



Leaf anatomy of the *Sesleria rigida* Heuffel ex Reichenb. (*Poaceae*) in Serbia

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ABSTRACT A detail description of the total variability of the characters of the leaf anatomy in different populations of taxon *S. rigida sensu lato* on the territory of Serbia was made. Morphometric analyses were performed on the cross-section of 521 tiller leaves collected from 21 populations of *S. rigida* in Serbia. Statistical data analyses were calculated for 27 morphometric characters. Descriptive statistics (mean, standard deviation, minimum, maximum and standard error, coefficient of variation) were calculated for each character state and correlative variability and variations in regard to the geographical gradients was performed to identify the trends in anatomical differentiation. Analysis of variance (ANOVA) was performed to identify significant variation between each character.

A detail analysis has shown that local populations are anatomically very well differentiated into the serpentine populations from western and carbonate populations from eastern Serbia.

KEY WORDS: *Poaceae*, *Sesleria rigida*, leaf-anatomy

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INTRODUCTION

The genus *Sesleria* Scop. (*Poideae*, *Sesleriae*) is an arcto-tertiary genus (ENGLER, 1879; MAY, 1991) that includes 27 species (DEYL, 1980) and builds a complicated group of similar and closely related taxa distributed mostly around Europe. The area of this genus is extended to the east to Caucasus, Lyban and Persia, to the north to Iceland, southern Scandinavia and Sankt-Petersburg, to the west to Spain and to the south to Morocco and Libya (MEUSEL *et al.*, 1965, DEYL, 1946). The Alps are considered to be the primary center of its development, while the Balkan Peninsula is considered as a secondary center of the evolution of this genus (DEYL, 1946). Recently the some group of species was the subject of formal anatomical studies which brought some new insights into taxonomy and systematics within the genus *Sesleria* (LYSÁK *et al.* 1997; DI PIETRO, 2007; ALEGRO, 2007).

The species *S. rigida* belongs to the section *Calcariae*, sub-section *Rigida*, whose center of origin is very probably located in the western part of the Balkan Peninsula (DEYL, 1946). In a wider sense, the taxon *S. rigida* is a Carpatho-Balkan's floristic element distributed in Romania, Hungary, Bulgaria, Serbia, Bosnia and Croatia (DEYL, 1946; TATIĆ, 1976; SEKULIĆ *et al.*, 1988). It grows in all the altitudinal zones, from lowlands to the Alpine regions, mostly on carbonates, but also on serpentine. The taxa *S. rigida* var. *degenii* Deyl, *S. rigida* var. *pancicii* Deyl, *S. rigida* subsp. *achtarovii* (Deyl) Deyl, *S. filifolia* Hoppe and *S. serbica* (Adam.) Ujhelyi are related to a widely considered species *S. rigida*. Their taxonomic and geographical characteristics are not clear enough, so much so that in the modern floristic literature they are neglected or almost exclusively considered as a synonyms or infraspecific taxa of the species *S. rigida* (DEYL 1946, 1980; DIKLIĆ & NIKOLIĆ, 1986; TATIĆ, 1976). Extremely rarely, the opinions may be

found in the modern botanical literature that the serpentine populations from western Serbia, Bosnia and Croatia represent “a good species” *S. serbica* as it was proposed by Ujhelyi (STEVANOVIĆ *et al.*, 1995; STEVANOVIĆ *et al.*, 2003; SEKULIĆ *et al.*, 1988).

As in case of many other grasses, in case of genus *Sesleria* also, the characters of the outside morphology of the vegetative and reproductive organs, due to their conservatism, are not sufficient for establishing satisfactory taxonomy at lower taxonomical level. In regard to that, today is generally accepted that the anatomical details, especially the ones referring to the leaf and embryo, when being used together, with a wide spectrum of other diagnostic characters, are indispensable for any satisfactory taxonomy of the grasses (ELLIS, 1976). In some genera (e.g., *Festuca*) the anatomical structure of the leaves is rather successfully employed for a relatively easy differentiation of a very closely related species. Since within the genus *Sesleria* there are also some very similar species, which based on their outside morphology of the vegetative and reproductive organs may be very difficultly differentiated, some authors have tried to differ them by analyzing the anatomy of their leaves (*S. calcaria* Opiz., *S. uliginosa* Opiz. – UJHELYI, 1938; *S. sadleriana* Janka, *S. varia* (Jacq.) Wettst. – UJHELYI AND FELFOLDY, 1948; *S. calcaria*, *S. coerulans* Friv., *S. uliginosa* – KOLÁŘ, 1930; *Sesleria calcaria*, *Sesleria tenuifolia* Schrad., *S. kalnikensis* Jáv., *S. sadlerana* Janka – STRGAR, 1966, 1980, 1985; *S. juncifolia* Suffren, *S. apennina* Ujhelyi, *S. calabrica* (Deyl) Di Pietro – DI PIETRO, 2007; *S. juncifolia*, *S. interrupta* Vis., *S. kalnikensis*, *S. ujhelyii* Strgar – ALEGRO, 2007). The results of these authors have generally pointed out the fact that in the related species of the genus *Sesleria* the characters of the leaf anatomy vary with gradual transitions and therefore it is very difficult to establish any significant qualitative difference of a higher diagnostic importance. Nevertheless, on the basis of the leaf anatomy, it was possible to separate several related taxa (*S. kalnikensis* vs. *S. tenuifolia*, *S. juncifolia* vs. *S. interrupta*, *S. juncifolia* vs. *S. apennina* vs. *S. calabrica*).

The aim of this study was, on the basis of formal morphometric research, to describe variability of anatomical structure of the leaves of the *S. rigida sensu lato* and to establish the existing trends in differentiation of its populations in the region of Serbia.

MATERIAL AND METHODS

Study area and plant sampling. Twenty one populations of *S. rigida* (521 individuals) were sampled for anatomical analysis. The samples were taken from two regions of Serbia: Western (9 population, 232 individuals) and Eastern Serbia (12 population, 289 individuals) (Table 1, Fig. 1).

The collected plant material was either dried out or fixed in 50% ethyl–alcohol solution and deposited in the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, Faculty of Biology, University of Belgrade (BEOU).

The studied region covers the northern-central location in the Balkan Peninsula. It belongs to the so-called „Balkan Serbia“, extended to the south of Sava and Danube rivers. The eastern borders of the studied region are composed of the mountains of the Carpatho-Balkan and the Rhodope’s systems, the western ones of the river Drina, and southern ones of the Kopaonik and Suva planina mts. (Fig 1.). In the most general orographic and geographical sense, the studied region covers some parts of the hilly region of the peri-Pannonian Serbia (Podrinje, Šumadija) and the mountain region within the Carpatho-Balkan, Rhodope and Dinaric mountain systems. In the altitudinal sense the studied region covers the space in between 100 and 1900 m.a.s. These basic geographical, but at the same time also ecological and regional units, have defined some important differences, both in the essential recent abiogenetic and historical-biogeographical characteristics of the habitats of the studied populations.

Depending on the geographical position, the studied populations grow in a very different climatic conditions (WALTER & LEITH, 1964; HORVAT *et al.*, 1974). Therefore, the populations in the hilly regions of western Serbia are influenced by the humid Atlantic climate which, via western parts of the Balkan Peninsula, particularly via Dinaric mountains, reach the western parts of Serbia. In this region there is a specific sub-type of the moderately-continental humid climate (type 2.1 sensu STEVANOVIĆ AND STEVANOVIĆ, 1995; VI 2b sensu WALTER AND LEITH, 1964), characterized by a relatively high quantity of precipitations in the course of the year (720 to 900 mm) and absence of a dry and semi-dry periods (occasionally, there are months with lower or higher number of semi-dry days). The moderately continental humid climate in western Serbia is also significantly influenced by the mountains, therefore it may be described as a transitional variety in between a moderately-continental and mountain climate of the Middle-European type (type 2.1/4.1 sensu STEVANOVIĆ & STEVANOVIĆ, 1995; VI 2b/X 1 sensu WALTER & LEITH, 1964). Contrary to the western populations, the ones that occur in eastern parts of Serbia are influenced by the continental climate from the east and Aegean variety of the Mediterranean climate from the south. This region is dominated by the sub-type of the semi-arid moderately-continental climate, also known as a sub-continental climate (type 2.2 sensu STEVANOVIĆ & STEVANOVIĆ, 1995; VI 3 sensu WALTER & LEITH, 1964). The characteristics of this sub-type of climate are relatively cold and moderately humid winters, while the summers

Table 1. Populations and number of individuals of *Sesleria rigida* Heuff. *sensu lato* in Serbia, used in this study. Vouchers are deposited in the herbarium of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOU).

