

MORPHOLOGICAL AND ANATOMICAL RESEARCHES ON *VIOLA ARVENSIS* MURRAY (*VIOLACEAE*)

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Abstract

The aim of this work was to carry out a morphological and anatomical study on the stem, leaf and stipel of *Viola arvensis* Murray by optical microscopy (OM) and scan electronic microscopy (SEM). We have shown some differential macroscopical characteristics that are necessary in order to identify this species and to emphasize some differential microscopical characteristics: the bifacial structure of the leaf and leaf's unicellular trichomes, the bifacial structure of the stipule, closely related to the leaf, the solid inclusions, the type of the vascular bundles, and we have made some micrometrical measurements. The images achieved by SEM means showed some differential characteristics concerning the trichomes and stomata and they represent elements of novelty in our country and abroad. Our results may serve as a criterion for interspecific differentiation and may have taxonomic value in the *Viola* genus.

Rezumat

Scopul acestei lucrări a fost efectuarea unui studiu morfologic și anatomic asupra tulpinii, frunzei și stipelei speciei *Viola arvensis* Murray cu ajutorul microscopiei optice (MO) și al microscopiei electronice de baleiaj (MEB). Au fost evidențiate caractere diferențiale macroscopice necesare identificării speciei, precum și caractere microscopice: structura bifacială a frunzei, formațiunile ei de apărare (peri tectori unicelulari), structura bifacială a stipelei, foarte asemănătoare cu cea a limbului foliar, incluziunile solide, tipul de fascicule conducătoare și au fost efectuate măsurători micrometrice. Imaginile realizate cu ajutorul MEB oferă date asupra unor caractere diferențiale ale perilor tectori și stomatelor și reprezintă elemente de noutate atât în țara noastră, cât și pe plan internațional. Rezultatele obținute pot servi drept criteriu de diferențiere interspecific și ar putea avea valoare taxonomică în cadrul genului *Viola*.

Keywords: *Viola arvensis*, optical microscopy, scan electronic microscopy.

Introduction

Viola genus contains about 500 species, widely spread all over the world and about 30 species are growing wild in Romania. While *Viola tricolor* L. (wild pansy) is considered the most important, there are known some other indigenous species: *V. arvensis* Murray, *V. odorata* L., *V. declinata* Waldst. et Kit. [2,11]. The aerial parts (*Violae tricoloris herba*) are used in traditional medicine to treat various skin conditions (eczema,

acne, pruritus), bronchitis and rheumatism. Their anti-inflammatory, expectorant and diuretic properties are due to the presence of the following active principles: saponins, flavonoids, mucilages, salicylic derivatives, carotenoids [5,15,16]. The cyclotides were recently identified and studied in some *Violaceae* species, those bioactive macrocyclic plant polypeptides have some important pharmacologic properties: anti-HIV, antitumor, uterotonic [4,7,9].

Although in traditional medicine the aerial parts of *V. tricolor* can be used together with the aerial parts of *V. arvensis* for the same properties [5], and in the European Pharmacopoeia the vegetal drug "*Violae herba cum flore*" contains the aerial parts of both species [1], the chemical composition of *Viola arvensis* was less studied [6,8,14].

In the scientific literature there is little information on the anatomy of *Viola species*, presented by Metcalfe and Chalk in 1965 [10], but there are mentioned only the main characters of some species from *Viola* genus: *V. robusta*, *V. luciae*, *V. maiensis*, *V. stoksii* and *V. cunninghamii*. *Viola tricolor* L. was less studied from the botanical point of view, there are histo-anatomical data on root, stem and leaf [3] and the electronic microscope scan analysis of the leaf's epidermis [13].

The aim of this paper was to complete these studies with morphological data on *Viola arvensis* by electronic microscope scan analysis on the leaf's epidermis and by optical microscope analysis of stem, leaf and stipule.

Material and methods

We have analysed the aerial parts of *Viola arvensis* Murray harvested from the spontaneous flora of Cluj county (Romania) in May 2005. Dried pieces of vegetal material (fragments of leaf) were used for scan electronic microscope analysis. Fresh pieces of vegetal material (fragments of stem, leaf and stipel) were fixed in alcohol:acetic acid (3:1) solution for 24 hours. Then they were washed with distilled water and processed according to the technique for inclusion into paraffin for the optical microscope analysis [12].

Scan electronic microscope analysis: dried 0.7x1 cm pieces of vegetal material were analysed using JEOL JSM-5510LV apparatus (Scanning Electron Microscope).

Optical microscope analysis: we used the histological technique for inclusion into paraffin, sectioned by means of American Optical microtome (serial 7µm sections), and the staining of the obtained sections with different

reagents: iodine green-alum carmine solution (R_1) and toluidine blue 0.2% water solution (R_2) [12]. The micrographs were taken using an Olympus BX51 microscope with digital camera.

Results and discussion

Stem transverse section of *Viola arvensis* is a typical primary structure for Dicotyledons. We observed the presence of epidermis with medium cells, but well differentiated, with obvious cuticle, the parenchymatic sub-epidermal tissue is an assimilatory tissue or chlorenchymatic parenchyma with small cells which sustain chloroplasts, the cortical parenchyma with bigger cells, 11 collateral type separated vascular bundles, with oval form in transverse section, and the xylem vessels well-represented orientated into radial series.

