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Seed Morpho-Anatomical Characters of Some *Cardamine* taxa from Turkey

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ABSTRACT

This manuscript includes morphological and anatomical characters of seeds of 7 taxa of *Cardamine* genus from Turkey and the importance of these characteristics from a systematic perspective. The outcomes demonstrate that the taxa vary in terms of seed shape and dimension. The seed dimension varies between 0.91 mm and 2.20 mm in length and between 0.41 mm and 1.65 mm in width, *Cardamine graeca* having the biggest and *C. tenera* having the smallest seeds. The seed surface ornamentation is categorized into five types: reticulate-alveolate, scalariform, reticulate, foveate and reticulate-foveate. The most common type is scalariform and reticulate, however; foveate, reticulate-foveate and reticulate-alveolate ornamentation types have been noticed to be taxon-specific. The testa is mostly occurred with 3 layers: the outer epidermis, the inner epidermis, and the parenchyma layer. However, *C. tenera* and *C. graeca* taxa have an additional inner testa layer under the outer testa. The shapes and thicknesses of the outer epidermis and the inner epidermis, presence of the inner testa and the parenchyma layer are very important characteristics that disclose inter-specific relations within the studied species. Moreover, a key is offered for the identification of the studied taxa based on seed characters.

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Türkiye'den Bazı *Cardamine* Taksonlarının Tohum Morfo-Anatomik Karakterleri

ÖZET

Bu makale Türkiye'den *Cardamine* cinsinin 7 taksonunun tohumlarının morfolojik ve anatomik karakterlerini ve bu karakterlerin sistematik bir bakış açısıyla önemini içermektedir. Sonuçlar taksonların tohum şekli ve boyut açısından farklı olduğunu göstermiştir. Tohum boyutları boyda 0.91 mm ile 2.20 mm ve ende 0.41 mm ile 1.65 mm arasında değişmiş, *Cardamine graeca* en büyük tohumlara, *C. tenera* ise en küçük tohumlara sahiptir. Tohum yüzey ornamentasyonu beş tipe ayrılır: retikulat-alveolat, scalariform, retikulat, foveat and retikulat-foveat. En yaygın tip scalariform ve retikulattır, buna karşın; foveat, retikulat-foveat ve retikulat-alveolat ornamentasyon tiplerinin taksona özgü olduğu not edilmiştir. Testa çoğunlukla 3 katmanla oluşmuştur: dış epidermis, iç epidermis ve parenkima katmanı. Bununla birlikte, *C. tenera* ve *C. graeca* taksonlarının dış testa altında ek bir iç testa tabakası vardır. Dış epidermis ve iç epidermisin şekilleri ve kalınlıkları, iç testa varlığı ve parenkima tabakası incelenen türler arası ilişkileri ortaya koyan çok önemli özelliklerdir. Ayrıca, incelenen taksonların tohum karakterlerine göre tanımlanması için bir anahtar sunulmuştur.

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INTRODUCTION

The family Brassicaceae is one of the greatest flowering plant families containing economic importance includes more than 340 genera and 3350 species the world (Al-Shehbaz, 1988; Karaismailoğlu, 2017). Taxa in this family are placed in worldwide, particularly in the North temperate areas and Southwestern and Central Asia. The family has 85 genera and 458 taxa in Turkey (Al-Shehbaz, 2012).

The genus *Cardamine* L. is taxonomically problematic, widespread genus with over 200 Arctic, alpine, and boreal taxa, and is one of the largest genera of the family in terms of number of species (Hewson, 1982; Al-Shehbaz, 1988; Webb et al., 1988; Al-Shehbaz et al., 2006; Carlsen et al., 2009). The number of acknowledged species differs noticeably among various researchers, because of the taxonomic complexity of the genus (Carlsen et al., 2009). As a result of the performed some studies such as Schulz (1903), Al-Shehbaz (1988) and Carlsen et al. (2009), it has seen that the diversity center of the genus is Eurasia. Genus has about 95 species in Eurasian, 48 species in China and 25 species in Europe involving the Caucasus (Carlsen et al., 2009). Some species of the genus are widely distributed, for example *C. hirsuta* L., *C. flexuosa* With., *C. parviflora* L. and *C. impatiens* L. The new taxa have been discovered since the first revision of the genus (Schulz, 1903); however, species restriction is impractical and the total number of species in *Cardamine* is still controversial (Carlsen et al., 2009). The first sectional division of genus (Schulz, 1903) has been condemned by some researchers for over-emphasis some morphological features (Al-Shehbaz, 1988; Rashid & Ohba, 1993; Carlsen et al., 2009). Some species in *Cardamine* have been analyzed widely with cytological and molecular markers (Lihova et al., 2000, 2003, 2004; Carlsen et al., 2009; Kučera et al., 2010; Karaismailoğlu, 2021). However, the taxonomic usability of the detailed morphological and anatomical characters in the genus has been ignored so far.

The seed coat features are known as important characteristics in taxonomical investigations of the Brassicaceae family (Karaismailoğlu & Erol, 2018). It is mainly used to resolve classification difficulties regarding closely correlated species, determine their evolutionary connections, and describe their adaptive importance (Kaya et al., 2011; Karaismailoğlu & Erol, 2018). Moreover, many studies have shown that macro and micro characters of the seeds has systematic significance in separating the taxa within the family Brassicaceae (Khalik and Maesen, 2002; Tantawy et al., 2004; Kaya et al., 2011; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a). Also, the anatomical features of the seed coat can contribute to solving the taxonomic problems regarding family. This statement has been supported by performed works on several genera of the family (Vaughan et al., 1976; Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a). However, there is no study on seed morphology and anatomy of the genus *Cardamine* from Turkey, where is one of the diversity centers of genus with 14 taxa (Mutlu, 2012), so far.

