

Micromorphological characters on nutlet and leaf indumentum of *Teucrium* sect. *Teucrium* (Lamiaceae) in Turkey

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Abstract: Leaf indumentum and nutlet surface micromorphology of 13 taxa belonging to the *Teucrium* sect. *Teucrium* was studied with a scanning electron microscope. We observed 5 trichome types on the nutlets and 8 trichome types on the leaves. Branched trichomes were not found on the nutlets. Nutlet shapes of the taxa were elliptic, obovate, oblong, or rotund, and their surfaces were generally alveolate with different ornamentations. Some nutlets had longitudinal ridges. The indumentum varied among unrelated species and showed some significant similarities within related species. In *Flora of Turkey* sect. *Teucrium* was divided into 2 groups according to leaf fragmentation and other morphological characters. The current study supports this grouping. The indumentum types on nutlets and leaves have a high taxonomical value for the species of the genus.

Key words: *Teucrium*, section *Teucrium*, Lamiaceae, micromorphology, scanning electron microscopy, leaf indumentum, nutlet surface, Turkey

1. Introduction

Teucrium L. belongs to the family Lamiaceae (represented by more than 260 species spread all over the world) and is a large and complex genus. It is mainly distributed in Europe, North Africa, and the temperate parts of Asia (Kastner, 1989; Abu-Assab and Cantino, 1993). The major area of distribution for this genus is the Mediterranean region, containing about 96% of all taxa of the genus (Cantino et al., 1992; Navarro and El Oualidi, 2000).

Teucrium species are shrubs, dwarf shrubs, and perennial, biennial, or annual herbs that grow in open, dry, rocky places (especially limestone and serpentine), on slopes, and in disturbed areas and tend to occupy exposed habitats (Kummerov, 1983).

Teucrium was represented by 27 species in *Flora of Turkey and the East Aegean Islands* (Ekim, 1982). Since then many new species and subspecies have been described in Turkey and their current number is 34 (46 taxa, 16 of which are endemic) (Duman, 2000; Dönmez, 2006; Dönmez et al., 2010; Dinç and Doğu, 2012; Dirmenci, 2012). *Teucrium* has been divided into 8 sections, distinguishable from each other by their calyx shape and inflorescence structure (Ekim, 1982).

Section *Teucrium* Benth. is 1 of the 8 sections and is distinguished by its calyces, which are not gibbous and obconical-campanulate, and its teeth, which are more or less equal. Leaves are entire to deeply dissected (Ekim, 1982). Sect. *Teucrium* has 11 species (13 taxa, 5 of which are endemic) in Turkey: *T. alyssifolium* Stapf, *T. brevifolium* Schreb., *T. creticum* L., *T. ekimii* H.Duman, *T. multicaule* Montbret & Aucher ex Benth., *T. orientale* L. [var. *glabrescens* Hausskn. ex Bornm., var. *orientale*, var. *puberulens* Ekim], *T. parviflorum* Schreb., *T. pestalozzae* Boiss., *T. pruinatum* Boiss., *T. pseudoroaniam* Parolly, Erdağ & Nordt, and *T. sandrasicum* O.Schwarz (Ekim, 1982; Duman, 2000; Dönmez, 2006; Dönmez et al., 2010; Dinç and Doğu, 2012).

The systematic and phylogenetic significance of the indumentum is well known in Lamiaceae and in related families such as Acanthaceae, Bignoniaceae, Plantaginaceae, Scrophulariaceae, and Verbenaceae (Metcalf and Chalk, 1950; El-Gazzar and Watson, 1968, 1970; Ahmad, 1974, 1978; Elias and Newcombe, 1979; Mathew and Shan, 1983; Abu-Assab and Cantino, 1987; Cantino, 1990; Rahn, 1992; Gairola et al., 2009). In some genera of Lamiaceae, the trichome morphology is helpful in infrageneric classification (Marin et al., 1994; Navarro

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and El Oualidi, 2000), as well as at specific and subspecific levels (Bini Maleci et al., 1992; Sebebe and Harley, 1992; Servettaz et al., 1992; Giuliani et al., 2008). Trichomes are widely distributed over the aerial reproductive and vegetative parts of the members of Lamiaceae and are ordinarily distinguished as glandular and nonglandular trichomes. Micromorphological characters, especially trichomes, are among the most useful taxonomic characters in *Teucrium* L. Their absence or presence and their typology have a significant role in the infrageneric classification of the genus. Several studies on the trichomes of *Teucrium* species are available (Antunes and Sevinato-Pinto, 1991; Bini Maleci and Servettaz, 1991; Bini Maleci et al., 1995; Navarro and El Oualidi, 2000; Grubestic et al., 2007; Dinç et al., 2008, 2009, 2011a, 2011b; Eshratifar et al., 2011; Dinç and Doğu, 2012; Kremer et al., 2012; Doğu et al., 2013).

The objective of the current study is to investigate the leaf and nutlet micromorphological characters of some species of sect. *Teucrium* and to determine the systematic value of these characters in specific and infraspecific segregation of the taxa.

