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Practicality of Seed Morpho-anatomical Characters for the Identification of Species *Alyssum* (Brassicaceae) in Turkey: a Systematic Approach

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ABSTRACT

In this work, morphological and anatomical features of seeds of 15 *Alyssum* taxa (*A. caricum*, *A. davisianum*, *A. filiforme*, *A. haussknechtii*, *A. murale* subsp. *murale* var. *murale*, *A. simplex*, *A. sibiricum*, *A. desertorum*, *A. hirsutum* subsp. *hirsutum*, *A. minutum*, *A. strictum*, *A. strigosum* subsp. *strigosum*, *A. szowitsianum*, *A. dasycarpum* and *A. linifolium* var. *linifolium*) from Turkey were examined with the cluster analysis, and the systematic importance of these characteristics was assessed. The findings exhibited that there were variations among the taxa with respect to seed form, color, and wing existence. The seed dimensions were between 0.43 - 2.12 mm in length, and between 0.34 - 2.05 mm in width. *A. davisianum* and *A. filiforme* were remarkably distinct from others in terms of seed dimension, the first having the smallest and the second having the largest seeds. The surface ornamentation was categorized as nine types; reticulate-foveate, scalariform, tuberculate, alveolate, reticulate-alveolate, aculate, ruminate, verrucate and rugose. The most common type was alveolate, however; tuberculate and rugose types were taxon specific. Also, formation and thicknesses in testa and endosperm layers were studied anatomically. Seed morphological and anatomical features with a few exclusions revealed variety and they were of the taxonomic significance in discrimination of the studied taxa.

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Türkiye'de *Alyssum* (Brassicaceae) Türlerinin Tanımlanmasında Tohum Morfo-anatomik Karakterlerinin Uygulanabilirliği: Sistematik Bir Yaklaşım

ÖZET

Bu çalışmada, Türkiye'den 15 *Alyssum* taksonunun (*A. caricum*, *A. davisianum*, *A. filiforme*, *A. haussknechtii*, *A. murale* subsp. *murale* var. *murale*, *A. simplex*, *A. sibiricum*, *A. desertorum*, *A. hirsutum* subsp. *hirsutum*, *A. minutum*, *A. strictum*, *A. strigosum* subsp. *strigosum*, *A. szowitsianum*, *A. dasycarpum* ve *A. linifolium* var. *linifolium*) tohumlarının morfolojik ve anatomik özellikleri küme analizi ile incelenmiş ve bu özelliklerin sistematik önemi değerlendirilmiştir. Bulgular, taksonlar arasında tohum şekli, rengi ve kanat varlığı açısından farklılıklar olduğunu ortaya çıkarmıştır. Tohum boyutları 0,43 - 2,12 mm uzunluğunda ve 0,34 - 2,05 mm genişliğinde olmuştur. *A. davisianum* ve *A. filiforme*, tohum boyutu açısından diğerlerinden oldukça farklıydı, birincisi en küçük, ikincisi en büyük tohumlara sahipti. Yüzey ornamentasyonları dokuz tip olarak sınıflandırılmıştır; ağsı-foveat, skalariform, tüberkülat, alveolat, ağsı-alveolat, akulat, ruminat, verrukat ve rugoz. En yaygın tip alveolattı, buna karşın; tüberkülat ve rugoz tipleri taksona özgüydü. Ayrıca testa ve endosperm tabakalarındaki oluşum ve kalınlıklar anatomik olarak incelenmiştir. Tohum morfolojik ve anatomik özellikleri, birkaç istisna dışında çeşitliliği ortaya koydu ve incelenen taksonların ayrımında taksonomik öneme sahipti.

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INTRODUCTION

Brassicaceae (Cruciferae) is one of the biggest Spermatophyta families, involving 340 genera and 3740 taxa dispersed through the areas mostly on mild areas of the Northern Hemisphere (Khalik and Maesen, 2002; Hohmann et al., 2015; Karaismailoğlu, 2017). The genus *Alyssum* L. has over 195 taxa in worldwide with major spread in Turkey and Eastern Europe (Warwick et al., 2006; Bülbül et al., 2019). The genus is represented by more than 100 taxa in Turkey (Güner et al., 2012).

The genus *Alyssum* and many genera in the family are taxonomically problematic, due to very variable in habit, fruit and floral morphology (Dudley, 1965; Karaismailoğlu, 2018). This situation triggers some difficulties in classification of the genus; and so extra characteristics are required in the classification of the genus in addition to traditional characters.

Micromorphological characters are systematically important in separation of the plant taxa (Brochmann, 1992), in defining of the evolutionary links and in explaining of the systematic difficulties (Khalik and Maesen, 2002; Karaismailoğlu, 2019a). Also, forms of the epidermal cells are excellent identification features at species level (Barthlott, 1981; Khalik and Maesen, 2002; Karaismailoğlu, 2019b). In addition, seed anatomical characteristics are utilized in separating of closely related taxa in the genera (Karaismailoğlu, 2016 and 2019a). However, there are only few systematic studies on seeds features of *Alyssum* (Bülbül et al., 2019; Şirin, 2019). Thus, the purpose of this investigation is to assess seed morpho-anatomical features of the examined *Alyssum* taxa, and their utilize in infrageneric delimitation.

MATERIAL and METHOD

The seeds belonging to 15 *Alyssum* taxa were used for the morpho-anatomical examinations. The samples were gathered from native inhabitants and collected in Siirt University Fauna and Flora center (SUFAF) (Table 1). Evaluations were made on 50 mature seeds for each taxon.

Morphological characters like shape, dimension and color of seeds were analyzed utilizing stereomicroscope and Kameram Imaging Software (KIS). To observe micromorphological structures, samples were arranged for Scanning Electron Microscopy (SEM) by sticking with silver-paste on the stub, and covered with gold (Karaismailoğlu, 2015).

