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WILD RASPBERRY FROM ECOLOGICALY SIGNIFICANT AREAS OF SERBIA – PHENOTYPIC VARIABILITY

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ABSTRACT. Inter-population differentiation of the species *Rubus idaeus* was analyzed based on the morpho-anatomical variability of its seven populations from the mountain regions of southwestern and southeastern Serbia. Results of the morphological analysis showed that the highest average height (123.40 cm) had individuals from the P3 study locality on Mt. Goč, while the lowest (77.00 cm) was from Mt. Kopaonik P5 locality. The longest and the widest leaves had representatives of the P1 population (9.25 and 9.20 cm). The smallest leaves had representatives from the Mt. Kopaonik P5 population (length 6,32 cm; width 5.11 cm). The height of the individual is influenced by ecological factors. PCA showed that the Mt. Golija P4 population is distinguished based on all the tested anatomical characters. In these individuals, the higher values for almost all anatomical examined characters were observed. The analysis of bioclimatic data shows that the highest annual amount of precipitation was recorded exactly for this population. It can be assumed that climate factors greatly contributed to shown morpho-anatomical traits. Observed variability could be explained as an adaptive response to different geographical and recent environmental factors.

Keywords: *Rubus idaeus*, morphology, leaf anatomy, bioclimatic data.

INTRODUCTION

Wild raspberry is a member of the Rosaceae family, which has about 3000 species, distributed in about 90 genera (POTTER *et al*., 2007). Based on analyses of six nuclear and four chloroplast genes, POTTER *et al.* 2007 subdivided Rosaceae into three subfamilies: Rosoideae, Dryadoideae and Spiraeoideae. Genus *Rubus* L. belongs to the subfamily Rosoideae (POTTER *et al*., 2007). It is one of the most diverse plant genera in the kingdom of plants and contains

12 subgenera (JENNINGS, 1988) with about 750 species (ROBERTSON, 1974; LU, 1983; GU *et al.*, 1993; THOMPSON, 1995).

There is data that the genus *Rubus* includes several thousand species (JENNINGS, 1988). The largest subgenus is *Idaeobatus* Focke 1910 (raspberries, 117 species), *Malachobatus* Focke 1910 (115, primarily Asian species), and *Rubus* = *Eubatus* Focke 1910 (blackberries – 132 species) (ALICE and CAMPBELL, 1999). Subgenus *Idaeobatus* includes species that are widespread in the Northern Hemisphere, mainly in Asia, Europe, Africa and North America (BALLINGTON *et al*., 1993). The two major subspecies of red raspberries are *Rubus idaeus* Linneus 1753 and *Rubus strigosus* (Michaux) Focke 1896. Raspberries grow in temperate climates and are used in the diet of the local human population. They are naturally growing at high altitudes (above 1000 meters), on wetlands (ÇEKIÇ and ÖZGEN, 2010).

Rubus idaeus is a perennial bush with a height of between 100 cm and 150 cm. The stem is erect, cylindrical, and grayish, with several small thorns on the surface. The leaves are pinnate of 5-7 leaflets or sometimes three, glabrous on the surface and very hairy on the abaxial side. The terminal leaflet is oblong or ovate and shallowly lobed, whereas stipules are fibrous or hairy. The cyme inflorescences are made of flowers that are usually lying down, composed of narrow white, glabrous and whitish petals. The fruit type is an aggregate (as drupetum), pale pink or light orange (TATIĆ, 1972).

There are very little data on morpho-anatomical examinations of leaves of wild raspberry (and ROWSON, 1960), as well as cultivated varieties (FELL and ROWSON, 1960; DONNELLY and VIDAVER, 1984), and the results of our research are even greater significance.

Considering the heterogeneity of *R. idaeus* habitats, the aim of this study was to quantify morphological variation (height and perimeter of a bush) among populations and to describe differentiation among populations based on multivariate statistics on characters related to the leaf morphology and anatomy. Also, relationships between morpho-anatomical and bioclimate variables were performed to evaluate the bioclimatic differentiation between the habitats.

MATERIAL AND METHODS

Plant material and morpho-anatomical analysis

Morpho-anatomical analysis was carried out on plant samples from seven populations collected in the mountain region of Serbia, from June to August 2016. The collected plant material was either placed in a herbarium, or fixed in 50% ethanol and deposited in the Herbarium at the Department of Applied Botany of the Faculty of Agriculture, University of Belgrade.

Morphological analysis (height and perimeter of a bush) was carried out directly, on study localities, while the length and width of the leaves were measured on herbarium samples.

For anatomical research leaf samples were stored in 50% ethanol. From each population, one leaf was taken from each of the 30 individuals. The largest and darkest colored leaves from each individual were selected for cross-transverse sections made by sliding microtome (5–10 μm) and permanent slides were prepared by a standard method for light microscopy (paraffin method) (RUZIN, 1999). Microslides were stained using Alcian Blue and Safranin (1% in 50% ethanol, w/v) for 24h. Image analysis of permanent slides was performed using a LEICA DM2000 microscope equipped with a digital camera LEICA DFC 320 and software IM1000. Observed anatomical parameters include the height of adaxial and abaxial epidermal cells, the total thickness othe f leaf, the thickness of palisade and spongy tissue, the length and width of the main vascular bundles, the length and width of the vascular bundles. The position of measurements was chosen by the method of random selection.