2. Materials and methods

Most of the materials used for this study were collected from natural habitats and different localities in Turkey by the authors. Some samples were provided by the ISTE herbarium (Table 1). Voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, İstanbul University (ISTE). The nutlets were preliminarily observed using a light microscope to make sure that they were of normal size and maturity. For scanning electron microscopy (SEM) analysis, at least 3 samples were prepared. The samples of nutlets and leaves were mounted on stubs and coated with gold before they were studied with an FEI Quanta 450 FEG-EDS scanning electron microscope.

Descriptions and abbreviations of indumenta based on Navarro and El Oualidi (2000) are as follows (Table 2).

1. Glandular trichomes (Simple glandular trichomes)

A: Short clavate glandular trichomes. Generally with 2 large and thin stalk cells or 3 stalk cells.

B: Subsessile glandular trichomes.

2. Nonglandular trichomes (simple, unbranched trichomes)

Table 1. Collection data of *Teucrium* taxa studied.

Taxon	Voucher number (ISTE)	Locality
<i>T. creticum</i>	101 441	C4 Mersin: Anamur, Bozyazı-Kaledibi, Maraş hill, 40–50 m, 05 June 2012, T. Özcan.
<i>T. sandrasicum</i>	101 449	C2 Muğla: Between Muğla and Fethiye, 170 m, 13 June 2011, T. Özcan, T. Dirmenci, E. Akçiçek.
<i>T. brevifolium</i>	101 442	C1 Muğla: Marmaris-Knidos, Datça peninsula, 30–100 m, 16 May 2012, T. Özcan, T. Dirmenci, O. Yıldırım.
<i>T. pestalozzae</i>	101 448	C3 Antalya: Between Antalya and Burdur, Çubuk Beli gateway, 950–1000 m, 17 May 2012, T. Özcan, T. Dirmenci, O. Yıldırım.
	45 034	C3 Burdur: Between Korkutelı and Burdur, 800 m, 24 June 1980, N. & E. Özhatay, E. Tuzlacı.
<i>T. ekimii</i>	101 450	C3 Antalya: Antalya: Kemer, Kuruçay stream, İçi, 100 m, 16 June 2011, T. Özcan, T. Dirmenci, E. Akçiçek.
	101 451	Kemer, Göynük, Beldibi-Kuruçay stream, 100 m, 17 May 2012, T. Özcan, T. Dirmenci, O. Yıldırım.
<i>T. alyssifolium</i>	101 443	C2 Muğla: Fethiye-Çameli road, Tuzla Beli gateway, 1440 m, 14 April 2011, T. Özcan, T. Dirmenci, E. Akçiçek.
	44 933	C2 Muğla: Köyceğiz, between Akköprü and Karabayır, 670 m, 22 June 1980, N. & E. Özhatay, E. Tuzlacı.
<i>T. pseudoroanum</i>	101 452	C3 Antalya: Kasaba-Dirgenler, east of Dirgenler, 100 m, 30.05.2013, T. Özcan, T. Dirmenci.
<i>T. multicaule</i>	101 446	B6 Sivas: Between Kangal and Divriği, 1140 m, 01 July 2012, T. Dirmenci, A. Akpulat.
<i>T. orientale</i> var. <i>orientale</i>	101 447	B6 Sivas: Between Kangal and Zara, 1280 m, 01 July 2012, T. Dirmenci, A. Akpulat.
<i>T. orientale</i> var. <i>glabrescens</i>	101 445	B6 Sivas: Between Divriği and İliç, Kemaliye crossroads, 876 m, 01 July 2012, T. Dirmenci, A. Akpulat.
<i>T. orientale</i> var. <i>puberulens</i>	101 453	C9 Artvin: Between Ardanuç and Ardahan, Bilbilan mountain, 2000 m, 27.08.2013, T. Dirmenci, B. Yıldız.
<i>T. pruinosum</i>	52426	B4 Ankara: Mamak, Hüseyingazi hill, 1000 m, 29 June 1980, K. Alpınar.
<i>T. parviflorum</i>	101 444	B6 Sivas: Between Divriği and İliç, 876 m, 01 July 2012, T. Dirmenci, A. Akpulat.

Table 2. Micromorphological characters, trichome types, distribution, and density of nutlet and leaves.