The cross-sections were obtained with an automatic microtome from the mid-section of seeds. The samples were put in FAA for 24 hours. Afterward, they were dehydrated via ethanol and xylene sets, and dyed with Hematoxylin-Eosin Y, and were covered with entellan to see anatomical elements (Karaismailoğlu, 2015). The anatomical features involving testa and endosperm were examined, and their pictures were taken with utilizing Olympus CX21FS1 light microscope and KIS.

The terminology of morpho-anatomical features of seeds was suitable with Stearn (Stearn,1985).

Clustering of taxa was done with applying the grouping assessment approach (UPGMA) as per 59 characteristics in Tables 2-3 [Characteristics utilized in statistical examination; seed color: brown or dark brown (1), clear brown (2), dark brown-black (3), shape: ellipticus (4), ovatus (5), circularis (6), circularis-transverse (7), ovatus-late (8), ovatus-transverse late (9), surface: coarse protrusions (10), smooth (11), slightly striped (12), reticulate (13), sizes: length (14), width (15), raphe presence (16), surface ornamentation: alveolate (17), scalariform (18), reticulate-alveolate (19), aculate (20), ruminate (21), verrucate (22), tuberculate (23), rugose (24), reticulate-foveate (25), anticlinal cell wall: raised (26), sunken (27), unclear (28), periclinal cell wall: concave (29), convex (30), unclear (31), epidermal cell structures: rectangular (32), pentagonal (33), alveolate (34), protrusion (35), polygonal (36), crushed polygonal (37), oval (38), unclear (39), the anatomical structures of outer epidermis of outer testa: flat (40), elongated rectangular (41), cubic (42), polygonal (43), oval (44), inner epidermis presence (45), inner epidermis structure: flat (46), rectangular (47), elongated rectangular (48), crushed (49), outer testa thickness (50), inner testa presence (51), inner testa structure: flat (52), rectangular (53), crushed (54), inner testa thickness (55), parenchyma structure: flat (56), rectangular (57), parenchyma thickness (58), presence of mucilage cell (59)]. Furthermore, the dissimilarity matrix of the analyzed taxa was created with MVSP (Kovach, 2007) (Table 4).

RESULTS

The seed features containing color, dimension, shape and surface characters are macromorphologically assessed (Figure 1 and Table 2). Seed colors of examined taxa are observed as brown, clear brown, dark brown, black-dark brown. Brown is the most

Table 1. The analyzed taxa and their locations.
Çizelge 1. Analiz edilen taksonlar ve lokasyonları.

No	Sections *Seksiyonlar*	Taxa *Taksonlar*	Location *Lokasyon*	Collection number *Koleksiyon numarası*
1	*Odontarrhena*	**Alyssum caricum* T.R.Dudley & Hub.-Mor.	Muğla, Marmaris, 13.8.2016	Karaismailoğlu 331
2	*Odontarrhena*	**A. davisianum* T.R.Dudley	Kütahya, Gediz, 16.6.2016	Karaismailoğlu 279
3	*Odontarrhena*	**A. filiforme* Nyár.	Gümüşhane, Kürtün, 13.7.2014	Karaismailoğlu 85b
4	*Odontarrhena*	**A. haussknechtii* Boiss.	Bolu, Abant, 21.5.2016	Karaismailoğlu 247
5	*Odontarrhena*	*A. murale* Waldst. & Kit. subsp. *murale* var. m*urale*	Gümüşhane, Kürtün, 13.7.2014	Karaismailoğlu 74b
6	*Odontarrhena*	*A. simplex* Rudolph	İstanbul, Büyükçekmece-Çatalca, 8.7.2016	Karaismailoğlu 312
7	*Odontarrhena*	*A. sibiricum* Willd.	Kütahya, Gediz, 24.6.2016	Karaismailoğlu 290
8	*Alyssum*	*A. desertorum* Stapf.	Ankara, Keçiören, 08.2016	Karaismailoğlu 337
9	*Alyssum*	*A. hirsutum* M.Bieb. subsp. *hirsutum*	Mersin, Mut, 27.7.2012	Karaismailoğlu 8
10	*Alyssum*	*A. minutum* Schlecht. ex DC.	Bursa, Uludağ, 1.7.2016	Karaismailoğlu 295
11	*Alyssum*	*A. strictum* Willd.	Niğde, Çamardı, 12.6.2016	Karaismailoğlu 271
12	*Alyssum*	*A. strigosum* Banks & Sol. subsp. *strigosum*	Bursa, Uludağ, 1.7.2016	Karaismailoğlu 296
13	*Alyssum*	*A. szowitsianum* Fisch. & C.A.Mey.	Gümüşhane, Zigana, 27.3.2015	Karaismailoğlu 115b
14	*Psilonema*	*A. dasycarpum* Stephan ex Willd.	Ankara, Haymana, 10.8.2016	Karaismailoğlu 330
15	*Meniocus*	*A. linifolium* Stephan ex Willd. var. *linifolium*	Konya, Cihanbeyli-Yavsan, 11.7.2016	Karaismailoğlu 316

* = endemic taxon
**= endemik takson*

Table 2. The seed macro and micro morphological features of the examined taxa (L=length, W=Width, +=present, -=absent, ±=standard deviation).
Çizelge 2. İncelenen taksonların tohum makro ve mikro morfolojik özellikleri (L=boy, W=en, +=mevcut, -=yok, ±= standart sapma).

