

HISTOANATOMICAL STUDY OF *SALVIA NUTANS* L.

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Summary

This paper presents anatomical aspects of the root, stem and leaf structure of *Salvia nutans* L. belonging to Lamiaceae family. Cross and paradermal sections were performed by the usual methods used in vegetal histology; the samples were stained using different staining methods and analysed with a BIOROM –T bright field microscope, equipped with a TOPICA 6001A video camera. The root in cross section exhibits a secondary structure, generated by the activity of two meristems: phellogen and vascular cambium. Cross section of the stem presents a primary structure that tends to become secondary through the xylem structure, both stem and leaf petiole disclose a two zones differentiated cortex and the same type of vascular bundles. The stem, petiole and blade present both glandular and non-glandular trichomes, the leaf blade is amphistomatic, ecrivifacial with a heterogeneous mesophyll and vascular bundles. Paradermal sections exhibit diacytic stomata. The mechanical tissue is well developed represented by collenchyma and sclerenchyma.

Keywords: anatomy, root, stem, leaf, secondary structure.

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Introduction

Salvia nutans L. belongs to the Lamiaceae family, is a perennial, herbaceous plant, indigenous to North America and spread in south and East of Europe.



Salvia nutans L.

The stem is erect, almost leafless, 40-45 cm high and moderately hairy. The leaves from the stem, though rare, possess

7.5 cm long petioles. The ovate cordate blade is 6-7 cm long, 2.5 cm wide with double crenate margins. The green leaves are ventrally glabrous or disperse hairy and dorsally tomentous (Ciocârlan & Costea, 1997).



Salvia nutans L.

The stem is branched, bearing 6.5 cm long inflorescences, with 2-3 pairs of nutant branches (Nyárády, 1942). The bilabiate violet blue flowers are grouped into panicle racemes. Corolla is 10-13 cm long and the bracts are small (Hedge 1972;

Răvăruț and Nyárády 1961). In the literature, knowledge of the anatomical characteristics of this species, are quite limited. This is the reason we considered that a study concerning the vegetative organs anatomy of *Salvia nutans* is welcome.

Material and methods

The species was collected from the arid coastlines of SW Conacu, Dobrudja, 43°58'54.648"N, 28°09'44.683"E, 100 m altitude, 29 VII 2009, G. Negrean & R. Bercu, with other rare species: *Bupleurum apiculatum*, *Cleistogenes bulgarica*, *Centaurea diffusa*, *Convolvulus cantabrica*, *Euphorbia dobrogensis*, *Koeleria lobata*, *Salvia nemorosa* subsp. *tesquicola*, *Salvia nutans*, *Taraxacum serotinum*, *Teucrium polium* subsp. *capitatum* etc.

The species was fixed in FAA 50 and transferred to alcohol 50%. For the

anatomical study, freehand sections were made on the root, stem and leaves. The samples were stained using different staining methods, such as alum-carmin and iodine green (the trasversal sections) and safranin 0,5% (the paradermal sections) (Bercu and Jianu, 2003). Histological observations and micrographs were performed with a BIOROM –T bright field microscope, equipped with a TOPICA 6001A video camera.

Results and discussions

Anatomically the root exhibits a secondary structure due to the activity of phellogen and vascular cambium. The one layered rhizodermis is replaced by 2-3 layers of protective cells containing suberin, phellem. This tissue is generated by the phellogen, composed of 1-2 layers of mighty flattened cells.

