



Leaf anatomical study of taxons *Salvia nemorosa* subsp. *tesquicola*, *Salvia nutans*, and *Salvia* × *Sobrogensis* from Dobrudja

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ABSTRACT: This paper describes aspects of the leaf anatomy of two species of *Salvia* L.: *Salvia nemorosa* L. subsp. *tesquicola* (Klokov & Pobeda.) Soó, *Salvia nutans* L. and a hybrid of the two plants - *Salvia* × *dobrogensis* Negrean. The objective was to highlight common anatomical characteristics and superiority of the hybrid, compared with its parental species. Differences were present in the structure of both the petiole and blade. For the petiole, differences concerned the degree of development of the external (collenchym and chlorenchym) and inner cortex. The vascular system in all species, comprised a great number of vascular bundles, with different levels of development of the conductive tissues. The mesophyll was heterogeneous, bifacial (*S. nemorosa* subsp. *tesquicola* and hybrid) and *S. nutans* ecvifacial. Numerous glandular and non-glandular trichomes (hairs) were present, different in structure, shape and size. Stomata were found on both upper and lower epidermis of the blade and were of the diacytic type, impressing, with an amphistomatic character. The vascular system of the mid-vein of the *Salvia* species was well developed, in particular those of the hybrid species. The petiole and blade anatomy of the two species of *Salvia* and their hybrid showed common and specific features indicating that, although the hybrid leaf is more developed anatomically than its parental species, the petiole has many features similar to that of *Salvia nutans* and the blade is very similar to that of *Salvia nemorosa*.

KEY WORDS: anatomy, leaf, *Salvia* species.

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INTRODUCTION

Salvia nemorosa L. subsp. *tesquicola* (Klokov & Pobeda.) Soó, *Salvia nutans* L. and a hybrid of the two plants - *Salvia* × *dobrogensis* Negrean are part of the Lamiaceae family. *Salvia nemorosa* subsp. *tesquicola* (Klok. & Pobed.) Soó is a perennial, herbaceous plant, drought resistant, native of Central and West Asia and grows in large groups. It is a species that readily hybridizes and hybrids of this species are difficult to identify. The 4-edged stem is 50-60 cm tall, erect, hairy, with sessile shoots at the axil of the leaves and branched to the tip. The lower lanceolate leaves are petiolate (2-5 cm long), hairy, pointed to the apex, with a cordate base, unequally crenate margins and reticulate

venation (DILCHER 1974). The lower leaves are 8 cm long, 2 cm wide and the middle and upper leaves are smaller (2.5 to 6 cm long, 0.7 to 1.5 cm wide). The green leaves are often rough, slightly hairy ventrally and dorsally almost glabrous, with short hairs along the veins. The species forms long inflorescences (± 17 cm), with smaller bracts than the hybrid and longer than *S. nutans*. The flowers, bilabiate with a violet corolla, are 8-13 cm long with purple bracts, slightly shorter than those of the hybrid (RĂVĂRUȚ & NYÁRÁDY 1961).

Salvia nutans L. is a perennial, herbaceous plant, indigenous to North America and also widespread in south and east Europe. The stem is erect, almost leafless, 40-45 cm high and moderately hairy. The leaves from the

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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 (CIOCARLAN & COSTEA 1997). The abundance of hairs on stems and petioles is intermediate between *S. nemorosa* and the hybrid. 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. The corolla is 10-13 cm long and the bracts are small (NYARADY & RĂVĂRUȚ 1961). The hybrid is also a perennial, up to 40 cm high, with branched stems to the tip, slightly nutant, very hairy compared to the parental species. The basal and lower ovate and petiolate leaves (4-5 cm long) are close with cordate bases and unequal crenate margins. The lamina is 8.5 cm in length and 5 cm wide. The flowers are slightly nutant, grouped in 3-6 racemes, each 13-20 cm long. The flowers are intermediate between the parents, abundant calyx hairy or variable hirsute. The stem, petioles, veins, lamina and calyx are lanuginose. The bracts of the inflorescences are larger than other species. From *Salvia nemorosa* sbsp. *tesquicola* the hybrid inherited the hairy stem mainly from the leaves and the \pm oblong-shaped lamina. From *S. nutans* it inherited few leaves, lamina with a cordate base and a slightly nutant inflorescence (NEGREAN 2011).

