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SESLERIA ULIGINOSA OPIZ – A COMPARATIVE STUDY OF LEAF ANATOMICAL TRAITS

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Abstract: The species *Sesleria uliginosa* is relatively common and widespread in Central (Hungary, Czech republic, Slovakia, Austria, Romania) and Northern Europe (Scandinavian peninsula), while in the Southeastern (Montenegro, Croatia, Bulgaria) and Southern Europe (Italy) it is a true natural rarity. Ecologically it is the typical species of calcareous swamps and by this feature it is an extreme quite singular within the whole genus. *S. uliginosa* can also grow on soils moist in spring but drying out later in summer, often at sunny stands, especially on clay soils.
According to Deyl, *S. uliginosa* belongs to Calcaria section, turma Uliginosa. The wax cover of the young leaves is typical for this species, but this pruining is nearly completely absent in the old leaves. It is closely related to *Sesleria heuflerana* Schur with which it shares some common characters – pruinos leaves, three floretted spikes and the occurrence in lower altitudes. But in the Balkan Peninsula it seems to have a far wider amplitude of its stands, so it can be found on the localities that reach up to the subalpine zone.
The aim of this study was to establish and describe the anatomical differentiation of populations of *S. uliginosa* from Romania, Hungary and Montenegro. The measurements were carried out on permanent handmade slides, prepared by the standard method for the light microscopy. To determine the significance of anatomical variation and differentiation, the following analysis were carried out: Principal component analysis (PCA), Canonical discriminant analysis (CDA) and cluster analysis by UPGMA method.

Keywords: *Sesleria uliginosa*, Poaceae, anatomy of leaves

Introduction

The taxon *S. uliginosa* was described by OPIZ (1836) in Flora of Bohemia, and the name was established for the populations of *S. caerulea* (L.) Ard. which are distributed in calcareous swamps. Over the time, the distinction between these two taxa became complicated, which led to many confusions [JANCHEN, 1960, 1965, 1966; PIGNATTI, 1982; ADLER, 1994].

A detailed analysis of the nomenclature conducted by FOGGI & al. [2001] confirmed the validity of *S. uliginosa* for the populations on wet habitats, and *S. caerulea* for dry grassland ones. These two species probably developed from common ancestor, but they represent independent evolutionary branches, and *S. uliginosa* is more related to *S. heuflerana* Schur [DEYL, 1946].

The species *S. uliginosa* belongs to the section *Calcariae*, turma *Uliginosa* [DEYL, 1946], and it is a European species with relatively limited and highly disjunctive areas. This species has a relatively continuous area in southern Scandinavia and the Baltic

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countries. The incidence in the rest of the area is scattered on different, often restricted areas (Romania, Montenegro, Bulgaria, Czech Republic and Italy).

Ecologically, it is one of the major indicators of wet habitats [HÁJEK & al. 2005]. By this feature it is an extreme, even singular, within the whole genus. *S. uliginosa* grows on soils moist in spring but drying out later in summer, often at sunny stands, especially on clay soils.

In his monography on the genus *Sesleria*, DEYL (1946) recognized the population from Montenegro – Moračke planine (Habitat: Montenegro, Korytan Rovački, leg. Rohlena VII. 1903) as a different entity, and described the variety *S. uliginosa* var. *rohlenae*. He also assumed that the variability of the Balkan localities on which *S. uliginosa* was recorded is large and that this hangs together with the age of these localities that is probably Tertiary, while the northern localities are of post-glacial origin [DEYL, 1946].

The basic aim of the present study was to quantify anatomical variation within and between populations of *S. uliginosa* from Romania, Hungary and Montenegro on the basis of multivariate statistics, and to determine whether there are clear anatomical differences between these populations.

Material and methods

Three populations from distant parts of the area (Romania, Hungary, Montenegro) were sampled for anatomical analyses. The plant material was either fixed in 50% ethyl–alcohol solution or dried out and deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade [BEOU].

