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Seed micromorphology of *Acanthophyllum* C.A.Mey. (Caryophyllaceae) Genus in Turkey

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Abstract: In this study, seeds of 5 species of *Acanthophyllum* in Turkey were examined with stereomicroscope and scanning electron microscope (SEM) to evaluate the systematic significance of seed microstructure. The seeds of *Acanthophyllum* were characterised by oblong or obovate shape. Seeds dorsal and ventral side are generally convex. The largest seeds are found in *A. acerosum* (2.45 mm in length and 1.16 mm in width). The smallest seeds are found in *A. verticillatum* (1.61 mm in length and 0.84 mm in width). Seeds surface of *Acanthophyllum* species are fine tuberculate arranged with elongate or polygonal cells like jigsaw puzzle. The shape of suture is sinuous, undulate or dentate. The colour of seed is reddish-brown or yellowish-brown. The radicle is on the one side of each seed and just out beyond the hilar notch. Colour of seeds, seed length and width, hilum length and width, suture shape, testa cell shape and the number of suture points per plate are important characters in differentiating *Acanthophyllum* species. By this study, similarities and differences between the species of *Acanthophyllum* genus are revealed and contributed to taxonomic studies.

Keywords: Caryophyllaceae, *Acanthophyllum*, Micromorphology, Seed, Scanning Electron Microscope (SEM)

Türkiye *Acanthophyllum* C.A.Mey. (Caryophyllaceae) cinsi tohum morfolojisi

Özet: Bu çalışmada Türkiye *Acanthophyllum* cinsine ait 5 türün tohum mikroyapısı sistematik önemini değerlendirmek için stereomikroskop ve taramalı elektron mikroskopu (SEM) ile incelenmiştir. Acanthophyllum tohumları oblong veya obovat şekli ile karakterizedir. Tohum dorsal ve ventral yüzeyleri genellikle konvekstir. En büyük tohumlar *A. acerosum'*da bulunur (2.45 mm uzunluğunda ve 1.16 mm genişliğindedir). En küçük tohumlar *A. verticillatum'*da bulunur (1.61 mm uzunluğunda ve 0.84 mm genişliğindedir). *Acanthophyllum* türlerinin tohumları, yapboz gibi uzun veya çokgen hücrelerle düzenlenmiş ince tüberküllüdür. Sütur şekli sinuous, undulate veya dentattır. Tohum rengi kırmızımsı kahverengi veya sarımsı kahverengidir. Radikula yalnızca bir tarafta ve hilum açıklığından daha ileride sonlanmaktadır. Tohum rengi, tohum uzunluğu ve genişliği, hilum uzunluğu ve genişliği, sütur şekli, testa hücrelerinin şekli sütur başına gözlenen diş sayısı *Acanthophyllum* türlerini ayırmada önemli karakterlerdir. Bu çalışmada, *Acanthophyllum* cinsi arasındaki benzerlikler ve farklılıklar ortaya çıkarılmış ve taksonomik araştırmalara katkıda bulunulmuştur.

Anahtar sözcükler: Caryophyllaceae, *Acanthophyllum*, Mikromorfoloji, Tohum, Taramalı elektron mikroskop (SEM)

Introduction

The family Caryophyllaceae is a large dicotyledonous family contains three subfamilies; Paronychioideae, Alsinoideae and Caryophylloideae (Bittrich, 1993). It contains 86 genera and almost 2200 species (Bittrich, 1993; Heywood, 1998). The genus *Acanthophyllum* having 60 species is generally distributed in the phytogeographic region of Irano-Turanian (Bittrich,1993). The genus *Acanthophyllum* C.A.Mey. belongs to the Caryophyllaceae family and is represented by 5 species: *A. acerosum* Sosn., *A. microcephalum* Boiss., *A. mucronatum* C.A.Mey., *A. verticillatum* C.A.Mey., and *A. oppositiflorum* Aytaç of which are endemic in Turkey (Aytaç, 2001).

