

CONTRIBUTIONS TO THE HISTO-ANATOMY OF THREE *EQUISETUM* SPECIES

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Abstract: This paper presents the histo-anatomical characteristics of the stem, nodal branches and leaves of three *Equisetum* species, *Equisetum pratense*, *Equisetum telmateia* and *Equisetum sylvaticum*, commonly known as “horsetail”. This study was elaborated using color photographs acquired from optical microscopy (OM) and scanning electron microscopy (SEM) observations. Using OM investigations, several structures have been evidenced: the epidermal wall cells, the outer cortex made of different types of tissues and the central cylinder organized into two regions: an external layer that enclosed vascular bundles and an internal layer represented by a large air cavity. The images achieved by SEM showed some differential characteristics concerning the trichomes and stomata but also the manner in which granules of silicon dioxide are spread throughout the surfaces of *Equisetum* species. These results could be used as a criterion for species differentiation as they have taxonomic value in the *Equisetum* family.

Keywords: *Equisetum* species, histo-anatomy, optical microscopy, scan electronic microscopy.

Introduction

The *Equisetum* family consists of approximately fifteen species and hybrids, spread worldwide from regions with an equatorial to those with a temperate or cold climate (Christenhusz and Chase, 2014). Nine of these species, commonly known as “horsetail”, are found in the Romanian flora (Sârbu et al., 2013). These are perennial plants which have spontaneously developed and are frequently found in forests, wet meadows and swamps (Grințescu, 1952).

According to literature, these species possess a horizontal, ramified and articulate rhizome; in addition, at every node possesses a small verticil of small scaly leaves that is welded at the base. The spores of this species are spherical, filled with tightly packed chloroplasts and four twisted elaters with a role in dissemination (Ancuceanu, 2013). Furthermore, the structure of the root reveals a single concentric bundle (with a single vessel) surrounded by a cortex (Carlquist, 2011). For the nodes there are vascular bundles arranged in a compact circular belt of xylem and phloem. The xylem consists of vessels

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with bordered pits and there is a continuity between bordered pits in the internodes and scalariform pits in the nodes. Regarding the structure of internodes, the xylem of the vascular bundles of all *Equisetum* species is reduced to a few isolated thick-walled vessels. Their walls consist of lignified rings or of wide-spaced scalariform pits. Parenchyma cells surround the phloem and within the area of xylem there is a large opening, the carinal canal, which occasionally contains tyloses (Schweingruber and Borner, 2018).

A remarkable characteristic of *Equisetum* species is their ability to take up and accumulate silicon in their tissues. Silica accumulates on the epidermis of the plants and it is also incorporated into the cell walls increasing their rigidity and stability (Husby, 2013).

Sterile stems belonging to the *Equisetum* genus are often used in traditional medicine. They are highly efficient in treating urinary tract infections, cardiovascular diseases, respiratory tract infections (Pallag et al., 2016), bleeding ulcer and rapid healing of bleeding wounds (Al-Snafi, 2017). Some experts believe that horsetail may be beneficial in preventing osteoporosis because silicon is a vital component for bone and cartilage formations (Schneider, 2005). Also, silicon increases the resistance against mold. Recent researchers noted that first response after fungi attack is higher in the presence of silicic acid (Labun et al., 2013).

The purpose of this study was to illustrate by comparison the histo-anatomical differences on three *Equisetum* species by electronic microscope scan analysis on the stem, nodal branches and leaves and by optical microscope analysis of the epidermal wall cells, the outer cortex and the center cylinder.

Materials and methods

Sterile stems of *Equisetum pratense* Ehrh., *Equisetum telmateia* Ehrh. (syn. *Equisetum maximum* Lam.) and *Equisetum sylvaticum* L. were used. *Equisetum pratense* and *Equisetum sylvaticum* were collected from the North-Eastern region of Romania (Neamț and Suceava county) in June-July 2017, while *Equisetum telmateia* was collected in July 2018 also from the North-Eastern region of Romania (Iași county). The freshly plants were fixed and preserved in 70% alcohol following to be sectioned with the hand microtome and with the help of botanic razor.

Superficial and cross sections were obtained from the stem and leaves of the plants, then colored using green iodine and ruthenium red and analyzed using the Novex Microscope. Furthermore, pictures were acquired using a microscope with an incorporated Olympus camera.

Scan electronic microscope analysis: samples were mounted on aluminum stubs and coated with gold at 25 nm thickness. Coated samples were observed on a Tescan Vega II scanning electron microscope at 30 kV.

