

## ANATOMICAL AND MICROMORPHOLOGICAL PARTICULARITIES OF VEGETATIVE ORGANS IN ENDEMIC *Erysimum wittmanii* Zaw. ssp. *wittmanii*

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**Abstract:** The *Erysimum wittmanii* ssp. *wittmanii* is an endemic biennial herbaceous species in South and East Carpathians. In this study the anatomical and micromorphological properties of this specie are investigated. Anatomical properties of root, stem and leaves were underlined. Roots acquire early in ontogenesis the secondary structure. Stems presents collateral vascular bundles both in primary and in secondary structure; cortical small bundles by concentric type, was observed on the cross sections from the top of the stem. The leaves are bifacial and amphystomatic. Scanning electron microscopy investigation regarding upper and lower epidermises of leaves in different developmental stages was carried out. Trichomes occur on both faces of young leaves. The tector hairs are multicellular, usually three-armed; rarely four-armed or two-armed (especially on the epidermis from the veins). The hairs cell walls are thin and the surface is smooth in the first developmental stages (on very young leaves); on the young and mature leaves the trichomes have thick-walled cells, densely covered by micro-papillae.

**Keywords:** endemic, stem, root, leaves, trichomes, SEM.

### INTRODUCTION

*Erysimum* (wallflowers) is a genus that includes about 180 species. It has recently described to a monogeneric cruciferous tribe, *Erysimeae* [7]. This tribe is distinguished by exclusively sessile, stellate and/or malpighiaceus (two-armed T-shaped) trichomes, yellow flowers and multi-seeds siliques.

*Erysimum wittmanii* ssp. *wittmanii* (*Brassicaceae*) is a South - Est Carpathian endemism [5, 13].

Anatomical investigation of some endemic species from *Brassicaceae* family and even on some *Erysimum* species was previously reported in the literature. Morphological, anatomical, ecological and phenological properties of *Erysimum amasianum*, which is endemic in Central Black Sea Region-Turkey was investigated by Cansaran and their collaborators (2007) [4]. Collenchyma is present in the stem, under the epidermis; the leaf is isobilateral with anisocytic stomata. In the same time, SEM data regarding pollen, fruit and seed coat from endemic *Erysimum pieninicum* (from Pieniny Mountains – Poland) was reported [11]. Tector hairs were described from the external surface of the siliqua; they are especially stellate, with three or four branches, rarely with two branches. An ultrastructural study of petal cells of *Erysimum cheirii* showing cellular structure and plastid development with a view to comparing the basic development with that of white *Arabidopsis* petals was made in 1999 [14].

There is not any study in the literature related with the structure and the ultrastructure of *Erysimum wittmanii*. In the present study, this endemic specie, of which the future is in danger [8], has been examined in micromorphological and anatomical aspects.

### MATERIALS AND METHODS

The plant material was collected from National Park Cheile Bicazului – Hasmaş in July 2008. For histo-anatomical analysis the vegetal material (whole plants in anthesis phase) was fixed and conserved in ethylic alcohol 70%. For anatomical analysis, cross

sections of root (middle, stem (top, middle and basis) and leaves (from the top and the middle of the stem) were used. Free hand sections were performed using a razor blade. The sections were coloured with Iodine Green and Ruthenium Red. The photos were taken with an Olympus E-330 photo camera, using an Olympus BX51 research microscope.

Scanning electron microscopy (SEM) investigations: the investigated material consists in small pieces of leaves in different developmental stages. Squares by 5 mm x 5mm was cut from the terminal part (under the inflorescence) (young leaves) and from the middle part of the stem (mature leaves). Very young leaves (2-3 mm) were excised from the axilar ramifications of the stem. The vegetal material were fixed in FEA (formol: ethylic alcohol 70%: acetic acid –5:90:5) for 48 hours, washed with distilled water and stored in 70% ethanol [2]. After dehydration in a graded ethanol series (80%, 90% and 100%) and acetone, the material was critical point dried with CO<sub>2</sub> (using a EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (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.

