Seed structure and its taxonomic implications for genus *Thlaspi* sensu lato sections *Nomisma*, *Thlaspi*, and *Pterotropis* (Brassicaceae)

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Abstract: We studied morphological and anatomical characteristics of seeds of 22 taxa in the sections *Nomisma*, *Thlaspi*, and *Pterotropis* of *Thlaspi* sensu lato from Turkey and the significance of these characters in a taxonomical context. The results show that the taxa differ from each other in terms of seed shape and color. The seed size ranges from 1.25 mm to 2.99 mm in length and from 0.66 mm to 2.16 mm in width, *Thlaspi rosulare* and *T. tatianae* having the largest and *T. annuum* having the smallest seeds. The seed surface ornamentation is classified into nine types: reticulate-areolate, scalariform, reticulate, reticulate-foveate, ruminate, ocellate, tuberculate, areolate, and colliculate. The most common type is reticulate-areolate, whereas areolate, colliculate, ocellate, and tuberculate ornamentation types were found to be taxon-specific. The testa is typically made up of four layers: the epidermis, the subepidermis, the sclerotic (or palisade) layer, and the parenchyma layer. The sclerotic (or palisade) layer may not be seen in some of the species. The structures and thicknesses of the epidermis, the subepidermis, the sclerotic (or palisade) layer, and the parenchyma layer are highly significant characters that reveal interspecific relationships within the examined taxa. The results are also compared with Meyer's findings and classification of 1973, 1979, 1991, and 2001, and taxon transfers are questioned. In addition, a key is provided for the identification of the examined taxa based on seed characteristics.

Key words: *Thlaspi*, sensu lato, Seed, scanning electron microscopy, UPGMA, Turkey, Cruciferae

1. Introduction

Thlaspi L. sensu lato (s.l.) is one of the largest genera of the family Brassicaceae. It is represented by 75 taxa worldwide (Appeal and Al-Shehbaz, 2003), and by 36 taxa belonging to 6 sections including *Nomisma* DC., *Thlaspi* L., *Pterotropis* DC., *Aptergium* Ledeb., *Carpoceras* DC., and *Syrenopsis* Hedge in Turkey (Hedge, 1965; Davis et al., 1988; Yıldırımlı, 2001).

The first comprehensive study on the sections *Nomisma*, *Thlaspi*, and *Pterotropis* of the genus *Thlaspi* was done by Hedge (1965). He identified 16 taxa, one of which was a questionable record (*T. tatianae*) for the flora of Turkey. With further floristic investigations, *T. leblebicii*, *T. praecox* subsp. *praecox*, *T. cariense*, *T. syriacum*, *T. aghricum*, and *T. watsonii* taxa were added to the flora of Turkey. The three sections we chose for this study are represented by 22 taxa (Davis et al., 1988; Yıldırımlı, 2001) in Turkey.

The classification of *Thlaspi* s.l. is still problematic. Based mostly on seed-coat anatomy, Meyer (1973, 1979) divided the genus into 12 genera (*Thlaspi* F.K.Mey. sensu stricto (s.s.) (with only 6 taxa in it), *Callothlaspi* F.K.Mey., *Kotschyella* F.K.Mey., *Neurotropis* (DC.) F.K.Mey.,

Microthlaspi F.K.Mey., *Noccaea* Moench, *Thlaspiceras* F.K.Mey., *Syrenopsis* Jaub. & Spach, *Atropatenia* F.K.Mey., *Vania* F.K.Mey., *Noccidium* F.K.Mey., *Masmenia* F.K.Mey.).

Meyer's classification was not accepted by many researchers because of its limiting taxonomical method (Greuter and Burdet, 1983; Appeal and Al-Shehbaz, 2003). Molecular phylogenetic studies such as those of Mummenhoff and Zunk (1991), Mummenhoff and Koch (1994), Mummenhoff et al. (1997a, 1997b), Koch et al. (1998), Koch and Mummenhoff (2001), Al-Shehbaz et al. (2006), and Koch et al. (2007) showed that the generic classification of *Thlaspi* in Meyer's work (1973) was not agreeable. As a result of family-wide molecular phylogenetic study (Warwick et al., 2010), the ten genera separated from *Thlaspi* s.l. by Meyer (1973) were placed in *Noccaea*. They were designated as synonyms of *Noccaea* (Al-Shehbaz, 2014). Despite numerous studies on the infrageneric and interspecific taxonomy of the genus, classification problems have not been resolved yet.

One of the biggest problems in the taxonomy of the genus is that most of the molecular phylogenetic studies rely on herbarium materials that usually contain

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incomplete samples or a single sample of a species, because they do not involve sufficient fieldwork. However, the presence of samples including flower, mature fruit, and seed for an accurate diagnosis of individuals belonging to the genus is fundamental. In addition to that, studies conducted so far contain few samples from Turkey, one of the major centers of species diversity of the genus. The presence of supposedly endemic taxa is also not certain because the ripe fruits or inflorescence structures of the species in Turkey are unknown. For these reasons, the genus *Thlaspi* sensu lato was examined in this study.

It is not possible to explain the evolutionary correlations among most of the taxa within the family Brassicaceae by merely examining morphological characteristics due to widespread convergence (Franzke et al., 2011). Therefore, it is necessary to study other characters of the taxa as well, to solve the taxonomic problems concerning closely related taxa.

The seed coat structure is considered as a significant character in systematics studies of the family. It is mostly used to solve classification problems concerning closely related taxa, establish their evolutionary relationships, and explain their adaptive significance (Kaya et al., 2011). In addition to that, many researchers agree on the taxonomic significance of macro and micro features of the seeds in discriminating the taxa within the family Brassicaceae (Khalik and Maesen, 2002; Tantawy et al., 2004; Kaya et al., 2011; Bona, 2013; Karaismailoğlu, 2016). Studying the anatomical structures of the seed coat may also provide a solution to the taxonomical problems concerning Brassicaceae. This approach was used in many studies conducted with various genera within the family (Vaughan et al., 1976; Moazzeni et al., 2007; Ghaempanah et al., 2013).

However, there are very few studies performed on the seed morphology and anatomy of the genus *Thlaspi* to date (Meyer, 1973, 1979, 1991; Fırat et al., 2014). The aim of this study is to analyze morphological and anatomical characteristics of seeds of 22 taxa within sections *Nomisma*, *Thlaspi*, and *Pterotropis* of the genus *Thlaspi* s.l. in Turkey, and to discuss taxonomic uses of these characteristics.

2. Materials and methods

The plant specimens used in this study were collected from various parts of Turkey during the fruiting period. In order to study the patterns of variation, specimens were collected from as many various parts of the existing distribution areas of the sections as possible (Table 1). The specimens were deposited at ISTF.

Macromorphological characters of the seeds including color, shape, size, and surface structures were examined with 100 seeds using an Olympus SZX7 stereomicroscope and Kameram Imaging Software (Figure 1; Table 2). For

micromorphological observations of the seeds including the surface ornamentation, anticlinal and periclinal cell walls, and the structure of epidermal cell, the specimens examined with a JEOL Neoscope-5000 scanning electron microscope (Figure 2; Table 3). For this procedure, they were mounted on the stub with silver epoxy and covered with platinum and gold.

Investigations of anatomical characters were performed on specimens preserved in 70% alcohol. Cross-sections were obtained through the middle of the seed with a fully automatic microtome (Thermo Shonda Met Finesse). Then they were passed through various series of alcohol and xylene and stained with hematoxylin and eosin Y in a staining device (ASC 720 Medite) and mounted in Entellan (Figure 3; Table 4) (Karaismailoğlu, 2015). Anatomical characters were observed using an Olympus CX21FS1 microscope and Kameram Imaging Software.

The terminology of morphological and anatomical characteristics was carried out in accordance with Stearn (1985), Meyer (1973, 1979, 1991), and Ghaempanah et al. (2013).

The data obtained from the studied parameters were assessed with SPSS. Duncan's multiple-range test was used to determine the statistical significance of variances among the values obtained for different taxa (SPSS Inc., 2006). Grouping of taxa was carried out utilizing the clustering analysis method (UPGMA) in accordance with 18 characters in Tables 2–4 (Figure 4). In addition, the similarity matrix of the examined taxa was formed in MVSP (Kovach, 2007) (Table 5).

3. Results

The seed characters of the examined taxa including color, shape, size, and surface structure are evaluated morphologically. Seed colors of the studied taxa are found to be light brown, dark brown, and dark brown-black. The most common color is light brown, seen in 13 of the examined taxa. We observed seeds of four shapes: ovatus, ovatus-late, ellipticus, and ellipticus-late. Ovatus and ellipticus are more frequent than the others, and the ovatus-late type is characteristic for *T. cataonicum*. The seed size ranges from 1.25 mm to 2.99 mm in length and from 0.66 mm to 2.16 mm in width. *T. rosulare*, *T. tatianae*, and *T. annuum* are notably different from the rest of the examined taxa in terms of seed size. Seed surface structures vary among the taxa: striped, smooth, reticulate, and coarse protrusions (Table 2; Figure 1).

The surface ornamentation, anticlinal and periclinal cell walls, and epidermal cell structures of the seeds are studied micromorphologically. The seed surface ornamentation is classified into nine types: reticulate-areolate, scalariform, reticulate, reticulate-foveate, ruminate, ocellate, tuberculate, areolate, and colliculate

Table 1. The studied taxa and their locations.

