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ANATOMY OF THE STEM AND LEAF IN *CALYSTEGIA SEPIUM* (L.) R. Br.

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Abstract: The article deals with the histo-anatomical characteristics of the stem and leaf in a voluble plant, namely *Calystegia sepium* (L.) R. Br. The anatomical characteristics of the stem and leaf (including the petiole) have been described and discussed. In literature are mentioned the morphological aspects of the plant but an anatomic study on the two main vegetative organs of this species is almost lacking. The results revealed that the anatomical structure of this plant justify its voluble nature, especially the mechanical tissue which is quite poorly represented in the stem and leaf and the distribution of the vascular elements in the stem. The strengthening of the stem is due to few sclerenchyma cells in the cortex and to the vascular system showing an irregularly development. The stele covers the major portion of the stem. Notable are the secondary xylem vessels with a large diameter and the cambium activity. As concerning the leaf, the closed collateral bundles of petiole and blade possess parencymatous sheaths. There are only some collenchyma cells on both sides of the mid rib of blade and under the petiole epidermis. Long one-celled slightly curved hairs on the petiole and blade surface are present.

Introduction

Calystegia sepium (L.) R.Br. or *Convolvulus sepium* L. (fam. *Convolvulaceae*), commonly called Hedge Convolvulus, Old Man's Night Cap or Hooded Bindweed, is a robust sprawling plant with large trumpet-shaped pale-lilac to white flowers that are approximately 5 cm in diameter. It is found abundantly throughout Romania [8]. This species may be distinguished from field bindweed by its two leafy, non-linear bracts that enclose and conceal the sepals [4, 9]. The leaves are large (3-13 cm) and arrow-head-shaped, somewhat thin and delicate in texture and hairless. They are arranged singly on alternate sides of the stem. Their axils spring the flower-stalks with only one large blossom, conspicuous white or pink [5]. The flowers are among the largest from their family. *Calystegia*, the name being derived from two Greek words signifying "beautiful covering" [1]. It specific name, *sepium*, is derived from the Latin *sepes*, a hedge, and refers to its place of growth. The flowers are in bloom from July to September, and like all the other species expand during sunshine and remain closed during dull weather. White Bindweed or *Convolvulus* twine around the supporting stem contrary to the sun, from right to left, and never otherwise; even if the gardener turn it in another direction, the plant, if unable to disengage itself and assume its natural bias, will eventually perish [6]. In literature there are mentioned the morphological aspects of this plants but studis on the anatomy of stem and leaf are almost lacking. The purpose of the paper was to show that the, stem and leaves, of this species, exhibit certain anatomical features of interest, in accordance with its valuable nature.

Material and Methods

Small pieces of mature stem and leaf were fixed in F.A.A. (formalin acetic-alcohol). Cross sections were performed, using the usual manual technique, clarified with chloral hydrate and stained with alum-carmine and iodine green. The samples were embedded in glicerine-jelly. The observations and microphotographs were performed with a BIOROM-T bright field microscope, equipped with TOPICA-1006A video camera. The micrographs were obtained directly from the microscope by means of video camera connected to the computer.

Results and Discussions

The juvenile plant possesses a primary structure, and later developing a secondary one which produces the thickening of stem. Cross sections of *Calistegia sepium* exhibits a more or less circular outline of shaped stem, hystologically differentiated into distinct regions.