MATERIAL AND METHODS

The species were 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 and R. Bercu, with other rare species: *Bupleurum apiculatum*, *Cleistogenes bulgarica*, *Centaurea diffusa*, *Convolvulus cantabrica*, *Euphorbia dobrogensis*, *Koeleria lobata*, *Salvia nemorosa* sbsp. *tesquicola*, *Salvia nutans*, *Taraxacum serotinum*, *Teucrium polium* sbsp. *capitatum* etc.

The plants were collected and conditioned in the lab using the usual botanical methods. Observations were made upon the hybrid and the two parental species. The material was submitted to the Botanical Institute Herbarium of Bucharest [BUC]. Nomenclature after Flora Europaea (HEDGE 1972) (www.euromed.org.uk) after Roumanian Flora (RĂVĂRUȚ & NYARADY 1961). The herbarium abbreviation follows HOLMGREN *et al.* (1990) and internet <http://www.theplantlist.org/>.

The species were fixed in FAA 50 and transferred to alcohol 50%. For the anatomical study, freehand sections were made on the leaves. The samples were stained using different staining methods, such as alum-carmin and iodine green (the transversal sections) and safranin 0.5% (the paradermal sections) (BERCU & JIANU 2003). Histological observations and micrographs were

performed with a BIOROM-T bright field microscope, equipped with a TOPICA 6001A video camera.

RESULTS AND DISCUSSION

Cross sections of the petiole belonging to the three species showed that the adaxial surface was almost flat to concave and the abaxial surface was convex (Fig. 1, A-C). The epidermis was composed of a single layer of more or less isodiametric cells, covered by an outer

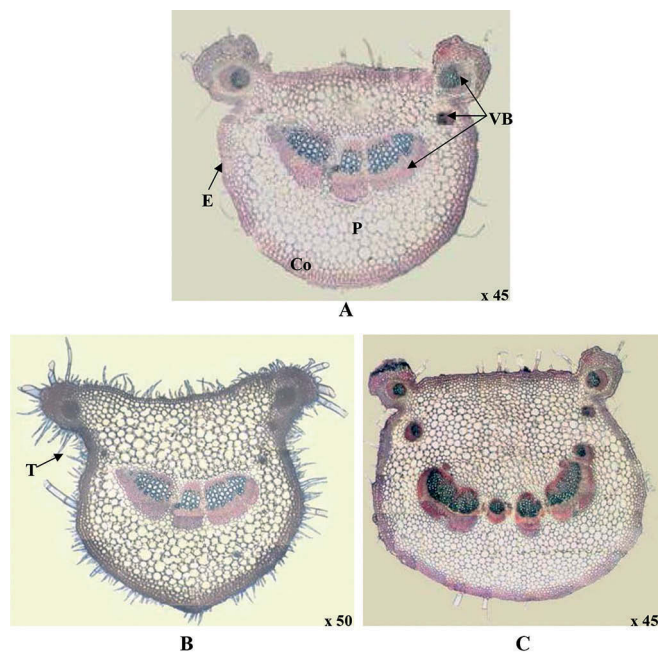


Fig. 1. Cross sections of the petiole of *Salvia nemorosa* subsp. *tesquicola* (A), *Salvia nutans* (B) and *Salvia x dobrogensis* (C) - ensemble: Co- collenchyma, E- epidermis, P- parenchyma, T- trichomes, VB- vascular bundle.