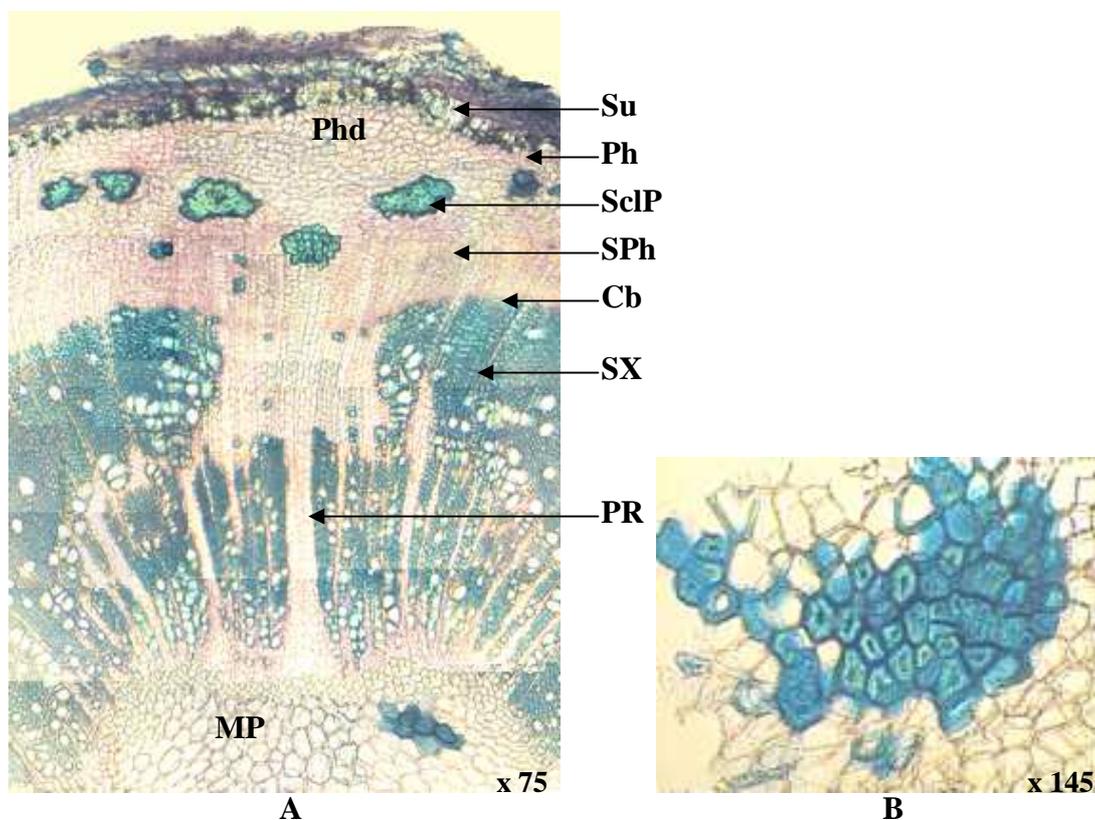


Fig. 1. Cross section of the root. Ensemble (A). Medullar parenchyma - details (B): Cb – cambium, MP – medullar parenchyma, Ph – phellogen, Phd – phelloderm, PR – pith ray, Su – suber, ScIP – sclerenchima pack, SPh – secondary phloem, SX – secondary xylem.

The parenchymatic, multilayered cells that grow inwards from the phellogen are representing the phelloderm (secondary cortex) (Bavaru and Bercu, 2002). The vascular cambium composed of 2-3 layers of mighty flattened cells, generates outwards the secondary phloem and inwards the secondary xylem and also medular rays. The secondary phloem is protected in the external area by packs of sclerenchymatic cells and also among the vascular elements of the phloem, isolated or groups of sclerenchyma cells occur (Fig. 1. A). The secondary xylem is more developed than the phloem, all its vascular elements are mighty lignified. In the internal area few elements of primary xylem can be observed. In the medullar parenchyma, centrally situated, packs of sclerenchymatic cells are present (Fig. 1. B).

The stem in cross section exhibits a circular-coasted configuration with 4 crests (Fig. 2. A). It has secondary structure due to its xylem anatomical development. The one-layered epidermis is composed of different shaped and sized cells with thickened external walls covered by cuticle (Batanouny, 1992). The presence of stomata, placed slightly above the epidermal level and trichomes interrupt the continuity of the epidermis. Like other members of the Lamiaceae, *Salvia nutans* possesses both peltate and capitate glandular trichomes, as well as non-glandular ones (Fig. 3. A-D). The latter are numerous, multicellular (up to 4 cells), uniseriate and unbranched. Multicellular trichomes are straight at the tip, few can be observed with a curved tip. As other salvia species (Kahraman et al., 2010), they are quite variable in length, some of them being large and placed mainly to the crest zone.