The aim of this investigation is to contribute to the seed morphological and anatomical characteristics of seven *Cardamine* taxa containing *C. bulbifera* Crantz, *C. hirsuta* L., *C. impatiens* var. *pectinata* (Pall. ex DC.) Trautv., *C. lazica* Boiss. & Bal., *C. tenera* Gmel ex Mey., *C. uliginosa* Bieb. and *C. graeca* L. from Turkey and to examine taxonomic uses of these characters at the interspecies level.

MATERIAL and METHODS

This study was planned with 50 seeds belonging to 10 individuals for each taxon taken from different regions of Turkey during the fruiting time. The examined taxa were deposited at SUFAF (Siirt University Flora and Fauna) and given in Table 1.

Table 1. The examined taxa and their locations
Çizelge 1. İncelenen taksonlar ve lokasyonları

Cardamine bulbifera Crantz	Ağrı, Patnos, Kizkapan village, stone slopes, 1650 m, 16.5.2015, Karaismailoğlu 160
C. hirsuta L.	Bursa, Uludağ, Aras valley, roadside stony areas, 1650 m, 2.7.2016, Karaismailoğlu 302
C. impatiens var. *pectinata* (Pall. ex DC.) Trautv.	Bolu, Abant, roadsides, 801 m, 30.4.2015, Karaismailoğlu 132b
C. lazica Boiss. & Bal.	Artvin, Hopa, roadsides, stone slopes, 350 m, 6.3.2015, Karaismailoğlu 100b
C. tenera Gmel ex Mey.	Kütahya, Gediz, Murat mountain, 1700 m, 23.6.2016, Karaismailoğlu 286
C. uliginosa Bieb.	İstanbul, Büyükçekmece, Beykent, meadows, 80-120 m, 6.7.2016, Karaismailoğlu 310
C. graeca L.	Muğla, Marmaris, Kırzeytin mountain, serpentine rocks, 490 m, 3.4.2015, Karaismailoğlu 122b

Macromorphological features of the seeds involving color, shape, dimension, and surface characters were analyzed by utilizing an Olympus SZX7 stereomicroscope and Kameram Imaging Software (Figure 1). Micromorphological examinations of the seeds involving surface ornamentation, anticlinal and periclinal cell walls, and the form of epidermal cell were studied with a JEOL Neoscope-5000 Scanning Electron Microscope (Figure 2). For micromorphological observations, seeds were pasted on the stub with silver adhesive and enclosed with platinum-gold.

Findings of anatomical features were obtained on samples well-preserved in 70% alcohol. Cross-sections

were taken from midpoint of seeds with an automatic microtome (Thermo Shonda Met Finesse). After, they were treated with series of alcohol and xylene, and dyed with hematoxylin in a staining tool (ASC 720 Medite) and covered with Entellan (Figure 3) (Karaismailoğlu, 2015, 2019a; Karaismailoğlu & Erol, 2018). Anatomical characteristics were detected using an Olympus CX21FS1 microscope and Kameram Imaging Software.

Figure 1. Seeds of the studied taxa: 1- *C. bulbifera*, 2- *C. hirsuta*, 3- *C. impatiens* var. *pectinata*, 4- *C. lazica*, 5- *C. tenera*, 6- *C. uliginosa*, 7- *C. graeca* (scales bars=1 mm)

Şekil 1. Çalışılan taksonların tohumları: 1- C. bulbifera, 2- C. hirsuta, 3- C. impatiens var. *pectinata, 4- C. lazica, 5- C. tenera, 6- C. uliginosa, 7- C. graeca (ölçekler=1 mm)*

The terminology of morphological and anatomical characters was performed in accordance with Stearn (1985), Ghaempanah et al. (2013) and Karaismailoğlu and Erol (2018).

The findings were evaluated with SPSS. Duncan's multiple-range test was utilized to define the statistical importance of variations among the quantitative values acquired for taxa (SPSS Inc, 2006). Grouping of taxa was performed with using the clustering assessment method (UPGMA) in accordance with 38 characters in Tables 2 (Figure 4) (Karaismailoğlu and Erol, 2018). Moreover, the similarity matrix of the examined taxa was created in MVSP (Kovach, 2007).

RESULTS

The seed features of the studied taxa involving color, shape, dimension, and surface from are macromorphologically assessed. Seed colors of the examined taxa are noticed as brown and its tones. *C. impatiens* var. *pectinata* and *C. lazica* taxa are distinctly different from other taxa with their light brown seeds. It has been seen six seed shapes: ovatus in *C. bulbifera*, ellipticus-rectangularis in *C. hirsuta*, rectangularis in *C.impatiens* var. *pectinata*, circularis-

rectangularis in *C. lazica*, ellipticus-late in *C. tenera* and *C. uliginosa*, and ellipticus in *C. graeca*. Seed shape is characteristic in studied taxa, except for *C. tenera* and *C. uliginosa*. The seed dimension varies between 0.91 mm and 2.20 mm in length and between 0.41 mm and 1.65 mm in width. *Cardamine bulbifera* and *C. graeca* are markedly separate from the rest of the studied taxa in terms of seed dimension. Seed surface structures are smooth, except for *C. graeca* that is of slightly striped. *Cardamine impatiens* var. *pectinata*, *C. tenera* and *C. uliginosa* have raphe on seeds, but not others (Figure 1 and Table 3).

