

SOME HISTOLOGICAL AND CYTOLOGICAL FEATURES OF FOUR HYDROPHYTES OCCURRING IN THE DANUBE DELTA (ROMANIA)

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Abstract: Some histological and cytological features of four hydrophytes occurring in the Danube Delta (Romania). The author describes some of the histological and cytological characteristics observed in the corm tissues of *Nuphar lutea* (L.) Sibth. & Sm., *Nymphaea alba* L., *Hydrocharis morsus-ranae* L. and *Trapa natans* L. Common cytological elements such as sphaeraphides, druses, chloroplasts and starch grains, have been reported in different tissues of the plants. Some of the interesting histological features such as trichomes (hairs), trichosclereids and idioblasts were described and discussed in detail.

Introduction

Nuphar lutea (L.) Sibth. & Sm., *Nymphaea alba* L., *Hydrocharis morsus-ranae* L. and *Trapa natans* L. are rooted hydrophytes with floating leaves, widespread in various aquatic habitats including the Danube Delta. The flowers of the water lily (*Nymphaea alba*) are large, showy, white and aromatic. The spatterdock (*Nuphar lutea*) flowers are yellow and "half-opened" at or above the water surface. The flowers in both plants are attached to rather thick, cylindrical peduncles that may be up to six feet in length. European frog-bit (*Hydrocharis morsus-ranae*) has thick, rounded, floating leaves about one inch in diameter, and flowers, with only three white petals, each with a yellow blotch. [7,10,11]. The Eurasian water chestnut (*Trapa natans* L.) was introduced to North America in 1874. It is an annual aquatic plant, growing in fresh water lakes and ponds or slowly flowing streams and rivers, up to 60cm deep, requiring sunny exposure and slightly acidic or neutral waters. The plant dislikes calcium rich water, but it prefers humus rich soil [6]. Initially, *Trapa natans* was cultured in Asia Gray's botanical garden at Harvard University in 1877. By 1879 it had escaped into local waters. This plant now occurs over a considerable part of Europe, the Caucasus and Siberia.

The authors dealing with plant anatomy mostly noticed the histological structure of the *Nymphaea alba* petiole, its aerenchyma and trichosclereids [1,2,3,12,13]. Others described the aerenchyma in the petiole of *Nuphar lutea* [4,9,13] and its physiological role in the life of the plant [9]. Data concerning the peduncle of the two Nymphaeaceae species dealt with and the petiole and leaf anatomy (including cyto-histological elements) of *Hydrocharis morsus-ranae* and *Trapa natans* are almost lacking.

Material and Methods

The plant material was collected during August from the Cardon channel in the Danube Delta. Small pieces of peduncle, petiole and blade were fixed in F.A.A. (formalin acetic-alcohol), clarified with chloral hydrate and stained with carmine alum, carmine and iodine green. The samples were embedded in glicerine-gelatine. The observations and microphotographs were performed with a BIOROM-T bright field light microscope, equipped with a TOPICA-1006A video camera, using the scanning technique.

Results and Discussions

Examinations of cross sections of *Nuphar lutea* and *Nymphaea alba* flower peduncles reveal an abundance of trichosclereids (star like hairs or internal hairs), present in the aerenchyma. According to Bouman and Houtuesen (1996) report, the trichosclereids of *Nuphar lutea* are located in the nodal tissue and on the uniseriate partitions (trabeculae) which delimit the air channels. They are poorly branched (3-4-branched star like hairs) with a short, root base. Each branch is short and thick and possesses calcium oxalate deposits (Fig. 1a, b) and protudes inside the air chambers [3].

In *Nymphaea alba* peduncle the trichosclereids differ from those of *Nuphar lutea*. They are numerous and spread everywhere on both sides of the aerenchyma tissue, mostly located between the air channels. The trichosclereids possess short bases and 5-6 long and thin branches, protudding into the air chambers. Calcium oxalate deposits are present too (Fig. 2a). In the collenchyma cortex of *Nymphaea alba*, just bellow the epidermis, long, unbranched trichosclereids occur (Fig. 2b).