Taxon	Nutlet			Trichomes		
	Color and shape	Length × width (mm)	Surface	Nutlet	Leaves	
					Adaxial	Abaxial
<i>T. creticum</i>	Brown, narrowly obovate-elliptic, obovate	1.8–3.2 × 1.3–1.8	Longitudinal ridges and alveolate, alveoli deep	B, F2	F5	I, B
<i>T. sandrasicum</i>	Dark brown, narrowly obovate-elliptic, oblong	3.2–4.2 × 1.5–2.0	Longitudinal ridges and alveolate, alveoli shallow	B	H	H
<i>T. brevifolium</i>	Light brown, elliptic, oblong	2.1–3.2 × 1.1–1.4	Longitudinal ridges and alveolate, alveoli shallow	B, F4	G	G
<i>T. pestalozzae</i>	Brown, narrowly obovate-elliptic	1.9–2.4 × 1.0–1.3	Longitudinal ridges and alveolate, alveoli deep	B, F4	G	G, B
<i>T. ekimi</i>	Light brown, obovate-broadly elliptic	2–2.7 × 1.2–1.4	Longitudinal ridges and alveolate, alveoli deep	B, F4	G	I, B
<i>T. abyssifolium</i>	Brown, rotund, broadly elliptic-obovate	3.2–4.1 × 2.2–3.1	Alveolate, alveoli shallow	B, F2	H, B	H, B
<i>T. pseudoroaniam</i>	Light brown, obovate-oblong	2.5–3.0 × 1.8–2	Longitudinal ridges and alveolate, alveoli shallow	B, F2, F5	I, B	I
<i>T. multicaule</i>	Light brown, elliptic-oblong	3.2–4.7 × 1.4–2.5	Alveolate, alveoli deep	B, F2, F4	G	H, B
<i>T. orientale</i> var. <i>orientale</i>	Brown-black, broadly oblong-elliptic	1.9–2.5 × 1.1–1.6	Alveolate, alveoli deep	B, F2, F4	F1, F2, B	F1, F2, B
<i>T. orientale</i> var. <i>puberulens</i>	Brown-black, broadly oblong-elliptic, obovate	2.2–2.4 × 1.3–1.5	Alveolate, alveoli deep	B, F4	F1	B
<i>T. orientale</i> var. <i>glabrescens</i>	Light brown, obovate-broadly elliptic, obovate	1.9–2.1 × 1.1–1.2	Alveolate, alveoli deep	A, B, F2	F4	B
<i>T. pruinosum</i>	Light brown, obovate, rotund	1.9–2.3 × 1.2–1.8	Alveolate, alveoli deep	B, F2, F4	F4, F5	F4, B
<i>T. parviflorum</i>	Light brown, broadly oblong-obovate	3.3–3.7 × 2–2.3	alveolate, alveoli deep	A, B, F2, F4	F2, B	F2, B

F: Thin-walled trichomes.

F1. Triangular, large, and thin-walled unicellular hairs, with ridges or micropapillae.

F2. Large, very thin-walled, 2(-5)-celled trichomes with an acute apical cell, with ridges and marked internodes.

F4. Short, thin-walled, 2-3-celled trichomes densely covered by micropapillae, with the apical cell elongated, slightly crumpled or falcate.

F5. Elongated and flexuose, with thin-walled 3-7(-11)-celled trichomes, with distinct internodes, the apical cell acute with micropapillae, the basal cell smooth, each cell transverse to the preceding one.

G: Thick-walled, 3(-5)-celled, falcate, erect or sometimes slightly curved, the basal cell with smooth trichomes.

H: Very long intertwined trichomes, fibrous-like, sometimes coalescent.

I: Vermiform hairs, thick-walled with elongated cells.

3. Results

Nutlet and leaf micromorphological characters of 13 taxa belonging to *Teucrium* sect. *Teucrium* in Turkey were examined in detail in this study.

3.1. Micromorphological characters of nutlets

Nutlets of *Teucrium* sect. *Teucrium* are light to dark brown or brown-black in color. Their sizes are 1.8-4.7 mm in length and 1.0-3.1 mm in width. The shapes are elliptic, obovate, oblong, or rotund. Nutlet surface is generally alveolate but there are some specific differences in depth among species. In some species, such as *T. creticum* and *T. multicaule*, the alveoli are deep, while in others, like *T. brevifolium* and *T. alyssifolium*, the alveoli are shallow. Some species, like *T. pestalozzae* and *T. ekimii*, have longitudinal ridges (Figures 1 and 2).

Nutlets are densely or sparsely covered with trichomes. Five different trichome types were observed on the nutlets (Table 2). The glandular trichomes are of A and B type. The nonglandular trichomes are of F2, F4, and F5 type. Branched trichomes were not found on the nutlets of sect. *Teucrium* (Figures 1 and 2).

B-type trichomes are distributed on the nutlets in all taxa. A-type trichomes are rarely located on the nutlets of *T. orientale* var. *glabrescens* and *T. parviflorum*. F2-type trichomes are present on the nutlet surfaces of *T. creticum*, *T. alyssifolium*, *T. pseudaroanium*, *T. orientale* var. *orientale*, *T. orientale* var. *glabrescens*, *T. multicaule*, *T. pruinatum*, and *T. parviflorum*. F4-type trichomes are distributed on the nutlets of *T. brevifolium*, *T. pestalozzae*, *T. ekimii*, *T. multicaule*, *T. orientale* var. *orientale*, *T.*

orientale var. *puberulens*, *T. pruinatum*, and *T. parviflorum*. F5-type trichomes are found only on the nutlets of *T. pseudaroanium* (Figures 1 and 2).

3.2. Micromorphological characters of leaves

The micromorphological characteristics and distribution of trichomes of *Teucrium* taxa in Turkey showed a considerable variation in trichome structure on the surfaces of leaves. Eight different unbranched trichome types were observed on the leaves of sect. *Teucrium*; no branched trichomes were found (Table 2).

