

ANATOMICAL INVESTIGATIONS ON ENDEMIC *CAMPANULA ROMANICA* SAVUL. AND THEIR ECOLOGICAL SIGNIFICANCE

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Abstract

Histo-anatomical properties of the vegetative organs in endemic *Campanula romanica* Savul. were presented. The structure of the root, rhizome, stem and leaves (basal and caulinar) was analysed with emphasis to the adaptation of this rare species to the environmental conditions. Moderate xeric features were observed at the anatomical level, despite the dry living area. These are correlated with the species life conditions and life cycle.

Introduction

Campanula L. is the largest genus within the family Campanulaceae, with between 350 and 500 species, growing in a wide range of habitats in the Northern Hemisphere (Fedorov and Kovanda 1976). This genus is mainly distributed in Eurasia and poorly represented in North America and Africa. A large concentration of *Campanula* species is found in the Eastern Mediterranean region and the Caucasus. In Romania, 25 species from Campanulaceae are identified (Ciocarlan 2009). *Campanula romanica* Savul. is a strictly endemic taxon in Romania (Ciocarlan 2009). It is listed in IUCN red list of threatened plants as "vulnerable" (Dihoru and Parvu 1987). It grows in steppe or forest-steppe areas, on limestone or carbonate bearing schists, in rock crevices. It is an isolated species, growing only in a single region of Romania, namely Dobrogea. Individuals are rare, but locally abundant and the species is not protected at all. The impact of human activities on natural ecosystems has resulted in the formation of new groups of rare species that were previously more abundant but are now rare (Celik *et al.* 2008).

Anatomical features of the species from Campanulaceae family have been less investigated. Most histo-anatomical data about the species of this family are found in the Metcalfe and Chalk (1983) monograph related to anatomy of dicotyledons. The literature has no information regarding the anatomical features of *C. romanica*.

Okak and Tokur (1996) analyzed the structure of the vegetative organs of *Campanula* spp., endemic in Turkey. Also, anatomical studies on other genera of the Campanulaceae family (*Asyneuma*) were made by Alçitepe (2008). Scanning electron microscopy investigations regarding some *Campanula* species were made on seeds (Alçitepe and Yildiz 2010) and on pollen grains (Potoğlu *et al.* 2008, Alçitepe 2012).

In this study, anatomical features of the vegetative organs of endemic *C. romanica*, which is under the danger of extinction, have been investigated in detail.

Materials and Methods

The plant material was collected from the Măcin Mountains, Tulcea county, Dobrogea Romania (45°06'52" N, 28°15'30" E, 215 m MLS) and from Chervantu Hill, Tulcea county, Dobrogea (45°06'50" N, 28°14'33" E, 150 m MLS) in July 2011. The geographic coordinates were recorded using eTrex Legend HCx GPS system. In this area, the average annual temperature is between 10 and 11°C and the average rainfall does not exceed 500 mm/year.

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A voucher specimen is stored in the Herbarium of Faculty of Biology, “Alexandru Ioan Cuza” Iasi University, Romania.

For histo-anatomical analysis the material was fixed in FEA and preserved in 70% ethyle alcohol. For each organ, five different individuals were considered. Free hand sections were performed using a razor blade. The sections were stained with ruthenium red and iodine green. Photomicrographs were taken with an Olympus E-330 camera, using an Olympus BX41 research microscope. The measurements of the anatomical features were made using the biometrical software from Nikon (NIR-Demonstration). Twenty five measurements were made for each parameter.

Results and Discussion

The root was found a secondary structure; the primary structure was found to be diarch. The secondary xylems occupy the central part of the root while primary xylems are still visible (Fig. 1A). It consists of vessels, scarce fibres and numerous parenchyma cells. The parenchymatic tissue form two wide lateral rays. The secondary phloem has visibly thickened cell walls. At the root periphery, a well developed periderm was observed. Anatomical measurements related to xylem vessels are presented in Table 1. The dimensions and density of the vessels, as well as the quantity of the parenchyma cells are directly linked with the available amount of water (Olson and Carlquist 2001). The dimensions of the vessels from the secondary xylem (with minimum diameters of $5.34 \pm 1.05 \mu\text{m}$ and maximum $11.63 \pm 1.72 \mu\text{m}$) are not likely to show a great deficiency of water in the vegetative period. However, as can be seen in Fig. 1A, narrow vessels are located in the external area of the secondary xylem, being formed right at the beginning of the dry season.

