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Evaluation of morphological diversity for some bilberry (*Vaccinium myrtillus* L.) populations from Banat mountains

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Abstract: Bilberry fruits have been harvested from ancient times for their nutritional and health benefits, being used in the food industry. The goal of this research was to assess the morphological diversity for some bilberry populations from Banat Mountains in order to obtain information about their potential for growth and development under different natural conditions of the region. The plants samples were selected from six sites with elevation from 750 to 1500 m. The highest differentiation between populations was recorded for stem diameter and branches number, while the lowest diversity between populations was observed for leaves size. According to al traits, the population from Sadova Noua was a separate one and expresses a diversity of 50% to other five populations. The morphological differentiation of plants from Sadova Noua population may be due the effect of particular environmental conditions like lowest elevation and/or high amount of nitrogen and potassium in the soil. The high morphological similarity of approximately 93.5% between the populations from Semenic and Cuntu was associated with their similarity regarding elevation, pH, nitrogen and potassium content of the soil. Besides other factors, some of the natural conditions like elevation or nutrient content of the soil it seems to have an important effect on growth and development of bilberry plants.

Key words

bilberry, morphological plant traits, population diversity.

Bilberry (*V. myrtillus)* plants usually grow in meadows and moist coniferous forests with acid soils, and its growth is favored by moderate shade and moderately humid conditions [4]. Generally, bilberry requires well-drained mineral soils with a medium fertility; even it can also grow in peatlands [13; 10]. Grow and development of bilberry in habitats with low nutrient availability is facilitated by the symbiosis with ericoid mycorrhiza [3; 12] and also the ability of plant to take up organic nitrogen [14].

Bilberry has high morphological plasticity; as such growth habit in terms of branching can differ greatly between habitats as a response to environmental conditions [19]. The aerial shoots of bilberry grow more vertically and have sharp branching angles in open habitats compared with the forest [15]. Clonally growth in bilberry is likely to be one of the main factors that constrain fruit and seed number [16].

The fruit yield in bilberry is correlated with current annual shoot size [20; 7] and is also linked with the ability of plants to produce new growth after biomass loss. Mannien et Peltola (2019) reported that bilberry fruit yield and fruit set increased after continuous picking, considering that picking probably accelerated the rejuvenation process of bilberry ramets, which may have a positive effect on bilberry performance in the long-term.

The bilberry plants have small fruit of about 5-9 mm in diameter, bluish black in colour, with many seeds [17]. Berry fresh weight is positively correlated with the seeds weight and with the number of seeds [18], meaning that cross-pollination has a positive effect on fruit yield. The berries are a rich source of various phenolic compounds, including large amounts of anthocyanins, with protective roles against various sources of stresses and also many important benefits for human health [6; 21].

Taking into account the high out crossing rate *V. myrtillus* is considered to be a mixed mating species. The dispersal of bilberry seeds and seedling occurrence in nature is associated with bird activity [2; 9]. The spatial distribution of the genetic diversity in a population is mainly influenced by the seed dispersal and by the clonally growth strategy [1]. Albert et al. (2004) considered that the patterns of the genetic variation in bilberry were mainly due to the mating system. In this regard, there is a low genetic variation between populations, due to seed dispersal and a low genetic variation within populations due to vegetative reproduction [11].

The goal of this research was to assess the morphological diversity for some bilberry populations

from Banat Mountains in order to obtain information about their potential for growth and development under different natural conditions of the region.

Material and Method

The research was performed in natural habitats of bilberry from five different sites from Banat Mountains (Muntele Mic, elevation 1500 m; Raul Lung, near Balosu-Zanoaga peak, elevation 1300 m; Semenic, elevation 1400 m; Sadova Noua, elevation 750 m; Cuntu, elevation 1400 m) and one site from Apuseni Mountains (Padis, elevation 1300 m).

Soil samples that included the O-layer were collected for chemical analysis in all sites. The soil analyses were made in the Laboratory of physico-chemical analysis soil-water-fertilizers from Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara. The pH ranged from 3,95 in Raul Lung to 4,95 in Padis, while the total nitrogen varied between 0.65% in Muntele Mic to 0.9% in Sadova Noua. Regarding the phosphorus content (ammonium lactate soluble P), the samples values ranged from 17,66 ppm at Semenic to 62,48 ppm for Padis, while the potassium content (ammonium lactate soluble K) showed values from 181 ppm in Muntele Mic to 454 ppm for Sadova Noua.

