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NONTRIVIAL VARIATIONS OF MORPHO-ANATOMICAL LEAF TRAITS IN NATURAL SOUTH-EASTERN POPULATIONS OF *VACCINIUM* SPECIES FROM CENTRAL BALKANS

IVANA BJEDOV[1*], DRAGICA OBRATOV-PETKOVIĆ[1], VERA RAKONJAC[2], DRAGANA SKOČAJIĆ[1], SRĐAN BOJOVIĆ[3], MILENA MARKOVIĆ[3], ZORA DAJIĆ – STEVANOVIĆ[2]

[1]University of Belgrade - Faculty of Forestry, Kneza Višeslava 1, 11000 Belgrade, Serbia
[2]University of Belgrade - Faculty of Agriculture, Nemanjina 6, 11080 Belgrade - Zemun, Serbia
[3]Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana142, 11000 Belgrade, Serbia

* Corresponding author, e-mail: ivana.bjedov@sfb.bg.ac.rs

Running title: *Bjedov et al. ……… Morpho-anatomical leaf traits in* Vaccinium *species*

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Morpho-anatomical characteristics of *Vaccinium myrtillus, V. uliginosum* and *V. vitis-idaea* leaves from several sites of the Central Balkans were examined. The aim of this study was to investigate for the first time morpho-anatomical leaf traits of these species in the studied populations and to identify traits that follow a specific trend along the gradients of climate factors. Leaf traits that discriminate *Vaccinium* species were as follows: depth of the adaxial cuticule (AdC), thickness of the palisade tissue (PT), thickness of the spongy tissue (ST), height of the abaxial epidermal cells (AbE), height of the abaxial cuticule (AbC) and leaf thickness (LT). Populations of *V. myrtillus* were characterized by the smallest, and populations of *V. vitis-idaea* by the highest values for AdC, PT, ST, AbE and LT. Additionally, AbC was significantly larger for *V. uliginosum* in comparison to two other species. On the basis of morpho-anatomical traits, intraspecific variability of the studied species , was explored by Principal Component Analysis (PCA), Cluster Analysis (CA) and Analysis of Variance (ANOVA). CA based on 10 morpho-anatomical traits showed that populations of *V. myrtillus* and *V. uliginosum* that grew at lower altitudes (characterized by higher mean annual temperature) are more similar to each other. Especially *V. myrtillus* was responsive to the elevational gradient and exhibited the highest plasticity in morpho-anatomical leaf traits. Populations of *V. vitis-idaea* had a different pattern of differentiation along the elevational gradient. CA showed that the populations at the lowest and at the highest altitudes were more similar according to the morpho-anatomical leaf traits, meaning that evergreen leaves were more resistant to environmental conditions.

Keywords: Climate factors, Cluster Analysis, elevational gradient, Serbia, intraspecific and interspecific variability, Analysis of Variance, Principal Component Analysis.

INTRODUCTION

Vaccinium myrtillus L., *V. uliginosum* L. and *V. vitis-idaea* L. are promising crops for the Central Balkans, and currently their only source are natural populations. Peripheral populations of *Vaccinium* species are threatened with decline due to over-exploitation by local people and to the ongoing suppression by a more competitive shrub *Juniperus communis* subsp. *alpina* (Tomićević et al., 2011; Bjedov, 2012). A previous study on genetic diversity (Bjedov et al., 2015) showed that *Vaccinium* populations in the Central Balkans are highly genetically differentiated. Because these populations are situated in the south-eastern marginal area of their geographic range and are highly fragmentarily distributed, there is a lack of gene flow and the genetic diversity is lower comparing with populations from central and northern Europe, where they have more continuous distribution. The reduced level of genetic variability due to the limited gene flow and genetic drift could cause loss of adaptive capacity and increase the risk of decline for populations of this autochthonous, economically important species.

The fundamental connection between environmental conditions and plant characteristics was recognized very early in the development of plant ecology (Cornwell and Ackerly, 2009). As a result of environmental filtering, mean values of the trait will differ among plant communities along an environmental gradient (Mayfield and Levine, 2010). The leaves are plant organs most exposed to the site conditions and the changes in their characters have been interpreted as adaptations to the local environment (Leymarie et al., 1999; Charles and David, 2003).

Adaptive plasticity is a result of direct selection, and the type of plasticity is of t great evolutionary and ecological interest. Variation in adaptive plasticity could be explained by environment or environment x genotype interaction (Alpert and Simms, 2002). Numerous studies provided evidence proving that variation in morphological and anatomical characteristics involves both genotypic differentiation and phenotypic plasticity aiming to maximize the overall fitness of the species (Castro-Díez et al., 1997; Cordell, 1998; Toivonen et al., 2014). The degree of variation in plant traits will influence the response of plants to climate change: phenotypic plasticity will allow short-term responses, while genetic differentiation may provide evolutionary responses to abiotic changes (Read et al., 2014).

Establishing the pattern of variation along the elevational gradient could be useful for future breeding efforts. Pato and Obeso (2012) found that along the elevational gradient (350 to 2000 m a.s.l.) *V. myrtillus* exhibited the most favorable vegetative performance at mid elevations, while maximum reproductive output was observed at higher elevations. Moreover, if the preferred traits along the elevational gradient of a species are known, it becomes possible to carry out common garden experiments aiming to establish whether the certain traits are genetically determined.

Plants do not respond directly to a change of altitude or latitude, but to a change in the range of environmental factors that are related to altitude or latitude (Read, 2014). As altitude increases, the mean annual air temperature and atmospheric pressure decrease and insolation ("clear sky turbidity") increases. However, moisture, sunshine duration, wind, season length, geology and even human land use show a more complex relationship with altitude (Körner, 2007).

Considering the above, models that predict functional characteristics of plants should not be developed in relation to altitude itself, but to the climate factors (such as the mean annual air temperature and annual precipitation).