The surface of the *Nuphar lutea* peduncle is covered by groups of simple, one-celled-hairs (Fig. 3a, b).

The trichosclereids and trichomes are absent in cross section of *Hydrocharis morsus-ranae* peduncles. A number of aerenchyma channels possess a diaphragmatic tissue, with large tanniniferous cells (Fig. 4a). Around the vascular bundles starch grains occur (Fig. 4b).

Transversal sections of *Trapa natans* petiole reveal a well developed aerenchyma with large air chambers. It is worth to mention the abundance of calcium oxalate druses, named by some authors sphaeraphides [2]. They may be found everywhere, but mostly on the trabeculae, facing the air chambers and in the diaphragmatic tissue as well (Fig. 5a, b). The same abundance of druses is a specific feature in the leaf mesophyll (palisade and spongy regions) of *Trapa natans* (Fig. 9a, b). Batanouny (1992) suggested that only on the base of substomatic cavities, are certain cells which bear druses but they can be observed in the spongy region too.

On the lower epidermis of *Trapa natans* leaf blade, at places, sharp, many-celled hairs are present (Fig. 9c), but in its meosophyll trichosclereids and idioblasts are absent.

In the petiole of *Nuphar lutea* branched trichosclereids are present, mostly in the nodal tissues. The branches are thin, long, almost equal in length, with sharp apices (Fig. 6a).

Cross sections of *Nymphaea alba* petiole reveal extremely numerous trichosclereids, comparatively with those found in *Nuphar lutea* petiole. They are placed, mostly, on the margins of air chambers [1,2,13]. As Batanouny (1992) reported the star like cells possess a large root base. Some of the branches may protude simultaneously into more than two air chambers, maintaining them open. Therefore, they have rather strange shapes (Fig. 6b, c, d).

In the palisadic and spongy regions of the water lily and spatterdock mesophyll, long branched idioblasts, of different shapes, are present [5,2,12], which bear angular crystals on their surfaces [8]. However, the idioblasts of *Nuphar lutea* mesophyll are longer and thinner than those found in *Nymphaea alba*. They portude into the spongy tissue (Fig. 7a; 8a) as earlier Sárkány & Szalai [12] suggested. Remarcable is the presence of trichoblasts in both Nymphaceae species spongy tissue (Fig. 7b; 8b). In the mesophyll of *Hydrocharis morsus-ranae* and *Trapa natans* these histological elements are absent.

Chloroplasts are present in the mesophyll of all four studied taxa (Fig. 7a; 8a; 9a). As a rule, in all species the upper epidermis continuity is broken by the presence of stomata with well developed substomatic cavities (Fig. 9a).

Conclusions

The present results indicate that the four hydrophytes dealt with possess such histological and cytological features that are in accordancance with the free floatting nature of their leaves.



Fig. 1: Trichosclereids in the peduncle of *Nuphar lutea* (L.) Sibth. & Sm. (a, b). X 280, X 235: AC - air chamber; COC - calcium oxalate crystals; NT - nodal tissue; T - trichosclereids. Orig.