Section	Taxa	Location	ISTF
Nomisma DC.	*Thlaspi arvense* L. (TH1)	Rize, İkizdere, Cimil, Güvenköy, open areas, meadows, 1850 m, 23.06.2014, Karaismailoğlu 54	41165
	T. huetii Boiss. (TH2)	Trabzon, Of, Halman village, around the hazelnut fields, 220 m, 07.05.2015, Karaismailoğlu 148	41166
Thlaspi L.	*T. orbiculatum* Stev. (TH3)	Artvin, Ardanuç, Peynirli village tops, steep slopes, 1663 m, 15.06.2015, Karaismailoğlu 201	41167
	T. kotschyanum Boiss. & Hohen. (TH4)	Kahramanmaraş, Göksun, Berit mountain, subalpine regions, humid fields, 2116 m, 19.06.2015, Karaismailoğlu 202	41168
	T. perfoliatum L. (TH5)	Gümüşhane, Kürtün, Karagöl highland road, around the hazelnut fields, 802 m, 07.03.2015, Karaismailoğlu 103	41169
	*T. microstylu*m Boiss. (TH6)*	Osmaniye, Zorkun road, Ulubaş-Fenk, 1216 m, 21.04.2016, Karaismailoğlu 221	41170
	T. annuum Koch (TH7)	Amasya, Taşova, Boraboy, village-lake, roadside rocky slopes, 881 m, 02.05.2015, Karaismailoğlu 143	41171
	T. bulbosum Spruner ex Boiss. (TH8)	Kahramanmaraş, Andırın, Meryemçil plateau, meadows, 1550 m, 20.06.2015, Karaismailoğlu 208	41172
	T. leblebicii Gemici & Görk (TH9)*	Muğla, Köyceğiz, Sandras mountain, Ağla village, roadside rocky slopes, 1262 m, 05.06.2015, Karaismailoğlu 192	41173
Pterotropis DC.	*T. ochroleucum* Boiss. (TH10)	Hatay, Dörtyol, Topaktaş-Karamezra plateau, Fagus forest, stony slopes, 1752 m, 24.04.2016, Karaismailoğlu 240	41174
	T. praecox Wulfen subsp. *praecox* (TH11)	Kırklareli, Dereköy road, roadside stony slopes, 520 m, 09.06.2015, Karaismailoğlu 197	41175
	T. cariense A.Carlström (TH12)*	Muğla, Marmaris, Kırzeytin mountain, serpentine, stony, 494 m, 03.04.2015, Karaismailoğlu 121	41176
	T. violascens Boiss. (TH13)*	Osmaniye, Düziçi, Dumanlı mountain, forest, 1259 m, 26.05.2015, Karaismailoğlu 181	41177
	T. densiflorum Boiss. & Kotschy (TH14)*	Kahramanmaraş, Ahır mountain, Ulucak hill, stony slopes, 1751 m, 20.06.2015, Karaismailoğlu 205	41178
	T. tatianae Bordz. (TH15)	Van, Güzeldere-Başkale, Güzeldere passage, wetland and swamp fields, 2651 m, 30.05.2015, Karaismailoğlu 187	41179
	T. cataonicum Reuter (TH16)	Adana, Saimbeyli, Obrukbaşı plateau, roadsides, open areas, meadows, 1472 m, 18.04.2015, Karaismailoğlu 124	41180
	T. syriacum Bornm. (TH17)*	Osmaniye, Hasanbeyli, stony slopes, 1215 m, 23.04.2016, Karaismailoğlu 222	41181
	T. elegans Boiss. (TH18)*	Osmaniye, Zorkun plateau, roadsides, open areas, 1104 m, 26.05.2015, Karaismailoğlu 178	41182
	T. rosulare Boiss. & Bal. (TH19)*	Niğde, Çamardı, Yelatan village tops, stony slopes, 2085 m, 25.05.2015, Karaismailoğlu 173	41183
	T. lilacinum Boiss. & Huet. (TH20)*	Bolu, Abant, slopes in the south of the lake, 1680 m, 21.05.2016, Karaismailoğlu 245	41184
	T. aghricum P.H.Davis & Kit Tan (TH21)*	Ağrı, Hamur-Tutak, steep slopes, meadows areas, 1605 m, 16.05.2015, Karaismailoğlu 162	41185
	T. watsonii P.H.Davis (TH22)*	Van, Hakkâri road, Güzeldere passage, hill and stony slopes, 2752 m, 02.07.2015, Karaismailoğlu 210	41186

* = Endemic taxon.
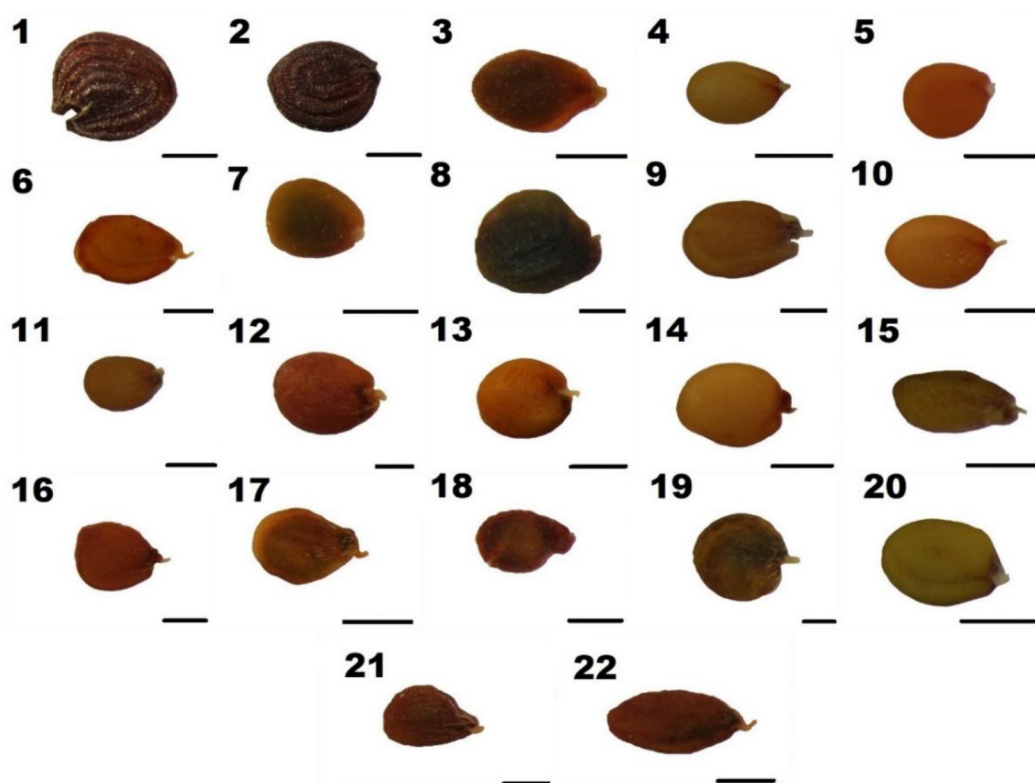
Figure 1. Seeds of the examined taxa: 1- *T. arvense*, 2- *T. huetii*, 3- *T. orbiculatum*, 4- *T. kotschyanum*, 5- *T. perfoliatum*, 6- *T. microstylum*, 7- *T. annuum*, 8- *T. bulbosum*, 9- *T. leblebicii*, 10- *T. ochroleucum*, 11- *T. praecox* subsp. *praecox*, 12- *T. cariense*, 13- *T. violascens*, 14- *T. densiflorum*, 15- *T. tatianae*, 16- *T. cataonicum*, 17- *T. syriacum*, 18- *T. elegans*, 19- *T. rosulare*, 20- *T. lilacinum*, 21- *T. aghricum*, 22- *T. watsonii* (scale bars = 1 mm).

(Table 3; Figure 2). The most common type is reticulate-areolate, observed in *T. microstylum*, *T. leblebicii*, *T. praecox* subsp. *praecox*, *T. cariense*, *T. elegans*, and *T. aghricum*. The second most common type is scalariform, seen in *T. annuum*, *T. perfoliatum*, *T. cataonicum*, and *T. syriacum*. The areolate (in *T. lilacinum*), colliculate (in *T. bulbosum*), ocellate (in *T. kotschyanum*), and tuberculate (in *T. orbiculatum*) ornamentation types are each represented by only one taxon.

The types of anticlinal cell walls in the examined taxa are sunken, raised, or indefinite. While sunken cell wall is a common feature among the reticulate, scalariform, and areolate ornamentation types, reticulate-foveate ornamentation types have an architecture consisting of interspaces surrounded by raised walls. There is no specific pattern observed in the anticlinal cell wall of the ruminate type. Periclinal cell walls are convex (in 11 taxa), concave (in 9 taxa), flat (in 2 taxa), or indefinite (in 2 taxa) in shape (Table 3). There is no correlation between the type of surface ornamentation and periclinal cell wall, except for the ruminate type whose cell walls do not have a definite shape. In addition to these, shapes of cells on the seed surfaces are very diverse: pentagonal-hexagonal, rectangular, circular, oval, crushed, polygonal cells, and indefinite. The most frequent cell type is polygonal, whereas circular and pentagonal-hexagonal are quite rare types (Table 3).

The results of the anatomical observations of the seeds are shown in Table 4 and Figure 3. Most of the seeds of the examined taxa consist of four layers, including the epidermis, the subepidermis, the sclerotic or palisade layer, and the endosperm. The epidermis layer is of either parenchymatic or sclerenchymatic type and consists of two layers including the epidermis and subepidermis.

In our observations from the cross-sections, the epidermis varies significantly in cell form (flat, rectangular, cubic, elongated rectangular, or polygonal), number of layers (1 or 2), wall structure (undulated or straight and thick or thin-walled), and cell type (regular or irregular cells) (Figure 3). The two most frequent forms are flat and rectangular, whereas the rarest one is the polygonal type (Table 4). The subepidermis comprises 1–8 layers of elongated rectangular, polygonal, flat, and cubic cells. The average thickness of the epidermis layers ranges from 41.07 µm (in *T. bulbosum*) to 115.21 µm (in *T. microstylum*). The sclerotic or palisade layer, which is generally a compressed