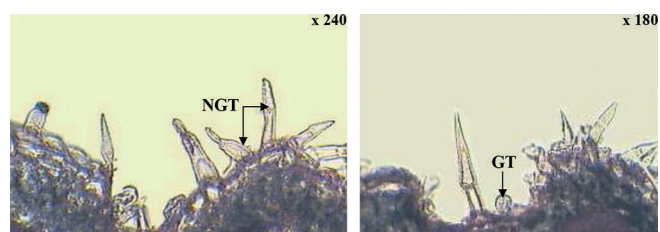


Fig. 2. Types of trichomes of *S. nemorosa* subsp. *tesquicola* petiole (A, B): NGT- non-glandular trichomes, GT- glandular trichome.

cuticle. Its continuity was interrupted by the presence of rare stomata arranged slightly above the epidermal level and numerous trichomes. Like other members of the Lamiaceae (KAHRAMAN *et al.* 2010), *S. nemorosa* subsp. *tesquicola*, *S. nutans* and their hybrid, carried both peltate and capitate glandular trichomes (JOHANSEN 1944), as well

as non-glandular ones. The leaf blade and petiole, as well as the stem, bore many non-glandular trichomes and less glandular ones compared with other plant organs.

The petiole non-glandular trichomes were multicellular (up to five cells), uniseriate and unbranched. Multicellular trichomes were straight or curved at the tip, quite variable in length, with some of them being large. Differences arose concerning the abundance and structure of non-glandular trichomes. The petiole of the hybrid had an abundance of trichomes, followed by *S. nutans* and fewer for *S. nemorosa* subsp. *tesquicola*.

A large percentage of *S. nemorosa* subsp. *tesquicola* non-glandular hairs had two- or three-celled stalks and were straight at the tip (Fig. 2, A). A few of these trichomes were longer and may have had a four-celled stalk. A large percentage of non-glandular trichomes of *S. nutans* (Fig. 3) were longer than those of *S. nemorosa* subsp. *tesquicola* and commonly were straight and three-celled. A small percentage of these trichomes were two- or four-celled. Some of them were curved at the tip, composed of two or three cells. Other trichomes, placed only to the wings area, were thicker and longer with four and even five cells. The hybrid possessed the largest percentage of non-glandular trichomes compared with those of the parental species, with a three- or four-celled stalk, mostly straight (Fig. 4, A, B). A smaller percentage of these trichomes were curved at the tip, compared with *S. nutans*. In addition to the other two species, the hybrid petiole had multicellular (of up to five cells) trichomes with ridges and marked internodes. Other trichomes, placed all over the petiole, were thicker and longer with four or five cells.

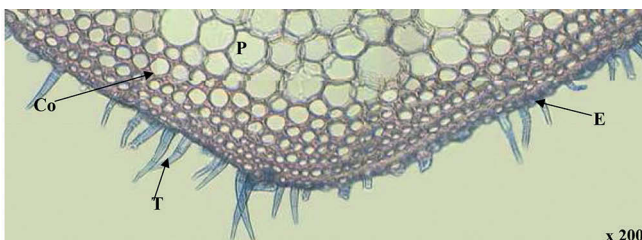


Fig. 3. Abaxial portion of *S. nutans* petiole cross section: Co- collenchyma, E- epidermis, P- parenchyma, T- trichomes.

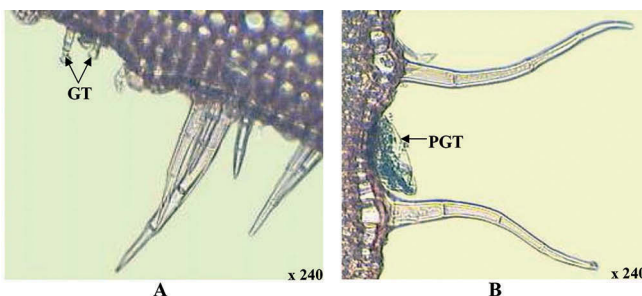


Fig. 4. Types of trichomes of *S. hybrid* petiole (A, B): GT- glandular trichomes, PGT- peltate glandular trichome.

