

LEAF ANATOMICAL STUDY OF THE GENUS *TEUCRIUM* L. (LAMIACEAE) IN IRAN

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Leaf anatomy of 10 species of the genus *Teucrium* L. in Iran were studied. Anatomical structure of leaves was compared in species. The most differences among species belong to the layers of palisadic and spongy parenchyma, thickness of leaf, cuticle of upper surface, size of epidermal cells and trichome types. These characters can be used in distinguishing species and infraspecific taxa and therefore have high taxonomical values.

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Key words. *Teucrium* L., Lamiaceae, leaf anatomy, Iran.

مطالعه تشریحی برگ در جنس توکریوم (مریم نخودی) از خانواده نعنای در ایران

مریم عشرتی فر، فارغ التحصیل رشته زیست شناسی علوم گیاهی دانشگاه تهران.

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در این تحقیق ساختار تشریحی برگ ۱۱ گونه و زیر گونه از جنس مریم نخودی در ایران مطالعه شده است. مهمترین اختلاف ها در این بین به تعداد لایه های پارانشیم نردبانی و اسفنجی، ضخامت برگ، ضخامت کوتیکول در سطح فوقانی، اندازه سلول های اپیدرمی و تیپ های مختلف کرک مربوط می شوند. این صفات دارای ارزش تاکسونومیکی بالایی بوده، می توانند به عنوان ابزاری مفید برای جداسازی گونه های مختلف این جنس محسوب گردند.

Introduction

The genus *Teucrium* L. is one of the genera of family Lamiaceae. This genus with about 100 species is distributed in areas like: Europe, North Africa and some parts of Asia, but the main distribution of the genus is in the Mediterranean region (Mabberley, 1997). *Teucrium* has 19 taxa in Iran which are mainly grown in Irano-Turanian region between 700-2000 meters above the sea level (Rechinger, 1982). Among them *Teucrium persicum* is the only endemic to Iran. *Teucrium* species are distributed in most regions of Iran. i.e. *T. persicum* is present only in elevations of southern regions as a Saharo-Sinidian element, while *T. hyrcanicum* grows in north of Iran which is a hyrcanian element. Some species namely *Teucrium polium* and *T. orientale* are widely distributed in steppes, arid and semiarid regions.

Based on Metcalf & Chalk (1957) there are general information about anatomical structure of Lamiaceae family. Also the trichome types and their taxonomical values in some species of *Teucrium* has been studied by Navarro & El oualidi (2000).

The present paper attempts to compare the species of *Teucrium* based on the important constant anatomical characters and evaluate their taxonomic implication.

Material and Methods

This study is based on the herbarium specimens of TUH and IRAN. The list of studied specimens is given in Table 1.

For anatomical studies, the leaves of 8 species and 6 subspecies were fixed in the proportion 1:1 of ethanol and glycerin. Cross sections were made at the middle of leaves with a razor; they were cleared with sodium

Table 1. Voucher specimens of *Teucrium* species used in this study.

