THE STUDY OF SOME ANATOMICAL FEATURES IN STEMS AND LEAVES OF MACROPHYTES (*HIPPURIS VULGARIS* L., *IRIS PSEUDACORUS* L. AND *NUPHAR LUTEA* (L.) SM.)

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Abstract: *The plants live under different environmental conditions and then they present specific adaptations (morphological, anatomical, physiological), changing the shape of their organs and their structures. The macrophytes are plants adapted to live in wet soil, either partially or totally submerged. The analysis of the morpho-anatomical structure of macrophytes shows their adaptations such as heterophylly, the development of aerenchyma tissues, the reduction of supporting tissues, the simplification of conducting vessels, the disposing of stomata only on the upper epidermis of the floating leaves, their absence in the case of submerged leaves etc. (ANTONESCU, 1951, PATRUT et al., 2005, LEME & SCREMIN-DIAS, 2014, NAKAYAMA & KIMURA, 2015, HUANG et al., 2017 etc.). In this paper, we present some anatomical aspects observed from a microscopic study of certain macrophytes: Hippuris vulgaris L. (mare's-tail), Iris pseudacorus L. (yellow flag iris), Nuphar lutea (L.) Sm. (yellow water lily). The sections were made on fresh material, according to the anatomical working rules (IANOVICI, 2009, ARSENE & NICOLIN, 2016). To highlight certain tissues we used staining methods. Sectioned organs were: stems of Hippuris, leaf lamina of Iris, petioles and leaf lamina of Nuphar. By microscopial study, we saw that aerenchyma tissues are very well developed in all three species; endoderma is well represented in mare's-tail stems; we found the idioblasts in the spongy clorenchyma of the leaf lamina of yellow water lily; we have noticed that the leaves of the yellow water lily have a bifacial structure and the leaves of the yellow iris have ecvifacial structure. Our observations are completed by data from other authors who have studied these species: SCHWEINGRUBER et al., 2013, KANE & ALBERT, 1987, GOLIBER & FELDMAN, 1990, GONTOVA & ZATYLNIKOVA, 2013, MARROTTE, 2011, KLIMENKO, 2012, BERCU & FAGARAS, 2008. We mention that some of our results are presented also as bachelor thesis (NEGRUȚ, 2018).*

Key words: *anatomical study, macrophytes, aerenchyma, stomata, idioblasts*

INTRODUCTION

Macrophytes are plants adapted to the aquatic or palustrine environment. Aquatic macrophytes often breed vegetatively as an adaptation to low water temperatures. Their body is protected against degradation under water by significant amounts of mucilaginous secretions. The root system is reduced or absent, because plants have direct access to the minerals in the water. The dichotomous ramification is characteristic of the roots of many macrophytes in nutrient-rich waters (HUANG *et al*., 2017).

The two major difficulties of submerged plants are to obtain the necessary light in the photosynthesis process and the gas exchange; therefore, most leaf modifications increase the absorption of light and the gas exchange.

One of the most popular adaptations is heterophylly, the particularity of producing leaves of various shapes. The submerged leaves are generally filiform, while the floating and aerial leaves have broad blades. In some species, leaf morphology varies depending on the season, as an adaptation to water temperature. At *Ceratophyllum demersum*, in autumn and winter, short and broad leaves are predominant, so that the plant can produce long and thin leaves in summer (ANTONESCU, 1951). NAKAYAMA & KIMURA (2015) also remind that, in submerged conditions, *Rorippa aquatica* develop deeply dissected leaves and, in terrestrial conditions, it develop simple leaves with smooth margins and may function as temperature sensors.

At the anatomical level there are specific adaptations such as (www.brainkart.com):

Roots (when present), stems and submerged leaves are protected by a thin cuticle because the tissues of these organs do not undergo drying.

The epidermis of the vegetative organs is generally composed of a single layer of thin-walled cells that provide a minimal protective role. The epidermal cells in the leaves contain chloroplasts and can function as a photosynthetic tissue (when the leaves and stems are very thin, e.g. *Hydrilla*), while internal tissues are often modified for storage. The submerged leaves do not have stomata, while the floating leaves bear stomata only on the upper epidermis. In amphibious plants, stomata can be distributed on all aerial parts.

The bark is well developed, has numerous aeriferous spaces; helps buoyancy and rapid gas exchange.