Studied localities

The studied localities belong to mountain regions of Serbia, known as ecologically significant areas (https://www.zzps.rs/wp/ekoloska-mreza/?script=lat), with plant communities rich in wild raspberries. Studied localities were Mt. Studena Planina (P1), Mt. Željin (P2), Mt. Goč (P3), Mt. Golija (P4), Mt. Kopaonik (P5), Mt. Ozren (P6) and Mt. Stara Planina (P7) (Tab. 1, Fig. 1).

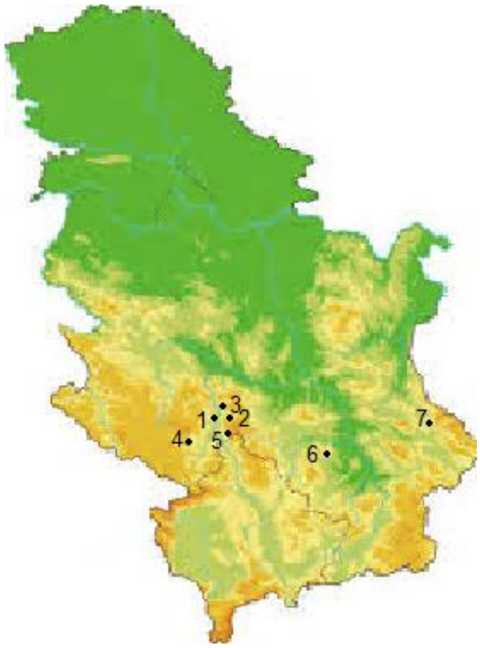

Figure 1. Geographical position of the studied localities: 1 – Mt. Studena Planina, 2 – Mt. Željin, 3 – Mt. Goč, 4 – Mt. Golija, 5 – Mt. Kopaonik, 6 – Mt. Ozren, 7 – Mt. Stara Planina.

Table 1. Geographical data of the studied localities.

Region	Locality	GPS	Elevation (m)	Study localities name
Southwestern Serbia (SWS)	P1	N- 43.51729933 E- 20.64440374	983	Polumir
	P2	N- 43.47037465 E- 20.82764226	1357	Ploča
	P3	N- 43.57179799 E- 20.73005118	675	Brezna
	P4	N- 43.19140735 E- 20.25105463	1432	Sebimilje
	P5	N- 43.18337160 E- 20.49538319	1985	Kopaonik
Southeastern Serbia (SES)	P6	N- 43.36538238 E- 21.53281832	931	Levovik
	P7	N- 43.361622 E- 22. 578614	1710	Balta Berilovac

P1- Mt. Studena Planina, P2- Mt. Željin, P3- Mt. Goč, P4-Mt. Golija, P5- Mt. Kopaonik, P6-Mt. Ozren, P7-Mt. Stara Planina.

The P1 occupies 54.4 km^2 named Mt. Studena Planina, an area confined by the flow of the rivers Ibar, Gokčanica, Rudnjačka Reka and Brezanska Reka. The highest peak of the locality is Kavgalia, at 1355 m a.s.l. (TATIĆ, 1969).

The P2, Mt. Željin, is a mountain in Serbia located between the valley of the river Ibar and the mountains: Kopaonik, Goč and Stolovi. The highest peak of the site is at 1785 m. This is among the water richest mountains in Serbia. The climate is suitable for growing raspberries and blackberries. The local population, besides livestock breeding, is engaged in fruit production (VASOVIĆ, 1988). The peak of Mt. Željin is known as the Landscape of exceptional qualities (Official Gazette of the R. of Serbia, no. 84/2022).

Mt. Goč extends west-east for a length of 10 kilometers. The highest peak is Krnjajela, at 1127 m (http://www.goc.rs/goc). The locality is known for forestry, livestock production, as well as recreation and sports tourism. Part of Mt. Goč (Gvozdac), where research was done, is a Strict Nature Reserve (Official Gazette of the R. of Serbia, no. 99/2014).

Mt. Golija is located between the mountains of Kopaonik and Javor. The highest peak is Jankov Kamen, at a height of 1833 meters. The mountain is built from a mosaic of ecosystems such as forests, meadows and lakes (PAPP and ERZBERGER, 2005). Due to the relic natural deciduous forest of the Balkan maple (*Acer heldreichii*), forming its own plant community, the ass. *Aceri heldreichii–Piceetum abietis* (MIŠIĆ and PANIĆ, 1989), and high presence of other types of deciduous and mixed forests (PANJKOVIĆ *et al.*, 2015), the part of the Mt. Golija of 53804 ha was proclaimed as a Special Nature Reserve and Man and Biosphere UNESCO natural property (Official Gazette of the R. of Serbia, no. 45/2001).

Mt. Kopaonik is a large massif, situated in central Serbia. In 1981 Mt. Kopaonik was proclaimed the National Park (PETROVIĆ *et al.*, 2016). The highest peak is the Pančić Peak at a height of 2017 m. The mountain is known for its very high floristic and vegetation diversity, with 1350 species of vascular plants, out of 91 endemic and 82 sub-endemic species (LAKUŠIĆ, 1995). The mountain is famous for its touristic capacities and especially winter tourism.

Mt. Ozren is a mountain in the vicinity of the small city of Sokobanja, in the eastern part of Serbia. Its highest peak is Leskovik, which is 1174 meters high. The part of the mountain, comprising 828 ha, is proclaimed the Nature Park „Ozrenske livade" (meaning "Ozren meadows") in 1971 (RANĐELOVIĆ and AVRAMOVIĆ, 2004).