From each site 20 randomly plants were selected, and were made measurements regarding the following traits: plant height (Ph) as a distance (cm) between the soil level and the top of main stem; stem diameter (Sd) at the basis of main stem (mm), measured with a digital caliper; branches number (Bn); leaf length (Ll) and leaf width (Lw), in the mid-part of leaves (mm); leaf shape index (Lsi) based of the ratio between length and width of leaves; leaf area (La) measured on 20 randomly selected leaves from different part of the plant, using Area Meter AM 300-002 ADC BioScientific Ltd. The bilberry plants were collected in 2019 during the fruit-ripening stage.

The statistical analysis of data was made using analysis of variance and means were separated by Least Significant Difference test according to Ciulca (2006). The similarity matrix was used for hierarchical clustering of populations based of the unweighted pair-group method with arithmetic averages (UPGMA), using the Neighbor program of the Phylip package, version 3.5c. In order to display in a single graph the performance of each population for each of the seven traits, the basic principle of the biplot technique developed by Gabriel (1971) was used.

Results and Discussions

Based of data from analysis of variance (Table 1) it can be observed that there are significant differences between the bilberry populations, according to all morphological traits. The highest variability between populations was observed for plant height and stem diameter, while in terms of leaf size and shape the variation between populations was smaller.

Table 1

Analysis of variance (ANOVA) for morphological traits in bilberry populations

Source of variation	DF	SS	MS	F value	Source of variation	DF	SS	MS	F value
Plant height					Leaf width				
Total	119	11669			Total	119	194.72		
Population	5	8337	1667.31	57.03**	Population	5	91.71	18.34	20.30**
Error	114	3333	29.23		Error	114	103.01	0.90	
Stem diameter					Leaf shape index				
Total	119	136.84			Total	119	1.43		
Population	5	100.60	20.12	63.29**	Population	5	0.50	0.10	12.11**
Error	114	36.24	0.32		Error	114	0.94	0.01	
Branches number					Leaf area				
Total	119	434.75			Total	119	111572		
Population	5	216.08	43.22	22.53**	Population	5	64335	12867	31.05**
Error	114	218.67	1.92		Error	114	47238	414	
Leaf length									
Total	119	388.89							
Population	5	150.77	30.15	14.44**					
Error	114	238.12	2.09						

The plant height for the six populations recorded amplitude of 27.33 cm, ranging between 24 cm for 'Cuntu' and 51.33 cm in the 'Raul Lung' population. Thus, it can be observed the plants from 'Raul Lung' population had a significantly higher value, with over 10 cm than the other populations. The populations from 'Muntele Mic' and 'Sadova Noua' had a similar size of this trait and also significantly higher values than 'Semenic' and 'Padis' populations, statistically undifferentiated. The plants of 'Cuntu' population had a significantly lower height than the others. At the intra-population level, there is a small

variability of this trait in 'Cuntu' and 'Sadova Noua', and a middle variability to others populations, respectively.

Regarding the stem diameter, the six populations recorded values from 3.11 mm in 'Cuntu' to 5.76 mm to 'Raul Lung' with amplitude of 2.65 m and a inter-populations variability of 22.57%. The highest inter-individual homogeneity of this trait was registered in 'Cuntu' population followed by 'Padis'. The highest intra-population variability and amplitude of stem diameter was observed in 'Semenic' population. Taking in to account the reciprocal comparisons between populations, a high significant value was noticed in Raul Lung followed by Padis population. The populations from 'Muntele Mic' and 'Semenic' registered close values of this trait and significantly higher compared to 'Sadova Noua' and 'Cuntu' populations.

The values of branches number at studied populations recorded generally a middle variability ($s_{\%}$ =19.86) and amplitude of 3.84, ranging from 5.33 in 'Padis' to 9.17 in 'Sadova Noua' population. The inter-individual variability for plant branching was higher in 'Raul Lung' and 'Padis' population and lower in 'Muntele Mic' and 'Semenic', respectively. Based of multiple comparisons it was observed that the population from Sadova Noua presented a degree of plant branching significantly higher to other populations, followed by 'Cuntu' and 'Raul Lung' populations..

Regarding the leaves length, the studied populations registered values from 15.29 mm at population from 'Muntele Mic' to 18.96 mm in case of 'Sadova Noua', associated with amplitude of 3.67 mm and a low inter-population variability (7.92%), and a low heterogeneity at intra-population level. The populations from 'Muntele Mic' and 'Raul Lung' presented the highest uniformity of leaves length. Considering the reciprocal comparison between populations, in 'Sadova Noua' population was noticed a significantly higher length of leaves, associated with differences over 2.3 mm to other populations.