Taxon	Locality
Sect. <i>Polium</i> <i>Teucrium polium</i> L.	Hamadan: Assad-abad, 1700 m, 13.6.1992, Attar, Dadjou, Okhovat & Mehdigholi, 14510-TUH. -Hormozgan: road of to Bandar-e Lengeh, 10 m, 22.4.1970, Ghahreman, 8333-TUH. -Mazandaran: Chalus, Hassan-abad, 40 m, 24.5.1967, Tregubov, 8337-TUH.
<i>T. stocksianum</i> Boiss.	Baluchestan: 20 km after Nik-shahr to Sarbaz, 1100 m, 10.4.1998, Ghahreman, Sheikh & Attar, 21577-TUH. -Hormozgan: Bandar-Abbas, Ghotbabad, Baghestan, Dametang, 650 m, 30.4.1987, Ghahreman & Mozaffarian, 5657-TUH.
Sect. <i>Chamaedrys</i> <i>T. chamaedrys</i> L.	Azerbaijan, Ahar, Kaleybar, 1580 m, 17.7.1993, Attar, 17255-TUH. -Mazandaran: 40 km to Amol, near Andovar village, 2500 m, 6.6.2000, Attar, Okhovat & Mehdigholi, 26347-TUH.
Sect. <i>Isotridon</i> <i>T. persicum</i> Boiss.	Bushehr: Bandar-e Taheri: Assalouyeh, 450 m, 21.3.2005, Attar & Ghahreman, 35725-TUH.
Sect. <i>Scordium</i> <i>T. scordium</i> subsp. <i>scordioides</i> Schreb.	West Azerbaijan: between Urumieh and Salmas, 1380 m, 16.7.1991, Mozaffarian, 70100-TUH.
<i>T. scordium</i> L. subsp. <i>serratum</i> (Benth.) Rech. f.	Khorassan: south of Birjand, Razg, 1800 m, 24.10.1997, Aliabadi, 22181-TUH.
<i>T. melissoides</i> Boiss. & Hausskn. ex Boiss.	Lorestan: on road to Andimeshk, Pol-e Dokhtar, 750 m, 28.10.1993, Ghahreman, Mozaffarian & Sheikholeslami, 17611-TUH.
Sect. <i>Stachyobotrys</i> <i>T. hyrcanicum</i> L.	Gilan: Bandar-e Anzali, -20 m, 25.5.1988, Mozaffarian & Maussoumi, 6906-TUH.
Sect. <i>Teucriis</i> <i>T. parviflorum</i> Schreb.	Lorestan: Khorramabad: road of Sefid-dasht, 50 km after bifurcation of Khorramabad-Sefid dasht, 1950m, 18.6.1998, 18.6.1998, Ghahreman, Ghaffari, & Attar, 21841-TUH. -Kurdistan: Marivan to Sanandaj, Gardane Garan, 1600 m, 8.7.1995, Ghahreman & Mozaffarian, 18324-TUH.
<i>T. oliverianum</i> Gingins	Bushehr: Darvahi village, near Shah-pour river, 1.4.1957, Mobayen, Yazdan-panah & Arefi, 8307-TUH;
<i>T. orientale</i> L. subsp. <i>glabrescens</i> (Hausskn. ex Bornm.) Rech. f.	Kermanshah: road of Mahidasht, 1539 m, 2.7.1996, Attar & Mirtadzanini, 19882-TUH.
<i>T. orientale</i> subsp. <i>gloeotrichum</i> Rech. f.	Lorestan: Khoramabad, Cham-Divan, Chal-e Ahmad, 1400 m, 20.5.1999, Veiskarami, 23934-TUH;
<i>T. orientale</i> subsp. <i>orientale</i> L.	East Azerbaijan: N.slopes of Mishou-Dagh, south of the main road, near Yam (S. of Marand), 1950m, 19.6.2001, D. Podlech, & Sh. Zarre, 55248-TUH. -Lorestan: 40km to Khorramabad: Razan Pass, 2050 m, 19.6.1998, Ghahreman, Attar & Ghaffari, 21848-TUH.
<i>T. orientale</i> subsp. <i>taylori</i> (Boiss.) Rech. f.	Bushehr: 6km from Khurmuj to Bushehr, 70 m, 4.3.1990, Ghahreman & Mozaffarian, 9533-TUH.

hypochlorite and stained with Methyl green and Bismarc brown colors. Transverse sections were observed and photographed by Light Microscope (VohoxAHSB3).

Results

The leaves of *Teucrium* are ovate-lanceolate to oblong, with obtuse or acute apex, cuneate at the base, dentate

or crenate at the margin, rarely pinnatifid or pinnatisect, shortly petiolate, canescent, with different types of indumentum on both surfaces, rarely green. Venation is reticulate. A total of 16 significant anatomical characters of leaf (lamina and midrib) were studied, they are presented in table 2. Figures 1-27 are shown lamina, midrib and trichome characteristics.

LAMINA (T. S.)

Lamina in the species of *Teucrium* mostly has a regular shape and arrangement. The following layers can be seen from up to down respectively: trichomes, cuticle layer, upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis, cuticle layer and trichomes. Among these characters there is another feature which is, the lower palisade parenchyma that is locating after spongy parenchyma. This feature is present only in four species: *T. polium*, *T. stocksianum*, *T. chamaedrys* and *T. persicum*. Thickness of lamina varies in different species, from 0.8 mm to 2.9 mm in *Teucrium hyrcanicum* and *T. oliverianum* respectively, generally the thickness of lamina is about 1.7 mm. Cuticle layer is presented in all species, but with different thicknesses. It is very thick in *T. orientale* subsp. *gloeotrichum*, *T. orientale* subsp. *orientale*, *T. orientale* subsp. *glabrescens*, *T. oliverianum* and *T. parviflorum*. The thickest is observed in *T. orientale* subsp. *gloeotrichum* (0.3 mm) and the thinnest in *T. hyrcanicum* (0.015 mm). Epidermis is one layered. The cells are variable in size and shape, which can be rounded or rarely rectangular. The cells of upper surface are usually larger than those in the lower surface. *T. scordium* subsp. *scordioides* has the largest cells that are irregularly arranged, shape of the cells in this species are rectangular to triangular. In *T. hyrcanicum*, cells are narrowly rectangular and suborbicular. *T. oliverianum* and *T. parviflorum* are nearly the same in shape of epidermal cells which both of them have rounded to rectangular cells. There is an unusual irregular shape of epidermal cells in *T. melissoides*. It has a complex of rounded, rectangular and triangular cells. The structure of palisade tissue is very important in the genus *Teucrium*. Based on Diane N. et al. (2003) the taxa of *Teucrium* are divided in to four types:

1. Bifacial type (B): This group is divided into two forms:

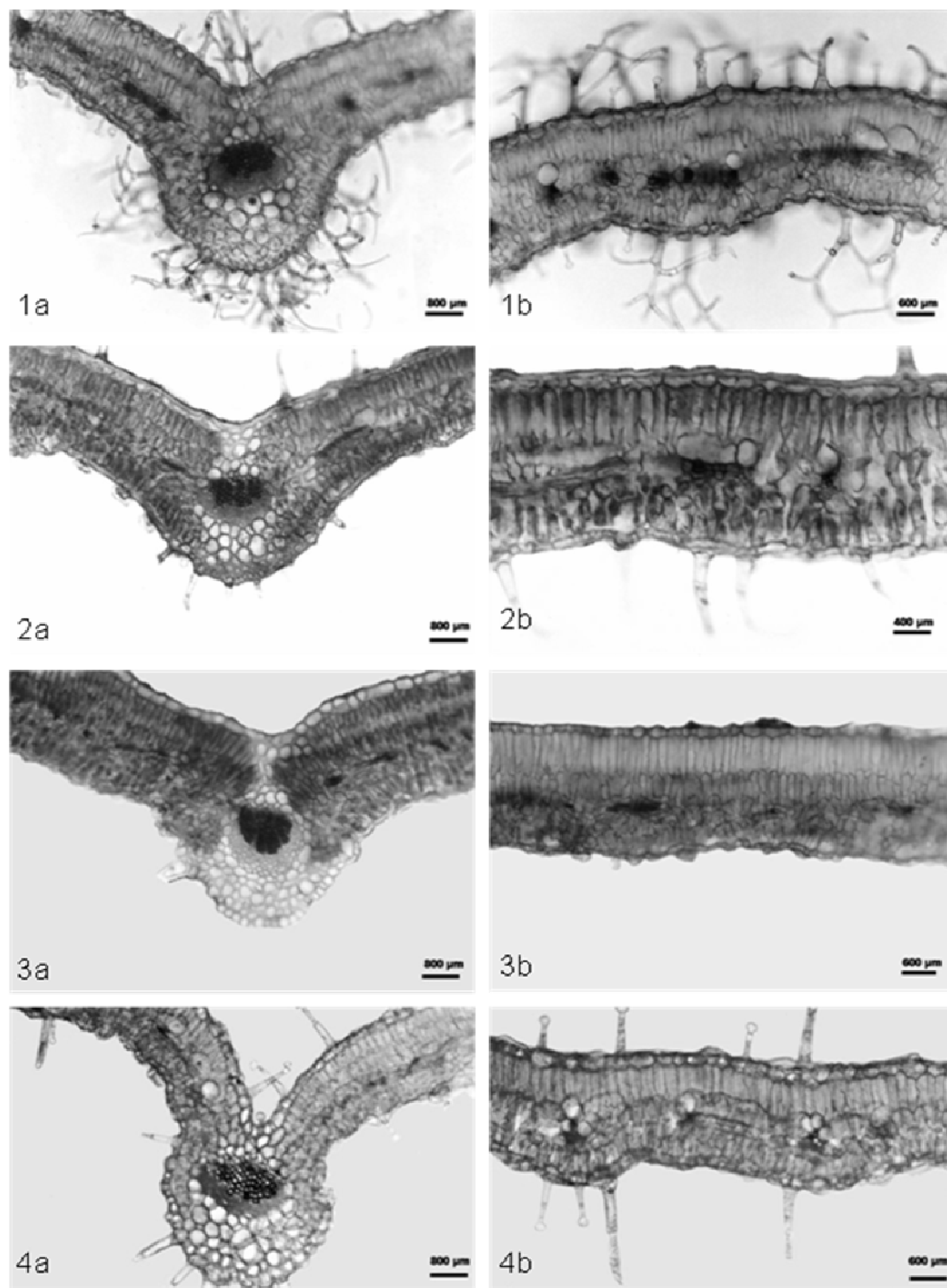
a: Palisadic parenchyma is 1-layered in upper surface and the rest region is occupied by spongy parenchyma. Bifacial type "a" is observed in *T. melissoides* (Fig.7b), *T. oliverianum* (Fig.10b), *T. parviflorum* (Fig.9b), *T. hyrcanicum* (Fig.8b), *T. orientale* in all sub-species (Figs.11b-14b).

b: Palisadic parenchyma is 2-layered, and the rest of the region is filled with spongy parenchyma and presents in *T. scordium* subsp. *scordioides* (Fig.5b).