The glandular trichomes are of two types. Some of them are typical peltate trichomes and others are capitate. Peltate trichomes of the Lamiaceae often comprise a broad head of several secretory cells (of

up to eight to sixteen cells), a short stalk and a basal epidermal cell (Hallahan, 2000). *S. chamelaeagnea* (Kamatou et al., 2006) has peltate trichomes, with up to sixteen head cells. The present study showed that *S. nutans* has peltate trichomes with a one-celled stalk, sunken in the epidermis and an eight-celled head arranged in a single circle which is in agreement with previous studies (Serrato-Valenti et al., 1997). However, in other species of the same family, such as *Origanum* species (Bosabalidis and Tsekos, 1984) and *Satureja thymbra* (Bosabalidis, 1990), a higher number of head cells are arranged in two concentric circles. Others are capitate trichomes with two-celled short stalk and a bicellular secretory head as well as those with unicellular short stalk and unicellular secretory head. Few glandular trichomes are composed of a longer one-celled-stalk with convex walls, a short one-celled neck and unicellular secretory head (Corsi and Bottega, 1999).

The cortex is represented by two zones. The external one is represented by 10-11 layers of collenchyma tissue placed only at the crest area and by 3-4 layers of chlorenchyma tissue between the crests. The inner zone presents 2-3 layers of parenchymatic cells, bigger than those of the external zone. Neither endodermis nor pericycle can be observed. The vascular system is represented by numerous open collateral bundles arranged on a single ring (Kahraman and Doğan, 2010). They are protected in the phloem area by groups (caps) of sclerenchymatic cells. The xylem elements are radially arranged in a cellulosic parenchyma. The vascular bundles are embedded in a slightly lignified tissue (Fig. 2. B). There are 4 large vascular bundles in the crest areas. On each side of a big vascular bundle, medium, small vascular bundles occur. Centrally, a medullar parenchyma is present (Fig. 2. A).

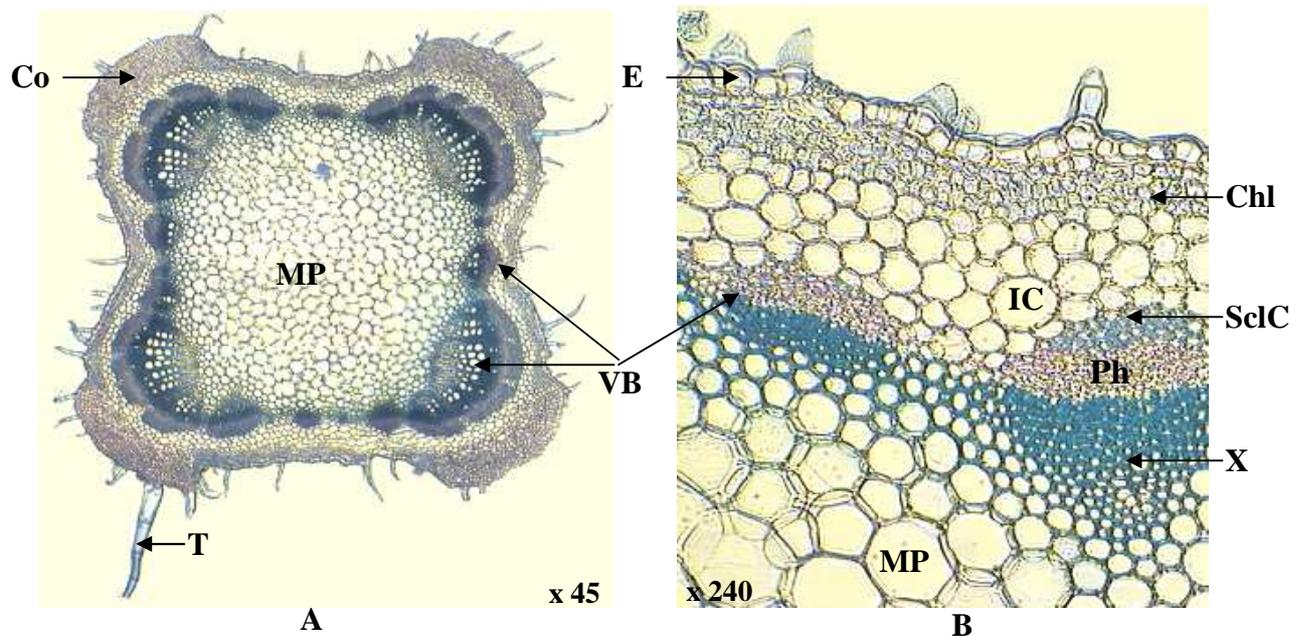


Fig. 2. Cross section of the stem. Ensemble (A) and details (B): Chl – chlorenchyma, Co – collenchyma, E – epidermis, IC – internal cortex, MP – medular parenchyma, Ph – phloem, SclC – sclerenchyma cells, T – trichome, VB – vascular bundles, X – xylem.