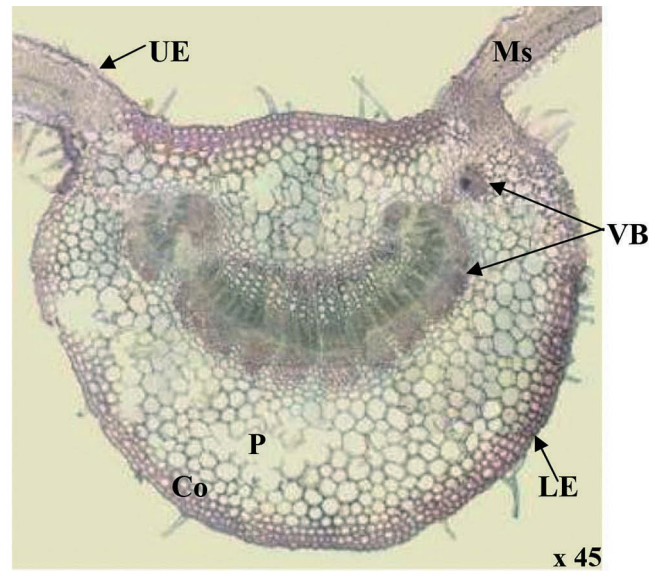


Fig. 5. Cross section of the *S. nemorosa* mesophyll – ensemble: Co- collenchyma, LE- lower epidermis, Ms- mesophyll, P- parenchyma, UE- upper epidermis, VB- vascular bundles.

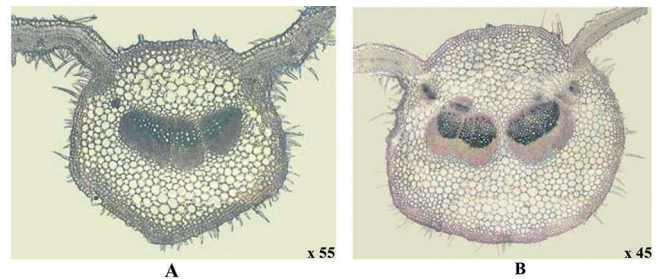


Fig. 6. Cross section of *S. nutans* (A) and *S. hybrid* (B) petiole – ensemble.

Few glandular trichomes were present on the surface of the petiole epidermis of all three species, compared with those of the mesophyll (KAHRAMAN & DOGHAN 2010). The glandular trichomes were of two types in all three taxa. Some of them were typical peltate trichomes (CORSI & BOTTEGA 1999). According to HALLAHAN 2000, peltate trichomes of the Lamiaceae often comprise a broad head of several secretive cells (of up to eight to sixteen cells), a short stalk and a basal epidermal cell. *S. chamelaeagnea* (KAMATOU *et al.* 2006) has peltate trichomes, with up to sixteen head cells. The present study showed that all three taxa had the peltate trichomes composed of a four- to eight-celled head in a single circle which is in agreement with a previous study (SERRATO-VALENTI *et al.* 1997). However, in other species of the same family, such as *Origanum* species (BOSABALIDIS & TSEKOS 1984) and *Satureja thymbra* (BOSABALIDIS 1990), a higher number of head cells are arranged in two concentric circles.