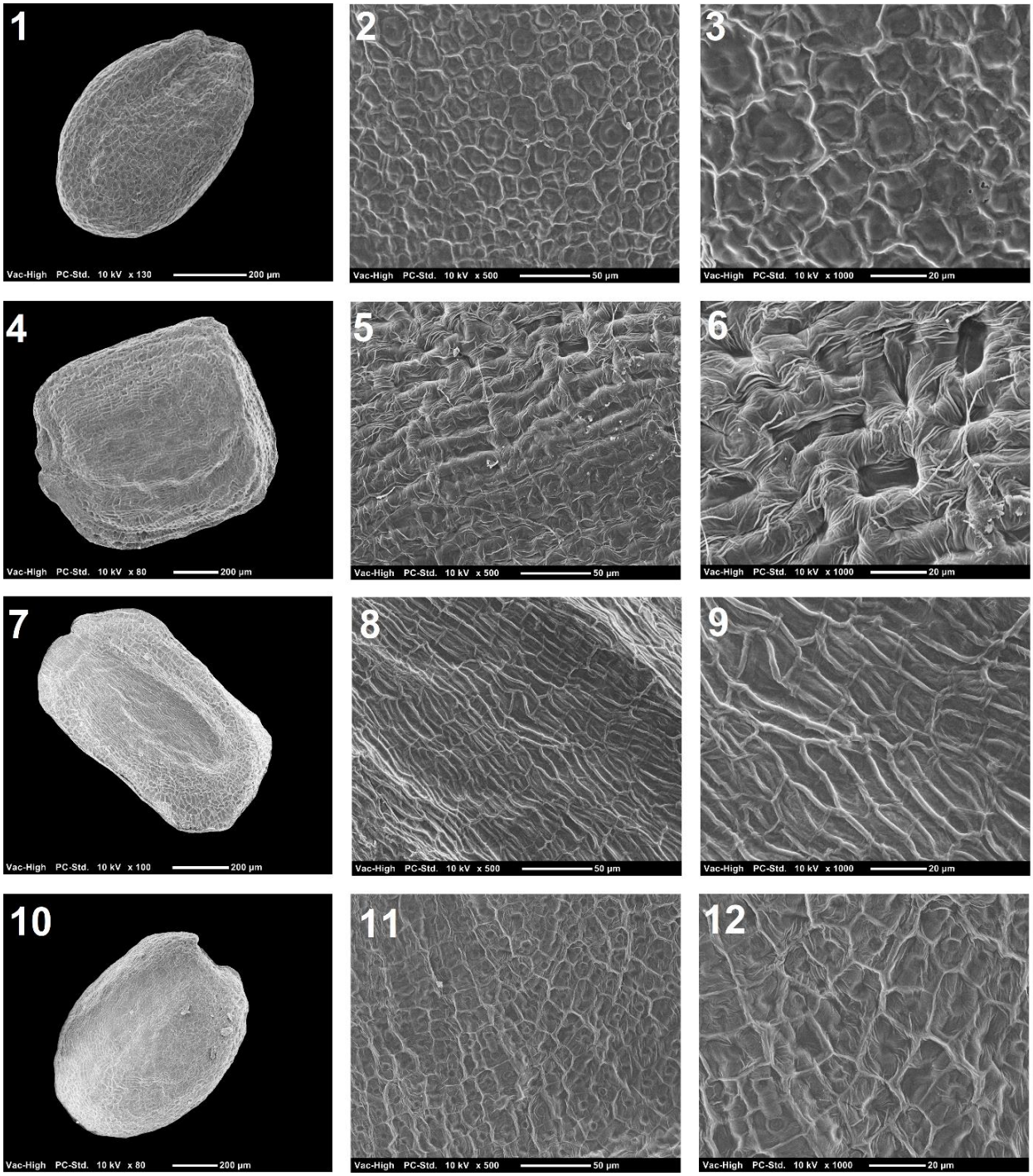

Figure 2. The micromorphological structures of the studied taxa: 1-3 *C. bulbifera*, 4-6 *C. hirsuta*, 7-9 *C. impatiens* var. *pectinata*, 10-12 *C. lazica*.

Şekil 2. Çalışılan taksonların mikromorfolojik yapıları: 1-3 C. bulbifera, 4-6 C. hirsuta, 7-9 C. impatiens var. *pectinata, 10-12 C. lazica.*