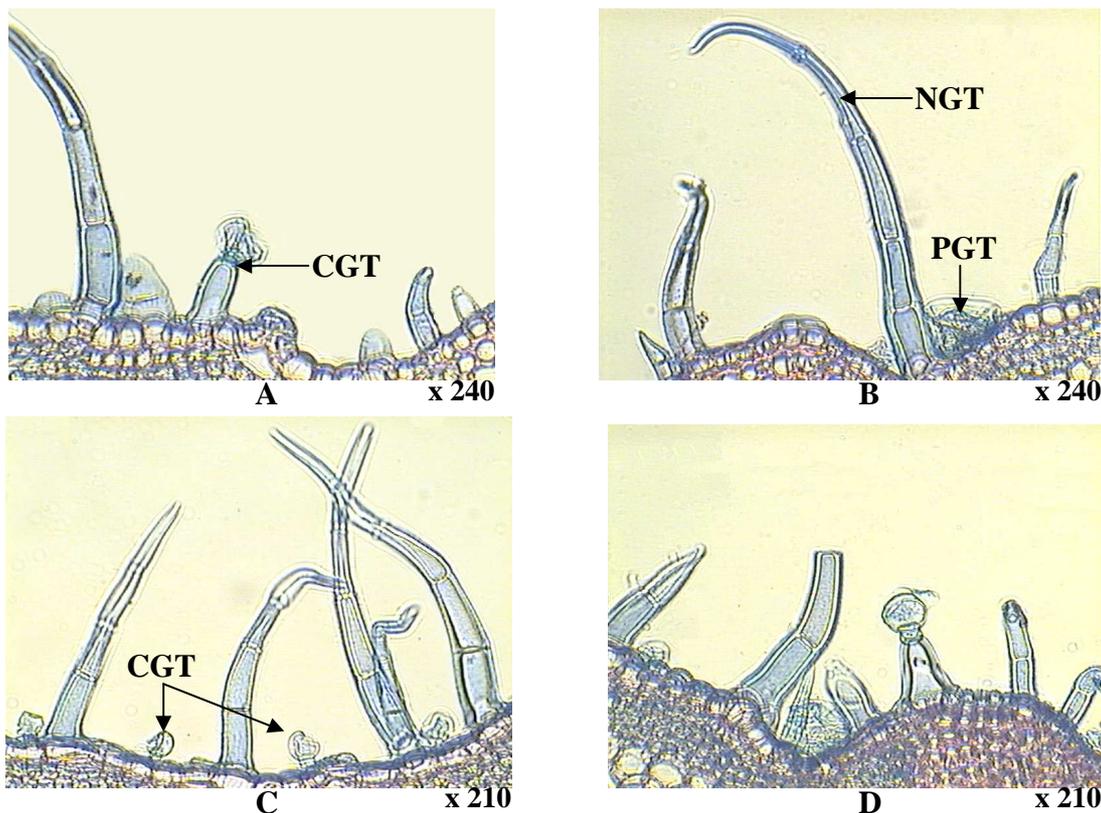


Fig. 3. Non-glandular and glandular trichomes (A-D): CGT – capitate glandular trichomes, NGT – non glandular trichome, PGT – peltate glandular trichome.

Cross section of the petiole discloses that, the adaxial surface is almost flat to concave, with two crests and the abaxial surface is convex (Fig. 4.). The

epidermis is composed of one-layer more or less isodiametric cells, covered by an outer cuticle. Its continuity is interrupted by the presence of rare stomata arranged slightly

above the epidermal level and numerous trichomes. The petiole presents the same type of non-glandular and glandular trichomes described at the stem epidermis, but the non-glandular ones are more abundant in the petiole (Fig. 5.). *S. nutans* petiole has rare peltate trichomes and numerous capitated trichomes with two-celled short stalk and a bicellular secretory head as well as those with unicellular short stalk and unicellular secretory head. The one celled-stalk with convex walls, a very short one celled neck and unicellular secretory head trichomes are few.