The peltate glandular trichomes of the three taxa petioles had a one-celled stalk, sunken in the epidermis

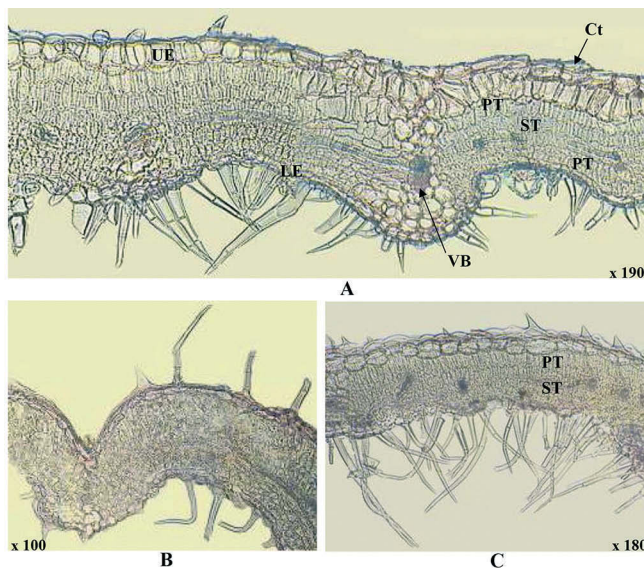


Fig. 7. Cross sections of the mesophyll of: *S. nutans* (A), *S. nemorosa* (B), *S. hybrid* (C): Ct- cuticle, LE- lower epidermis, PT- palisade tissue, ST- spongy tissue, UE- upper epidermis, VB- vascular bundles.

(Fig. 4, B). Others were capitate glandular trichomes (a large percentage), composed of a basal epidermal cell, unicellular or bicellular to multicellular stalk, the latter of variable length, a neck cell and a large, cutinized, unicellular or bicellular secretive head (Fig. 2, B). The bicellular glandular trichomes had a large slightly convex cell and a shorter one, followed by the unicellular secretive head.

S. nemorosa subsp. *tesquicola* petioles had a reduced number of trichomes compared with the other two species. Trichomes which had a one-celled stalk and a unicellular or bicellular secretive head were frequent, the latter being shorter than the others. *S. nutans* petioles had rare peltate glandular cells and numerous glandular trichomes with a two-celled stalk and the bicellular secretory head as well as those with a unicellular stalk and unicellular secretive head (Fig. 3). The few glandular trichomes were longer, composed of a three- or two-celled stalk (the latter sometimes having convex walls) and a unicellular secretive head.

An abundance of all types of glandular trichomes were present in the petiole of the hybrid, especially those with a two-celled stalk which might have had convex walls and unicellular head.

The petiole cortex was differentiated into two zones. The external one was hypodermis represented by a chlorenchyma, alternating with collenchyma tissue and the large inner region, parenchymatous in nature (BAVARU & BERCU 2002). Differences arose concerning the number of hypodermal layers. The petiole collenchyma of *S.*

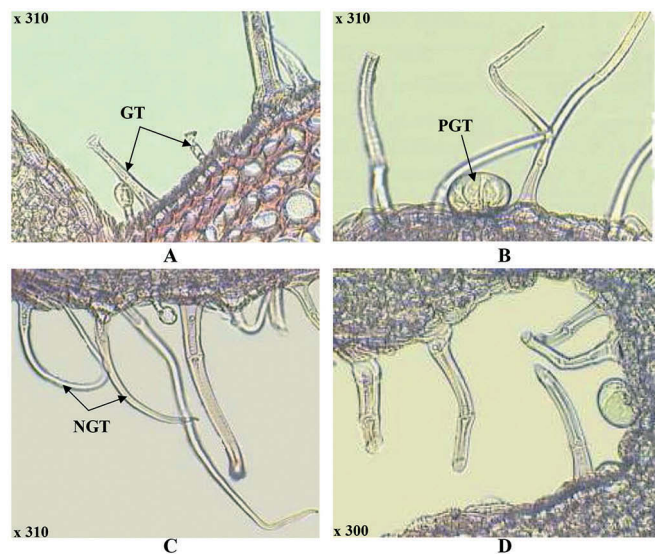


Fig. 8. Types of trichomes from upper (A, B) and lower (C, D) epidermis of *S. nemorosa* subsp. *tesquicola*: GT- glandular trichomes, NGT- non- glandular trichomes, PGT- peltate glandular trichomes.