However, the outermost layer of cells – epidermis – consists of closely arranged cells, covered by thick cuticle. Its continuity is interrupted by the presence of one-celled hairs (Fig. 1 g). Under the epidermis there extremely reduced is the cortex (2-3 layers of thin-walled round and oval cells with intercellular spaces). The cortical cells may be photosynthetic in nature due to the presence of chloroplasts in them. Cortical cells in abaxial region are larger than those of the other parts of the stem. A number of cortical cells are oxaliferous (druses) (Fig. 1a, c, d). The innermost uniseriate layer of cells is the starch sheath (endodermis). It is well defined supported by one layered of pericycle, belonging to the stele. Some of the pericycle cells are sclerenchymatous. The vascular system consists of secondary phloem and secondary xylem separated by rectangular cambium cells. The stem structures, especially the vascular system, have an irregular development because of its highly enlarged lateral regions [3, 7, 8]. Both the abaxial and adaxial regions of the stem are poorly developed whereas the lateral region is well developed (Fig. 1a) due to the increased activity of cambium that forms more xylem towards the inner side and less phloem towards the outer side. Batanouny [2] reported that the secondary growth of climbing plants is initiated by the formation of a single layered cambium, which later becomes several layered. Secondary phloem forms a ring of phloem elements. Sieve cells, companion cells and few phloem parenchyma cells are distinctly present. The secondary xylem exhibits (in the lateral zones) a thick ring of conjoint collateral and open vascular bundles separated by large parenchyma cells (mostly in the adaxial zone). Secondary xylem consists of large vessels and xylem parenchyma. The smaller vessels are located in inner position whereas the larger ones face the phloem elements. Bellow the secondary xylem a ring of primary xylem is present (Fig. 1a, d, e). The metaxylem elements are located towards the outer side and protoxylem elements towards the pith. Remarkable is the presence of some phloem packets, placed in the peripheral pith region (Fig. 1e). These phloem islands, as other authors [10] noticed for *Convolvulaceae* species are separated from the protoxylem elements by a number of medullary cells. The packets of phloem resulted from the bicollateral bundles of the primary structure [11]. The phloem packets, as Batanouny [2] reported, are important since they retain and continue their function even during unfavorable condition. These phloem islands serve to supply food for the axillary buds, which on return of favorable conditions, developed into branches. The pith consists of large parenchyma cells, enclosing intercellular spaces. Some portions are broken by the development of secondary xylem, forming soon a large lacuna (Fig. 1f).

Transversalsection of the petiole reveals a plane or slightly concave face and a convex one such as other *Convolvulaceae* species do [12]. The epidermis consists of one layer of cutinized cells, covered, externally, by a thick cuticle. The epidermis continuity is broken by the presence of one-celled hairs and stomata (Fig. 2b). Under the epidermis is the cortex, differentiated into two zones. The outer region is a collenchyma tissue (4-5 layers of collenchyma cells), the inner part consists of large polygonal parenchymatous cells, some of them larger than the other, enclosing intercellular spaces and secretory elements (Fig. 2a, c, d). The stele consists of three collateral vascular bundles, two small and one larger, the latter placed in the center of the petiole (Fig. 2a). The centrally located one is a collateral vascular bundle with a number of xylem vessels in radial arrangement, separated by parenchyma cells. The phloem consists of few phloem elements and in places druses are present (Fig. 2e). The vascular bundle is surround by parenchyma sheath. The small vascular bundles consist of few xylem and phloem elements. They are placed to the peripheral regions of the petiole (Fig. 2f).