The petiole cortex is differentiated into two zones. The external one is represented by a chlorenchyma, alternating with collenchyma tissue and a large parenchymatous inner region. *S. nutans*

petiole has abaxial 3-4 layers of collenchymatous cells whereas adaxial 1-2 layers. Lateral 2-3 layers of chlorenchyma cells are present. In the crests this tissue is well developed, there are 5-6 layers of collenchyma cells. The parenchymatous region is represented abaxial and adaxial by 7-8 layers of cells.

In the parenchyma there are two to three large vascular bundles in the centre and two to three small subsidiary bundles towards the petiole crests. The vascular tissues lie along a shallow arc and their arrangement is open collateral. In the phloem area the vascular bundles are protected by discontinues groups of sclerenchymatous cells. The vascular bundles are surrounded and separated by parenchymatous cells (Figs. 4., 5.).

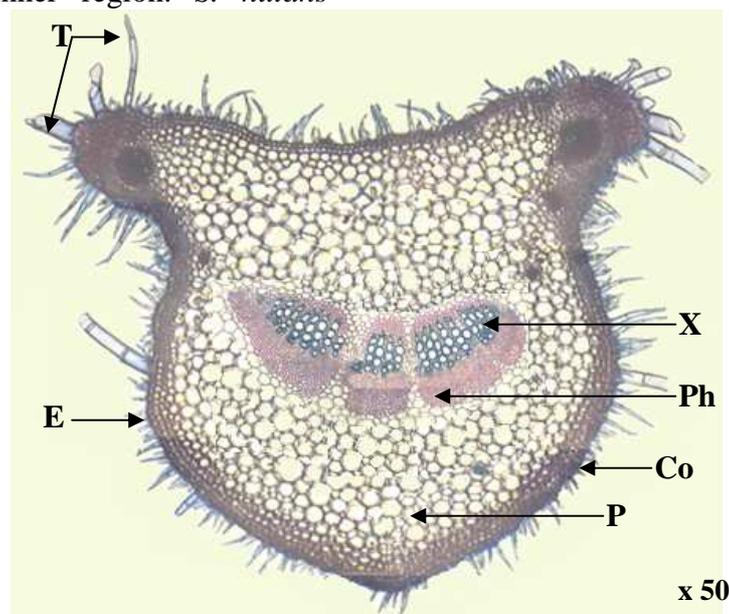


Fig. 4. Cross section of the petiole - ensemble: Co – collenchyma, E – epidermis, P – parenchyma, Ph – phloem, T – trichomes, X – xylem.

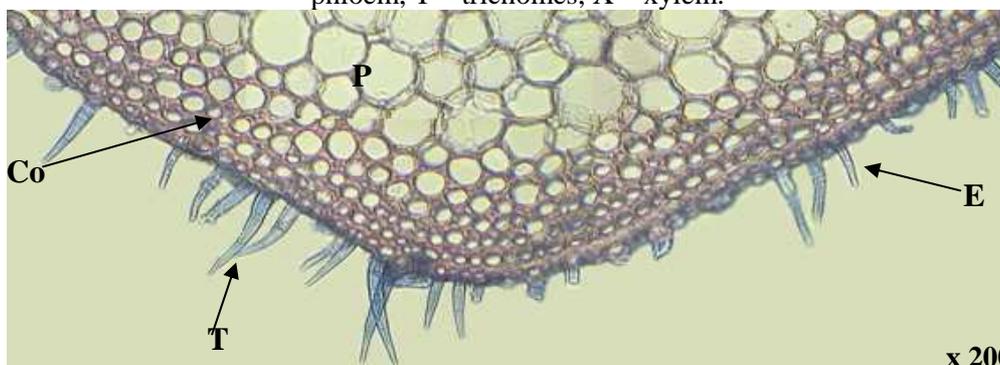


Fig. 5. Abaxial portion of the petiole in cross section: Co- collenchyma, E- epidermis, P- parenchyma, T- trichomes.