| Group | Population | Locality | Coordinate | Altitude | Habitat | Substratum | Voucher | Individuals |
|--------|------------|------------------------------------|----------------------|----------|--|--------------|---------------------------------------|-------------|
| Gr_I | E1 | Derdap's gorge, Golubac | 44.6497 N, 21.6642 E | 150 | rocky crevices (Asplenieta trichomanes) | limestone | Kuzmanović, N. 28738 | 27 |
| | E2 | Canyon of Lazareva reka, Vidikovac | 44.0285 N, 21.9533 E | 450 | rocky grounds (Festuco-Brometea) | limestone | Lakušić, D., Kuzmanović, N. 27087 | 32 |
| | E3 | Canyon of Lazareva reka, Kotlovi | 44.0265 N, 21.9529 E | 300 | rocky crevices (Asplenieta trichomanes) | limestone | Lakušić, D., Kuzmanović, N. 27099 | 10 |
| | E4 | Canyon of Lazareva reka, entrance | 44.0235 N, 21.9492 E | 350 | rocky crevices (Asplenieta trichomanes) | limestone | Lakušić, D., Kuzmanović, N. 27098 | 11 |
| | E5 | Jelašnica's gorge | 43.2784 N, 22.0661 E | 350 | rocky crevices (Asplenieta trichomanes) | limestone | Kuzmanović, N. 27444 | 28 |
| Gr_II | E6 | Veliki krš, submit | 44.1711 N, 22.0874 E | 1150 | rocky grounds (Festuco-Brometea) | limestone | Lakušić, D. 27198 | 25 |
| | E7 | Rtanj, northern slopes | 43.7791 N, 21.8928 E | 1400 | rocky crevices (Asplenieta trichomanes) | limestone | Lakušić, D. 27637 | 8 |
| | E8 | Suva planina, Sokolov kamen | 43.2145 N, 22.11 E | 1300 | rocky grounds (Festuco-Brometea) | limestone | Lakušić, D. 27529 | 31 |
| | E9 | Rtanj, Šiljak | 43.7762 N, 21.8933 E | 1550 | subalpine pasture (Festuco-Seslerietea) | limestone | Lakušić, D. 27635 | 32 |
| | E10 | Suva planina, Devojački grob | 43.1882 N, 22.1607 E | 1400 | subalpine pasture (Festuco-Seslerietea) | limestone | Kuzmanović, N. 27441 | 29 |
| | E11 | Suva planina, Sokolov kamen | 43.2103 N, 22.1149 E | 1500 | subalpine pasture (Festuco-Seslerietea) | limestone | Lakušić, D. 27522 | 30 |
| | E12 | Suva planina, Trem | 43.1834 N, 22.1714 E | 1800 | subalpine pasture (Festuco-Seslerietea) | limestone | Kuzmanović, N. 27440 | 26 |
| Gr_III | W1 | Brdjani's gorge | 43.9934 N, 20.421 E | 300 | rocky grounds (Festuco-Brometea) | serpentinite | Kuzmanović, N. 27197 | 25 |
| | W2 | Vujan | 43.9858 N, 20.4478 E | 550 | rocky grounds (Festuco-Brometea) | serpentinite | Lakušić, D. 28744 | 28 |
| | W3 | Maljen, Ljuti krš | 44.1256 N, 19.9981 E | 950 | black pine forests (Seslerio-Pinetum nigrae) | serpentinite | Kuzmanović, N. et al. 27445 | 28 |
| Gr_IV | W4 | Tara planina, Paljevine | 43.8769 N, 19.4168 E | 950 | black pine forests (Erico-Pinetum nigrae) | serpentinite | Lakušić, D. 27106 | 26 |
| | W5 | Mokra gora 1 | 43.8286 N, 19.5268 E | 900 | black pine forests (Seslerio-Pinetum nigrae) | serpentinite | Kuzmanović, N. 28740 | 26 |
| | W6 | Mokra gora 2 | 43.7319 N, 19.6431 E | 800 | black pine forests (Seslerio-Pinetum nigrae) | serpentinite | Kuzmanović, N. 28739 | 26 |
| | W7 | Zlatibor | 43.8142 N, 19.5132 E | 1000 | black pine forests (Seslerio-Pinetum nigrae) | serpentinite | Kuzmanović, N. 28741 | 30 |
| | W8 | Kopaonik, Treska | 43.2604 N, 20.7854 E | 1600 | subalpine pasture (Festuco-Seslerietea) | serpentinite | Jakovljević, K., Kuzmanović, N. 28735 | 28 |
| | W9 | Kopaonik, Nebeske stolice | 43.2605 N, 20.8298 E | 1850 | subalpine pasture (Festuco-Seslerietea) | serpentinite | Lakušić, D. 27617 | 15 |

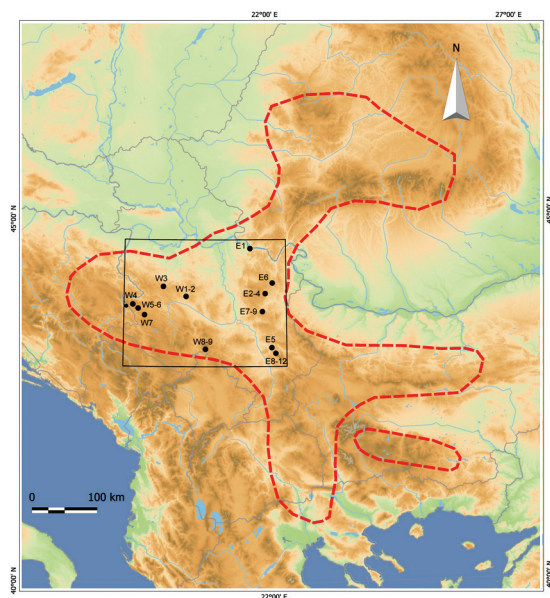


Fig. 1. Distribution of populations of *S. rigida* Heuff. *sensu lato* in Serbia used in this study. Legend: General distribution of *Sesleria rigida* Heuff. according to MEUSEL et al., 1965 (interrupted line), study area (black box) and position of populations used in this study (black dots – for details see Table 1).

are warm and dry (semi-dry). Total annual precipitation is between 620 and 760 mm, with its optimum in May and June. The dry period is absent, while the semi-dry one lasts two to three months. Due to a mostly mountain relief of the studied region, the basic types of climates, dominating in the hilly region, significantly change along the altitudinal gradient, so much so that in the mountain-high mountain regions we may talk about an individual mountain climate, which is, due to geographical position of the mountain, differentiated in two basic types. A humid mountain climate of the Alpine type (type 4.1 sensu STEVANOVIĆ & STEVANOVIĆ 1995; X 1 sensu WALTER & LEITH, 1964) is characteristic for the mountains of western Serbia with total precipitation in between 1100 and 2000 mm per year, and the continental mountain climate for the eastern parts of Serbia (type 4.2 sensu STEVANOVIĆ & STEVANOVIĆ, 1995; X 1 sensu WALTER & LEITH, 1964), with the annual precipitation between 850 and 1400 mm of water deposits.

In geological sense the analyzed populations are clearly differentiated in the western ones, growing on the ultra-basic silicates (peridotite, serpentine) and the eastern ones which grow exclusively on the basic carbonate rocks (limestone, dolomites).

Finally, different populations inhabit the spaces of different biogeographical units. The basic biogeographical differentiation of the analyzed populations can be observed in the vertical profile. Therefore, the hilly-

mountain populations are located on the territory of the Middle-European biogeographical region, while the high-mountain populations make part of the territory of the Middle-South-European mountain biogeographical region. In addition, the hilly-mountain populations make part of the territories of the Illyrian (western Serbia) and Western-Moesian (eastern Serbia) biogeographical provinces, while the high-mountain populations make part of the territories of the Dinnaric (western Serbia) and Balkan (eastern Serbia) biogeographical provinces (STEVANOVIĆ, 1995).

Leaf cross-section. Anatomical analyses of the leaves were done on the permanent hand made slides, prepared by the standard method for the light microscopy. Cross-sections of the tiller leaves were cleared in Parazone and thoroughly washed before staining in safranin (1 % w/v in 50 % ethanol) and alcian blue (1 % w/v, aqueous).

Morphometric analysis. The measurements were performed on the cross-section of 521 tiller leaves (each obtained from different individuals) collected in the field. 27 characters (Fig. 2) of the statistical analysis were grouped in two categories: I. metric characters (22) and II. ordinal characters (5).

I. Metric characters: 1. Width of the tiller leaf blades (TL_W), 2. Thickness of the tiller leaf blades in zone of the central rib (TL_To), 3. Largest thickness of the tiller leaf blades (TL_T1), 4. Distance between the middle and largest leaf blade thickness point of tiller leaf (TL_T2), 5. Width of the central rib of tiller leaf (TL_Rc_W), 6. Height of the trichome of abaxial side of tiller leaf (TL_Trab_H), 7. Height of the trichome of adaxial side of tiller leaf (TL_Trad_H), 8. Height of the central vascular bundle of tiller leaf (TL_VBC_H), 9. Width of the central vascular bundle of tiller leaf (TL_VBC_W), 10. Height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H), 11. Width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W), 12. Height of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScSC_H), 13. Height of the sclerenchyma strand/girder in widest zone of tiller leaf (TL_ScS1_H), 14. Surface of the sclerenchyma of tiller leaf (TL_ScS_Ar), 15. Surface of the tiller leaf blades (TL_B_Ar), 16. Number of the sclerenchyma strands on adaxial side of tiller leaf (TL_ScSad_No), 17. Number of the sclerenchyma strands on abaxial side of tiller leaf (TL_ScSab_No), 18. Number of the sclerenchyma girders on adaxial side of tiller leaf (TL_ScGad_No), 19. Number of the sclerenchyma girders on abaxial side of tiller leaf (TL_ScGab_No), 20. Number of the major vascular bundles of tiller leaf (TL_VB2_No), 21. Number of the minor vascular bundles of tiller leaf (TL_VB3_No), 22. Dimension of the bulliform cells of tiller leaf (TL_BC_H).

II. Ordinal characters: 23. Shape of tiller leaf blade (TL_Sh) (1 – round, 2 – “V” shape, 3 – oblong), 24.

Indumentum of adaxial side of tiller leaf (TL_Ind_ad) (0 – glabrous, 1 – slightly hairy, 2 – hairy, 3 – very hairy), 25. Indumentum of abaxial side of tiller leaf (TL_Ind_ab) (0 – glabrous, 1 – slightly hairy, 2 – hairy, 3 – very hairy), 26. Type of the sclerenchyma on adaxial side of tiller leaf (TL_Sc_ad) (0 – absent, 1 – regularly interrupted, 2 – irregularly interrupted, 3 – continual), 27. Type of the sclerenchyma on abaxial side of tiller leaf (TL_Sc_ab) (0 – absent, 1 – regularly interrupted, 2 – irregularly interrupted, 3 – continual).

Descriptive statistics (mean, standard deviation, minimum, maximum and standard error, coefficient of variation) were calculated for each character state and correlative variability and variations in regard to the geographical gradients was performed to identify the trends in anatomical differentiation. Analysis of variance (ANOVA) was performed to identify significant variation between each character. Statistical analyses were performed using the package Statistica 5.1 (StatSoft 1996).

RESULTS

General characteristics of the leaf anatomy. Based on the measurements of the anatomical characteristics of the cross-sections of the leaves of the sterile rosettes (tiller leaves) of the studied populations of taxon *S. rigida sensu lato*, a detail description was made covering the total variability of populations of this species on the territory of Serbia (Table 2, Fig. 3).