There are some investigations carried out on seed morphology of different Caryophyllaceae genera by scanning electron microscopy (SEM). Such studies were applied for example to *Arenaria* L. (Wofford, 1981; Wyat,1984), *Bolanthus* (Ser.) Reichb. (Koç and Hamzaoğlu, 2015), *Bufonia* L. (İlçim and Behçet, 2013), *Dianthus* L. (Vural, 2008; İlçim et al. 2013; Hamzaoğlu

and et al.,2015), *Gypsophila* L. (Fedatova and Artzhanova, 1992; Kovtonyuk, 1994; El Naggar, 2004; Amini et al. 2011; Ataşlar and Ocak, 2004), *Minuartia* L. (Celebioğlu et al., 1983), *Moehringia* L. (Minuto et al., 2006, 2011), *Paronychia* Miller (Ocana et al., 1997; Kaplan et al., 2009), *Pseudostellaria* Pax (Xia et al., 2011), *Sagina* L. (Crow, 1979), *Silene* L. (Melzheimer, 1977; Aeschimann, 1984; Yıldız and Çırpıcı, 1998; Hong et al., 1999; Liden et al., 2000; Yıldız, 2006; Yıldız and Erik, 2010; Yıldız and Dadandı 2009; Mitra and Gholipour 2013; Dadandı and Yıldız 2015), *Stellaria* L. (Mahdavi et al., 2012) and *Spergularia* (Pers.) J.Presl & C.Presl. (Memon et al., 2010); *Velezia L* (Poyraz and Ataşlar 2010). Kanwal et al. (2012) studied 59 species distributed in 21 genera of the Caryophyllaceae family in Pakistan. Yıldız (2002) have been studied 17 species belonging in 12 genera of the Caryophyllaceae family distributed in Turkey. Brem et al. (2011) have been studied 15 taxa of Caryophyllaceae. In this research, the micromorphological seed features of micromorphological seed features of *Acanthophyllum* were studied in order to provide more detailed descriptions for future works.

Material and methods

Specimens were collected in 2014 and 2016 from the localities given in "Table 1". At least 30 seeds samples for each species were examined in stereomicroscope and micrographs were taken with Olympus SZ2-LGB digital imaging system. After the examination of each species, detected for seed shape, colour, and size. For SEM, seeds were directly mounted on stubs and covered with gold. The surface ornamentations of seeds were examined in detail with Jeol Tescan MAIA3 XMU model electron microscope in Bartın University Central Research Laboratory. The seed terminology of Stearn (1992), Bojnansky and Fargasova (2007), Punt et al. (2007), Amini et al. (2011), and Mitra and Gholipour (2013) were used.

Table 1. List of investigated taxa and localities.

Collector Number	Species	GPS	Altitude (m)	Collection Date	Habitat	Phytogeographic Region	Localities
Armağan 5693	*Acanthophyllum verticillatum*	39°09'29.6"N 39°29'01.7"E	970	24.7.2014	Steppe, Hillside	Irano-Turanian	B7Tunceli: Tunceli, 10. km from Tunceli to Ovacık (Munzur Valley)
Armağan 5966	*Acanthophyllum oppositiflorum*	38°53'41.2"N 39°14'52.9"E	875	13.8.2014	Steppe	Irano-Turanian Endemic	B7Tunceli: Pertek, 9. km from Pertek to Çemişgezek, 2.5 km before Singeç Bridge
Demir 790	*Acanthophyllum mucronatum*	38°21'4.21"N 43°45'11.9"E	1980	26.7.2007	Steppe	Irano-Turanian	B9Van: Gürpınar, between Zernek dam and Çörekli village
Armağan 6758	*Acanthophyllum microcephalum*	37°47'47.5"N 44°05'04.2"E	1825	31.7.2016	Naked Hillsides	Irano-Turanian	C10Van: Başkale, 33 km from Başkale to Hakkari
Armağan 6759	*Acanthophyllum acerosum*	37°47'47.5"N 44°05'04.2"E	1825	31.7.2016	Naked Hillsides	Irano-Turanian	C10Van: Başkale, 33 km from Başkale to Hakkari

Results

The morphological characters of the seeds from the studied species of *Acanthophyllum* were given below. Data obtained by stereomicroscope and electron microscope were reviewed in "Tables 2", as well as in "Figures 1, 2, 3".

