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Research Article

NOTES ON LEAF AND STEM ANATOMY OF *Thlaspi* sensu lato

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Abstract: In this study, anatomical characteristics of leaves and stems of 19 taxa of *Thlaspi* sensu lato, 9 of which are endemic to Turkey, were investigated in order to determine taxonomic relationships among the studied taxa. The anatomical characteristics of all taxa were determined and assessed using the cluster analysis and the principal component analysis. The transverse sections of the leaves and stems exhibited various diagnostic characters in terms of the pattern of epidermal cell and mesophyll layers, the number and size of vascular bundles, and the thickness of the cortex and endodermis. Important differences were detected in number, size, and index of stomata, and in epidermal cell wall structures in the adaxial and abaxial surfaces. The results showed that the compared anatomical characteristics among taxa are partially compatible with their sectional delimitation in their traditional rank in The Flora of Turkey and the East Aegean Islands. The results also point out that some taxonomic re-arrangements may be required.

Özet: Bu çalışmada, 9'u Türkiye için endemik olan 19 *Thlaspi* sensu lato taksonunun yaprak ve gövde anatomik karakterleri, taksonlar arasındaki taksonomik ilişkileri belirleme amacıyla tanımlanmıştır. Tüm taksonların anatomik karakterleri belirlendi ve kümeleme analizi ve temel bileşen analizi kullanılarak değerlendirildi. Yaprakların ve gövdelerin enine kesitleri epidermal hücre ve mezofil tabakalarının yapıları, vasküler demetlerin sayısı ve boyutu, korteks ve endodermisin kalınlığı açısından çeşitli teşhis karakterleri gösterdi. Ayrıca, adaksiyal ve abaksiyal yüzeylerde stomaların sayı, boyut ve indeksinde ve epidermal hücre duvarı yapılarında önemli farklılıklar saptandı. Bu sonuçlar taksonlar arasındaki karşılaştırılmış anatomik özelliklerin Türkiye Florası ve Doğu Ege Adaları'ndaki geleneksel sıralamasındaki seksiyon sınırlamaları ile kısmen uyumlu olduğunu göstermektedir. Elde edilen veriler ayrıca bazı taksonomik yeniden düzenlemelerin gerekli olabileceğine işaret etmektedir.

Introduction

Brassicaceae is a large and important plant family in terms of economically significant species and model organisms such as *Arabidopsis* Heynh. and *Brassica* L. The family has approximately 338 genera and 3709 species worldwide, primarily in temperate zones of the Northern Hemisphere (Al-Shehbaz *et al.* 2006). In Turkey, Brassicaceae includes 571 species with 65 subspecies, 24 varieties and 660 taxa belonging to 91 genera (Al-Shehbaz *et al.* 2007). *Thlaspi* L. is known to be one of the largest genera of Brassicaceae and it has 75 species distributed mainly in Eurasia (Al-Shehbaz 1986, Appel & Al-Shehbaz 2003). The genus, represented by 36 taxa at various levels, is diverse in Turkey. Twenty-one of these taxa are endemic to Turkey, making the endemism rate of the genus 58% (Davis 1988, Karaismailoğlu & Erol 2019).

The classification in generic and subgeneric categories of *Thlaspi* sensu lato is quite confused with complex taxonomy and nomenclature. The traditional understanding of *Thlaspi* was radically changed with Meyer's works (1973, 1979, 1991, 2001), which were based on seed coat anatomy. Meyer divided the genus into 12 genera and maintained only six taxa in *Thlaspi* (*Thlaspi* sensu stricto). Many researchers (Greuter & Raus 1983, Greuter *et al.* 1986, Al-Shehbaz 1986, Artelari 2002, Appel & Al-Shehbaz 2003, Al-Shehbaz 2014, Karaismailoğlu & Erol 2018) opposed this classification by considering it as not practical for taxonomy and its extensive-scale use extremely limited (Al-Shehbaz 2014). In the following years, remarkable molecular phylogenetic studies such as Mummenhoff & Koch (1994), Zunk *et al.* (1996), Mummenhoff *et al.* (1997a, 1997b), Koch *et al.* (1998), Koch *et al.* (2001) clearly showed that the classification of Meyer (1973, 1979) was baseless (Al-Shehbaz 2014). However, despite the numerous works on the infrageneric and interspecific taxonomy of the genus, problems have yet to be elucidated.

Metcalfe & Chalk (1957) showed that the important distinctive anatomical characters of Brassicaceae include epidermal cell type, stoma type and structures of the vascular bundles, which may provide insight into many taxonomical characters demonstrated to be significant in the species classification (Stace 1984). Many such data on these characters have been extensively used in taxonomical assignments (Liu & Zhu 2011, Selvi & Paksoy 2013, Ozcan *et al.* 2015, Karaismailoğlu 2016, 2019).

There is a lack of data in literature on anatomical features of *Thlaspi* taxa except those of seed anatomy (Karaismailoğlu & Erol 2019). This paper offers the first comprehensive assessment of the systematic importance of leaf and stem anatomy in the examined *Thlaspi* taxa.

Materials and Methods

Samples

The examined taxa were collected from various phytogeographic regions of Turkey (Table 1). Voucher specimens are deposited in the Istanbul University Science Faculty Herbarium (ISTF).

Anatomical preparations

Anatomical studies were made using specimens preserved in 70% alcohol. Cross sections were taken with a fully automatic microtome (Thermo Shonda Met Finesse) from the stem and cauline leaves. Subsequently, they were taken through alcohol and xylene series, stained with hematoxylin (Harris-RRSP67-E) in a staining device (ASC 720 Medite) and covered with Entellan for examination of the anatomical structures (Karaismailoğlu 2015a, 2015b, Karaismailoğlu & Güner 2019). The stomatal density of leaf surfaces was enumerated using surface sections taken by hand. Anatomical characters were observed using an Olympus CX21FS1 microscope and Kameram Imaging Software.

The terminology used for anatomical characters of the leaves follows Wilkinson (1979) and Stace (1984).

Table 1. The locality and collection data details of the examined taxa.