Anatomically, the leaf blade consists of upper epidermis, lower epidermis, mesophyll and vascular system. Upper and lower epidermis are monolayered, each cell different shape and size, slightly elongated tangentially. Epidermal lateral cell walls and the external ones are slightly cutinized and the latter are protected by cuticle. The upper epidermis cells are larger than lower ones. (Fig. 6.).

The non-glandular trichomes are similar to those of the petiole. In addition are present trichomes with ridges and marked internodes in the lower epidermis. The same types of glandular trichomes such as those of the petiole are present in the blade, being more abundant in the lower epidermis (Fig. 8., A-D). Most abundant are the short two-celled stalk and bicellular secretory head trichomes.

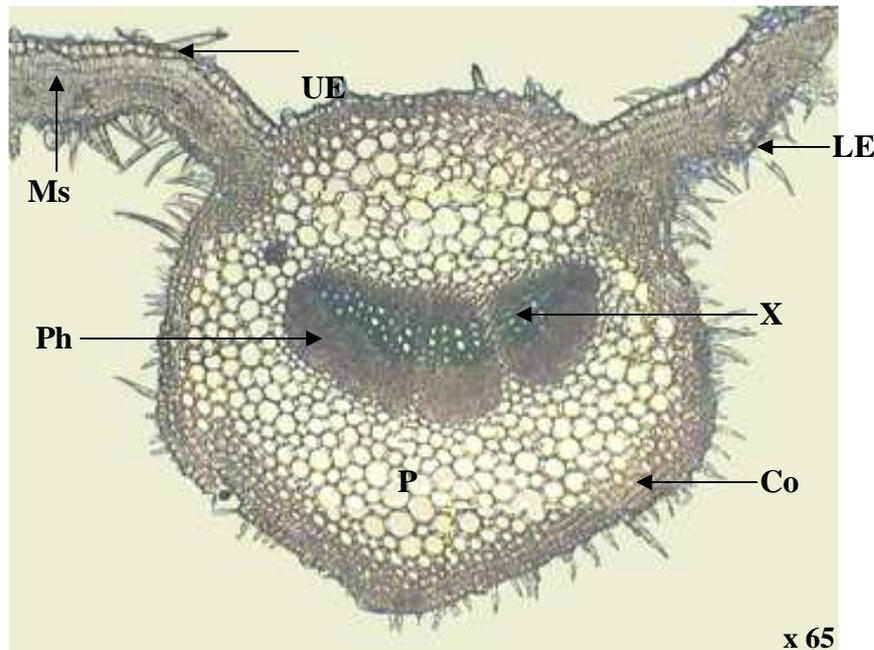


Fig. 6. Cross section of the blade - ensemble: Co – collenchyma, LE – lower epidermis, Ms – mesophyll, Ph – phloem, UE – upper epidermis, X - xylem.