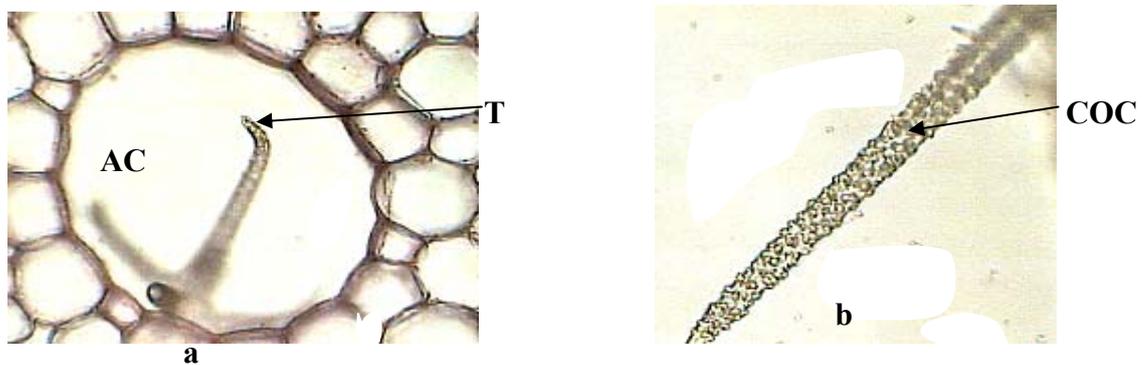


Fig. 2: Air chamber with trichosclereid of *Nymphaea alba* L. peduncle (a). X 125. Unbranched trichosclereid of *Nymphaea alba* L. peduncle (b). X 300: AC- air channel; COC- calcium oxalate crystals; T- trichosclereid. Orig.

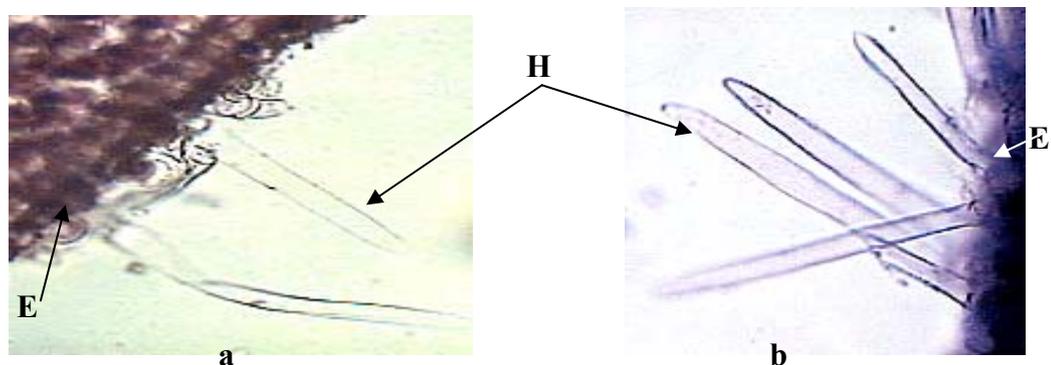


Fig. 3: One-celled hairs from the surface of *Nuphar lutea* (L.) Sibth. & Sm. peduncle (a, b). X 180; X 235: E- epidermis; H- hairs. Orig.

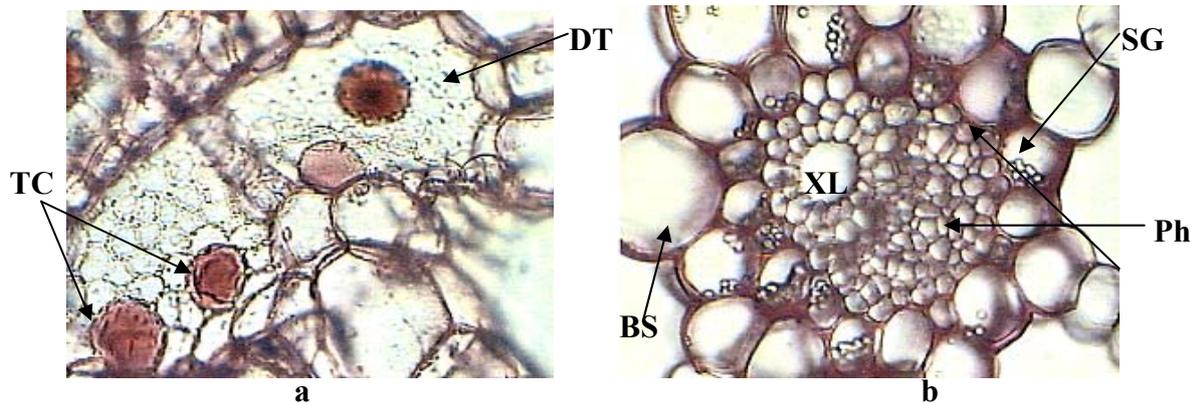


Fig. 4: Cross sections of *Hydrocharis morsus-ranae* L. peduncle. Air chamber with diaphragmatic tissue and tanniferous cells (a). X 238. Inter vascular bundle (b). BT- bundle sheath; DT- diaphragmatic tissue; Ph- phloem; SG- starch grains; TC- tanniferous cells; XL- xylem lacuna. Orig.