Section	Taxa	Locality	Collection number
Nomisma DC.	*T. arvense* L. (T1)	Samsun, Kavak-Akdağ, agricultural field edges, 766 m, 2.5.2015	Karaismailoğlu 139
	T. huetii Boiss. (T2)	Artvin, Şavşat, Ciritdüzü village, roadside, stony areas, 2050 m, 10.07.2014	Karaismailoğlu 66
Thlaspi L.	*T. orbiculatum* Stev. (T3)	Artvin, Ardanuç, above Peynirli village, steep slopes, 1663 m, 15.06.2015	Karaismailoğlu 201
	T. kotschyanum Boiss. & Hohen. (T4)	Kahramanmaraş, Göksun, Berit Mountain, humid areas, 2116 m, 19.06.2015	Karaismailoğlu 202
	T. perfoliatum L. (T5)	Tekirdağ, Tekirdağ-Kırklareli, roadsides and inclined slopes, 273 m, 21.03.2015	Karaismailoğlu 109
	T. annuum Koch (T6)	Amasya, Boraboy, village-lake, inclined slopes, 881 m, 02.05.2015	Karaismailoğlu 143
	T. bulbosum Spruner ex Boiss. (T7)	Kahramanmaraş, Andırın, Meryemçil plateau, grassland, 1633 m, 21.06.2015	Karaismailoğlu 209
	T. leblebicii Gemici & Görk (T8)*	Muğla, Köyceğiz, Sandras Mountain, Ağla village, roadsides, stony slopes, 1262 m, 05.06.2015	Karaismailoğlu 192
Pterotropis DC.	*T. ochroleucum* Boiss. (T9)	Hatay, Dörtyol, Topaktaş-Karamezra plateau, Fagus forest, stony slopes, 1752 m, 24.04.2016	Karaismailoğlu 240
	T. violascens Schott & Kotschy (T10)*	Osmaniye, Düziçi, Dumanlı Mountain, forest, 1259 m, 26.05.2015	Karaismailoğlu 181
	T. densiflorum Boiss. & Kotschy (T11)*	Kahramanmaraş, Ahir Mountain, Ulucak hill, stony slopes, 1751 m, 20.06.2015	Karaismailoğlu 205
	T. cataonicum Reuter (T12)*	Adana, Saimbeyli, Obruk plateau, grassland, 1472 m, 18.04.2015	Karaismailoğlu 124
	T. elegans Boiss. (T13)*	Osmaniye, Düziçi, near Haruniye, open fields and inclined slopes, roadside, 797 m, 19.04.2015	Karaismailoğlu 130
	T. rosulare Boiss. & Balansa (T14)*	Niğde, Çamardı, Yelatan village, stony slopes, 2085 m, 25.05.2015	Karaismailoğlu 173
	T. praecox Wulfen subsp. *praecox* (T15)	Kırklareli, Dereköy, roadsides, stony areas, 520 m, 09.06.2015	Karaismailoğlu 197
	T. cariense A. Carlström (T16)*	Muğla, Marmaris, Kırzeytin Mountain, serpentine rocks, 494 m, 05.06.2015	Karaismailoğlu 190
	T. tatianae Bordz. (T17)	Van, Güzeldere-Başkale, Güzeldere pass, summit, wetlands, 2651 m, 30.05.2015	Karaismailoğlu 186
	T. aghricum P.H. Davis & Kit Tan (T18)*	Ağrı, Hamur-Tutak, meadow, inclined slopes, 1605 m, 16.05.2015	Karaismailoğlu 162
	T. watsonii P.H. Davis (T19)*	Van, Güzeldere-Başkale, Güzeldere pass, summit, 2752 m, 02.07.2015	Karaismailoğlu 210

* Endemic to Turkey

Statistical analysis

The data obtained from the examined parameters were evaluated with the SPSS (Statistical Package for the Social Sciences) and MVSP (Multi Variate Statistical Package) computer programs. The Duncan multiple-range test of the SPSS was used to determine the statistical importance of differences among the quantitative values obtained for different taxa in Tables 2-4 (SPSS 2006). Grouping of taxa was carried out utilizing the clustering analysis method (UPGMA) of the MVSP in accordance with the 36 characters in Tables 2-4 (Fig. 5). Principal component analysis (PCA) ordination of the MVSP was also performed (Fig. 6 and Table 5) (Kovach 2007).

Results

The comparative anatomical characteristics of the leaves and the stems are presented in Tables 2-4 and Figs 1-4, demonstrating important differences among the taxa. Tables 2-3 and Figs 1-3 show the anatomical features of the leaves and Table 4 and Fig. 4 show the anatomical characters of the stems of the examined taxa.

The cross-sections of the leaves feature a thin cuticle, which is more prominent in T4 and T6 than in other taxa, on the adaxial and abaxial epidermis. Epidermal cells are isodiametric and mostly range from square to rectangular in shapes, rarely polygonal. The thickness of the adaxial epidermis layer varies between 28.12 X 7.19 µm (T3) and 3.21 X 5.16 µm (T11), while the abaxial epidermis layer ranges from 69.77 X 6.14 µm (T4) to 4.08 X 2.98 µm (T1) (Table 2 and Fig. 1). The adaxial epidermis cells in most taxa are thicker than abaxial epidermis cells.

Most of the examined taxa have unifacial-type or two-layered, rarely three-layered (equifacial in T2 and T9) mesophyll with an arrangement of 2-4 layers of palisade parenchyma and 3-5 layers of spongy parenchyma with small intercellular gaps (Fig. 1). Mesophyll thickness ranges from 65.22 to 152.31 µm. It is widest in T17 and narrowest in T16 (Table 2). Most of the taxa appear to have straight anticlinal cell walls in epidermal layers, but T4, T7, T13, T17 and T19 have sinuous cell walls. The anticlinal cell walls of most of the taxa are sinuous on the abaxial surface, excluding T4, T15 and T16, which shows straight anticlinal cell walls.

Midrib dimensions varies from 50.12 (T7) to 95.91 µm (T8) in length, from 45.18 (T11) to 102.41 µm (T16) in width (Table 2). Midrib sizes are generally bigger in the taxa of the *Pterotropis* section than in the *Nomisma* and *Thlaspi* sections. The midribs are usually oval in shape; however, this varied, being circular to elliptical with a convex abaxial midrib surface in T10, T12, T16 and T18. Collateral vascular bundles are organized in a single row and enclosed with parenchymatic sheath cells.

Three different types of epidermal cells were observed on the leaf surfaces: irregular, polygonal, and rectangular (Figs 2-3). The stomatal index is between 12.37 (T3) and 63.01 (T5) on the adaxial surface, and 10.18 (T7) and 51.55 (T5) on the abaxial surface (Table 3). The stomata type of researched taxa were defined as anisocytic, and rarely anomocytic, which appeared to be on the same level as the epidermis. Stoma size differed substantially among the examined *Thlaspi* taxa. The largest stomatal size was found on the adaxial and abaxial surfaces of T18 and T17, whilst the smallest ones were observed on the adaxial and abaxial surfaces of T3 (Table 3, Figs 2-3)

The one-layer epidermis consisting of flat, square, or rectangular cells, with a thin cuticle (0.4-2 µm) on the outside was observed in stem cross sections. The surface is hairless, but there are protrusions in T1 and T2 (in the *Nomisma* section only) (Table 4 and Fig. 4). The epidermis layer is amphistomatic. The cortex consists of 3-12 parenchyma layers with thin- or thick-walled, regularly flat, or circular cells. Its thickness ranges from 14.46 (in T3) to 116.85 (in T11) µm (Table 4). Starch granules were observed in the cortex parenchyma. The endodermis layer located under the cortex consists of 1 or rarely 2 seriate flat-shaped cells, varying between 8.07 µm (T19) and 28.75 µm (T7) in length and between 3.84 (T10) and 12.87 µm (T11) in width. The number of bundles ranges from 5-6 (T5 and T7) to 14-16 (T4) (Table 4 and Fig. 4). This number is notably larger in taxa of the *Pterotropis* section compared to *Nomisma* and *Thlaspi*. The dimensions of the vascular bundles are 70.53-130.84 µm in length, and 40.62-156.06 µm in width (Table 4). The interfascicular region was found between vascular bundles in some of the examined taxa. Generally, cambium cells were not obvious. Some of the examined taxa also have a layer of scleranchymatic cells between 31.14 and 102.45 µm thick. The pith consists of polygonal or circular parenchymatic cells.

