Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
2017, volume 102, livre 4, pp. 162-169
Youth Scientific Conference "Kliment's Days", Sofia 2016

LEAF EPIDERMIS MORPHOLOGY OF *SORBUS UMBELLATA* (DESF.) FRITSCH (ROSACEAE)

TSVETA GANEVA

Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski"
Corresponding author: tsveta_ganeva@yahoo.com

Keywords: leaf epidermis, stomata, trichomes, quantitative analysis, *Sorbus umbellata*

Abstract: Leaf epidermis morphology of *Sorbus umbellata* (Desf.) Fritsch was described and a comparison between the two subspecies - ssp. *umbellata* and ssp. *koevessii* (Pénzes) Kárpáti was made. Cuticle ornamentation, epidermis cells, stomata and trichomes were observed by light and scanning electron microscopy and analyzed. The length and the width of the epidermal cells and the stomata were measured and the numbers of stomata and trichomes were counted. All data were processed by the statistical variation method. In result, differences in the upper epidermis cells outlines, smaller sizes of both upper and lower epidermis cells, higher stomata density and increased trichome density on the lower surface were found in the leaves of *S. umbellata* ssp. *koevessii* compared to ssp. *umbellata*. The observed epidermis peculiarities were probably adaptations to the local environment conditions.

INTRODUCTION

The large family Rosaceae Juss. consists of trees, shrubs and herbs that have worldwide distributions, but are more concentrated in the Northern Hemisphere. Traditionally and widely adopted until recently classification of Rosaceae was based on the fruit type and had four subfamilies – Spiraeoideae, Rosoideae, Prunoideae and Maloideae. Now, it is thought that fruit types are not good indicators of relationships within the family and a new classification based on molecular data delimited three subfamilies – Dryadoideae, Rosoideae and Spiraeoideae. All members of Maloideae as subtribe Pyrinae are included within the latter (Pot t er

et al., 2007; Campbell et al., 2007). The Pyrinae species are an economically significant group of woody plants cultivated for their valuable fruit as well as for their ornamental beauty. Genus *Sorbus* L. includes more than 250 species that are widespread mainly in temperate areas (Phipps et al., 1990; Aldasoro et al., 1998). There is great variability of leaf shape and other morphological features such as flowers, fruits and pollen that impedes the taxonomic differentiation.

Sorbus umbellata (Desf.) Fritsch is native to the southeastern parts of Europe. In Bulgaria, it occurs in the forests of Znepole and Vitosha regions, West Frontier, Rila, Pirin and Rhodope mountains, from 600 m to 1500 m alt. *Sorbus umbellata* ssp. *umbellata* is present in most of the same distribution area, whereas *S. umbellata* ssp. *koevessii* (Pénzes) Kárpáti is found only above Gotse Delchev town in Pirin mountains (Valev, 1973). The aim of this study is to determine the leaf epidermis structure of *S. umbellata*, using light and scanning electron microscopy, and to estimate the features of taxonomic value.

MATERIALS AND METHODS

The leaf epidermis of *S. umbellata* ssp. *umbellata* and *S. umbellata* ssp. *koevessii* (Pénzes) Kárpáti were observed and analyzed by light and scanning electron microscopy (SEM). Preparations were made from herbarium specimens (4 of *S. umbellata* ssp. *umbellata* and 2 of *S. umbellata* ssp. *koevessii*), preserved in the collections of Sofia University "St. Kliment Ohridski", Faculty of Biology (SOM): SOM-43587, SOM-147364, SOM-265091, SOM-36543 and University of Forestry - Sofia. Cuticle membranes from the middle part of the mature leaves after maceration procedure with Jeffrey solution as modified by Stace (1965) were used for measurements of the length and the width of the epidermis cells and the stomata, and for stomata and trichomes counting. The slides were examined with light microscope Amplival (Carl Zeiss) and the measurements were made at magnification x 400 using ocular micrometer. All data were processed by the statistical variation method performed using SigmaStat and SigmaPlot software. Kolmogorov-Smirnov test, Kruskal-Wallis H test and Dunn's test were applied for comparing two independent samples of different sample sizes (Sokal and Rohlf, 1997; Zar, 1999). Preparations for SEM were made from herbarium material without any treatment. The samples were sputtered with gold in vacuum-evaporator Jeol JFC-1200 fine coater and observed by Jeol JSM-5510 microscope.

RESULTS AND DISCUSSION

The micromorphology observations of *S. umbellata* leaf surface using SEM revealed smooth cuticle with sparse granular wax on the upper epidermis (figs. 1, 2) and fine-striated cuticle on the lower one (fig. 3).

