ANATOMICAL PECULIARITIES OF THE VEGETATIVE ORGANS FROM TWO SPECIES OF THE GENTIANACEAE FAMILY

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Abstract: In this article, two species within the Gentianaceae family: Gentiana cruciata L. and Gentianopsis ciliata (L.) Ma (Gentiana ciliata L., Gentianella ciliata (L.) Borkh.) have been studied. Some anatomical features of the two species were highlighted, features which have theoretical value and contribute to the enrichment of the existing data on the anatomy of the Gentianaceae family. The results from the present study confirm the known characteristics from the classical works, but also bring new elements, highlighted in the root bark and leaf epidermis.

Key words: Gentianaceae family, anatomical peculiarities, root exodermis, stomatal apparatus, traditional medicine

Introduction

The Gentianaceae family is represented by eight genera in Romanian flora, Gentiana genus including the most and best known species. The Gentianopsis genus, proposed in 1951 by Ma, comprises a single species, G. ciliata. In the “Flora of Romania” (Ţopa, 1961) the species was listed in the Gentiana genus and the “Flora of the USSR” (Grossheim, 1951) was included in Gentianella genus. In the most subsequent revisions, the newly created genus, Gentianopsis, was accepted (Ciocârlan, 2000; Czerepanov, 2007; Ştefan and Oprea, 2007). Initially both genera, Gentianopsis and Gentianella were based only on the basis of morphological criteria, but anatomical caryological, chemical, pollinic and molecular evidences were subsequently brought (Gielly and Taberlet, 1996). Nowadays, in many parts of its distribution area, Gentianopsis ciliata is considered in decline or a rare species - in the UK, Netherlands, Switzerland, France (Kéry and Matthies, 2004; Oostermeijer et al., 2002).

Numerous species within Gentianaceae family have ornamental value, especially those belonging to the Gentiana genus, but also present pharmaceutical interest due to its interesting phytochemical properties. In the pharmaceutical preparations, the species from Gentianaceae family are used for their high content of iridoids which determine the bitter characteristic taste. They are still used in the preparation of bitter, traditionally used as a remedy against the decrease of appetite and fever, and still included in many "tonic” recipes (bitters). In medicine, Gentiana cruciata (cross gentian) is less common, but Butură (1979) mentions that it is utilized in traditional medicine for external use (leaves are used for wound healing) or intern use (roots are used for digestive system diseases). The species has similar uses in mountainous areas of Bosnia (Tuka et al., 2011), and in 1819 it was mentioned in a London medical dictionary (Parr, 1819). Monitoring of this species in order to reconsider its pharmaceutical properties had as result its protection by law in Hungary and Lithuania (Baričević et al., 2004).

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In literature, there are numerous anatomical studies on representatives of this family especially on species recognized used in medicinal purposes (Tüzün et al., 2011). Recent works give important information in order to clarify the ontogenetic mechanisms involved in root development (Sotníková and Lux, 2003) or in the structure of the stomatic system (Stanescu et al., 2010).

Materials and methods

The studied material was collected from the wild, in July 2012, and is represented by two taxa of the Gentianaceae family. Gentiana cruciata L. (cross gentian), harvested from Scăriţa Belioara geo-botanical reserve (Alba county), is a perennial, xero-mesophytic species, with Eurasian areal. It presents a 1-2 cm thick rhizome with fasciculated roots. Gentianopsis ciliata (L.) Ma (Gentiana ciliata L., Gentianella ciliata (L.) Borkh.), harvested from Valea Ierei (Cluj county), is a biannual-perennial, oligotrophic, xero-mezophytic species, with central European – sub-Mediterranean areal. It has a fusiform rhizome with ramifications from the adventive buds and the middle and upper leaves are described as uninerved (Grossheim, 1952; Țopa, 1961).

The vegetative organs of the two taxa have been processed in accordance with histology conventional methods (Toma (eds.), 2000), using dual staining with green iodine and red ruthenium. Preparations obtained were analyzed with an Optika optical microscope and the results were illustrated with images obtained using a Nikon camera.

Results and discussions

The young root (Fig. 1) has a typical primary structure. The rhizoderm, devoid of absorbent hairs, is made up of cells with thick outer wall, indicating that the roots form mycorrhizae. The bark starts with a uniseriate exoderm composed of hexagonal cells with thickened walls, obviously the internal one in a smaller proportion. Cortical cellulose parenchyma presents 4-5 layers of cells with irregular shape, with dimensions generally centripetally decreasing, together with numerous and large intercellular spaces. In time, most of them are destroyed by the walls lysis, so there are many endogenous cavities. Secondary endoderm include radial elongated cells in which are observed, in the majority of cases, one or more tangentially dividing walls. The central cylinder contains conductive xylem fascicles (composed of vessels with thickened and lignified walls) and conductive phloem fascicles very poorly represented, all implanted in a cellulosic mass. The cylinder may be di- or tetrarch.

