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MICROMORPHOLOGICAL AND PALYNOLOGICAL INVESTIGATIONS REGARDING THE ENDEMIC SPECIES *CAMPANULA CARPATICA* JACQ.

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Abstract: Micromorphology and palinology of *Campanula carpatica*, endemic for the Carpathian Mountains, have been investigated. Leaves, different parts of the flowers and pollen grains were investigated from micromorphological point of view.
Keywords: *Campanula carpatica*, leaf, pollen, SEM

Introduction

The genus *Campanula* contains more than 200 species in Europe, according to the still valid literature, for Europe (Fedorov and Kovanda, 1976). In Romania, over 27 species from *Campanulaceae* genus are identified (Ghişa et al., 1964).

Campanula carpatica Jacq. is endemic in the Carpathian Mountains, being found in the Romania, but also in the north part of Carpathians (in Poland, Slovakia or Cehia). In Romania, in the Red List it is considered a rare plant (Olteanu et al., 1994). Systematic position of this species is not well established. According to Zlatko and collaborators (2008) results, this species is closer to the “isophylloid” group (agg. *C. waldsteiniana*) than to sect. Rapunculus where it traditionally belongs (Eddie et al., 2003; Shulkina et al., 2003).

C. carpatica is used as ornamental plant, through their decorative elements: big blue flowers; it can be used to decorate calcareous rockeries and abrupt embankments (Mardari et al., 2009).

C. carpatica was studied now for the first time for micromorphological point of view (SEM analyses). Erdelská (2001) examined the stigmatic trichomes of *C. carpatica* using SEM. Another paper was focused on in vitro propagation of this species (Sriskandarajah et al., 2001; Frello et al., 2002).

In this study, we aimed to give detailed knowledge about *C. carpatica* from micromorphological and palynological point of view.

Materials and methods

The plant material was collected from the Cheile Bicazului – Hasmas natural reservation, located close to the city of Bicaz (Romania) (N 46º 50' 18", E 25º 50' 19" at an average altitude of 1,267 m) in July 2011. A voucher specimen is stored in the Herbarium of Faculty of Biology, Al. I. Cuza Iaşi University, Romania (I).

Scanning electron microscopy (SEM) investigations – Portions of leaves at different developmental stages developmental stages and floral parts were fixed in FEA (formol: ethylic alcohol: acetic acid) for 48 hours, stored in 70% ethanol (Johansen, 1940). After dehydration in a graded ethanol series, the material was dissected and critical point dried with CO2 (using a EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold

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(30 nm) (using a EMS 550X Sputter Coater) and, finally, examined in a scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 kV. The measurements of the stomata were made using the biometrical software from Nikon (NIR-Demonstration). Fifty measurements were made for each parameter. Stomatal index calculation was performed according to the formula: Stomatal Index (%) = (NS x 100/(EC + NS)); where NS is the number of stomata and EC is the number of epidermal cells.

Results and discussions

C. carpatica is a clump-forming perennial with rounded to ovate (Brickel and Zuk, 1997) or cordate (Gadella, 1964), toothed, basal leaves 2.5– 5 cm long.

The leaf epidermis cells are polygonal with straight or slightly undulated anticlinal walls. The stomata are present on both surfaces (Fig. 1 A, B, C, D). The stomatal apparatus is by anomomocytic type. The stomata dimensions are higher on lower epidermis, both in young and mature leaves. The stomatal index (and implicit the stomata density) is higher on lower epidermis (Table 1). Moderate stomatal index and stomata dimensions are commonly associated with ecological adaptations on low water stress (Yang and Wang, 2001). Although *C. carpatica* on the rocks (where water is easily lost through drainage), their habitat is located in areas characterized by quite high levels of precipitation. Therefore its structural characters show only moderate adaptations to drought (average xeromorfism).