Taxa *Taksonlar*	**Color** *Renk*	**Shape** *Şekil*	**Seed surface** *Tohum yüzeyi*	**Seed dimensions** *Tohum boyutları*		**Seed ornamentation** *Tohum ornamentasyonu*	**Anticlinal cell wall** *Antiklinal hücre duvarı*	**Periclinal cell wall** *Periklinal hücre duvarı*	**Epidermal cell form** *Epidermal hücre formu*
				L (mm)	**W (mm)**				
A. caricum	Dark Brown	Ellipticus	Coarse protrusions	1.32±0.18	1.05±0.12	Scalariform	Raised	Concave	Rectangular or Pentagonal
A. davisianum	Dark Brown	Ovatus	Smooth	0.43±0.08	0.34±0.06	Scalariform	Sunken	Convex	Rectangular
A. filiforme	Clear Brown	Circularis	Smooth	2.12±0.18	2.05±0.12	Alveolate	Sunken	Concave	Alveolate
A. haussknechtii	Brown	Ovatus	Slightly striped	0.75±0.24	0.47±0.16	Reticulate-Alveolate	Raised	Concave	Polygonal or Alveolate
A. murale* subsp. *murale* var. m*urale	Brown	Circularis-Transverse	Smooth	2.07±0.15	1.91±0.08	Aculate	Sunken	Convex	Protrusion
A. simplex	Black-Dark Brown	Ovatus-transverse late	Smooth	1.22±0.12	1.07±0.08	Ruminate	Unclear	Unclear	Unclear
A. sibiricum	Brown	Ovatus-late	Reticulate	1.02±0.04	0.95±0.04	Verrucate	Sunken	Convex	Oval
A. desertorum	Clear Brown	Circularis	Smooth	1.15±0.14	1.09±0.15	Tuberculate	Sunken	Convex	Unclear
A. hirsutum* subsp. *hirsutum	Dark Brown	Ovatus	Smooth	1.41±0.15	1.21±0.12	Aculate	Sunken	Convex	Alveolate
A. minutum	Brown	Ovatus-transverse late	Reticulate	1.38±0.18	1.29±0.15	Tuberculate	Sunken	Convex	Unclear
A. strictum	Brown	Circularis	Reticulate	1.08±0.06	1.01±0.04	Alveolate	Sunken	Concave	Oval
A. strigosum* subsp. *strigosum	Black-Dark Brown	Ovatus	Smooth	1.93±0.12	1.41±0.15	Verrucate	Sunken	Concave	Unclear
A. szowitsianum	Clear Brown	Ellipticus	Smooth	1.09±0.18	0.95±0.10	Rugose	Sunken	Convex	Crushed polygonal
A. dasycarpum	Brown	Ellipticus	Slightly striped	1.02±0.21	0.79±0.18	Reticulate-Foveate	Sunken	Convex	Polygonal
A. linifolium* var. *linifolium	Dark Brown	Ellipticus	Reticulate	1.28±0.21	1.05±0.10	Ruminate	Unclear	Unclear	Unclear

Table 3. The seed anatomical features of the examined taxa (+=present, -=absent, ±standart deviation).
Çizelge 3. İncelenen taksonların tohum anatomik özellikleri (+=mevcut, -=yok, ±= standart sapma).

Taxa *Taksonlar*	**Outer testa** *Dış testa*			**Inner testa** *İç testa*		**Parenchyma layer** *Parenkima tabakası*	
	Outer epidermis structures *Dış epidermis yapısı*	**Inner epidermis structure** *İç epidermis yapısı*	**Thickness** *Kalınlık* (µm)	**Structure** *Yapı*	**Thickness** *Kalınlık* (µm)	**Structure** *Yapı*	**Thickness** *Kalınlık* (µm)
A. caricum	1-2 layers, flat cells	-	28.39±2.06	-	-	1 layer, flat cells	20.18±1.24
A. davisianum	1 layer, elongated rectangular cells	1 layer, flat cells	59.86±4.03	-	-	1 layer, flat cells	33.85±2.39
A. filiforme	2-3 layers, polygonal cells	1 layer, flat cells	122.77±5.86	-	-	1 layer, flat cells	25.42±1.88
A. haussknechtii	1 layer, flat cells	2-3 layer, flat cells	67.42±3.24	-	-	1 layer, flat cells	27.17±0.99
A. murale subsp. ***murale*** var. m***urale***	1 layer, cubic or polygonal cells	1 layer, flat cells	56.27±2.51	-	-	1 layer, flat cells	19.76±2.54
A. simplex	1 layer, oval cells	1 layer, flat cells	69.27±3.46	1 layer, flat cells	22.38±1.28	1 layer, flat cells	27.52±2.41
A. sibiricum	1-2 layers, polygonal cells	1 layer, flat cells	72.19±2.45	1 layer, flat cells	39.72±2.87	1 layer, flat cells	35.28±3.17
A. desertorum	1 layer, cubic cells	1 layer, flat cells	50.24±2.11	-	-	1 layer, flat cells	21.16±3.07
A. hirsutum subsp. ***hirsutum***	2-3 layers, polygonal cells	1-2 layers, elongated rectangular cells	133.15±4.64	-	-	1 layer, flat cells	32.14±1.14
A. minutum	1 layer, elongated rectangular cells	1 layer, elongated rectangular cells	86.25±3.35	-	-	1 layer, flat cells	24.83±1.56
A. strictum	1 layer, elongated rectangular cells	1 layer, elongated rectangular cells	130.21±4.98	1 layer, rectangular cells	24.26±2.15	1 layer, flat cells	24.76±2.05
A. strigosum subsp. ***strigosum***	2-3 layers, polygonal cells	1-2 layers, crushed cells	138.49±3.77	-	-	1 layer, rectangular cells	29.44±3.29
A. szowitsianum	1-2 layers, polygonal cells	1-2 layers, elongated rectangular cells	115.89±2.54	1 layer, crushed cells	19.21±2.54	1 layer, flat cells	50.66±2.97
A. dasycarpum	1 layer, elongated rectangular cells	1 layer, flat cells	67.81±5.42	-	-	1 layer, flat cells	24.47±1.10
A. linifolium var. ***linifolium***	2-3 layers, polygonal cells	-	60.28±3.88	-	-	1 layer, flat cells	22.19±0.77

Table 4. The dissimilarity matrix of the examined taxa.
Çizelge 4. *Çalışılan taksonların benzemezlik matrisi.*