Voucher specimens:

Romania, Transilvanija, Brašov, Prejmer, alkaline wet grassland, 511 m, 25.7382 E, 45.73085 N, (*Kuzmanović N*., *Comanescu P*. *30336*, 27.04.2010, BEOU).

Hungary, Budapest, Soroksar botanic garden, wet grassland, 110 m, 19.154327 E, 47.400341 N, (*Barina Z*. *32879*, 26.04.2011, BEOU).

Montenegro, Durmitor, Crno Jezero, Seslerietum uliginosae, 1399 m, limestone, 19.096156 E, 43.147777 N (*Lakušić D*. *24439*, 31.05.2007, BEOU)

Anatomical analyses of the leaves were done on the permanent handmade 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). The measurements were performed on the cross-section of 30 tiller leaves, each obtained from different individuals (10 per population). All measurements were performed using the software Leica Q Win. 17 characters were measured and subjected to statistical processing (Tab. 1).

Descriptive statistics (mean, minimum, maximum, standard deviation and coefficient of variation) were calculated for each character state. To determine the significance of anatomical variation and differentiation, the following statistical analyses were carried out: Principal component analysis (PCA), Cannonical discriminant analysis (CDA) and cluster analysis by UPGMA method based on Mahalanobis distances. Statistical analyses were performed using the package Statistica 5.1 [StatSoft 1996].

Results

MORPHOLOGICAL CHARACTERISTICS OF PLANTS

In its general habitus *S. uliginosa* is rather variable, but pruined young leaves are typical for all the individuals.

In his paper *Sesleria* studien, UJHELY (1938) gave a detail description of the leaf anatomy of *S. uliginosa*. He analyzed the differences between *S. uliginosa* and *S. caerulea* and found that the anatomical structure of the leaves is a very good distinguishing character between these two species, and that the anatomical differences are as great as between distant groups.

The leaves of the analyzed populations are in the cross section slightly rolled around the central nerve or unrolled and flat. The width of the leaves varies from 1176.7 to 2159.0 μm, with 7-13 vascular bundles. Sclerenchyma is discontinued and is present mostly in the form of sclerenchyma girders. In the zone of the central vascular bundle the sclerenchyma is organized exclusively in the form of a sclerenchyma girder. Bulliform cells are present in all the analyzed individuals. Small thinned out hairs were observed on the edge of some leaves (Fig. 1).

STATISTICAL DATA ANALYSIS

Coefficient of Variation

The analysis of variation of particular anatomical characters in populations of *S. uliginosa* was performed to establish that the highest number of characters show a moderate degree of variability (CV=10-30% – Tab. 1). Within the group of a highly variable characters, whose coefficient of variation (CV %) is higher than 30% is only surface of the sclerenchyma of tiller leaf (TL_ScS_Ar) – 33.2%. In the group of stable characters whose coefficient of variation is below 10% is only thickness of the tiller leaf blades in zone of the central rib (TL_To) – 8.8%.

Principal component analysis (PCA)

First three PCA axes account 72.06% of the total variability. Most of the variation was explained by the first axis (49.15%), 14.76% by the second and only 8.14% by the third. As it can be inferred from the component loadings, expressing character correlation with the axes, several characters are responsible for the differentiation along the first axes: width of the tiller leaf blades (TL_W), largest thickness of the tiller leaf blades (TL_T1), height of the central vascular bundle of tiller leaf (TL_VBC_H), width of the central vascular bundle of tiller leaf (TL_VBC_W), height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H), width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W), width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W), surface of the sclerenchyma of tiller leaf (TL_ScS_Ar), surface of the tiller leaf blades (TL_B_Ar) (Tab. 1).

On the principal component analysis (PCA) diagram the individuals from Montenegro are mostly grouped in the bottom-right quadrant with two individuals in

upper and bottom-left quadrant. The individuals form Hungary are grouped in the upper-left quadrant with one individual in bottom-left and one in upper-right quadrant. The individuals from Romania are scattered in two quadrants – upper-right and bottom-left (Fig. 2).