Table 2

Means, standard error of mean and coefficient of variation for morphological traits in bilberry populations

Population	Plant height (cm)		Stem diameter (mm)		Branches number		Leaf length (mm)	
	$\bar{x} \pm s_{\bar{x}}$	S%	$\bar{x} \pm s_{\bar{x}}$	S%	$\bar{x} \pm s_{\bar{x}}$	S%	$\bar{x} \pm s_{\bar{x}}$	S%
'Muntele Mic'	37.50±2.31 b	15.06	4.59±0.23 c	12.39	6.33±0.33 c	12.89	15.29±0.45 c	7.16
'Raul Lung'	51.33±4.06 a	19.39	5.76±0.30 a	12.63	7.00±0.86 bc	29.97	15.67±0.43 c	6.68
'Semenic'	32.67±2.26 c	16.95	4.30±0.33 c	18.67	8.83±0.48 a	13.23	15.95±0.58 bc	8.97
'Cuntu'	24.00±0.86 d	8.74	3.11±0.11 e	8.52	7.67±0.61 b	19.64	16.60±0.53 b	7.77
'Sadova Noua'	40.67±1.48 b	8.89	3.63±0.22 d	14.66	9.17±0.65 a	17.48	18.96±0.85 a	10.98
'Padis'	33.67±1.87 c	13.63	5.36±0.23 b	10.70	5.33±0.56 d	25.62	16.66±0.68 b	9.97
$LSD_{5\%;\ 1\%;\ 0.1\%}$	3.39; 4.48; 5.76		0.35; 0.47; 0.60		0.87; 1.15; 1.48		0.91; 1.20; 1.54	
Population	Leaf width (mm)		Leaf shape index		Leaf area (mm^2)			
	$\bar{x} \pm s_{\bar{x}}$	S%	$\bar{x} \pm s_{\bar{x}}$	S%	$\bar{x} \pm s_{\bar{x}}$	S%		
'Muntele Mic'	9.61±0.22 bc	5.66	1.60±0.04 d	6.01	88.19±16.36d	45.44		
'Raul Lung'	9.55±0.20 bc	5.12	1.65±0.05 cd	6.85	108.00±5.59bc	12.69		
'Semenic'	9.16±0.41 c	11.09	1.74±0.03 ab	4.08	105.10±4.82 c	11.23		
'Cuntu'	9.43±0.60 bc	15.46	1.77±0.04 a	5.43	106.39±5.18 c	11.92		
'Sadova Noua'	11.80±0.59 a	12.26	1.61±0.04 d	5.36	162.48±11.06a	16.68		
'Padis'	9.92±0.26 b	6.37	1.71±0.05 bc	6.55	120.08±3.75 b	7.62		
$LSD_{5\%;\ 1\%;\ 0.1\%}$	0.60; 0.79; 1.01		0.06; 0.08; 0.1		12.75; 16.87; 21.70			

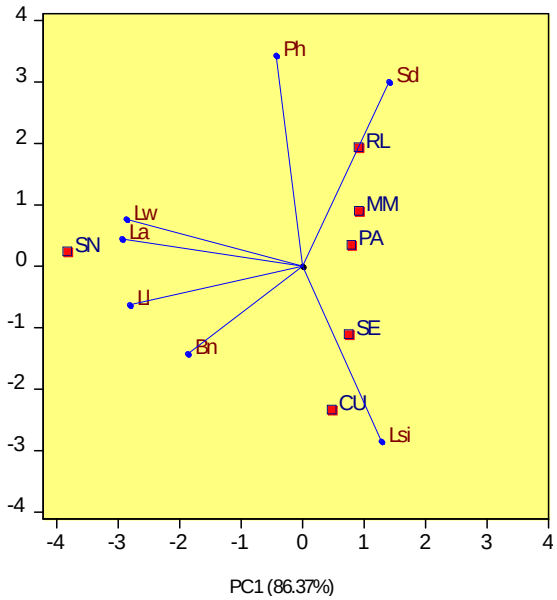
* Different letters indicates significance at p 0.05:

The leaves width of the six populations registered amplitude of 2.64 mm, ranging from 9.16 mm in 'Semenic' to 11.80 mm for 'Sadova Noua' population, on the background of a small variation between populations. Thus, it was observed that in case of 'Sadova Noua' population the leaves width was significantly higher to other populations with about 2 mm. The differences between other populations for this trait were smaller and generally no significant. At intra-population level it was noticed a low variability of this trait in 'Muntele Mic', 'Raul Lung' and 'Padis', and a middle variability for other populations, respectively.