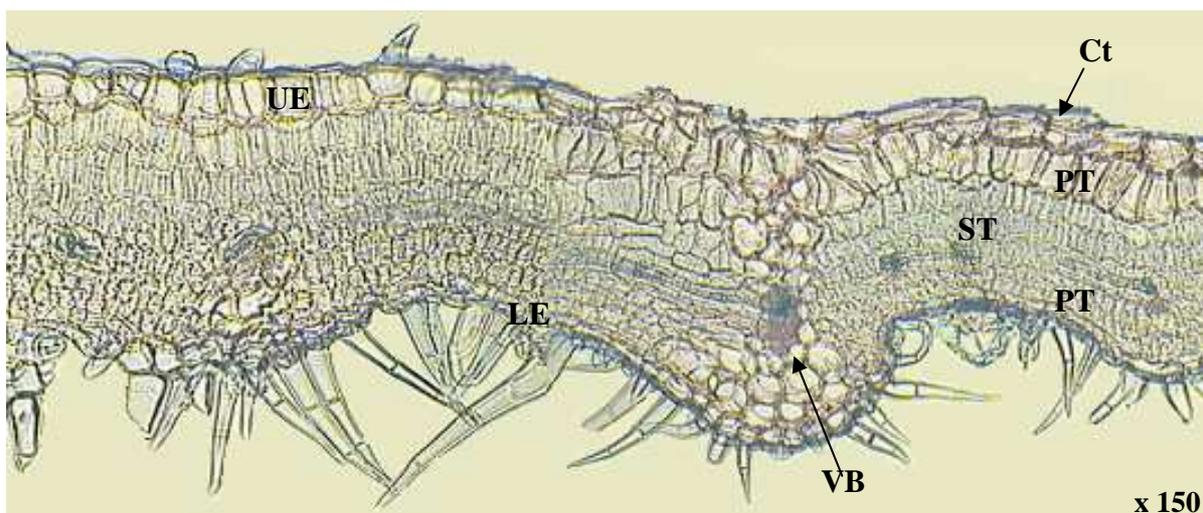


Fig. 7. Portion of the mesophyll in cross sections: Ct- cuticle, LE- lower epidermis, PT- palisade tissue, ST- spongy tissue, UE- upper epidermis, VB- vascular bundles.