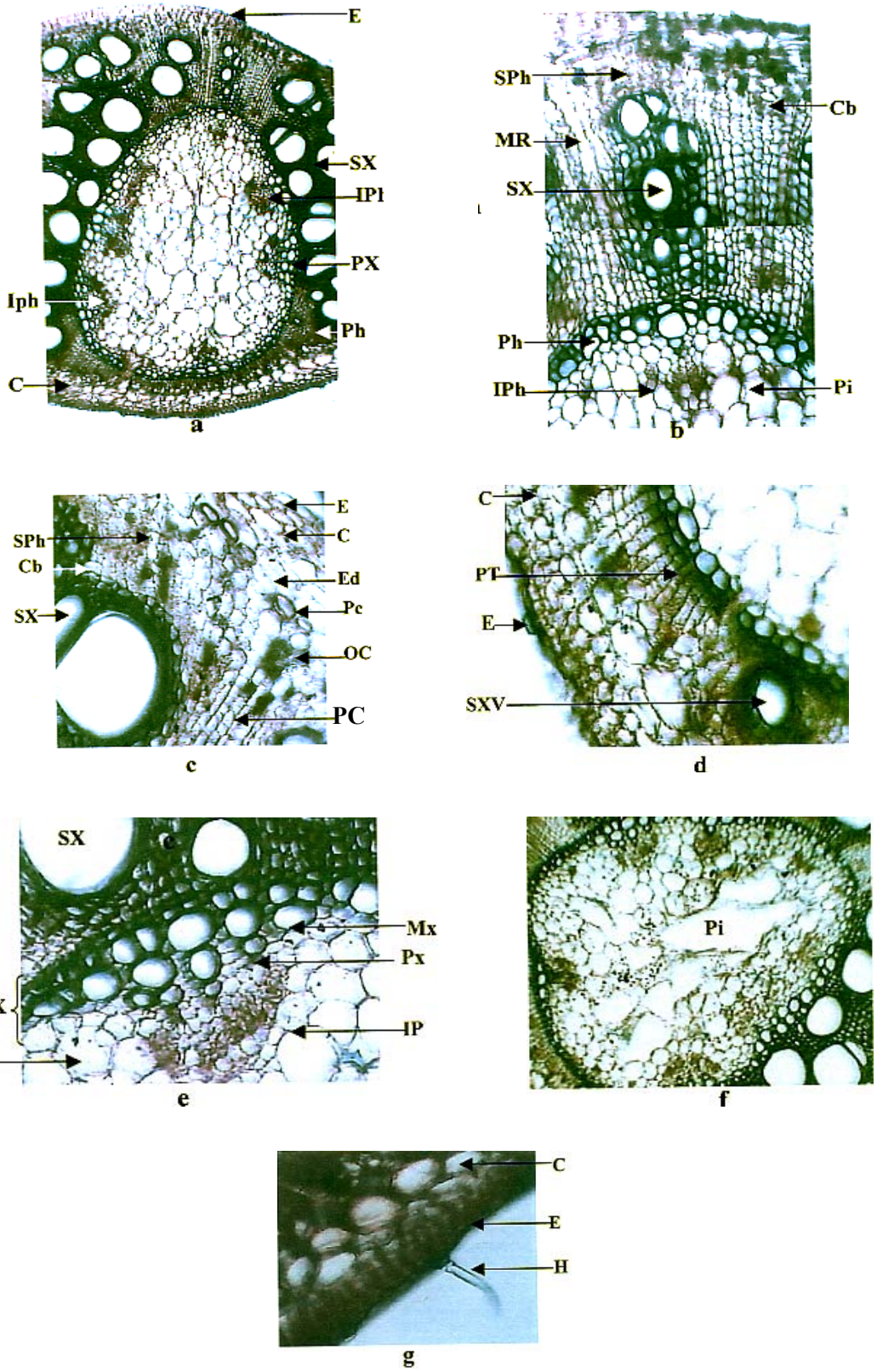

Fig 1: Cross section of the stem. General view

(a). X 64. Part of the adaxillary region (b, c). Part of the abaxillary region (d). Part of the stem with included phloem (e). X 140. The pith (f). X 58 A hair of the stem (g). X 140: C- cortex, Cb- cambium, E- epidermis, Ed- endodermis, H- hair, IPh- included phloem, OC- oxaliferous cells, MxV- metaxylem Bessel, Pc- pericycle, Ph- phloem, Pi- pith, PC- parenchyma cells, PT- primary tissue, PxV- protoxylem vessel, PX- primary xylem, SPh- secondary phloem; SX- secondary xylem, SXV- secondary xylem vessel (orig).