### RESULTS

**The root:** at the analysed level, the root has secondary structure (Fig. 1A). Periderm consists of three different tissues: phellem (3-5 layers of cells with relative thin walls), phellogen (1 layer) and phelloderm (1-2 layers). Cortex is thin, parenchymatic under the periderm. Secondary phloem is ring – shaped; it consists especially in parenchyma cells and grouped sieve tubes and companion cells. Cambium is 1-2 layered. Secondary xylem occupies a wide area. In their external part the fibres are numerousness, with thick and intense lignified walls. In the central part parenchyma cells could be observed between xylem vessels.

**The stem:** in cross section the stem is circular, with numerousness irregular ribs (Fig. 1B). At their top, the

stem has primary structure. The epidermis consists in a single cells layer with thick cutinized external walls; the insertion points of the tector hairs could be observed. The stomata are localised at the same level with the epidermis cells (Fig. 1E). The cortex is exclusively by parenchymatic type, the cells are isodiametrical, larger in the internal part. The mechanic tissue (collenchyma) is missing. The vascular bundles are distributed on an irregular ring (Fig. 1B). Cortical bundles are visible at this level (Fig. 1D). No mechanical tissue is visible at the bundles periphery.

In the middle part of the stem the largest bundles slightly passed to secondary structure. The first conducting elements of the phloem and belonging to the protophloem are already crushed (Fig. 1F). The same phenomenon is observed on the protoxylem vessels. At the xylem level, the rays have already cells with moderate lignified walls (Fig. 1C). This feature is visible even between small vascular bundles from the top of the stem (Fig. 2A).

At the stem basis the secondary structure is complete (Fig. 2B). The vascular bundles present sclerenchyma strands at their phloemic pole (Fig. 2C). The sclerenchymatic cells are smaller in the secondary phloem vicinity and larger, with unlignified walls at the external side. The secondary xylem consists predominantly by vessels; the fibres have strongly lignified walls. The rays (both intrafascicular than interfascicular) have cells with very thick and lignified walls (Fig. 2A & 2C). The pith has also sclerified cells.

**The leaves:** There is a single layered epidermis on the upper and lower surface of the leaf. There are ramified hairs on both surfaces. The mesophyll is dorsiventral, with 2 – 3 layers of well - defined, homogenous cells in the palisade parenchyma (Fig. 2E & 2F). Spongy parenchyma cells are 5 - 6 layered. Stomata are present in both epidermis (amphistomatic leaves). Vascular bundle is collateral type (Fig. 2D).

**Micromorphological investigations:** very young, young and mature leaves were investigated using scanning electron microscopy.

On the very young leaves the cuticular surfaces are smooth, without visible striations. The tector hairs have no specifically wart-like protuberances (Fig. 3A).

On the young leaves, the stomata, by anisocytic types, are present in both epidermises (Fig. 3B & 3E). At a higher magnification the striate cuticle, consisting of the striae running parallel with the longitudinal axis of the leaf (epidermis cells) or with stomata walls (subsidiary cells) become visible (Fig. 3C & 3F). The leaf epidermises are covered by three-armed (Fig. 3B), rarely two-armed (Fig. 3D) (on veins epidermis) trichomes. The arms cells are right or curved (Fig. 3B & 3E) and have acute ends. The hairs are more densely on the lower epidermis.