Adaxial and abaxial surfaces of the leaves of *Teucrium* taxa have same or different types of trichomes. The abaxial and adaxial surface trichome morphologies of *T. brevifolium*, *T. sandrasicum*, *T. alyssifolium*, *T. parviflorum*, and *T. orientale* var. *orientale* are completely the same but other taxa are partially or completely different (Figures 3-5).

B-type trichomes generally occur on the leaf surfaces of most taxa, but not on those of *T. sandrasicum* and *T. brevifolium* (Figure 3).

F1-type trichomes are present on both leaf surfaces of *T. orientale* var. *orientale* and on the adaxial surface of *T. orientale* var. *puberulens*. F2-type trichomes are distributed on the adaxial and abaxial surfaces of *T. orientale* var. *orientale* and *T. parviflorum*. F4-type trichomes are located on the adaxial surface of *T. orientale* var. *glabrescens*. They are also located on both leaf surfaces of *T. pruinatum*. F5-type trichomes are located on the adaxial sides of *T. creticum* and *T. pruinatum* (Figures 3-5).

H-type trichomes are located on both leaf surfaces of *T. alyssifolium* and *T. sandrasicum*. They are also present on the abaxial surface of *T. multicaule* (Figures 3 and 4).

G-type trichomes are distributed on both leaf surfaces of *T. brevifolium* and *T. pestalozzae*. They are also present on the adaxial surface of *T. ekimii* and *T. multicaule* (Figures 3 and 4).

I-type trichomes are present on the abaxial surface of *T. ekimii* and *T. creticum*, and on both leaf surfaces of *T. pseudaroanium* (Figures 3 and 4).

In this study the nutlet and leaf features of *T. pestalozzae*, *T. ekimii*, *T. orientale* var. *puberulens*, *T. pruinatum*, and *T. multicaule* are reported in detail for the first time. The leaf features of *T. alyssifolium* and *T. pseudaroanium* are also described in detail for the first time (Figures 1-4).

4. Discussion

Trichomes are among the most useful taxonomic characters in the genus *Teucrium*. Their absence or presence and their typology can be used as taxonomic markers in the