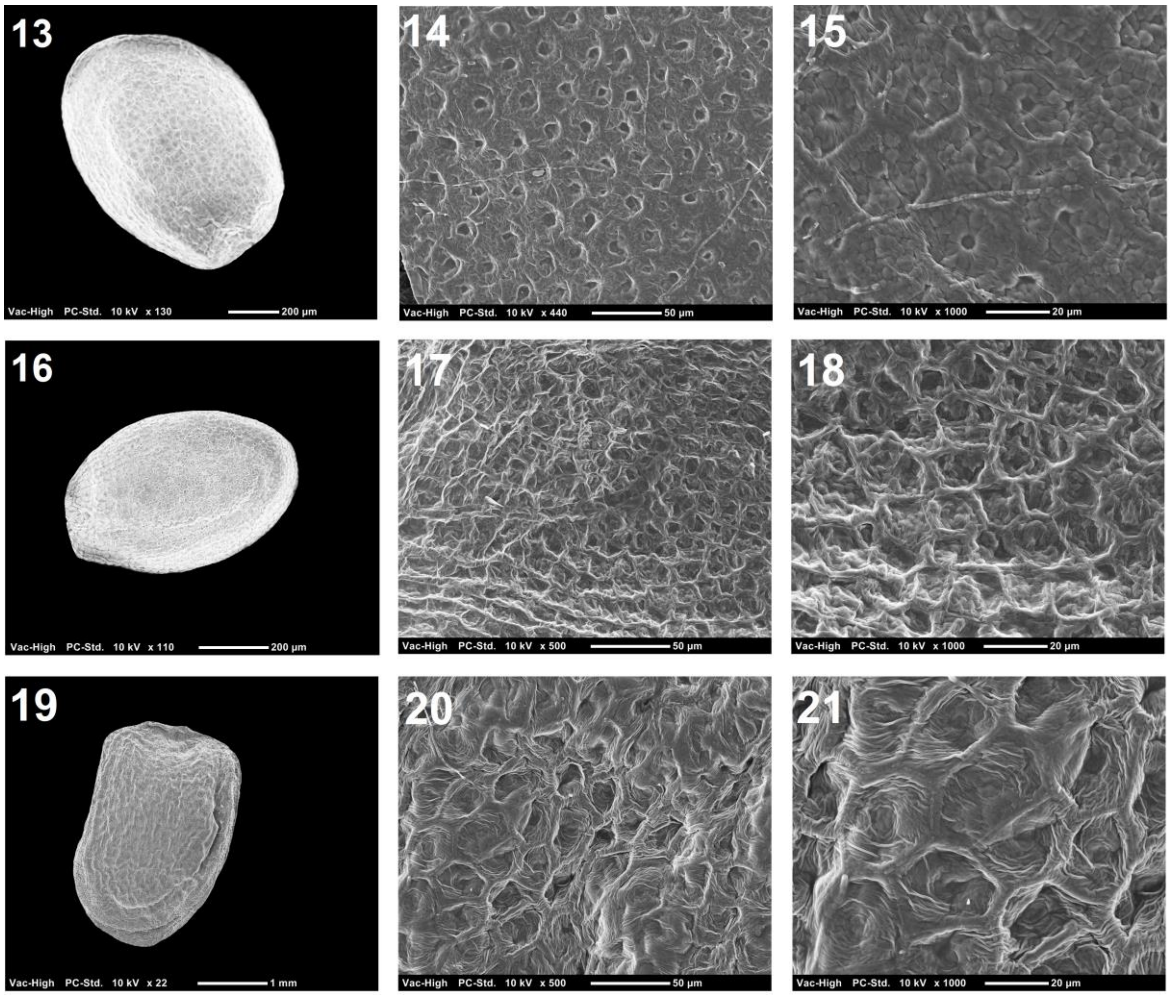

Figure 2. The micromorphological structures of the studied taxa: 13-15 *C. tenera*, 16-18 *C. uliginosa*, 19-21 *C. graeca.*

Şekil 2. Çalışılan taksonların mikromorfolojik yapıları: 13-15 C. tenera, 16-18 C. uliginosa, 19-21 C. graeca.

Table 2. Characters used in statistical analysis and their codes

Çizelge 2. İstatistik analizlerde kullanılan karakterler ve kodları

Characters *(Karakterler)*	**Codes** *(Kodlar)*
Seed color	Dark brown or brown (1), Light brown (2)
Seed shape	Ovatus (3), Ellipticus-rectangularis (4), Rectangularis (5) Circularis-rectangularis (6), Ellipticus-late (7), Ellipticus (8)
Seed surface	Smooth (9), Slightly striped (10)
Seed size	Length (11), Width (12)
Raphe	Presence (13)
Surface ornamentation	Reticulate-alveolate (14), Scalariform (15), Reticulate (16), Foveate (17), Reticulate-foveate (18)
Anticlinal cell walls	Raised (19)
Periclinal cell walls	Convex (20), Concave (21)
Epidermal cell structure	Polygonal (22), Alveolar (23), Rectangular (24)
Anatomical structure of the outer epidermis	Flattened (25), Rectangular (26), Cubic (27)
Anatomical structure of the inner epidermis	Flat (28), Rectangular (29), Elongated rectangular (30), Outer testa thickness (31)
Inner testa structures	Presence (32), Crushed (33), Flat (34), Thickness (35)
Parenchyma cell structures	Flat (36), Thickness (37)
Mucilage cell	Presence (38)