Mt. Stara Planina belongs to the northern part of the Balkan Mountain system. In Serbia, the massif extends over the southeastern part of the country. The altitude ranges from 300 to 2168 meters. The highest peak is Midžor at 2169 m. In 1997 the western part of Mt. Stara Planina was enacted for strict protection due to "a natural merit of the first class". Because of its geographic position and paleogeographic history, Mt. Stara Planina represents the first, among six biodiversity hotspots in Europe (JAKŠIĆ, 2008). In the past, the area was known for sheep production and export of a famous sheep hard cheese, but in the last decades agriculture collapsed, and many villages disappeared while the rest is inhabited by a human population older than sixty (PEETERS and DAJIĆ, 2006).

Bioclimatic characteristics of the investigated localities

Data from the WorldClim database (HIJMANS *et al*., 2005; https://www.worldclim.org/data/v1.4/worldclim14.html) were used to show the climatic characteristics of the investigated localities. It is a global climate database, which contains data based on temperature values and rainfall from about 3.500 national meteorological stations that are included in the world network.

Statistical analysis

Statistical analyses were performed with the software packages SPSS for Windows (version 10.0) and STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA). All measurements were carried out on thirty samples per population, and the results are presented as mean. Statistical analysis was performed via descriptive statistics and One-way ANOVA, as well as, multivariate statistical analysis by PCA. To establish relationships between morpho-anatomical and bioclimate variables cluster analysis (UPGMA) was performed to evaluate the bioclimatic differentiation between the habitats of the seven populations. Each location was characterized by 19 bioclimatic parameters, extracted from the WorldClim set of global climate layers. An analysis of variance (K independent sample test) was performed to determine the relationship between the examined anatomical parameters and altitude.

RESULTS AND DISCUSSION

Results of morphological analysis (height and perimeter of a bush, as well as, length and width of the leaves) are presented in Tab. 2.

The highest average height had individuals from the P3 (123.40 cm), while the lowest average height had individuals from the P5 (77.00 cm). The lowest value was less than those quotes by TATIĆ (1972) for *Rubus idaeus*. For example, in localities P5 and P2, measurements were made on individuals that grow surrounded by conifers. On the contrary, individuals whose environment were deciduous representatives such as beech (*Fagus* sp.), and oak (*Quercus* sp.) were higher (P1 and P3). The highest perimeter of the bush was recorded in individuals from the P1 (59.33 cm), while the smallest perimeter had individuals from the P5 locality (29.07 cm).

Mature leaves (those that are the largest and darkest colored) of red raspberry were fivefoliate or sevenfoliate, sometimes trifoliate. Our investigations showed that the longest and the widest leaves had representatives of the P1 population (9.25 and 9.20 cm). The smallest leaves had the representatives from the P5 population (length 6,32 cm; width 5.11 cm). Individual leaflets have sharply pointed apices, with the margin which is coarsely serrate-dentate. The lower surface of the leaves is white (due to the presence of non-glandular trichomes), which is an important taxonomic character that is visible even after drying the leaves. Dried leaves are quite pleasantly aromatic. Similar findings for morphological characters (leaf shape and size) are recorded by FELL and ROWSON (1960).

One way ANOVA showed that there was a statistically significant difference between seven analyzed populations for all morphological characters examined ($p<0.05$).

PCA showed that the P1 population is distinguished based on all the investigated characters (Fig. 2). In these individuals, the longest and widest leaves were recorded, as well as individuals with the largest perimeter.

In Tab. 3 are presented results of descriptive statistics of nine anatomical parameters of wild raspberry leaves. The epidermis of the adaxial and abaxial sides of the leaves was single-layered. The highest epidermal cells (adaxial and abaxial) had the individuals from the P4 population (15.21 and 9.76 µm). Cells' height of the adaxial epidermis was almost twice as high as the abaxial. This population is distinguished by other measured characteristics: it has the thickest leaves (96.46 µm), the palisade and spongy tissue are the thickest in these representatives (41.92 and 33.15 µm), and the leaves of this population have the longest and widest all vascular bundles. The mesophyll is always differentiated into palisade and spongy tissues. The cells of the palisade tissue were placed upright on the surface of the adaxial epidermis. Their shape varies from relatively elongated ellipsoid to cone shapes. Spongy tissue cells are oval to irregular, with less chloroplast and larger intercellular than in palisade tissue (Fig. 10). Thick mesophylls provide the leaf with higher photosynthetic capacity per unit of area, but also enhance the competition among cells for CO_2 and light (THE , 2007). GIVNISH (1979) predicted thick leaves to be selected in environments with high availability of both resources and where their absorption rates by the mesophyll cells were low. This would happen when transpiration is high, and when photosynthesis is low (CASTRO-DIEZ *et al*., 2000). Vascular bundles in mesophyll were collaterally closed. The main vascular bundle was always the largest. Observed anatomical variability represented an adaptive response to different geographical and environmental factors, as suggested also by STEVANOVIĆ and STEVANOVIĆ (1985) for species *Teucrium montanum* or for *Solidago* genus and related Asteraceae species by ANDERSON and CREECH (1975). Fig. 3 shows micrographs of cross-sections of wild raspberry leaves from different localities.

Table 2. Morphometric data of populations of *Rubus idaeus* and descriptive statistics for all measured morphometric characters.