The unweighted pair group method with arithmetic mean (UPGMA) dissimilarity clustering dendrogram for 36 anatomical characters of leaves and stems of 19 *Thlaspi* taxa is presented in Fig. 5, and their infraspecific correlations in Tables (2-4). The dendrogram distinguishes the two major clusters and subsets from all other examined species. T11, T17, T14, T8, T13 and T7 form the first main cluster at a distance coefficient of about 25.0, while 13 other taxa make up the second main cluster at a distance coefficient of about 22.0. As indicated in the dendrogram, T11, T16, T5, T9 and T19 are markedly different from other taxa based on the examined characteristics (Tables 2-4). The clades included closely correlated taxa such as T3-T4, T1-T2 and T9-T19, consistent with the traditional taxonomic rank of *Thlaspi* taxa in Turkey.

The principal component analysis (PCA) ordination and dissimilarity matrix according to the anatomical characteristics of leaves and stems are given in Table 5 and Fig. 6. The closest and most distant taxa are defined. T9 and T19 are the most closely related taxa (dissimilarity percentage: 9.46), whereas T9 and T11 are the most distant taxa (dissimilarity percentage: 32.00) (Table 5 and Fig. 5). Additionally, the cumulative variance value of principal components reached 68.27% (Axis 1: 47.55%, Axis 2: 20.72%).

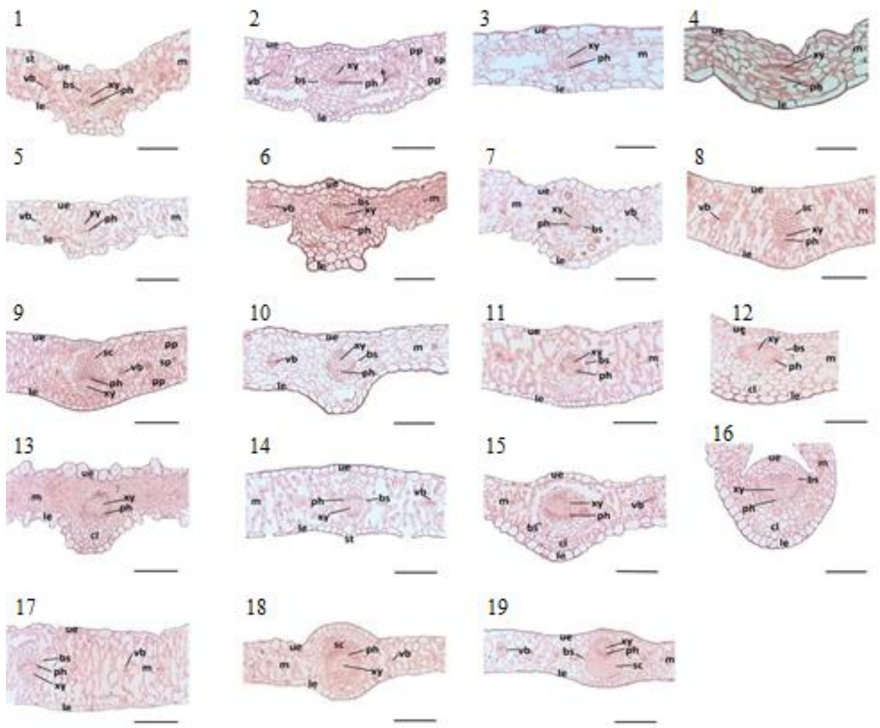

Fig. 1. Leaf cross sections of **1:** *T. arvense*, **2:** *T. huetii*, **3:** *T. orbiculatum*, **4:** *T. kotschyanum*, **5:** *T. perfoliatum*, **6:** *T. annuum*, **7:** *T. bulbosum*, **8:** *T. leblebicii*, **9:** *T. ochroleucum*, **10:** *T. violascens*, **11:** *T. densiflorum*, **12:** *T. cataonicum*, **13:** *T. elegans*, **14:** *T. rosulare*, **15:** *T. praecox* subsp. *praecox*, **16:** *T. cariense*, **17:** *T. tatianae*, **18:** *T. aghricum*, **19:** *T. watsonii*. (**ue:** upper epidermis, **le:** abaxial epidermis: **vb:** vascular bundle**, bs:** bundle sheath, **ph:** phloem, **xy:** xylem, **st:** stoma, **sc:** sclerenchymatic cells, **m:** mesophyll, **cl:** collenchyma, scale bars: 100 µm.)

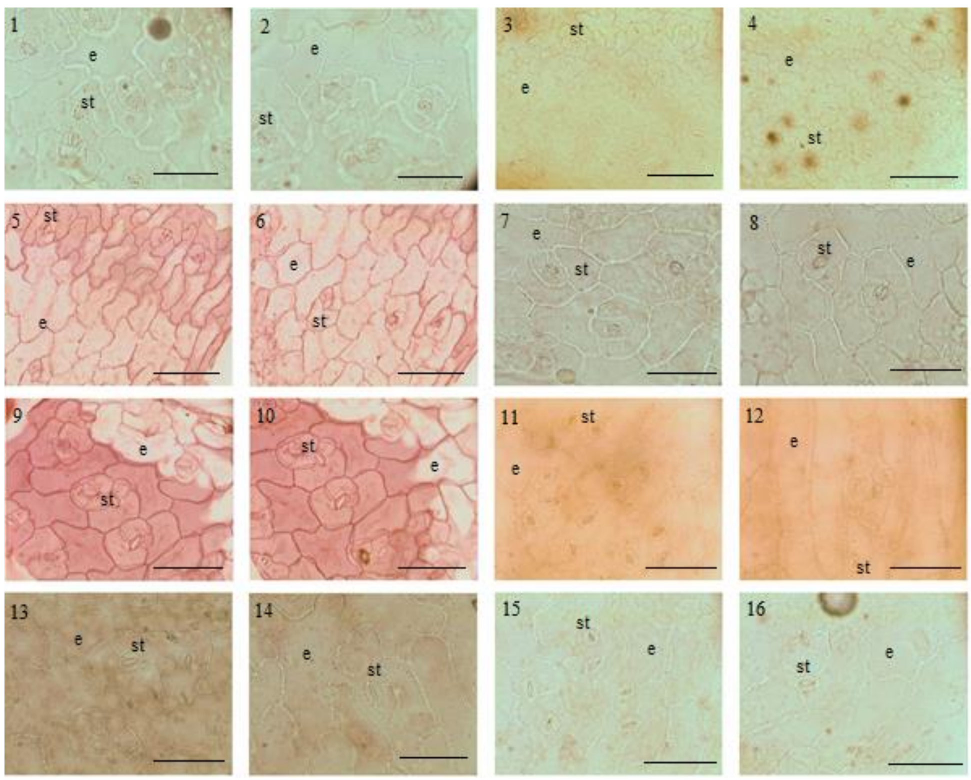

Fig. 2. Leaf surface patterns of **1-2:** *T. arvense*, **3-4:** *T. huetii*, **5-6:** *T. orbiculatum*, **7-8:** *T. kotschyanum*, **9-10:** *T. perfoliatum*, **11-12:** *T. annuum*, **13-14:** *T. bulbosum*, **15-16:** *T. leblebicii* (**e:** epidermis, **st:** stoma, scale bars: 100 µm, odd numbers show adaxial surfaces, even numbers show abaxial surfaces).