Because water density can support their body, aquatic plants have less developed mechanical and conductive tissues. Some plants (e.g. *Nuphar* sp.) develop a particular type of star-shaped cells, called (astro-) sclereids. These cells provide mechanical support to plants. Generally, in submerged organs, mechanical tissues are absent. In conducting tissues, in submerged forms, xylem vessels are less frequent (in many cases there are only tracheids), while in amphibious forms, the xylem and the phloem are well developed.

Aeriferous tissues are very well represented; the gas exchange necessary for their metabolism is produced here. Due to oxygen deficiency, the body of some macrophytes can account for up to 70% of intercellular spaces. Some water plants have a well-developed aerenchyma in their roots as well (e.g. mangroves have roots with pneumatophores).

MATERIAL AND METHODS

The species considered for the anatomical study were: *Hippuris vulgaris* L., *Iris pseudacorus* L. and *Nuphar lutea* (L.) Sm. The sections were made on fresh material in the botany laboratory, according to the anatomical working rules. The following materials were transversely severed: *Hippuris* stems, *Iris* leaves, *Nuphar* petioles and leaves. Their examination was performed using the Krüss optical microscope. To highlight certain constituents, we have used the colouring technique. We used Genoese reagent for coloring. Photos were taken using a Sony camera. Each species is accompanied by a brief botanical description, made after SĂVULESCU, 1952-1976.

RESULTS AND DISCUSSIONS

Perennial species, *Hippuris vulgaris* L. (horsetail), belonging to the *Hippuridaceae* family, is a frequently semi-submerged plant of 15-45 cm. The stem is cylindrical, spongy, narrowed at the bottom. The submerged leaves, of about 8 cm, are flipped over and attached to the stem. The floating leaves are linear, of about 10 cm, more or less acute, perpendicular to the stem. Both types of leaves are arranged in whorls of 8-16. The flowers are small, axillary, with rudimentary cover. The gynoecium is inferior, and the androecium has only one stamen. The fruit is a drupe type.

The horsetail grows in plain and hill areas, in stagnant and flowing waters, preferring those with muddy substrate rich in limestone. In Arctic regions, the species is fodder (SĂVULESCU, 1952-1976).

Anatomical aspects. Figure 1 shows the stem of *Hippuris vulgaris* in cross-section. It presents only the primary structure (it does not form a secondary structure), in which the following areas are differentiated: epidermis, bark and central cylinder. On the outside, a one-layer epidermis with thin walls, devoid of cuticle and bristles, protects the stem. The green colour of the stem is given by the assimilator parenchyma, with visible chloroplasts in the outer layers. The bark, the area between the epidermis and the central cylinder, consists of several layers of parenchyma cells with distinct disposition and large and numerous aeriferous cavities (Figure 3).

The bark area actually forms an aeriferous parenchyma. The inner layer of the bark, the endodermis, surrounds the central cylinder and is observable in Figure 2, coloured in yellow. In the central cylinder, we observe the conducting vascular bundles surrounded by the parenchyma (Figure 2). The woody, slightly lignified vessels exhibit scalar shape thickness and the floem consists of small groups of sieve tubes and parenchyma cell (SCHWEINGRUBER *et al.*, 2013). The cambium is missing. The pith parenchyma is well developed, observable in the central area of the section.

KANE & ALBERT (1987) observed distinct morphological and anatomical differences between leaves produced on submerged and aerial shoots on *Hippuris vulgaris*. Analysis of the leaf plasticity, GOLIBER & FELDMAN (1990) say that recent evidence shows that abscisic acid regulates leaf development.

Iris pseudacorus L. (water flag) is a perennial species of the *Iridaceae* family, of 70-150 cm. It shows clearly compressed stems. The ensiform leaves are 1.5-2.5 cm. Slightly membranous leaflets are lanceolate, acuminate and crenate. Flower ovary has three edges and three longitudinal grooves. The outer perigonial flowers are yellowish, broad-ovate, with basal streaks. They are punctuated with burgundy or sometimes are blackish. Internal flowers are small, linear-lanceolate, with elongate-ovoid stigmata, and dark brown stamens with yellowish filaments. The fruits are cylindrical, rostrate pods (SĂVULESCU, 1952-1976).

The water flag prefers swampy habitats. The species is used successfully in artificial arrangements, being decorative by flowers.

Anatomical aspects. The leaves are unicoloured, almost vertical, which means they receive equal light on both epidermis. Figures 4-7 show some anatomical features of the foliage. Figure 4 is a portion of the extremity area of the leaf blade, and Figure 5, the area of the median veins. Figures

6-7 show the conducting vascular bundles. They are collateral-closed bundles (i.e. without procambium between floem and xylem), specific to the monocotyledons.