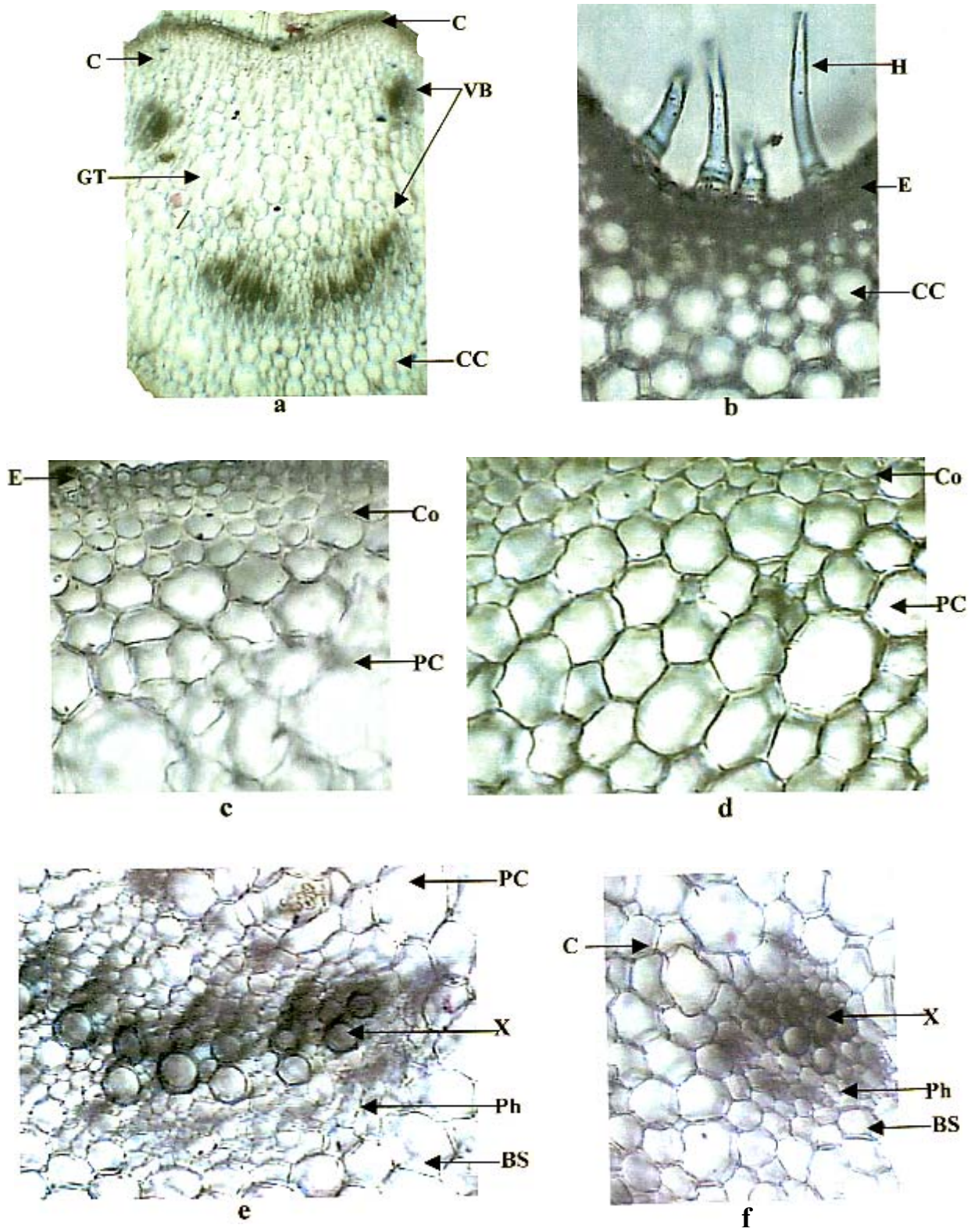

Fig. 2: Cross sections of the petiole. General view

(a). X 51. Portion with epidermis and hairs (b). Portions with epidermis and cortex (c, d). The large vascular bundle of the stele (e). X 187. One of the small vascular bundles of the stele (f). X 240: BS- bundle sheath, Ct- cuticle, C- cortex, Co- collenchyma, CC- collenchyma cells, E- epidermis, GT- ground tissue, H- hairs, Ph- phloem, PC- parenchyma cells, VB- vascular bundle(s), X- xylem (orig.).

