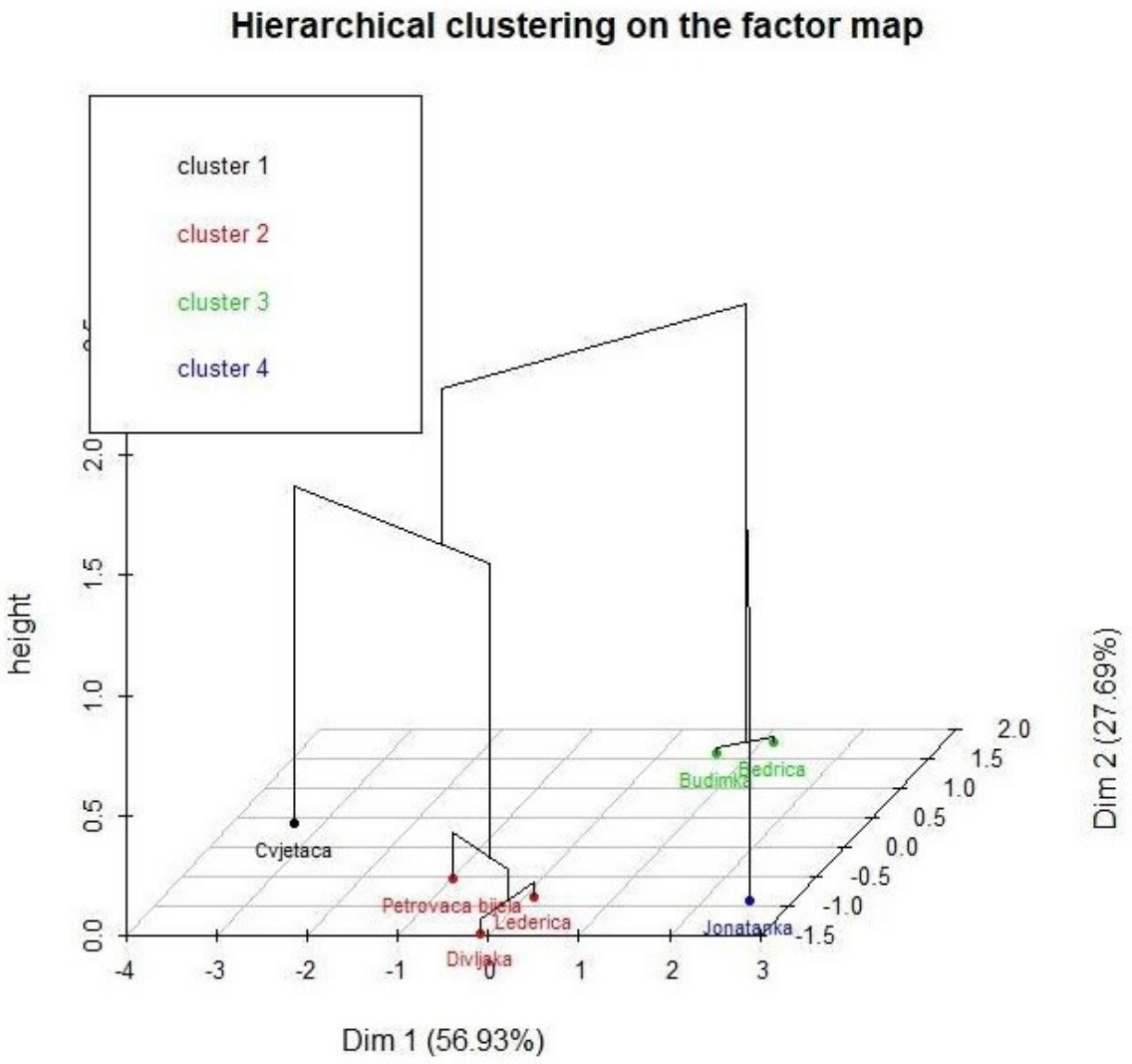

Figure 4. *Dendrogram created on the basis of the Euclidean matrix - Euclidean distance between average values for 5 quantitative traits in 7 autochthonous apple varieties*

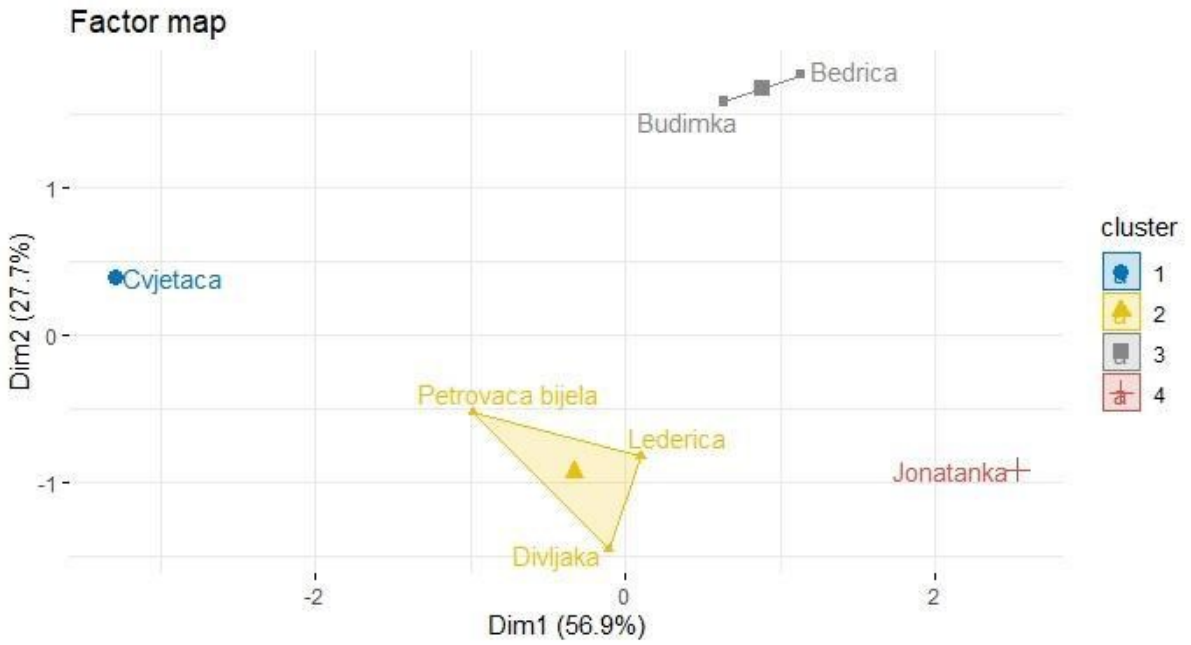

Figure 5. *Distribution of 7 analyzed autochthonous apple varieties into separate clusters using hierarchical cluster analysis over factor analysis of data*

Conclusions

Analysis of morphological characteristics of fruits of selected autochthonous apple varieties Petrovača bijela, Budimka, Ljutika-Divljaka, Ledarica, Bederica, Ljepocvjetka-Cvjetača and Jonatanka from Stipanići Tomislavgrad indicates the existence of diversity that can serve as a starting material for further selection work.

The tested autochthonous apple varieties can serve as a valuable genetic material in the breeding programs, which can be used in order to create new apple varieties, as well as for the expansion in the commercial plantations.

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