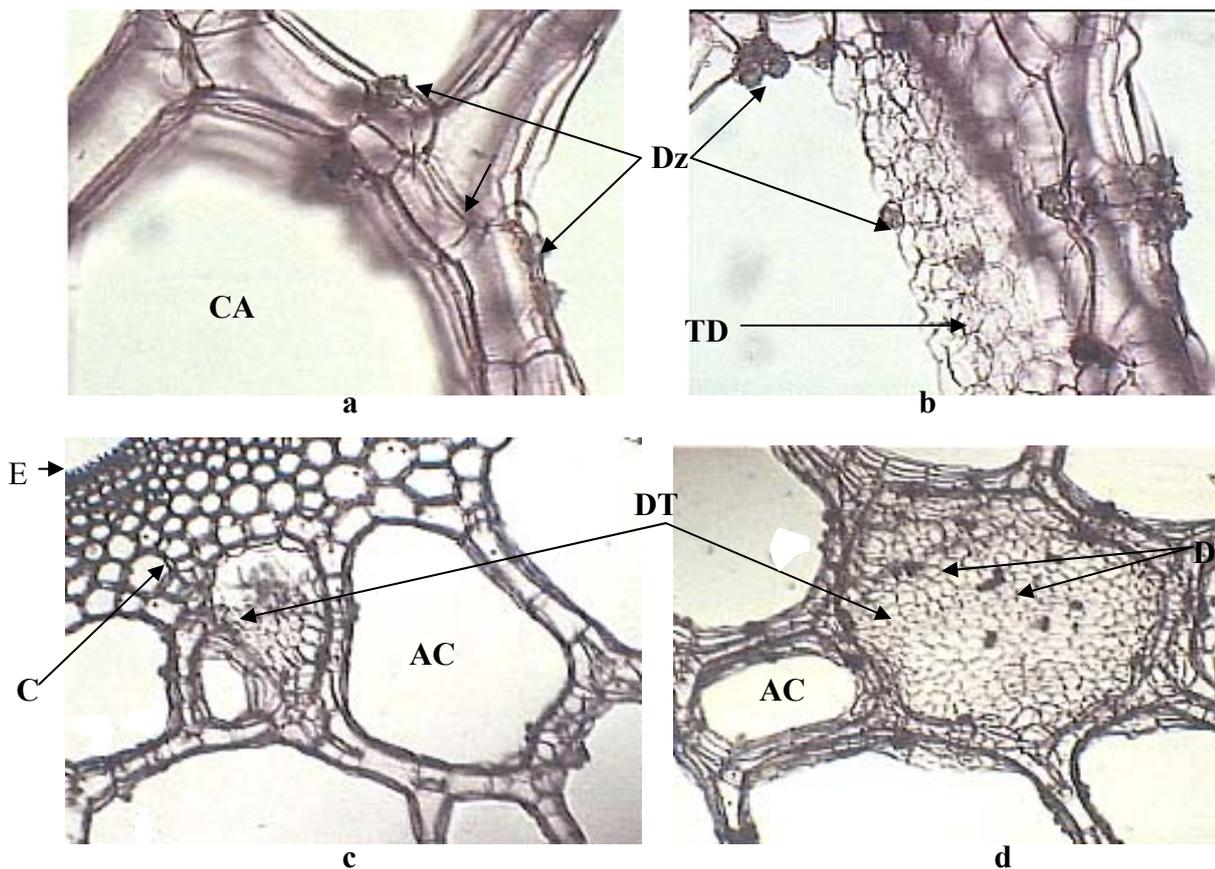


Fig. 5: Cross sections of the petiole of *Trapa natans* L.. Portions with trabeculae and druses (a, b). X 200. Portion with epidermis, cortex and aerenchyma (c). X 104. Portion with diaphragmatic tissue (d). X 49: AC – air chamber; C- cortex; E- epidermis; D – druses, DT- diaphragmatic tissue. Orig.