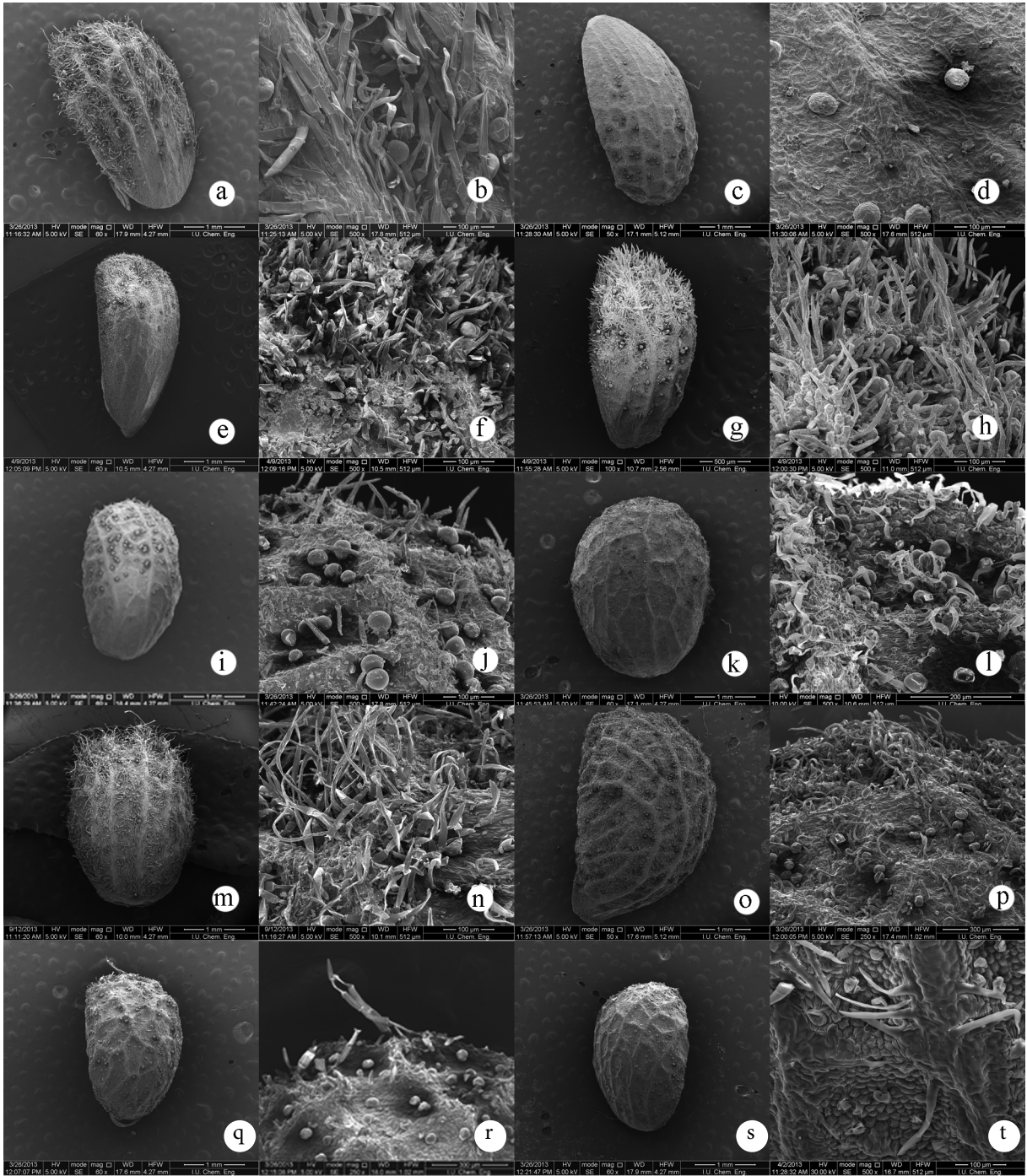


Figure 1. SEM micrographs of nutlets of *Teucrium* sect. *Teucrium*: *T. creticum* (a, b), *T. sandrasicum* (c, d), *T. brevifolium* (e, f), *T. pestalozzae* (g, h), *T. ekimii* (i, j), *T. alyssifolium* (k, l), *T. pseudaroanum* (m, n), *T. multicaule* (o, p), *T. orientale* var. *orientale* (q, r), *T. orientale* var. *puberulens* (s, t) (scale bars: b, d, f, h, j, n, t = 100 µm; l = 200 µm; p, r: 300 µm; g = 500 µm; a, c, e, i, k, m, o, q, s = 1 mm).

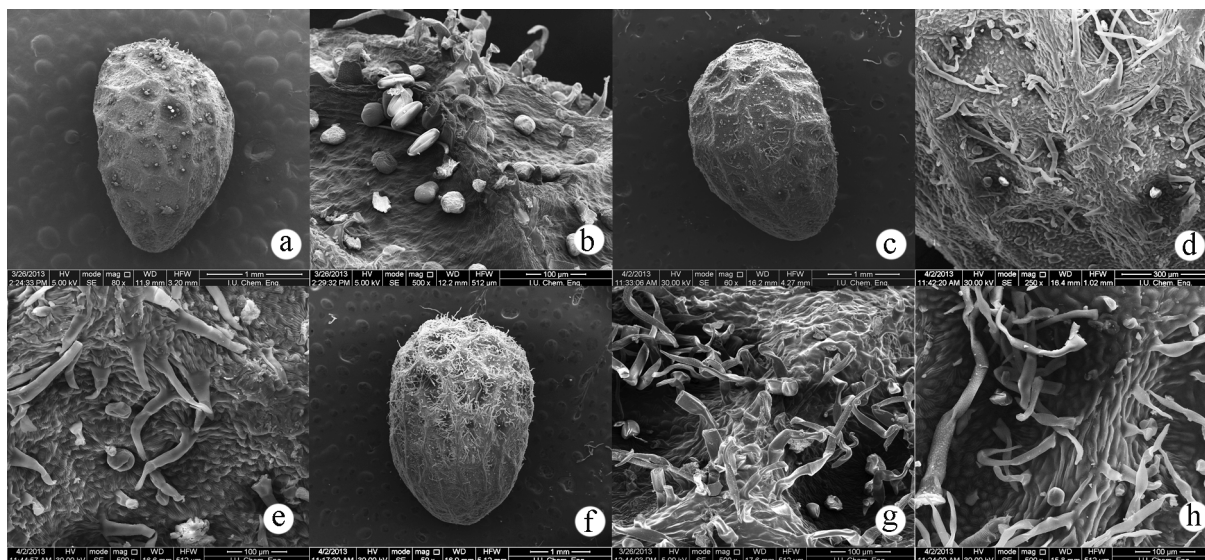


Figure 2. SEM micrographs of nutlets of Turkish *Teucrium* sect. *Teucrium*: *T. orientale* var. *glabrescens* (a, b), *T. pruinosum* (c–e), *T. parviflorum* (f–h) (scale bars: b, e, g, h = 100 μ m; d = 300 μ m; a, c, f = 1 mm).

infrageneric classification of the genus *Teucrium* (Bini Maleci and Servettaz, 1991; Marin et al., 1994; Navarro and El Oualidi, 2000; Torke, 2000).

Navarro (2000) considered sects. *Teucrium* and *Teucriopsis* the most distinct sections of the genus. Marin et al. (1994) and Harborne et al. (1986) suggested that sect. *Teucrium* is totally separate from the other sections of the genus. The summarized results of nutlet characteristics show the presence of glandular and nonglandular trichomes in all sect. *Teucrium* taxa. These morphological results are supported by phytochemistry. A study of flavonoids in European *Teucrium* found that flavonol glycosides were characteristic of the section *Teucrium* but absent from all other taxa (Jeffrey et al., 1986).

In *Flora of Turkey* (Ekim, 1982), the section *Teucrium* was divided into 2 groups based on leaf fragmentation and other morphological characters. In the first group most of the leaves are 2–3 pinnatifid (e.g. *T. multicaule*, *T. orientale*, *T. parviflorum*, and *T. pruinosum*). In the second group the leaves are entire or rarely subentire (*T. alyssifolium*, *T. brevifolium*, *T. creticum*, *T. ekimii*, *T. pestalozzae*, *T. pseudaroanum*, *T. sandrasicum*).