The leaves are in the cross section rolled around the central nerve, and in their form they vary from the oval ones to the ones with an expressive “V” form (Fig. 3). Surface of the leaf blades ranges from 137,32 to 960,33 mm². Width of the leaf blades varies from 0,6 to 1,7 mm. Thickness of the leaf blades in zone of the central nerve varies from 0,152 to 0,329 mm, and the largest thickness from 0,140 to 0,384 mm. On the adaxial side of the leaf there is only a central rib whose width varies from 0,08 to 0,24 mm. The mesophyll is not differentiated to a spongy and palisade tissues. It is built of the chlorenchyma cells which fulfill all the space not covered by sclerenchyma or vascular bundles and their sheath layer. The vascular bundles make just one row and they are located in mesophyll, close to the epidermis of the adaxial side of the leaf. They are different in size, therefore it is easy to differentiate the “major” (big) and “minor” (small) bundles which alternate in a regular way one after the other, going from the central nerve towards the margin of the leaf. All the bundles have elliptic form and are surrounded by one layer of the cells, making the sheath of the vascular bundle. The major vascular bundles have clearly differentiated big tracheas. Number of major vascular bundles varies from 3 to 9. Minor vascular bundles are small, without or with hardly noticeable big tracheas. Number of minor vascular bundles varies from

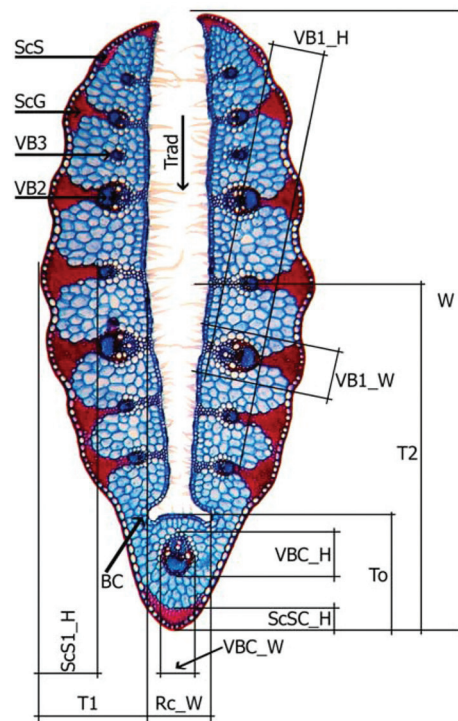


Fig. 2. Tiller leaf cross-section of *S. rigida* in Serbia-measured characteristics.

Legend: BC - bulliform cells; Rc_W - width of the central rib; ScG - sclerenchyma girder; ScS - sclerenchyma strand; ScS1_H - height of the sclerenchyma strand/girder in widest zone of leaf; ScSC_H - height of the sclerenchyma strand of central vascular bundle; T1 - largest thickness of the leaf blade; T2 - distance between the middle and largest leaf blade thickness point; To - thickness of the leaf blades in zone of the central rib; Trad - trichome of adaxial side; VBC_H - height of the central vascular bundle; VBC_W - width of the central vascular bundle; VB1_H - height of the largest lateral vascular bundle; VB1_W - width of the largest lateral vascular bundle; VB2 - major vascular bundle; VB3 - minor vascular bundle; W - width of the leaf blade.

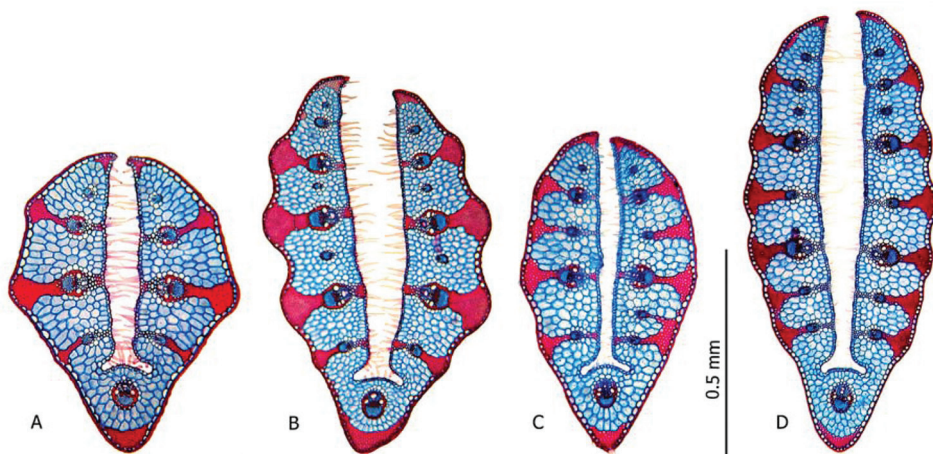
4 to 12. Height of the central vascular bundle varies from 0,053 to 0,126 mm. Width of the central vascular bundle varies from 0,042 to 0,099 mm. Height of the largest lateral vascular bundle varies from 0,052 to 0,138 mm and width of the largest lateral vascular bundle varies from 0,040 to 0,114 mm.

Sclerenchyma is discontinued. It is organized in the form of sclerenchyma girders and strands (Fig. 3). The sclerenchyma girders are, as a rule, located from the adaxial to the abaxial side of the leaf, in connection with the sheath of the vascular bundles, and therefore making a very strong lateral connection between the adaxial and abaxial sides. Occasionally, the sclerenchyma girders do not form a complete lateral connection, but from the adaxial to the

Fig. 3. Tiller leaf cross-section of *S. rigida* Heuff. *sensu lato* in Serbia

Legend:

- A – Đerdap's gorge,
 B – Suva planina, Sokolov kamen,
 C – Brdjani's gorge,
 D – Zlatibor



abaxial side of the leaf they descend only to the vascular bundles. On the contrary, the sclerenchyma strands do not touch the vascular bundles, but in a form of a free and more or less regular formations they are located under the epidermis, exclusively within the zone of the vascular elements and in the margin of the leaf. Occasionally, the sclerenchyma strands reach deeper into the mesophyll, forming stronger structures that do not reach the vascular bundles. The sclerenchyma strands on adaxial side of leaf are generally absent, while on abaxial side of leaf they are always present. Number of the sclerenchyma strands on abaxial side of leaf varies from 1 to 6. Sclerenchyma girders appear both on adaxial and abaxial side of leaf in a similar number. Number of sclerenchyma girders on adaxial side of leaf varies from 5 to 14, and on abaxial side of leaf from 4 to 14. Occasionally, the sclerenchyma strands and girders on abaxial side of the leaf are mutually connected, forming sclerenchyma strands extending in a parallel way with the epidermis of the leaf (Fig. 3). Extremely rarely, the sclerenchyma strands are registered on the adaxial side of the leaf as well. Discontinuation of sclerenchyma can be regular and irregular. The type of discontinuation is defined by a pattern and number of the sclerenchyma girders and strands on the adaxial and abaxial side of leaf along the part of the leaf blade on both sides of the central nerve. Regular discontinuation means equal number of sclerenchyma parts (girders and/or strands) along the part of the leaf blade on both sides of the central vascular bundle. Irregular discontinuation implies unequal number of sclerenchyma parts on both sides of the central vascular bundle and/or mutual connection of the sclerenchyma parts. Height of the sclerenchyma strand of central vascular bundle varies from 0,015 to 0,075 mm. Within the zone of the central vascular bundle the sclerenchyma is organized exclusively in a form of a sclerenchyma strand, located on abaxial side of the leaf. Surface of the sclerenchyma varies

from 13,29 mm² to 152,13 mm². Occasionally, in mesophyll some colorless cells can be observed, within the zone of sclerenchyma girders on the adaxial side of leaf.

On the adaxial side of leaf in all the analyzed populations a presence of moderately to highly densely distributed simple hairs is observed, whose height varies from 0,022 to 0,145 mm. The thinned out hairs on the abaxial side of leaf, whose height varies from 0,008 to 0,079 mm, are almost exclusively present in the plants from the western part of Serbia (Fig. 7).

Bulliform cells, located only in the basis of the central vascular bundle of the adaxial side of leaf, are present in the highest number of the analyzed leaves. Dimension of the bulliform cells, expressed as a relative ratio of the heights of bulliform cells and neighboring cells of the epidermis of the adaxial side of leaf, varied in range from 0,88 to 6,68.

Statistical data analysis

Coefficient of Variation. The analysis of variation of particular anatomical characteristics in populations of the taxon *Sesleria rigida sensu lato* in Serbia was performed to establish that the highest number of characters show a moderate degree of variability (CV=10-30 % - Table 2). Within the group of a highly variable characters, whose coefficient of variation (CV%) is higher than 30 %, are the following: number of the sclerenchyma strands on adaxial side of leaf (58.07 %), number of the sclerenchyma girders on adaxial side of leaf (53.10 %), number of the sclerenchyma girders on abaxial side of leaf (46.02 %), height of the sclerenchyma strand/girder in widest zone of leaf (42.03 %), surface of the sclerenchyma of leaf (41.58 %), height of the trichome of abaxial side of leaf (38.64 %), type of the sclerenchyma on abaxial side of leaf (35.36 %), type of the sclerenchyma on adaxial side of leaf (34.97 %) and surface of the leaf blades (34.03 %). Contrary to them,