The seed shape was generally reniform in the family Caryophyllaceae but *Acanthophyllum* species have oblong or oblong-obovate. Seeds dorsal and ventral side are generally convex. The largest seeds are found in *A. acerosum* (2.45 mm in length and 1.16 mm in width). The smallest seeds

are found in *A. verticillatum* (1.61 mm in length and 0.84 mm in width). *Acanthophyllum* species have tuberculate seed surface patterns. Under high magnification, the pattern was characterized by distinct hills, which had narrow and flattened ends. Shape of suture on tubercle showed differentiation in detailed investigation with high magnification. The shape of suture was sinuous, undulate or dentate. The colour of seed was reddish-brown or yellowish-brown. The radicle was on the one side of each seed and just out beyond the hilar notch. Number of suture points per plate of *Acanthophyllum* species were 5-17.

Acanthophyllum acerosum Sosn.

Seed shape is generally oblong with straight sides and rounded ends. Seeds have slightly convex dorsal side and smooth ventral sides with two or three shallow longitudinal grooves. The radicle is on the one side of each seed and just out beyond the hilar notch. Seed surface of *A. acerosum* is fine tuberculate arranged with elongate or polygonal cells like the jigsaw puzzle. Suture outline of testa cell is sinuous or smoothly sinuous. The colour of seed is dark reddish-brown. Seed size is 2.45 (2.18-2.61) mm long and 1.16 (1.05-1.31) mm wide. Testa cell size is approximately 20 x 80 µm. Number of suture points per plate were 8-12.Hilum region is 328.7 (241.1-407) µm long and 165.6 (101-286.2) µm wide.

Acanthophyllum microcephalum Boiss.

Seed shape is generally oblong with straight sides and rounded ends. Seeds have slightly convex dorsal and ventral side. The radicle is on the one side of each seed and just out beyond the hilar notch. Seed surface of *A. microcephalum* is fine tuberculate arranged with elongate or polygonal cells like the jigsaw puzzle. Suture outline of testa cell is sinuous or smoothly sinuous. The colour of seed is dark reddish-brown. Seed size is 2.24 (2.10-2.33) mm long and 1.06 (0.92-1.21) mm wide. Testa cell size is approximately 25x75 µm. Number of suture points per plate were 6-11. Hilum region is 310.1(300-319.3) µm long and 169.2(119.2-219.1) µm wide.

Acanthophyllum mucronatum C.A.Mey.

Seed shape is generally oblong with straight sides and rounded ends. Seeds have slightly convex dorsal and ventral side. The radicle is on the one side of each seed and just out beyond the hilar notch. Seed surface of *A. mucronatum* is fine tuberculate arranged with elongate or polygonal cells like the jigsaw puzzle. Suture outline of testa cell is smoothly sinuous. The colour of seed is dark reddish-brown. Seed size is 2.25 (2.17-2.34) mm long and 1.01 (0.85-1.03) mm wide. Testa cell size is approximately 30x65 µm. Number of suture points per plate were 5-9. Hilum region is 320.1(310.5-329.3) µm long and 166.7(112.2-221) µm wide.

Acanthophyllum oppositiflorum Aytaç

Seed shape is generally oblong-obovate with straight sides and rounded ends. The radicle is on the one side of each seed and just out beyond the hilar notch. Seeds have convex dorsal and ventral side. Seed surface of *A.oppositiflorum* is fine tuberculate arranged with elongate or polygonal cells like the jigsaw puzzle. Suture outline of testa cell is undulate. The colour of seed is shiny or oily-looking, golden to bright yellowish with a reddish circular marking over the hilar zone. Seed size is 1.70 (1.52-2.01) mm long and 1.08 (0.94-1.04) mm wide. Testa cell size is approximately 40x80 µm. Number of suture points per plate were 9-13.Hilum region is 331.2 (289.4-364) µm long and 288.2 (212-412.5) µm wide.

Acanthophyllum verticillatum C.A.Mey.