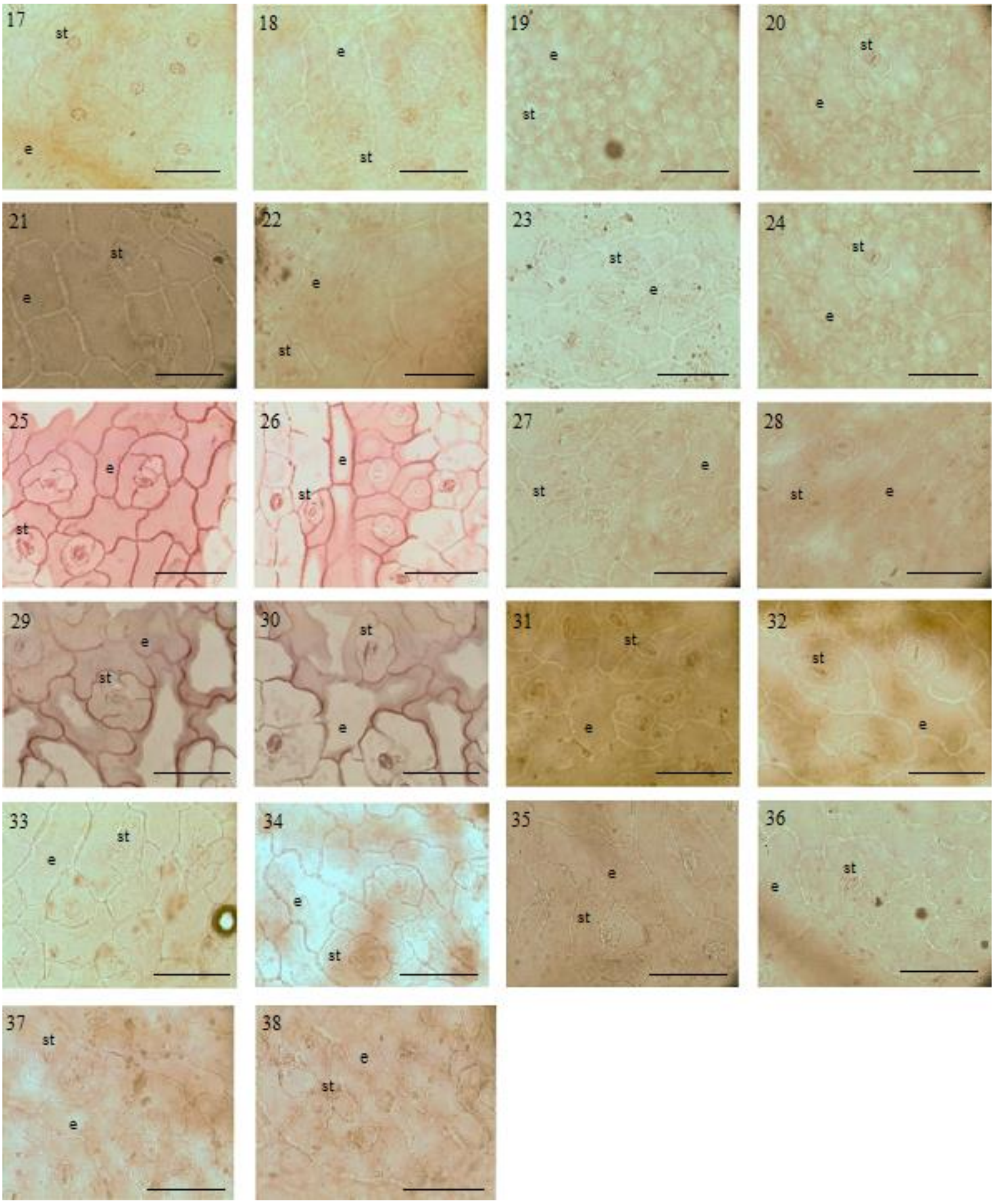

Fig. 3. Leaf surface patterns of **17-18:** *T. ochroleucum*, **19-20:** *T. violascens*, **21-22:** *T. densiflorum*, **23-24:** *T. cataonicum*, **25-26:** *T. elegans*, **27-28:** *T. rosulare*, **29-30:** *T. praecox* subsp. *praecox*, **31-32:** *T. Cariense*, **33-34:** *T. tatianae*, **35-36:** *T. aghricum*, **37-38:** *T. watsonii* (**e:** epidermis, **st:** stoma, scale bars: 100 µm, odd numbers show adaxial surfaces, even numbers show abaxial surfaces).