Table 2. Basic statistic parameters of analyzed populations of *Sesleria rigida* in Serbia (Legend: Valin N – Number of measured cases, Mean – Mean value, Min. – Minimum value, Max. – Maximum value, Std. Dev. – Standard Deviation, Std. Error – Standard Error, CV% – coefficient of variation, F - distance between individual distributions, p - probability of error)

| | Valid N | Mean | Min. | Max. | Std. Dev. | Std. Err. | CV% | F | P |
|---|---------|-------|-------|--------|-----------|-----------|------|------|-------|
| *Width of the tiller leaf blades (in μm) | 517 | 968,9 | 599,5 | 1673,2 | 175,3 | 7,7 | 18,1 | 28,0 | 0,000 |
| *Distance between the middle and largest leaf blade thickness point of tiller leaf (in μm) | 517 | 556,0 | 357,7 | 922,9 | 96,0 | 4,2 | 17,3 | 24,9 | 0,000 |
| *Thickness of the tiller leaf blades in zone of the central rib (in μm) | 517 | 230,3 | 152,5 | 328,7 | 29,4 | 1,3 | 12,8 | 24,4 | 0,000 |
| *Largest thickness of the tiller leaf blades (in μm) | 517 | 226,7 | 139,6 | 383,8 | 36,2 | 1,6 | 16,0 | 49,0 | 0,000 |
| **Width of the central rib of tiller leaf (in μm) | 517 | 143,9 | 80,8 | 238,5 | 23,9 | 1,0 | 16,6 | 14,7 | 0,000 |
| *Height of the trichome of abaxial side of tiller leaf (in μm) | 188 | 20,6 | 7,6 | 78,7 | 8,0 | 0,6 | 38,6 | 3,0 | 0,004 |
| *Height of the trichome of adaxial side of tiller leaf (in μm) | 521 | 64,2 | 22,3 | 145,3 | 17,5 | 0,8 | 27,2 | 8,5 | 0,000 |
| *Height of the central vascular bundle of tiller leaf (in μm) | 517 | 82,0 | 52,6 | 125,9 | 11,8 | 0,5 | 14,4 | 18,3 | 0,000 |
| *Width of the central vascular bundle of tiller leaf (in μm) | 517 | 67,1 | 42,4 | 99,5 | 9,3 | 0,4 | 13,8 | 20,0 | 0,000 |
| *Height of the largest lateral vascular bundle of tiller leaf (in μm) | 517 | 85,6 | 52,5 | 138,0 | 13,4 | 0,6 | 15,7 | 33,7 | 0,000 |
| *Width of the largest lateral vascular bundle of tiller leaf (in μm) | 517 | 69,5 | 39,5 | 114,0 | 11,4 | 0,5 | 16,4 | 47,1 | 0,000 |
| *Height of the schlerenchyma strand of central vascular bundle of tiller leaf (in μm) | 516 | 37,0 | 14,8 | 74,9 | 9,8 | 0,4 | 26,4 | 12,6 | 0,000 |
| *Height of the schlerenchyma strand/girder in widest zone of tiller leaf (in μm) | 180 | 26,4 | 9,9 | 80,8 | 11,1 | 0,8 | 42,0 | 2,6 | 0,005 |
| *Surface of the schlerenchyma of tiller leaf (in mm^2) | 517 | 45,5 | 13,3 | 152,1 | 18,9 | 0,8 | 41,6 | 0,0 | 0,000 |
| *Surface of the tiller leaf blades (in mm^2) | 517 | 330,3 | 137,3 | 960,3 | 112,4 | 4,9 | 34,0 | 0,0 | 0,000 |
| Number of the schlerenchyma strands on adaxial side of tiller leaf | 516 | 0,0 | 0,0 | 4,0 | 0,3 | 0,0 | 58,1 | 1,4 | 0,177 |
| Number of the schlerenchyma strands on abaxial side of tiller leaf | 516 | 3,5 | 1,0 | 6,0 | 0,8 | 0,0 | 23,7 | 8,0 | 0,000 |
| *Number of the schlerenchyma girders on adaxial side of tiller leaf | 516 | 8,4 | 5,0 | 14,0 | 1,6 | 0,1 | 53,1 | 30,8 | 0,000 |
| *Number of the schlerenchyma girders on abaxial side of tiller leaf | 516 | 7,9 | 4,0 | 14,0 | 1,6 | 0,1 | 46,0 | 46,9 | 0,000 |
| *Number of the major vascular bundles of tiller leaf | 517 | 4,9 | 3,0 | 9,0 | 0,9 | 0,0 | 19,3 | 11,4 | 0,000 |
| *Number of the minor vascular bundles of tiller leaf | 517 | 6,5 | 4,0 | 12,0 | 1,3 | 0,1 | 24,5 | 6,4 | 0,000 |
| *Shape of tiller leaf blade | 517 | 2,3 | 2,0 | 3,0 | 0,5 | 0,0 | 20,1 | 16,6 | 0,000 |
| *Indumentum of adaxial side of tiller leaf | 517 | 2,7 | 1,0 | 3,0 | 0,5 | 0,0 | 19,2 | 3,6 | 0,000 |
| *Indumentum of abaxial side of tiller leaf | 517 | 0,4 | 0,0 | 2,0 | 0,5 | 0,0 | 7,3 | 93,8 | 0,000 |
| *Type of the schlerenchyma on adaxial side of tiller leaf | 518 | 1,3 | 1,0 | 2,0 | 0,4 | 0,0 | 35,0 | 1,8 | 0,042 |
| Type of the schlerenchyma on adaxial side of tiller leaf | 518 | 1,3 | 1,0 | 2,0 | 0,5 | 0,0 | 35,4 | 1,8 | 0,053 |
| *Dimension of the bulliform cells of tiller leaf (expressed as a relative ratio of the heights of bulliform cells and neighboring cells of the epidermis) | 520 | 2,1 | 0,9 | 6,7 | 0,5 | 0,0 | 26,4 | 5,0 | 0,000 |