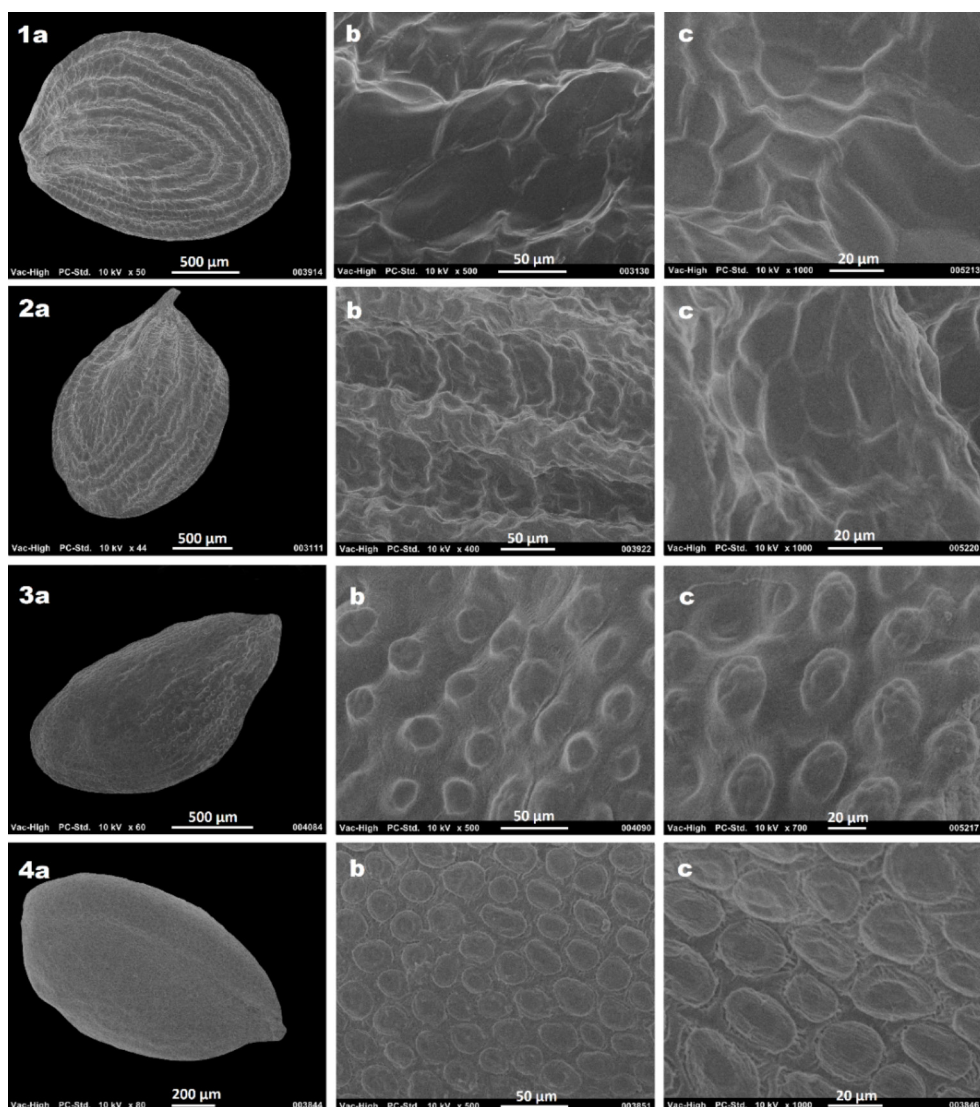**Table 2.** Macromorphological features of the seeds of the examined taxa.

Taxa	Examined seed number	Color (1)	Shape (2)	Seed surface (3)	Seed dimensions		L/W (6)
					Length (L) (mm) (4)	Width (W) (mm) (5)	
Thlaspi arvense	100	Dark brown-black	Ovatus	Striped	2.40 ± 0.10b	1.85 ± 0.15b	1.29
T. huetii	100	Dark brown-black	Ellipticus-late	Striped	1.72 ± 0.18de	1.55 ± 0.15bc	1.10
T. orbiculatum	100	Dark brown	Ellipticus	Smooth or slightly reticulate	1.61 ± 0.11de	1.15 ± 0.15d	1.40
T. kotschyanum	100	Light brown	Ovatus	Slightly reticulate	1.07 ± 0.13gh	0.72 ± 0.08ef	1.48
T. perfoliatum	100	Light brown	Ellipticus-late	Striped or smooth	1.04 ± 0.16gh	0.91 ± 0.12de	1.14
T. microstylum	100	Light brown	Ovatus	Smooth	1.84 ± 0.16d	1.27 ± 0.13cd	1.44
T. annuum	100	Light brown	Ovatus	Smooth or slightly striped	1.25 ± 0.15fg	0.85 ± 0.15de	1.47
T. bulbosum	100	Dark brown-black	Ovatus	Smooth or slightly striped	2.25 ± 0.15b	1.56 ± 0.16bc	1.44
T. leblebicii	100	Light brown	Ellipticus	Smooth or slightly striped	2.32 ± 0.18b	1.51 ± 0.19c	1.53
T. ochroleucum	100	Light brown	Ovatus	Smooth	1.54 ± 0.16def	0.76 ± 0.13ef	2.02
T. praecox subsp. *praecox*	100	Light brown	Ovatus	Smooth	1.42 ± 0.12def	0.91 ± 0.10de	1.56
T. cariense	100	Light brown	Ellipticus	Slightly striped	2.81 ± 0.19ab	2.05 ± 0.15ab	1.37
T. violascens	100	Light brown	Ellipticus	Slightly striped	1.62 ± 0.13de	1.15 ± 0.15d	1.40
T. densiflorum	100	Light brown	Ovatus	Smooth or slightly striped	1.60 ± 0.10de	1.08 ± 0.12de	1.48
T. tatianae	100	Light brown	Ellipticus	Smooth	1.44 ± 0.06ef	0.66 ± 0.07ef	2.18
T. cataonicum	100	Dark brown	Ovatus-late	Reticulate	1.63 ± 0.13de	0.89 ± 0.11de	1.83
T. syriacum	100	Dark brown	Ovatus	Slightly striped	1.50 ± 0.20def	0.89 ± 0.17de	1.68
T. elegans	100	Light brown	Ellipticus	Slightly striped	1.76 ± 0.16d	1.05 ± 0.15de	1.67
T. rosulare	100	Dark brown	Ellipticus	Smooth or slightly striped	2.99 ± 0.21a	2.16 ± 0.16a	1.38
T. lilacinum	100	Light brown	Ellipticus	Smooth	1.65 ± 0.15de	0.98 ± 0.12de	1.68
T. aghricum	100	Dark brown	Ovatus	Smooth or coarse protrusions	2.18 ± 0.18bc	1.35 ± 0.15cd	1.61
T. watsonii	100	Dark brown-black	Ellipticus	Smooth	2.30 ± 0.20b	1.08 ± 0.18de	2.12

Mean value ± standard deviation; means with different letters are significant at P = 0.05 according to Duncan's multiple range test.

tissue under the epidermis layers, has 1–8 layers of flat, crushed, or rectangular cells. Its thickness ranges from 12.78 μm (in *T. kotschyanum*) to 88.69 μm (in *T. huetii*). Exceptionally, *T. violascens, T. cataonicum*, and *T. aghricum* do not have the sclerotic or palisade layer (Table 4; Figure 3). The endosperm of the studied taxa is 1- or 3-layered and consists of cells that are mostly flat and rarely cubic or polygonal. Endosperm thickness varies between 16.79 μm and 63.60 μm; the widest endosperm is noted in *T. ochroleucum* and the narrowest in *T. orbiculatum* (Table 4). Mucilage cells are seen in epidermis or subepidermis layers of seeds some of the examined taxa (Table 4; Figure 3).

Figure 2. The micromorphological structures of the examined taxa: *T. arvense*: 1- a, b, and c; *T. huetii*: 2- a, b, and c; *T. orbiculatum*: 3- a, b, and c; *T. kotschyanum*: 4- a, b, and c.

The numerical analysis of the seed morphological and anatomical characteristics permits the form of a dendrogram, which shows the variances or similarities among the examined taxa. A dendrogram is constructed as a result of the cluster analysis of the examined taxa of *Thlaspi* based on the variation of 18 characters in 22 taxa. The cophenetic correlation coefficient is calculated to find the relation between the dendrogram and similarity matrix (Figure 4; Table 5). The cophenetic correlation between the coefficient matrix (similarity matrix) and tree matrix (dendrogram) has been calculated as 0.65, symbolizing a good match.

Our cluster analysis divided the taxa into 2 major clusters of A and B: Cluster A1 includes TH1, TH2, TH13, and TH21. Cluster A2 includes TH3–TH9, TH15, and TH17. Cluster B1 includes TH16 and TH20. Cluster B2 comprises TH10, TH11, TH12, TH14, TH18, TH19, and TH22. TH18 has formed a clade outside these clusters in the dendrogram (Figure 4). TH4 and TH6 are the most closely correlated taxa (similarity coefficient: 0.99), while TH4 and TH18 are the most distantly correlated taxa (similarity coefficient: 0.10) (Table 5). Cluster A2 contains the highest number of taxa compared to other clusters.