On the mature leaves, the number of the trihomes is

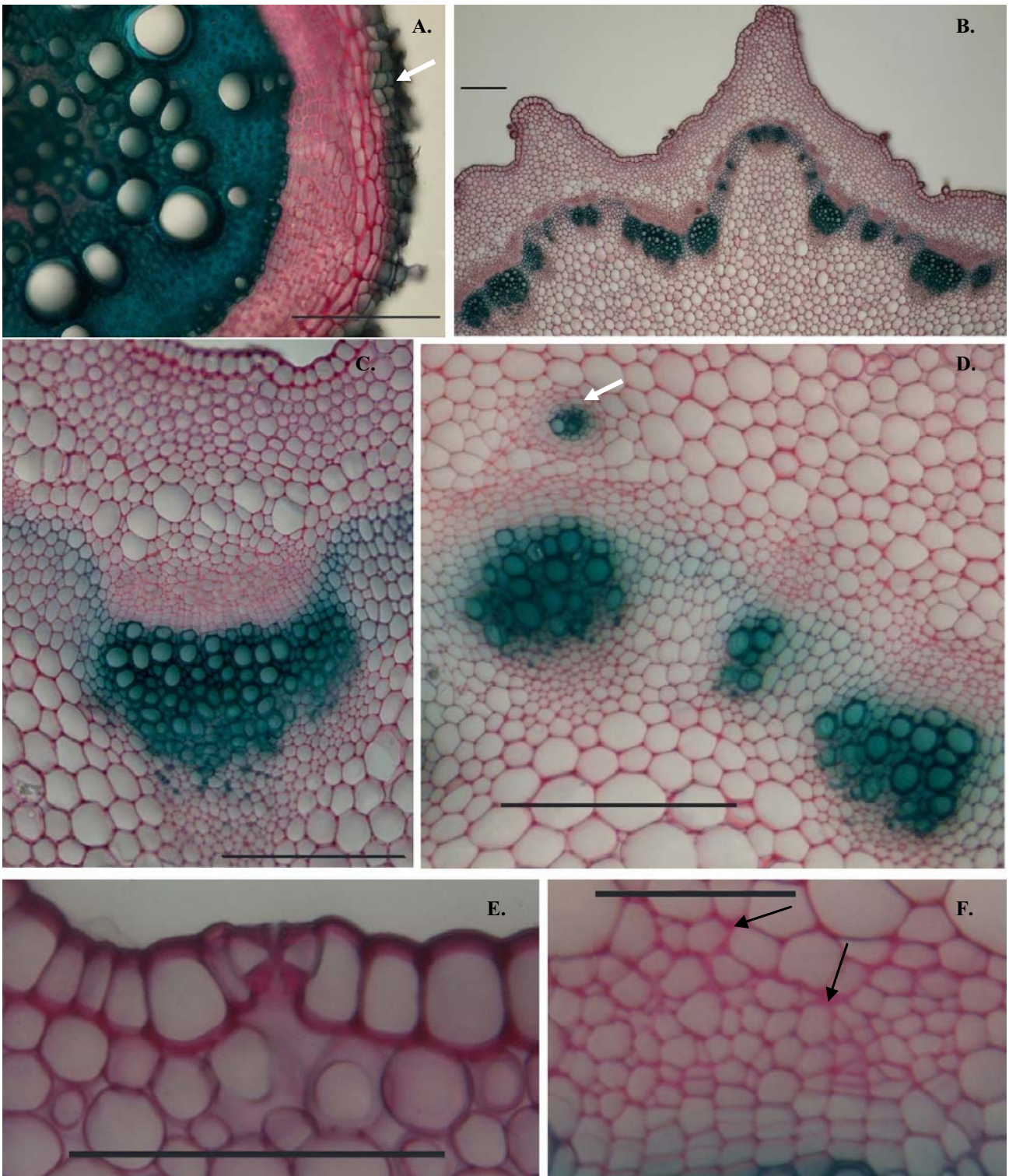
reduced comparative with the young leaves (Fig. 3G). They have thick-walled cells, densely covered by micro-papillae. The two-armed trichomes are numerous especially in the mid-vein region (Fig. 3H). The increase of the number of two-armed hairs on the leaves from the middle part of the stem comparatively with the leaves from the upper part demonstrates the continuous histogenesis of these formations. Trichome walls have densely distributed protuberances (larges and small) with rounded tips. The epicuticular wax consists in angular rodlets (Fig. 3I).