The rhizome has also secondary structure (Fig. 1B). In the secondary wood, parenchyma cells arranged in regular rows are predominant. The phelogen arises from deep layers of cortical parenchyma. The parenchymatous tissues (both from pith and secondary xylem) are well developed, related to their role in starch storage. *C. romanica* overwinter through rhizomes, so the storage tissues are better represented here than in roots.

Cross sections of the stem were taken at three levels: top, middle and base of the stem. At the upper part of the stem (Fig. 1C), the cross section has a circular shape. The epidermis is unilayered, the cortex is thin (4 - 5 cells layers), the primary endodermis is visible. The vascular tissues have secondary structure. The wood shows few vessels and a large amount of sclerenchyma fibres.

At the middle of the stem (Fig. 1D), the secondary wood is well developed (with thick-walled lignified fibres). The epidermis has isodiametric cells, with the external walls covered by a thick striate cuticle; small, unicellular tector hairs, also with walls, could be observed. The presence of a thick cuticle is interpreted as an adaptation to drought with regard to the formation of an efficient transpiration barrier (Burghardt *et al.* 2008).

At the stem basal part (Fig. 1E, F), the phelogen arise from the external layers of the secondary phloem, inside the central cylinder. The primary endodermis (with Casparian thickness) is visible at all levels.

The occurrence of the endodermis in aerial organs is uncommon in angiosperms (Gutenberg 1943). It usually remains in stage 1 with Casparian strips (Lersten 1997). The presence of the endodermis is noticed also by Metcalfe and Chalk (1979) in some members of Campanulaceae. But their functional role in the stem is not clear. It is considered an atavism by Lersten (1997), which, if it did not interfere with function, could be tolerated by the plant organ.

Table 1. Anatomical characteristics (μm) of different tissues in transverse section.

	Root	Rhizome	Stem (base)	Stem (middle)	Stem (top)	Basal leaf	Caulinar leaf
Pxv					9.4 \pm 1.53	5.03 \pm 0.92	4.42 \pm 0.56
Sxv - L	11.63 \pm 1.72	13.7 \pm 2.6	9.53 \pm 3.22	8.06 \pm 2.33			
Sxv - S	5.34 \pm 1.05	6.31 \pm 1.59					
Ep H			12.94 \pm 1.35	11.01 \pm 1.81	12.07 \pm 1.59	19.07 \pm 3.92*	15.07 \pm 3.73*
						13.41 \pm 3.53**	17.09 \pm 3.03**
Ep W			15.45 \pm 5.74	11.21 \pm 2.45	10.74 \pm 2.65	25.24 \pm 7.4*	21.53 \pm 5.74*
						12.8 \pm 3.65**	16.05 \pm 3.41**
Cc D			16.2 \pm 3.93	10.66 \pm 1.85	7.03 \pm 1.98		
Pp (H)						27.68 \pm 3.2	34.27 \pm 4.09
Sp (D)						9.88 \pm 1.68	9.49 \pm 2.57
Lt						126.06 \pm 8.12	120.99 \pm 7.06

Pxv: primary xylem vessels, Sxv - L: secondary xylem vessels - large, Sxv - S: secondary xylem vessels - small, Ep H: epidermic cell's height, Ep W: epidermic cells width (* upper epidermis, ** lower epidermis), Cc D: cortical cells diameter, Pp (H): palisade parenchyma cells (High), Sp (D): spongy parenchyma cells (diameter) and Lt - lamina thickness.