Taxa *Taksonlar*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
A. caricum **(1)**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. davisianum **(2)**	**1.06**	0	-	-	-	-	-	-	-	-	-	-	-	-	-
A. filiforme **(3)**	4.23	3.76	0	-	-	-	-	-	-	-	-	-	-	-	-
A. haussknechtii **(4)**	4.85	8.43	6.25	0	-	-	-	-	-	-	-	-	-	-	-
A. murale **subsp.** ***murale*** **var.** ***murale*** **(5)**	3.54	3.61	1.89	7.24	0	-	-	-	-	-	-	-	-	-	-
A. simplex **(6)**	4.19	8.08	11.75	10.75	6.67	0	-	-	-	-	-	-	-	-	-
A. sibiricum **(7)**	3.68	5.65	4.27	6.08	5.39	8.18	0	-	-	-	-	-	-	-	-
A. desertorum **(8)**	8.95	2.19	5.16	6.74	5.63	8.43	4.15	0	-	-	-	-	-	-	-
A. hirsutum **subsp.** ***hirsutum*** **(9)**	2.01	4.59	1.15	6.18	1.88	8.11	3.58	4.32	0	-	-	-	-	-	-
A. minutum **(10)**	2.48	4.96	4.32	5.34	5.18	8.54	3.14	5.58	3.19	0	-	-	-	-	-
A. strictum **(11)**	5.86	4.57	4.09	6.33	5.93	8.46	1.88	5.51	2.97	1.21	0	-	-	-	-
A. strigosum **subsp.** ***strigosum*** **(12)**	4.21	4.83	4.11	6.03	6.16	7.76	1.86	6.03	3.27	1.09	1.29	0	-	-	-
A. szowitsianum **(13)**	7.38	7.27	5.21	7.46	5.35	9.46	5.19	2.01	4.76	4.62	4.19	6.84	0	-	-
A. dasycarpum **(14)**	12.67	11.32	12.65	11.42	11.64	**12.95**	11.73	11.51	12.09	11.95	11.18	12.33	11.54	0	-
A. linifolium **var.** ***linifolium*** **(15)**	10.52	9.89	9.55	9.74	8.96	8.87	9.02	8.55	8.61	8.69	8.44	8.51	8.49	7.55	0

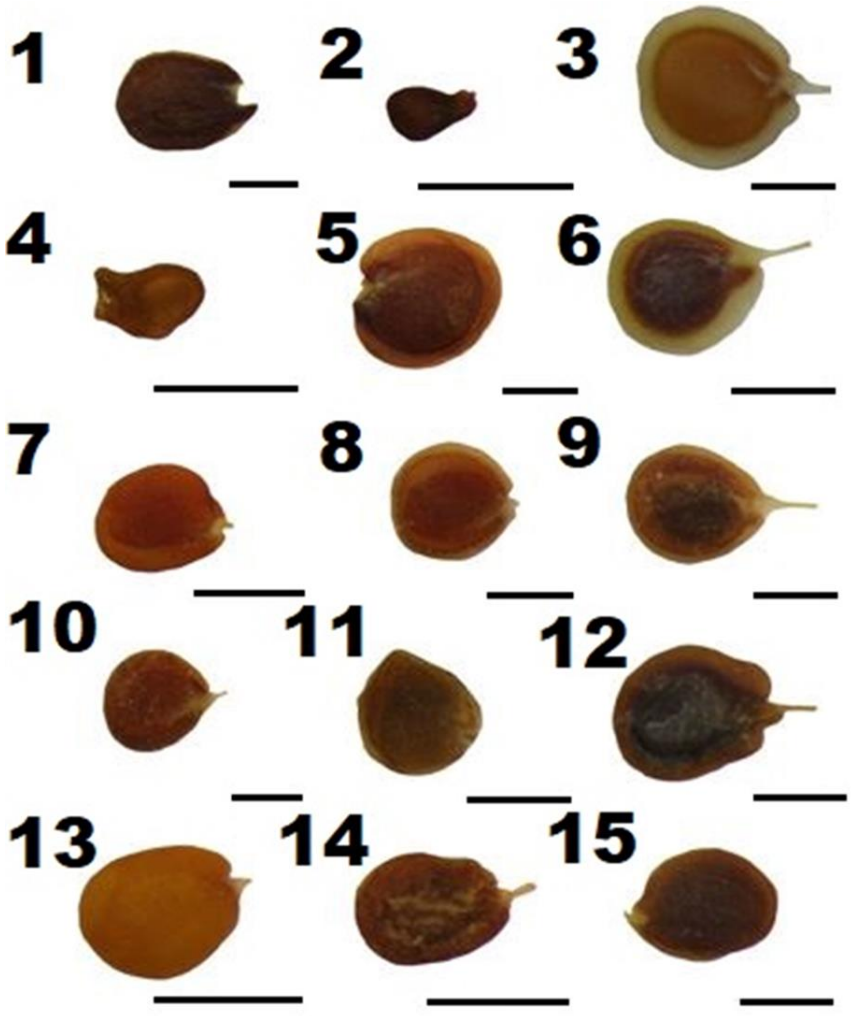

Figure 1. The seeds of the analyzed taxa; 1: *Alyssum caricum*, 2: *A. davisianum*, 3: *A. filiforme*, 4: *A. haussknechtii*, 5: *A. murale* subsp. *murale* var. *murale*, 6: *A. simplex*, 7: *A. sibiricum*, 8: *A. desertorum*, 9: *A. hirsutum* subsp. *hirsutum*, 10: *A. minutum*, 11: *A. strictum*, 12: *A. strigosum* subsp. *strigosum*, 13: *A. szowitsianum*, 14: *A. dasycarpum*, 15: *A. linifolium* var. *linifolium* (Scale bars=1 mm).