Seed shape is generally oblong with straight sides and rounded ends. Seeds have slightly convex dorsal side and ventral sides. The radicle is on the one side of each seed and just out beyond the hilar notch. Seed surface of *A. verticillatum* is distinct tuberculate arranged with elongate cells. Suture outline of testa cells are zip-shape sometimes dentate. The colour of seed is yellowish-brown to dull pale orange with a slightly darker hilar end. Seed size is 1.61 (1.33-2.08) mm long and 0.84 (0.71-0.96) mm wide. Testa cell size is approximately 30 x 60 µm. Number of suture points per plate were 12-17. Hilum region is 328.4 (227-426.2) µm long and 184.8 (105.5-267.9) µm wide.

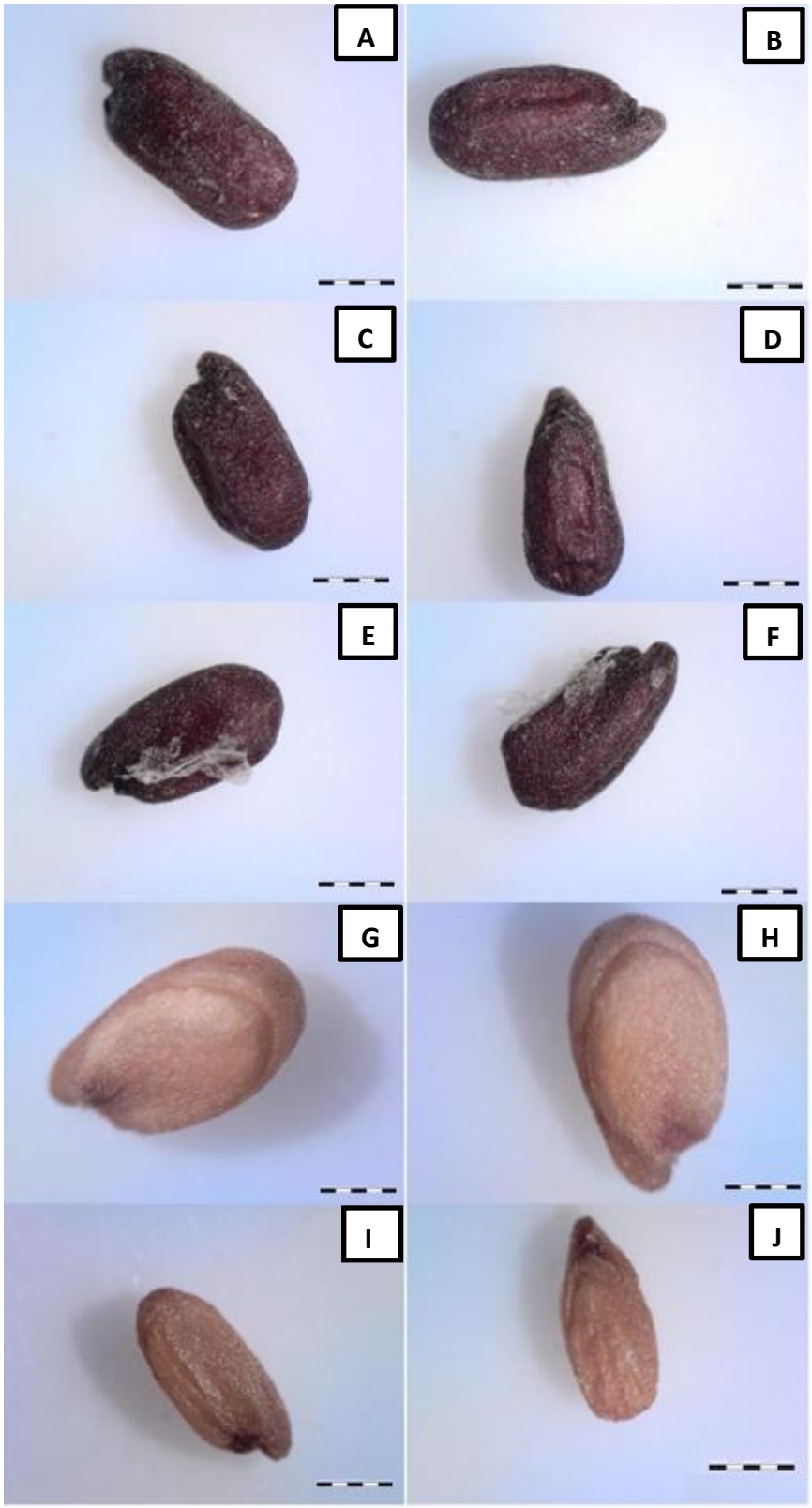

Figure 1. Stereomicroscope photos of seed of *Acanthophyllum* species: *A.acerosum* A-B, *A. microcephalum* C-D, *A. mucronatum* E-F, *A.oppositiflorum* G-H and *A. verticillatum* I-J. Scala bars: 1 mm