Regarding the shape of leaves, the six populations registered value of shape index between 1,61 in 'Sadova Noua' to 1,77 in 'Cuntu', with a low amplitude and inter-population variability. The highest inter-individual homogeneity for this trait was recorded in 'Semenic' population, under a low inter-population variability. Given the reciprocal comparisons between populations, it was found that the leaves of 'Cuntu' population had a more elongated shape, while the leaves of 'Muntele Mic', 'Raul Lung' and 'Sadova Noua' populations had a more ovate shape.

The values of average leaf area registered generally a middle variability ($s_{\%}$ =19.28) and

amplitude of 57.62 mm^2, ranging from 88.19 in 'Muntele Mic' to 162.48 mm^2 for 'Sadova Noua' population. The inter-individual variability for this trait was very height in 'Muntele Mic', while at 'Padis' population a high uniformity of leaf area was found. Based of multiple comparisons it was observed that in 'Sadova Noua' population the leaf area was significantly higher to other populations, followed by 'Padis' population whose leaves had an area significantly superior to the leaves of 'Semenic' and 'Cuntu' populations.



MM-'Muntele Mic'; RL-'Raul Lung'; SE-'Semenic'; CU-'Cuntu'; SN-'Sadova Noua';PA-'Padis'
Ph-Plant height; Sd-Stem diameter; Bn-Branches number; Ll-Leaf length: Lw-Leaf width; Lsi-Leaf shape index; La-Leaf area

Fig. 1. Biplot based of first two principal components for morphological traits in bilberry populations

The biplot based of first two principal components (Figure 1) express 99.66% from the variability of the seven morphological traits. Depending on the position of the populations to the vectors of different traits, the dimensions related to them were expressed. As such, it was observed that 'Sadova Noua' population presented the highest values of all traits. In case of 'Cuntu' population, the high values of leaves size and branches number were associated with a plant height above mean and a low stem diameter. At 'Raul Lung', 'Muntele Mic' and 'Padis' populations, the highest values of plant height and stem diameter were registered, under a low branching degree and leaves size. The plants of 'Semenic' and 'Cuntu' populations were characterized by a higher degree of branching, a low height and thinner branches, associated with low size of leaves and an elongated shape thereof.

Based on the cosine of the angles between the vectors of the different traits (Figure 1), one can make appraisals on the relationships between them. Thus it is observed that the stem diameter had the highest contribution on the variability of plants height, amid a positive correlation between them. The number of branches showed a correlation and a negative influence on plant height of the studied bilberry populations, respectively.

Table 3

Matrix of similarity coefficients between bilberry populations for analyzed morphological traits

Population	**1**	**2**	**3**	**4**	**5**	**6**
1. 'Muntele Mic'	1					
2. 'Raul Lung'	0.9168	1				
3. 'Semenic'	0.8291	0.8134	1			
4. 'Cuntu'	0.7412	0.6291	0.9348	1		
5. 'Sadova Noua'	0.5159	0.4849	0.5128	0.4809	1	
6. 'Padis'	0.8767	0.8696	0.8352	0.804	0.5065	1

According to the data presented in Table 3, it was noted that in terms of the seven morphological traits, the highest phenotypic similarity exists between populations: 'Semenic' and 'Cuntu' (93.48 %),

'Muntele Mic' si 'Raul Lung' (91.68), respectively. As well, 'Padis' population registered a phenotypic similarity of 87*88% to 'Muntele Mic' and 'Raul Lung' populations. A high level of morphological differentiation was observed between populations: 'Sadova Noua' and 'Cuntu' (51.91 %); 'Sadova Noua' and 'Raul Lung' (51.51 %); 'Sadova Noua' and 'Padis' (49.35 %).