In a cross-section of a mature root (Figs. 2-4), the edification of the secondary structure (due to the cambial activity) is observed. The rhizoderm is destroyed for the most part, and only sporadically can be observed large, isolated cells. The bark appears thin compared to the diameter of the section. The exoderm presents cells more tangentially elongated due to numerous anticline divisions, with uniformly thickened walls. The cortical parenchyma is throttled because of exo - and endoderm development, so that it is reduced to 2-3 layers of cells with thin walls, in process of destruction. Secondary endoderm is well defined and presents greatly tangentially elongated cells through numerous radial divisions (often over 20); the Caspary bands remain visible in the side initial walls of the cells. The central cylinder occupies about 80% of the root area, contains a massif of xylem, well
represented by vessels of various diameters, with thickened and lignified walls, among which there are a few libriform elements. At the exterior of this massif there is an irregular phloem ring.

The presence of endoderm cells with numerous anticlinal walls is a characteristic of the Gentianaceae family, mentioned in all the classical works (Metcalfe and Chalk, 1972). The presence of exoderm with tangentially elongated cells in which there are anticlinal division walls is less mentioned (El-Shanawany et al., 2004), although its characteristics (alongside endoderm features) are involved in with the secondary root development (Seago and Fernando, 2013). In Gentianopsis ciliata, alongside anticlinal divisions, endoderm cells with transversal or oblique divisions can be observed also (Lux and Luxová, 2001). The number of the observed anticlinal walls exceeds the number mentioned in the treaty of Metcalfe, but such observations have been previously made in Gentiana asclepiadea.

Secondary roots formed on pericicle account shows that initially the central cylinder is diarch and then is tetrarch at least in the main root.

**The rhizome** (Figs. 5, 6) is approximately circular shaped, protected by epidermal tall cells in Gentiana cruciata and tangentially elongated in Gentianopsis ciliata. Central cylinder is separated by the cellulosic bark through the endoderm only in Gentiana cruciata. The vascular system is represented by a xylem massif in Gentiana cruciata, which in Gentianopsis ciliata is fragmented and in its structure presents xylem vessels dispersed in the cellulosic parenchyma and libriform respectively.

The cross section through the middle region of the stem (Figs. 7-10) has a hexagonal shape with two pairs of opposite ribs in Gentianopsis ciliata and oval, slightly irregular in Gentiana cruciata. The epidermis contains isodiametric cells, with internal and external walls thickened; the latter dished and covered by a thick cuticle. At Gentianopsis ciliata the cells have the internal wall less thickened and cuticle is smooth, while the cuticle is laced in Gentiana cruciata and the hypodermic stratum is collenchymatized.

The bark is better developed (8-9 cell layers) in Gentiana cruciata comparing to Gentianopsis ciliata (5-6 cell layers), and in the latter case the layers are loose because of the large intercellular spaces and endogenous cavities occurrence. The endoderm is of a secondary type, with the Caspary bands visible in the side walls. In Gentiana cruciata there is no special stratum delimiting and defining the bark on the inside.

The central cylinder starts with a phloem ring which in the above mentioned species is separated from the xylem ring by 4-5 layers of cambial elements. The xylem ring consists of libriform elements prevailing outside and vessels extended (the primary timber) from place to place towards the axis center in Gentianopsis ciliata, forming a discontinuous ring. In the center there are islands of intra-axial phloem arranged on a ring, well developed and with more numerous elements in Gentiana cruciata compared with Gentianopsis ciliata. In this species the stem axis is occupied by medulla composed of cellulosic parenchyma with large cell interspaces, which begins to lyse in the center, creating a medullar canal. In Gentiana cruciata the medulla is thicker (covering almost 50% of the stem) and presents within scattered islands of phloem, which, in some cases, have 1-2 elements of xylem parenchyma.

**The leaf** - the epidermis (Figs. 11, 12) of the leaf lamina highlights, in both species, large cells with corrugated walls, the corrugations having about the same magnitude both in the upper epidermis and the bottom epidermis. Around the ribs, the cells are polygonal shaped and more tangentially elongated.
The stomata are very numerous in lower epidermis of both species and very rare in the upper epidermis in *Gentianopsis* so, the lamina is hypostomatic in *Gentiana cruciata* and amphistomatic in *Gentianopsis ciliata*. In *Gentiana cruciata*, the anomocytic type is predominant, while in *Gentianopsis ciliata*, the anyzocytic type prevails, but both types are present in both taxa. At *Gentianopsis ciliata*, in the lower epidermis, intertwined stomata are frequently met (sometimes even with a common wall), and more rarely, diacytic stomata type can be observed. The guard cells in anyzocitic or diacytic stomata type are smaller (shorter), while in the anomocytic type they are much higher (4-5 times longer than in the previous case).