4. Discussion
The morphological characters of the seeds provide precise information about the evolutionary relationships of flowering plants (Corner, 1976). The surface, color, and size of the seeds of the family Brassicaceae are valuable characters in discriminating the taxa (Barthlott, 1981; Koul
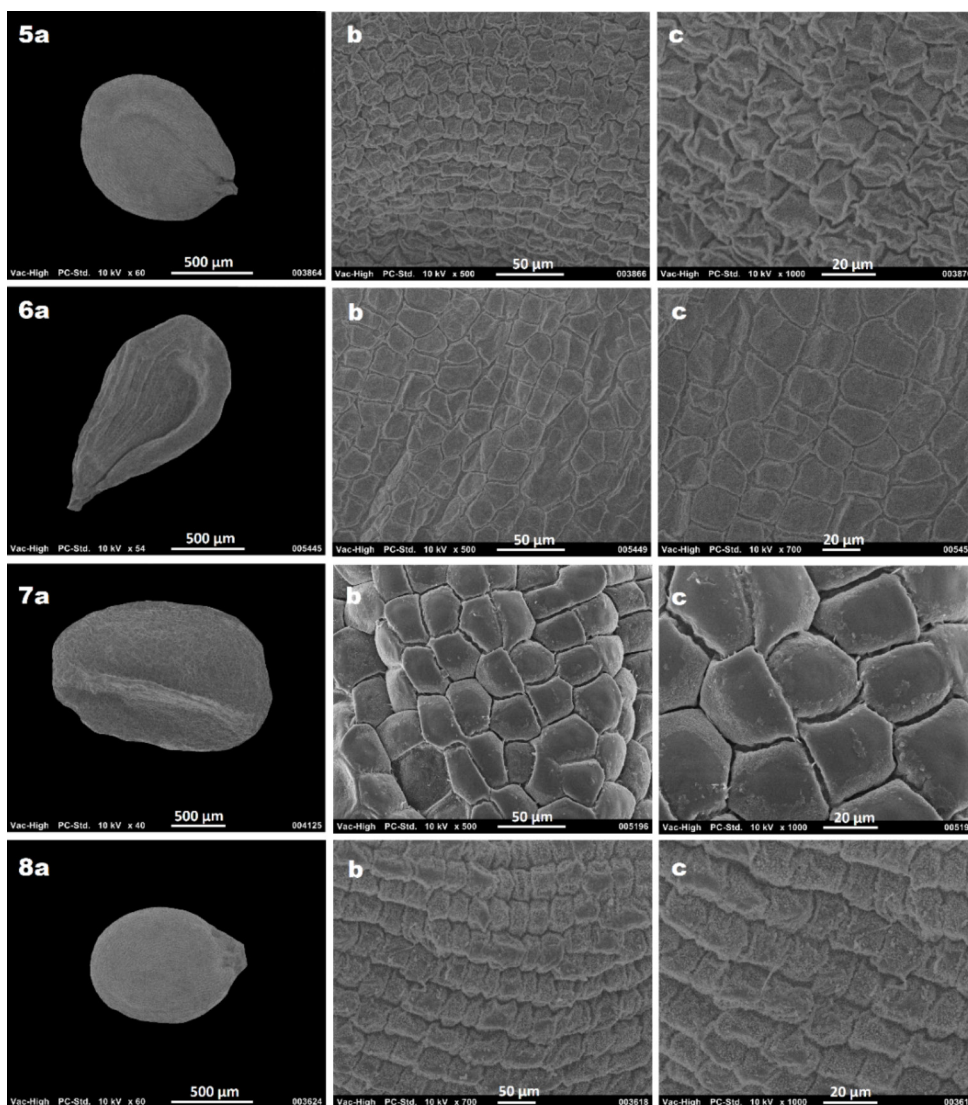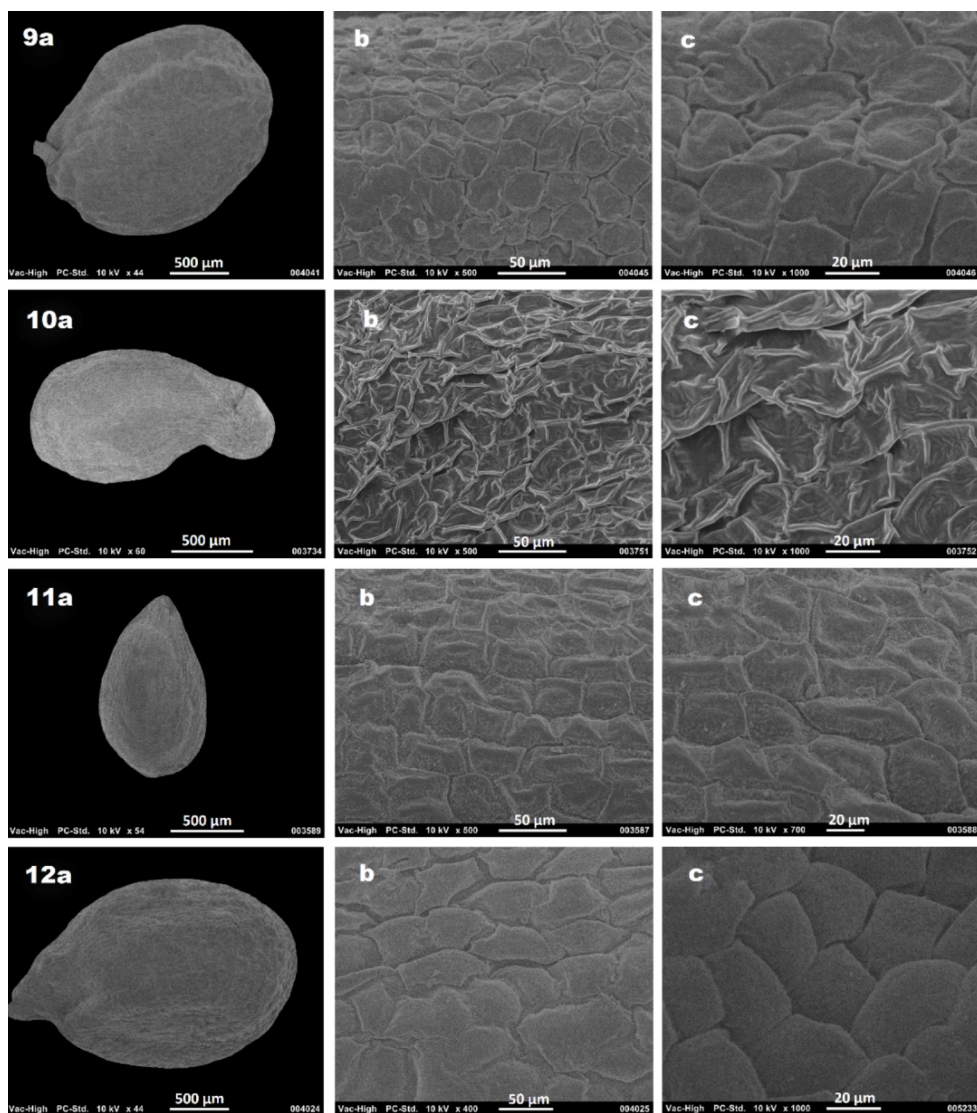
Figure 2. The micromorphological structures of the examined taxa: *T. perfoliatum*: 5- a, b, and c; *T. microstylum*: 6- a, b, and c; *T. annuum*: 7- a, b, and c; *T. bulbosum*: 8- a, b, and c.

et al., 2000). Vaughan and Whitehouse (1971), who studied morphological characteristics of the seeds of 200 species belonging to 90 genera within Brassicaceae, pointed out that the macromorphological characteristics can be used to solve problems in the systematics of the taxa. Moreover, the characters that can be identified with eyesight or a simple magnifier are a 'handheld' powerful tool for a field botanist.

Macromorphological characteristics of seeds of the *Thlaspi* s.l. taxa vary among the species. However, the taxa examined in our study are not very diverse in terms of seed color. Light brown dominates the genus, whereas a small number of taxa have dark brown and dark brown-black seeds. The color is effective in separating some of the closely related species, although it is not very effective at the genus level. Population appearances, floristic characteristics, and

siliques of *T. orbiculatum* and *T. kotschyanum* are similar, but the seeds of *T. orbiculatum* are dark brown while the seeds of *T. kotschyanum* are light brown. At the section level, *Nomisma* has dark brown-black seeds, whereas *Thlaspi* and *Pterotropis* have seeds that are various shades of brown and thus they cannot be separated by use of this character.

Seed surface morphology reflects natural selection and adaption. Therefore, it has systematic significance at the genus and species levels (Brochmann, 1992; Koul et al., 2000). In this study, we observed that seed surfaces of the examined taxa are in various forms: striped, smooth, reticulate, or irregular protrusions. The variety of seed surface has been found to be different, so seed surface is a reliable character in separating the taxa from each other.

Figure 2. The micromorphological structures of the examined taxa: *T. leblebicii*: 9- a, b, and c; *T. ochroleucum*: 10- a, b, and c; *T. praecox* subsp. *praecox*: 11- a, b, and c; *T. cariense*: 12- a, b, and c.

The macromorphological findings of this study are consistent with the previous studies conducted with exomorphic characters of seeds of the family Brassicaceae (Khalik and Maesen, 2002; Pınar et al., 2009; Kasem et al., 2011; Kaya et al., 2011; Bona, 2013).

Comparative studies of the micromorphological structures on the surfaces of the seeds are very important in terms of taxonomy. Heywood (1971) suggested the importance and effectiveness of scanning electron microscopy in solving systematic problems. Many researchers reported that seed microstructures are beneficial characters to use in discriminating the taxa within the family Brassicaceae (Barthlott, 1981; Koul et al., 2000; Kasem et al., 2011; Kaya et al., 2011).

In this study, we used scanning electron microscopy to solve problems in systematics of the taxa, establish evolutionary relationships, and show the adaptive importance of the seed coat. Nearly all the examined taxa have been studied for the first time, except for *T. perfoliatum* (micropapillate) and *T. aghricum* (rugulate) (Kasem et al., 2011; Fırat et al., 2014). We found 9 seed surface ornamentation types: reticulate, reticulate-foveate, reticulate-areolate, areolate, tuberculate, ocellate, scalariform, collicul ate, and ruminate.