Some authors (Lens et al., 2004; Ponikierska et al., 2004) studied variation in leaf traits of *Vaccinium* species in different environmental conditions. However, such types of investigation were not performed for *Vaccinium* populations on the south-east edge of their geographic distribution. In this paper, an investigation of anatomical and morphological characteristics of leaves of *V. myrtillus, V. uliginosum* and *V. vitis-idaea* from several peripheral populations in Serbia is presented. Starting from the hypothesis that there are differences in response of morpho-anatomical leaf traits to the certain environmental factors between *Vaccinium* populations, we defined the following aims of the study: 1. to investigate for the first time morpho-anatomical leaf traits of these species in populations in the Central Balkans, and 2. to identify traits that follow a specific trend along the gradients of climate factors.

MATERIAL AND METHODS

PLANT MATERIAL

The investigation was performed in the Central Balkans, in different parts of Serbia. The studied sites are located in mountainous regions in west (Divčibare and Kamena gora), central (Kopaonik Mt.), and southeast Serbia (Stara planina Mt. and Vlasina). The altitudes of chosen habitats ranged from 960 m to 1820 m. Five populations of *V. myrtillus*, four populations of *V. vitis-idaea* and three populations of *V. uliginosum* were sampled from several sites within the territory of the Central Balkans (Table 1). In order to minimize the likelihood of clone sampling, young leaves were collected from individuals that were 30 m apart. Voucher specimens were deposited in the herbarium of the Faculty of Forestry, University of Belgrade (HFFBU/2016/*Vaccinium* anatomy). Forty plants of the same age were randomly chosen from each population. In order to describe the investigated characteristics of the leaves, ten leaves were taken from the middle part of the stems of each individual plant and measured shortly afterward.

MORPHO-ANATOMICAL ANALYSIS

As already mentioned, the altitudes of chosen habitats ranged from 960 m to 1820 m. Kofidis et al. (2007) found that leaves are plant organs that undergo significant changes due to a combined effect of altitude and season. In accordance with this, the following morphological traits of the leaves were measured: leaf length (LL), leaf width (LW), leaf surface area (LA) and leaf length to width ratio (L/W). The LA was calculated by counting the grid, projecting a leaf on a piece of grid paper and counting the numbers of squares. The cross sectioned specimens were measured for seven anatomical traits: depth of the adaxial cuticule (AdC), height of the adaxial epidermal cells (AdE), thickness of the palisade tissue (PT), thickness of the spongy tissue (ST), height of the abaxial epidermal cells (AbE), height of the abaxial cuticule (AbC) and leaf thickness (LT).

Sample preparation for light microscopy

The fresh leaves were fixed in 50% alcohol. The cross sections of the leaves were made using the standard paraffin method for light microscopy (Jensen, 1962; Blaženčić, 1990). Sections 7–12 μm thick were prepared using a LEICA SM 2000 R microtome, and were stained with safranin and alcian blue. The mounted sections were then analyzed and imaged using a LEICA DMLS microscope equipped with a digital camera LEICA DC 300 and LEICA imaging software.

Statistical analysis

For assessment of intraspecific variations in leaf traits, statistical analysis was conducted on three data sets, for every species separately. The data were tested for normality using Shapiro and Wilk test and were log10-transformed before analysis, when necessary, homogeneity of variance was checked with Levene's test. Welch's test was used for features with non-homogeneous variance.

The overall structure and variability of the data were assessed by Principal Component Analysis (PCA). PCA was performed on the sets of all leaf traits (Table 2). LT represents the sum of the following traits: AdC, AdE, PT, ST, AbE and AbC, and therefore was omitted from analysis. Cluster Analysis (CA) was performed based on mean values of the examined traits, using "Single-linkage" method with Euclidean distances, to assess similarities between populations. Variability of morpho-anatomical leaf traits among populations was assessed by one-way analysis of variance (ANOVA). To relate the leaf traits with climatic factors of the studied populations, ordinary linear regression was used, where means of traits represented response variables and means of climate data represented explanatory variables. Climate data for the localities of the studied populations were taken from "WorldClim" database (http://www.worldclim.org/current) at the spatial resolution of 1 km^2 (Levitt 1972). "WorldClim" database includes 19 climatic variables, of which mean annual temperature (MAT) and total annual precipitation (MAP) were used because they are readily interpretable and uncorrelated variables (Toivonen et al., 2014). Statistical analysis was carried out with the following software: STATA 12, R 3.2.3., MINITAB 17 and Microsoft EXCEL 2010.

RESULTS

Morpho-anatomical features of leaves and interspecific variability of *Vaccinium* species

In all studied species the leaves are considered as typically bifacial. Both adaxial and abaxial leaf sides of the studied species are covered with a single-layered epidermis and cuticle. The stomata are randomly distributed and visible only on the lower, abaxial side of the leaf (Fig. 1).

Our results indicate that in Serbian populations, the mean values of morphological traits of *V. myrtillus* leaves ranged as follows: LA from 85.3 to 155.9 mm^2, LL from 13.6 to 17.3 mm, LW from 7.1 to 11.3 mm and L/W from 1.4 to 2.3. In *V. uliginosum* populations, LA ranged from 83.0 to 99.9 mm^2, LL from 12.1 to 15.2 mm, LW from 7.0 to 7.9 mm and L/W from 1.6 to 2.2. For *V. vitis-idaea* the following ranges were recorded: LA from 92.8 mm^2 to 118.1 mm^2, LL from 11.3 mm to 13.4 mm, LW from 7.6 mm to 8.3 mm and L/W from 1.4 to 1.8 (Table 2). In *V. myrtillus* populations mean values of anatomical leaf traits were the following: AdC from 1.03 to 1.19 mm, AdE from 12.3 to 14.9 mm, PT from 31.1 to 37.6 mm, ST from 62.0 to 75.1 mm, AbC from 9.5 to 11.7 mm, AbE from 0.87 to 0.96 mm and LT from 118.2 to 137.8 mm. Mean values of anatomical leaf traits of *V. uliginosum* populations were recorded in the following ranges: for AdC from 1.79 to 2.16 mm, for AdE from 14.8 to 17.6 mm, for PT from 49.0 to 51.5 mm, for ST from 104.2 to 106.5 mm, for AbC from 14.8 to 16.4 mm, for AbE from 1.59 to 1.69 mm and for LT from 190.0 to 193.0 mm. In *V. vitis-idaea* populations, AdC ranged from 5.48 to 5.94 mm, AdE from 13.4 to 16.5 mm, PT from 63.2 to 90.7 mm, ST from 216.8 to 253.1 mm, AbC from 11.3 to 13.2 mm, AbE from 2.9 to 3.6 mm and LT from 316.1 to 360.3 mm (Table 2).