He assumed that Casparian strip formation is stimulated by darkness. This hypothesis is not checked in this case, because the analyzed species living in strong light conditions.

The stem structure shows more xerophytic features than the root and rhizome. The aeriferous spaces are reduced in all parenchymatous tissues, especially in the external parts of the stem. The xylem fibres have thick and well lignified walls, the vessels are rare. The development of mechanical tissue, with cells having lignified walls, is a clear feature of xerophytism.

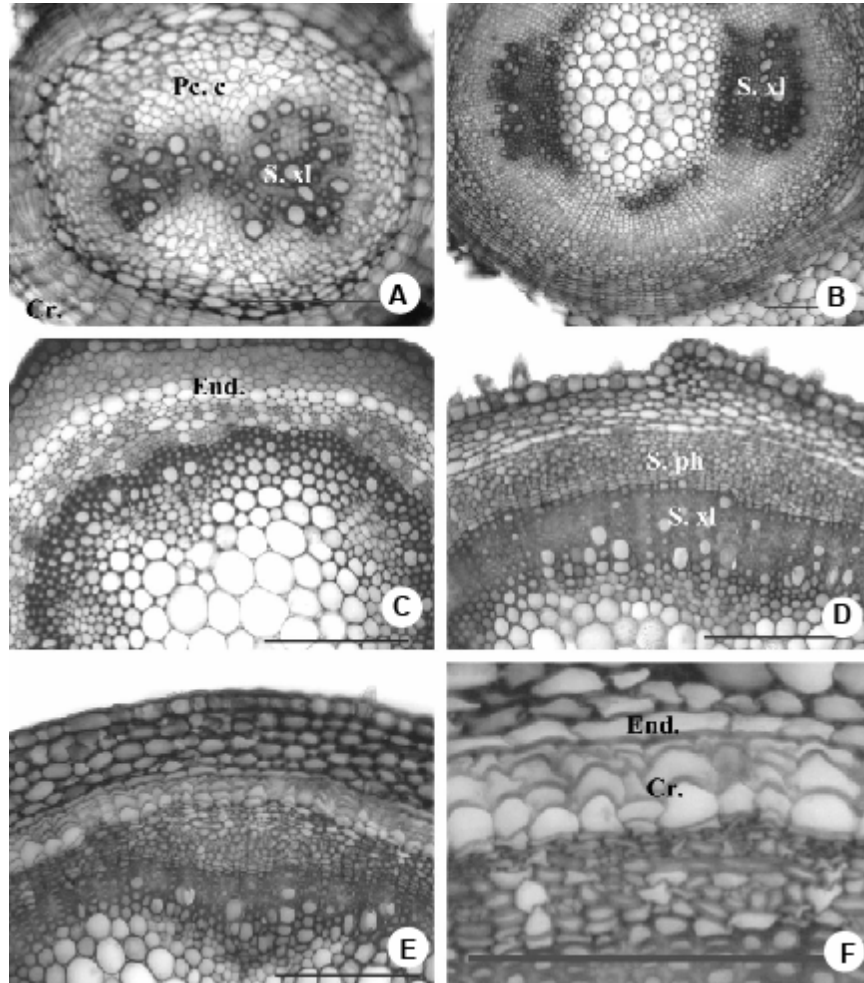


Fig. 1. Cross-section through vegetative axial organs. A. root with secondary structure, B. rhizome. Stem: C. upper part, D. middle part, E, F. basal part (Cr. cork, End. endodermis, Pc. c: parenchyma cells, S. ph: secondary phloem and S. xl: secondary xylem) (Bars: 100 μ m).