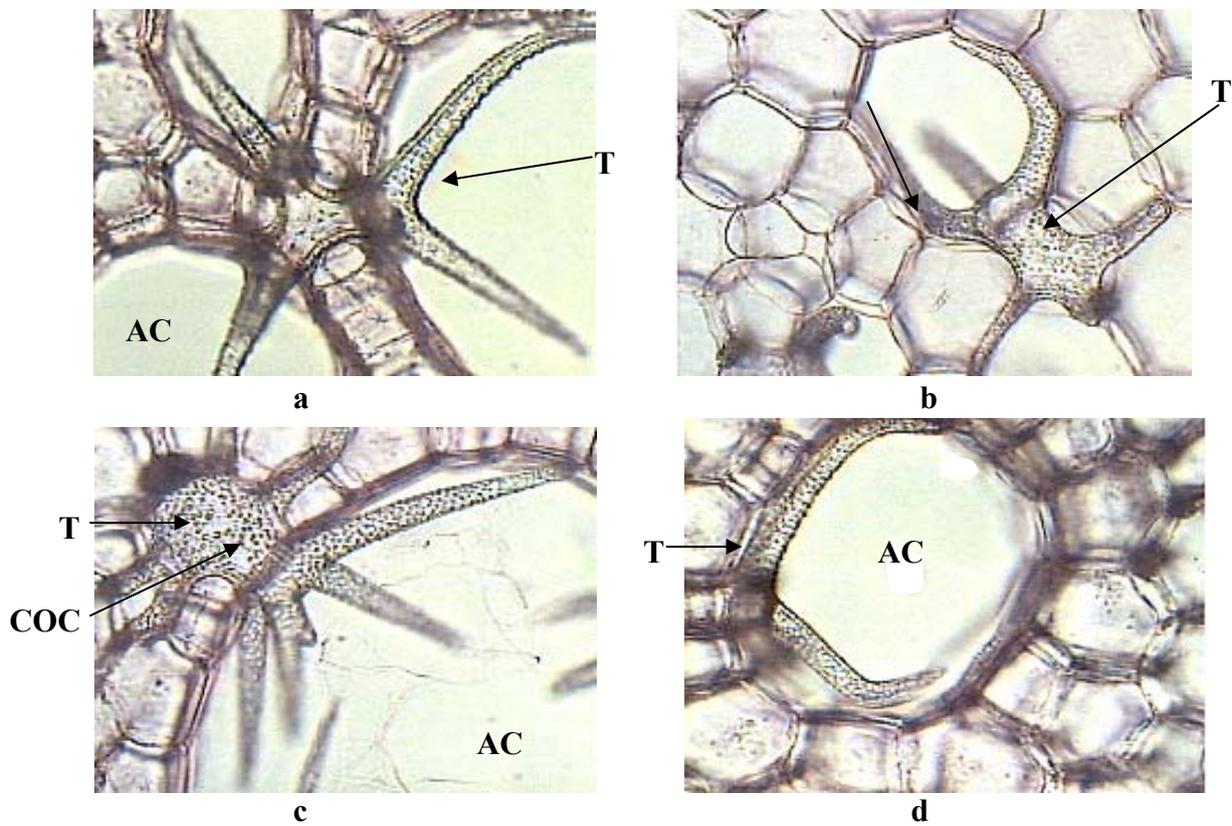


Fig. 6: Cross sections of the petiole of *Nuphar lutea* (L.) Sibth. & Sm. Trabeculae with star shaped cells (a). X 240. Portions of aerenchyma of *Nymphaea alba* L. (b, c, d). X 125: AC- air channel; COC- calcium oxalate crystals; T- trichosclereids. Orig.