Cross sections of the dorsiventral blade exhibit heterogenous mesophyll, differentiated into palisade and spongy tissues. The mesophyll lies between the upper and the lower epidermis and possesses druses of calcium oxalate and glandular hairs (Fig. 3c, d). The upper epidermis consists of regularly arranged simple cells, covered by a thick cuticle. Right to the mid-rib vein the upper epidermis forms a crest filled with collenchyma cells (Fig. 3a). The palisade cells (one layer) contain numerous chloroplasts. The compactly arranged spongy tissue encloses small intercellular spaces. The lower unistratose epidermis, such as the upper one consists of cells covered by thin cuticle. Its continuity is broken by the presence of stomata (Fig. 3c). The vascular system of the mid-rib is a collateral bundle such as the petiole. Bellow the mid rib a number of slightly collenchymatous cells with mechanical role is present (Fig. 3b). The blade possesses a bifacial structure close by a unifacial one.

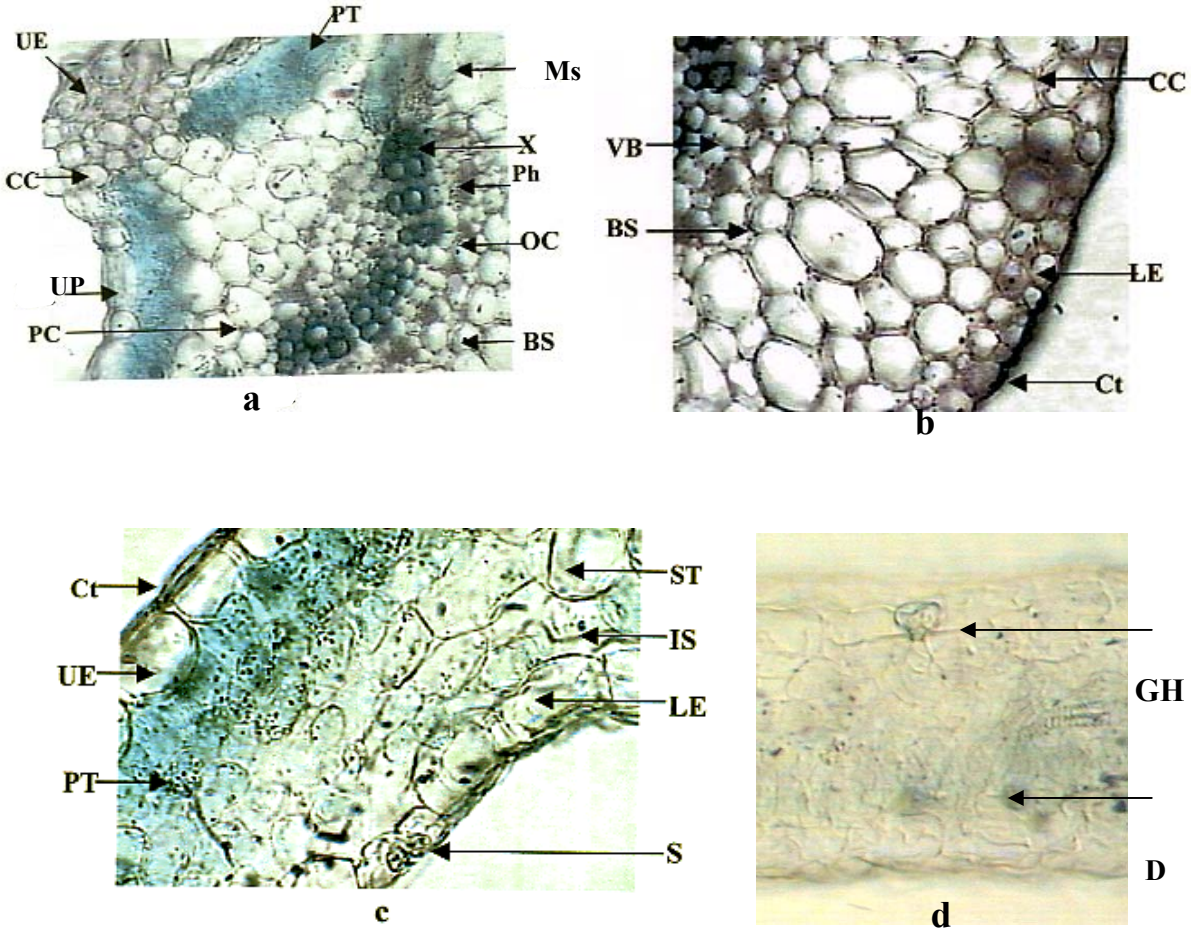

Fig. 3: Cross section of the blade. Portion with upper epidermis and mid rib vein
(a). Portion with lower epidermis (b). Portion with mesophyll (c). X 200. Glandular hair (d). X 280: BS- bundle sheath, Ct- cuticle, CC- collenchymatic cells, D- druses, GH- glandular hairs, IS- intercellular spaces, LE- lower epidermis, Ms- mesophyll, OC- oxaliferous cell, Ph- phloem, PC- parenchyma cells, PT- palisade tissue, S- stoma, ST- spongy tissue, UE- upper epidermis, VB- vascular bundle, X- xylem (orig.).

Conclusions

The present results revealed some anatomical characteristics in accordance with the plant voluble nature. The epidermis cells of the stem and leaf have their outer walls covered by a thick cuticle and bears one-celled, long and curved hairs for the plant support. The cortex is extremely reduced and devoid of thin walled parenchymatous cells. However a number of cortical cells are assimilatory in nature, other possesses druses. Cambium irregularly developed vascular tissues in