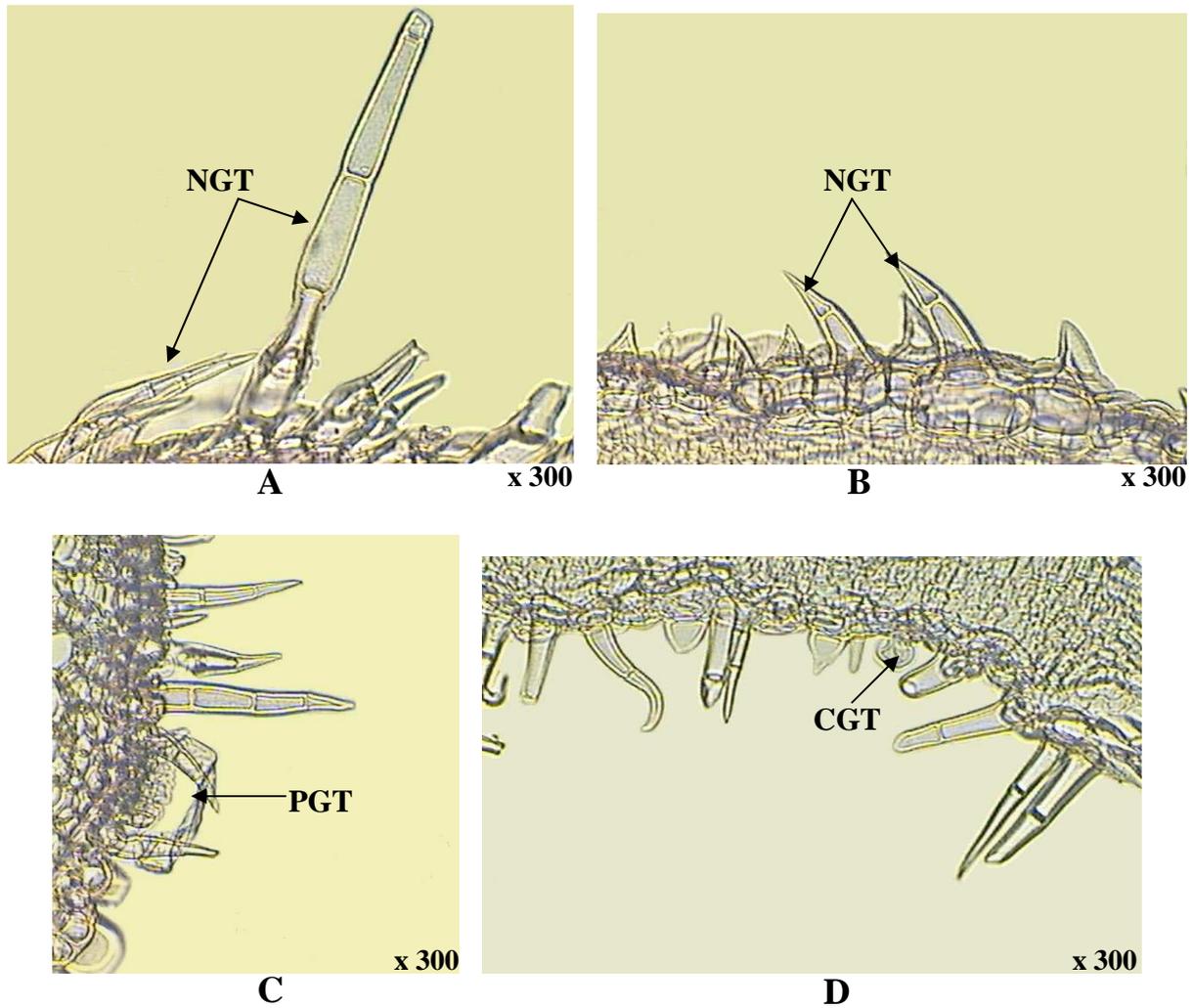


Fig. 8. Types of trichomes in transections of the upper (A, B) and lower (C, D) epidermis: CGT – capitate glandular trichomes, NGT – non-glandular trichomes, PGT – peltate glandular trichomes.

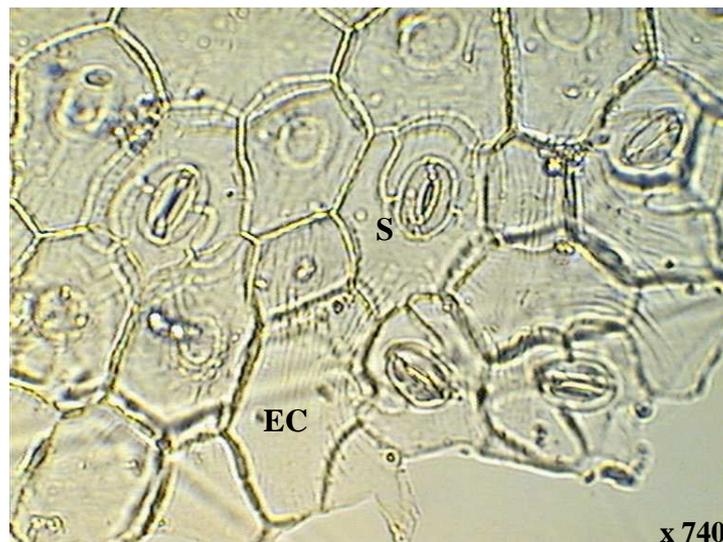


Fig. 9. Paradermal section of the lower epidermis: EC – epidermal cell, S - stomata.

The leaf blade is ecvifacial and amphistomatic and comprises 2 layers of palisade tissue just below the upper epidermis and as well the lower epidermis and 1-2 layers of spongy tissue between them. The vascular bundles of the vein are embedded in the mesophyll (Fig. 7.). They are anatomically similar to the ones described in the petiole but with a typical foliar arrangement of the conductive elements. The midrib is adaxially flat and abaxially convex. It is represented by 1-2 big vascular bundles and 1 or 2 small ones. In the midrib area, 1-2 layers of collenchyma tissue are present, just beneath the upper epidermis and 2-3 layers can be observed beneath the lower one (Fig. 6.) (Webb and Carlquist, 1964).

Paradermal sections of the blade disclose straight-walled epidermal cells, glandular and non-glandular trichomes and numerous diacytic stomata (Dilcher, 1974) especially in the lower epidermis (Fig. 9).

Conclusions

The anatomical structure of the root is a secondary one, represented by the known sequence of tissues. Remarkable is the presence of sclerenchyma tissue in the medullar parenchyma.

The stem in cross section exhibits a secondary structure due to the development of the xylem. The epidermis possesses a number of stomata and trichomes. The vascular bundles are orderly arranged in a single ring forming an eustel.

The petiole epidermis presents fewer stomata than the stem epidermis but more numerous trichomes, especially non-glandular ones. Both stem and petiole cortex is differentiated in two zones. The vascular bundles of the petiole are similar with those described in the stem.

The leaf blade is ecvifacial, amphistomatic and presents a heterogeneous mesophyll. Stomata and trichomes are more abundant at the lower epidermis of the blade. Both epidermises possess glandular and non-glandular trichomes, which are more abundant than those of the petiole.

The vascular bundles are similar to those of the petiole but the arrangement of the conductive elements is typical to a foliar structure. The paradermal section of the epidermises exhibits a diacytic stomata and straight walled epidermal cells.

The mechanical tissue is well developed in all plant organs and is represented by collenchyma and sclerenchyma tissue.

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