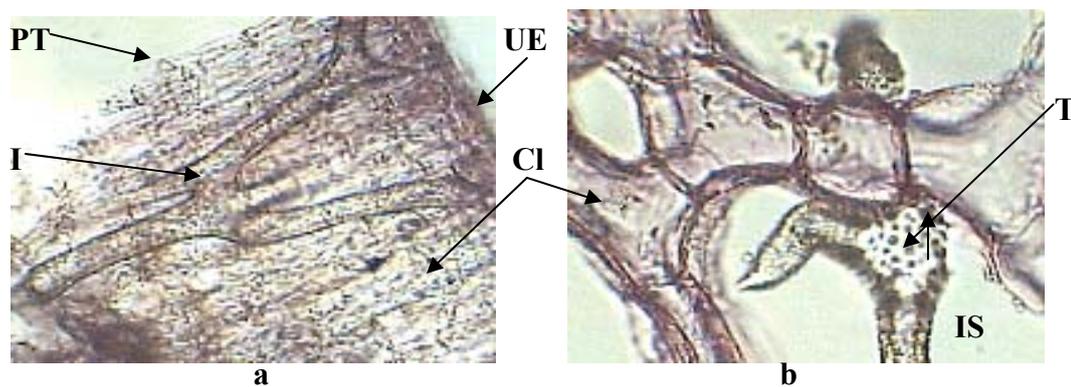


Fig. 7: Cross sections of *Nuphar lutea* (L.) Sibth. & Sm leaf blade. Portion of palisade tissue (a). X 44; Portion of the spongy tissue (b). X 161: CI- chloroplasts; I- idioblast; IS- intercellular space; PT- palisade tissue; T- trichosclereid; UE- upper epidermis. Orig.

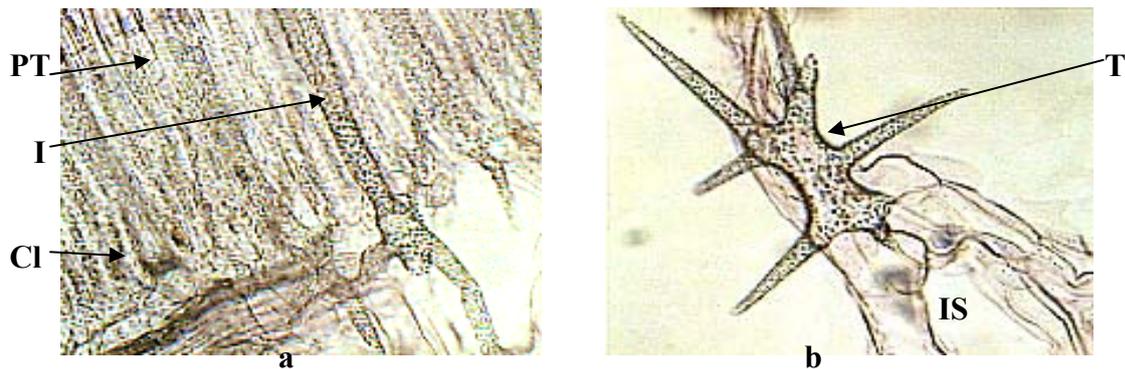


Fig. 8: Cross sections of *Nymphaea alba* L leaf blade. Portion of the mesophyll with palisade tissue (a). X 95. Portion of the spongy tissue (b). X 200: Cl- chloroplasts; I - idioblast; IS- intercellular space; PT- palisade tissue; T- trichosclereid; UE- upper epidermis. Orig.