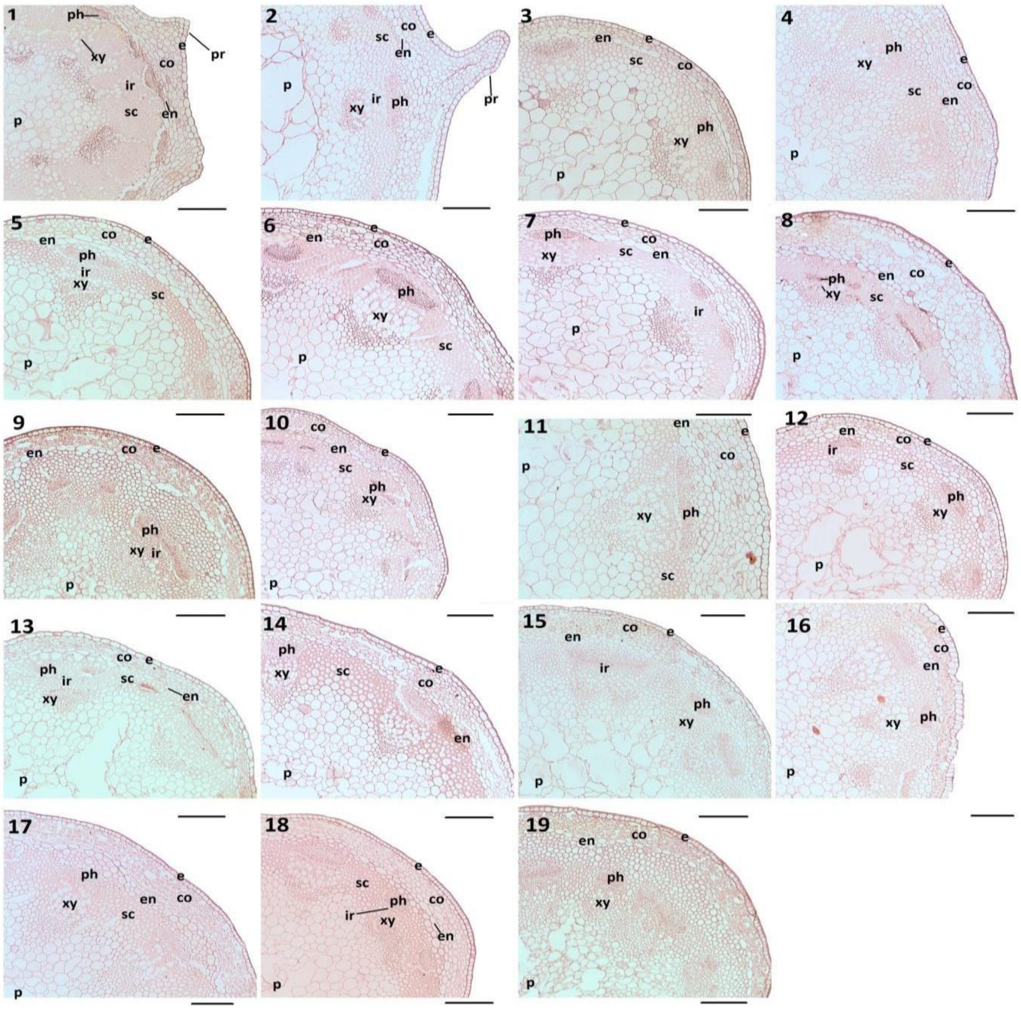

Fig. 4. Stem anatomical patterns of **1:** *T. arvense*, **2:** *T. huetii*, **3:** *T. orbiculatum*, **4:** *T. kotschyanum*, **5:** *T. perfoliatum*, **6:** *T. annuum*, **7:** *T. bulbosum*, **8:** *T. leblebicii*, **9:** *T. ochroleucum*, **10:** *T. violascens*, **11:** *T. densiflorum*, **12:** *T. cataonicum*, **13:** *T. elegans*, **14:** *T. rosulare*, **15:** *T. praecox* subsp. *praecox*, **16:** *T. cariense*, **17:** *T. tatianae*, **18:** *T. aghricum*, **19:** *T. watsonii*. (**e:** epidermis, **co:** cortex, **en:** endodermis, **ph:** phloem, **xy:** xylem, **ir:** interfascicular region, **sc:** sclerenchymatic region, **p:** pith, scale bars: 100 µm.)

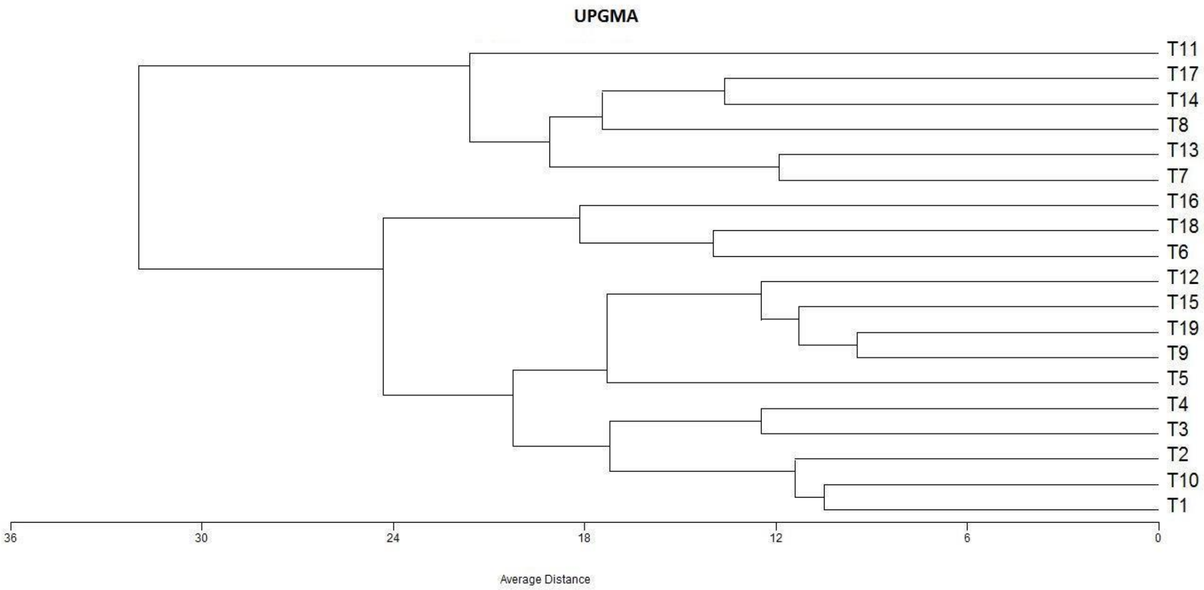

Fig. 5. The dendrogram obtained with UPGMA of the examined taxa (See Table 1 for taxa abbreviations).

Table 2. Anatomical characteristics of leaves of the examined taxa. Outcomes represent mean values ± standard deviation; means with different letters are significant at p = 0.05 level (Duncan's multiple-range test), for taxa abbreviations see Table 1 (L: Length, W: Width).