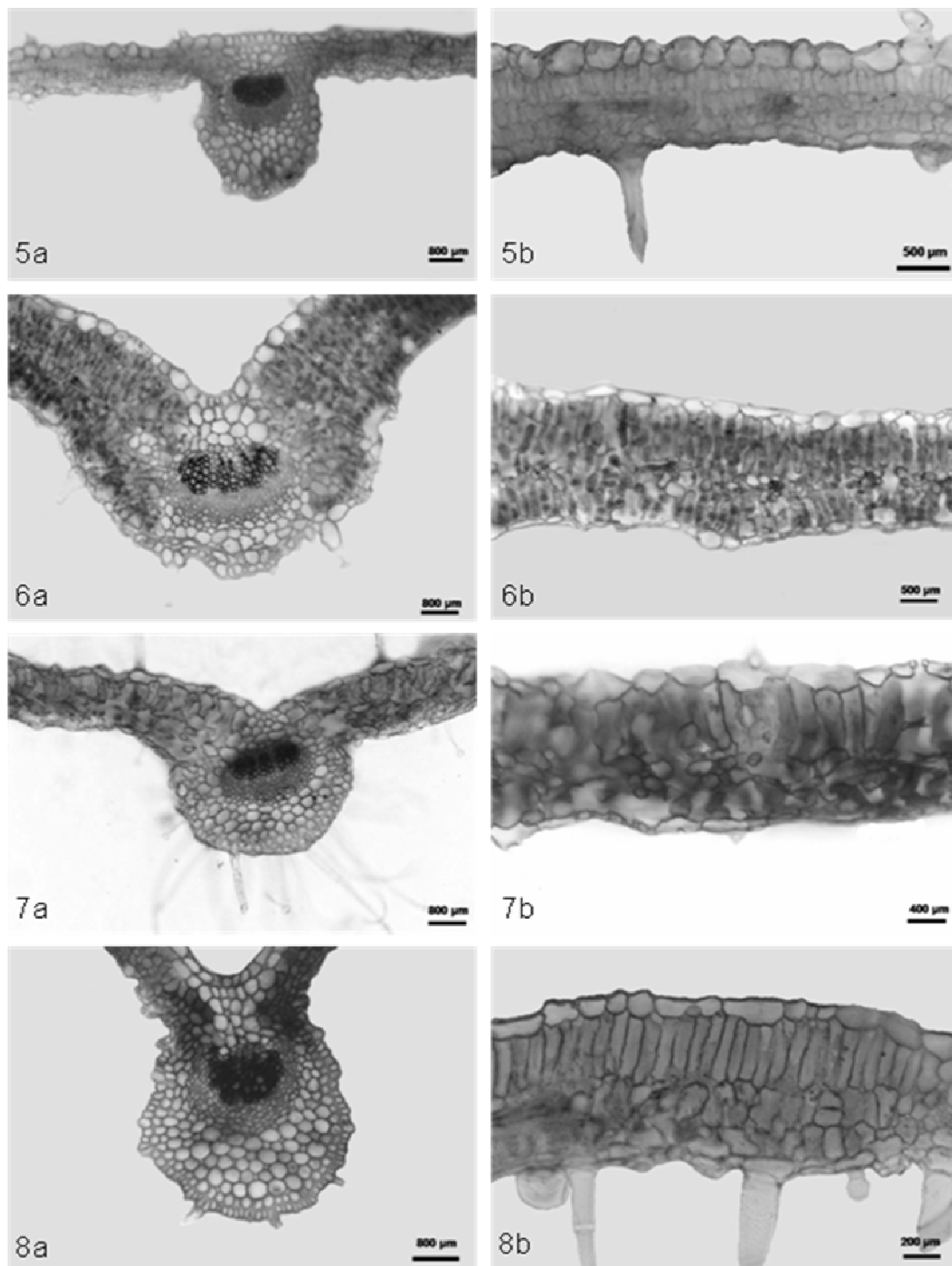
2. Subbifacial type (B+): In this type, palisade parenchyma is one-layered in upper surface, also incompletely one-layered parenchyma is present in lower surface. This type is only found in *T. stocksianum* (Fig.2b).

Table 2. Leaf anatomy and distribution of foliar trichome types in the genus *Teucrium* L. Midrib: **Sh**, shape (1=rounded, 2= subrounded, 3= suboval); **Cth**, cuticle thickness (mm); **Upth**, upper parenchyma thickness (mm); **Lpth**, lower parenchyma thickness (mm); **Sc**, sclerenchyma cells (mm); **Xth**, xylem thickness (mm); **Phth**, phloem thickness (mm); **Lam**, lamina thickness (mm); **Cth**, cuticle thickness (mm); **La**, leaf anatomy (B=bifacial, B+=subbifacial, I=isolateral 1-layered, Ih = isolateral heterogenous); **UPl**, upper palisade parenchyma length (mm); **LPl**, lower palisade parenchyma length (mm); **Spr**, spongy parenchyma rows; **Gt**, glandular trichomes (+=present, -=not-present); **Tt**, foliar trichome types (1=unbranched unicellular, 2=unbranched multicellular, 3=non-stalked glands, 4=stalked unicellular glands, 5=stalked multicellular glands, 6=branched).