Şekil 1. Analiz edilen taksonların tohumları; 1: Alyssum caricum, 2: A. davisianum, 3: A. filiforme, 4: A. haussknechtii, 5: A. murale subsp. murale var. murale, 6: A. simplex, 7: A. sibiricum, 8: A. desertorum, 9: A. hirsutum subsp. hirsutum, 10: A. minutum, 11: A. strictum, 12: A. strigosum subsp. strigosum, 13: A. szowitsianum, 14: A. dasycarpum, 15: A. linifolium var. linifolium (Ölçekler= 1mm).

ordinary color, noticed in 6 of the studied taxa. 6 different seed shapes are found: ellipticus, ovatus, circularis, circularis-transverse, ovatus-late and ovatus-transverse late. Ovatus and ellipticus are the more common than other taxa. Ovatus-late and circularis-transverse types are characteristic for *A. sibiricum* and *A. murale* subsp. *murale* var. *murale*, respectively (Figure 1 and Table 2). The seed dimension varies between 0.43 - 2.12 mm in length, between 0.34 - 2.05 mm in width. *Alyssum davisianum* and *A. filiforme* are markedly distinct from other taxa in terms of seed dimension. Seed surface structure has demonstrated differences: smooth in 8 taxa, reticulate in 4 taxa, slightly striped in 2 taxa and coarse protrusion in 1 taxon (Figure 1 and Table 2). In addition, the presence of raphe has an important role in separation of some taxa with the same population appearance.

The seed ornamentation types, periclinal-anticlinal cell wall structures, and epidermal cell forms of the examined taxa are micromorphologically analysed. Seed ornamentation has recorded in 9 different types: alveolate, scalariform, reticulate-alveolate, aculate, ruminate, verrucate, tuberculate, rugose and reticulate-foveate (Figure 2 and Table 2). The most common types are scalariform, alveolate, aculate, ruminate, verrucate and tuberculate (each is seen in 2 taxa). Some types such as reticulate-alveolate (in *A. haussknechtii*), rugose (in *A. szowitsianum*) and reticulate-foveate (in *A. dasycarpum*) are specific to only 1 taxon. The anticlinal cell walls are in raised, sunken and unclear structures. The anticlinal cell wall of the ruminate ornamentation type does not contain special structure. Periclinal cell wall is convex (8 taxa), concave (5 taxa), or unclear (2 taxa) in form (Table 2). Also, the cell forms on surface are very distinct: rectangular, pentagonal, alveolate, protrusion, polygonal, crushed polygonal, oval, and unclear. The most common cell form is unclear, while protrusion is the rarest type (Table 2).

The outcomes of the seed anatomical examination are displayed in Figure 3 and Table 3. Generally, the seeds of the analysed samples are comprised of 4 layers, involving the outer epidermis, the inner epidermis (outer testa), the inner testa, and the endosperm. The outer epidermis consists of the elongated rectangular, flat, cubic, polygonal, and oval cell shapes, with the number of layers varying between 1 and 3 (Table 3). The most frequent type is polygonal, while the rarest one is the oval form (Figure 3). The inner epidermis consists of 1-3 layers of flat, crushed, and elongated rectangular cells. There is no the inner epidermis layer in *A. caricum* and *A. linifolium* var. *linifolium* taxa. The thickness of the epidermis layers (outer testa) varies between 28.39 µm (in *A. caricum*) - 138.49 µm (in *A. strigosum* subsp. *strigosum*). The inner testa, which is mostly a squeezed tissue below the outer testa layers, has 1-layered of flat, rectangular, or crushed cells in the studied 4 taxa (*A. strictum*, *A. simplex*, *A. szowitsianum* and *A. sibiricum*). Thickness of this layer ranges from 19.21 µm (in *A. szowitsianum*) to 39.72 µm (in *A. sibiricum*) (Table 3). The endosperm of the analysed samples is 1-layered, and contains mainly flat and rarely rectangular cells. Endosperm thickness ranges from 19.76 µm to 50.66 µm. The broadest endosperm is observed in *A. szowitsianum*; however, *A. murale* subsp. *murale* var. *murale* is of the narrowest. Mucilage cells are noticed in the outer testa layers of seeds of the examined taxa, outside of *A. caricum* (Figure 3 and Table 3).

The statistical evaluation of the seed morpho-anatomical features allows the produce of a dendrogram revealing the similarities and differences between the taxa studied. A dendrogram is created as a consequence of the cluster testing based on the difference and similarity of 59 characteristics in 15 Alyssum taxa. The co-phenetic relationship coefficient has computed to obtain the correlation between the dissimilarity matrix and dendrogram (Table 4 and Figure 4). The co-phenetic relationship between dissimilarity matrix and dendrogram has been determined as 0.59. The cluster analysis show that there are 2 main clusters as A and B: Cluster A1 contains *A. linifolium* var. *linifolium*, *A. szowitsianum* and *A. desertorum*. Cluster A2 consists of 2 subsets as A21 (*A. hirsutum* subsp. *hirsutum*, *A. filiforme*, *A. murale* subsp. *murale* var. *murale*, *A. davisianum*, *A. caricum*) and A22 (*A. sibiricum*, *A. strictum*, *A. minutum* and *A. strigosum* subsp. *strigosum*). Cluster B contains *A. haussknechtii* and *A. simplex*. *Alyssum dasycarpum* has created a clade branch outside of clusters in dendrogram (Figure 4). *A. caricum* and *A. davisianum* are the most closely related taxa (dissimilarity coefficient: 1.06), as *A. dasycarpum* and *A. simplex* are the most distantly related taxa (dissimilarity coefficient: 12.95) (Table 4).

DISCUSSION

The seed morphological data such as color, dimension and surface are valuable in explaining evolutionary relationships, solving taxonomic problems and separating closely related taxa in the family Brassicaceae (Vaughan and Whitehouse, 1976; Corner, 1976; Karaismailoğlu, 2016, 2019a, 2019b). The seed macromorphological features have demonstrated differences among the *Alyssum* species. The color is helpful in separating of some closely correlated taxa in terms of population appearance, flower and fruit characters, like *A. strictum* (brown) - *A. szowitsianum* (clear brown), *A. simplex* (black-dark brown) - *A. sibiricum* (brown) and *A. desertorum* -