*ANOVA effects significant at $p < 0.05$

Table 3. Correlations of analyzed anatomical characters of *Sesleria rigida* in Serbia (Marked correlations are significant at $p < 0.05$)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 TL_W | 1.00 | 0.95 | 0.71 | 0.76 | 0.57 | 0.09 | 0.52 | 0.53 | 0.38 | 0.66 | 0.66 | 0.62 | 0.35 | 0.86 | 0.95 | -0.12 | -0.23 | 0.65 | 0.41 | 0.61 | 0.60 | -0.03 | 0.14 | -0.09 | 0.31 | 0.08 |
| 2 TL_T2 | 0.95 | 1.00 | 0.73 | 0.79 | 0.53 | -0.02 | 0.52 | 0.52 | 0.33 | 0.66 | 0.66 | 0.61 | 0.37 | 0.83 | 0.93 | -0.06 | -0.25 | 0.52 | 0.35 | 0.59 | 0.48 | 0.08 | 0.01 | 0.29 | 0.12 | |
| 3 TL_To | 0.71 | 0.73 | 1.00 | 0.64 | 0.44 | -0.05 | 0.50 | 0.72 | 0.45 | 0.66 | 0.60 | 0.74 | 0.34 | 0.65 | 0.73 | -0.11 | -0.13 | 0.19 | -0.07 | 0.28 | 0.22 | -0.01 | -0.06 | -0.09 | 0.30 | 0.37 |
| 4 TL_T1 | 0.76 | 0.79 | 0.64 | 1.00 | 0.52 | -0.10 | 0.48 | 0.53 | 0.41 | 0.75 | 0.67 | 0.60 | 0.60 | 0.89 | 0.89 | -0.04 | -0.19 | 0.49 | 0.21 | 0.59 | 0.30 | 0.26 | 0.00 | -0.10 | 0.27 | 0.05 |
| 5 TL_Re_W | 0.57 | 0.53 | 0.44 | 0.52 | 1.00 | -0.06 | 0.43 | 0.64 | 0.68 | 0.65 | 0.62 | 0.42 | 0.40 | 0.58 | 0.63 | -0.19 | -0.03 | 0.34 | 0.00 | 0.49 | 0.11 | 0.20 | -0.01 | -0.16 | 0.43 | -0.06 |
| 6 TL_Trab_H | 0.09 | -0.02 | -0.05 | -0.10 | -0.06 | 1.00 | -0.04 | 0.12 | 0.10 | -0.01 | 0.08 | -0.08 | -0.20 | 0.05 | 0.00 | -0.17 | 0.00 | 0.19 | 0.14 | 0.08 | 0.34 | -0.01 | 0.29 | -0.15 | 0.24 | -0.23 |
| 7 TL_Trad_H | 0.52 | 0.52 | 0.50 | 0.48 | 0.43 | -0.04 | 1.00 | 0.55 | 0.57 | 0.55 | 0.56 | 0.50 | 0.24 | 0.48 | 0.55 | 0.09 | 0.03 | 0.30 | 0.18 | 0.32 | 0.10 | 0.01 | 0.18 | -0.24 | 0.08 | 0.16 |
| 8 TL_VBC_H | 0.53 | 0.52 | 0.72 | 0.53 | 0.64 | 0.12 | 0.55 | 1.00 | 0.79 | 0.82 | 0.78 | 0.56 | 0.31 | 0.57 | 0.58 | -0.13 | 0.10 | 0.14 | -0.17 | 0.35 | 0.06 | 0.23 | 0.08 | -0.27 | 0.40 | 0.10 |
| 9 TL_VBC_W | 0.38 | 0.33 | 0.45 | 0.41 | 0.68 | 0.10 | 0.57 | 0.79 | 1.00 | 0.68 | 0.69 | 0.36 | 0.25 | 0.44 | 0.46 | -0.13 | 0.27 | 0.08 | -0.20 | 0.31 | 0.05 | 0.20 | 0.07 | -0.32 | 0.29 | -0.08 |
| 10 TL_VB1_H | 0.66 | 0.66 | 0.66 | 0.75 | 0.65 | -0.01 | 0.55 | 0.82 | 0.68 | 1.00 | 0.89 | 0.65 | 0.41 | 0.74 | 0.75 | -0.11 | -0.14 | 0.32 | 0.01 | 0.49 | 0.20 | 0.19 | -0.10 | -0.27 | 0.35 | 0.16 |
| 11 TL_VB1_W | 0.66 | 0.66 | 0.60 | 0.67 | 0.62 | 0.08 | 0.56 | 0.78 | 0.69 | 0.89 | 1.00 | 0.52 | 0.31 | 0.66 | 0.71 | -0.10 | -0.06 | 0.29 | 0.10 | 0.40 | 0.26 | 0.10 | 0.06 | -0.26 | 0.31 | 0.10 |
| 12 TL_ScSC_H | 0.62 | 0.61 | 0.74 | 0.60 | 0.42 | -0.08 | 0.50 | 0.56 | 0.36 | 0.65 | 0.52 | 1.00 | 0.28 | 0.65 | 0.64 | -0.13 | -0.28 | 0.28 | 0.06 | 0.25 | 0.29 | 0.05 | -0.02 | -0.16 | 0.22 | 0.22 |
| 13 TL_ScS1_H | 0.35 | 0.37 | 0.34 | 0.60 | 0.40 | -0.20 | 0.24 | 0.31 | 0.25 | 0.41 | 0.31 | 0.28 | 1.00 | 0.54 | 0.46 | -0.12 | -0.16 | 0.37 | 0.11 | 0.38 | -0.08 | 0.00 | -0.01 | -0.22 | 0.19 | -0.05 |
| 14 TL_ScS_Ar | 0.86 | 0.83 | 0.65 | 0.89 | 0.58 | 0.05 | 0.48 | 0.57 | 0.44 | 0.74 | 0.66 | 0.65 | 0.54 | 1.00 | 0.93 | -0.07 | -0.22 | 0.64 | 0.27 | 0.65 | 0.49 | 0.17 | 0.08 | -0.20 | 0.37 | -0.04 |
| 15 TL_B_Ar | 0.95 | 0.93 | 0.73 | 0.89 | 0.63 | 0.00 | 0.55 | 0.58 | 0.46 | 0.75 | 0.71 | 0.64 | 0.46 | 0.93 | 1.00 | -0.08 | -0.20 | 0.61 | 0.34 | 0.65 | 0.51 | 0.06 | 0.08 | -0.12 | 0.29 | 0.05 |
| 16 TL_ScSab_No | -0.12 | -0.06 | -0.11 | -0.04 | -0.19 | -0.17 | 0.09 | -0.13 | -0.13 | -0.11 | -0.10 | -0.13 | -0.12 | -0.07 | -0.08 | 1.00 | 0.22 | -0.22 | 0.08 | 0.04 | 0.00 | 0.03 | 0.17 | 0.28 | -0.31 | 0.01 |
| 17 TL_ScSad_No | -0.23 | -0.25 | -0.13 | -0.19 | -0.03 | 0.00 | 0.03 | 0.10 | 0.27 | -0.14 | -0.06 | -0.28 | -0.16 | -0.22 | -0.20 | 0.22 | 1.00 | -0.26 | -0.38 | -0.06 | -0.09 | 0.23 | 0.31 | -0.03 | -0.24 | -0.16 |
| 18 TL_ScGab_No | 0.65 | 0.52 | 0.19 | 0.49 | 0.34 | 0.19 | 0.30 | 0.14 | 0.08 | 0.32 | 0.29 | 0.28 | 0.37 | 0.64 | 0.61 | -0.22 | -0.26 | 1.00 | 0.65 | 0.60 | 0.55 | -0.04 | 0.31 | -0.12 | 0.21 | -0.20 |
| 19 TL_ScGad_No | 0.41 | 0.35 | -0.07 | 0.21 | 0.00 | 0.14 | 0.18 | -0.17 | -0.20 | 0.01 | 0.10 | 0.06 | 0.11 | 0.27 | 0.34 | 0.08 | -0.38 | 0.65 | 1.00 | 0.44 | 0.46 | -0.18 | 0.20 | 0.14 | 0.00 | -0.14 |
| 20 TL_VB2_No | 0.61 | 0.59 | 0.28 | 0.59 | 0.49 | 0.08 | 0.32 | 0.35 | 0.31 | 0.49 | 0.40 | 0.25 | 0.38 | 0.65 | 0.65 | 0.04 | -0.06 | 0.60 | 0.44 | 1.00 | 0.34 | 0.33 | 0.09 | 0.09 | 0.39 | -0.19 |
| 21 TL_VB3_No | 0.60 | 0.48 | 0.22 | 0.30 | 0.11 | 0.34 | 0.10 | 0.06 | 0.05 | 0.20 | 0.26 | 0.29 | -0.08 | 0.49 | 0.51 | 0.00 | -0.09 | 0.55 | 0.46 | 0.34 | 1.00 | -0.16 | 0.26 | -0.01 | 0.22 | -0.05 |
| 22 TL_Sh | -0.03 | 0.08 | -0.01 | 0.26 | 0.20 | -0.01 | 0.01 | 0.23 | 0.20 | 0.19 | 0.10 | 0.05 | 0.00 | 0.17 | 0.06 | 0.03 | 0.23 | -0.04 | -0.18 | 0.33 | -0.16 | 1.00 | 0.00 | 0.18 | 0.16 | -0.21 |
| 23 TL_Ind_ad | 0.14 | 0.08 | -0.06 | 0.00 | -0.01 | 0.29 | 0.18 | 0.08 | 0.07 | -0.10 | 0.06 | -0.02 | -0.01 | 0.08 | 0.08 | 0.17 | 0.31 | 0.31 | 0.20 | 0.09 | 0.26 | 0.00 | 1.00 | 0.17 | -0.11 | -0.36 |
| 24 TL_Sc_ad | -0.09 | 0.01 | -0.09 | -0.10 | -0.16 | -0.15 | -0.24 | -0.27 | -0.32 | -0.27 | -0.26 | -0.16 | -0.22 | -0.20 | -0.12 | 0.28 | -0.03 | -0.12 | 0.14 | 0.09 | -0.01 | 0.18 | -0.17 | 1.00 | -0.03 | 0.13 |
| 25 TL_Sc_ab | 0.31 | 0.29 | 0.30 | 0.27 | 0.43 | 0.24 | 0.08 | 0.40 | 0.29 | 0.35 | 0.31 | 0.22 | 0.19 | 0.37 | 0.29 | -0.31 | -0.24 | 0.21 | 0.00 | 0.39 | 0.22 | 0.16 | -0.11 | -0.03 | 1.00 | -0.14 |
| 26 TL_BC_H | 0.08 | 0.12 | 0.37 | 0.05 | -0.06 | -0.23 | 0.16 | 0.10 | -0.08 | 0.16 | 0.10 | 0.22 | -0.05 | -0.04 | 0.05 | 0.01 | -0.16 | -0.20 | -0.14 | -0.19 | -0.05 | -0.21 | -0.36 | 0.13 | -0.14 | 1.00 |

the group of stable characters with a coefficient of variation below 10 % includes only a character of indumentum of abaxial side of leaf (7.29 %).

Correlative variability. The analysis of correlation of characters of leaf anatomy has shown that the highest number of characters are statistically significantly correlated (Table 3). The basic group of the highly correlated characters (coefficient of correlation $> 0,6$) is made of: width of the leaf blades–distance between the middle and largest leaf blade thickness point–thickness of the leaf blades in zone of the central rib–largest thickness of the leaf blades–height and width of the largest lateral vascular bundle–height of the sclerenchyma strand of central vascular bundle–surface of the leaf blades–surface of the sclerenchyma of leaf–number of the sclerenchyma girders on adaxial side of leaf–number of the major vascular bundles. Within this group, an extremely high level of correlation ($>0,9$) was shown by the following characters: width of the leaf blades–distance between the middle and largest leaf blade thickness point–surface of the leaf blades–surface of the sclerenchyma of leaf. In addition to the basic group of a highly correlated characters, in some lower degree (coefficient of correlation $> 0,3$) are correlated also the following characters: height of the trichome of adaxial side of leaf–height and width of the central vascular bundle–height of the sclerenchyma strand/girder in widest zone–number of the sclerenchyma girders on abaxial side of leaf–number of the minor vascular bundles–type of the sclerenchyma on abaxial side of leaf.

Such results of correlated analysis unmistakably point out that with the increase of basic dimensions of the leaf (width–thickness–surface) the dimensions of almost all other characters of leaf anatomy are regularly increased. The exception to this rule is made only by the following characters: number of the sclerenchyma strands on adaxial and abaxial side of leaf, height of the trichome of abaxial side of leaf, shape of leaf blade, indumentum of adaxial side of leaf, type of the sclerenchyma on adaxial side of leaf and dimension of the bulliform cells whose values are not significantly dependant on variations of dimensions of the whole leaf.

Variations in regard to the geographical gradients.

A detail analysis has shown that local populations differentiate among themselves on the anatomical level, as well as that the registered variations show more or less regular trends in the horizontal („east-west“, „north-south“) and vertical gradients (Fig. 4-8). On the basis of the results of these analyses, four groups of populations may be defined, showing similar trends of variation of anatomical characters of the leaf. The 1st group is composed of populations from the gorges and canyons of eastern Serbia

(Đerdap's gorge–Canyon of Lazareva reka–Jelašnica's gorge), 2nd group is composed of populations from the mountain region of eastern Serbia (Veliki Krš–Rtanj–Suva planina), 3rd group is composed of populations from the gorges and mountain region of western Serbia (Brdjani's gorge–Vujan–Maljen) and the 4th group of populations from the mountain region of western Serbia (Tara–Mokra Gora–Zlatibor–Kopaonik).

In the 1st group of populations (Đerdapska klisura gorge– Canyon of Lazareva reka–Jelašnica gorge), all the characters referred to the leaf size (width of the leaf blades, distance between the middle and largest leaf blade thickness point, thickness of the leaf blades in zone of the central rib, largest thickness of the leaf blades, width of the central rib, surface of the leaf blades) have their smallest dimensions in the furthest northern population in Đerdap's gorge, while with a slight increase of the altitude, going towards the south, dimensions of these characters are regularly increased, in a way that the biggest ones occur in the furthest southern population, in Jelašnica's gorge (Fig. 4). Within the mountain populations from eastern Serbia (2nd: Veliki Krš–Rtanj–Suva planina), with an increase of the altitude the dimensions of these characters are also increased, in a way that the smallest values are found in the individuals of population from Veliki krš, and the highest ones in the individuals from the mt. Suva planina. Almost identical trends of changes of these characters occur within the plants from western Serbia. Within the 3rd group (Brdjani's gorge–Vujan–Maljen) the smallest dimensions of all the characters occur within the population from Brdjani's gorge, while they are regularly increased with the increase of altitudes on Vujan and Maljen. In identical way as in the group of mountain populations from eastern Serbia, within the mountain populations in western Serbia (4th group: Tara–Mokra Gora–Zlatibor–Kopaonik) the increase of altitude provokes also the increase of dimensions of all the characters, therefore the smallest dimensions are noted within the population from Tara mountain, and the biggest ones within the populations from Kopaonik.