## DISCUSSIONS

Metcalf and Chalk (1979) [12] gave general information regarding the vegetative organs structure in different members of *Brassicaceae* family. There was no information about this species anatomical feature. Cansaran and their collaborators (2007) [4] shows anatomical characteristics of another endemic *Erysimum* specie – *E. amasianum*. They are some anatomical similarities between *Erysimum* and *Alyssum* species [4, 9, 10]. Unlike *E. amasianum*, *E. wittmanii* have no collenchyma under the stem epidermis. This lack of a specific mechanical tissue (collenchyma) for many *Brassicaceae* family members is compensated by early lignification of the medullar rays cells walls at the xylem level. Another character which differentiates this genus (*Alyssum* and *Erysimum*) is the trichomes types: stelate in *Alyssum* 3-4 branched or malpighiaceus (T-shaped) in *Erysimum*. This difference was supported also by genetically investigations [1]. The pattern of trichome evolution across the family may represent numerous innovations of trichome branching, but ultimately careful developmental and molecular genetic studies are needed to make a more confident assessment of trichome evolution [1].

It appeared that the high distribution of trichomes in *E. wittmanii* leaves could be regarded as an adaptation associated with relative arid life conditions (rocky areas). The trichomes were assumed to affect transpiration by influencing the water diffusion boundary layer of the transpiring leaf surface. In addition, they might also indirectly influence the water economy of the leaves or young stems through temperature [3]. Similar features involved in species resistance to water deficit have also been observed in xeromorphic species [6].

The leaf mesophyll is bilateral, in relation with the leaf position on the stem. This feature is rather related with morphological and ecological peculiarities of a species than with the genus relationships. The stomata are by anisocytic types, what is usually reported in the *Brassicaceae* family.