Dimensions (cm)	Population	Mean	Min.	Max.	Std. Dev.	Coef. of Var.	Std. Err.
Height of the bush	**P1**	104.67	40	220	44.62	42.63	8.15
	P2	84.67	40	180	39.54	46.70	7.22
	P3	123.40	60	200	40.74	33.01	7.44
	P4	100.67	40	170	31.18	30.97	5.69
	P5	77.00	50	100	12.36	16.05	2.26
	P6	91.00	40	150	28.06	30.84	5.61
	P7	96.83	40	180	36.04	37.22	6.58
Perimeter of the bush	**P1**	59.33	40	80	12.51	21.08	2.28
	P2	39.17	20	70	12.94	33.03	2.36
	P3	29.97	20	45	6.77	22.61	1.24
	P4	33.67	20	60	10.08	29.94	1.84
	P5	29.07	20	45	5.90	20.28	1.08
	P6	43.40	20	60	10.97	25.27	2.19
	P7	42.17	20	80	13.18	31.25	2.41
Length of leaf	**P1**	9.20	6.42	12.02	1.48	16.05	0.27
	P2	6.34	4.46	10.14	1.28	20.16	0.23
	P3	7.13	4.30	10.36	1.50	20.98	0.27
	P4	7.24	5.24	10.18	1.42	19.60	0.26
	P5	6.32	4.18	8.78	1.15	18.15	0.21
	P6	7.35	5.40	11.52	1.52	20.65	0.30
	P7	7.00	5.50	11.30	1.36	19.39	0.25
Width of leaves	**P1**	9.25	6.54	13.42	1.40	15.10	0.26
	P2	6.92	4.44	10.26	1.54	22.32	0.28
	P3	5.81	4.40	7.64	0.78	13.39	0.14
	P4	5.47	4.16	7.14	0.86	15.72	0.16
	P5	5.11	4.10	6.30	0.63	12.41	0.12
	P6	5.90	4.48	8.14	0.97	16.45	0.19
	P7	5.43	4.40	7.78	0.76	13.95	0.14
Ratio (Length of leaves/width of leaves)	**P1**	1.00	0.81	1.66	0.15	14.86	0.03
	P2	0.93	0.76	1.31	0.13	13.88	0.02
	P3	1.22	0.95	1.60	0.16	13.41	0.03
	P4	1.33	0.99	1.71	0.18	13.89	0.03
	P5	1.23	0.94	1.50	0.14	11.30	0.03
	P6	1.24	0.97	1.62	0.12	9.89	0.02
	P7	1.29	1.09	1.53	0.11	8.20	0.02

Abbreviations: Mean – average value, Min. – minimum, Max. – maximum, Std. Dev. – standard deviation, Coef. of Var. – coefficient of variation, Std. Err. – standard error of the mean.

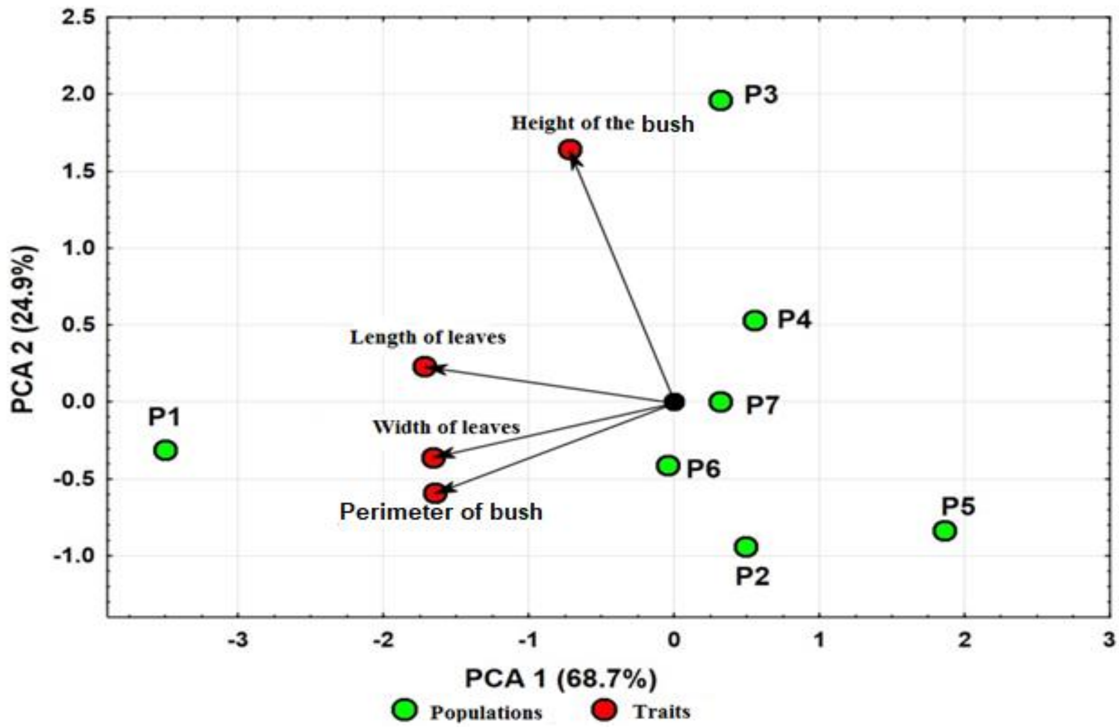

Figure 2. PCA score and loading biplot based on the morphological parameters of wild raspberry leaves from seven populations (P1-7)