Classic vegetal anatomy treaties consider the anomocytic type as characteristic for *Gentianaceae*, but recent studies show that stomatic apparatus is more varied. Patel & al. (1981) included the anomocytic, actinocytic, anyzocytic, paracytic, diacytic types and stomata with a single guard cell. The presence of anyzocytic type was not highlighted on *Gentiana cruciata* material collected in Iran (Zarinkamar, 2007), this type being especially cited for *Centaurium* species (Metcalfe and Chalk, 1972; El-Shanawany et al., 2004). The presence of cytoplasmic connections between the guard cells of adjacent stomata is mentioned by Gill & Nyawuame (1990) which states that this is a rare phenomenon, observed by them in *Gentianaceae*, *Oleaceae* and *Solanaceae*.

The cross section of lamina, in both species (Figs. 13-16), have a ribbon shape, with very strong median rib on the abaxial side. Both epidermis include polygonal cells with external wall covered with a thin cuticle; from place to place in lower epidermis there are visible stomata. The median rib comprises one or two conductive fascicles consisting of numerous xylem vessels and libriform elements arranged in an arc, with thickened and lignified walls. The phloem is present outside the xylem arc, consisting of sieve tubes and annex cells, in *Gentianopsis ciliata* only to the ventral side. Also in this species is observed a layer of parenchymal cell surrounding the vascular fascicle, reminding of a parenchyma sheath. The sheath is clearly evident in both species, in the secondary ribs.

The mesophyll is differentiated in both species, the lamina having a bifacial dorsiventral structure. The palisade tissue is un- or bi-stratified in *Gentiana cruciata*, with typical tall cells while in *Gentianopsis ciliata* is uni-stratified with almost squared cells. The lacunar tissue, in both cases, consists of un-uniform small cells, with large intercellular spaces, so the overall look is very lax.

It is worth noting that in the lamina of *Gentianopsis ciliata* were observed both secondary and tertiary ribs. They occupy a small percentage of leaf lamina section, are represented by very few elements and never come into direct contact with any of the epidermis. Their presence requests a clarification of the term "uninerved" used in the „Romanian Flora” and „USSR Flora”, in the species description, since it is valid only in for the macroscopic perspective.

**Conclusions**

The two analyzed species present specific characters for the botanic family to they belong, but some new particularities were observed. Thus, the root presents in the secondary structure not only endoderm with numerous radial divisions, but also exoderm, which explains the development way of this organ.
In cross section the *Gentianopsis ciliata* leaf has numerous secondary ribs, so that the term "uninerved" used to describe species in „Flora of Romania” and „Flora of Russia” is only valid for macroscopic perspective.

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Explanation of the plates

Plate I
Fig. 1. Gentiana cruciata - cross section through a primary root – tetrarch cylinder (x 20)
Fig. 2. Gentiana cruciata - cross section through a root (x 20)
Fig. 3. Gentiana cruciata - cross section through a root - anticlinal walls (x 20)
Fig. 4. Gentianopsis ciliata - cross section through a root - exoderm with tangentially elongated cells (x 20)
Fig. 5. Gentiana cruciata - cross section through a rhizome (x 10)
Fig. 6. Gentianopsis ciliata - cross section through a rhizome (x 20)
Fig. 7. Gentiana cruciata - cross section through a middle stem - collenchymatized hypodermis (x 20)
Fig. 8. Gentiana cruciata - cross section through a middle stem - lack of endoderm (x 20)

Plate II
Fig. 9. Gentiana cruciata - cross section through a middle stem - islands of phloem (x 20)
Fig. 10. Gentianopsis ciliata - cross section through a middle stem - presence of endoderm (x 10)
Fig. 11. Gentiana cruciata - superficial section of lower epidermis of lamina - anomocytic type stomata (x 20)
Fig. 12. Gentianopsis ciliata - superficial section of lower epidermis of lamina - different type stomata (x 20)
Fig. 13. Gentiana cruciata - cross section through a leaf lamina - middle vein (x 20)
Fig. 14. Gentianopsis ciliata - cross section through a leaf lamina - middle vein (x 20)
Fig. 15. Gentiana cruciata - cross section through a leaf lamina - secondary vein (x 20)
Fig. 16. Gentianopsis ciliata - cross section through a leaf lamina - secondary vein (x 20)
PLATE I

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8