Nutlet and leaf micromorphology supports this grouping. The nutlets of the second group of taxa are characterized by longitudinal ridges. G-, H-, and I-type trichomes are present on the leaves of the second group of species and *T. multicaule*. F-type trichomes are found on the leaves of members of the first group and *T. creticum*. F2- and F4-type trichomes are distributed on the nutlets of the first group of taxa.

The B type is the most general type present on the nutlets and leaves of *Teucrium* taxa, except for the leaves of *T. sandrasicum* and *T. brevifolium*. The B type is generally present on the abaxial surface of leaves. Dinç et al. (2008) reported that only the B type was found on the nutlets of *T. sandrasicum*, which is confirmed by our findings. This is the most distinguishing feature of *T. sandrasicum* from other taxa of sect. *Teucrium*.

In the current study, A-type trichomes were only found on the nutlets of *T. orientale* var. *glabrescens* and *T. parviflorum*. However, Parolly and Eren (2007) found A-type trichomes on the nutlets of *T. pseudaroanum*. Type F5 is present on the adaxial side of leaves of *T. creticum* and *T. pruinosum*, and on the nutlets of *T. pseudaroanum*. Our results supported the results of Parolly and Eren (2007), who also found type F5 on *T. pseudaroanum* nutlets. This dissimilarity is important for differentiating these 3 taxa from the others.

G-type trichomes are present on 4 taxa. According to the results of Navarro and El Oualidi (2000), G-type trichomes were only found in sect. *Chamaedrys* and were absent in sect. *Teucrium*. Our findings thus seem to differ.

H-type trichomes can be seen on *T. sandrasicum* and *T. alyssifolium*. Our results supported the results of Dinç et al. (2008) about *T. sandrasicum*. The only difference is that the trichome density of the upper epidermis is not as high as that of the lower epidermis.

Micromorphological differences between leaves and nutlets allow differentiating the varieties of *T. orientale*, which is in agreement with the morphological observations