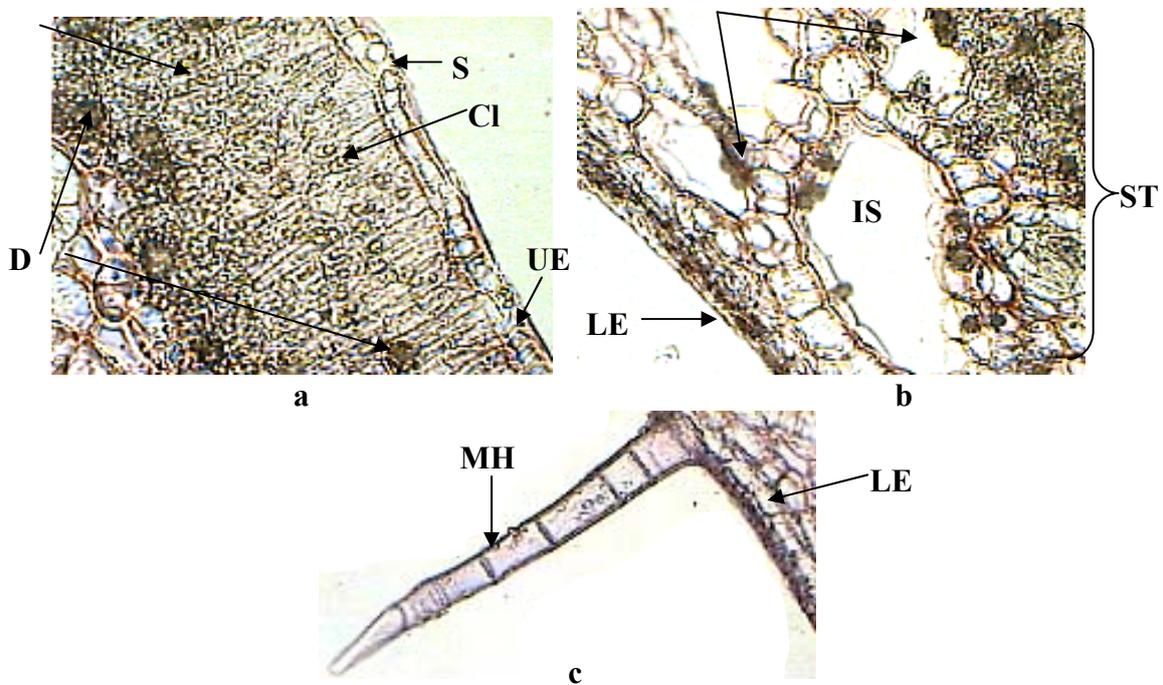


Fig. 9: Cross sections of *Trapa natans* L leaf blade. Portion of the mesophyll with palisade tissue (a). X 179. Portion of the spongy tissue (b). X 194. Many-celled hair of the lower epidermis (c). X 213: Cl- chloroplasts; D- druses; IS- intercellular space; LE- lower epidermis; PT- palisade tissue; S- stomata; ST- spongy tissue; UE- upper epidermis. Orig.

The ground tissues in their peduncles and petioles, consist entirely of aerenchyma encircling large air channels. Characteristically, the petiole and peduncle of *Nuphar lutea* and *Nymphaea alba* possess trichosclereids which protrude into the air channels. The trichosclereids are of various shapes and sizes, branched or simple (*Nuphar lutea*) and bear calcium oxalate crystal deposits.

Trichosclereids are absent in *Hydrocharis morsus ranae* peduncle and in *Trapa natans* petiole. A characteristic cytological feature for the leaves of *Trapa natans* (petiole and mesophyll) is the abundance of sphaerophytes.

Specifically, the peduncle of *Hydrocharis morsus ranae* possess tanniferous cells, spread everywhere in its aerenchyma.

A characteristic feature of *Nuphar lutea* and *Nymphaea alba* mesophyll is the presence of long branched idioblasts. Such cells are not typical for *Trapa natans* and *Hydrocharis morsus ranae* mesophyll. Note the presence of one-celled-hairs at the surface of the peduncle of *Nuphar lutea*, serving probably for the plants defence.

The mechanical tissue is absent but its role is fulfilled by the trichosclereids, distributed in the peduncle and petiole aerenchyma and of idioblasts in the blade mesophyll as well.

The commonest cytological elements were also documented, such as starch grains (around the vascular bundles and cortex of peduncle and petiole) and chloroplasts in the mesophyll of leaves.

Note the presence of stomata with large substomatic cavities in all leaf blades investigated. Stomata are present only among the upper epidermal cells, as usual in the leaves of epistomatic type.