Taxa	Leaf								
	Adaxial Epidermis	Midrib			Xylem	Phloem	Trachea	Mesophyll	Abaxial Epidermis
	L (1) X W (2) (µm)	Bundle Sheath (3)	Sclerenchyma (4)	L (5) X W (6) (µm)	Height (7) (µm)	Height (8) (µm)	Diameter (9) (µm)	Thickness (10) (µm)	L (11) X W (12) (µm)
T1	9.13±0.16c X 5.02±0.22bc	present	absent	67.15±2.45f X 59.48±3.18h	44.16±2.08de	5.16±0.54d	3.17±0.12c	99.23±4.04g	4.08±2.32i X 2.98±1.02h
T2	6.71±2.14ef X 3.41±0.14c	present	absent	64.05± 0.15g X 62.17±0.21h	32.59±0.19h	11.04±0.22a	2.24±0.08d	108.75±2.72e	4.12±1.08i X 6.02±0.51e
T3	28.12±4.91a X 7.19±2.03b	present	absent	59.74±0.26h X 47.25±1.09k	20.42±0.37i	9.05±0.33a	4.12±0.22a	115.32±0,29d	18.26±4.97b X 4.35±0.24fg
T4	24.27±2.75b X 6.17±1.98bc	absent	absent	61.47±2.15h X 65.79±1.35g	29.27±0.98h	7.16±0.28ab	3.15±0.11c	103.95±4.17f	69.77±10.98a X 6.14±0.22de
T5	6.18±0.39e X 4.27±1.45bc	present	absent	58.45±2.21h X 51.12±1.23j	31.04±1.17h	3.11±1.74de	3.01±0,09c	98.14±0.21g	6.78±0.25h X 6.54±0.65de
T6	5.17±0.22ef X 7.11±0.19b	present	absent	62.17±1.01h X 82.24±1.18c	45.16±0.54d	4.17±0.24e	2.22±0.36d	79.16±1.23hi	4.98±0.33i X 7.11±0.31d
T7	5.42±0.16e X 5.21±0.11bc	present	absent	50.12±1.07k X 46.22±0.89k	42.07±0.56e	5.63±0.21d	3.07±0.15c	105.18±0.21f	9.44±0.12e X 6.25±0.16de
T8	10.22±2.01de X 5.08±0.25bc	absent	present	95.91±2.16a X 68.24±0.98f	20.16±0.15i	4.01±0.12e	1.15±0.04f	108.11±0.34e	6.35±0.29i X 2.56±0.14h
T9	7.72±2.03e X 3.28±1.35c	present	present	91.14±2.54b X 70.46±1.14e	36.15±0.44g	5.18±0.10d	1.19±0.06ef	107.08±0.97ef	5.04±0.22i X 3.03±0.18h
T10	8.91±0.26e X 3.25±0.12c	present	absent	87.14±0.33c X 56.59±0.89h	60.18±0.35a	9.21±2.09a	2.34±0.11d	101.12±3.79fg	8.72±0.07f X 3.21±0.08h
T11	3.21±0.21g X 5.16±0.17bc	present	absent	54.45±0.14i X 45.18±0.27l	39.47±2.15ef	4.18±0.14e	2.02±0.08d	137.27±4.13b	3.49±0.16i X 5.64±0.22e
T12	5.19±0.27f X 3.25±0.16c	present	absent	51.17±1.17k X 81.14±0.21cd	46.28±0.44d	6.17±0.46bc	1.39±0.18e	126.39±2.12c	10.69±0.27d X 6.75±0.17de
T13	4.71±0.26f X 7.49±0.22b	absent	absent	69.27±0.59ef X 53.81±1.34i	43.19±1.18de	5.21±0.11d	1.27±0.07e	99.18±1.17fg	4.27±0.12i X 6.51±0.14de
T14	6.28±0.21e X 4.57±0.16c	present	absent	81.57±0.28d X 50.24±0.11j	41.19±0.27f	5.67±0.22d	2.18±0.04d	110.25±0.22e	6.52±0.25i X 4.86±0.36f
T15	10.65±0.44de X 6.79±0.52b	present	absent	70.56±0.87e X 88.95±1.96b	48.56±1.85bc	7.19±0.26ab	2.38±0.06d	102.91±0.98f	21.16±0.08b X 16.35±2.32b
T16	7.12±.23e X 11.47±0.33a	present	absent	68.79±1.45ef X 102.41±1.22a	40.11±0.41f	4.56±0.21e	3.77±0.11b	65.22±2.18i	8.23±0.41g X 23.23±0.94a
T17	12.11±0.89d X 6.22±0.41b	present	absent	90.53±2.11b X 51.48±0.99j	45.29±1.33d	5.11±0.33de	2.56±0.29cd	152.31±3.32a	6.65±0.33hi X 5.14±0.17f
T18	20.15±0.77c X 7.14±0.30b	absent	present	61.82±1.33h X 82.46±0.85c	52.35±2.77b	8.27±0.92a	3.02±0.35c	80.54±1.08h	12.05±0.19c X 8.75±0.33c
T19	10.63±0.19de X 5.27±0.27bc	present	present	53.13±0.39j X 46.29±0.45kl	35.84±0.95g	6.28±0.19b	2.11±0.12d	97.35±1.54g	8.19±0.21g X 6.28±0.22de

Table 3. Characteristics of leaf surface patterns of the examined taxa. Outcomes represent mean values ± standard deviation; means with different letters are significant at p = 0.05 level (Duncan's multiple-range test), for taxa abbreviations see Table 1 (L: Length, W: Width).

Taxa	Adaxial epidermis						Abaxial epidermis					
	Anticlinal cell wall	Shape of epidermal cells	Stomata				Anticlinal cell wall	Shape of epidermal cells	Stomata			
			L (µm)	W (µm)	Number per mm^2	Index			L (µm)	W (µm)	Number per mm^2	Index
	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
T1	Undulated	Irregular	30.76±1.21g	21.22±0.76j	81±2c	33.19±0.15ef	Undulated	Irregular	27.29±0.89i	20.96±0.44h	36±4f	31.45±0.18de
T2	Sinuate	Irregular	30.22±0.37g	19.27±0.22lm	36±2g	28.68±0.27h	Sinuate	Irregular	25.05±0.33jk	19.16±0.31j	27±2g	22.63±0.09i
T3	Undulated	Irregular	22.14±0.23k	17.42±0.33n	36±2g	12.37±0.11p	Sinuate	Irregular	21.23±0.26l	16.98±0.22k	27±4g	10.89±0.32p
T4	Undulated	Polygonal	26.51±0.17j	20.05±0.12jk	45±2f	34.62±0.15d	Undulated	Polygonal	32.17±0.28e	21.12±0.11h	36±2f	21.96±0.25j
T5	Undulated	Irregular	30.89±0.25g	16.18±0.31o	99±2b	63.01±0.08a	Undulated	Irregular	29.17±0.22g	18.02±0.15k	90±2a	51.55±0.20a
T6	Undulated	Polygonal	31.15±0.22g	24.28±0.35n	135±5a	48.76±0.24b	Straight	Rectangular	27.62±0.37i	20.19±0.23i	36±4f	27.16±0.12fg
T7	Undulated	Irregular	34.53±0.44f	22.62±0.23i	45±2f	33.33±0.15ef	Straight or Undulated	Irregular	26.44±0.18j	16.48±0.29kl	18±2h	10.18±0.28r
T8	Undulated	Irregular	38.56±0.37d	24.51±0.26h	72±4cd	44.15±0.33g	Undulated	Irregular	31.28±0.51ef	19.27±0.41j	45±4e	20.11±0.15l
T9	Undulated	Irregular	36.41±0.20e	22.78±0.39i	82±4c	32.05±0.13e	Straight	Polygonal	26.11±0.79ij	21.03±0.38h	65±3c	21.21±0.18k
T10	Undulated	Polygonal	28.16±0.39hi	25.67±0.20g	74±5cd	33.87±0.41e	Undulated	Polygonal	30.14±0.25g	23.09±0.30f	40±4ef	25.29±0.10h
T11	Straight	Polygonal	29.75±0.16g	19.49±0.14l	40±4efg	18.41±0.30o	Straight or Undulated	Polygonal	26.38±0.11i	19.27±0.08j	10±2i	12.58±0.27o
T12	Undulated	Irregular	28.54±0.33h	21.16±0.25j	80±6c	20.84±0.27mn	Sinuate	Irregular	25.17±0.14j	21.04±0.47h	70±4bc	32.29±0.41d
T13	Undulated	Irregular	25.89±0.11jk	21.41±0.17j	51±3e	18.76±0.19no	Straight or Undulated	Polygonal	27.09±0.23i	29.45±0.51c	75±5b	27.61±0.24f
T14	Undulated	Irregular	40.16±0.25c	28.14±0.26d	74±2cd	27.46±0.21i	Straight	Polygonal	38.27±0.11d	25.38±0.34d	76±2b	30.55±0.12e
T15	Sinuate	Irregular	38.41±0.35d	27.36±0.23e	51±4e	23.19±0.16l	Sinuate	Irregular	24.75±0.21k	20.77±0.18h	45±4e	19.80±0.14m
T16	Undulated	Irregular	41.07±0.14bc	26.61±0.35f	100±4b	38.59±0.33c	Undulated	Irregular	38.99±0.14c	25.23±0.39de	36±4ef	33.33±0.19c
T17	Undulated	Irregular	42.21±0.29b	33.15±0.26b	47±2ef	20.98±0.22m	Sinuate	Irregular	45.40±0.30b	42.01±0.11a	40±2ef	21.08±0.27kl
T18	Undulated	Irregular	45.10±0.11a	39.08±0.22a	70±4cd	24.51±0.08k	Undulated	Irregular	46.34±0.33a	41.24±0.35b	24±2g	15.52±0.35n
T19	Undulated	Irregular	31.79±0.18g	31.17±0.15c	72±2cd	25.49±0.15j	Undulated	Irregular	28.47±0.11h	22.62±0.27fg	60±4cd	40.11±0.46b

Table 4. Anatomical characteristics of stems of the examined taxa. Outcomes represent mean values ± standard deviation; means with different letters are significant at p = 0.05 level (Duncan's multiple-range test), for taxa abbreviations see Table 1 (L: Length, W: Width).