In our research, significant differences were determined between populations in all 11 analyzed characteristics for *V. myrtillus*, in 7 traits (AdC, AdE, AbE, LA, LL, LW and L/W) for *V. uliginosum* and in 9 traits (AdE, PT, ST, AbE, AbC, LT, LA, LL and L/W) for *V. vitis-idaea* (Tables 3). Considering our findings, *V. myrtillus* exhibited the highest plasticity in morpho-anatomical leaf traits along the elevation gradient, comparing to two other species. Moreover, according to the analysis (Table 2), *Vaccinium* species were discriminated by the following leaf traits: AdC, PT, ST, AbE and LT. Populations of *V. myrtillus* had significantly lower values of the analyzed features: AdC, PT, ST, AbE and LT, comparing to two other species. The highest values of these traits were recorded for *V. vitis-idaea.* Additionally, AbC was significantly larger for *V. uliginosum* in comparison with two other species.

INTRASPECIFIC VARIABILITY OF *VACCINIUM* SPECIES

Population variability of the studied *Vaccinium* species was explored on the basis of morpho-anatomical leaf traits by Principal Component Analysis (PCA), Cluster Analysis (CA) and Analysis of Variance (ANOVA). The coefficients of variation (V) of the studied leaf traits are shown in Table 2. AdE and AbE were the most varied traits of *V. myrtillus*, while LA, AdE and AbE were the most varied traits of *V.uliginosum*. For the evergreen species *V. vitis-idaea,* PT, AbC and LA had the highest coefficients of variation.

The results of PCA revealed that there is no clear differentiation of populations within all three *Vaccinium* species (Fig. 2). Partial differentiation of Vm3 and Vm5 populations from Vm1, Vm2 and Vm4 populations in relation to the first main axis can be seen on Fig. 2A. The presented PCA result is confirmed by Cluster Analysis (Fig. 3A). Two populations from Mt. Stara Planina (Vm1 and Vm4) together with the population of Mt. Kopaonik (Vm2) formed one group, while the populations Vm3 and Vm5 were clearly separate (Fig. 4A). Principal Component Analysis (PCA) and Cluster Analysis (CA) performed on the basis of the material representing *V. uliginosum* showed no differentiation of the analyzed populations (Fig. 2B, Fig. 3B). According to CA, Vu1 and Vu2 populations are more similar, and Vu3 population is the most distinct (Fig. 3B). On the graphical representation of the first and second principal axes (Fig. 2C), partial differentiation of *V. vitis-idaea* populations can be seen. Cluster Analysis revealed two groups: Vvi1 and Vvi4 populations are more similar (forming one group) and the population Vvi2 and Vvi3 are separated and form another group (Fig. 3C).

ANOVA showed that all 11 studied morpho-anatomical traits clearly differentiated between *V. myrtillus* populations (Table 3). Statistically significant differences between *V. uliginosum* populations were determined for 7 traits: AdC, AdE, AbE, LA, LL, LW and L/W, and between *V. vitis-idaea* populations for 9 traits: AdE, PT, ST, AbE, AbC, LT, LA, LL and L/W (Table 3).

RELATIONSHIP BETWEEN MORPHO-ANATOMICAL LEAF TRAITS OF *VACCINIUM* SPECIES AND CLIMATIC FACTORS

Populations Vm3 and Vm5 that grow at lower altitudes were more similar and they were separated into one group (Table 1, Fig. 2A, Fig. 3A). They were characterized by greater LA and LW, comparing to the populations Vm1, Vm2 and Vm4 that grow at higher altitudes (Table 1). Due to the lower MAT and lower resource availability, populations at higher altitudes are exposed to stressful conditions. For *V. myrtillus*, a positive linear trend was recorded between LA and MAT. In the case of *V. Uliginosum,* it was found that population Vu3 that grows at lower altitudes was separated from populations Vu1 and Vu2 that grow at higher altitudes (Table 1, Fig. 3B). A negative linear trend was found between L/W and MAT, then between AdC and MAP, and positive linear trends between AdE and MAP. Also, a positive trend between AdE and MAP, as well as between AbE and MAP was found in *V. vitis-idaea* populations, AbE and MAP, and PT and MAT.

DISCUSSION

MORPHO-ANATOMICAL LEAF TRAITS OF STUDIED *VACCINIUM* POPULATIONS AND INTERSPECIFIC DIFFERENTIATION

The highest AdC, AbC, PT, ST and LT, were recorded for *V. vitis-idaea* populations. *V. vitis-idaea* was characterized by the highest AdC, PT, ST, AbE and LT, comparing to two other species. This was expected considering that *V. vitis-idaea* is an evergreen species. The evergreen sclerophyllous leaves are characterized as being thicker than mesomorphic leaves, commonly exhibiting smaller intercellular spaces and the area/volume ratio, as well as a strongly developed palisade parenchyma (Larcher, 1995; Morecroft and Woodward, 1996). On the other hand, the smallest AdC, PT, ST, AdE and LT in comparison with two other species were recorded for *V. myrtillus* populations. *V. uliginosum* stands out with larger AbC, comparing to two other species.