the stem, causing volution of stem. So the lateral region of the stem has a secondary structure and is well developed whereas the abaxial and adaxial zones are poorly developed, being the zone for the plant winding. Note the abundance of the xylem elements with large diameter and the thickness of the secondary xylem elements. Large parenchymatous cells are present between the vascular tissues (in adaxial position). A number of primary vascular elements are differentiated around the medullary parenchyma. The mechanical tissue of the stem is poorly developed but it is sufficient to keep it in almost vertical position. It is represented by some pericycle sclerenchyma cells and by the secondary vascular tissue itself. The leaf stereom is well developed and consists of collenchyma tissue in the cortex of petiole and a number of collenchyma cells between the mid rib of the blade. The petiole vascular system, such as the veins, is represented by collateral vascular bundles, protected by a parenchyma sheath. The lower epidermis is interrupted by the presence of stomata.

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ANATOMIA TULPINII ŞI A FRUNZEI DE *CALYSTEGIA SEPIUM* (L.) R. Br.

(Rezumat)

Calystegia sepium (L.) R.Br. sau *Convolvulus sepium* L. (fam. Convolvulaceae) este o plantă volubilă robustă cu o corolă infundibuliformă de culoare pal-liliachie spre albă cu un diametru de aproximativ 5 cm. Florile sale sunt unele dintre cele mai mari ai familiei sale. Este larg răspândită în Romania [9]. Specia se distinge uşor de alte plante din familia sa prin bractei stufoase ce ascund în parte caliciul (1, 4, 5, 9]. Frunzele sale sunt mari (3-13 cm), sagitate, păroase dar cu textură delicată. După cum afirma Ann Pratt [6] planta se răsuceşte în jurul suportului contrar luminii solare (diferit de alte plante volubile), de la dreapta spre stânga, niciodată invers chiar dacă grădinarul o întoarce în altă direcţie. În literatură sunt prezentate mai mult aspectele morfologice ale speciei şi mai puţin cele anatomice ale tulpinii şi frunzei. Scopul lucrării este de a înfăţişa unele caractere anatomice particulare ce justifică natura volubilă a acestei plantei.