The constitutive tissues of the leaf blade are the epidermis, the mesophyll, the conducting tissue represented by several vascular bundles and the supporting elements (sclerenchyma sheath at bundle level).

The leaf blade has a homogeneous structure, showing palisade chlorenchyma layers towards both epidermis. The parenchyma between them (poorly developed chlorenchyma, according to GONTOVA & ZATYLNIKOVA, 2013) shows circular air cavities, the central one being obviously larger and ovoid-elongated. The conducting vascular bundles in front of the cavities are smaller, and those located in areas with uninterrupted parenchyma are larger, with visible sclerenchyma sheaths.

In the comparative morpho-anatomical study, conducted on *Iris sibirica* L. and *Iris pseudacorus* L., GONTOVA & ZATYLNIKOVA (2013) showed, among others, tetracitic and idioblast type stomatal apparatuses in the subepidermic layers.

Nuphar lutea (L.) Sm. (*Nympheaceae* family) is an aquatic plant with a repent rhizome, with triangular petioles and long peduncles, widened towards the base. The usual green and rarely brown foliage is ovate-incised-cordate, 10-30 cm long. Yellow odorant flowers, 3-6 cm in diameter. Sepals are persistent, usually 5, but can sometimes vary between 4-7. On the outside, they are green, and on the inside, yellowish. 10-20 yellow petals, obovate, shorter than sepals. Numerous stamens with folded anthers. The stigma's disk is deep, sometimes crater-like. Conical fruit, 3-4 cm, with seeds weighing 450 mg and containing much starch (SĂVULESCU, 1952-1976).

Grows in stagnant or flowing waters with a muddy substrate.

The rhizomes are edible, medicinal, or have household uses (for dying, tanning, etc.). Leaves can be consumed as fodder. Flowers can be used in food (for preparing jams). It is used successfully in the artificial arrangements of ponds and gardens in wetlands.

Anatomical aspects. Figures 8-12 show some anatomical features of the yellow lily leaf blade. It has a bifacial structure, being arranged in a palisade chlorenchyma towards the upper epidermis, and in a spongy chlorenchyma towards the lower one, the cells leaving large aeriferous spaces (cavities) between them.

In the spongy chlorenchyma there are isolated, branched (star-shaped) sclereids, called idioblasts, which penetrate into the intercellular spaces. Although sclereids have been studied by several authors in literature, their importance is still discussed; some believe that these cells confer resistance to tissues (in our case, we suppose they have the role of supporting spongy chlorenchyma). MARROTTE (2011) studied the utility of *Nymphaeaceae* sclereids in paleoenvironmental research and shows that sclereids from *Nuphar lutea* can be a useful indicator of its presence.

In front of the median vein, we can see the conducting vascular bundles. In the severed material, we identified 4 such bundles, of which 3 were larger. In the extremity of the leaf, the stomatal apparatuses are clearly visible (figures 11-12).

We mention that our sections were executed on terrestrial leaves of water lily. The anatomy of terrestrial, floating and submerged leaves differs, given by the different depth and physio-chemical characteristics of the environment in which they develop; floating leaves do not have cuticle and stomata at the epidermal level, the parenchyma is not differentiated, they do not have sclereids (KLIMENKO, 2012). The same author found differences between the photosynthetic apparatus of the floating leaves and the submerged leaves at the level of mesophyll cell chloroplasts, content of pigments, and chlorophyll fluorescence parameters. The anatomical features of the plant organs are in accordance with its floating nature and remarkable are the differences between asterosclereids aerenchyma in peduncle and blade (BERCU & FAGARAS, 2008).

The anatomy of the petiole is similar to that of a primary structure stalk (Figures 13-14). In the severed material, the following tissues are distinguished under the epidermis: collenchyma, chlorenchyma, fundamental parenchyma, large aeriferous cavities, and eight conducting vascular bundles. The seven outer bundles are arranged on a single circle, and in the centre of the section one bundle is observed. Each bundle has its own endoderm, the petioles having such bundles being called polystelics (ARSENE, 2004).