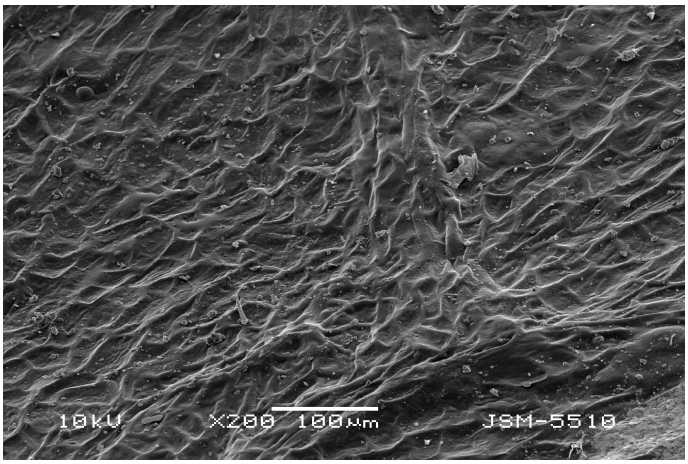

Fig. 1. *S. umbellata* ssp. *umbellata* – upper epidermis (SEM).

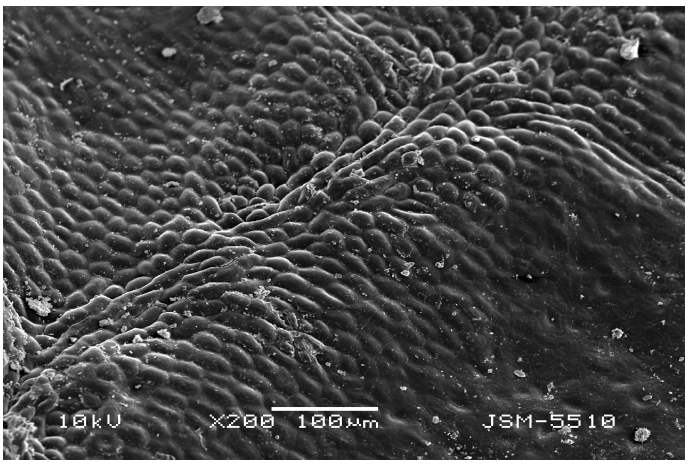

Fig. 2. *S. umbellata* ssp. *koevessii* – upper epidermis (SEM).

Striations were found radial to stomata and trichome bases, and on the guard cells. The upper epidermis cells were polygonal with curved in ssp. *umbellata* or straight in ssp. *koevessii* anticlinal cell walls (figs. 1, 2), while the lower ones were irregular with curved to undulate anticlinal cell walls (figs. 3, 4). The observed trichomes were simple unicellular non-glandular. On the upper surface they were distributed sparsely, mainly above veins, whereas thick indumentum covered the lower surface, especially in ssp. *koevessii* (fig. 4). The stomata were regularly distributed on the lower epidermis and conform to the anomocytic type.

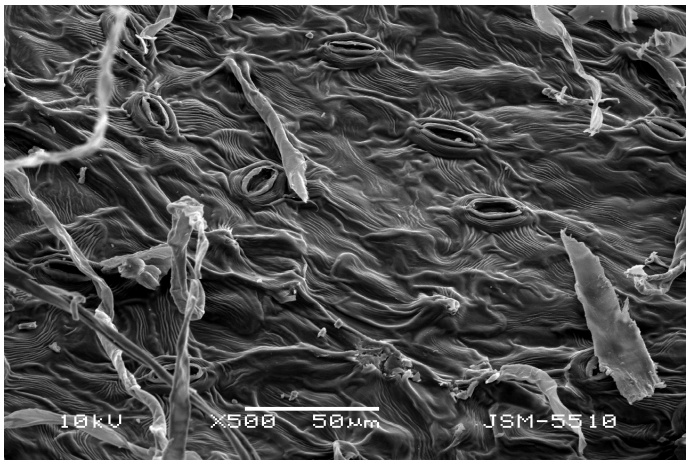

Fig. 3 *S. umbellata* ssp. *umbellata* – lower epidermis (after trichome removal) (SEM).