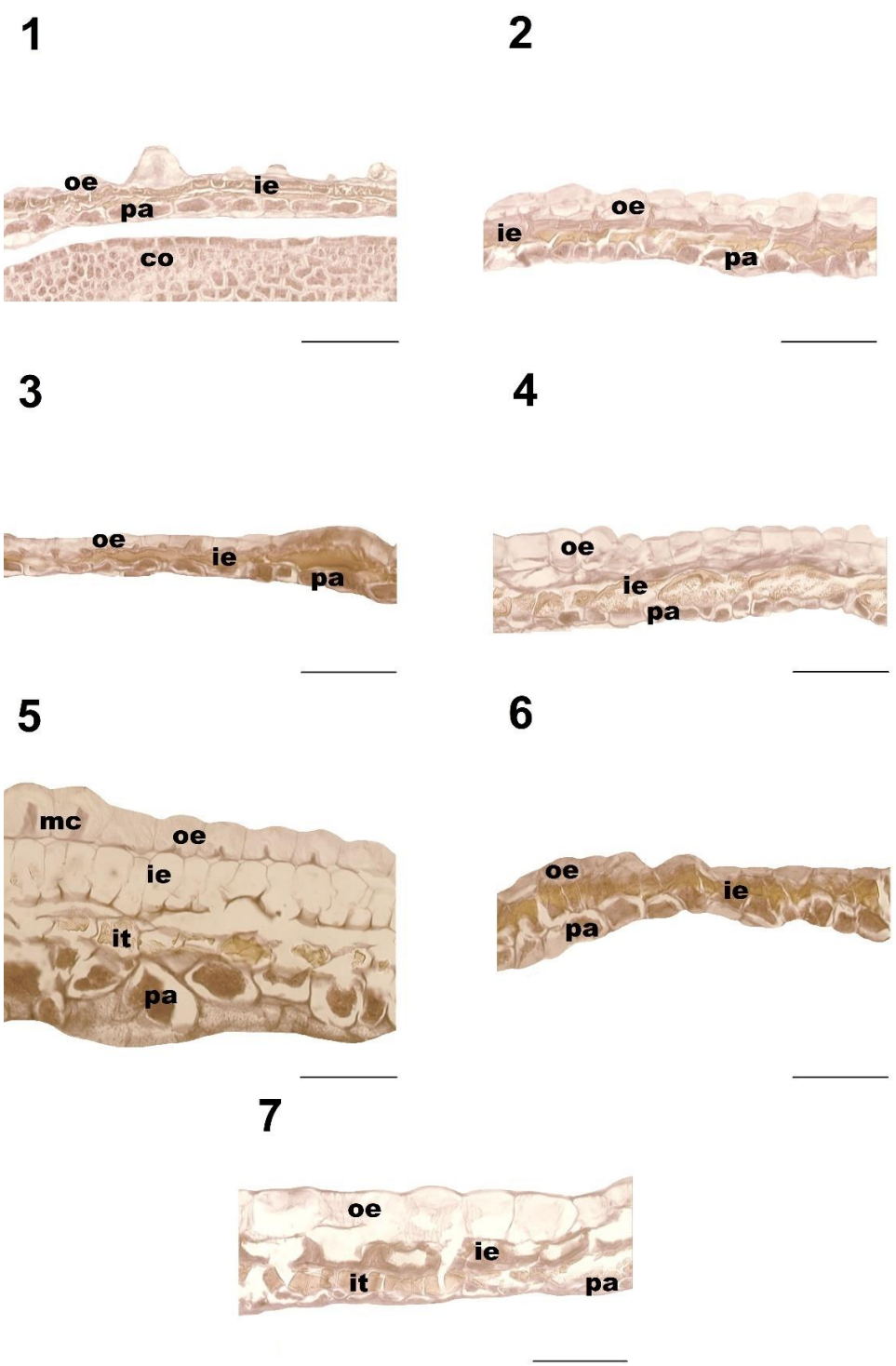

Figure 3. The anatomical structures of the seed testa of the studied taxa: 1- *C. bulbifera*, 2- *C. hirsuta*, 3- *C. impatiens* var. *pectinata*, 4- *C. lazica*, 5- *C. tenera*, 6- *C. uliginosa*, 7- *C. graeca* (oe: outer epidermis, ie: inner epidermis, it: inner testa, co: cotyledon, pa: parenchyma, mc: mucilage cells, scale bars: 100 µm)

Şekil 3. Çalışılan taksonların tohum testalarının anatomik yapıları: 1- C. bulbifera, 2- C. hirsuta, 3- C. impatiens var. *pectinata, 4- C. lazica, 5- C. tenera, 6- C. uliginosa, 7- C. graeca (oe: dış epidermis, ie: iç epidermis, it: iç testa, co: kotyledon, pa: parenkima, mc: musilaj hücreleri, ölçekler: 100 µm)*

The surface ornamentation types, anticlinal-periclinal cell walls, and epidermal cell forms of the seeds are micromorphologically researched. The seed surface ornamentation is categorized into five types: reticulate-areolate, scalariform, reticulate, foveate, reticulate-foveate (Figure 2 and Table 3). The most common types are reticulate and scalariform, seen in *C. hirsuta*, *C. impatiens* var. *pectinata*, *C. lazica* and *C. graeca*. The reticulate-alveolate (in *C. bulbifera*),

foveate (in *C. tenera*), and reticulate-foveate (in *C. uliginosa*) types are each characterized by solely one taxon. While the forms of anticlinal cell walls in the studied taxa are raised, periclinal cell walls are concave except *C. bulbifera* (convex). Moreover, cell shapes on surfaces are very different: polygonal, alveolar and rectangular. The most common cell type is polygonal, whereas rectangular is quite rare types (Table 3).