INTRASPECIFIC DIFFERENTIATION OF STUDIED *VACCINIUM* POPULATIONS ALONG ENVIRONMENTAL GRADIENT

Numerous studies have shown a positive trend between LA and MAT, as well as between LA and MAP (Cordell et al., 1998; Chabot and Hicks, 1982; Toivonen, 2014). Furthermore, Kofidis et al. (2007) studied the leaf size of *Clinopodium vulgare* and found that the leaves of plants that grew at the lowest altitudes were the tiniest in August and October, compared to those species that grew at higher altitudes. In the case of the studied species of *V. myrtillus*, the length and width of the leaves cannot be related to the change in altitude of the localities from which the plants were sampled. However, in the case of the measured "leaf area" character, it can be stated that individuals from the localities at the lowest altitudes of Divčibare (960 m) and Kamena gora (1226 m) had the highest measured values of this character, compared to plants sampled from localities situated at higher altitudes: Krvave bare (Stara planina - 1820 m), Srebrnac (Kopaonik -1707 m) and Kopren (Stara planina - 1709 m). However, *V. vitis-idaea* populations Vvi3 and Vvi4, which are located at lower altitudes, are not separated in relation to the other two that are found at higher altitudes (Table 1, Fig. 3C). The reason for this may be different response to the elevational gradient between deciduous (*V. myrtillus* and *V. uiginosum*) and evergreen species (*V. vitis-idaea*). According to some authors (Kikuzawa, 1995; Castro-Díez, 1997; Cornelissen, 1999; Körner and Diemer, 1987), evergreen leaves acquire their physical resistance in relation to environmental conditions.

According to some authors (Morecroft and Woodward, 1996; Cordell et al., 1998; Kao and Chang, 2001), very often, a smaller size of leaves of species that grow at higher altitudes is caused by low air temperatures. In the studied *V. myrtillus* populations, the lowest values for the character "leaf area" were found in populations sampled from the localities Kopren (Vm1), Krvave bare (Vm4) and Srebrnac (Vm2), where the mean annual, minimum and maximum temperatures are lower in relation to other localities.

Membrives et al. (2003) found that species that grow in areas with higher mean annual precipitation have longer leaves. The results obtained for *V. myrtillus* show the same trend. The longest leaves in this species were found at the localities of Divčibare and Kamena gora, where the annual amount of precipitation is higher in relation to other analyzed localities. However, this correlation between precipitation and leaf length cannot be commented for the two other species (*V. vitis-idaea* and *V. uliginosum*).

Körner and Diemer (1987) found that plants that grow at higher altitudes have greater LT and PT, compared to plants growing at lower altitudes. In *V. vitis-idaea* populations a positive trend between PT and MAT was found, which means that populations at higher altitudes have lower PT, contrary to the assertion of Körner and Diemer (1987). Development of models that predict the leaf traits along the environmental gradient is a major challenge because numerous factors are involved (abiotic and biotic).

CONCLUSION

This is the first record of morpho-anatomical leaf traits of *Vaccinium* populations on the south-east edge of their geographic distribution. There are discriminative leaf traits among *Vaccinium* species, especially between *V. vitis-idaea* populations and populations of two other species.

This research has shown that there are differences in response of morpho-anatomical leaf traits to the elevation gradient between deciduous species *V. myrtillus* and *V. uliginosum,* and evergreen species *V. vitis-idaea*. Considering our findings, *V. myrtillus* exhibited the highest plasticity in morpho-anatomical leaf traits along the elevational gradient, when compared to two other *Vaccinium* species. Also, it can be concluded that some of the variability of AdC, AdE and AbE in *V. uliginosum* and *V. vitis-idaea* populations can be predicted with MAP.

This might be related with a large distribution area of the species, abundance of its populations, as well as abundance of individuals within the populations. Our findings are relevant for further common garden experiments on the studied localities that will improve breeding efforts for *Vaccinium* species.

AUTHORS’ CONTRIBUTIONS

I.B. and D.S. carried out field investigation. D.O.P. and Z.D.S. supervised the findings of this work. I.B. wrote the manuscript with support from D.O.P., Z.D.S., V.R., M.M., V.R. and S.B. performed statistical analysis of data. All authors discussed the results and contributed to the final manuscript.

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TABLE 1. Location, climate and habitat data on the studied populations of the genus *Vaccinium*.

Population	Number of individuals per population	Number of leaves per individual	Localities	Altitude [m.a.s.l.]	GPS data	MAT[a] [ºC]	Tmin[a] [ºC]	Tmax[a] [ºC]	MAP[a] [mm]
Vm1, Vvi3	40	10	Kopren (Stara planina)	1709	N 43°19´56.88˝ E 22°47´48.43˝	3,2	-9.0	17,1	806
Vm2, Vu2	40	10	Srebrnac (Kopaonik)	1707	N 43°18´59.04˝ E 20°50´06.19˝	3,7	-8,5	17,4	978
Vm3	40	10	Kamena gora	1228	N 43°17´15.01˝ E 19°33´53.88˝	6,7	-6,6	21,0	1095
Vm4, Vu1, Vvi1	40	10	Krvave bare (Stara planina)	1820	N 43°23´07.05˝ E 22°44´18.18˝	2,4	-9,7	15,9	846
Vm5	40	10	Divčibare	960	N 44°07´49.12˝ E 20°00´55.42˝	7,4	-6,1	21,6	889
Vu3, Vvi4	40	10	Babin zub (Stara planina)	1568	N 43°22´42.88˝ E 22°37´37.09˝	4,1	-8,4	18,1	787
Vvi2	40	10	Pajino preslo (Kopaonik)	1817	N 43°16´36.61˝ E 20°49´20.23˝	3,2	-8,9	16,7	1002

Vm – *Vaccinium myrtillus,* Vu – *V. uliginosum,* Vvi – *V. vitis – idaea,* MAT – mean annual temperature; Tmin – minimum annual temperature; Tmax – maximum annual temperature**;** MAP – mean annual precipitation, [a] Data obtained from „WorldClim“ database at a spatial resolution of 30 arc s (Hijmans *et al.*, 2005).