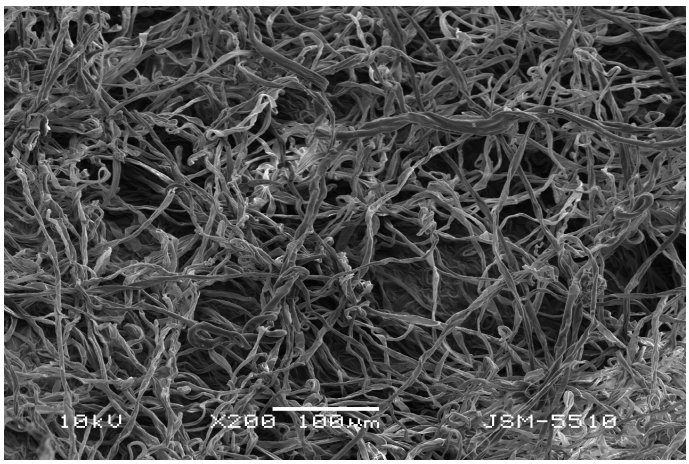

Fig. 4 *S. umbellata* ssp. *koevessii* – lower epidermis (SEM).

The results of the quantitative analyses of the epidermis features were represented in tables (tables 1, 2) and box plot graphics (fig. 5). After data comparison, statistically significant differences (p =<0.001) were found for the length and the width of both upper and lower epidermis cells, stomata density and trichome density on the lower epidermis between the two investigated subspecies. The differences in the median values among the treatment groups were greater than would have been expected by chance. Almost twice higher stomata density was counted in *S. umbellata* ssp. *koevessii* (223/mm^2) compared to ssp. *umbellata* (109/mm^2). Furthermore, extremely high trichome density was counted on the lower surface of *S. umbellata* ssp. *koevessii* (715/mm^2).

Table 1 *S. umbellata* ssp. *umbellata* – quantitative analysis of the epidermis features*.

S. umbellata ssp. umbellata	*n*	$\bar{x}$	$s_{\bar{x}}$	STD	CV	min	max	Me
UEC length (µm)	30	49.2	1.91	10.44	21.2	28.7	69.7	49.2
UEC width (µm)	30	31.8	1.03	5.67	17.8	20.5	41.0	32.8
LEC length (µm)	30	37.6	1.46	7.99	21.3	24.6	61.5	36.9
LEC width (µm)	30	21.7	0.60	3.31	15.3	16.4	28.7	20.5
St length (µm)	30	29.5	0.59	3.21	10.9	24.6	41.0	28.7
St width (µm)	30	21.7	0.52	2.83	13.0	16.4	28.7	20.5
St num/mm^2	30	108.2	3.36	18.41	17.0	72.3	138.6	108.4
Tr UE num/mm^2	30	5.4	1.24	6.78	125.0	0.0	30.1	6.0
Tr LE num/mm^2	30	209.2	8.48	46.42	22.2	120.5	283.1	204.8

Table 2 *S. umbellata* ssp. *koevessii* – quantitative analysis of the epidermis features*.

S. umbellata ssp. koevessii	*n*	$\bar{x}$	$s_{\bar{x}}$	STD	CV	min	max	Me
UEC length (µm)	30	28.6	1.04	5.67	19.9	20.5	41.0	28.1
UEC width (µm)	30	21.7	0.52	2.83	13.0	16.4	24.6	20.5
LEC length (µm)	30	22.8	0.80	4.40	19.3	16.4	32.8	24.6
LEC width (µm)	30	13.7	0.53	2.91	21.2	8.2	20.5	12.3
St length (µm)	30	28.6	0.87	4.77	16.7	20.5	41.0	28.7
St width (µm)	30	20.6	0.41	2.25	10.9	16.4	24.6	20.5
St num/mm^2	12	222.9	9.11	31.57	14.2	180.7	301.2	216.9
Tr UE num/mm^2	30	6.8	1.03	5.65	82.7	0.0	24.1	6.0
Tr LE num/mm^2	12	714.4	18.19	63.00	8.8	584.3	771.1	731.9

* n – total number of measurements; $\bar{x}$ – mean; $s_{\bar{x}}$ – standard error of mean; STD – standard deviation; CV – coefficient of variation; min – minimum value; max – maximum value; Me – median; UE – upper epidermis; LE – lower epidermis; C – cells; St – stomata; Tr – trichome; num – number.