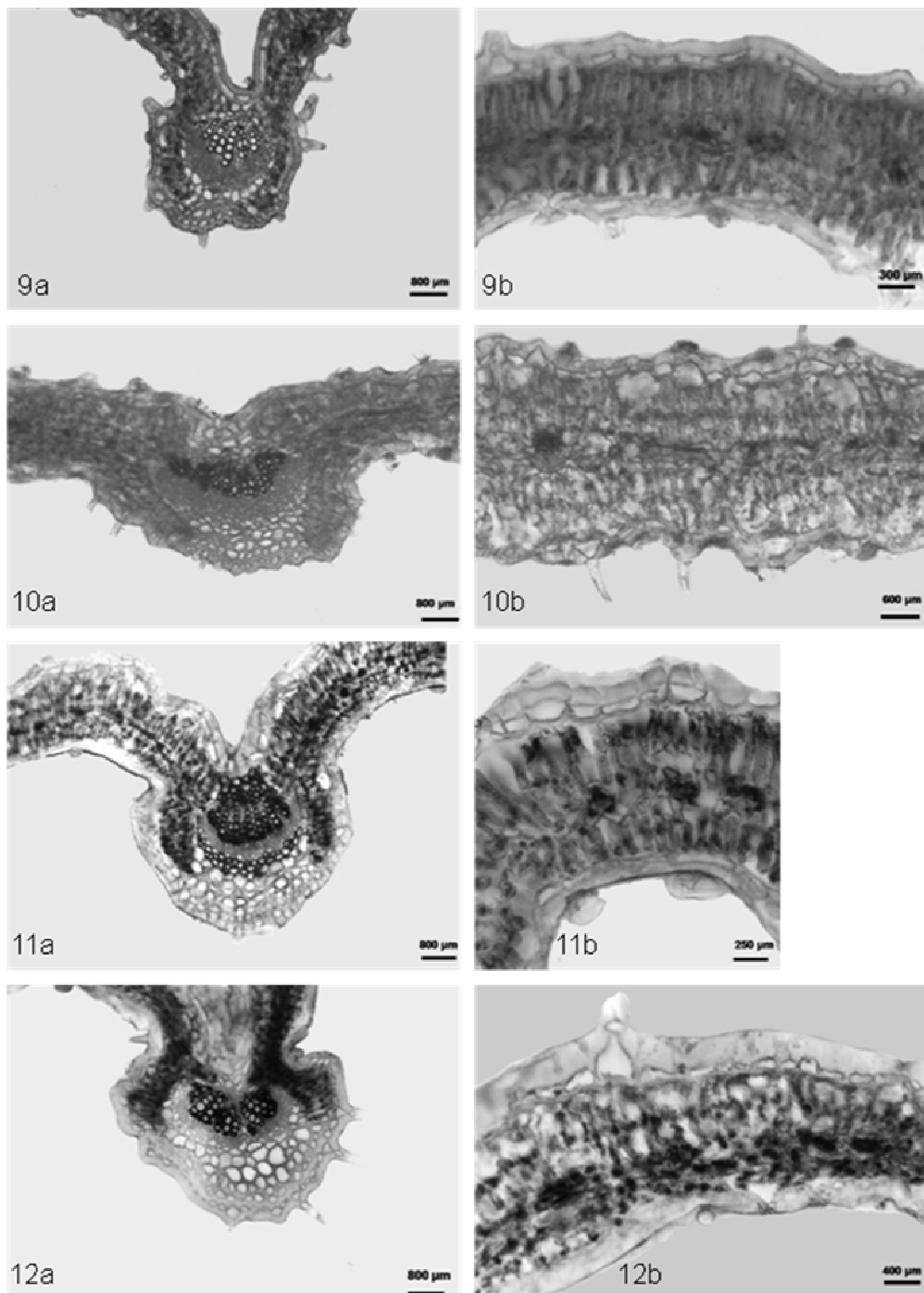
Taxon	Midrib						Lamina									
	Sh.	Cth.	Upth.	Lpth.	Sc.	Cc.	Xth.	Phth.	Lth.	Cth.	La.	Upl.	Lpl.	Spr.	Gt.	Tt.
<i>T. polium</i>	2	0.05	0.9	0.8	-	0.6	0.8	0.4	2.2	0.05	Ih	0.9	0.5	0.6	+	3,4,5,6
<i>T. stocksianum</i>	2	0.1	0.9	0.8	-	0.2	0.5	0.4	1.75	0.07	B+	0.5	0.6	0.4	+	2,3,4,5
<i>T. chamaedrys</i>	2	0.025	1.4	0.8	-	0.2	0.9	0.5	2.5	0.025	Ih	1.2	0.4	0.6	+	2,3
<i>T. persicum</i>	2	0.05	0.8	1	-	0.4	0.6	0.4	1.7	0.025	I	0.5	0.4	0.4	+	1,2,3,4,5
<i>T. scordium</i> subsp. <i>scordioides</i>	2	0.025	0.7	1	-	0.3	0.55	0.5	1.05	0.025	B	0.5	-	0.3	+	2,3,4,5
<i>T. scordium</i> subsp. <i>serratum</i>	3	0.05	1	0.8	-	0.5	0.7	0.35	1.9	0.025	Ih	0.9	-	0.8	+	3,4,5
<i>T. melissoides</i>	2	0.025	0.7	0.5	-	0.4	0.4	0.5	1.3	0.025	B	0.7	-	0.5	+	2,3,4,5
<i>T. hyrcanicum</i>	2	0.015	1.1	1	-	0.2	0.8	0.5	0.8	0.015	B	0.35	-	0.3	+	2,3,4,5
<i>T. parviflorum</i>	1	0.1	0.3	0.4	-	0.2	0.8	0.5	1.4	0.1	B	0.7	-	0.4	+	1,2,3
<i>T. oliverianum</i>	3	0.2	0.7	0.4	-	0.3	0.6	0.4	2.9	0.2	B	1	-	1.1	+	1,2,3
<i>T. orientale</i> subsp. <i>glabrescens</i>	2	2	0.2	0.6	0.3	0.4	1.5	0.35	1.5	0.15	B	0.6	-	0.5	+	2,3,4,5
<i>T. orientale</i> subsp. <i>gloeotrichum</i>	2	0.25	0.2	0.8	-	0.4	0.7	0.3	1.8	0.3	B	0.6	-	0.5	+	2,3,4,5
<i>T. orientale</i> subsp. <i>orientale</i>	2	0.1	0.4	0.9	-	0.15	0.8	0.4	2	0.1	B	0.6	-	0.7	+	2,3,5
<i>T. orientale</i> subsp. <i>taylori</i>	3	0.05	0.4	0.8	-	0.15	0.5	0.3	2.1	0.05	B	0.7	-	1	+	2,3