TABLE 2. Average values of the examined leaf characteristics of *Vaccinium* species. The coefficient of variation is given in parentheses.

Population	AdC (µm)	AdE (µm)	PT (µm)	ST (µm)	AbC (µm)	AbE (µm)	LT (µm)	LA (mm^2)	LL (mm)	LW (mm)	L/W
Vm1	1,05 ^{f} (14,6)	12,3 ^{d} (22,3)	37,6 ^{d} (19,3)	75,1 ^{d} (21,2)	10,8 ^{c} (13,3)	0,93 ^{de} (27,3)	137,8 ^{e} (14,4)	95,7 ^{cde} (24,2)	14,6 ^{d} (7,2)	8,4 ^{b} (13,8)	1,8 ^{d} (15,0)
Vm2	1,19 ^{e} (18,1)	14,8 ^{bc} (20,2)	34,4 ^{de} (20,9)	71,4 ^{de} (21,3)	11,9 ^{c} (13,5)	0,96 ^{d} (22,0)	134,6 ^{ef} (14,2)	85,3 ^{f} (22,9)	16,2 ^{b} (12,5)	7,1 ^{ef} (13,4)	2,3 ^{a} (19,4)
Vm3	1,11 ^{ef} (19,3)	13,6 ^{bcd} (24,0)	31,1 ^{f} (18,3)	62,0 ^{f} (24,3)	9,5 ^{e} (17,9)	0,89 ^{e} (24,9)	118,2 ^{g} (13,5)	155,9 ^{a} (20,1)	17,3 ^{a} (13,7)	11,3 ^{a} (11,0)	1,5 ^{efg} (14,9)
Vm4	1,19 ^{e} (28,5)	13,0 ^{cd} (34,3)	33,1 ^{ef} (25,4)	66,1 ^{ef} (22,9)	11,3 ^{cd} (31,3)	0,93 ^{de} (28,6)	125,7 ^{fg} (18,6)	89,8 ^{f} (22,3)	13,6 ^{e} (13,6)	8,3 ^{bc} (14,1)	1,7 ^{de} (22,8)
Vm5	1,03 ^{f} (13,0)	14,9 ^{b} (26,1)	32,3 ^{ef} (15,4)	62,1 ^{ef} (23,4)	11,7 ^{cd} (17,1)	0,87 ^{e} (25,6)	123,0 ^{g} (14,8)	149,9 ^{a} (15,2)	15,3 ^{c} (8,8)	11,2 ^{a} (9,7)	1,4 ^{h} (6,1)
Vvi1	5,48 ^{a} (22,9)	13,4 ^{cd} (11,1)	63,2 ^{b} (33,5)	219,6 ^{a} (14,5)	11,5 ^{cd} (30,3)	2,90 ^{b} (19,9)	316,1 ^{c} (13,5)	92,8 ^{de} (23,5)	13,4 ^{e} (13,3)	7,6 ^{de} (13,0)	1,8 ^{d} (9,1)
Vvi2	5,80 ^{a} (18,1)	16,5 ^{a} (20,2)	64,7 ^{b} (20,9)	230,7 ^{a} (21,3)	13,2 ^{b} (13,5)	3,10 ^{ab} (22,0)	334,1 ^{bc} (14,2)	118,1 ^{b} (22,9)	11,6 ^{f} (12,5)	7,9 ^{cd} (13,4)	1,5 ^{fg} (19,4)
Vvi3	5,83 ^{a} (17,0)	13,9 ^{bc} (15,9)	72,3 ^{b} (43,6)	253,1 ^{a} (14,0)	11,9 ^{cd} (21,2)	3,15 ^{ab} (13,2)	360,3 ^{a} (13,3)	113,8 ^{b} (30,8)	11,3 ^{f} (12,6)	8,3 ^{bc} (18,6)	1,4 ^{gh} (23,7)
Vvi4	5,94 ^{a} (18,5)	13,6 ^{bcd} (19,7)	90,7 ^{a} (25,5)	216,8 ^{b} (14,9)	11,3 ^{cd} (41,7)	3,60 ^{a} (20,6)	342,0 ^{ab} (13,6)	108,2 ^{bc} (29,6)	13,1 ^{e} (10,1)	7,8 ^{cd} (16,4)	1,7 ^{d} (21,9)
Vu1	1,96 ^{c} (18,6)	14,8 ^{bc} (28,5)	50,7 ^{c} (20,6)	106,1 ^{c} (23,8)	14,8 ^{a} (20,9)	1,69 ^{c} (24,3)	190,0 ^{d} (16,0)	85,0 ^{f} (22,9)	15,1 ^{cd} (16,7)	7,0 ^{f} (16,7)	2,2 ^{b} (25,4)
Vu2	1,79 ^{d} (16,6)	17,6 ^{a} (21,3)	49,0 ^{c} (14,4)	106,5 ^{c} (20,9)	16,4 ^{a} (21,0)	1,61 ^{c} (26,5)	193,0 ^{d} (12,6)	99,9 ^{cd} (14,3)	15,2 ^{cd} (16,4)	7,9 ^{cd} (10,8)	1,9 ^{c} (18,8)
Vu3	2,16 ^{b} (20,8)	14,8 ^{bc} (26,0)	51,5 ^{c} (24,1)	104,2 ^{c} (24,2)	15,7 ^{a} (26,2)	1,59 ^{c} (27,3)	190,0 ^{d} (18,4)	83,0 ^{f} (36,0)	12,1 ^{f} (14,5)	7,7 ^{cde} (23,4)	1,6 ^{def} (26,4)

AdC – depth of the adaxial cuticule, AdE – height of the adaxial epidermal cells, PT – thickness of the palisade tissue, ST – thickness of the spongy tissue, AbC – height of the abaxial cuticule, AbE – height of the abaxial epidermal cells, LT – leaf thickness, LA – leaf surface area, LL – leaf length, LW – leaf width, L/W – leaf length to width ratio.