nemorosa subsp. *tesquicola*, abaxial and adaxial, was composed of 2-3 layers of cells whereas the crests had 3-4 layers of cells. Chlorenchyma tissue was represented by 2-3 layers of cells placed on the lateral sides. The rest of the petiole was occupied by a many-layered parenchymatous tissue. *S. nutans* petiole had 3-4 abaxial layers of collenchymatous cells whereas the adaxial surface had 1-2. In the crests, this tissue were well developed (6-5 layers of collenchyma cells) and lateral 2-3 layers of chlorenchyma cells. The parenchymatous region was more developed compared with that of *S. nemorosa* subsp. *tesquicola*, with 7-8 abaxial and adaxial layers of cells. The hybrid petiole abaxial surface in the crests area 6-7 layers of collenchyma cells and 3-4 adaxial layers. The basic tissue was highly-developed (18-19 adaxial and 10-11 abaxial layers of parenchyma cells). In the parenchyma there were two to three large vascular bundles in the centre and two to four small subsidiary bundles in the petiole wings. The vascular tissues lay along a shallow arc and their arrangement was open collateral. The vascular bundles were protected on the phloem side by discontinuous sclerenchymatous groups of cells. Vascular bundles were surrounded by parenchyma and also separated by parenchymatous cells. The hybrid had the greatest number of vascular bundles (Fig. 1, C).

Anatomically, the leaf blade of all three taxa had an upper epidermis, lower epidermis and mesophyll (Figs. 5, 6). The upper and lower epidermis was monolayered, with each cell a different shape and size, slightly elongated tangentially. Epidermal external cell walls were slightly cutinized and protected by a cuticle (BATANOUNY

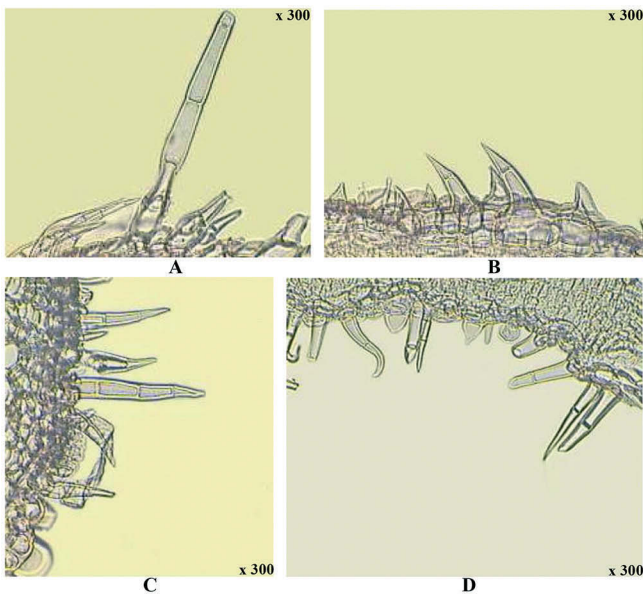


Fig. 9. Upper (A, B) and lower (C, D) epidermis types of trichomes from transections of *S. nutans* blade: GT- glandular trichomes, NGT- non- glandular trichomes, PGT- peltate glandular trichomes.