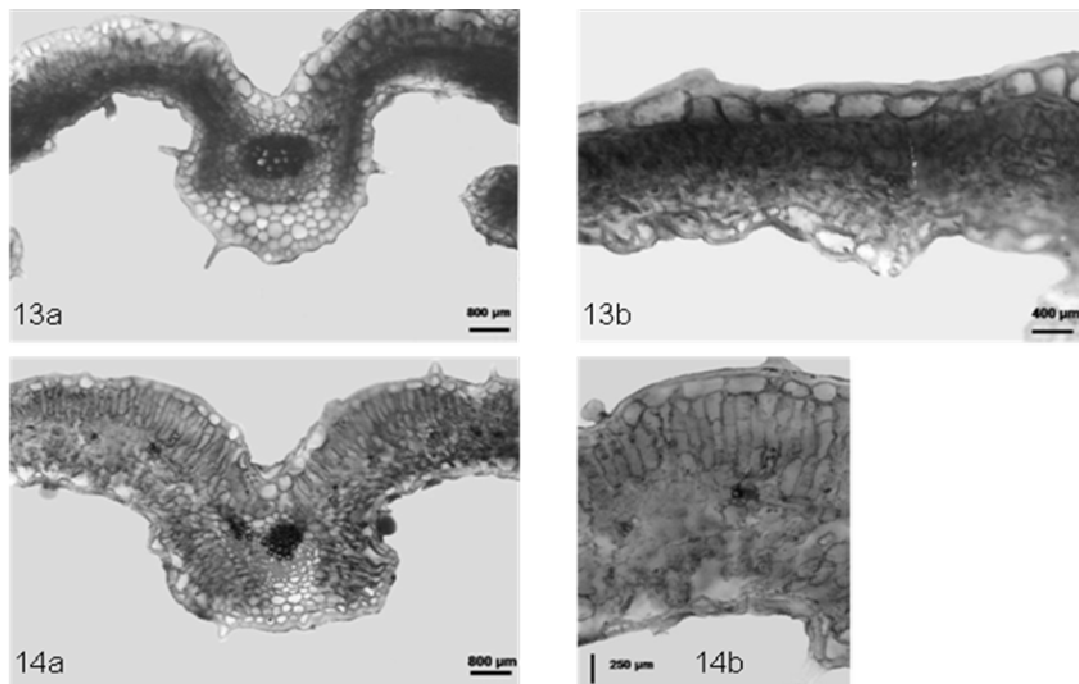
Figs. 1-4. Transversal sections of midrib and lamina. (1) *Teurcium polium*; a. subrounded midrib, b. isobilateral heterogenous lamina; (2) *T. stocksianum*: a. subrounded midrib, b. subbifacial lamina; (3) *T. chamaedrys*: a. subrounded midrib, b. isobilateral heterogenous lamina; (4) *T. persicum*: a. subrounded midrib, b. isobilateral one-layered lamina.