The outcomes of the anatomical examinations of the seeds are demonstrated in Figure 3 and Table 4. The seed testa of the studied taxa consists of the outer epidermis and inner epidermis (outer testa), rarely inner testa and parenchyma layers. The epidermis layers in outer testa are of either parenchymatic or sclerenchymatic form and occur with two layers involving the outer epidermis and the inner epidermis. According to the obtained observations from the cross-sections, the outer epidermis differs considerably in cell shape (flattened, rectangular or cubic), and wall formation (undulated or straight and thick or thin-walled) (Figure 3). The two most common types are flattened and cubic, however; the rarest one is the rectangular form (Table 4). The inner epidermis includes 1 layer of flat, rectangular and elongated rectangular cells. The average thickness of the outer testa varies between 25.64 µm (in *C. impatiens* var. *pectinata*) and 98.75 µm (in *C. tenera*). The inner testa, which is a compressed tissue under the outer testa, has 1–2 layers of flat, or crushed cells. This layer is seen only in 2 taxa studied, *C. graeca* and *C. tenera*. Its thickness ranges from 21.19 µm (in *C. graeca*) to 48.25 µm (in *C. tenera*) (Figure 3 and Table 4). The parenchyma layer of the examined taxa is 1-layered and comprises of flat cells. The thickness of the parenchyma layer ranges from 11.18 µm to 44.65 µm; the largest endosperm is observed in *C. tenera* and the thinnest in *C. graeca* (Table 4). In addition, mucilage cells are observed in epidermis layers in seeds of *C. tenera* unlike other taxa (Figure 3 and Table 4).

The numerical assessment of the seed morphological and anatomical features allows the form of a dendrogram, which reveals the variations or resemblances among the studied taxa. A dendrogram is created as a consequence of the cluster analysis of the examined taxa of *Cardamine* built on the difference of 38 characteristics in 7 taxa. The cophenetic correlation coefficient is designed to discover the correlation between the dendrogram and dissimilarity matrix (Table 5 and Figure 4). The cophenetic correlation between the dissimilarity matrix and dendrogram has been computed as 0.68, representing a good match. As a result of the cluster analysis, 2 clusters have emerged; A and B. Cluster A consists of *C. graeca*, *C. impatiens* var. *pectinata* and *C. lazica*. Cluster B contains *C. bulbifera*, *C. tenera*, *C. hirsuta* and *C. uliginosa* (Figure 4). *C. hirsuta* and *C. tenera* are the most closely related taxa (dissimilarity coefficient: 0.88), as *C. graeca* and *C. uliginosa* are the most distantly related taxa (dissimilarity coefficient: 2.39) (Table 5).

DISCUSSION

The morphological features of the seeds offer valuable data regarding the evolutionary relations of the angiosperms (Corner, 1976). The seed surface, shape, color, and dimension in Brassicaceae family are precious features in separation of the taxa (Barthlott, 1981; Koul et al., 2000; Karaismailoğlu and Erol, 2018). *Cardamine* taxa vary among the species in terms of the seed morphological characters. Seed shape is almost sufficient to distinguish among the examined taxa, it is the same in only *C. tenera*-*C. uliginosa* taxa, different in others. Seed color in the studied taxa is brown and its tones. *C. impatiens* var. *pectinata* and *C. lazica* are easily distinguished from other taxa by having light brown seeds. Seed surface morphology is of the taxonomic importance at the genus and species levels (Brochmann, 1992; Karaismailoğlu and Erol, 2018). *C. graeca* is separated with its slightly striped seed surfaces from other taxa, which have a smooth surface. The macromorphological results of this research are compatible with the former works performed with macromorphological features on seeds of Brassicaceae family (Khalik and Maesen, 2002; Kasem et al., 2011; Kaya et al., 2011; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a, 2019b; Şirin and Karaismailoğlu, 2020).

The significance and efficiency of scanning electron microscopy in explaining of the taxonomic difficulties (Heywood, 1971). Many studies have shown that seed micromorphological features are helpful characteristics to utilize in discriminating of the taxa in Brassicaceae family (Barthlott, 1981; Koul et al., 2000; Kasem et al., 2011; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2016, 2019a, 2019b; Şirin and Karaismailoğlu, 2020). In this work, it has utilized scanning electron microscopy to explain difficulties in taxonomy of taxa, and reveal adaptive significance of seed coat. All of the studied taxa have been examined for the first time. 5 different surface types have found: reticulate, reticulate-foveate, foveate, reticulate-alveolate, and scalariform. Reticulate seed ornamentation type is the most common in Brassicaceae, as indicated in this work (Tantawy et al., 2004). The scalariform ornamentation type has found in the genus *Thlaspi* L., *Aethionema* W.T. Aiton (Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a). Earlier seed exomorphic investigations have demonstrated that the structures of anticlinal-periclinal cell walls are well identification factors at the species level (Barthlott, 1981; Tantawy et al., 2004). Also, the structures of anticlinal-periclinal cell

Table 3. Macro and micro morphological characters of the seeds of the studied taxa.
Çizelge 3. *Çalışılan taksonların tohumlarının makro ve mikro morfolojik karakterleri*

Taxa *Taksonlar*	**Color** *Renk*	**Shape** *Şekil*	**Seed surfaces** *Tohum yüzeyleri*	**Seed Sizes*** *Tohum boyutları* **L (mm)**	**W (mm)**	**Raphe presence** *Rafe varlığı*	**Seed surface ornamentations** *Tohum yüzey ornamentasyonları*	**Anticlinal cell wall** *Antiklinal hücre duvarı*	**Periclinal cell wall** *Periklinal hücre duvarı*	**Epidermal cell structures** *Epidermal hücre yapıları*
C. bulbifera	Light Brown	Ovatus	Smooth	1.03±0.15bc	0.41±0.04d	-	Reticulate-Alveolate	Raised	Convex	Polygonal and Alveolar cells
C. hirsuta	Brown	Ellipticus-Rectangularis	Smooth	1.09±0.10b	0.95±0.08b	-	Scalariform	Raised	Concave	Rectangular cells
C. impatiens var. *pectinata*	Light Brown	Rectangularis	Smooth	0.92±0.08c	0.63±0.04c	+	Scalariform	Raised	Concave	Rectangular cells
C. lazica	Light Brown	Circularis-Rectangularis	Smooth	1.05±0.06b	0.91±0.04b	-	Reticulate	Raised	Concave	Polygonal cells
C. tenera	Dark Brown	Ellipticus-Late	Smooth	0.91±0.08c	0.84±0.06b	+	Foveate	Raised	Concave	Alveolar cells
C. uliginosa	Brown	Ellipticus-late	Smooth	1.14±0.21b	1.03±0.10b	+	Reticulate-Foveate	Raised	Concave	Polygonal and Alveolar cells
C. graeca	Brown	Ellipticus	Slightly striped	2.20±0.18a	1.65±0.12a	-	Reticulate	Raised	Concave	Polygonal cells