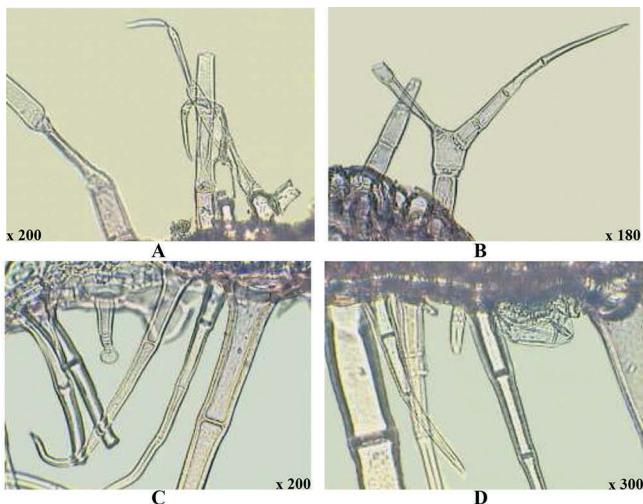


Fig. 10. Upper (A, B) and lower (C, D) epidermis types of trichomes from transections of the *hybrid* blade: GT- glandular trichomes, NGT- non- glandular trichomes, PGT- peltate glandular trichomes.

1992). The upper epidermis cells were larger than lower epidermis cells. Both epidermises possessed glandular and non-glandular trichomes (Figs. 8, 9, 10). The non-glandular trichomes were similar to those of the petiole. In addition, multicellular trichomes were present with ridges and marked internodes (the hybrid upper epidermis and lower epidermis of the other two species) (Fig. 10, A, B).

The same types of glandular trichomes such as those of the petiole were present in all three species, being more abundant in the lower epidermis. Although, the long glandular trichomes composed of a two- or three-celled

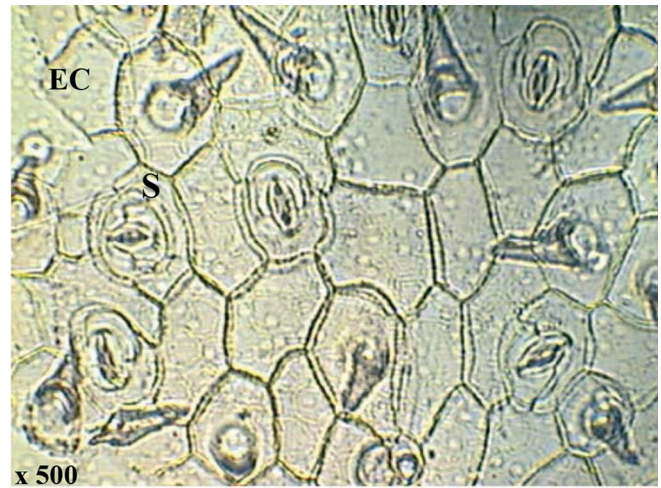


Fig. 11. Paradermal section of the lower epidermis of *S. nemorosa*: EC- epidermal cell, S- stoma.



Fig. 12. Paradermal section of the lower epidermis of *S. nutans*: EC- epidermal cell, S- stoma.



Fig. 13. Paradermal section of the lower epidermis of the hybrid: EC- epidermal cell, S- stoma.

stalk and unicellular secretive head were found mostly in *Salvia* × *dobrogensis* (Fig. 10, C, D) and were very rare and poorly developed in *Salvia nemorosa* (Fig. 8, A). These types of glandular trichomes were absent in *S. nutans* blade (Fig. 9, A-D). Unlike the glandular hairs of the petiole in both blade epidermises, short two-celled stalk and bicellular secretive head trichomes were still present. These glandular trichomes were most common in *S. nutans* blades (Fig. 9, A-D). Glandular trichomes composed of a long two-celled stalk, a short neck cell and a unicellular secretive head also frequently occurred in *S. nemorosa* and the hybrid (Fig. 10, C)