Tulpina în cursul perioadei de vegetaţie trece printr-o structură primară (în primele faze de dezvoltare) şi una secundară în cursul căreia se realizează îngroşarea tulpinii. Secţiunile transversale efectuate prin tulpina matură de *Clystegia sepium* înfăţişează un contur aproape circular, mai dilatat în părţile sale laterale, datorită dezvoltării inegale a structurilor componente, zona de cambiu producând mai mult lemn secundar în părţile laterale ale tulpinii decât în rest. Alte zone (adaxială dar mai ales abaxială) rămân neîngroşate, determinând caracterul volubil al acestei plante, ca şi a altor specii din aceeaşi familie [3, 8]. La exterior are epiderma unistratificată, acoperită de cuticulă (Fig. 1, a-d). Sub epidermă se găseşte cortexul, extrem de redus urmat de o teacă amiliferă. Unele dintre celulele cortexului conţin cloroplaste, altele sunt celule oxalifere. Periciclul este unistratificat, parte dintre celulele sale au pereţii îngroşaţi, sclerificaţi (Fig. 1c). Sistemul vascular este reprezentat, în părţile sale laterale printr-un manşon gros de fascicule conducătoare, compacte, mixte, colateral deschise, separate, pe alocuri, (porţiunea adaxială) de celule parenchimatice, ce dau suplete plantei [7]. Ţesutul conducător secundar este alcătuit din vase cu diametrul

mare de meta- şi protoxilem, interconectate prin parenchim lemnos şi ţesut liberian secundar (slab dezvoltat) (Fig. 1a). Acesta este format din vase liberiene însoţite de celule anexe şi parenchim liberian. Elementele de lemn şi liber secundar sunt separate de zona de cambiu. Porţiunea adaxială şi abaxială sunt mai puţin dezvoltate, aceasta din urmă fiind locul de înfăşurare a tulpinii, partea flexibilă. Se ramarcă inelul de xylem primar ce înconjoară măduva (Fig. 1e, f). Se evidenţiază prezenţa, în zona periferică a măduvei, a unor grupuri de liber rămase din fasciculele bicolaterale ale structurii primare a plantei. Ele sunt separate de lemnul primar prin câteva celule din parenchimul medular [10, 11]. Rolul lor a fost descris şi de Batanouny [2].

Peţiolul prezintă în secţiune transversală, o suprafaţă plană sau uşor concavă şi una convexă [12]. La exterior se găseşte epiderma unistratificată urmată de cortex, diferenţiat în două zone. Zona externă este formată din câteva straturi de colenchim angular, urmat de regiunea internă de natură parenchimatică (Fig. 2c, d). Stelul este reprezentat prin trei fascicule colaterale închise cu lemnul dispus radial, separate de celule parenchimatice (Fig. 2e, f). Limbul frunzei bifaciale are mezofil heterogen, diferenţiat în ţesut palisadic şi lacunos. În mezofil se găsesc druze şi peri glandulari (Fig. 3c, d). În dreptul nervurii mediane epiderma superioară formează o creastă în care se găsesc celule colenchimatizate (Fig. 1a). Ţesutul vascular al nervurii mediane este reprezentat printr-un fascicul colateral asemănător cu cel din peţiol, (Fig. 3a). Între nervura mediană şi epiderma inferioară se găsesc celule cu pereţii colenchimatizaţi, cu rol mecanic. Continuitatea epidermei inferioare este întreruptă de prezenţa stomatelor (Fig. 3c).