Group of characters referred to vascular elements (number of the major vascular bundles, number of the minor vascular bundles, height of the central vascular bundle, width of the central vascular bundle, height of the largest lateral vascular bundle, width of the largest lateral vascular bundle) show very similar trends in variations as a group of characters referred to the leaf dimensions (Fig. 5). For example, within the 1st group (Đerdap's gorge–Canyon of Lazareva reka–Jelašnica's gorge) all the characters with the exception of the width of the central vascular bundle show an increase in number and in their dimensions, going from north towards the south of the areal. The smallest numbers and dimensions of vascular bundles occur in the individuals from the Đerdap's gorge, while on

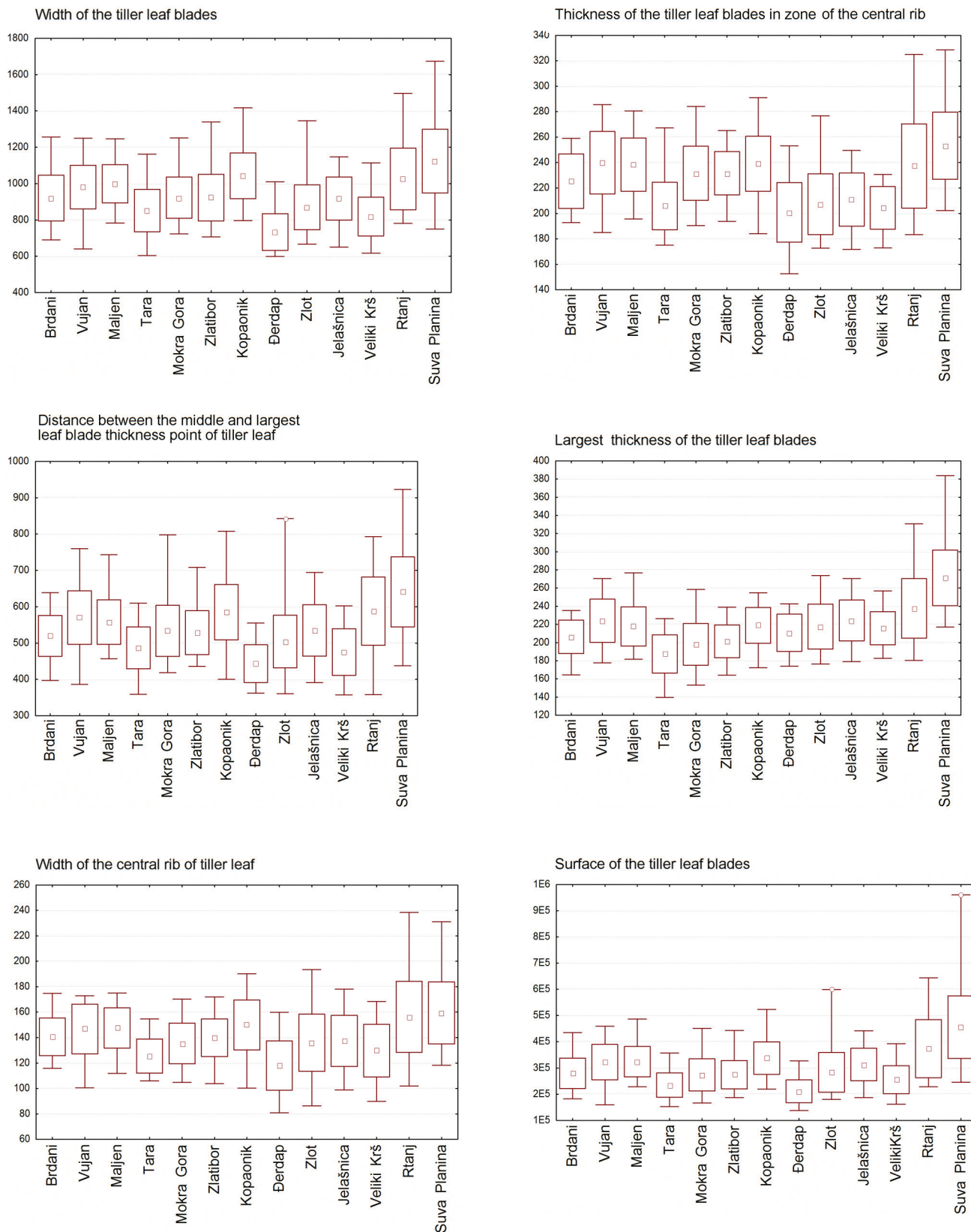


Fig. 4. Box and whisker plots of basic statistic parameters of leaf dimension – all measures in μm (Legend: Middle point = Mean, Box = Mean \pm SD, whisker = Min-Max, \circ = Outliers)

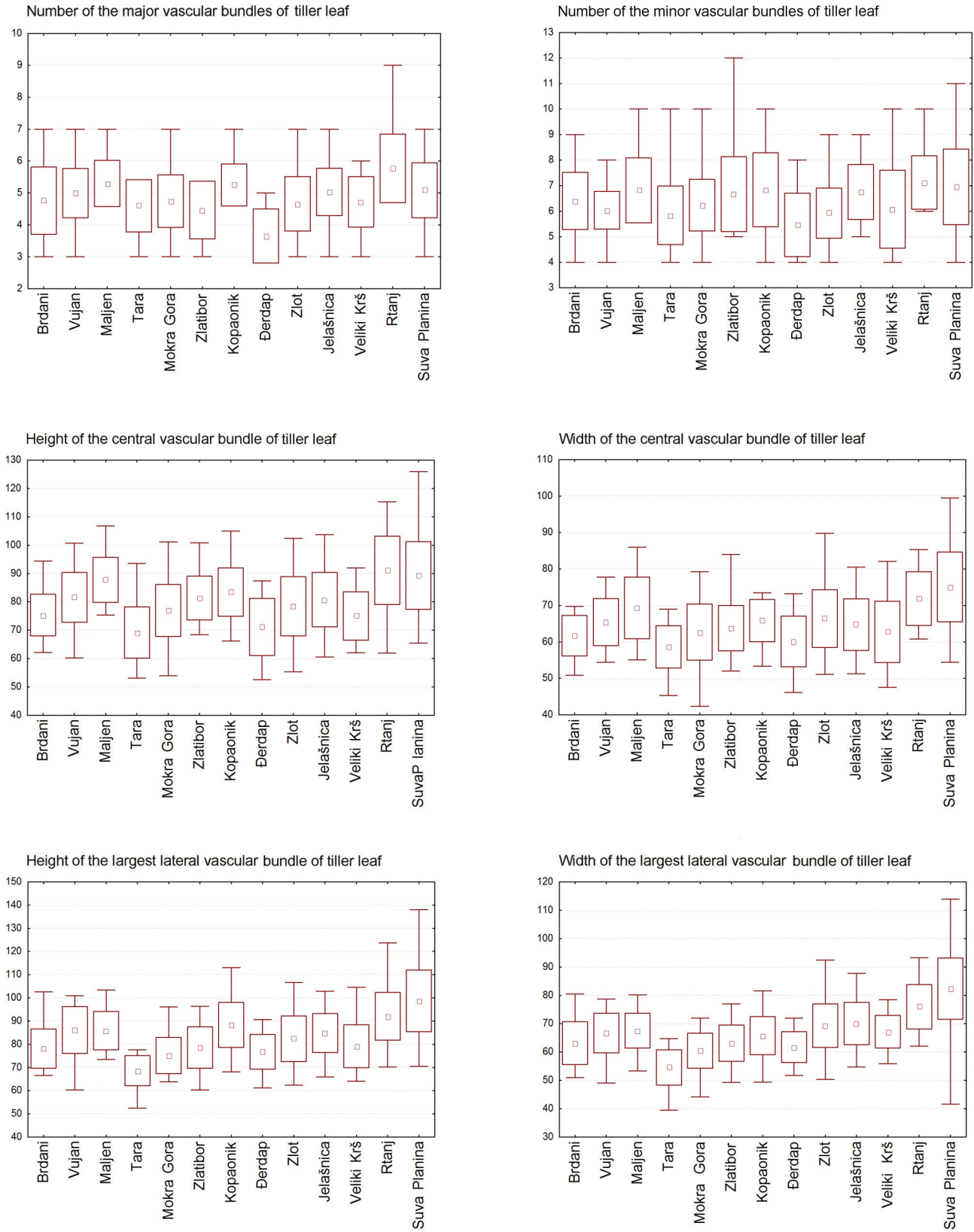


Fig. 5. Box and whisker plots of basic statistic parameters of vascular bundles – all measures in μm (Legend: middle point = Mean; box = Mean±SD; whisker = Min-Max)

the contrary, in the individuals from the furthest southern population, from Jelašnica's gorge, the highest numbers and dimensions were registered. Within the populations of eastern Serbia (2nd group: Veliki Krš–Rtanj–Suva planina), all the characters show a regular pattern in increase of their dimensions with the increase of the altitude, in a way that all the smallest values are registered in the population from Veliki krš. Minor exception to this trend is shown by the following characters: number of the major and the minor vascular bundles which are the highest within the representatives of the population from Rtanj, and a bit smaller in the population from the Suva planina mt. The same trend, with some exceptions, was registered within the plants from western Serbia. In both groups (3rd group: Brdjani's gorge–Vujan–Maljen, 4th group: Tara–Mokra Gora–Zlatibor–Kopaonik), the lowest values were registered noted in the Brdjani's gorge (3rd group) and on Tara (4th group), and the highest on Maljen (3rd group) and Kopaonik (4th group). The exceptions to these rules are shown only by the following characters: number of the major and the minor vascular bundles which within the plants from Vujan (3rd group) and Zlatibor (4th group) have smaller values in regard to the populations from lower altitudes from Brdjani's gorge and from Tara. In addition, the character height of the largest lateral vascular bundle deviates from the general trend, because within the populations from Vujan and Maljen it has approximately similar values.