Results and discussions

The internode

The epidermis of *Equisetum pratense* and *Equisetum telmateia* consists of isodiametric thick-walled cells, the exterior wall being faintly creased. The walls of the epidermis and the stomata cells are impregnated with silicon dioxide. In the case of *Equisetum sylvaticum*, the epidermis was found to have isodiametric cells with strongly

thickened walls, the outer walls heavily silicified. Each ridge presents 1-2 short unicellular trichomes with obtuse tops (Plate I, Fig. 1).

The external cortex of *Equisetum pratense* is dense and made of different types of tissues: a parenchymatous tissue that encloses large, thin-walled cells; the inner cortex with numerous large vallecular canals (13); and a cortical layer, made out of small walled cells. In contrast, *Equisetum telmateia* has a parenchymatous tissue that encloses small, thin-walled cells; the inner cortex is made of more numerous and larger vallecular canals (18) than *Equisetum pratense*, but the cortical layer is similar, made out of small walled cells. *Equisetum sylvaticum* is found to be composed of chlorenchyma mechanical cords arranged into 3-4 layers in the ridges and 1-2 layers in the areas located between ridges. The inner layer of the cortex was found to be a typical endodermis, with primary cells that possessed visible Casparian strips on the radial walls. The cortex did not present vallecular canals (Plate I, Fig. 1).

In *Equisetum pratense* and *Equisetum telmateia*, the central cylinder starts with a single-layered pericycle; the central cylinder contains vascular bundles and a central area represented by a large pith cavity. On further observation, a bundle contains a group of xylem vessels on its flanks and a carinal canal beneath; the carinal canal in contact with 1-2 xylemic vessels in between the small parenchyma cells that surrounded it. The only difference between these two species is the number of vascular bundles, which is bigger for *Equisetum telmateia*. For *Equisetum sylvaticum*, the central cylinder started as a single-layered pericycle which continues with a vascular bundles and a center represented by a large pith cavity. The bundle was separated by a fundamental parenchymatous cell layer larger than those which formed the cortical layer. On further observation a bundle portrayed a group of wooden vessels on its flanks and an carinal canal; the carinal canal was traversed by 1-2 wooden vessels in between the small parenchyma cells that surrounded it (Plate I, Fig. 1).

Compared to the other hollow tube plants, the *Equisetum* species are characterized by special anatomy and physiology features that allow them to resist mechanically collapse due to the change in the status of cell water during the growing season (Zajackowska et al., 2017).

Upon analysis under the electronic microscope numerous elongated cells with the external wall impregnated with silicon dioxide were detected for all species (Plate I, Fig. 2).

The nodal branches

Equisetum pratense has the shape of a cross with highly prominent ridges and silicified walls, *Equisetum telmateia* has a circular shape with 8 prominent ridges and also silicified walls; *Equisetum sylvaticum* has the shape of a star with five prominent ridges, and in between five deep valleculae.

The epidermis, for all the studied species, was made out of isodiametric, slightly elongated cells, which were disposed in a tangential pattern.

The cortex of *Equisetum pratense* and *Equisetum sylvaticum* was made out of chlorenchymatous tissue cords on the tips of the ridges and parenchymatous tissue on the inner layer. The difference between these two and *Equisetum telmateia* was the presence of five big vallecular canals near the stele (Plate I, Fig. 3).

The central cylinder of *Equisetum telmateia* and *Equisetum sylvaticum* begins with a unilayered pericycle that supports five vascular bundles. Their composition is similar to

those present within internode. The stele of *Equisetum pratense* begins with a unilayered pericycle that sustains four vascular bundles. The bundles enclosed xylemic vessels on their flanks and a small, radial shaped carinal canal of an elliptical or triangular form (Plate I, Fig. 3).

Upon analysis under the microscope, the following were detected: the epidermal cells near to the ridges have a prominent external wall; numerous stomata can be seen in the spaces between the ridges (Cullen and Rudall, 2016). All of the epidermal cells have a silicified external wall. Similar to this, stomata also have silicon dioxide granules (Plate I, Fig. 4).

The leaf

The leaf structure is similar with other *Equisetum* species, being small, united around the stem and forming a common sheath. The outline of the transversal section is semicircular.

From the histo-anatomical point of view, all these three species of *Equisetum* are characterized by slightly elongated epidermal cells of a tangential pattern. In addition, their walls are noticeable thickened and silicified. A cord of mechanical fibers resided in the middle zone of the tissue, under both epidermises. The fundamental parenchyma was found to have isodiametric cells without chloroplasts (Plate II, Fig. 5).

The analysis performed with the microscope revealed numerous long, unicellular trichomes and stoma impregnated with silicon dioxide in its walls (Plate II, Fig. 6).