The perimedullary parenchyma has big cells, disposed into 2 or 3 layers, and in the central part of the stem is a big central lacuna. *Viola arvensis* stem structure is asymmetric, determined by the 3 edges, the bundles are disposed separately. *Viola arvensis* stem transverse sections are presented in figure 1a and figure 1b.

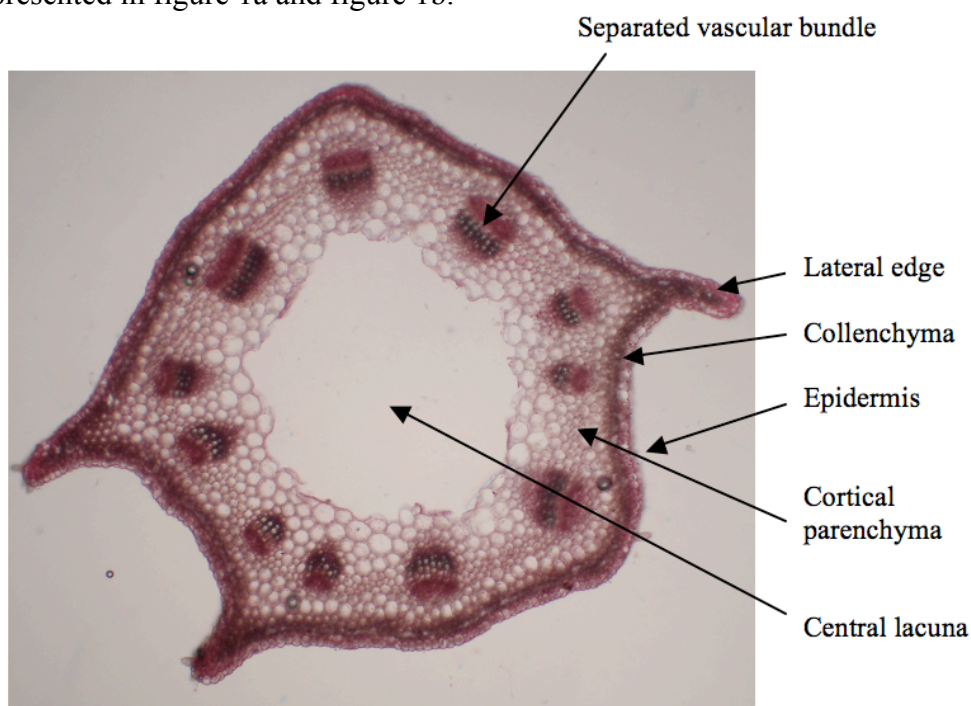


Figure 1a.

Viola arvensis stem transverse section, 4x objective (in R_1)

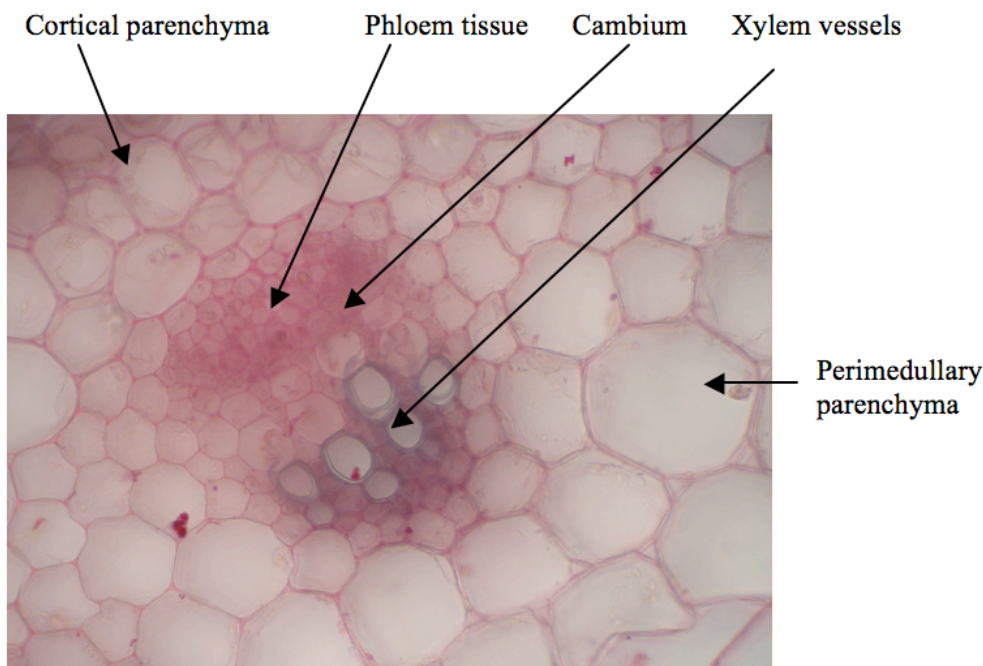


Figure 1b.