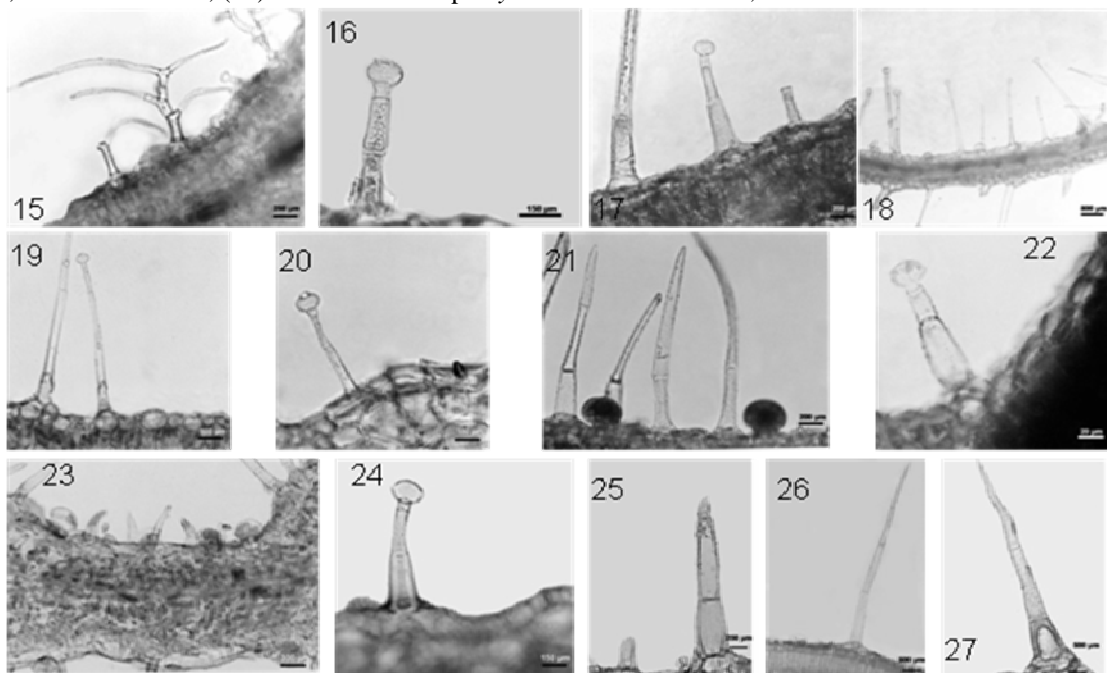
Figs. 5-8. Transversal sections of midrib and lamina. (5) *Teucrium scordium* subsp. *scordioides*: a. subrounded midrib, b. bifacial lamina; (6) *T. scordium* subsp. *serratum*: a. suboval midrib, b. isobilateral heterogenous lamina; (7) *T. melissoides*: a. subrounded midrib, b. bifacial lamina; (8) *T. hyrcanicum*: a. subrounded midrib, b. bifacial lamina.



Figs. 9-12. Tb. bifacial lamina; (10) *Teucrium oliverianum*: a. suboval midrib, b. bifacial lamina; (11) *T. orientale* subsp. *glabrescens*: a. subrounded midrib, b. bifacial lamina; (12) *T. orientale* subsp. *gloeotrichum*: a. subrounded midrib, b. bifacial lamina; ransversal sections of midrib and lamina. (9) *T. parviflorum*: a. rounded midrib.



Figs. 13-14. Transversal sections of midrib and lamina (13) *Teucrium orientale* subsp. *orientale*: a. subrounded midrib, b. bifacial lamina; (14) *T. orientale* subsp. *taylori*: a. suboval midrib, b. bifacial lamina.



Figs. 15-27. Transversal sections of trichomes. (15) *Teucrium polium*: branched trichomes; (16) *T. stocksianum*: unbranched multicellular trichomes; (17) *T. persicum*: stalked multicellular glands; (18) *T. scordium* subsp. *scordioides*: unbranched multicellular trichomes, sessile and stalked glands; (19) *T. scordium* subsp. *serratum*: stalked multicellular glands; (20) *T. melissoides*: stalked multicellular glands; (21) *T. hyrcanicum*: unbranched multicellular trichomes and sessile glands; (22) *T. parviflorum*: stalked multicellular glands; (23) *T. oliverianum*: unbranched multicellular trichomes and sessile glands; (24) *T. orientale* subsp. *gloeotrichum*: stalked multicellular glands; (25) *T. orientale* subsp. *glabrescens*: unbranched multicellular trichomes; (26) *T. chamaedrys*: unbranched multicellular trichomes; (27) *T. orientale* subsp. *orientale*: unbranched multicellular trichome.

3. Isobilateral, one-layered (I): This type including one-layered palisade parenchyma in both surfaces and the middle space is occupied by spongy parenchyma presents in *T. persicum* (Fig. 4b).

4. Isobilateral, heterogeneous (Ih): Including two-layered palisade parenchyma in upper surface and one-layered palisade parenchyma in lower surface. This type is observed in, *T. scordium* subsp. *serratum* (Fig. 6b.), *T. polium* (Fig. 1b) and *T. chamaedrys* (Fig. 3b).