Silicon deposits were observed in all plant regions: internode, nodal branches and leaf. Numerous structures were silicified including cell walls and stomata at varying stages of differentiation (Law and Exley, 2011). According to literature, silicon is one of the most common biogenic mineral after carbonates (Sola-Rabada et al., 2016) and it helps raise the plant health by creating stronger and more resistant structures (Volkov et al., 2019). Furthermore, horsetails incorporate much silicon into their stem tissue and external ridges and give the stems rough and abrasive character (Husby, 2013).

Despite the low number of species in the current flora (15), the ability of the *Equisetum* to withstand different environmental conditions is remarkable. This environmental resistance is due to morpho-functional adaptations that allow perturbation tolerance. These species offer insights into the way their older ancestors lived and how they managed to survive in tropical regions (Husby, 2013).

The hydraulic architecture of *Equisetum* stems has not yet been fully elucidated, although the carinal canals in each internode seem to provide reduced resistance through internodes, a role analogous to angiosperm vessels. Such a function would be of significant importance during the elongation of the internodes when the xylem pathways are not functional (Leroux et al., 2011; Xia et al., 1993). Leroux et al. (2011), discovers a cell wall matrix that lifts carinal canals, which could facilitate water transport. Husby (2013), notes that young stems guttate much more readily and show less conductive sensitivity to environmental factors than mature stems, which may indicate differences in the physiology of water transport.

Conclusions

Equisetum species were used traditionally for tuberculosis, as a catarrh in the kidney and bladder regions, as a hemostatic for profuse menstruation, nasal, pulmonary and gastric hemorrhages, for brittle fingernails and loss of hair, for rheumatic diseases, gout, poorly healing wounds and ulcers, swelling and fractures and for frostbite (Al-Snafi, 2017).

The histo-anatomical studies on the stem, nodal branch and leaf of *Equisetum pratense*, *Equisetum telmateia* and *Equisetum sylvaticum* were achieved by optical microscope analysis and scanning electronic microscope analysis. The described characters could have taxonomic value in the Equisetaceae family (Christenhusz and Chase, 2014).

The differences from the observed species appeared in cross sections of the sterile stems and the nodal branches. On the stem level, the main differences are the amount of silicified collenchyma on the edges and the presence of vallicular canals for *Equisetum pratense* and *Equisetum telmateia*. On the nodal branches level, the most important difference is the shape of cross section that every species has: cross for *Equisetum pratense*, star for *Equisetum sylvaticum* and 8 edges for *Equisetum telmateia*. Also, *Equisetum telmateia* is the only specie that has vallicular canals near the stele.

Furthermore, the species revealed important information on the presence and location of silicon dioxide formations. Silicon salts and other bioactive components will be the subject of further studies. Also, the chemical composition allows them to become a promising resource for making plant extracts or separating components of important biological value (Stănescu et al., 2014).

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EXPLANATION OF THE PLATES

PLATE I

Fig. 1. Transversal section from the internode of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

Fig. 2. Front view from the internode of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

Fig. 3. Transversal section from the nodal branch of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

Fig. 4. Front view from the nodal branch of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

PLATE II

Fig. 5. Transversal section from the leaf of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

Fig. 6. Front view from the leaf of:

- a. *E. pratense*
- b. *E. sylvaticum*
- c. *E. telmateia*

PLATE I

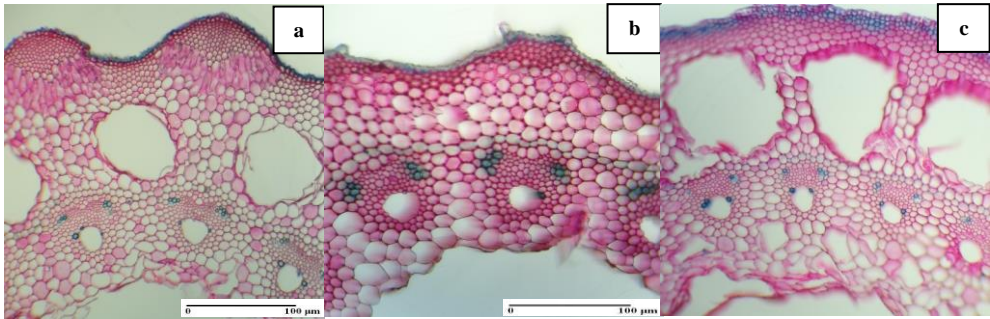


Figure 1

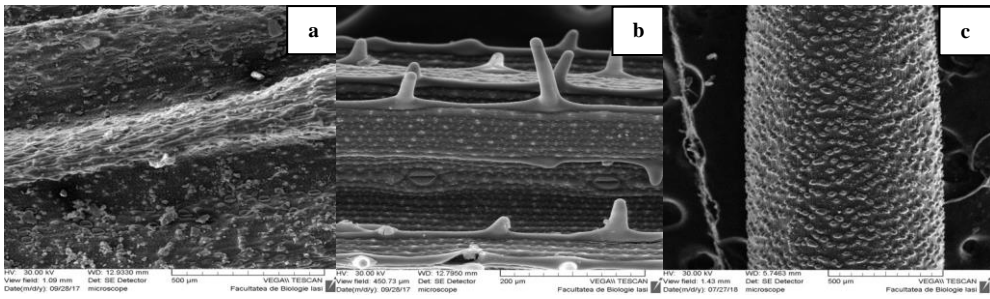


Figure 2

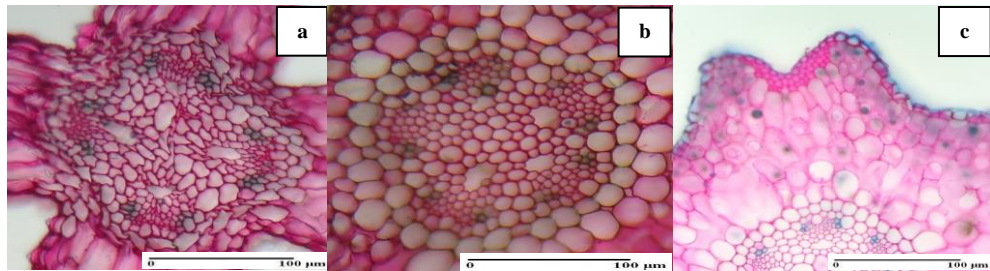


Figure 3

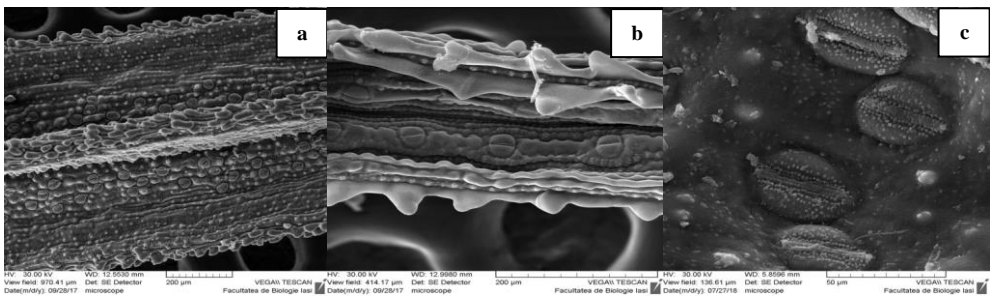


Figure 4

PLATE II

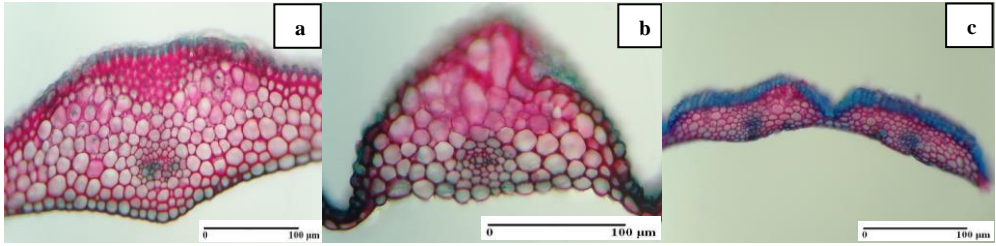


Figure 5

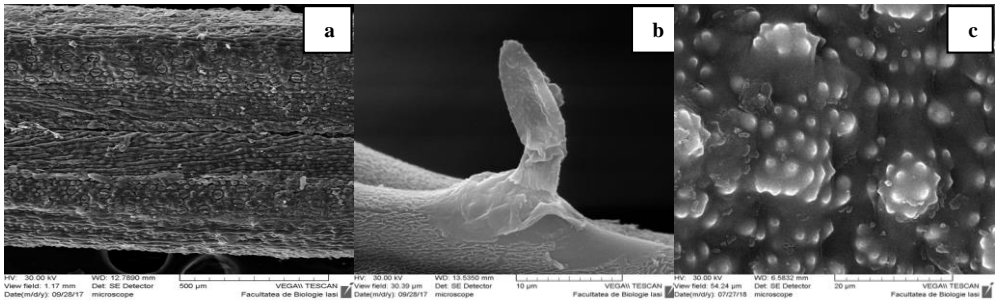


Figure 6