Canonical discriminant analysis (CDA)

The canonical discriminant analysis (CDA) resulted in two differentiated groups, which are completely separated along the first axis – population from Montenegro as one group, and populations from Hungary and Romania as the other group (Fig. 3).

Along the second axis, the populations from Hungary and Romania are clearly separated also.

A clear differentiation between the population from Montenegro and populations from Hungary and Romania is shown also by the UPGMA cluster analysis, in which two completely separated clusters were formed (Fig. 4).

Discussion

The material for the analyses was collected from the habitats that are typical for *S. uliginosa*, from Romania – Braşov (alkaline wet grassland, altitude 510 m), Hungary – Budapest (alkaline wet grassland, altitude 110 m) and Montenegro – Durmitor (shore of Crno Jezero – Lake, altitude 1459 m). But the population from Durmitor is very particular because it is several months during a year under the water!

The most significant differences in leaf anatomy between the population from Durmitor and populations from Braşov and Budapest are observed in the height of the largest lateral and central vascular bundle, largest thickness of the tiller leaf blades, height of the sclerenchyma strand of central vascular bundle, surface of the sclerenchyma of tiller leaf and number of the minor vascular bundles (Tab. 2).

DEYL also noticed that the population from Montenegro (Moračke planine) is different from other populations of *S. uliginosa* that he had seen, and described a variety *S. uliginosa* var. *rohlenae*, pointing out that the populations of *S. uliginosa* on the Balkan peninsula are probably from Tertiary, while nothern ones are of post glacial origin [DEYL, 1946].

It is possible that all this together (specific life cycle and historical circumstances) led to the formation and expression of the differencies in leaf anatomy (maybe also genetical differencies) of the population from Durmitor in regard to the populations from Budapest and Braşov.

Finally, we can conclude that the populations of *S. uliginosa* from Montenegro, growing on the specific highmountain habitat, show a significant degree of anatomical differentiation from the lowland populations from Hungary and Romania. Furthermore, if we consider that the leaf anatomical characters have a significant diagnostic character within the genus *Sesleria* [KOLÁŘ, 1930; UJHELYI, 1938; UJHELYI & FELFOLDY, 1948; STRGAR, 1966, 1980, 1985; DI PIETRO, 2007; ALEGRO, 2007, KUZMANOVIĆ & al. 2011], the obtained results support Deyl's opinion that populations from Montenegro represent a separate taxon [DEYL, 1946]. Because only the leaf anatomical characters were analyzed in this paper**,** and sample of population was restricted**,** a question of a definite

taxonomic status of the Montengrine populations can be solved only following a detailed comparative morphological study of the reproductive organs, as well as a comprehensive molecular and phylogenetic study, which is in progress.

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Tab. 1. Basic and multivariate statistic parameters of all analyzed populations of *S. uliginosa*. All measures in µm (STD – standard deviation, CV% - coefficient of variation, Comp.1, 2, 3 – principal components, **bold** components with loadings >0.7)

Characters	Min	Mean	Max	STD	CV %	Comp.1	Comp.2	Comp.3
Width of the tiller leaf blades (TL_W)	1176.7	1695.5	2159	268.5	15.8	**0.86**	0.35	-0.17
Distance between the middle and largest leaf blade thickness point of tiller leaf (TL_T2)	636	888.5	1104.2	123.3	13.9	0.67	0.25	-0.37
Thickness of the tiller leaf blades in zone of the central rib (TL_To)	160.8	205.9	238.9	18.2	8.8	0.61	-0.21	-0.25
Largest thickness of the tiller leaf blades (TL_T1)	165.7	220.8	295.3	35.5	16.1	**0.82**	-0.28	0.32
Width of the central rib of tiller leaf (TL_Rc_W)	142.1	241	326.9	49.4	20.5	0.67	-0.01	-0.36
Height of the central vascular bundle of tiller leaf (TL_VBC_H)	60.4	82.4	101.4	11.0	13.4	**0.78**	-0.44	0.07
Width of the central vascular bundle of tiller leaf (TL_VBC_W)	47.9	66.8	87.3	11.0	16.5	**0.78**	-0.13	0.02
Height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H)	64.6	89.8	120.7	15.5	17.3	**0.79**	-0.34	0.35
Width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W)	49.4	70	95	12.1	17.2	**0.79**	-0.13	0.28