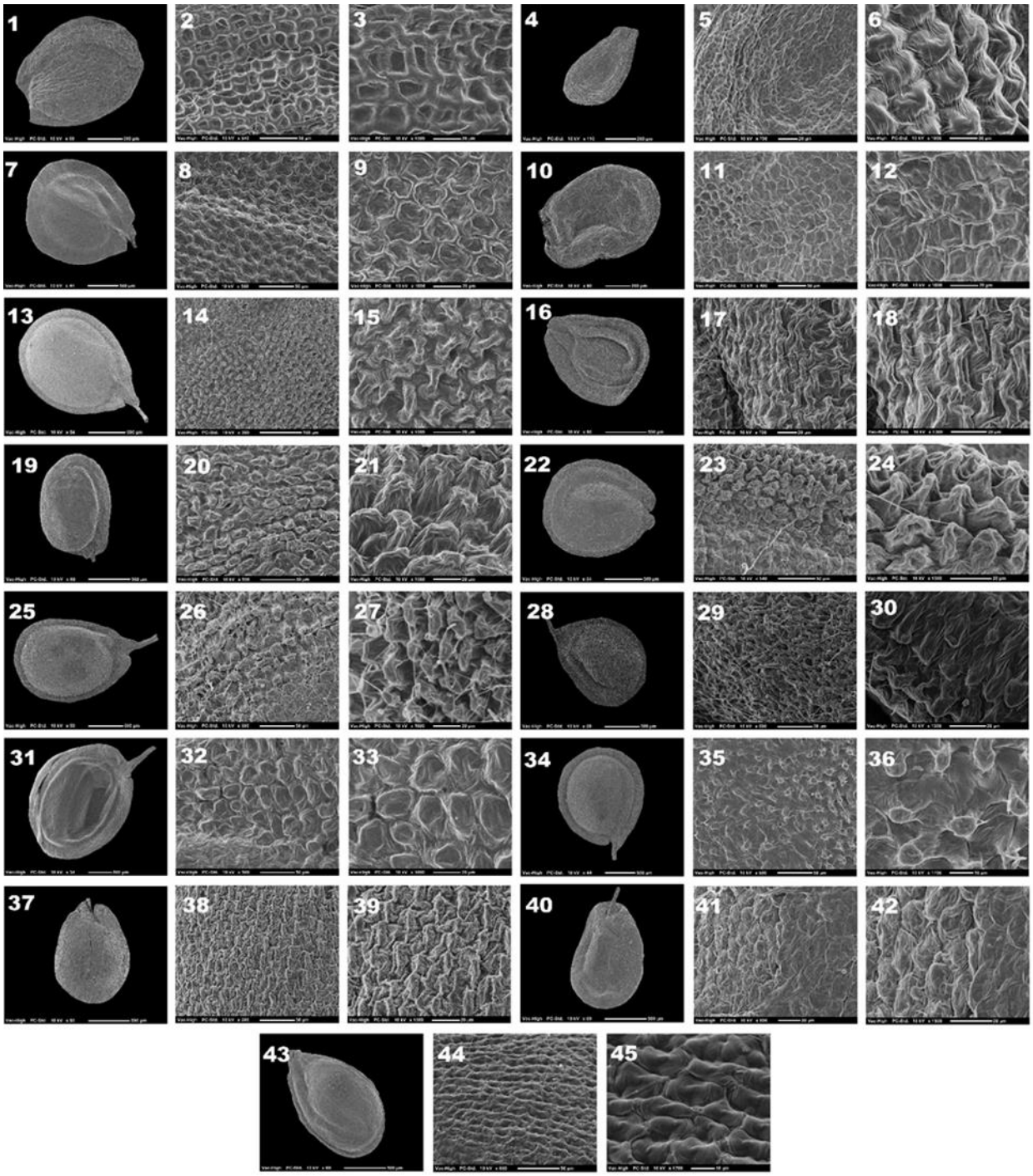

Figure 2. SEM pictures of the examined taxa: 1-3: *Alyssum caricum*, 4-6: *A. davisianum*, 7-9: *A. filiforme*, 10-12: *A. haussknechtii*, 13-15: *A. murale* subsp. *murale* var. *murale*, 16-18: *A. simplex*, 19-21: *A. sibiricum*, 22-24: *A. desertorum*, 25-27: *A. hirsutum* subsp. *hirsutum*, 28-30: *A. minutum*, 31-33: *A. strictum*, 34-36: *A. strigosum* subsp. *strigosum*, 37-39: *A. szowitsianum*, 40-42: *A. dasycarpum*, 43-45: *A. linifolium* var. *linifolium*.

Şekil 2. İncelenen taksonların SEM resimleri: 1-3: Alyssum caricum, 4-6: A. davisianum, 7-9: A. filiforme, 10-12: A. haussknechtii, 13-15: A. murale subsp. murale var. murale, 16-18: A. simplex, 19-21: A. sibiricum, 22-24: A. desertorum, 25-27: A. hirsutum subsp. hirsutum, 28-30: A. minutum, 31-33: A. strictum, 34-36: A. strigosum subsp. strigosum, 37-39: A. szowitsianum, 40-42: A. dasycarpum, 43-45: A. linifolium var. linifolium.