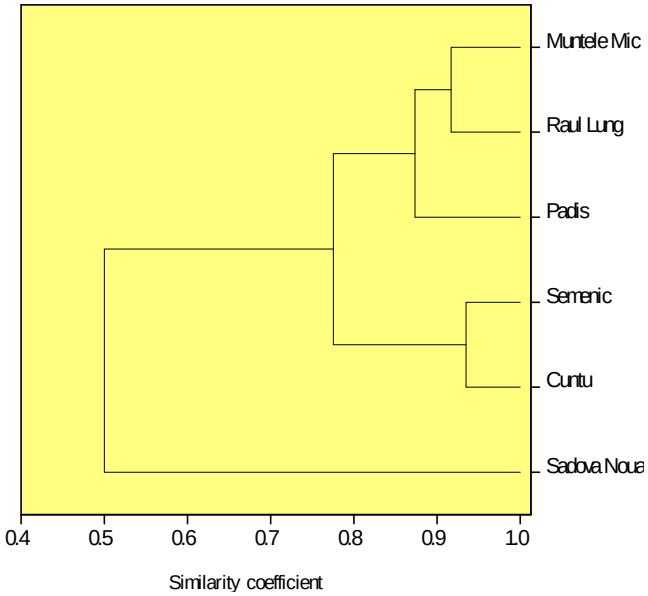


Fig. 2. UPGMA clustering of bilberry populations based on morphological traits

Depending on the morphological similarity for the seven traits, the populations were hierarchically classified into three clusters, among which there is an average diversity of about 50% (Figure 2). The first group was made up of populations from 'Muntele Mic' and 'Raul Lung' phenotypically similar to approximately 92% and to an extent of about 87% with 'Padis' population, respectively. The second cluster was composed by 'Semenic' and 'Cuntu' populations which show a morphological similarity of approximately 93.5%, and diversity about 21% to the populations of the first cluster. The population from 'Sadova Noua' represents a separate cluster, and expresses a similarity of 49.69% to the populations from second cluster and 50.24% to those of the first cluster.

Table 4

Analysis of variance for bilberry populations concerning the diversity based of morphological traits

Population	Between groups		Within groups		F Test
	SS	DF	SS	DF	
'Muntele Mic'	215.88	1	56.65	5	19.05**
'Raul Lung'	219.09	1	46.55	5	23.53**
'Semenic'	267.98	1	58.37	5	22.96**
'Cuntu'	291.67	1	80.19	5	18.19**
'Sadova Noua'	180.63	1	95.89	5	9.42*
'Padis'	256.35	1	72.08	5	17.78**

Considering the results shown in Table 4, it was observed that the populations from 'Cuntu' and 'Semenic' generates the largest morphological differences, having high and significant contributions of 18.72-20.37 % to the total variability recorded on the basis of the dendrogram from Figure 2. The population from 'Sadova Noua' had the lowest contribution (12.62%) to all variability between the three clusters. At the level of the first cluster the 'Padis' population had the highest contribution (41.12%) to intra-population diversity, while al the level of the second cluster the population from 'Cuntu' was highlighted by a contribution of 57.87%.

Table 5

Analysis of variance for morphological traits of bilberry populations

Trait	Between groups		Within groups		F Test
	SS	DF	SS	DF	
Plant height	1.280	1	3.724	4	1.37
Stem diameter	3.578	1	1.379	4	10.38*
Branches number	3.758	1	1.219	4	12.33*
Leaf length	1.462	1	3.521	4	1.66
Leaf width	0.304	1	4.696	4	0.26
Leaf shape index	0.736	1	4.264	4	0.69
Leaf area	0.659	1	4.341	4	0.61

Regarding the analysis of variance for morphological traits of bilberry populations (Table 5), it was observed that high and significant values of the variance were recorded for stem diameter and branches number, which show a high ability for differentiation between the three groups of populations. The lowest diversity between populations of different clusters was observed for leaves size.

Conclusions

The highest differentiation between populations was recorded for stem diameter and branches number, while the lowest diversity between populations was observed for leaves size. Stem diameter had the highest contribution on the variability of plants height, amid a positive correlation between them. The number of branches showed a negative effect on plant height of the studied bilberry populations, respectively.

According to al seven morphological traits, the population from 'Sadova Noua' was a separate one and expresses a diversity of approximately 50% to other five populations. The morphological differentiation of plants from 'Sadova Noua' population may be due the effect of particular environmental conditions like lowest elevation and/or high amount of nitrogen and potassium in the soil. The high morphological similarity of approximately 93.5% between the populations from 'Semenic' and 'Cuntu' was associated with the similarities of natural conditions regarding elevation, pH, nitrogen and potassium content of the soil.

Besides other factors, some of the natural conditions like elevation or nutrient content of the soil it seem to have an important effect on growth and development of bilberry plants.

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