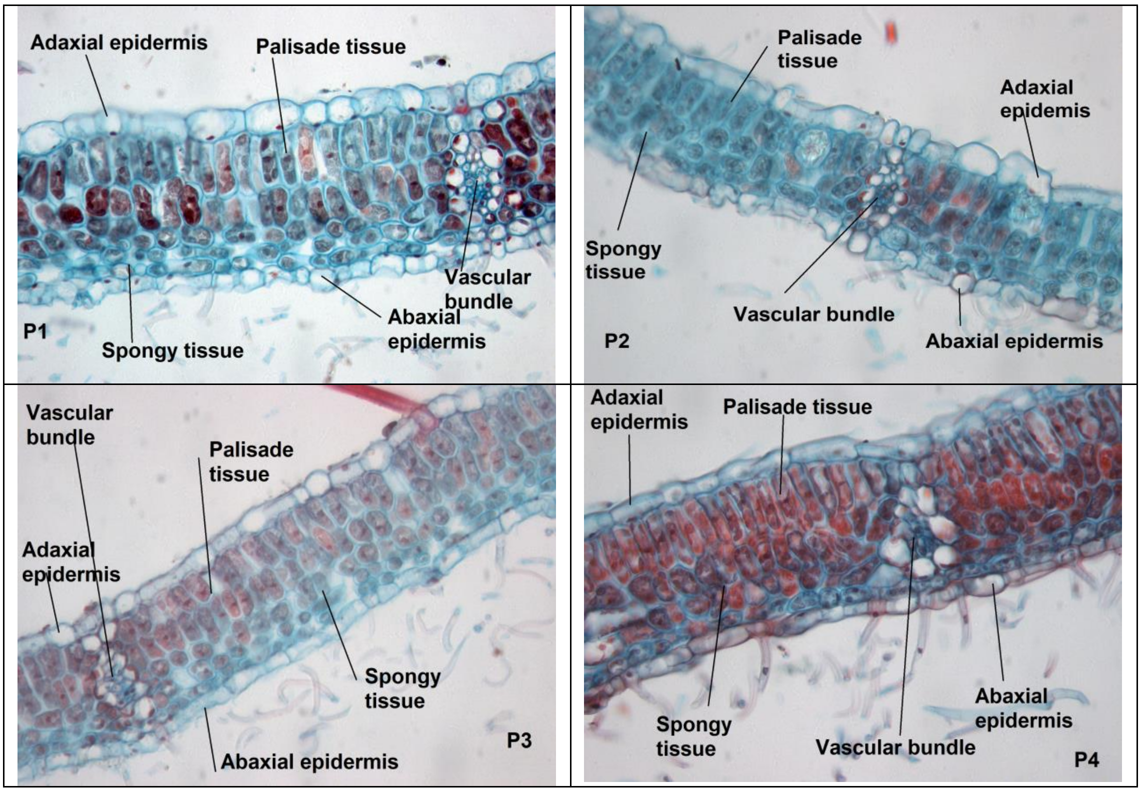

Figure 3. Cross section of wild raspberry leaves from P1, P2, P3 and P4 populations.

Table 3. Data for anatomical analysis of *Rubus idaeus* and descriptive statistics, for all measured morphometric characters.

Traits (μm)	Population	Mean	Min.	Max.	Std. Dev.	Coeff. of Variation	Std. Error
Height of adaxial epidermal cells	P1	12.86	5.80	21.40	3.14	24.42	0.44
	P2	13.04	8.90	19.60	1.94	14.85	0.27
	P3	12.39	7.90	18.20	2.29	18.51	0.32
	P4	15.01	9.10	22.30	3.04	20.24	0.43
	P5	13.64	8.80	20.80	2.42	17.71	0.34
	P6	11.53	6.30	17.00	2.90	25.11	0.41
	P7	12.95	9.40	20.40	2.48	19.13	0.35
Height of abaxial epidermal cells	P1	8.34	4.10	17.60	2.59	31.03	0.37
	P2	7.77	5.20	12.10	1.45	18.61	0.20
	P3	8.68	6.30	11.60	1.44	16.61	0.20
	P4	9.76	6.30	15.40	2.10	21.52	0.30
	P5	7.82	4.70	12.30	1.54	19.74	0.22
	P6	7.75	5.00	11.00	1.37	17.71	0.19
	P7	7.86	4.10	11.90	1.78	22.60	0.25
Thickness of leaf	P1	75.63	47.20	106.90	10.12	13.38	1.43
	P2	83.89	60.70	104.30	11.52	13.73	1.63
	P3	75.16	42.80	96.90	11.85	15.77	1.68
	P4	96.46	56.00	134.90	19.19	19.90	2.71
	P5	85.26	60.10	114.20	14.00	16.42	1.98
	P6	67.73	41.80	95.60	11.72	17.31	1.66
	P7	83.92	53.10	122.00	14.11	16.81	2.00
Thickness of palisade tissue	P1	26.33	12.30	38.40	6.65	25.28	0.94
	P2	34.66	16.40	55.90	11.12	32.08	1.57
	P3	27.21	11.30	49.10	9.37	34.45	1.33
	P4	41.93	19.50	70.10	13.10	31.23	1.85
	P5	34.80	16.70	56.60	11.22	32.24	1.59
	P6	20.12	8.70	34.60	6.42	31.91	0.91
	P7	37.41	13.50	56.60	10.57	28.26	1.50
Thickness of spongy tissue	P1	26.72	8.80	46.50	8.48	31.75	1.20
	P2	27.34	14.50	45.60	7.00	25.62	0.99
	P3	25.50	12.30	38.70	7.34	28.77	1.04
	P4	33.15	17.60	61.60	10.90	32.89	1.54
	P5	27.74	15.70	47.50	7.16	25.82	1.01
	P6	28.03	12.60	43.70	7.67	27.35	1.08
	P7	26.52	14.20	49.70	6.89	25.98	0.97
Length of the main vascular bundles	P1	237.17	164.10	311.90	36.28	15.30	5.13
	P2	351.65	260.10	487.30	54.88	15.61	7.76
	P3	377.82	271.40	479.60	71.77	19.00	10.15
	P4	408.02	254.70	573.30	103.46	25.36	14.63
	P5	354.09	252.50	495.70	54.64	15.43	7.73
	P6	220.21	155.00	285.80	31.14	14.14	4.40
	P7	315.24	209.70	399.70	53.00	16.81	7.50
Width of the main vascular bundles	P1	245.14	136.50	384.30	72.07	29.40	10.19
	P2	389.52	264.50	493.20	70.55	18.11	9.98
	P3	393.07	202.80	491.20	90.23	22.96	12.76
	P4	423.70	265.70	602.20	120.75	28.50	17.08
	P5	393.56	268.90	495.60	70.36	17.88	9.95
	P6	241.15	168.20	332.10	43.19	17.91	6.11
	P7	361.97	237.40	499.70	79.53	21.97	11.25