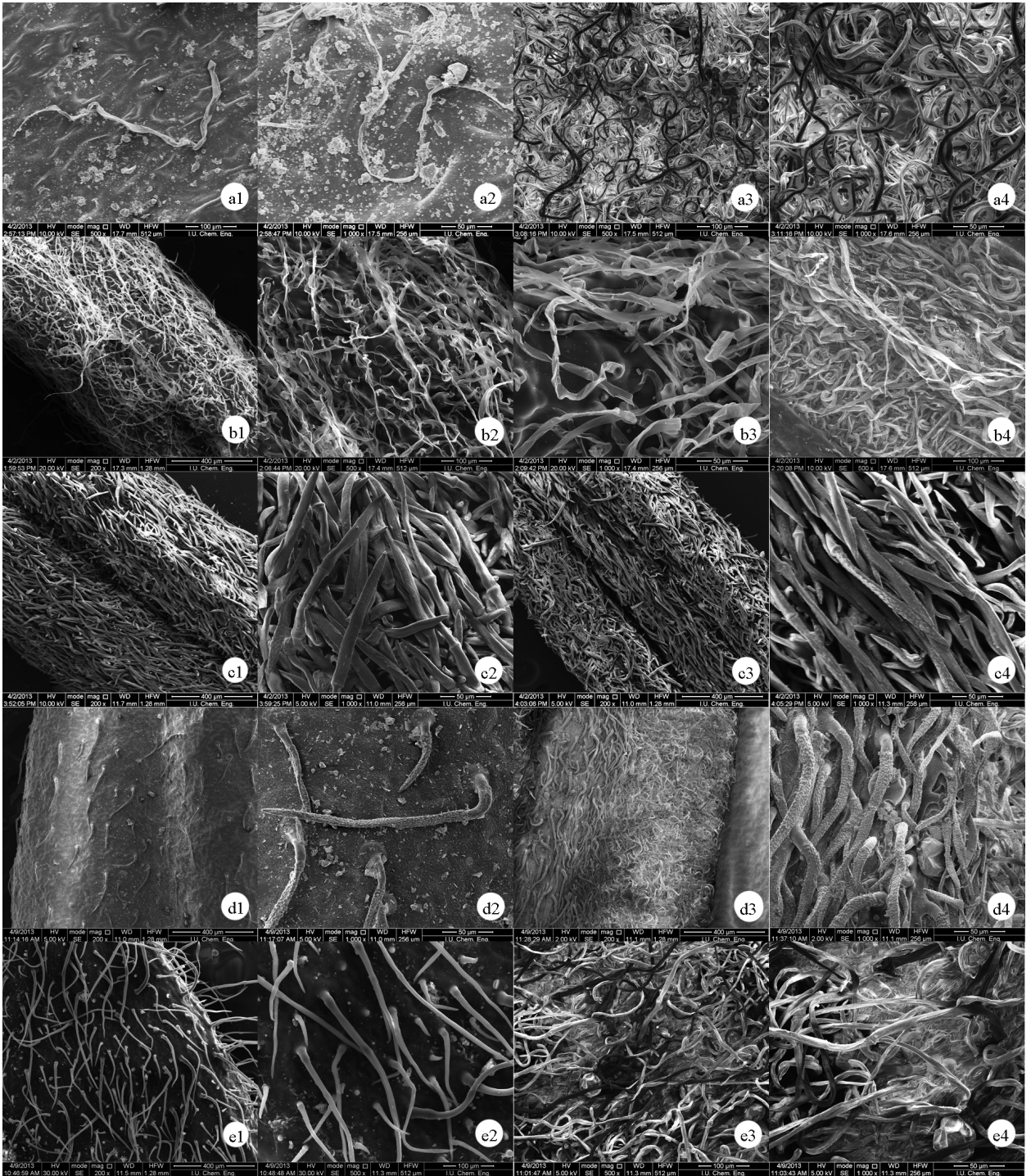


Figure 3. SEM micrographs of leaves of *Teucrium* sect. *Teucrium*, adaxial surface (1–2) and abaxial surface (3–4): *T. creticum* (a), *T. sandrasicum* (b), *T. brevifolium* (c), *T. pestalozzae* (d), *T. ekimii* (e) (scale bars: a2, a4, b3, c2, c4, d2, d4, e4 = 50 µm; a1, a3, b1, b4, e1, e3: 100 µm; b1, c1, c3, d1, d3, e1 = 400 µm).