Tantawy et al. (2004) showed that reticulate is the most common seed ornamentation type and reticulate-areolate is the second most common type in the family Brassicaceae, as shown in our study. The tuberculate
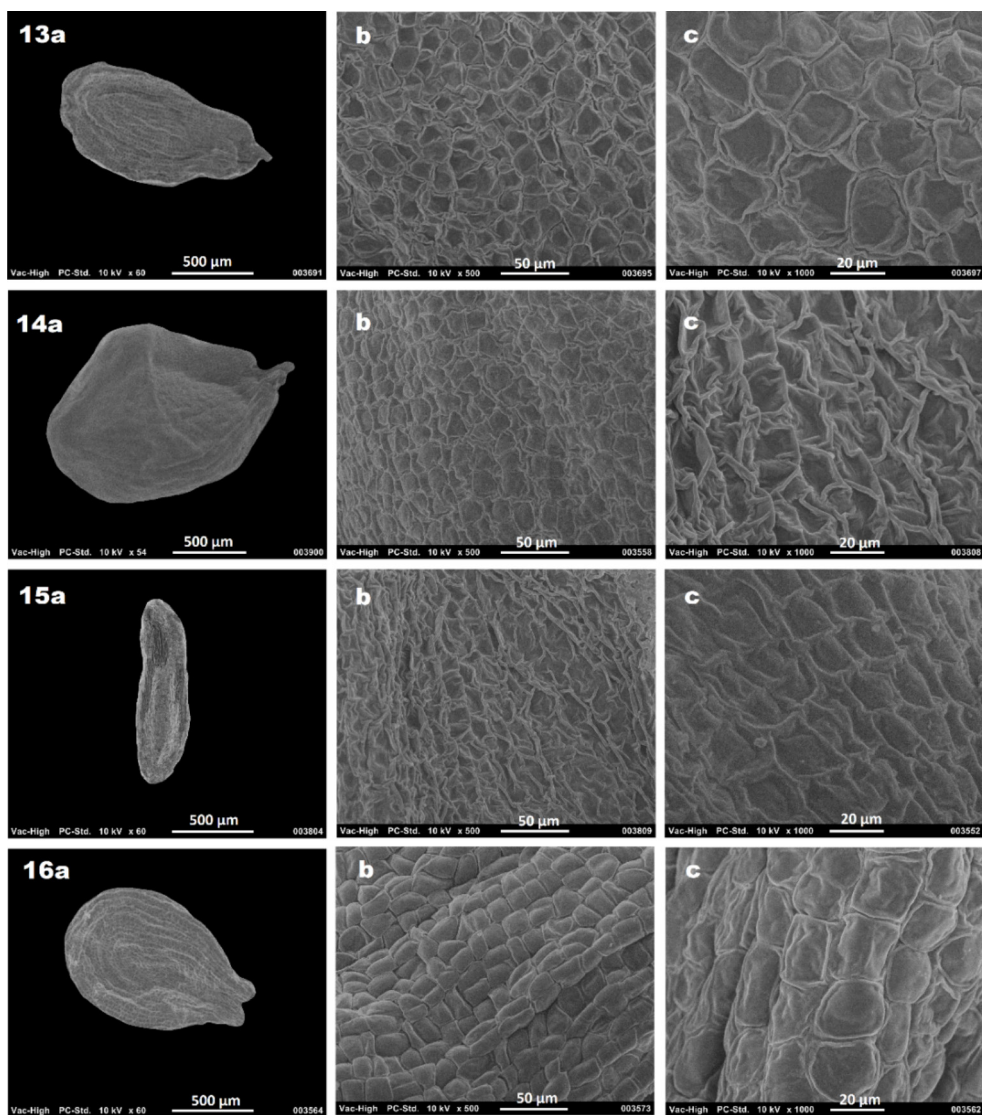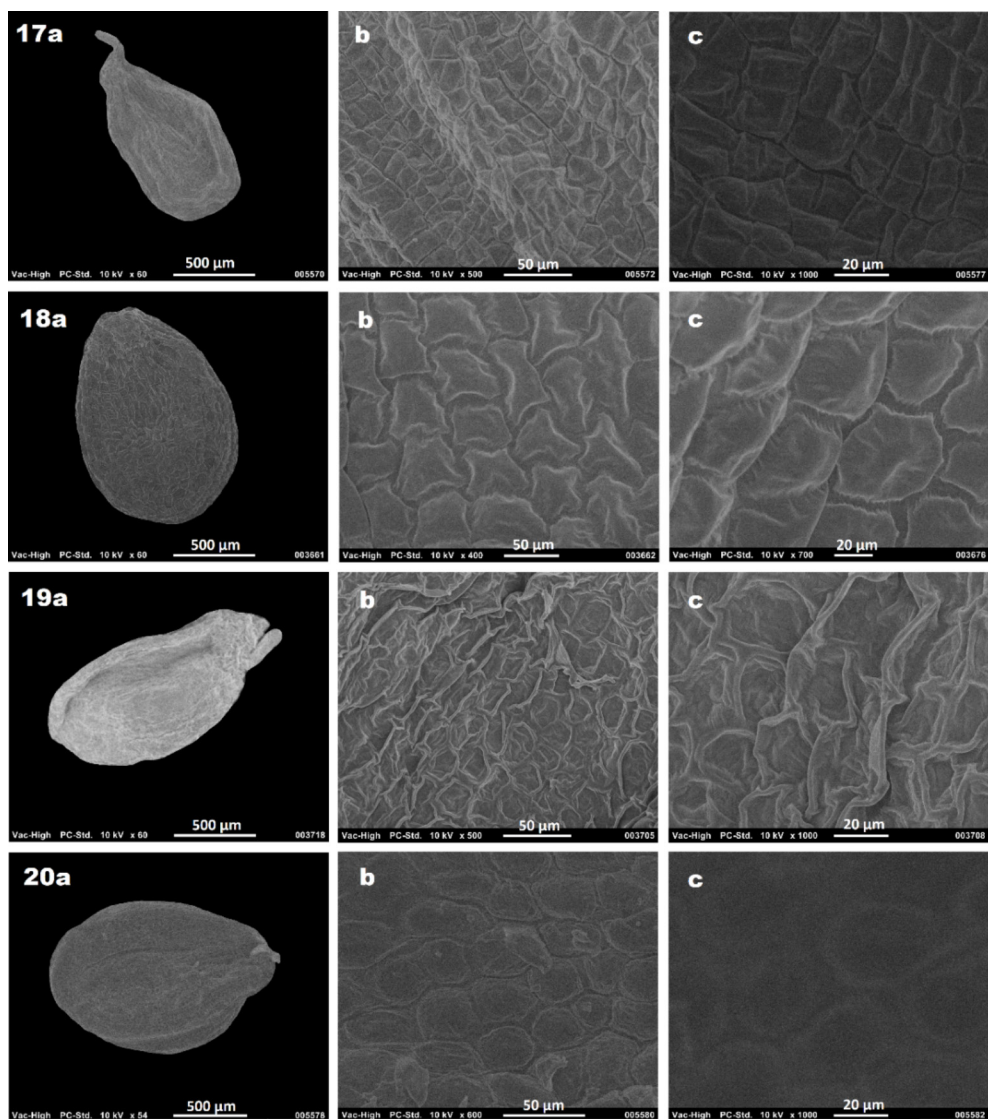
Figure 2. The micromorphological structures of the examined taxa: *T. violascens*: 13- a, b, and c; *T. densiflorum*: 14- a, b, and c: *T. tatianae*: 15- a, b, and c; *T. cataonicum*: 16- a, b, and c.

ornamentation type was seen in *Erysimum* L., *Alyssum* L., *Lobularia* Desv., and *Camelina* Crantz before (Murley, 1951). The ocellate type was noted in *Carricthera* DC. (Koul et al., 2000) and *Hesperis* L. (Pınar et al., 2009). The ruminate ornamentation type was found in the genus *Sisymbrium* L., and another common type was collicate, which was observed in some species of the genus *Isatis* L. (Moazzeni et al., 2007).

Two closely related taxa in the section *Nomisma*, *T. arvense* and *T. huetii*, have different surface ornamentation types: reticulate-foveate and reticulate, respectively. We found that seed surface ornamentation is an excellent character in discriminating the taxa of the section *Thlaspi*, except for *T. leblebicii* and *T. microstylum* as they

have the same ornamentation type (reticulate-areolate). The ornamentation types vary (6 types). In the section *Pterotropis*, ornamentation types are quite diverse, and the different appearance of the surface ornamentation in closely related taxa has increased the importance of systematic use of this character in the section.

Previous seed surface studies showed that the appearances of anticlinal and periclinal cell walls are good diagnostic parameters at the species level within the genus (Barthlott, 1981; Khalik and Maesen, 2002; Tantawy et al., 2004). The structures of anticlinal and periclinal cell walls are also effective in separation of the taxa. The type of the anticlinal cell wall is raised, sunken, or indefinite, and the type of the periclinal cell wall is flat, concave, convex,

Figure 2. The micromorphological structures of the examined taxa: *T. syriacum*: 17- a, b, and c; *T. elegans*: 18- a, b, and c: *T.* rosulare: 19- a, b, and c; *T. lilacinum*: 20- a, b, and c.

and indefinite. While the types of anticlinal and periclinal cell walls vary among the taxa of the section *Nomisma*, these characters are similar among the taxa of the section *Thlaspi*, the anticlinal cell wall being sunken and the periclinal cell wall being convex or concave in all the taxa. Taxa of *Thlaspi* are diverse in terms of the epidermal cell structure. Epidermal cells can be pentagonal-hexagonal, circular, oval, crushed, or polygonal forms. Nearly all the taxa in *Nomisma* and *Thlaspi* have different cell shapes, except for *T. bulbosum* and *T. leblebicii*, both of which have polygonal cells. In general, scanning electron microscope studies showed that the detailed examination of seed characteristics of the *Thlaspi* taxa is very useful in separating species from each other.

Studies of the anatomy of the seed coats of the family Brassicaceae have shed light on taxonomic problems (Vaughan et al., 1976; Ghaempanah et al., 2013). It has been reported that the seed coat structures can be used as a reliable character in the classification and identification of the taxa as well as the explanation of evolutionary and phylogenetic relationships among the taxa (Koul et al., 2000). The anatomical structures of the types of some seed coats in the family Brassicaceae were described by Vaughan et al. (1976), Meyer (1973, 1979, 1991), and Moazzeni et al. (2007). However, few studies adapted a phylogenetic approach to investigate the assumed correlations and evolutionary relationships among the taxa with a comparative examination of anatomical structures of seed coats.
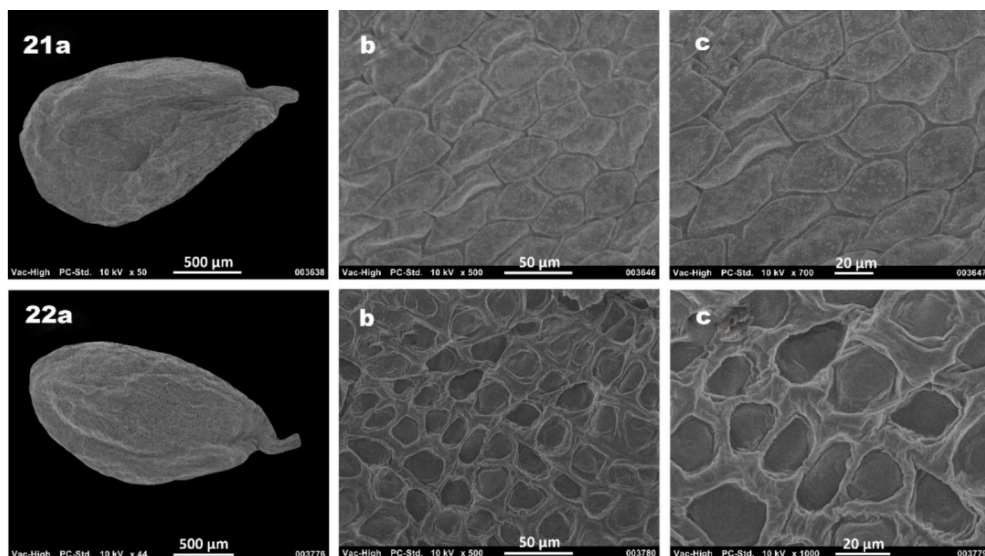
Figure 2. The micromorphological structures of the examined taxa: *T. aghricum*: 21- a, b, and c; *T. watsonii*: 22- a, b, and c.

Table 3. Micromorphological features of the seeds of the examined taxa.