The basal and caulinar leaves were investigated (Fig. 2). The basal leaves have a petiole - with a nearly circular shape, with prominent adaxial side and modified by two latero-adaxial wings in cross section. It has one large vascular bundle in the middle of the homogenous parenchyma and two very small bundles in the lateral parts. A sclerenchymatic tissue is observed

in the inner part of the phloem from the central bundle. The leaf's lamina has bifacial heterofacial structure. The stomata cells are present on the surfaces of both epidermis and are often located at the same level as the epidermal cells. The palisade parenchyma has slightly elongated cells, 1 - 2 layered. The palisade cells are higher ($34.27 \pm 4.09 \mu\text{m}$) in the caulinar leaves, receiving a greater amount of light, than in the basal leaves ($27.68 \pm 3.2 \mu\text{m}$). The spongy parenchyma cells are 6 - 7 layered with small intercellular spaces. The midrib region is slightly prominent at the abaxial and adaxial sides. Only one vascular bundles (collateral type) is visible in the midrib (without mechanical tissues). The caulinar leaves are sessile, with thinner foliar lamina ($120.99 \pm 7.06 \mu\text{m}$) than the basal ones ($126.06 \pm 8.12 \mu\text{m}$).

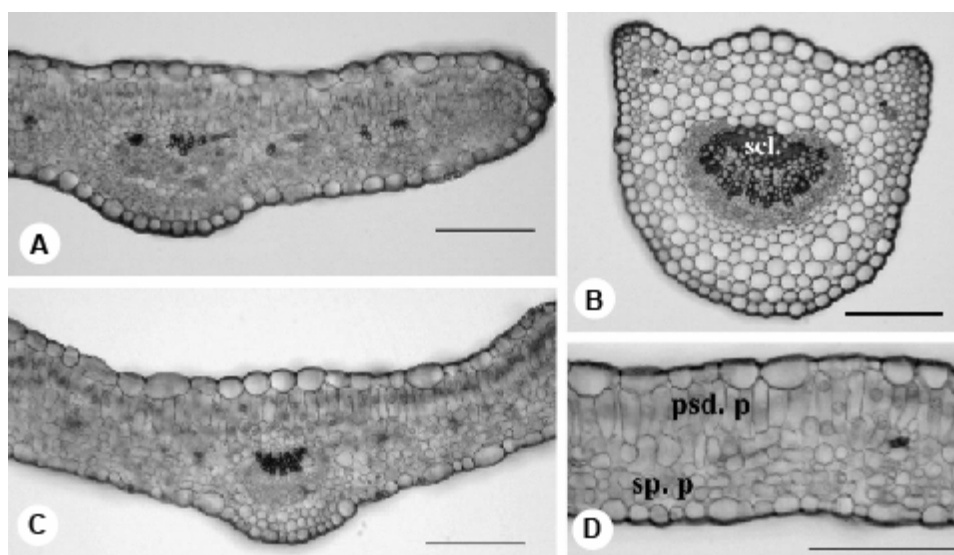


Fig. 2 A-D. Cross-section through the leaves: A. basal leaf, lamina. B. basal leaf, petiole. C. caulinar leaf, mid rib. D. caulinar leaf, area between ribs (sp. p: spongy parenchyma, scl: sclerenchyma, psd. p: palisade parenchyma) (Bars: $100 \mu\text{m}$).

Leaves are the most important organs of the plants, which can change their structure to adapt to the environmental conditions. *C. romanica* leaves show some traits of xerophytic characters (Dickison 2000) such as thick cuticle, thickened external epidermis cell walls, compact palisade parenchyma, small aeriferous spaces. However, some typical characters such as localized stomata at the same level with the epidermis cells and not below the blade surface, weak mechanical tissue are missing.

The lack of strong xeromorphic characteristics of the leaves of plants living in semi-arid habitats was also noticed in *Ecbalium elaterium* and *Adonis vernalis* (Christodoulakis *et al.* 2011, Gostin 2011).

Macin Mountains has a continental climate with Mediterranean influences in higher areas and steppe features in their south. Summers are hot and dry, long and dry autumns and cool-wet winters with little humidity. Generally, across the mountains, there is a wetter climate than surrounding areas. Water from precipitation, retained in the cracks of rocks and valleys steppe vegetation maintain a microclimate that favours the development of a rich phytocoenosis (Pisota and Bogdan 2005, Gerakis *et al.* 1975). These environmental conditions could explain the

moderate xeric features which were found in vegetative organ structure of *C. romanica*. From this point of view, *C. romanica* should be included in “drought-escaping” group species, rather than in “drought-enduring” group species, according to Dickison (2000).