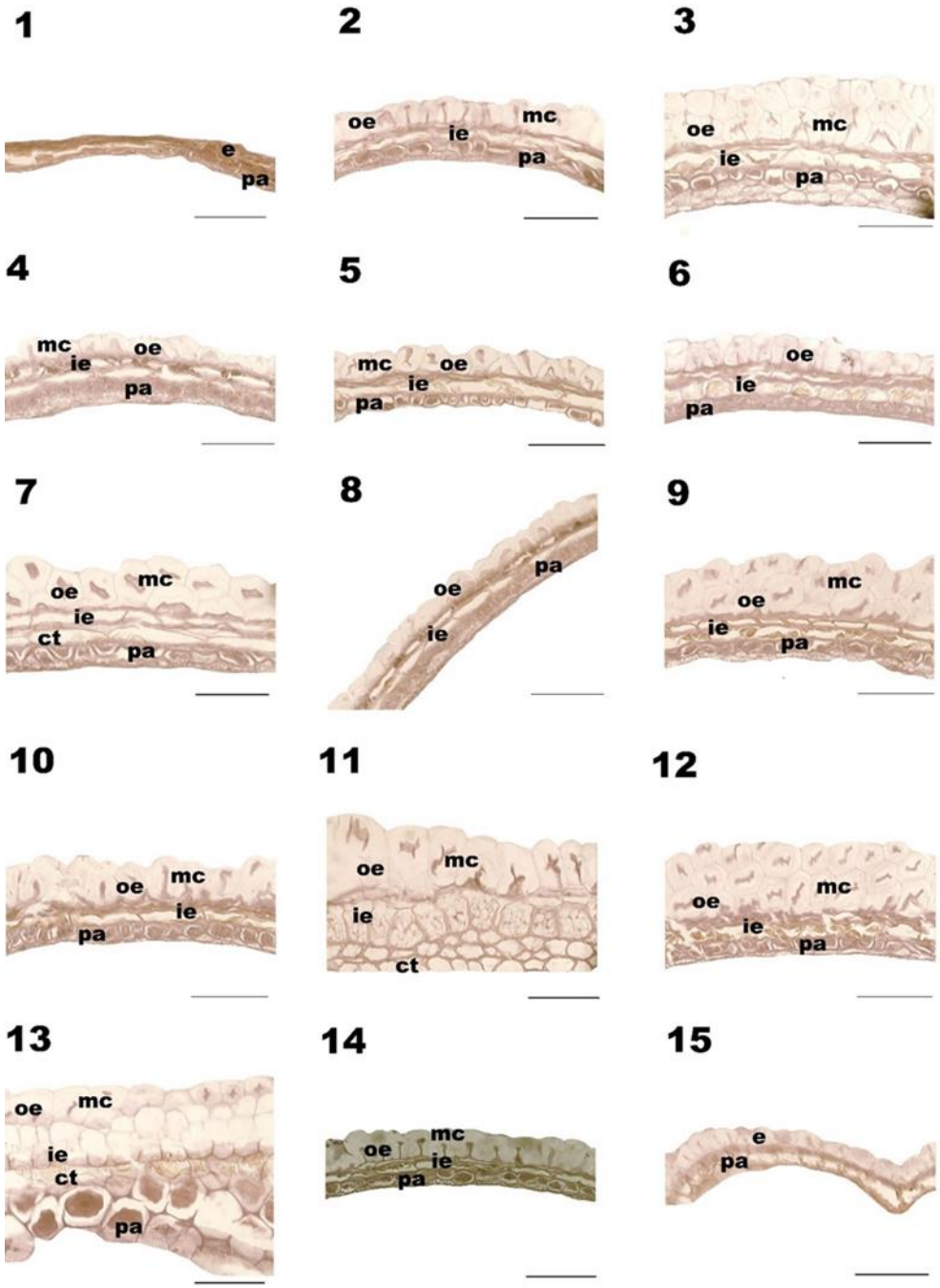

Figure 3. The anatomical structures of the seeds of the analyzed taxa; 1: *Alyssum caricum*, 2: *A. davisianum*, 3: *A. filiforme*, 4: *A. haussknechtii*, 5: *A. murale* subsp. *murale* var. *murale*, 6: *A. simplex*, 7: *A. sibiricum*, 8: *A. desertorum*, 9: *A. hirsutum* subsp. *hirsutum*, 10: *A. minutum*, 11: *A. strictum*, 12: *A. strigosum* subsp. *strigosum*, 13: *A. szowitsianum*, 14: *A. dasycarpum*, 15: *A. linifolium* var. *linifolium* (e: epidermis, oe: outer epidermis, ie: inner epidermis, pa: parenchyma, ct: compressed tissue=inner testa, mc: mucilages cells, scale bars=100 µm).

Şekil 3. Analiz edilen taksonların tohumlarının anatomik yapıları; 1: Alyssum caricum, 2: A. davisianum, 3: A. filiforme, 4: A. haussknechtii, 5: A. murale subsp. murale var. murale, 6: A. simplex, 7: A. sibiricum, 8: A. desertorum, 9: A. hirsutum subsp. hirsutum, 10: A. minutum, 11: A. strictum, 12: A. strigosum subsp. strigosum, 13: A. szowitsianum, 14: A. dasycarpum, 15: A. linifolium var. linifolium (e: epidermis, oe: dış epidermis, ie: iç epidermis, pa: parenkima, ct: baskılanmış doku=iç testa, mc: musilaj hücreleri, ölçekler=100 µm).

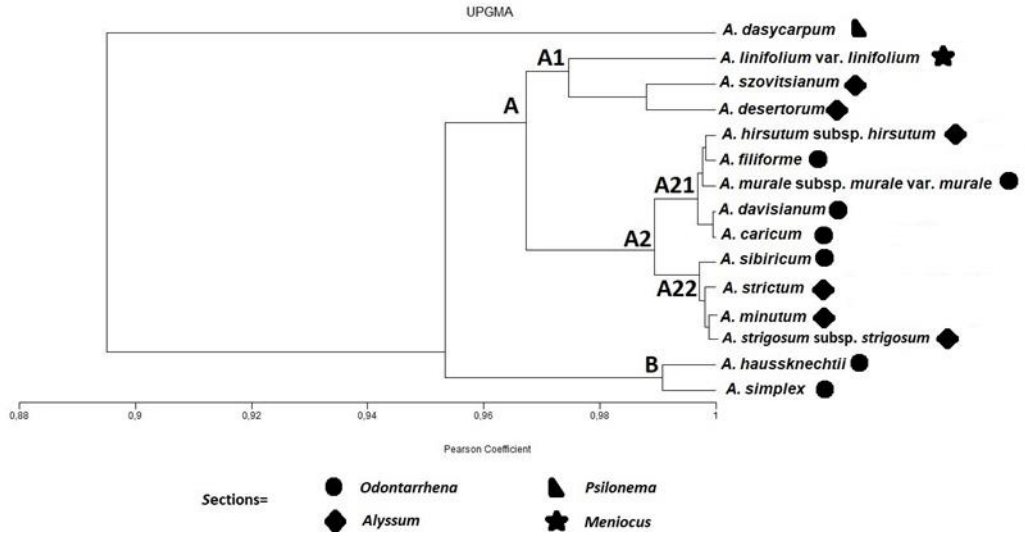

Figure 4. The dendrogram created with UPGMA of studied taxa.
Şekil 4. Çalışılan taksonların UPGMA ile oluşturulan dendrogramı.