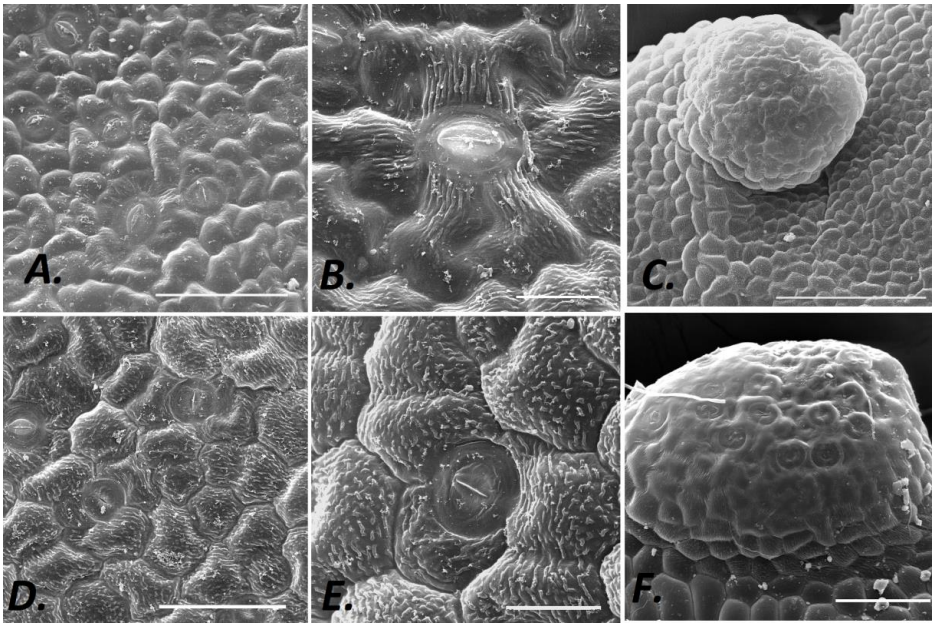

Figure 1. SEM microphotographs of an young leaf A, B - Upper epidermis (A – general view, B – detail with a stomata), C – Hydatode from an young leaf, D, E - Lower epidermis (D – general view, E – detail with a stomata), F – Hydatode from a mature leaf (A, D - Scale bar = 50 μm, B, E- Scale bar = 20 μm, C- Scale bar = 200 μm, F - Scale bar = 100 μm)

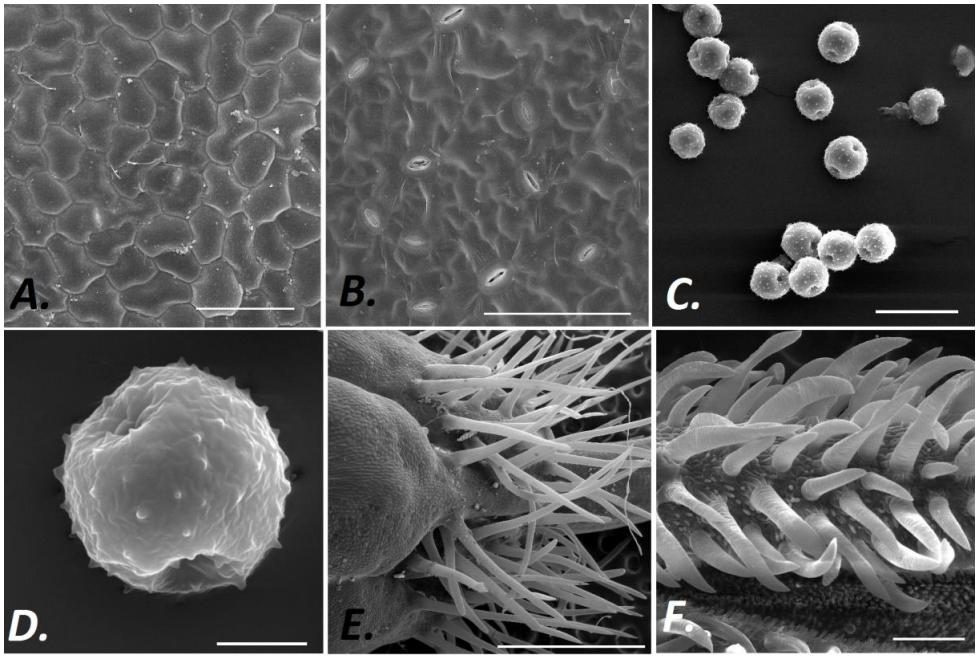

Figure 2. A, B - SEM microphotographs of an mature leaf: A - Upper epidermis B - Lower epidermis; C, D – Pollen grains, E – Base of the calyx (unicellular tector hairs could be observed), F - Stigmatic trichomes (A, B - Scale bar = 100 µm, C - Scale bar = 50 µm, D - Scale bar = 10 µm, E - Scale bar = 1 mm, F - Scale bar = 500 µm)

Table 1. Measurements of different anatomical parameters of the *Campanula carpatica* leaf epidermis: St h – height of the stomatal cells, St w – width of the stomatal cells , SI – stomatal index

		St h (µm)	St w (µm)	SI
Young leaf	Upper epidermis	17.77±1.61	17.27±1.63	8.82
	Lower epidermis	19.51±1.97	15.29±1.56	23.85
Mature leaf	Upper epidermis	28.73±2.25	18.77±1.62	4.07
	Lower epidermis	29.2±2.51	21.81±3.09	18.06

There are different cuticle ornamentations and wax depositions on the adaxial and abaxial leaf surface under SEM (Figs. 1, 2). The cuticle from the upper epidermis of young leaf has rough striations. On the lower epidermis the cuticle has rough parallel striations, especially in the stomata vicinity. The mature leaf presents generally smooth cuticle surface (especially on the lower epidermis). The tector hairs are absents from all analysed leaves; they are presents only at the sepal basis (Fig. 2 E). Also, stigmatic trichomes (or pollen collecting hairs) were observed on the flower stigma (Fig. 2 F).

The leaf margin is serrated, and hydathodes could be observed on the marginal dentations (Fig. 1 C, F); they occupy a definite position on the leaf in relation to the conducting system. The hydathodes role seems to be the pressure relief, to prevent water

accumulation in leaves of shade, while the thicker cuticle greatly reduces transpiration (Rea, 1921).

The pollen grains were triporate, oblato-sphaeroidal. The exine sculpture was rugulate-scabrate (Fig. 2 C, D). The pollen surface was covered with supratectal spinules. Rough surface is closely related to entomophile pollination of this species. In taxonomy, the sculpture types of pollens have valid morphological features.

Conclusions

The micromorphological characteristics of *C. carpatica* provide additional clarification regarding how it adapts to living environment. The absence of obvious xerophytic characters shows that the plant has access to almost all the vegetation period at a moderate water resource.

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