Table 3. continue

Traits (µm)	Population	Mean	Min.	Max.	Std. Dev.	Coeff. of Variation	Std. Error
Length of the vascular bundles	P1	38.12	25.20	73.10	10.21	26.79	1.44
	P2	39.28	18.70	60.50	10.18	25.91	1.44
	P3	35.64	25.10	60.40	7.10	19.92	1.00
	P4	43.72	25.80	69.80	9.77	22.34	1.38
	P5	39.62	17.30	63.20	10.68	26.95	1.51
	P6	30.92	21.10	51.90	6.24	20.17	0.88
	P7	39.06	25.80	62.40	8.41	21.54	1.19
Width of the vascular bundles	P1	26.54	16.40	45.90	6.91	26.03	0.98
	P2	24.39	14.90	42.20	5.64	23.11	0.80
	P3	21.78	16.10	30.50	3.23	14.81	0.46
	P4	28.60	17.60	44.70	6.07	21.23	0.86
	P5	24.38	15.90	43.10	6.02	24.68	0.85
	P6	20.82	11.00	31.80	4.62	22.20	0.65
	P7	24.76	14.50	43.40	6.11	24.68	0.86

Abbreviations: Mean – average value, Min - minimum, Max - maximum, Std. dev. standard deviation, Std. err. standard error of the mean

Leaf variability in *R. idaeus* can be considered an adaptive advantage of habitats marked by strong variations of sun radiation, air humidity and temperature and wind exposition, which has been proven for other species (SANTIAGO and KIM, 2009).

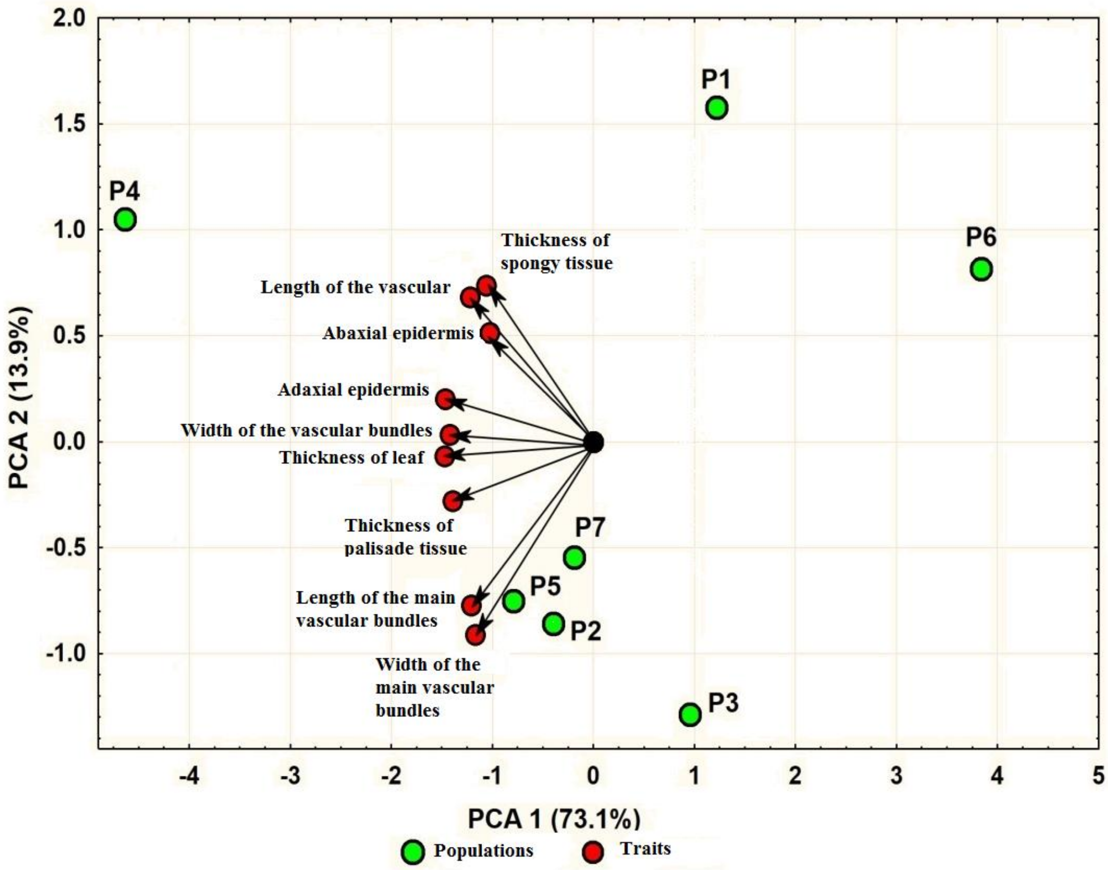

Figure 4. PCA score and loading biplot based on the anatomical parameters of wild raspberry's leaf from seven populations (P1-7).