CONCLUSIONS

Following the anatomical study of the species under consideration, we have noticed the following:

- cuticle absent or poorly developed in aquatic species (*Hippuris vulgaris* and *Nuphar lutea*);
- well developed aeriferous tissues (in all analysed species, with large cavities in *Iris pseudacorus* leaves),
- well-represented endoderm with a role in water control (*Hippuris vulgaris*);
- the presence of isolated sclereids (in poorly developed tissues) to support aeriferous parenchyma (*Nuphar lutea*);
- stomata disposed on the upper epidermis, in species with floating leaves;
- leaves with bifacial structure, in floating leaves (water lily), and equifacial structure, in the submerged or palustrine species (water flag).

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Figure 1. Stem in cross-section, overall view (4x10, without colouring)

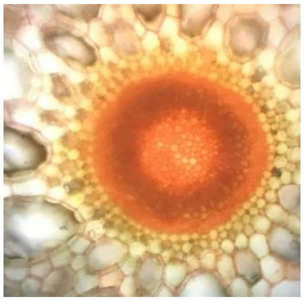

Figure 2. Central cylinder - detail (10x10, colouring with Genoese reagent)

Figure 3. Cortex with parenchyma cells and aeriferous cavities - detail (40x10, colouring with Genoese reagent)

Figure 4. Extremity of the water flag leaf, in cross-section (4x10, without colouring)

Figure 5. Median area of the leaf blade, in cross-section (4x10, colouring with Genoese reagent)

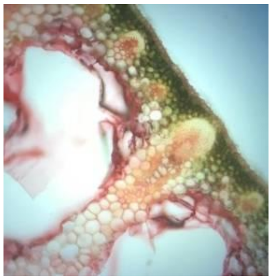

Figure 6. Conducting vascular bundles, in the palisade chlorenchyma (10x10, colouring with Genoese reagent)

Figure 7. Detail of a vascular bundle, supported by a sclerenchyma sheath (40x10, colouring with Genoese reagent)

Figure 8. Cross-section through the yellow lily leaf, overall image (10x10, colouring with Genoese reagent)

Figure 9. Conducting vascular bundles and branched sclereids, in the background (10x10, colouring with Genoese reagent)

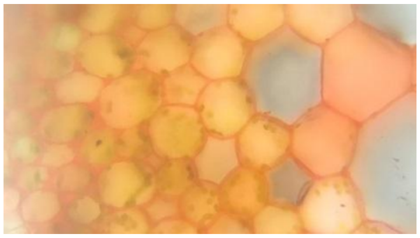

Figure 10. Parenchyma and aeriferous cavities (40x10, colouring with Genoese reagent)

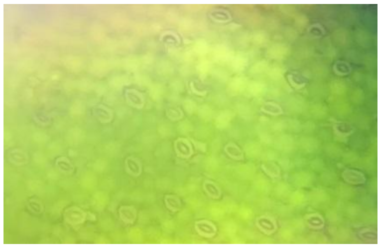

Figure 11. Detail of stomatal apparatuses (40x10)

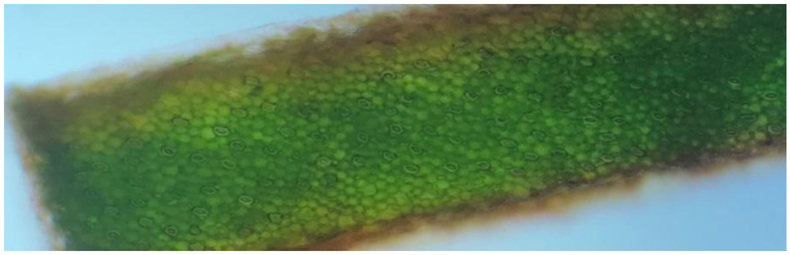

Figure 12. Reniform stomata on the upper epidermis of the yellow lily leaf (4x10)

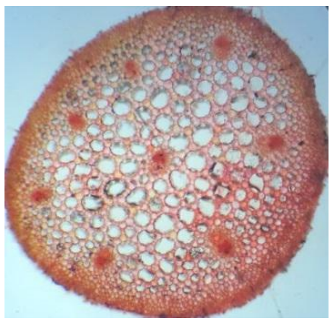

Figure 13. Petiole, cross-section

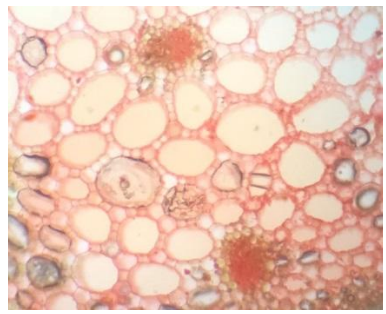

Figure 14. Detail of the leaf petiole: conductive vascular bundles and aeriferous cavities