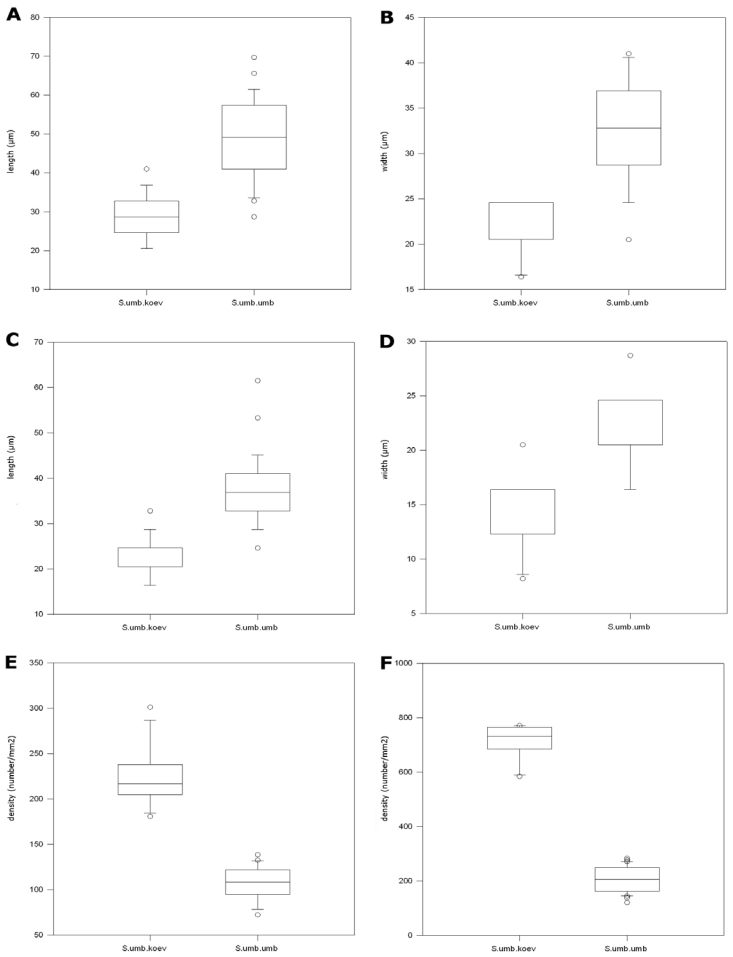

Fig. 5 Box plots of the numerical data of *S. umbellata* ssp. *umbellata* and ssp. *koevessii* epidermis features. For better graphical visualization, the medians and the most important percentiles – 25th, 75th, 10th and 90th were presented. A. Upper epidermis cells (UEC) – length (H=38.380)*^{,1,2}; B. UEC – width (H=34.976)*^{,1,2}; C. Lower epidermis cells (LEC) – length (H=38.332)*^{,1,2}; D. LEC – width (H=39.702)*^{,1,2}; E. Stomata density (H=25.247)*^{,1,2}; F. Trichome density on lower epidermis (H=25.163)*^{,1,2}. *H – value of the Kruskal-Wallis H test; ^{1}degrees of freedom (d.f.), d.f.=1; ^{2}probability (p), p =<0.001

Leaf epidermis study of *S. umbellata* displayed little differences in the structure between ssp. *umbellata* and ssp. *koevessii*. Both subspecies shared same characteristics – cuticle ornamentation, epidermis cells form, stomata form and size, stomata type, and trichome type. However, in result of the quantitative analysis, significant differences were found. *Sorbus umbellata* ssp. *koevessii* had smaller length and width of the epidermis cells, higher stomata density, and higher trichome density on the lower epidermis compared to ssp. *umbellata*. Stomata density is an important ecophysiological parameter (Ceulemans et al., 1995) though it is strongly affected by different environmental factors (Tichá, 1982; Woodward and Bazzaz, 1988). Soil water deficiency and poor ecological conditions may result in an increase of stomata density per unit area (Gindel, 1969), that contributes to a better control of transpiration under drought stress (Bosabalidis and Kofidis, 2002). Trichomes on the leaf surface have a multitude of functions but their adaptive significance can depend on the environment (Press 1999). Dense trichomes protect the underlying cells against ultraviolet radiation damage (Karabourniotis et al., 1995), insect herbivores and act as a barrier against water loss (Woodman and Fernandes, 1991). Considering the observed micromorphology of the *S. umbellata* ssp. *koevessii* leaf surface and the morphometric values of stomata and trichome density, it is presumed that the epidermis structure variations were due to the local ecological conditions.

CONCLUSIONS

Sorbus umbellata ssp. *umbellata* and ssp *koevessii* shared same epidermis structure. However, differences in the upper epidermis cells outlines, smaller sizes of both upper and lower epidermis cells, higher stomata density and increased trichome density on the lower surface were found in the leaves of ssp. *koevessii* compared to ssp. *umbellata*. The displayed epidermis peculiarities were probably adaptations to the local environment conditions.

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