The influence of the environment (solar radiation, air temperature and humidity) on the anatomical characteristics (foliar architecture, blade and petiole epidermal and internal anatomy) of the leaf is also recorded for the species *Elaeagnus angustifolia*, by KLICH (2000), which is in agreement with our findings. PCA showed that the P4 population is distinguished from all others based on all tested characters (Fig. 4). In these individuals, the highest values for almost all anatomically examined characters were observed. On the other hand, the P6 population is characterized by the thinnest leaves compared to samples from other populations, as well as the smallest values of other tested parameters. This result can be explained by the fact that P6 population grows in darker places, surrounded by beech forests, with increased air humidity.

Bioclimatic data

By analyzing the climatic parameters for the fifty years (1950-2000) shown in Table 4, the average annual temperature (BIO1) in the studied localities ranges from 4.6°C (P7) to 9.8°C (P5). The widest temperature range is characteristic for the population P5 (29.9°C), while the narrowest range is recorded in the population P7 (26.9°C). The mean temperature of the wettest quarter (BIO8) ranges from 5.3°C (P5) to 10°C (P3), and the mean temperature of the driest quarter (BIO9) ranges from -4.0°C to 0.1°C (P5). The highest annual rainfall (BIO12) is characteristic for population P4 (1005 mm), while the lowest rainfall in the observed period was for population P6 (751 mm). Precipitation of the wettest quarter (BIO16) is fairly uniform and varies from 238 mm (P6) to 285 mm (P2), while the rainfall of the driest quarter (BIO17) has a slightly larger amplitude, from 156 mm (P6) to 221 mm (P4). The average amount of precipitation in the warmest quarter (BIO18) is the lowest population P6 (209 mm), while the highest is in the population P3 (255 mm). The lowest precipitation during the coldest quarter (BIO19) was recorded in population P6 (167 mm), while the highest amount was characteristic of population P4 (237 mm).

The analysis of bioclimatic data shows that the highest annual amount of precipitation was recorded for population P4, while on the other side the lowest annual amount of precipitation was recorded in population P6 (Tab. 4). It can be assumed that climate factors (primarily the amount of precipitation) have greatly contributed to such results, which can be seen in Fig. 5. The variability of morphoanatomical characteristics of wild raspberry leaves may indicate adaptation to environmental factors, especially solar radiation, humidity and temperature, as well as exposure to wind. The influence of external factors on the anatomical characteristics of the leaves of other plant species from the area of Serbia was also noted, which is in accordance with the presented results. VASIĆ and DUBAK (2012) showed changes in the anatomical characteristics of leaves from different altitudes and different habitat conditions. Since leaf samples of wild raspberries were taken from different altitudes, we can conclude that altitude had a significant impact on the variation of anatomical characteristics of raspberry leaves as in juniper. It can also be assumed that the type of substrate significantly influenced the obtained results, considering that the populations in SWS are mainly found on serpentinites, while in SES limestone dominates. Thus, KUZMANOVIĆ *et al*. (2012) showed that the geological background significantly affects the anatomy of the leaf.

Table 4. Bioclimatic parameters of the investigated localities.

Climatic parameters	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**
Mean annual temperature (BIO1) (°C)	7.5	6.0	8.7	6.7	9.8	7.5	4.6
Range of mean monthly temperatures (BIO2) (°C)	8.7	8.2	9.3	8.4	9.5	8.9	8.3
Isothermality (BIO2/BIO7) (* 100) (BIO3) (%)	30	29	31	30	31	31	30
Temperature seasonality (standard deviation * 100) (BIO4)	6945	6780	7105	6747	7190	6985	6632
Maximum temperature of the warmest month (BIO5) (°C)	22.3	20.4	24.0	21.1	25.5	22.7	18.8
Minimum temperature of the coldest month (BIO6) (°C)	-6.1	-7.0	-5.3	-6.5	-4.4	-5.9	-8.1
Annual temperature range (BIO5-BIO6) (BIO7) (°C)	28.4	27.4	29.3	27.6	29.9	28.6	26.9
Mean temperature of the wettest quarter (BIO8) (°C)	14.3	12.6	15.7	10.0	5.3	14.3	11.1
Mean temperature of the driest quarter (BIO9) (°C)	-0.4	-1.8	-0.8	-2.3	0.1	-1.8	-4.0
Mean temperature of the warmest quarter (BIO10) (°C)	16.1	14.4	17.4	15.0	18.6	16.1	12.8
Mean temperature of the coldest quarter (BIO11) (°C)	-1.8	-3.0	-0.8	-2.3	0.1	-1.8	-4.0
Annual precipitation (BIO12) (mm)	884	905	838	1005	907	751	764
Precipitation of the wettest month (BIO13) (mm)	100	102	96	104	94	87	92
Precipitation of the driest month (BIO14) (mm)	56	58	52	69	63	48	48
Seasonality of precipitation (coefficient of variation) (BIO15)	17	17	18	12	13	18	21
Precipitation of the wettest quarter (BIO16) (mm)	277	285	266	279	252	238	252
Precipitation of the driest quarter (BIO17) (mm)	180	188	168	221	200	156	158
Precipitation of the warmest quarter (BIO18) (mm)	247	255	237	248	217	209	225
Precipitation of the coldest quarter (BIO19) (mm)	192	199	178	237	216	167	173

P1 – Mt. Studena Planina, P2 – Mt. Željin, P3 – Mt. Goč, P4 – Mt. Golija, P5 – Mt. Kopaonik, P6 – Mt. Ozren, P7 – Mt. Stara Planina.