*Average value ± standard deviation; means with dissimilar letters are important at P = 0.05 by Duncan's multiple range test, + =present, - =absent, L=length, W=width.
**Ortalama değer ± standard sapma; Farklı harflere sahip ortalamalar Duncan'ın çoklu aralık testine göre P = 0.05'te önemlidir, + =var, - =yok, L=uzunluk, W=genişlik.*

Table 4. Testa anatomical characters of the studied taxa
Çizelge 4. *Çalışılan taksonların testa anatomik karakterleri.*

Taxa *Taksonlar*	**Outer testa** *(Dış testa)*			**Inner testa** *(İç testa)*		**Parenchyma layer**		**Presence or absence of mucilage cells**
	Outer epidermis structures *Dış epidermis yapıları*	**Inner epidermis structures** *İç epidermis yapıları*	**Thickness* (µm)** *Kalınlık*	**Structure** *Yapı*	**Thickness* (µm)** *Kalınlık*	**Structure** *Yapı*	**Thickness* (µm)** *Kalınlık*	*Musilaj hücrelerinin varlığı veya yokluğu*
C. bulbifera	1 layer, flattened cells	1 layer, flat cells	32.17±2.54e	-	-	1 layer, flat cells	24.16±1.09b	-
C. hirsuta	1 layer, cubic cells	1 layer, rectangular cells	51.29±2.35c	-	-	1 layer, flat cells	23.79±1.35b	-
C. impatiens var. *pectinata*	1 layer, rectangular cells	1 layer, flat cells	25.64±2.21f	-	-	1 layer, flat cells	15.84±2.16c	-
C. lazica	1 layer, cubic cells	1 layer, flat cells	44.93±1.78d	-	-	1 layer, flat cells	16.11±1.38c	-
C. tenera	1 layer, cubic cells	1 layer, elongated rectangular cells	98.75±9.56a	1-2 layer, crushed cells	48.45±2.56a	1 layer, flat cells	44.65±5.65a	-
C. uliginosa	1 layer, flattened cells	1 layer, elongated rectangular cells	55.62±2.55bc	-	-	1 layer, flat cells	40.09±2.35a	-
C graeca	1 layer, cubic cells	1 layer, flat cells	60.15±4.17b	1 layer, flat cells	21.19±2.09b	1 layer, flat cells	11.18±0.58d	-

*Average value ± standard deviation; means with dissimilar letters are important at P = 0.05 by Duncan's multiple range test, + =present, - =absent.
**Ortalama değer ± standard sapma; Farklı harflere sahip ortalamalar Duncan'ın çoklu aralık testine göre P = 0.05'te önemlidir, + =var, - =yok.*

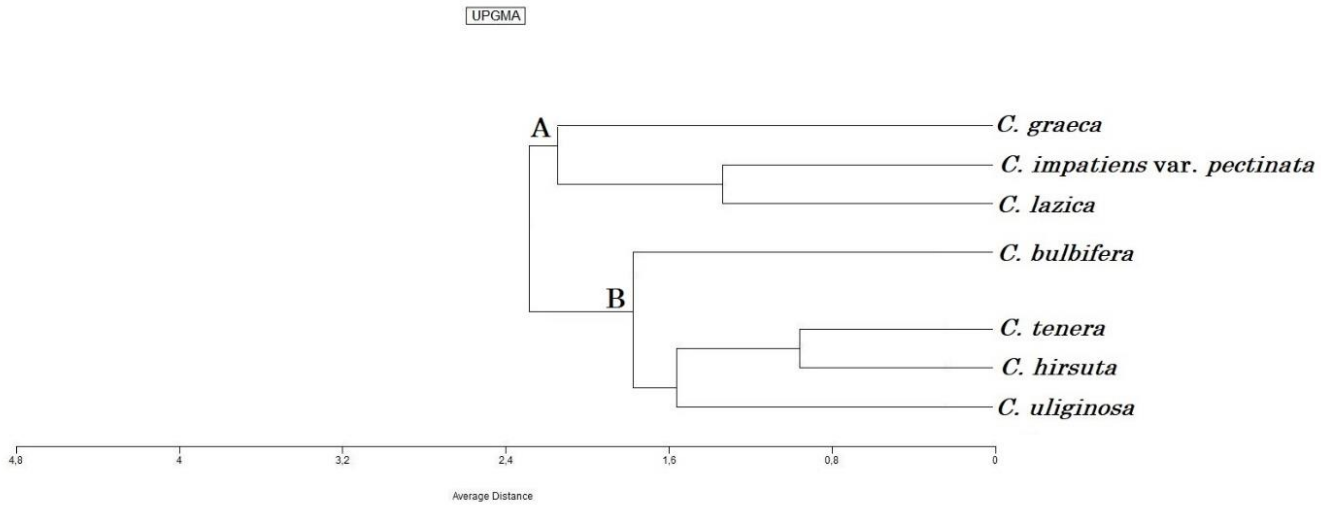

Figure 4. Cluster analysis of the studied taxa.
Şekil 4. Çalışılan taksonların kümeleme analizi

Table 5. The dissimilarity matrix of the studied taxa
Çizelge 5. İncelenen taksonların benzemezlik matrisi.