Vm1 – *Vaccinium myrtillus* population from Kopren; Vm2 – *V. myrtillus* population from Srebrnac, Vm3 – *V. myrtillus* population from Kamena gora; Vm4 – *V. myrtillus* population from Krvave bare; Vm5 – *V. myrtillus* population from Divčibare; Vu1 – *V. uliginosum* population from Krvave bare; Vu2 – *V. uliginosum* population from Srebrnac; Vu3 – *V. uliginosum* population from Babin zub; Vvi1 – *V. vitis – idaea* population from Krvave bare; Vvi2– *V. vitis – idaea* population from Pajino preslo; Vvi 3 – *V. vitis – idaea* population from Kopren and Vvi4 – *V. vitis – idaea* population from Babin zub. Significance level: $P<0,05$.

TABLE 3. Results of ANOVA comparing the means of the investigated *Vaccinium* populations from Serbia.

Species	Leaf trait	n	df b.	df w.	F	*p*
V. myrtillus	AdC[a]	200	4	97	4.67	0.0017 **
	AdE[a]	200	4	97	7.48	0.000 ***
	PT[a]	200	4	96	7.08	0.000 ***
	ST[b]	200	4	195	6.65	0.000 ***
	AbE[b]	200	4	195	4.21	0.0027 **
	AbC[b]	200	4	195	3.09	0.017 *
	LT[b]	200	4	195	10.83	0.000 ***
	LA[b]	200	4	195	100.88	0.000 ***
	LL[a]	200	4	96	21.39	0.000 ***
	LW[b]	200	4	195	132.52	0.000 ***
	L/W[a]	200	4	88	93.26	0.000 ***
V. uliginosum	AdC[a]	120	2	73	28.11	0.000 ***
	AdE[a]	120	2	117	7.81	0.001 **
	PT[a]	120	2	75	1.58	0.2122
	ST[b]	120	2	117	0.30	0.7412
	AbE[b]	120	2	117	3.16	0.046 *
	AbC[b]	120	2	117	0.64	0.5315
	LT[b]	120	2	117	0.50	0.6078
	LA[b]	120	2	70	17.10	0.000 ***
	LL[a]	120	2	117	26.84	0.000 ***
	LW[b]	120	2	72	7.26	0.001 **
	L/W[a]	120	2	75	24.84	0.000 ***
V. vitis-idaea	AdC[a]	160	3	83	1.93	0.1311
	AdE[a]	160	3	80	24.52	0.000 ***
	PT[a]	160	3	156	17.87	0.000 ***
	ST[b]	160	3	156	9.95	0.000 ***
	AbE[b]	160	3	84	11.13	0.000 ***
	AbC[b]	160	3	85	4.61	0.005 **
	LT[b]	160	3	156	5.68	0.001 **
	LA[b]	160	3	84	12.79	0.000 ***
	LL[a]	160	3	85	24.36	0.000 ***
	LW[b]	160	3	84	1.59	0.1972
	L/W[a]	160	3	82	22.37	0.000 ***

"a" – features from heterogeneous variance (unequal variances), "b" – features from heterogeneous variance (equal variances), n – number of individuals, df b. – degrees of freedom between groups, df w. – degrees of freedom within groups. Significant differences are indicated: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

FIGURES:

Fig. 1. Transverse sections of leaves of *V. myrtillus* (A), *V. uliginosum* (B) and *V. vitis-idaea* (C) observed by light microscope (40x magnification). AdC - adaxial cuticule, AdE - adaxial epidermal cells, PT - palisade tissue, ST - spongy tissue, AbC - abaxial cuticule, AbE - abaxial epidermal cells, VB – vascular bundle.

Fig. 2. Results of discrimination analysis (PCA) for *V. myrtillus* (A), *V. uliginosum* (B) and *V. vitsi-idaea* (C). Vm1 – *Vaccinium myrtillus* population from Kopren; Vm2 – *V. myrtillus* population from Srebrnac, Vm3 - *V. myrtillus* population from Kamena gora; Vm4 - *V. myrtillus* population from Krvave bare; Vm5 - *V. myrtillus* population from Divčibare; Vu1 – *V. uliginosum* population from Krvave bare; Vu2 - *V. uliginosum* population from Srebrnac; Vu3 - *V. uliginosum* population from Babin zub; Vvi1 – *V. vitis – idaea* population from Krvave bare; Vvi2– *V. vitis – idaea* population from Pajino preslo; Vvi 3 – *V. vitis – idaea* population from Kopren and Vvi4 – *V. vitis – idaea* population from Babin zub.

Fig. 3. Cluster analysis (CD) dendrogram for *V. myrtillus* (A), *V. uliginosum* (B) and *V.vitis-idaea* (C). Vm1 – *Vaccinium myrtillus* population from Kopren; Vm2 – *V. myrtillus* population from Srebrnac, Vm3 - *V. myrtillus* population from Kamena gora; Vm4 - *V. myrtillus* population from Krvave bare; Vm5 - *V. myrtillus* population from Divčibare; Vu1 – *V. uliginosum* population from Krvave bare; Vu2 - *V. uliginosum* population from Srebrnac; Vu3 - *V. uliginosum* population from Babin zub; Vvi1 – *V. vitis – idaea* population from Krvave bare; Vvi2– *V. vitis – idaea* population from Pajino preslo; Vvi 3 – *V. vitis – idaea* population from Kopren and Vvi4 – *V. vitis – idaea* population from Babin zub.