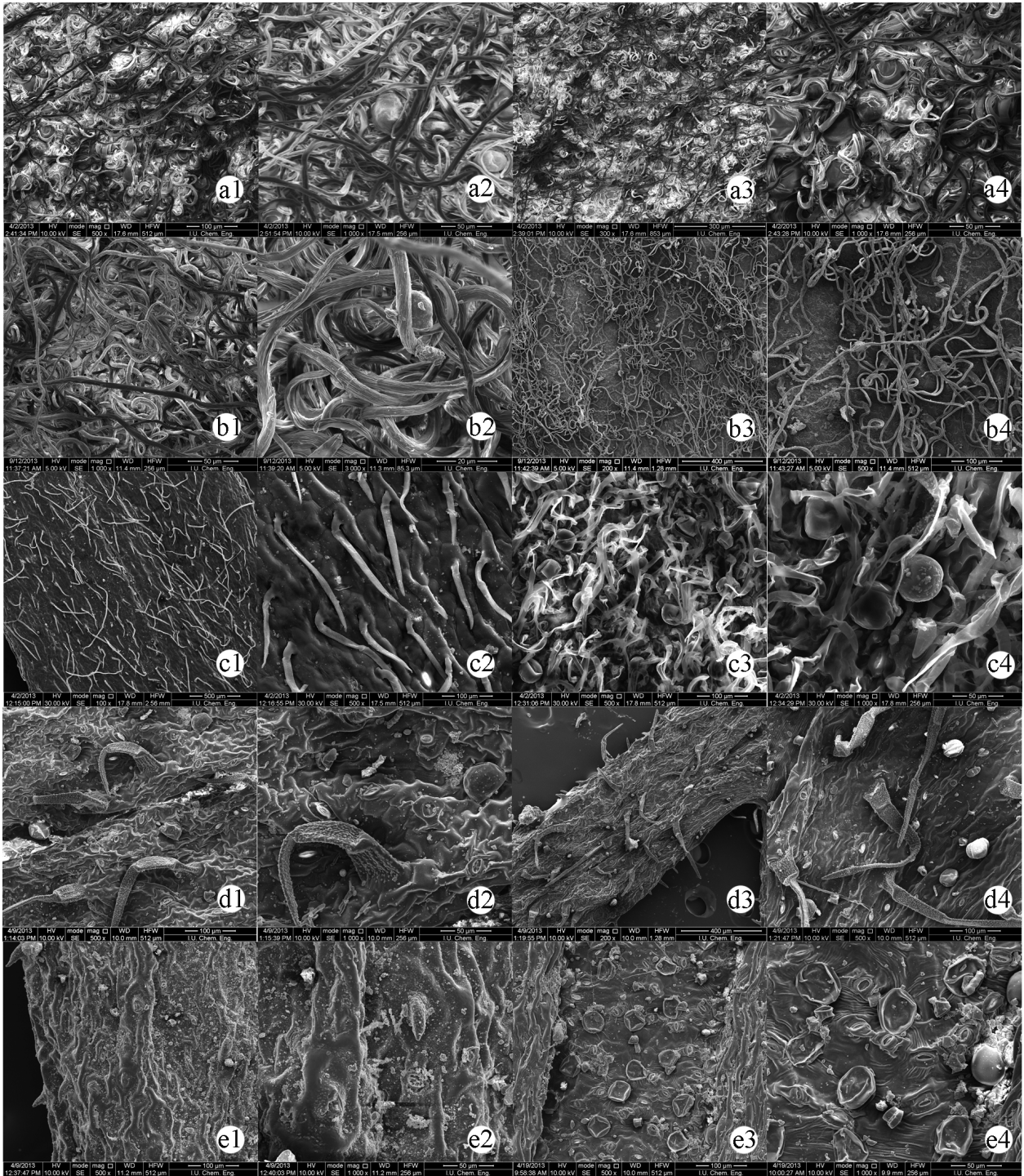


Figure 4. SEM micrographs of leaves of *Teucrium* sect. *Teucrium*, adaxial surface (1–2) and abaxial surface (3–4): *T. alyssifolium* (a), *T. pseudaroanum* (b), *T. multicaule* (c), *T. orientale* var. *orientale* (d), *T. orientale* var. *puberulens* (e) (scale bars: b2 = 20 µm; a2, a4, b1, c4, d2, e2, e4 = 50 µm; a1, b4, c2, c3, d1, d4, e1, e3 = 100 µm; a3 = 300 µm; b3, d3 = 400 µm; c1 = 500 µm).

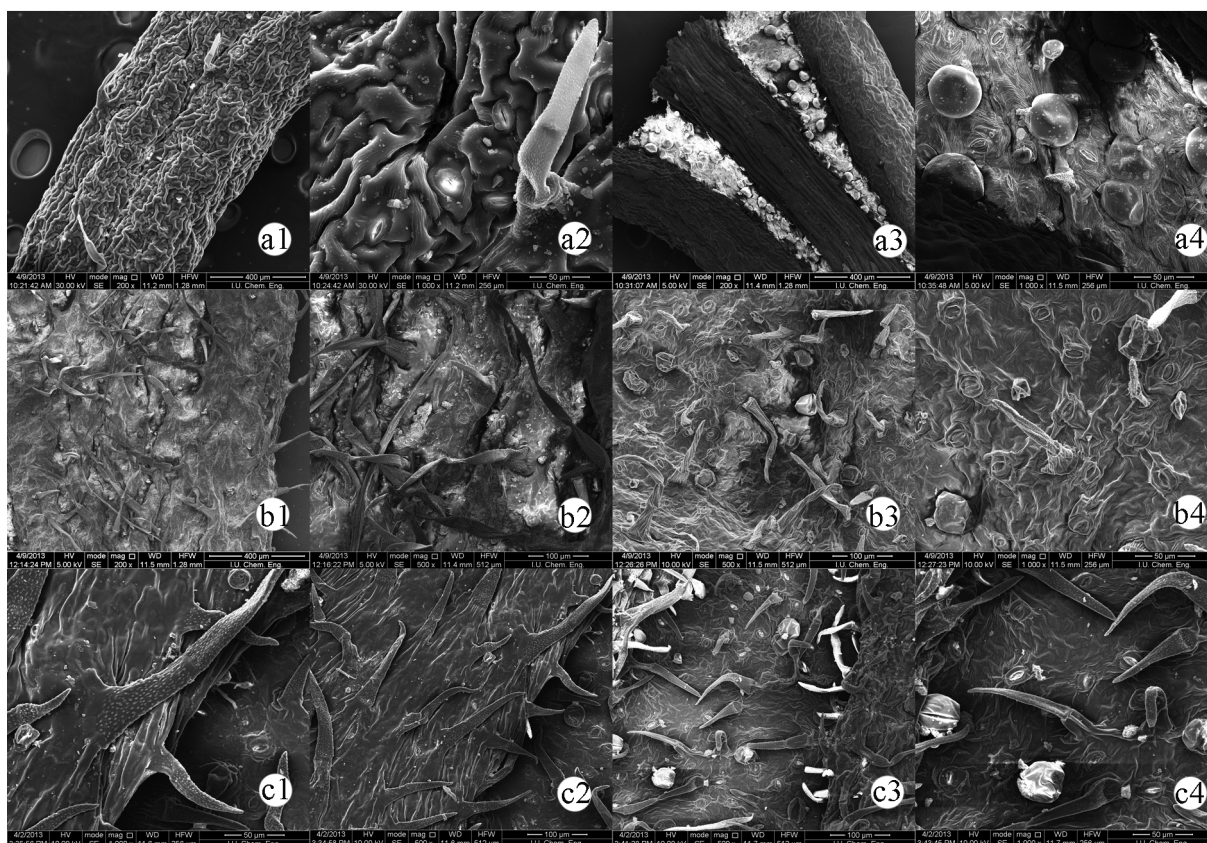


Figure 5. SEM micrographs of leaves of *Teucrium* sect. *Teucrium*, adaxial surface (1–2) and abaxial surface (3–4): *T. orientale* var. *glabrescens* (a), *T. pruinosum* (b), *parviflorum* (c) (scale bars: a2, a4, b4, c1, c4 = 50 μ m; b2, b3, c2, c3 = 100 μ m; a1, a3, b1 = 400 μ m).

described in *Flora of Turkey and the East Aegean Islands* for each variety studied. *T. orientale* varieties have different trichomes types. On the nutlet surface A-type trichomes are only found in var. *glabrescens*. On the leaves F1-type trichomes are present in var. *orientale* and var. *puberulens*. F2 is only found in var. *orientale*, and the F4 type is only present in var. *glabrescens*.

The data presented here show that the nutlet and the leaf microcharacters are more useful in separation of *Teucrium* taxa of sect. *Teucrium*.

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