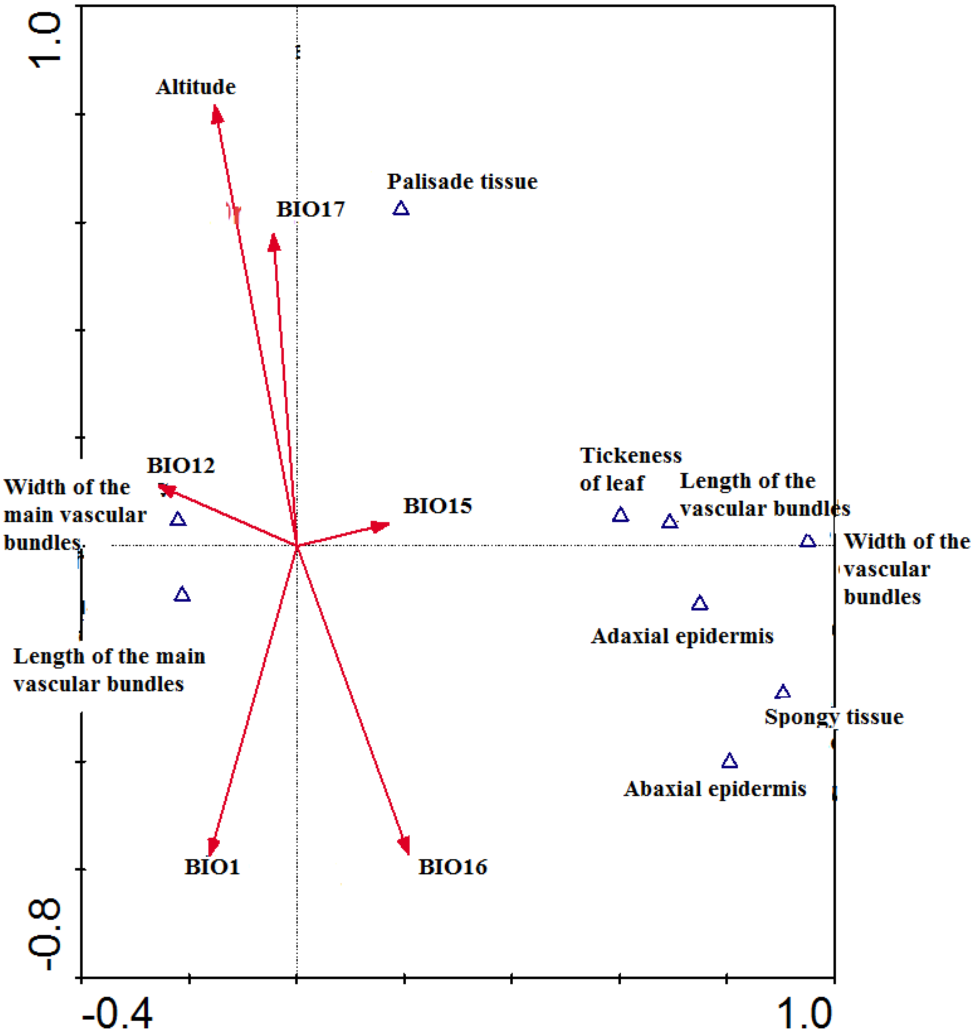

Figure 5. Correlation of bioclimatic and tested morpho-anatomical parameters.

To determine the relationship between the examined anatomical parameters and altitude, an analysis of variance (K independent sample test) was performed. Based on the obtained results (Tab. 4), we can conclude that the altitude is statistically significant for most of the analyzed anatomical parameters (eg. leaf thickness, the height of palisade tissue, length, and width of vascular bundles).

Table 4. Statistical dependence of *R. idaeus* L. anatomical parameters and altitude.

	***p*-value**
Height of adaxial epidermis	**0.000**
Height of abaxial epidermis	1.000
Thickeness of leaf	**0.000**
Thickenes of palisade tissue	**0.000**
Thickenes of spongy tissue	0.092
Length of the main vascular bundles	**0.012**
Width of the main vascular bundles	**0.001**
Length of the vascular bundles	**0.000**
Width of the vascular bundles	**0.012**

K independent sample test**,** Correlation is significant at the 0.05 level

The influence of altitude on anatomical characteristics has been confirmed before, for many different types of plants. GONUZ and ÖZORGUCU (1999) found a significant influence of altitude on anatomical and morphological parameters (plant height, aerial fresh and dry weights) of *Nepeta binaludensis* Jamzad. Similar to the obtained results, ZARINKAMAR *et al.* (2011) found statistically significant differences in the anatomical characteristics of the same species (*Crocus sativus*) from different altitudes, given the clear links between a certain altitude and climatic parameters.

The results of our research performed in the region of Serbia indicated that variation of morpho anatomical characteristics is correlated with the high ecological plasticity of wild raspberry populations.

CONCLUSION

Based on the obtained results, we can conclude that habitat conditions as well as the surrounding types of forest significantly affect the differences in the tested morpho anatomical characteristics of the leaves. There is a difference between populations surrounded by conifers and populations surrounded by deciduous forests. Also, climatic factors (primarily the amount of precipitation) are a very important factor that has determined the difference between populations. Therefore, it is assumed that the obtained differences are a response to different environmental conditions.

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