Height of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScSC_H)	27.2	57.6	90.2	16.1	28	0.66	-0.3	0.25
Width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W)	75.3	117.7	154	23.0	19.6	**0.74**	-0.05	-0.46
Surface of the sclerenchyma of tiller leaf (TL_ScS_Ar)	12849.1	29123.6	49189.4	9674.6	33.2	**0.89**	0	0.22
Surface of the tiller leaf blades (TL_B_Ar)	163445	305938	475260	83901.9	27.4	**0.94**	0.21	0.03
Number of the major vascular bundles of tiller leaf (TL_VB2_No)	4	4.7	6	0.7	15.2	0.55	0.54	-0.16
Number of the minor vascular bundles of tiller leaf (TL_VB3_No)	3	5.3	8	1.1	21.7	0.19	**0.79**	0.32
Total number of the vascular bundles (TL_VB_No)	7	10	13	1.5	15	0.41	**0.86**	0.17
Dimension of the bulliform cells of tiller leaf (TL_BC_Ha)	28.5	41.3	64.6	9.1	22.1	0.24	-0.22	-0.49

Tab. 2. Anatomical differences between analyzed population of *S. uliginosa*. All measures in µm

	Budapest	Brašov	Durmitor
Width of the tiller leaf blades (TL_W)	(1177 -) 1243 - 1751 (- 1873)	(1497 -) 1579 - 1822 (- 1873)	(1514 -) 1629 - 2149 (- 2159)
Distance between the middle and largest leaf blade thickness point of tiller leaf (TL_T2)	(636 -) 660 - 912 (- 1034)	(730 -) 813 - 963 (- 969)	(931 -) 925 - 1056 (- 1104)
Thickness of the tiller leaf blades in zone of the central rib (TL_To)	(161 -) 175 - 217 (- 235)	(188 -) 193 - 226 (- 238)	(190 -) 199 - 225 (- 239)
Largest thickness of the tiller leaf blades (TL_T1)	(173 -) 172 - 218 (- 253)	(198 -) 211 - 282 (- 295)	(166 -) 193 - 249 (- 250)
Width of the central rib of tiller leaf (TL_Rc_W)	(171 -) 181 - 240 (- 273)	(142 -) 179 - 294 (- 311)	(223 -) 242 - 311 (- 327)
Height of the central vascular bundle of tiller leaf (TL_VBC_H)	(60 -) 62 - 78 (- 84)	(85 -) 87 - 98 (- 101)	(77 -) 81 - 89 (- 89)
Width of the central vascular bundle of tiller leaf (TL_VBC_W)	(48 -) 48 - 66 (- 79)	(59 -) 64 - 80 (- 87)	(55 -) 63 - 80 (- 83)
Height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H)	(65 -) 66 - 86 (- 93)	(83 -) 92 - 115 (- 121)	(74 -) 79 - 102 (- 104)
Width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W)	(49 -) 50 - 70 (- 82)	(66 -) 67 - 85 (- 95)	(58 -) 64 - 84 (- 91)
Height of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScSC_H)	(27 -) 34 - 62 (- 71)	(61 -) 63 - 83 (- 90)	(37 -) 41 - 64 (- 75)
Width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W)	(75 -) 79 - 118 (- 142)	(99 -) 104 - 134 (- 141)	(107 -) 117 - 154 (- 154)
Surface of the sclerenchyma of tiller leaf (TL_ScS_Ar) / 100	(134 -) 160 - 268 (- 295)	(273 -) 285 - 440 (- 492)	(128 -) 202 - 392 (- 394)
Surface of the tiller leaf blades (TL_B_Ar) / 100	(1724 -) 1819 - 3008 (- 3607)	(2645 -) 2826 - 3836 (- 4003)	(1634 -) 2454 - 4413 (- 4753)
Number of the major vascular bundles of tiller leaf (TL_VB2_No)	(4 -) 4 - 5 (- 6)	(4 -) 4 - 5 (- 6)	(4 -) 4 - 6 (- 6)
Number of the minor vascular bundles of tiller leaf (TL_VB3_No)	(4 -) 5 - 6 (- 7)	(4 -) 4 - 6 (- 6)	(3 -) 4 - 7 (- 8)
Total number of the vascular bundles (TL_VB_No)	(8 -) 9 - 11 (- 12)	(8 -) 9 - 10 (- 10)	(7 -) 8 - 13 (- 13)
Dimension of the bulliform cells of tiller leaf (TL_BC_Ha)	(31 -) 31 - 49 (- 65)	(31 -) 35 - 52 (- 60)	(29 -) 31 - 51 (- 58)