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References

- Alçitepe E 2008. Morphological, anatomical and palynological studies on *Asyneuma michauxioides* (Campanulaceae). *Biologia* **63**(3): 338-342.
- Alçitepe E 2012. Comparative pollen morphology of sect. *Quinqueloculares* (Campanulaceae) in Turkey. *Biologia* **67**(5): 875-882.
- Alçitepe E and Yildiz K 2010. Taxonomy of *Campanula tomentosa* Lam. and *C. vardariana* bocquet from Turkey. *Turk. J. Bot.* **34**: 231-240.
- Burghardt M, Burghardt A, Gall J, Rosenberger C and Riederer M 2008. Ecophysiological adaptations of water relations of *Teucrium chamaedrys* L. to the hot and dry climate of xeric limestone sites in Franconia (Southern Germany). *Flora* **203**: 3-13.
- Celik S, Uysal T and Menemen Y 2008. Morphology, anatomy, ecology and palynology of two *Centaurea* species from Turkey. *Bangladesh J. Bot.* **37**(1): 67-74.
- Christodoulakis NS, Kollia K and Fasseas C 2011. Leaf structure and histochemistry of *Ecballium elaterium* (L.) A. Rich. (squirting cucumber). *Flora* **206** (3): 191-197.
- Ciocârlan V 2009. Flora ilustrată a României, Pteridophyta et Spermatophyta. Editura Ceres București. pp. 1141.
- Dickison WC 2000. Integrative Plant Anatomy. Academic Press, San Diego. pp. 533.
- Dihoru G and Pârva C 1987. Plante endemice în flora României, Editura Ceres București. pp. 180 pp.
- Fedorov A and Kovanda M 1976. Campanulaceae. *In: Flora Europaea*, Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM and Webb DA (Eds), pp. 74-93. Cambridge University Press.
- Gerakis PA, Guerrero FP and Williams WA 1975. Growth, water relations and nutrition of three grassland annuals as affected by drought. *J. Appl. Ecol.* **12**(1): 125-135.
- Gostin IN 2011. Anatomical and micromorphological peculiarities of *Adonis vernalis* L. (Ranunculaceae). *Pak. J. Bot.* **43**(2): 811-820.
- Guttenberg H von 1943. Die physiologischen Scheiden. *In: Handbuch der Pflanzenanatomie*, Linsbauer K (Ed), pp. 1-217. Gebrüder Borntraeger, Berlin.
- Lersten NR 1997. Occurrence of endodermis with a Casparian strip in stem and leaf. *The Botanical Review* **63**: 265-272.
- Metcalf CR and Chalk L 1983. Anatomy of the Dicotyledons. Clarendon Press, Oxford. pp. 330.
- Ocak A and Tokur S 1996. Anatomical investigations on *Campanula* L. taxa that distributed in (B3) Eskisehir Region. *Turk. J. Bot.* **20**: 221-231.
- Olson ME and Carlquist S 2001. Stem and root anatomical correlations with life form diversity, ecology, and systematics in *Moringa* (Moringaceae). *Bot. J. Linn. Soc.* **135**: 315-348.
- Pisota I and Bogdan O 2005. Clima Podisul Dobrogei de Nord, Geografia Romaniei. Editura Academiei Romane, Bucuresti. pp. 170.
- Potoğlu EI, Ocak A and Pehlivan S 2008. Pollen morphology of some Turkish *Campanula* spp. and their taxonomic value. *Bangladesh J. Bot.* **37**: 33-42.
- Roquet RC 2008. Evolució, sistemàtica i biogeografia de *Campanula* L. i relacions filogenètiques amb gèneres afins de Campanulàcies. Thesis, Universitat Autònoma de Barcelona. pp. 158.

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