Taxa	Seed surface ornamentation (7)	Anticlinal cell wall (8)	Periclinal cell wall (9)	Epidermal cell structure (10)
Thlaspi arvense	Reticulate-foveate	Sunken	Flat or convex	Pentagonal-hexagonal cells
T. huetii	Reticulate	Sunken or raised	Convex	Rectangular cells
T. orbiculatum	Tuberculate	Sunken	Convex	Circular cells
T. kotschyanum	Ocellate	Sunken	Flat or concave	Oval cells
T. perfoliatum	Scalariform	Sunken	Concave	Crushed cells
T. microstylum	Reticulate-areolate	Sunken	Concave	Polygonal cells
T. annuum	Scalariform	Sunken	Convex	Rectangular cells
T. bulbosum	Colliculate	Sunken	Convex	Polygonal cells
T. leblebicii	Reticulate-areolate	Sunken	Concave	Polygonal cells
T. ochroleucum	Ruminate	Indefinite	Indefinite	Indefinite
T. praecox subsp. *praecox*	Reticulate-areolate	Sunken	Convex	Polygonal cells
T. cariense	Reticulate-areolate	Sunken	Convex	Polygonal cells
T. violascens	Reticulate	Sunken	Concave	Polygonal cells
T. densiflorum	Reticulate	Raised	Concave	Polygonal cells
T. tatianae	Ruminate	Indefinite	Indefinite	Indefinite
T. cataonicum	Scalariform	Sunken	Concave	Rectangular cells
T. syriacum	Scalariform	Sunken	Convex	Rectangular cells
T. elegans	Reticulate-areolate	Sunken	Convex	Polygonal cells
T. rosulare	Reticulate-foveate	Raised	Concave	Polygonal cells
T. lilacinum	Areolate	Sunken	Convex	Oval cells
T. aghricum	Reticulate-areolate	Sunken	Convex	Polygonal cells
T. watsonii	Reticulate-foveate	Raised	Concave	Polygonal cells
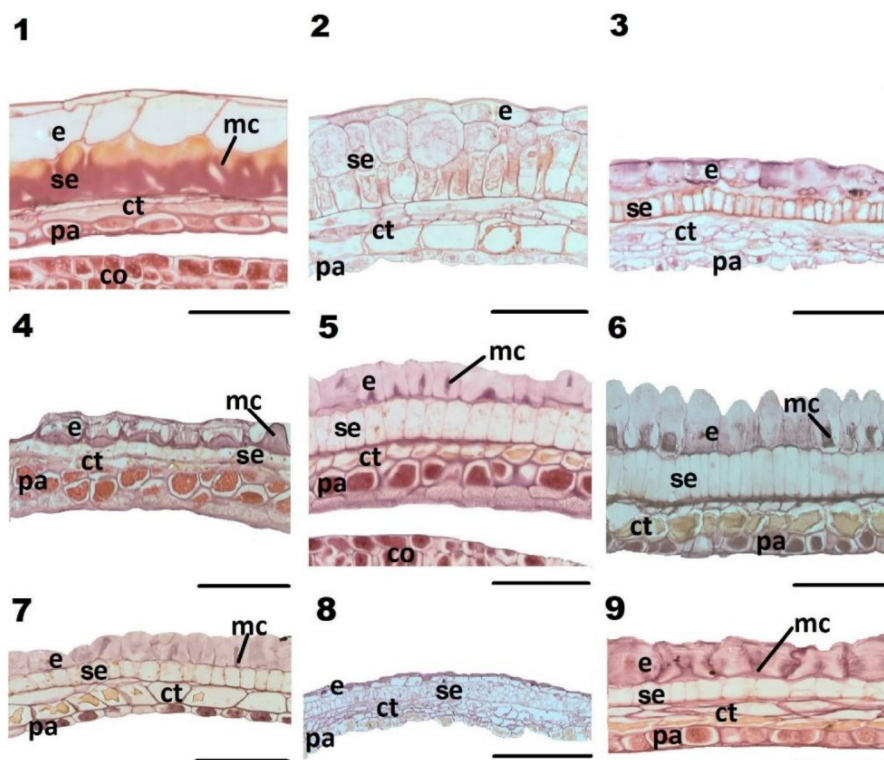
Figure 3. The testa structures of seeds of the examined taxa: 1- *T. arvense*, 2- *T. huetii*, 3- *T. orbiculatum*, 4- *T. kotschyanum*, 5- *T. perfoliatum*, 6- *T. microstylum*, 7- *T. annuum*, 8- *T. bulbosum*, 9- *T. leblebicii* (scale bars = 100 μm, e = epidermis, se = subepidermis, ct = compressed tissue = sclerotic or palisade layer, pa = parenchyma, mc = mucilage cell, co = cotyledon).

The seed coat generally consists of 4 layers (Bouman, 1975). These layers are the epidermis, the subepidermis, the sclerotic or palisade layer, and the parenchymatic layer (Ghaempanah et al., 2013).

In this study, anatomical structures of seed coats of *T. kotschyanum*, *T. microstylum*, *T. microstylum*, *T. annuum*, *T. leblebicii*, *T. ochroleucum*, *T. cariense*, *T. violascens*, *T. tatianae*, *T. cataonicum*, *T. syriacum*, *T. elegans*, and *T. watsonii* have been studied in detail for the first time, and it is discussed whether the anatomical structures of the seeds can be used for all the examined taxa to solve related taxonomic problems. The seed coat originates from differentiated layers such as the epidermis, the subepidermis, the sclerotic or palisade layer, and the parenchymal layer, as can be seen in the literature survey of the seed coat structure of the examined taxa.

Vaughan and Whitehouse (1971) studied the anatomical structures of seed coats of 200 species belonging to 90 genera in the family Brassicaceae and discussed their use as taxonomic characters and found 15 different types of epidermis cells in their study. In this work, we found that the type of the epidermis fairly varies among the taxa. This 1-layer epidermis can consist of flat, cubic, rectangular,

or polygonal cells. While flat cells are frequently seen, polygonal cells are rare in the examined taxa.

The structure of the sclerotic or palisade layer of the examined taxa has also been thoroughly studied for the first time in this study as well. The type of the sclerotic or palisade layer, which is a compressed tissue under the epidermis layers, shows considerable variation among the taxa. The sclerotic or palisade layer consists of 1–8 layers and its cells are be flat, crushed, or rectangular in the examined taxa except for *T. violascens*, *T. cataonicum*, and *T. aghricum*, which do not have the sclerotic or palisade layer. The sclerotic or palisade layer is very effective and useful in separating closely related taxa. In the section *Nomisma*, the sclerotic or palisade layer of *T. arvense* consists of one layer, while that of *T. huetii* consists of 2 or 3 layers of flat cells. In the section *Thlaspi*, *T. kotschyanum* consists of 1–2 layers of flat cells, while *T. orbiculatum* consists of 6–8 layers of flat cells. In the section *Pterotropis*, *T. violascens* lacks the sclerotic or palisade layer, while *T. densiflorum* has one composed of 1–2 layers of oval or flat cells. The sclerotic or palisade layer consists of 2–3 layers of flat cells in *T. lilacinum*, whose population appears like *T. watsonii*, the sclerotic or palisade layer of which consists of 4–6 layers of flat cells.
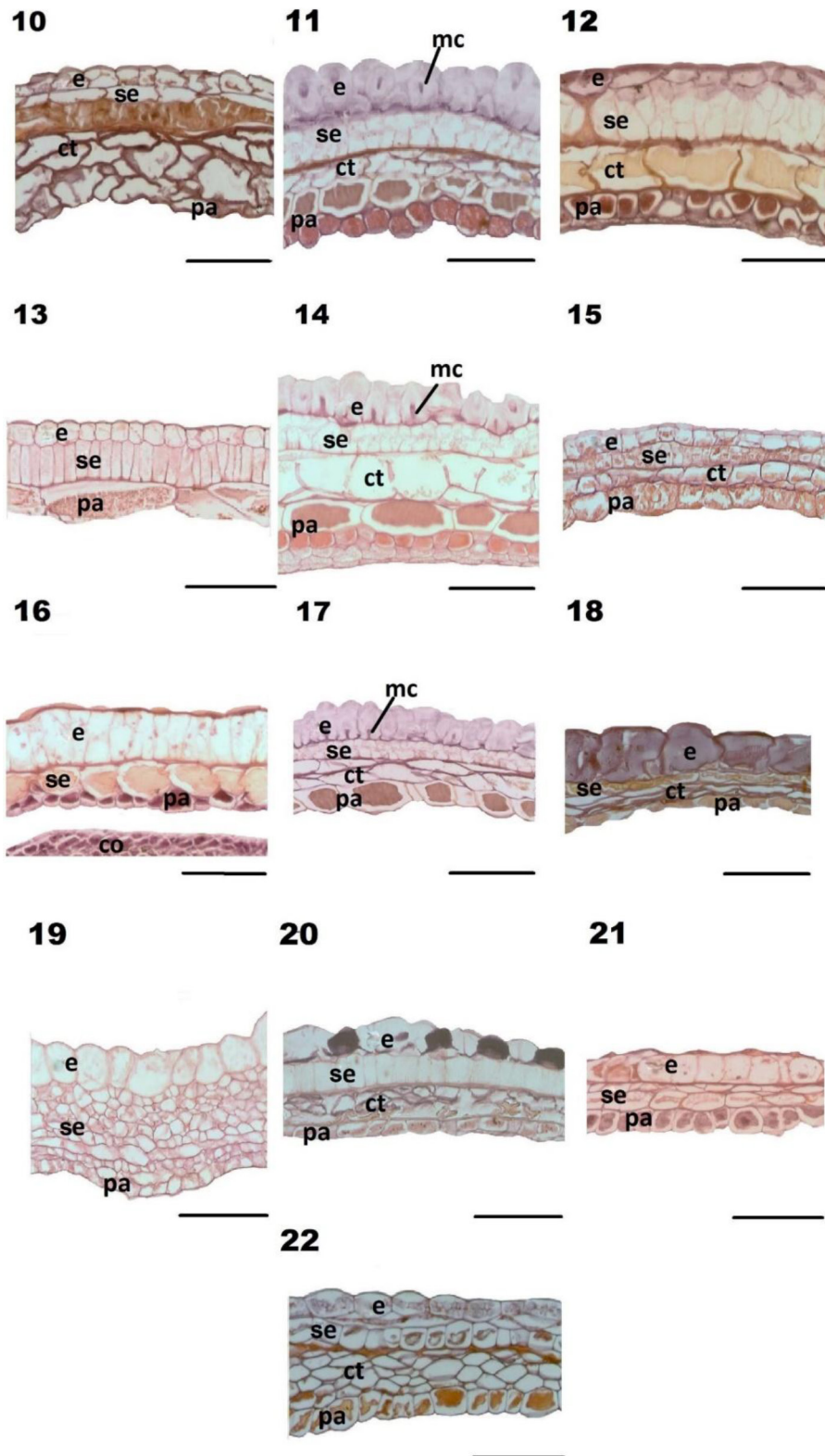
Figure 3. The testa structures of seeds of the examined taxa: 10- *T. ochroleucum*, 11- *T. praecox* subsp. *praecox*, 12- *T. cariense*, 13- *T. violascens*, 14- *T. densiflorum*, 15- *T. tatianae*, 16- *T. cataonicum*, 17- *T. syriacum*, 18- *T. elegans*, 19- *T. rosulare*, 20- *T. lilacinum*, 21- *T. aghricum*, 22- *T. watsonii* (scale bars = 100 μm, e = epidermis, se = subepidermis, ct = compressed tissue = sclerotic or palisade layer, pa = parenchyma, mc = mucilage cell, co = cotyledon).