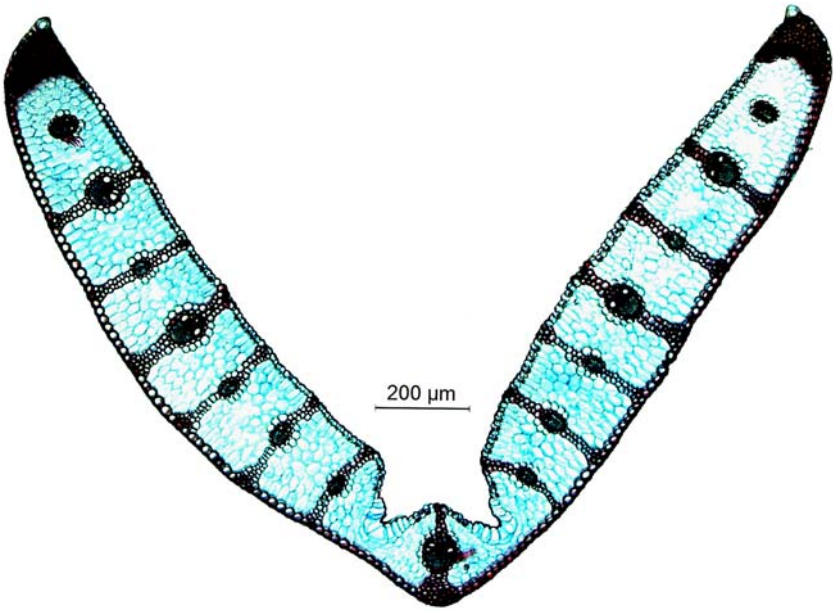

Fig. 1. Tiller leaf cross section of *S. uliginosa* from Montenegro

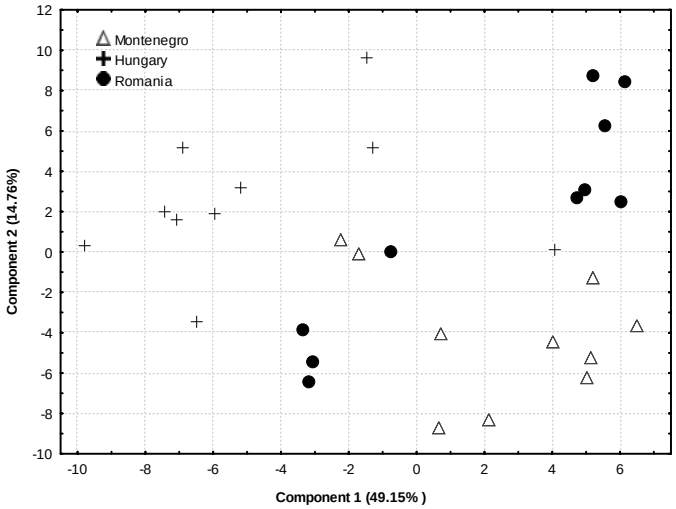

Fig. 2. Results of Principal Component Analysis (PCA) plotted along the first two discriminant axes