Viola arvensis stem transverse section, vascular bundle, 20x objective (in R₂)

Leaf transverse sections of *Viola arvensis* showed a bifacial structure (figure 2a), the palisade tissue from the upper surface has 1-2 layers, and between the cells there are intercellular spaces. The epidermal cells are big, with a well-developed cuticle. The stomata are disposed in both epidermis, and in the central surface of the foliar limb, between the palisade and lacunose tissues there are many cells with crystals of calcium oxalate, bigger than the adjacent cells. The vascular bundles from the nervure are collateral, with series of xylem vessels orientated towards the superior epidermis and phloem bundles towards the inferior part.

Comparing with the leaf structure of *Viola tricolor* [13], we can observe more cells with calcium oxalate and that the epidermal cells are transformed into sharp unicellular trichomes.

The stipule transverse section (figure 2b) showed a bifacial structure, with big epidermal cells, the palisade parenchyma continuing in the principal nervure area, where cells are transformed into trichomes and the stomata are disposed in both epidermis. The thickness of the assimilator tissue from stipules is smaller than the one of the leaf.

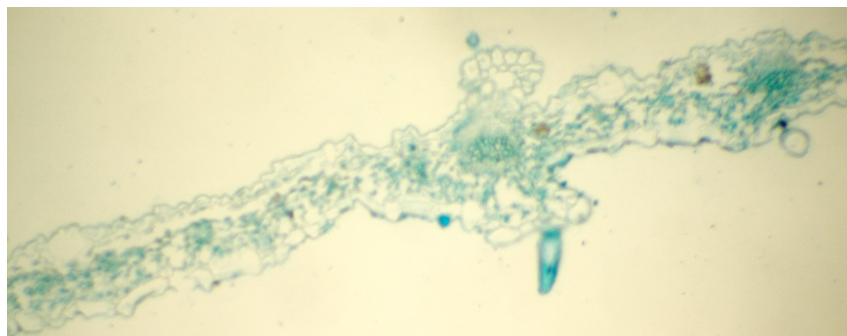


Figure 2a.

V. arvensis leaf transverse section, 10x objective (in R₂)

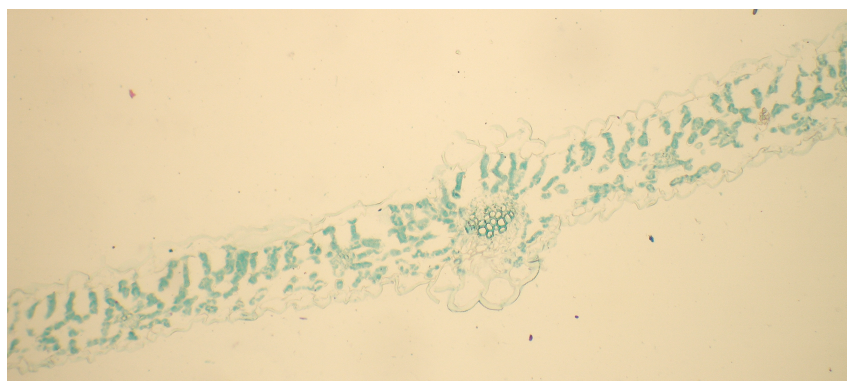


Figure 2b.

V. arvensis stipule transverse section, 10x objective (in R₁)

The images obtained by SEM show some differential characters that haven't been mentioned in the literature until now: unicellular trichomes, more frequent in the nervure area (figure 3a), 100-125 μ m long (figure 3b).

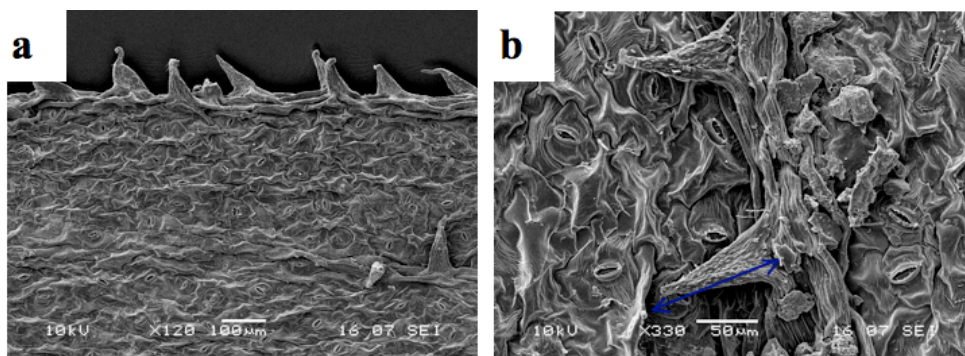


Figure 3.

Scan electronic microscopy images for *V. arvensis* leaf: trichomes (a), micrometrical measurements (b)

Conclusions

This is the first anatomo-morphological study on the stem, leaf and stipule of *Viola arvensis*, achieved by optical microscope analysis and scan electronic microscope analysis. The described characters could have taxonomic value, completing our previous studies on *Viola* species and may have taxonomic value in the *Viola* genus.

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