Figure 1

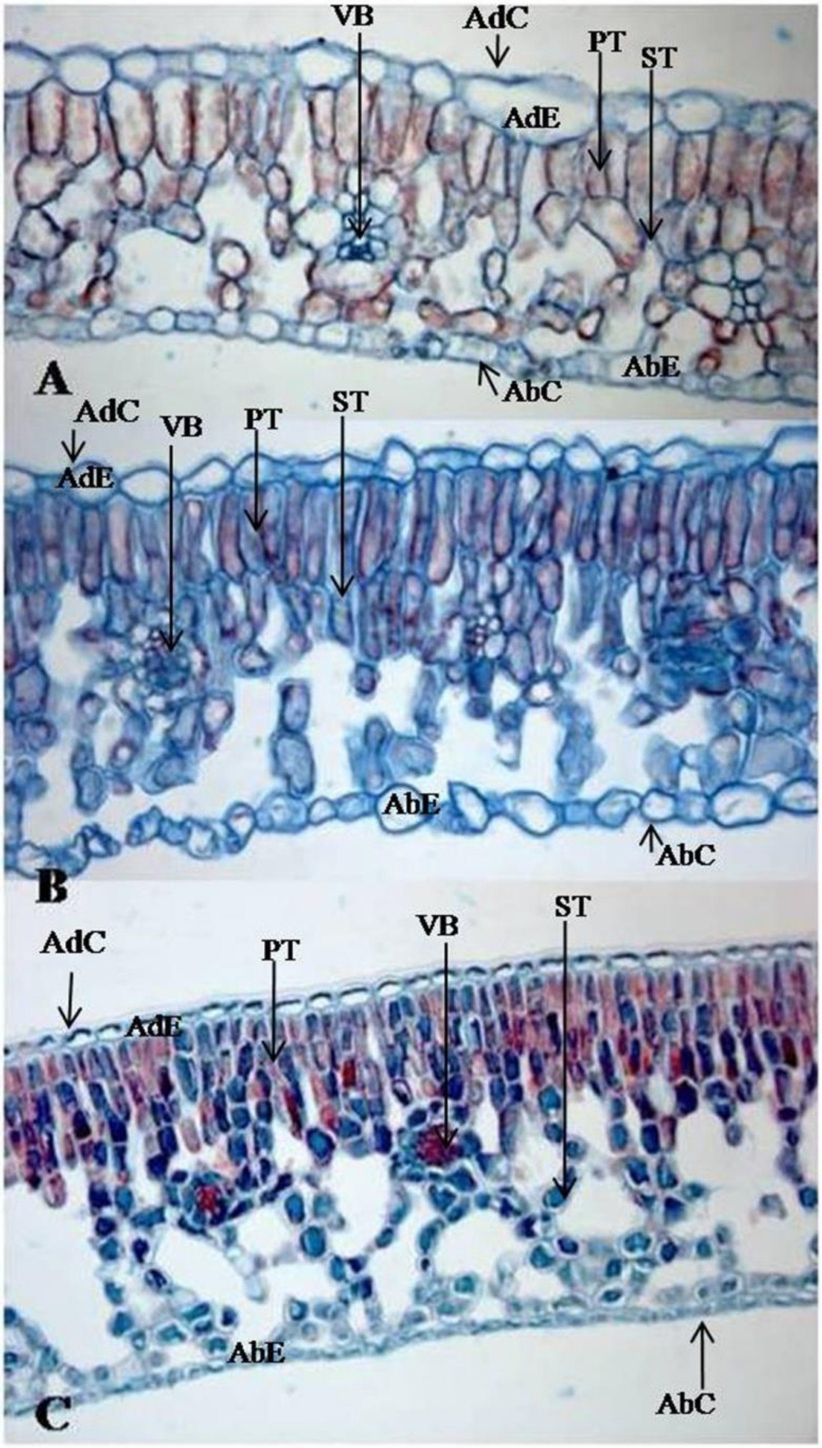

VB
AdC
PT
ST
AdE
A
AbC
AbE
AdC
VB
PT
ST
AdE
AbE
AbC
B
AdC
PT
VB
ST
AdE
AbE
AbC
C

Figure 2

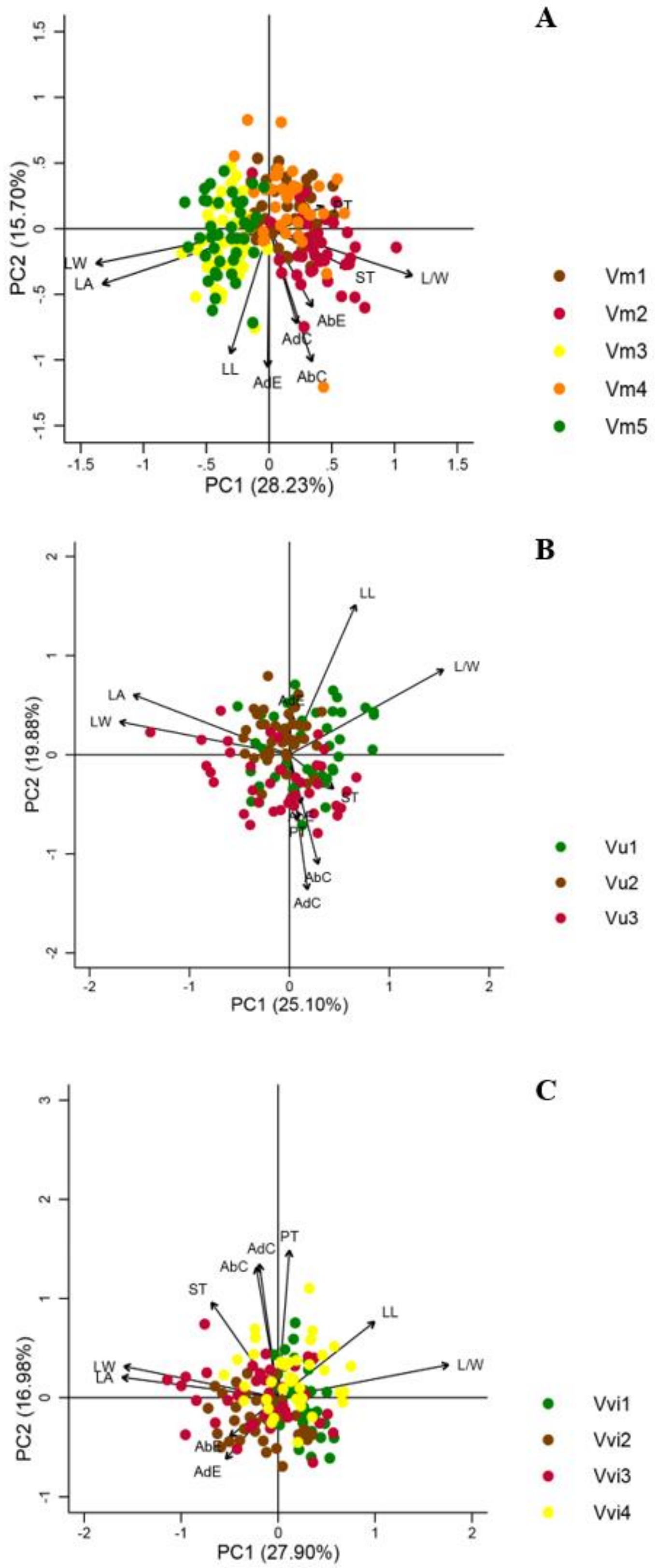
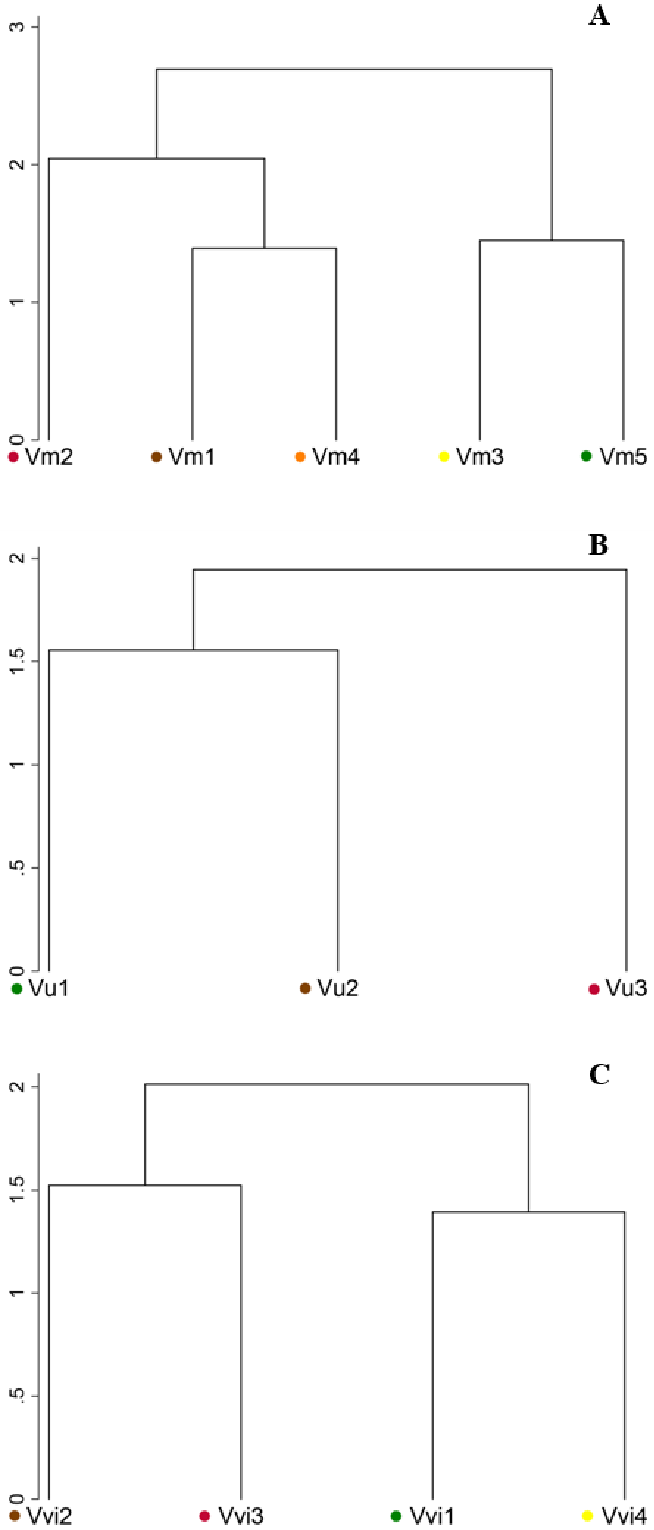

A
PC2 (15.70%)
PC1 (28.23%)
LW
LA
LL
AdE
AbC
AdC
AbE
ST
L/W
PT
Vm1
Vm2
Vm3
Vm4
Vm5
B
PC2 (19.88%)
PC1 (25.10%)
LL
L/W
LA
LW
ST
AbC
AdC
Vu1
Vu2
Vu3
C
PC2 (16.98%)
PC1 (27.90%)
PT
AdC
AbC
ST
LL
L/W
LW
LA
AdE
Vvi1
Vvi2
Vvi3
Vvi4

Figure 3


A
3
2
1
0
Vm2
Vm1
Vm4
Vm3
Vm5
B
2
1.5
1
.5
0
Vu1
Vu2
Vu3
C
2
1.5
1
.5
0
Vvi2
Vvi3
Vvi1
Vvi4