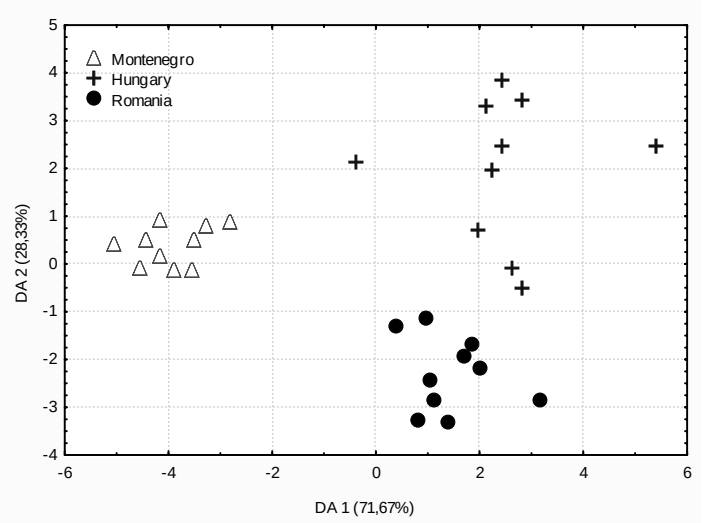

Fig. 3. Results of Canonical Discriminant Analysis (CDA) plotted along the first two discriminant axes

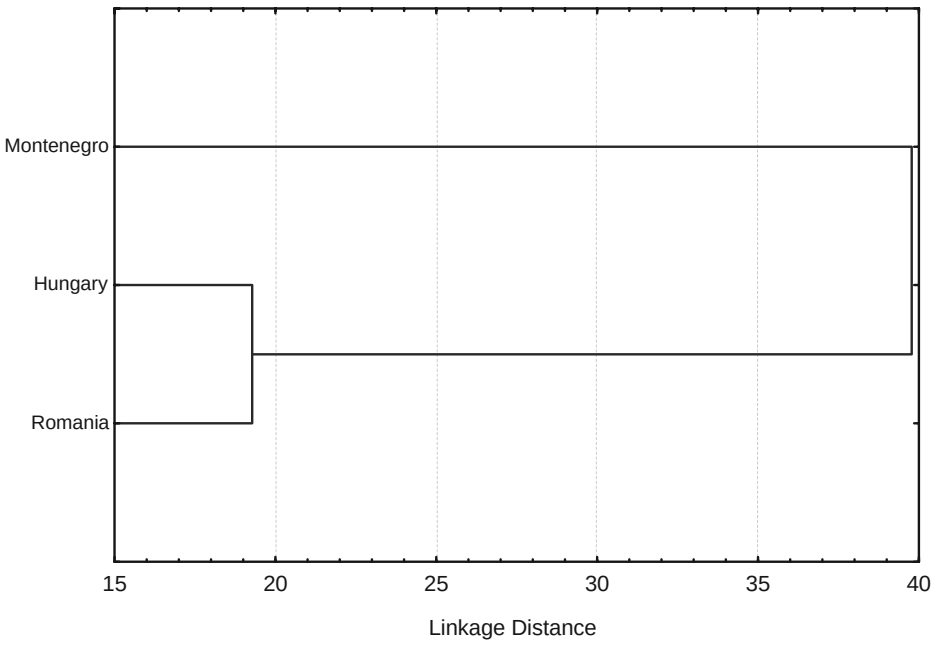

Fig. 4. Results of cluster analysis (UPGMA) based on Mahalanobis distances