The leaf blade of *S. nemorosa* subsp. *tesquicola* and hybrid was dorsiventral and amphistomatous. It comprised 2–3-layered palisade and 2–3-layered spongy tissues (Fig. 7, B, C). This feature is also present in other *Salvia* species, such as *S. officinalis*, *S. apiana*, *S. mellifera* (WEBB & CARLQUIST, 1964). The *S. nutans* leaf blade comprised 2 layers of palisade tissue just bellow the upper epidermis as well as lower epidermis and 1-2 layers of spongy tissue between them (Fig. 7, A). The vascular bundles of the vein were embedded in the mesophyll. They were anatomically similar to those described in the petiole but with a typical foliar arrangement of the conductive tissues. The midrib was adaxially flat and abaxially convex. It was represented by a single vascular bundle in *S. nemorosa* subsp. *tesquicola* blades (Fig. 5), and 1-2 vascular bundles in *S. nutans* (Fig. 6, A). The hybrid midrib possessed two large well-developed vascular bundles and several small ones (Fig. 6, B). Next to the vascular system of the midrib a variable number of collenchyma layers were present. Therefore, adaxial to the midrib of *S. nemorosa* subsp. *tesquicola* the blade mesophyll had 4-5 layers of collenchyma, *S. nutans* had 1-2 and the hybrid had 2-3 layers. Abaxially, 2-3 layers of collenchymatous cells were present for the first two species and 4-5 layers for the hybrid.

Paradermal sections of the three taxa blade showed straight-walled epidermal cells, glandular and non-glandular trichomes and numerous stomata, especially in the lower epidermis (Fig. 11, 12, 13).

CONCLUSIONS

Organization of the leaf of the three sage taxa followed the same structural plan but both similarities and differences were present between the two species *S. nemorosa* subsp. *tesquicola*, *S. nutans*, and their hybrid. Distinct differences arose in the structure of stems, in the stem bark and the vascular system. Anatomically, the most developed petiole belonged to *Salvia* × *dobrogensis* (the hybrid), followed by *S. nutans* and *S. nemorosa* subsp. *tesquicola*.

Both leaf epidermises of the three species had straight-walled cells and diacytic stomata. The leaf mesophyll

was heterogeneous in all three species. The leaves were amphistomatous and dorsiventral in *Salvia nemorosa* subsp. *tesquicola* and the hybrid and amphistomatous with two palisade tissues bellow both epidermises in *S. nutans*. The petiole and blade of all three species bore a large number of non-glandular and glandular trichomes that varies in their morphology. Mechanical tissue – collenchyma and sclerenchyma – was present in both the petiole and blade.

Many of the hybrid leaf tissues were well-developed, compared with the two parental species. The petiole anatomy of the hybrid *Salvia* × *dobrogensis* had several similarities with *S. nutans*. In contrast, the blade features were more similar to those of *Salvia nemorosa* subsp. *tesquicola*.

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- www.euromed.org.uk = Flora Europaea internet
<http://www.theplantlist.org/> = The Plant List Data

 REZIME

Anatomska studija listova *Salvia nemorosa* subsp. *tesquicola*, *Salvia nutans*, i *Salvia* × *Dobrogensis*

Rodica BERCU, Gavril NEGREAN, Livia BROASCĂ

U ovom radu dati su podaci o lisnoj anatomiji *Salvia nemorosa* L. subsp. *tesquicola* (Klokov & Pobeda.) Soó, *Salvia nutans* L. i hibrida ova dva taksona - *Salvia* × *dobrogensis* Negrean. Radjena je komparativna atomska analiza. Razlike se uočavaju i kod structure latica i lisnih ploča. Latice se razlikuju po stepenu razvijenosti spoljašnjeg i unutrašnjeg korteksa. Vaskularni sistem obuhvata kod svih studiranih taksona veliki broj snopića, sa različito razvijenim provodnim tkivom. Mezofil je heterogen dvoličan (*S. nemorosa* subsp. *tesquicola* i hibrid) dok je kod *S. nutans* ujednačeno razvijen. Prisutno je mnogo glandularnih i neglandularnih trihoma, različitih po strukturi, obliku i veličini. Stome su prisutne na obe lisne strane i dijakritičnog su tipa, uronjene su i imaju amfistomatični karakter. Vaskularni sistem nerava lista je naročito razvijen kod hibrida. Postoje jasne atomske razlike hibridnog taksona u odnosu na parentalne taksone.

Ključne reči: anatomija, list, *Salvia*