Table 4. Testa anatomical features of the examined taxa (mean value ± standard deviation; means with different letters are significant at P = 0.05 according to Duncan's multiple range test, + = present, - = absent).

Taxa	Examined seed number	Epidermis layers		Thickness (μm) (13)
		Epidermis structures (11)	Subepidermis structures (12)	
Thlaspi arvense	100	1 layer, large flat cells	1 layer, elongated rectangular cells	101.16 ± 0.26c
T. huetii	100	1 layer, flat cells	2 layer, elongated rectangular cells	109.87 ± 0.54b
T. orbiculatum	100	1 layer, rectangular cells	1 layer, elongated rectangular cells	51.02 ± 0.33l
T. kotschyanum	100	1 layer, flat cells	1 layer, polygonal cells	49.58 ± 0.17m
T. perfoliatum	100	1 layer, cubic cells	1 layer, elongated rectangular cells	59.87 ± 0.32jk
T. microstylum	100	1 layer, flat cells	1 layer, elongated rectangular cells	115.21 ± 0.42a
T. annuum	100	1 layer, elongated rectangular cells	1 layer, rectangular cells	72.04 ± 0.19h
T. bulbosum	100	1 layer, flat cells	1–2 layers, polygonal cells	41.07 ± 0.62n
T. leblebicii	100	1 layer, cubic or polygonal cells	1 layer, elongated rectangular cells	88.24 ± 0.77e
T. ochroleucum	100	1 layer, flat cells	1 layer, flat cells	95.06 ± 0.36cd
T. praecox subsp. *praecox*	100	1 layer, cubic cells	2 layers, crushed polygonal cells	103.06 ± 0.34c
T. cariense	100	1 layer, flat cells	1–2 layers, elongated rectangular cells	93.37 ± 2.04d
T. violascens	100	1 layer, cubic cells	1 layer, elongated rectangular cells	80.71 ± 0.65f
T. densiflorum	100	1 layer, elongated rectangular cells	2 layers, cubic cells	74.26 ± 0.39h
T. tatianae	100	1 layer, rectangular cells	1 layer, elongated rectangular cells	57.51 ± 0.22k
T. cataonicum	100	1–2 layers, elongated rectangular cells	1 layer, large flat cells	113.31 ± 1.18ab
T. syriacum	100	1 layer, flat cells	1 layer, elongated rectangular cells	64.19 ± 2.21ij
T. elegans	100	1 layer, cubic cells	1 layer, flat cells	66.11 ± 0.29i
T. rosulare	100	1 layer, flat cells	6–7 layers, flat cells	107.19 ± 0.97b
T. lilacinum	100	1 layer, cubic cells	1 layer, elongated rectangular cells	74.41 ± 1.21h
T. aghricum	100	1 layer, elongated rectangular cells	1–2 layers, flat cells	73.10 ± 0.33h
T. watsonii	100	1 layer, flat cells	1–2 layers, cubic cells	63.75 ± 0.41j

Table 4. (Continued).

Taxa	Sclerotic or palisade layer		Parenchyma layer		Presence or absence of mucilage cells (18)
	Structure (14)	Thickness (μm) (15)	Structure (16)	Thickness (μm) (17)	
Thlaspi arvense	1 layer, flat cells	18.50 ± 2.90j	1 layer, flat cells	24.29 ± 0.16i	+
T. huetii	2–3 layers, flat or rectangular cells	88.69 ± 2.52a	1 layer, flat cells	19.89 ± 0.25kl	-
T. orbiculatum	6–8 layers, flat cells	63.07 ± 7.03d	1 layer, flat cells	16.79 ± 0.27m	-
T. kotschyanum	1–2 layers, flat cells	12.78 ± 0.34l	1 layer, flat cells	54.87 ± 0.19b	+
T. perfoliatum	1 layer, flat cells	18.82 ± 2.04j	1 layer, flat cells	32.64 ± 0.44g	+
T. microstylum	1 layer, flat cells	48.27 ± 2.13f	1 layer, flat cells	36.08 ± 0.33f	+
T. annuum	1–2 layers, rectangular cells	16.37 ± 1.19jk	1 layer, cubic or flat cells	30.34 ± 0.23h	+
T. bulbosum	6–8 layers, crushed cells	31.84 ± 1.13g	1 layer, flat cells	21.10 ± 0.26jk	-
T. leblebicii	2–3 layers, crushed cells	30.12 ± 1.41gh	1 layer, cubic cells	33.16 ± 0.15g	+

Table 4. (Continued).

T. ochroleucum	2–4 layers, crushed cells	72.10 ± 3.77c	3–5 layers, polygonal cells	63.60 ± 0.25a	-
T. praecox subsp. *praecox*	3–4 layers, flat cells	80.29 ± 1.58b	1 layer, flat cells	34.21 ± 0.27fg	+
T. cariense	1 layer, flat cells	72.26 ± 1.19c	1–2 layers, flat or cubic cells	46.25 ± 1.29c	-
T. violascens	-	-	1 layer, large flat cells	42.46 ± 0.30d	-
T. densiflorum	1–2 layers, oval or flat cells	62.79 ± 1.01d	1 layer, large flat cells	35.61 ± 0.23f	+
T. tatianae	1–2 layers, rectangular cells	29.02 ± 0.36h	1 layer, large flat cells	39.13 ± 0.32e	-
T. cataonicum	-	-	1 layer, flat cells	21.45 ± 1.08jk	-
T. syriacum	2–3 layers, flat cells	27.82 ± 1.17i	1 layer, flat cells	39.67 ± 0.89e	+
T. elegans	4–6 layers, crushed cells	27.06 ± 1.61i	1 layer, flat cells	20.79 ± 0.56jk	-
T. rosulare	1–2 layers, flat cells	21.00 ± 0.53j	1–2 layers, flat cells	46.13 ± 0.41c	-
T. lilacinum	2–3 layers, flat cells	27.82 ± 1.17i	1 layer, flat cells	23.29 ± 0.97ij	+
T. aghricum	-	-	1 layer, flat cells	29.96 ± 0.12h	-
T. watsonii	4–6 layers, flat cells	56.58 ± 0.93de	1 layer, flat cells	30.06 ± 0.94h	-
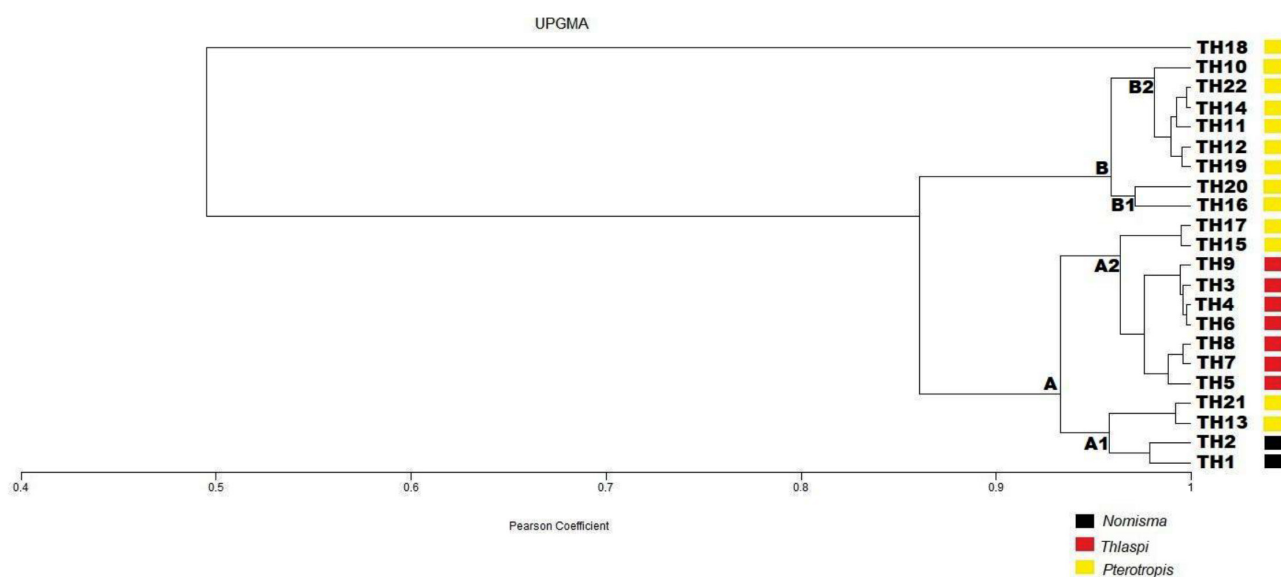
Figure 4. Cluster analysis of the examined taxa (see Table 1 for the taxa abbreviations).

The structure and thickness of the parenchyma of seed coats of the taxa has also been examined thoroughly for the first time in this study, and it is found that its characteristics vary among the taxa. The parenchymal layer consists of 1 or more layers of flat, cubic, or polygonal cells. The most common cell type is flat, whereas cubic and polygonal cells are less common types.

Despite the frequently mentioned convergence of fruits and flowers in the family Brassicaceae, epidermis layers, sclerotic or palisade layers, and parenchymal layers of the seed coats of the examined taxa have proven to be more useful characters than the traditional ones used in

classification of *Thlaspi*. Meyer (1973, 1979, 1991, 2001, 2006) had similar findings in his anatomical observations of some species of *Thlaspi* including a limited number of taxa from Turkey. However, Meyer's taxa transfers based on seed coat anatomy are contradictory.

According to Meyer (1979), anatomy of the seed coat is an important and useful additional character in the *Thlaspi* complex, and it could improve the classification of this huge genus. *Thlaspi* is distinguished by Meyer with 12 genera, which are not close according to anatomical features of the epidermis layers of the seed coat, and a few species (6 taxa) remaining in sensu stricto have similar

Table 5. The similarity matrix of the examined taxa.