Within the group of characters referred to the sclerenchyma elements (Fig. 6), with few exemptions, were not established similar trends in variations as in the previous two groups of characters. The most expressed trends of dimensions' increase in correlation with the increase of altitude were noted within the following characters: surface of the sclerenchyma of leaf within the 1st, 2nd and 3rd group of populations, number of the sclerenchyma girders on adaxial side of leaf within the 1st, 3rd and 4th group of populations, number of the sclerenchyma girders on abaxial side of leaf within the 1st group of populations and the height of the sclerenchyma strand/girder in widest zone within the 3rd group of populations. Other characters per groups of populations have not shown more significant variation and regularity. The most expressive differences and deviations from the general trend were noted in the character: number of the sclerenchyma strands on adaxial side of leaf which are completely absent in some populations (Vujan, Maljen, Zlatibor, Canyon of Lazareva reka, Veliki krš). Also, significant deviations are noted in the character: number of the sclerenchyma girders on adaxial and abaxial side of leaf which show significant differences between the eastern (1st and 2nd group) and western populations (3rd and 4th group). In general, the number of sclerenchyma

girders is much higher within the plants from western Serbia in regard to the plants from eastern Serbia. As an exception, within this group of characters referred to sclerenchyma elements the negative trends were also recorded, i.e., the trends of decrease of dimensions with the increase of altitude. This was recorded only in the case of the character: height of the sclerenchyma strand/girder in widest zone of leaf within the plants of the 1st and 4th groups of populations.

Within the groups of characters referred to trichomes no significant regularity in variations was found in vertical gradients (Fig. 7). However, as well as in the case of number of sclerenchyma girders, the indumentum and the height of the trichome of abaxial side of leaf show very significant differences between the eastern (1st and 2nd groups) and western populations (3rd and 4th groups). Namely, trichomes on the abaxial side of the leaf are present in all the populations from western Serbia, while within the plants from eastern Serbia they are almost completely absent (except in some samples from Canyon of Lazareva reka and Jelašnica's gorges). Also, some regularities may be observed in the very dimensions of the trichomes. For example, the height of the trichome of abaxial side of leaf in the plants from the 3rd group of populations (Brdjani's gorge–Vujan–Maljen) is to some extent smaller in regard to the plants from the 4th group (Tara–Mokra Gora–Zlatibor–Kopaonik), while the height of the trichome of adaxial side of leaf is generally bigger in the plants from eastern (1st and 2nd groups of populations) in regard to the plants from western Serbia (3rd and 4th groups of populations). In addition, the height of the trichome of adaxial side of leaf in the plants from eastern Serbia shows slight regularity in increase of its dimensions with the increase of the altitude.

Finally, neither the bulliform cells have shown any significant regularity in variation of their dimensions in the vertical profile of the studied region. Some regularity was established in regard to the east-west gradient, since some larger bulliform cells were registered in the plants from eastern Serbia in regard to the plants from western Serbia (Fig. 8).

Analysis of variances (ANOVA). Analysis of variances has shown that almost all the characters statistically significantly contribute to differentiation of the analyzed populations (Table 2). The characters that have not shown any statistical significance in anatomical differentiation are only the following: number of the sclerenchyma strands on adaxial side of leaf and type of the sclerenchyma on abaxial side of leaf. The highest significance in differentiation have shown the following characters: width of the leaf blades, distance between the middle and largest leaf blade thickness point, thickness of the tiller leaf blades in zone

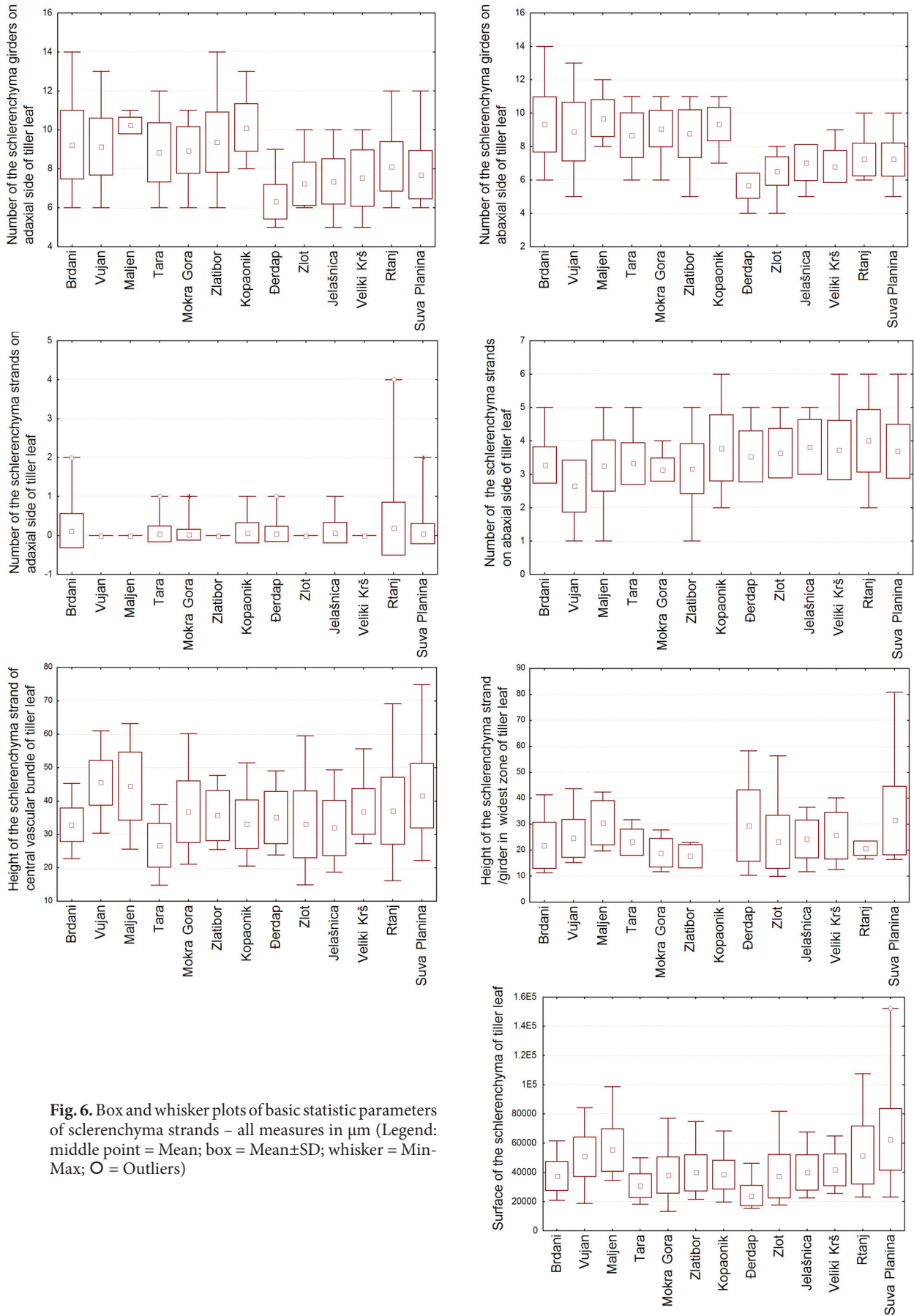


Fig. 6. Box and whisker plots of basic statistic parameters of sclerenchyma strands – all measures in μm (Legend: middle point = Mean; box = Mean \pm SD; whisker = Min-Max; O = Outliers)

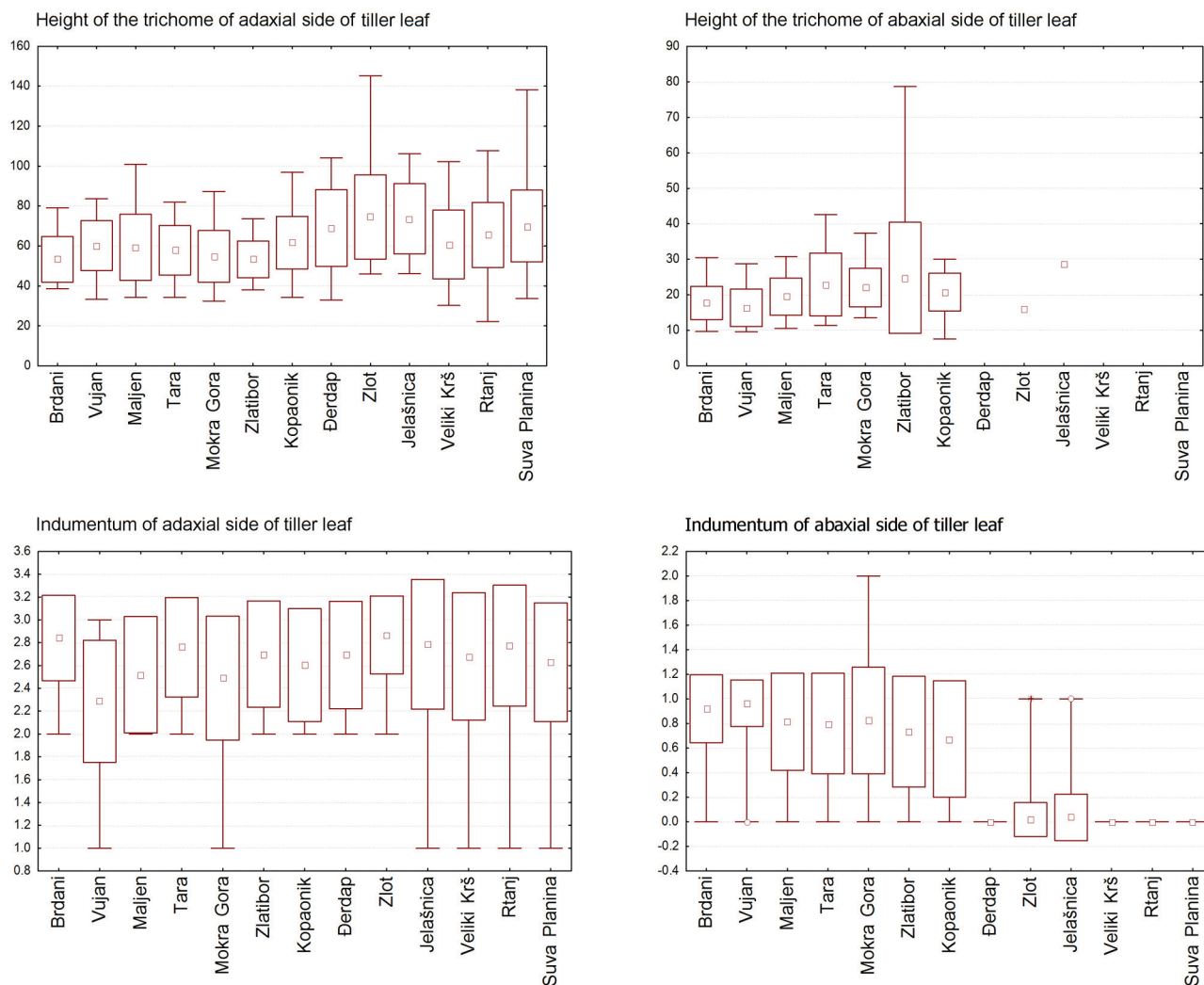


Fig. 7. Box and whisker plots of basic statistic parameters of trichomes – all measures in μm (Legend: middle point = Mean; box = Mean \pm SD; whisker = Min-Max; \square = Outliers)