Taxa *(Taksonlar)*	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C. bulbifera **(1)**	0	-	-	-	-	-	-
C. hirsuta **(2)**	1.87	0	-	-	-	-	-
C. impatiens **var. *pectinata*** **(3)**	2.11	2.33	0	-	-	-	-
C. lazica **(4)**	2.13	2.17	1.45	0	-	-	-
C. tenera **(5)**	1.88	0.88	2.24	1.57	0	-	-
C. uliginosa **(6)**	1.95	1.44	1.82	1.59	1.36	0	-
C. graeca **(7)**	2.29	2.35	2.16	2.36	2.31	2.39	0

walls are active in discrimination of the taxa. The type of the anticlinal cell wall is raised, as the type of the periclinal cell wall is concave or convex. Taxa of *Cardamine* are different in terms of epidermal cell shapes. Epidermal cells are polygonal, rectangular or alveolar types. Generally, scanning electron microscope works have revealed that the comprehensive assessment of seed structures of the *Cardamine* taxa is considerably valuable in discriminating taxa from each other.

The performed works on the seed coat anatomy in the Brassicaceae have used to overcome of the taxonomic difficulties (Vaughan et al., 1976; Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a, 2019b). It has been informed that the seed coat characters can be utilized as consistent characteristics in the taxonomy of the taxa (Koul et al., 2000; Karaismailoğlu and Erol, 2018). The seed testa structures belonging to some genera in Brassicaceae have anatomically defined by Vaughan et al. (1976), Meyer (1973, 1991), Karaismailoğlu and Erol (2018), Karaismailoğlu (2019a, 2020). The seed coat mostly comprises 4 layers, which are the epidermis, the subepidermis, the sclerotic or palisade, and the parenchymatic layers (Bouman, 1975; Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018). In this work, anatomical structures of seed coats of the examined taxa have been analyzed in detail for the first time, and it is debated whether the anatomical features of the seeds are able to be utilized for the studied taxa to resolve reported systematic difficulties. The seed coat occurs from discriminated layers as the outer epidermis, the inner epidermis (outer testa) and the parenchymal layer. Contrary to the literature information, there is no inner testa except for *C. tenera* and *C. graeca* taxa.

The anatomical works performed on seed coats of species belonging various genera in Brassicaceae have showed that seed epidermis cell types can be used as a reliable taxonomic character. This character has been found in 15 different types by Vaughan and Whitehouse (1971), and 4 different types by Karaismailoğlu and Erol (2018). In this research, the type of the epidermis quite differs among the taxa. This 1-layered epidermis layers can occur from flat, rectangular, cubic or elongated rectangular. As flat and cubic cells are commonly observed, rectangular cells are the rarest in the studied taxa. The inner testa is the sclerotic or palisade structure, which is a compressed tissue under the outer testa. This layer is

found in only two of the studied taxa (*C. tenera* and *C. graeca*). It consists of 1–2 layers and its cells are be flat or crushed.

The parenchyma thickness of seed coats of the taxa has also been systematically analyzed for the first time in this work, and it is noticed that its features differ among the taxa. The parenchyma thickness ranges from 11.18 µm (*C. graeca*) to 44.65 µm (*C. tenera*). However; The number of layers and cell shape are the same in all taxa examined. Notwithstanding the often mentioned convergence on flowers and fruits in Brassicaceae, epidermis layers, and parenchymal thickness of the seed testa of the studied taxa have demonstrated to be more useful characters than the traditional ones utilized in taxonomy of *Cardamine*. Karaismailoğlu and Erol (2018) had similar outcomes with studying anatomically of *Thlaspi* L. from Turkey. Also, the presence of mucilage cells distinguishes *C. tenera* from others.

A dendrogram was created to assess the morphological and anatomical features of the seeds of the studied *Cardamine* taxa with UPGMA cluster analysis. The dendrogram, showing 2 main groups, was partly similar with the results of Cullen (1965). The morphological and anatomical characters of the seeds have maintained the characters utilized in the ranking of *Cardamine* taxa in the flora of Turkey (Figure 4).

In conclusion, studying the morphological and anatomical characteristics of seeds of the studied taxa of *Cardamine* offers major assists in terms of the systematics of taxa within the genus.

Key to examined *Cardamine* taxa, based on seed characteristics

1. Seed color is light brown 2
2. Seed surface ornamentation is scalariform *C. impatiens* var. *pectinata*
2. Seed surface ornamentation is reticulate .*C. lazica*
1. Seed color is brown or dark brown 3
3. Seed shape is ovatus *C. bulbifera*
3. Seed shape is ellipticus, ellipticus-late, ellipticus-rectangularis 4
4. Seed surface ornamentation is scalariform *C. hirsuta*
4. Seed surface ornamentation is foveate, reticulate, reticulate-foveate 5
5. Outer testa consists of flattened cells *C. uliginosa*
5. Outer testa does not consist of flattened cells .6
6. Mucilage cells are presence *C. tenera*
6. Mucilage cells are absence *C. graeca*

Statement of Conflict of Interest

Author has declared no conflict of interest.

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