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UNELE PARTICULARITĂȚI HISTOLOGICE ȘI CITOLOGICE LA PATRU HIDROFITE DIN DELTA DUNĂRII (ROMÂNIA)

(Rezumat)

Lucrarea de față își propune evidențierea unor particularități histologice și citologice prezente în țesuturile unor organe ale cormului la patru specii hidrofite din Delta Dunării: *Nuphar lutea* (L.) Sibth. & Sm., *Nymphaea alba* L. (fam. Nymphaeaceae), *Hydrocharis morsus-ranae* L. (fam. Hydrocharitaceae) și *Trapa natans* L. (fam. Trapaceae).

Secțiunile transversale efectuate prin peduncul floral de *Nuphar lutea* și *Nymphaea alba* înfățișează prezența, în tot aerenchimul și în scoarță, a trichosclereidelor (celule stelate), care la *Nuphar lutea* sunt mai puțin ramificate, decât cele ale speciei *Nymphaea alba*. Aceste formațiuni histologice sunt prezente, în special pe marginea canalelor aerifere, ramificațiile lor pătrunzând în lumenul canalelor. Ramificațiile trichosclereidelor pedunculului floral al nufărului galben sunt mai scurte și mai groase decât cele ale nufărului alb (Fig. 1a, b; 2a) La *Nymphaea alba*, în scoarța colenchimatică se găsesc și trichosclereide neramificate (Fig. 2b). Pe trichosclereide se observă depuneri de cristale de oxalat de calciu. Din loc în loc la suprafața pedunculului sunt prezente grupuri de peri tectori unicelulari simpli la *Nuphar lutea* (Fig. 3a, b).

În aerenchimul pedunculului de *Hydrocharis morsus-ranae* nu sunt prezente trichosclereidele dar în țesutul diafragmatic și, în special, pe trabecule se observă prezența unor celule taninifere mari (Fig. 3a).

Secțiunile transversale prin pețiolul de *Trapa natans* prezintă un țesut aerifer bine dezvoltat. În unele canale aerifere se observă țesutul diafragmatic (Fig. 5c, d). Caracteristic pentru pedunculul de *Trapa natans* este prezența unui număr neobișnuit de mare de druze mari de oxalat de calciu atât pe marginea canalelor aerifere cât și în țesutul diafragmatic (Fig. 5 a-d).

Pețiolul frunzelor celor două Nymphaeaceae, pe secțiuni transversale, prezintă un țesut aerifer bine reprezentat în care se găsesc, de asemenea, trichosclereide. Acestea sunt patru-ramificate la *Nuphar lutea* (Fig. 6a) și mai bogat ramificate la *Nymphaea alba*. Ramificațiile lor sunt lungi, subțiriri și ascuțite la vârf. La *Nymphaea alba* ramificațiile pătrund, simultan, în mai mult de două canale aerifere (Fig. 6b, c).

În mezofilul frunzelor de *Nuphar lutea* și *Nymphaea alba*, caracteristică este prezența idioblastelor lungi și ramificate ce străbat aproape întreg mezofilul frunzelor și care suplinesc, ca și trichosclereidele, rolul țesutului mecanic, aproape inexistent la aceste plante (Fig. 7a; 8a). În țesutul lacunos al mezofilului se pot observa și celule stelate (Fig. 7b; 8b). Idioblastele sunt absente în limbul frunzelor de *Trapa natans* și *Hydrocharis morsus-ranae*.

Specific mezofilului de *Trapa natans* este, ca și în pețiol, abundența druzelor de oxalat de calciu (Fig. 9a, b), prezente atât în țesutul palisadic, cât și în cel lacunos. În scoartă și în jurul fasciculelor vasculare interne se observă grăunțioare de amidon (Fig. 4b). Stomatele sunt prezente numai la nivelul epidermei superioare, frunzele fiind epistomatice. Ele se caracterizează prin prezența unor largi camere substomatice (Fig. 9a). La nivelul epidermei inferioare a laminei frunzei de *Trapa natans*, din loc în loc se găsesc peri pluricelulari simpli (Fig. 9).

Formațiunile histologice și citologice asigură împreună cu structura lor anatomică adaptarea acestor plante la viața acvatică natantă