Taxa	Epidermis cells		Cortex Thickness (µm) (28)	Endodermis cells		Vascular bundles		Interfascicular region (35)	Scleranchymatic region thickness (36)
	Structure (25)	L(26) X W(27) (µm)		Structure (29)	L (30) X W (31) (µm)	Number (32)	Sizes L(33) X W(34)		
T1	flat cells	14.16±0.59b X 9.81±0.12b	59.45±0.97e	large flat cells	12.14±1.45h X 5.66±0.19fg	10-12b	104.13±0.78f X 41.22±0.44m	clearly	102.45±1.56a
T2	rectangular cells	9.87±0.22d X 7.65±0.18d	74.59±0.81c	flat cells	9.88±0.55i X 4.99±1.11fg	9-10bc	95.18±2.07g X 48.46±1.22l	clearly	60.48±1.97e
T3	flat cells	10.83±0.22d X5.96±0.21g	14.46±0.16l	large flat cells	15.12±0.21f X 8.26±0.14c	6-8d	107.19±2.23e X 60.85±1.54k	unclearly	43.41±1.05i
T4	square-shaped cells	5.02±0.21k X 4.84±0.19ij	48.74±2.10fg	large flat cells	12.87±0.12h X 6.12±0.23f	14-16a	110.54±0.88d X 68.96±2.05j	unclearly	48.91±0.88h
T5	thick-walled flat cells	8.77±0.22f X 4.09±0.37k	39.10±1.14h	1 or 2 seriate large flat cells	24.05±1.22b X 8.74±0.44bc	5-6de	102.85±0.77fg X 83.24±1.33h	clearly	31.14±0.63l
T6	flat cells	5.48±0.21j X 3.91±0.12k	52.46±1.08f	large flat cells	12.05±0.19h X 7.43±0.10d	7-9cd	106.11±1.54ef X 102.85±0.77de	unclearly	82.41±3.46cd
T7	thick-walled flat cells	10.63±0.12d X 6.32±0.16f	25.61±0.49k	flat cells	28.75±0.86a X 6.99±0.21e	5-6de	108.77±1.21de X 112.86±0.86c	clearly	34.78±2.46k
T8	thick-walled flat cells	7.55±0.86g X 5.31±0.12h	105.46±0.27b	large flat cells	14.73±0.92f X 6.02±0.12f	8-9cd	70.53±1.32k X 105.09±1.27d	unclearly	48.97±2.13h
T9	thick-walled square-shaped cells	6.96±0.12hi X 6.59±0.24ef	38.76±0.95hi	large flat cells	18.65±0.26e X 7.83±0.15c	12-14ab	77.85±0.92j X 40.62±0.77m	clearly	-
T10	flat cells	8.07±0.22g X 4.88±0.08ij	44.61±0.74g	flat cells	9.41±0.17i X 3.84±0.34g	10-12b	81.50±3.17ij X 46.41±2.91l	unclearly	54.64±0.89g
T11	square-shaped cells	9.21±0.22de X 8.94±0.11c	116.85±2.38a	flat cells	14.15±0.22fg X 12.87±0.25a	5-6de	124.41±1.47b X 156.06±2.85a	unclearly	83.55±1.14c
T12	flat cells	10.23±0.84d X 7.49±0.13d	37.45±0.51i	flat cells	14.66±0.31fg X 6.54±0.21ef	10-12b	84.66±0.77i X 59.52±0.65k	clearly	46.11±0.39hi
T13	flat cells	19.16±0.36a X 6.78±0.21ef	42.75±0.36h	large flat cells	25.16±0.24b X 7.42±0.21d	6-8d	104.11±2.51fg X 124.04±1.08b	clearly	39.41±0.65j
T14	thick-walled flat cells	12.16±0.17c X 6.88±0.27e	49.56±0.21f	large flat cells	15.08±0.21f X 8.27±0.15c	7-9cd	123.81±1.24bc X 91.73±0.85g	unclearly	97.44±0.86b
T15	flat cells	9.54±0.12d X 6.61±0.08f	47.81±0.39f	flat cells	10.85±0.33h X 7.21±0.24de	10-12b	95.12±0.89g X 74.18±0.65i	clearly	-
T16	elongated rectangular cells	7.15±0.25h X 15.36±0.16a	29.96±0.37j	large flat cells	18.49±0.22e X 9.17±0.21b	10-12b	130.84±0.86a X 102.39±0.77de	unclearly	-
T17	rectangular cells	8.47±0.12fg X 5.09±0.10hi	71.25±2.52d	large flat cells	20.35±0.12c X 7.84±0.29c	9-10bc	120.54±2.18c X 95.17±0.72f	unclearly	56.22±0.29f
T18	rectangular cells	8.14±0.15g X 5.86±0.12g	58.91±1.46e	large flat cells	20.07±0.32cd X 8.19±0.39c	7-9cd	106.45±0.59e X 102.16±0.46de	clearly	67.18±0.37d
T19	thick-walled flat cells	11.02±0.12d X 6.91±0.15e	51.04±2.32f	flat cells	8.07±0.12j X 5.47±0.12fg	10-14ab	88.43±0.18h X 46.63±0.32l	clearly	-