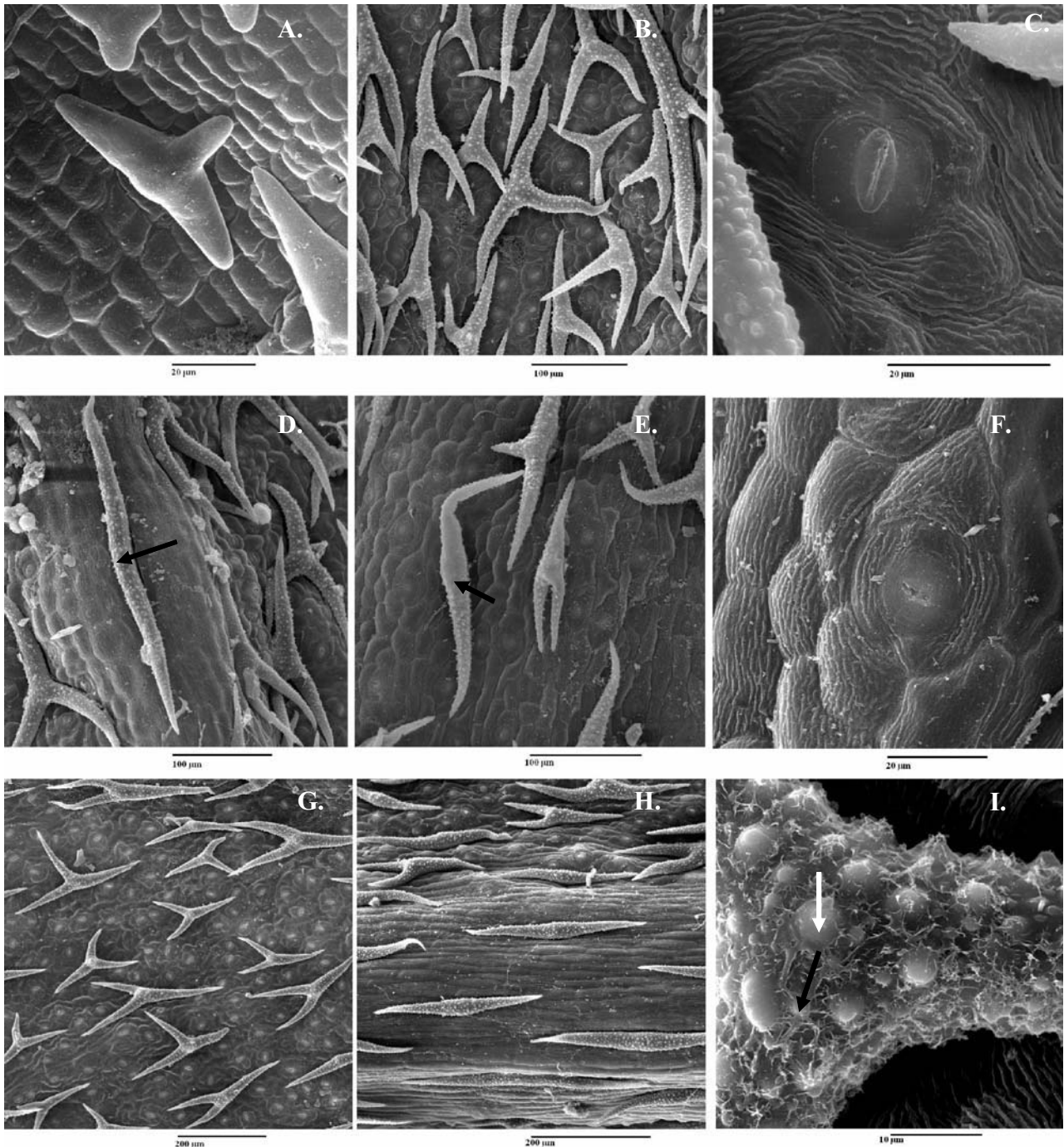


**Figure 1.** Micrographs of the cross sections through the root and stem of *Erysimum wittmanii*: A – section through the root with secondary structure (white arrow indicate the phellem); B – cross section through the middle of the stem – primary structure and early passing to secondary structure could be observed; C – cross section through the middle of the stem - detail with a vascular bundle; D – cross section through the top of the stem with primary structure (white arrow indicate a small cortical bundle); E – detail from the stem epidermis; F – detail from the vascular bundle phloem (early passing to secondary structure) – crushes protophloem vessels could be observed (black arrows) (scale bar A – D – 100  $\mu$ m, E – 50  $\mu$ m, F – 25  $\mu$ m) (original).





**Figure 2.** Micrographs of the cross sections through stem and leaves of *Erysimum wittmanii*: A - cross section through the top of the stem with primary structure – detail from a small vascular bundle; B – cross section through the stem basis with the secondary structure (black arrow indicate the sclerenchyma at the phloem pole; white arrow indicate the lignified secondary rays) ; C - cross section through the stem basis – detail from a vascular bundle; D – cross section through a leaf from the upper part of the stem – detail from midvein (white arrow indicate the unique vascular bundle); E – cross section through a leaf from the upper part of the stem (white star – palisade parenchyma, black star – spongy parenchyma); F – cross section through a leaf from the middle part of the stem (scale bar – 100 μm) (original).



**Figure 3.** Field emission scanning electron micrographs of the leaves of *Erysimum wittmanii*: A – very young leaf – ramified tector hairs with thin, smooth walls could be observed; B – D – views of the surface of the lower epidermis of a leaf from the top of the stem (young leaf): B – tector hairs between veins; C – stomata; D – two –armed tector hairs from a large vein (arrow); E – F - views of the surface of the upper epidermis of a leaf from the top of the stem: E –two-armed tector hair (arrow), F – stomata; G – I - views of the surface of the lower epidermis of a leaf from the middle part of the stem: G – tector hairs between veins; H – tector hairs from the mid-vein; I – detail from a trichome surface (white arrow – large protuberance, black arrow – small protuberance) (original).

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