(clear brown) *A. hirsutum* subsp. *hirsutum* (dark brown). Seed surfaces mirror ecological knowledge and adaptation of the species. So, they are diverse among species and are of the systematic importance (Brochmann, 1992; Karaismailoğlu, 2015, 2016, 2019a; Karaismailoğlu and Erol, 2018). In this work, seed surfaces of the studied species are in different structures: slightly striped, reticulate, smooth or coarse protrusions. This diversity has been found to be one of the reliable characters that distinguish taxa from each other. The obtained macro-morphological outcomes are coherent with the earlier findings, which are concerned on seed exomorphic features realized on some genus within the family (Kaya et al., 2011; Bona, 2013; Karaismailoğlu and Erol, 2018; Bülbül et al., 2019; Karaismailoğlu 2019a, 2019b; Şirin, 2019; Şirin and Karaismailoğlu, 2020).

The seed micromorphological features are of the high taxonomic significance (Karaismailoğlu and Erol, 2018; Şirin and Karaismailoğlu, 2020). The significance and effectiveness of scanning electron microscopy in resolving systematic problems within the family Brassicaceae has been shown in many studies performed on seed microstructures (Kaya et al., 2011; Kasem et al., 2011; Bona, 2013; Bülbül et al., 2019; Karaismailoğlu 2019b). The tested taxa have been analyzed for the first time, except for *A. caricum* (reticulate), *A. sibiricum* (reticulate-foveate) and *A. strictum* (ruminate) (Bülbül et al., 2019). In this work, 9 different ornamentation types have found as alveolate, scalariform, reticulate-alveolate, aculate, ruminate, verrucate, tuberculate, rugose and reticulate-foveate. The most common types are scalariform, alveolate, aculate, ruminate, verrucate and tuberculate (each is seen in 2 taxa), as reported in Tantawy et al. (2004) and Karaismailoğlu and Erol (2018). The surface ornamentation type is effective in separating of some closely related taxa; *A. strictum* (alveolate) - *A. szowitsianum* (rugose), *A. simplex* (ruminate) - *A. sibiricum* (verrucate) and *A. desertorum* (tuberculate) - *A. hirsutum* subsp. *hirsutum* (aculate). Earlier seed morphology reports have indicated that the structures of the anticlinal-periclinal cell walls are great problem-solving parameters within genus (Barthlott, 1981; Tantawy et al., 2004; Bona, 2013). Also, the forms of anticlinal-periclinal cell walls are useful in discrimination of taxa. As, anticlinal cell wall is in raised, sunken or unclear structures, the form of periclinal cell wall is concave, convex or unclear. *Alyssum* species are mostly distinct in terms of the epidermal cells, which may be rectangular, pentagonal, alveolate, protrusion, polygonal, crushed polygonal, oval and unclear structures. Generally, scanning electron microscope findings have revealed that the exhaustive analysis of seed features of *Alyssum* species is very helpful in identification and separation of taxa.

Studies of seed testa anatomy in the Brassicaceae family have enabled systematic problems to be overcome (Vaughan et al., 1976; Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a; Karaismailoğlu and Erol, 2020; Şirin and Karaismailoğlu, 2020). The testa anatomical features of some seed belonging to family have been reported by Vaughan et al. (1976), Karaismailoğlu and Erol (2018) and Karaismailoğlu (2019a). But still there are not enough studies explaining evolutionary relationships and including a phylogenetic perspective. The seed testa anatomical structures of the studied taxa have been revealed for the first time in this study. The seed testa mostly contains of 4 layers as the outer epidermis, the inner epidermis (outer testa), the inner testa, and the endosperm

(Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018). Vaughan and Whitehouse (1971) have analyzed the testa anatomical forms of seeds of 200 taxa belonging to 90 genera in the family and debated their usage as systematic characteristics, and discovered 15 form of epidermis cells. Besides, Karaismailoğlu and Erol (2018) have reported 4 different type in the seeds of *Thlaspi*. In this study, the outer and inner epidermis structures quite differ among taxa. They contain the elongated rectangular, flat, cubic, polygonal, crushed and oval cell shapes, with 1-3 layered. As flat and polygonal cells are commonly observed, oval cells are uncommon in the studied taxa. The inner testa has 1-layered of flat, rectangular or crushed cells in the studied 4 taxa (*A. sibiricum*, *A. simplex*, *A. szowitsianum* and *A. strictum*). The endosperm parenchyma of seed has showed differences among taxa in terms of structure and thickness. The endosperm of taxa is 1-layered and contains mostly flat and seldom rectangular cells. The widest endosperm is observed in *A. szowitsianum*; however, *A. murale* subsp. *murale* var. *murale* has the narrowest. Mucilage cells are noticed in the outer testa layers of seeds of the examined taxa, outside of *A. caricum*. Furthermore, mucilage presence on seed surfaces is acceptable as an ecological response to water shortage (Young and Martens, 1991). Since, the examined taxa are not observed in wetlands.

CONCLUSION

A dendrogram is designed to assess the seed morpho-anatomical features of the studied *Alyssum* taxa with UPGMA test. The dendrogram, representing 2 main groups, is somewhat congruent with the results of Dudley (1965). The seed morpho-anatomical variations have been seen in inter-species level, especially in closely related taxa like *A. strictum* - *A. szowitsianum*, *A. simplex* - *A. sibiricum* and *A. desertorum* - *A. hirsutum* subsp. *hirsutum*. As a result, assessing the seed morpho-anatomical characters of the studied taxa of *Alyssum* presents substantial contributions to the taxonomy of the genus.

Conflicts of Interest

No conflict of interest was declared by the author.

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