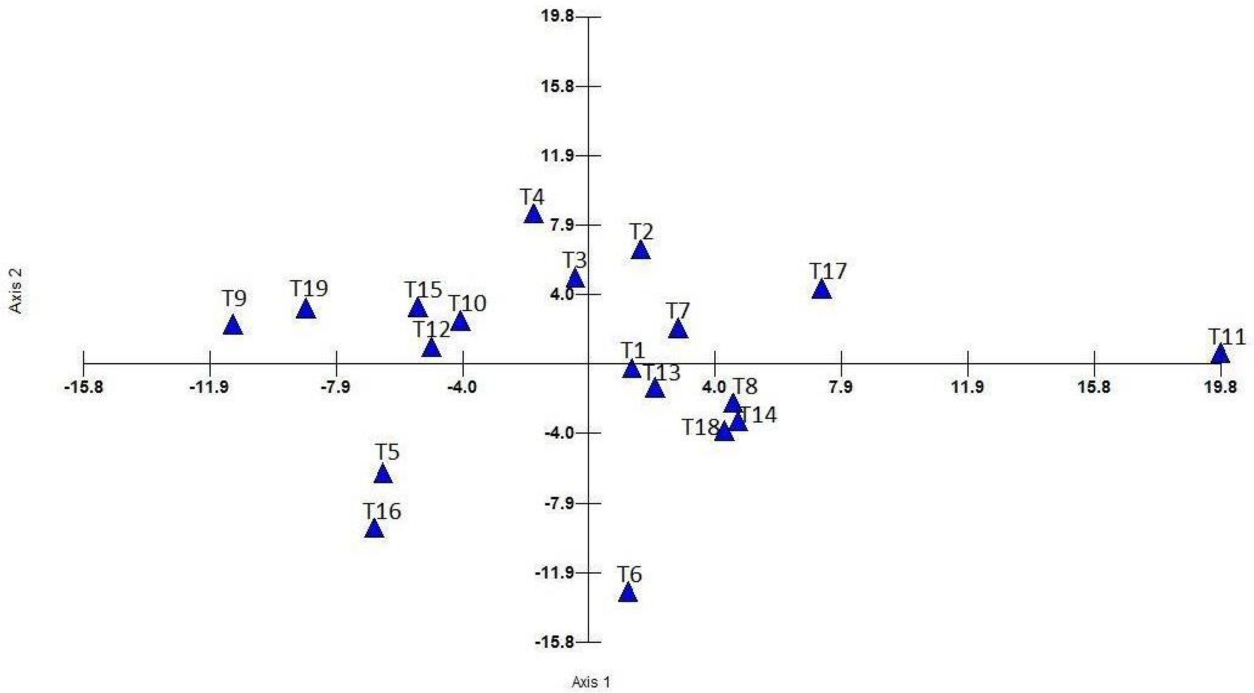

Fig. 6. Principal component analysis of the examined taxa (See Table 1 for taxa abbreviations).

Discussion

Thlaspi s.l. is represented by 36 taxa, including species and subspecies, found in six sections in the Flora of Turkey and the East Aegean Islands (Flora of Turkey) (Hedge 1965, Davis 1988, Yıldırımlı 2001). The present work assesses the significance of anatomical characteristics and evaluates the correlations among leaf and stem anatomy. The anatomical structures of the leaves and stems of the examined *Thlaspi* taxa were investigated in detail for the first time in this study.

Generally, the examined taxa have a unifacial mesophyll, rarely bifacial and made up of 2-5 layers of palisade parenchyma and 3-6 layers of abaxial spongy parenchyma, or equifacial. Metcalfe & Chalk (1957) formerly found that bifacial mesophyll is most common in the Brassicaceae family, but unifacial, bifacial and equifacial types were commonly observed in the present study. These types of leaf mesophyll, except unifacial, are widespread in correlated genera, such as bifacial in *Alyssum* L. (Orcan & Binzet 2002), bifacial and equifacial in *Ricotia* L. (Selvi & Paksoy 2013) and bifacial in *Aubrieta* Adans (Karaismailoğlu 2016).

All but three (*T. huetii*, *T. densiflorum* and *T. praecox* subsp. *praecox*) of the examined taxa have undulated anticlinal cell walls (Fig. 1). These three taxa (straight or sinuate) grow at low altitudes and humid zones, while the 13 taxa with undulated anticlinal cell walls are found in higher altitudes and arid habitats. According to Stace (1984), epidermal cells with undulated or straight outlines are widespread in xeromorphic and mesomorphic plants. Another study reported that most epidermal cells of leaves in dicotyledons have sinuate anticlinal cell walls, which may be caused by pressure exerted on the walls during cell development (Orcan & Binzet 2002).

The studied *Thlaspi* taxa grow at various altitudes between 270 and 2750 m and in different ecological conditions. While the mesophyll layer in the taxa growing at low altitudes is quite loose, it is denser in appearance of the parenchyma cells at high altitudes. This shows that high altitude and inadequate water can trigger further development of the mesophyll parenchyma, and consequently enhance photosynthetic activity (Fahn 1990, Ozcan *et al.* 2015).

All the examined taxa are of the amphistomatic type. Stomatal density differs significantly between the abaxial and adaxial surfaces of leaves (Table 3). The density is clearly higher on adaxial surfaces than abaxial surfaces in most of the examined taxa. The exceptions are T12-T14, T17 and T18 taxa, which exhibited the opposite. This result is compatible with several previous studies, including Orcan & Binzet (2002), Arambarri *et al.* (2006), Ozcan *et al.* (2015) and Karaismailoğlu (2016). Stomata are mostly anisocytic (Cruciferae type) or rarely anomocytic (Ranunculaceae type) in the examined taxa. These types of stomata were formerly found in the family Brassicaceae by Metcalfe & Chalk (1957), Cansaran *et al.* (2007), Selvi & Paksoy (2013) and Karaismailoğlu (2016, 2019).

The stems of herbaceous *Thlaspi* taxa generally produce no secondary tissues. Cortex is composed of flat or circular parenchyma in 3-12 layers. A thick bundle sheath covers the adaxial side of the phloem and xylem.

All vascular bundles were of the collateral type. The inner part of the pith breaks down in the late or early phases of primary growth, like in some other Brassicaceae family members (Metcalfe & Chalk 1957).

Yentür (2003) showed that the arrangement of bundles provides useful information in comparative anatomical investigations. The number of vascular bundles in the stem is usually between 5 and 16 and the bundles are arranged in a single ring. Selvi & Paksoy (2013) and Karaismailoğlu (2016) reported that vascular bundles are distributed in a circular manner in one ring in stems of some Brassicaceae species. Clustered scleranchymatic cells were positioned on the adaxial and abaxial sides of the vascular bundles in most of the examined taxa (Fig. 4), except for T9, T15, T16 and T19 (Fig. 4 and Table 4). The presence or absence of secretory channels is quite significant in comparative anatomical studies (Makbul *et al.* 2011). Also, the content, distribution and presence or absence of secretory channels were shown to differ among the plant taxa (Milan *et al.* 2006). This work found that there are a few secretory networks in the stem cortex near the vascular bundles of the examined taxa.

A dendrogram was formed to assess the anatomical characteristics of the leaves and stems of the examined *Thlaspi* taxa using UPGMA cluster analysis. The dendrogram indicating two major clusters was partially compatible with Hedge (1965), Davis (1988) and Yıldırımlı (2001), where 36 taxa were placed into six sections. The anatomical differences between the leaves and stems were observed at the species level, but showed no correlation at section level. It seems that anatomical features are not in full agreement with the available classification, nevertheless it proves to be valuable data. That is, the anatomical characters of leaves and stems partially supported the features utilized in the distribution of *Thlaspi* species in The Flora of Turkey.

Principal component analysis can provide data about the variability of the used anatomical characters. The high cumulative variance values of principal components indicate that the characters investigated can be used to elucidate variances among the studied *Thlaspi* taxa. Dissimilarity ratios among the taxa were defined. Accordingly, the closest relationship was seen between T9 and T19, while the most distant relationship was found between T9 and T11.

Conclusion

This study questions the usefulness of the studied characteristics in the infrageneric delimitation in *Thlaspi*. The compared anatomical characteristics among the examined *Thlaspi* taxa are partially in accordance with their sectional delimitation given in The Flora of Turkey. However, the findings showed that new arrangements may be necessary for the systematic positions of some taxa. The leaf and stem anatomical characteristics are helpful for distinctions based on morphology. This study is a preliminary study to determine the usefulness of the examined anatomical characters. Further investigations that include all the taxa of the genus are required to define all variations and obtain a better systematic understanding of the genus.

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