The palisade tissue varies in thickness from 0.3 to 1.1 mm. The average thickness of this layer among species is 0.6 mm. The thickest spongy parenchyma is recognized in *T. oliverianum*. In all taxa the surfaces are densely or loosely covered by different indumentum. In this study, six types of trichomes and glands including those of Diane et al. (2003) are recognized:

1. Simple unbranched unicellular trichomes, including one cell that is long or short.
2. Simple unbranched multicellular trichomes, including two or more cells that form a long trichome with different shapes.
3. Branched trichomes: this type is only found in *T. polium*, including multicellular stipe and multicellular branches.
4. Sessile glands, that have swelling cell.
5. Stalked unicellular glands, including one-cellular stipe and one terminal spherical cell.
6. Stalked multicellular glands, with multicellular stipe and one-cellular spherical head.

Midrib

Generally three shapes can be defined for these species: rounded, subrounded and suboval. Most of the species has subrounded shapes of midrib. While three taxa have suboval shape, *T. parviflorum* is the only species which has rounded shape midrib. The cuticle thickness varies from 0.015 mm in *T. hyrcanicum* to 2 mm in *T. orientale* subsp. *glabrescens*. Upper parenchyma thickness also has some differences among the species, it has a range between 0.2 to 1.4 mm. Lower parenchyma thickness is from 0.2 mm to 1 mm. Vascular system is single and inserted in central part. Thickness of xylem layer varies from at least 0.4 mm in *T. melissoides* to the at most 1.5 mm in *T. orientale* subsp. *glabrescens*, but most of the species have 0.7-0.8 mm thickness. On the contrary of Xylem thickness the Phloem thickness in all species are very close together and about 0.4-0.5 mm. Sclerenchymatous fibers has not been found around the vascular system except in *T. orientale* subsp. *glabrescens*. Palisadic parenchyma is observed in midrib especially in upper surface in most species except in *T. melissoides*, *T.*

scordium and *T. hyrcanicum*. The amount of palisadic parenchyma in midrib region of different species is variable. Collenchyma tissue which is located under epidermis is present in most species with angular cell type. Thickness of them is 0.15 to 0.6 mm but the average is 0.4 mm.

Discussion

The genus *Teucrium* L. can be divided into four specific types based on the structure of palisadic tissue: Bifacial types a and b, which according to table 2 presents in most of the species, subbifacial, that can be seen only in *T. stocksianum* which is distributed in southeast of Iran, Isobilateral one-layered that is specified for *T. persicum* and at last Isobilateral heterogenous which exists in three following species: *T. polium*, *T. chamaedrys* and *T. scordium* subsp. *serratum*. According to the indumentum there are two different groups among the species of this genus. The first group includes only *T. polium*. This species has branched trichomes, an inclusive beautiful character which is present only in *T. polium* (fig. 16). The other group includes the rest of the species and more or less they have different types of trichomes such as unbranched uni- and multicellular trichomes and non-stalked, stalked uni- and multicellular glands. There are also three different midrib shapes: rounded, subrounded and suboval. While most of the species have subrounded midrib, *T. parviflorum* is the only species which has rounded shape of midrib and also the following species have the suboval shape: *T. oliverianum*, *T. scordium* subsp. *serratum* and *T. orientale* subsp. *taylori*. There are some other anatomical characters that can be used in distinguishing the species like thickness of cuticle and lamina, upper and lower parenchyma thickness in midrib and presence or absence of the sclerenchyma tissue in midrib. The thinnest lamina and cuticle belonged to *T. hyrcanicum* which is distributed in Caspian forests that is a wet temperate region. Also in this species palisadic parenchyma is one-layered and dense spongy parenchyma is present. In the species of the arid and semiarid regions thickness of cuticle and lamina is more than the other species, palisadic parenchyma is two or three-layered, also palisadic cells are long. There is one exception in this genus too, and this belongs to *T. orientale* subsp. *glabrescens* which is the only species that has sclerenchyma tissue in its midrib as a protective tissue.

There are some anatomical characters among two subspecies belonging to *T. scordium* which make the difference between them. The shape of midrib in *T. scordium* subsp. *scordioides* is subrounded, but in *T. scordium* subsp. *serratum* is suboval. In this character

T. scordium subsp. *serratum* is very similar to *T. oliverianum*. While the lamina in *T. scordium* subsp. *scordioides* is bifacial, *T. scordium* subsp. *serratum* has the isobilateral heterogeneous lamina which makes this taxon similar to *T. polium* and *T. chamaedrys*. Regarding to table 2 it can be obtained that there are some specific similarities between most of the species which are morphologically close to each other. There are some characters like midrib shape (Sh.) and lower parenchyma thickness (Lph.), lower palisade parenchyma length (Lpl.) and spongy parenchyma rows (Spr.) in lamina and also the trichomes which are common among *T. polium*, *T. stocksianum*, *T. chamaedrys* and *T. persicum*. Some other similarities also can be observed between close species. In conclusion it is obtained that leaf anatomy of *Teucrium* is of high systematic value and prove the classification of this genus based on morphological results.

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