of the central rib, largest thickness of the leaf blades, height and width of the central vascular bundle, height and width of the largest lateral vascular bundle, number of the sclerenchyma girders on adaxial and abaxial side of leaf and indumentum of abaxial side of leaf.

DISCUSSION

Carpatho-Balkan species *S. rigida*, in a wider sense, is relatively frequent on the territory of Serbia within the vegetation of the open Alpine and Sub-Alpine high mountain pastures, mountain rocky grounds, and slightly less frequent in the light coniferous or dark deciduous forests. Its habitats were recorded at altitudes between 100 and 1900 m.a.s., both on the basic-carbonate and ultra-basic serpentinite and peridotite substratum.

In accordance with a relatively wide range of climatic conditions and types of habitats inhabited by *S. rigida* on the territory of Serbia, this plant shows a significant degree of inter-population variability, on the level of characteristics of a leaf anatomy. A detail analysis has shown that local populations on the anatomic level are more or less differentiated in between themselves, as well as that the registered changes show more or less regular trends in horizontal and vertical profiles. The most expressed trends of variability are represented by the characters referred to dimensions of the leaf and vascular elements, where was observed a clear trend of increase of general dimensions in correlation with an increase of altitude and shifting of populations to the south. Namely, the smallest dimensions of these characters were recorded within the furthest northern populations, located at the lowest altitudes, and

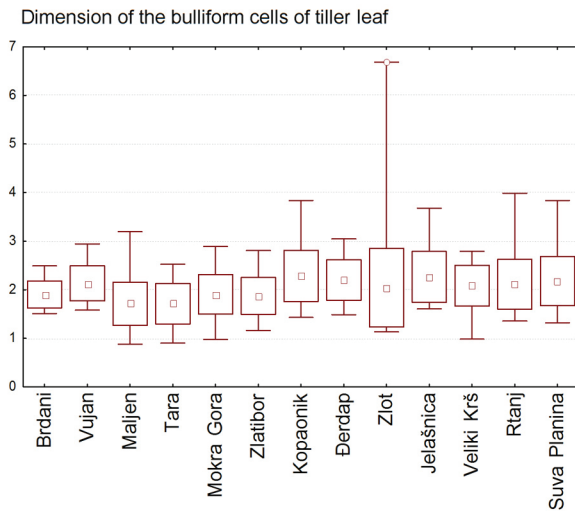


Fig. 8. Box and whisker plots of basic statistic parameters of bulliform cells - expressed as a relative ratio of the heights of bulliform cells and neighboring cells of the epidermis of the adaxial side of leaf (Legend: middle point = Mean; box = Mean \pm SD; whisker = Min-Max; \circ = Outliers)

the highest ones within the furthest southern populations at the highest altitudes. The characters referred to the sclerenchyma elements, trichomes and bulliform cells have not shown significant regularities in variability of their dimensions in the vertical profile.

Although the formal measurement was not performed, it was observed that the length of leaf of the analyzed individuals in the vertical profile descends in a regular way. The longest leaves, which are usually laid down on the ground, occur in the plants from the gorges and canyons and from the warm mountain regions. On the contrary, the Alpine plants have much shorter, erected (rigid) and often extremely sharp-thistly leaves. These changes in the length of leaf are correlated with the statistically significant increase of thickness and width of the leaf in the cross-section. These two trends in changes of dimensions of the leaf led to significant changes in correlation of the surface and volume. Namely, the leaves of the plants inhabiting the warmest conditions of the habitats on the lowest altitudes have got long and thin leaves, meaning that the total leaf surface is increased in regard to its volume. On the contrary, the plants from the coldest conditions have got the shortest and thickest leaves, meaning that the total volume of the leaf is increased in regard to its surface. These trends are to be related with a well-known ecological

Table 4. Basic differences between populations of *Sesleria rigida* from West and East Serbia

| | West Serbia | | | | East Serbia | | | |
|---|-------------|--------|--------|----------|-------------|--------|--------|----------|
| | Mean | Min. | Max. | Std.Dev. | Mean | Min. | Max. | Std.Dev. |
| Largest thickness of the tiller leaf blades | 207,65 | 139,64 | 276,80 | 23,89 | 241,89 | 174,13 | 383,83 | 37,19 |
| Height of the trichome of adaxial side of tiller leaf | 57,28 | 32,47 | 100,85 | 13,02 | 69,70 | 22,26 | 145,28 | 18,63 |
| Height of the largest lateral vascular bundle of tiller leaf | 80,31 | 52,48 | 113,08 | 10,70 | 89,76 | 61,21 | 137,97 | 13,86 |
| Width of the largest lateral vascular bundle of tiller leaf | 63,05 | 39,50 | 81,51 | 7,52 | 74,68 | 41,60 | 113,95 | 11,31 |
| Number of the sclerenchyma girders on adaxial side of tiller leaf | 9,42 | 6,00 | 14,00 | 1,41 | 7,50 | 5,00 | 12,00 | 1,28 |
| Number of the sclerenchyma girders on abaxial side of tiller leaf | 9,12 | 5,00 | 14,00 | 1,34 | 6,90 | 4,00 | 10,00 | 1,06 |
| Indumentum of abaxial side of tiller leaf | 0,81 | 0,00 | 2,00 | 0,41 | 0,01 | 0,00 | 1,00 | 0,08 |

rule by which the members of certain taxon that inhabit the cold regions have got increased volume/surface ratio in regard to the members of the same taxon that inhabit the warmer regions. This rule is referred to the significance of the surface/volume ratio of the body for maintaining the body temperature. The speed of eliciting of body's warmth depends on the body surface, therefore, as a rule, the organisms that live in colder conditions, have better surface/volume ratio, in terms of surface decrease and volume increase.

Analysis of variability of particular anatomical characters was performed to establish that the highest number of characters have moderate degree of variability and that as a highly variable ones single out only the characters referred to the number, size and surface of the sclerenchyma elements, dimension of trichomes and surface of the leaf blades. The highest number of characters has shown statistically significant level of correlative variances. Generally speaking, by increasing of basic dimensions of the leaf (width-thickness-surface), the dimensions of almost all other characters of the leaf anatomy are also being increased. Exception to this rule was found only in the following characters: number of the sclerenchyma strands, height of the trichome, shape of leaf blade, type of indumentum and sclerenchyma as well as dimension of the bulliform cells whose values do not significantly depend on the changes of dimensions of the whole leaf.

Moreover, by performing the comparative analysis it was found that the analyzed populations on the territory of Serbia are anatomically very well differentiated into the serpentine populations from western Serbia and carbonate populations from eastern Serbia. In addition, this analysis also proved that in populations from eastern Serbia there is a clear trend of differentiation to the populations that inhabit gorges and canyons and the populations that inhabit the highest mountain regions, which point out to the fact that in this part of the area has already occurred certain degree of anatomic differentiation in the vertical profile. The most expressed differences between the western and eastern populations occur in the characters of the indumentum, sclerenchyma girders, dimensions of the vascular bundles and the width of the leaf. (Table 4). The abaxial side of the leaf from eastern Serbia is, as a rule, always glabrous, while the leaves of the plants from western Serbia are always covered by short trichomes on their abaxial side. In addition, the leaves of the plants from eastern Serbia almost always have a bit longer trichomes than the leaves of the plants from western Serbia. The expressed differences occur also in number of sclerenchyma girders, which appear always in higher number in the plants from western Serbia. Contrary to the sclerenchyma girders, the width of the leaf and dimensions

of the lateral vascular bundles have shown higher values in the plants from eastern Serbia in regard to the plants from western Serbia.

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REZIME

Anatomija listova vrste *Sesleria rigida* Heuffel ex Reichenb. (Poaceae) u Srbiji

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Uradu je prikazan opis ukupne varijabilnosti anatomije listova u različitim populacijama vrste *S. rigida sensu lato* na području Srbije. Morfometrijske analize su obuhvatile 27 karkatera na uzorku od 521 lista sa pojedinačnih individua iz 21 populacije. Obrada podataka je uključila deskriptivnu statistiku (srednja vrednost, standardna devijacija, minimum, maksimum, standardna grešk, koeficijent varijacije) za svaki morfometrijski karakter, analizu varijansi, korelacionu analizu i analizu variranja karaktera u odnosu na geografske gradijende, čime je bilo moguće ustanoviti osnovne trendove u anatomskej diferencijaciji analiziranih populacija. Detaljne analize su pokazale da lokalne populacije značajno variraju u anatomskej smislu, kao i da je generalno moguće uočiti jasnu diferencijaciju zapadnih serpentinitskih populacija u odnosu na istočne populacije koje se javljaju na karbonatnoj podlozi.

KLJUČNE REČI: *Poaceae*, *Sesleria rigida*, anatomija lista