Taxa	TH1	TH2	TH3	TH4	TH5	TH6	TH7	TH8	TH9	TH10	TH11	TH12	TH13	TH14	TH15	TH16	TH17	TH18	TH19	TH20	TH21	TH22
TH1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH2	0.86	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH3	0.73	0.91	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH4	0.75	0.72	0.82	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH5	0.85	0.84	0.75	0.39	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH6	0.77	0.92	0.81	0.99	0.91	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH7	0.68	0.85	0.74	0.33	0.98	0.95	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH8	0.86	0.95	0.94	0.68	0.92	0.94	0.87	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH9	0.95	0.90	0.81	0.42	0.97	0.94	0.91	0.93	1	-	-	-	-	-	-	-	-	-	-	-	-	-
TH10	0.85	0.92	0.91	0.67	0.93	0.92	0.90	0.98	0.91	1	-	-	-	-	-	-	-	-	-	-	-	-
TH11	0.85	0.79	0.96	0.71	0.89	0.90	0.83	0.92	0.91	0.95	1	-	-	-	-	-	-	-	-	-	-	-
TH12	0.81	0.76	0.93	0.70	0.92	0.95	0.91	0.96	0.93	0.94	0.90	1	-	-	-	-	-	-	-	-	-	-
TH13	0.91	0.66	0.56	0.11	0.95	0.84	0.96	0.74	0.93	0.80	0.74	0.73	1	-	-	-	-	-	-	-	-	-
TH14	0.82	0.94	0.95	0.73	0.90	0.91	0.87	0.91	0.81	0.94	0.93	0.78	0.74	1	-	-	-	-	-	-	-	-
TH15	0.90	0.84	0.83	0.52	0.93	0.65	0.72	0.96	0.72	0.97	0.92	0.76	0.90	0.94	1	-	-	-	-	-	-	-
TH16	0.64	0.73	0.51	0.10	0.91	0.91	0.94	0.71	0.93	0.74	0.76	0.73	0.91	0.73	0.82	1	-	-	-	-	-	-
TH17	0.92	0.87	0.82	0.42	0.79	0.96	0.91	0.92	0.95	0.92	0.92	0.95	0.91	0.93	0.94	0.81	1	-	-	-	-	-
TH18	0.22	0.33	0.25	0.10	0.36	0.39	0.44	0.54	0.43	0.33	0.22	0.25	0.39	0.43	0.45	0.41	0.46	1	-	-	-	-
TH19	0.92	0.83	0.72	0.30	0.98	0.68	0.96	0.86	0.98	0.84	0.82	0.88	0.93	0.86	0.94	0.95	0.96	0.46	1	-	-	-
TH20	0.91	0.92	0.83	0.45	0.92	0.96	0.91	0.94	0.90	0.92	0.92	0.92	0.91	0.92	0.94	0.93	0.92	0.45	0.92	1	-	-
TH21	0.94	0.72	0.57	0.11	0.94	0.90	0.92	0.78	0.91	0.73	0.75	0.71	0.92	0.74	0.82	0.97	0.90	0.31	0.94	0.92	1	-
TH22	0.84	0.95	0.91	0.74	089	0.84	0.82	0.96	0.90	0.97	0.92	0.93	0.72	0.98	0.93	0.71	0.92	0.32	0.85	0.91	0.73	1

anatomical structures of the seed coat. All the distinct species placed within *Thlaspi* showed completely different structures; the epidermis has additional properties or lacks additional properties.

According to Meyer (1991), the epidermis of taxa of *Thlaspi* s.s. consists of large cells; it is not flattened and has special structures, whereas the subepidermis consists of thick-walled cubic cells. In our study, we found that the epidermis of *T. arvense* and *T. huetii*, two taxa of the section *Nomisma*, is composed of large flattened cells. Also, the subepidermis consists of 1 or 2 layers of elongated rectangular cells, in contrast to the cubic form reported by Meyer. Also, he reported that the epidermal cells of the seed coat of *T. lilacinum* (*Pterotropis*) are glass-shaped, whereas we observed that the epidermis consists of cubic cells. Special structures such as the mucilage cells embedded in the epidermis were also observed.

Meyer (1973, 1979, 1991, 2001) defined the genus *Thlaspi* as a complex and regarded the genus *Noccaea* as the most primitive taxon that he included in *Thlaspi* s.l. because of its flat and unspecialized epidermis cells. However, the epidermis of *Atropatenia* is composed of very flat cells as in the genus *Noccaea*, but Meyer regarded *Atropatenia* as a genus in his classification and thus contradicted himself. The same contradiction is seen in the classification of the genus *Noccidium*.

Most of the species of Brassicaceae do not contain a mucilage layer on epidermal cells during maturation (Vaughan and Whitehouse, 1971; Western et al., 2000). There are epidermal cells that produce mucilage in the center and enclose seeds during imbibition. Mucilage is produced during seed development in the epidermis of the seed coat of the examined taxa of *Thlaspi*. Mucilage cells were found in *T. arvense* of the section *Nomisma*; in *T. kotschyanum*, *T. perfoliatum*, *T. microstylum*, *T. annuum*, and *T. leblebicii* of the section *Thlaspi*; and in *T. praecox* subsp. *praecox*, *T. densiflorum*, *T. syriacum*, and *T. lilacinum* of the section *Pterotropis*.

Mucilage cells were most commonly found in the taxa of the section *Thlaspi*. The presence or absence of mucilage cells is effective in separating closely related taxa such as *T. arvense* and *T. huetii* (*Nomisma*), *T. kotschyanum* and *T. orbiculatum* (*Thlaspi*), and *T. lilacinum* and *T. watsonii* (*Pterotropis*), hence its importance in the classification of the taxa. Also, the production of mucilage is correlated with the distribution of the population and the colonization of new habitats for many taxa of the genus *Thlaspi*. Moreover, mucilage production on the seed surface can be considered an ecological adaptation to water insufficiency (Young and Martens, 1991) because mucilage cells are not found in the taxa growing in wet fields such as meadows and swamps.

A dendrogram was formed to evaluate the morphological and anatomical characters of the seeds of

the examined *Thlaspi* taxa with UPGMA cluster analysis. The dendrogram, indicating two major clusters, was partially compatible with the findings of Hedge (1965), Davis et al. (1988), and Yıldırımlı (2001), where 36 taxa were placed into six sections. The seed morphological and anatomical differences have been observed at the species level and section level. It seems that morphological and anatomical properties are suitable for the available classification, except for some exceptions. Namely, the morphological and anatomical characteristics of the seeds have supported the features used in the distribution of *Thlaspi* species in the flora of Turkey (Figure 4).

In conclusion, examining the morphological and anatomical characters of seeds of the examined taxa of *Thlaspi* provides significant contributions to the systematics of species within the genus. The differences between this study and previous studies on the subject can be listed as follows:

1- Meyer's taxa transfers in *Thlaspi* s.l. based on seed coat anatomy were questionable because the origins of the specimens were not known. The specimens used in previous studies on the taxa found in Turkey did not involve flowers, mature fruits, or seeds. Our study was based on field observations, and the specimens were collected with their flowers, mature fruits, and seeds in accordance with the rules.

2- A detailed morphological and anatomical study was performed for the first time, covering all the taxa in the studied sections.

3- The testa structure of the studied taxa of *Thlaspi* s.l. showed prominent micromorphological and anatomical variations and thus provided significant contributions to the systematics of the species within the genus. These prominent features enabled easier identification of morphologically similar taxa such as *T. arvense-T. huetii*, *T. violascens-T. densiflorum*, and *T. lilacinum-T. watsonii.*

4- The results were evaluated with statistical analysis and their reliability was determined.

5- A key was provided for the identification of the examined taxa based on seed characteristics.

Key to examined *Thlaspi* taxa from Turkey, based on seed characters

1. Seeds are dark brown-black 2
2. Seed shapes are ovatus 3
3. Seed surfaces are clearly striped .... (*T. arvense*)
3. Seed surfaces are smooth or slightly striped ....
.... (*T. bulbosum*)
2. Seed shapes are ellipticus or ellipticus-late 4
4. Seed surfaces are striped .... (*T. huetii*)
4. Seed surfaces are smooth .... (*T. watsonii*)
1. Seeds are light or dark brown 5
5. Seeds are light brown 6

6. Seed shapes are ovatus 7
7. Seed surfaces are slightly reticulate ....
.... (*T. kotschyanum*)
7. Seed surfaces are smooth or slightly striped 8
8. Seed surface ornamentations are reticulate or reticulate-areolate 9
9. Reticulate .... (*T. densiflorum*)
9. Reticulate-areolate 10
10. Periclinal cell walls are concave .... (*T. microstylum*)
10. Periclinal cell walls are convex ....
.... (*T. praecox* subsp. *praecox*)
8. Seed surface ornamentations are scalariform or ruminate 11
11. Scalariform .... (*T. annuum*)
11. Ruminate .... (*T. ochroleucum*)
6. Seed shapes are ellipticus or ellipticus-late 12
12. Seed surface ornamentations are reticulate, areolate or reticulate-areolate 13
13. Epidermal cells are oval .... (*T. lilacinum*)
13. Epidermal cells are polygonal 14
14. Epidermis consists of flat cells .... *T. cariense*)
14. Epidermis consists of cubic or polygonal cells 15
15. Subepidermis consists of flat cells .... (*T. elegans*)
15. Subepidermis consists of rectangular cells 16
16. The sclerotic or palisade layer is present ....
.... *T. leblebicii*)
16. The sclerotic or palisade layer is absent ....
.... *T. violascens*)
12. Seed surface ornamentations are scalariform and ruminate 17
17. Scalariform .... (*T. perfoliatum*)
17. Ruminate .... (*T. tatianae*)
5. Seeds are dark brown 18
18. Seed shapes are ellipticus 19
19. Seed surface ornamentation is tuberculate ....
.... (*T. orbiculatum*)
19. Seed surface ornamentation is reticulate-foveate .....
.... (*T. rosulare*)
18. Seed shapes are ovatus or ovatus-late 20
20. Seed surface is smooth .... (*T. aghricum*)
20. Seed surface is reticulate or slightly striped 21
21. Epidermis consists of rectangular cells ....
.... (*T. cataonicum*)
21. Epidermis consists of flat cells .... (*T. syriacum*)

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