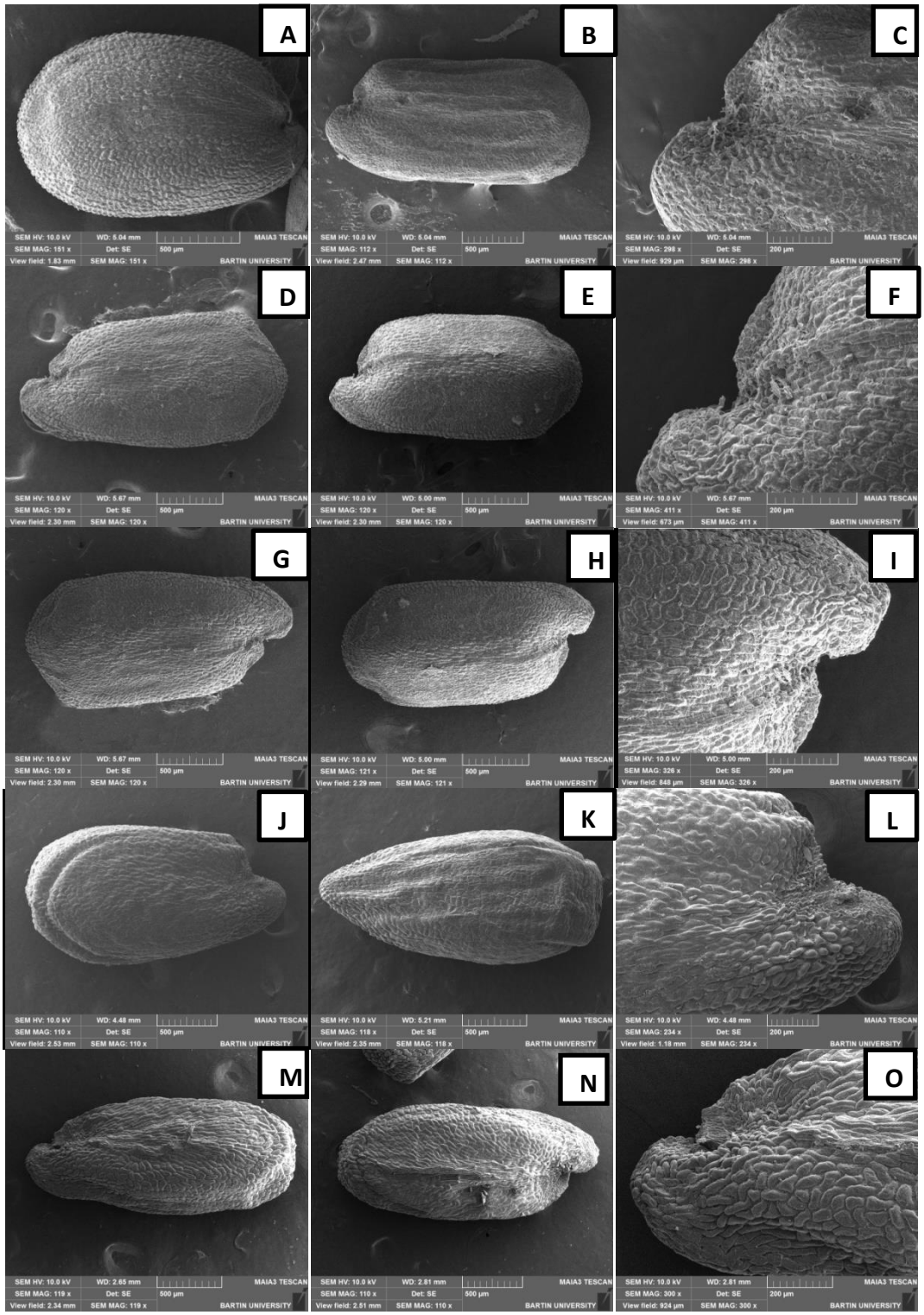

Figure 2. SEM photos of seed of *Acanthophyllum* species*: A.acerosum A-C, A. microcephalum* D-F, *A.mucronatum* G-I, *A. oppositiflorum* J-K *and A.verticillatum* I-J Scale bars: A,B,D,E,G,H,J,K,M &N = 500 µm; C,F,I,L& O = 200 µm.

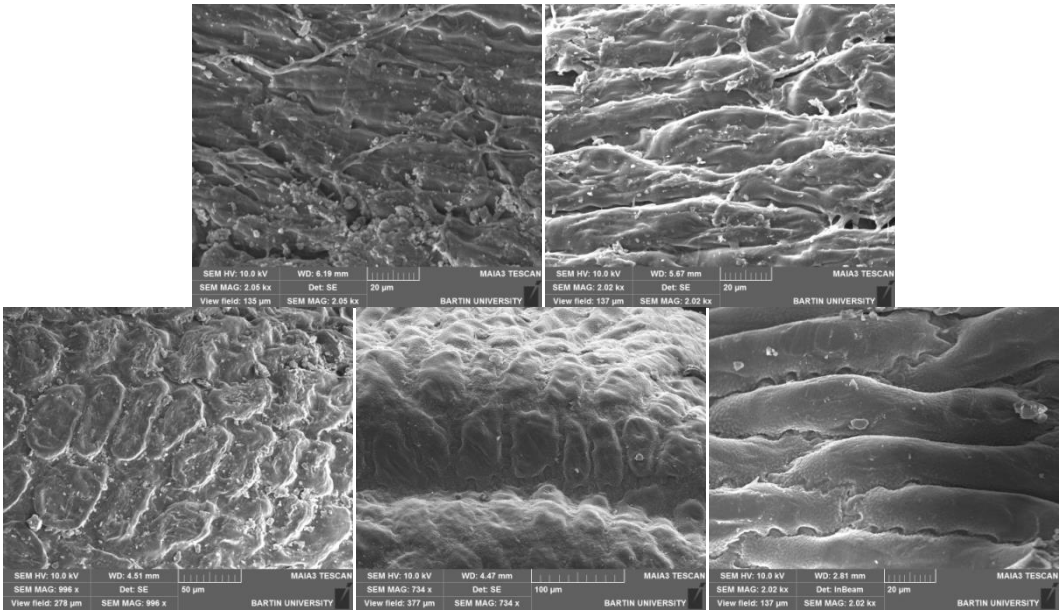

Figure 3. SEM photos of seed surface granulation and suture outline of *Acanthophyllum* species: *A. acerosum* A, *A. microcephalum* B, *A. mucronatum* C, *A. oppositiflorum* D and *A. verticillatum* E.

Table 2. Seed morphological characters of *Acanthophyllum* species.

	A. acerosum	*A. microcephalum*	*A. mucronatum*	*A. oppositiflorum*	*A. verticillatum*
Seed shape	Oblong	Oblong	Oblong	Oblong-obovate	Oblong
Seed length(mm)	2.45 (2.18-2.61)	2.24 (2.10-2.33)	2.25 (2.17-2.34)	1.70 (1,52-2.01)	1.61 (1,33-2.08)
Seed width(mm)	1.16 (1.05-1.31)	1.06 (0,92-1.2)	1.01 (0.85-1.03)	1.08 (0.94-1.04)	0.84 (0.71-0.96)
Seed of L/W ratio	2.11	2.12	2.22	1.57	1.90
Shape of dorsal surfaces	Slightly convex	Slightly convex	Slightly convex	Convex	Slightly convex
Shape of ventral surfaces	Smooth	Slightly convex	Slightly convex	Convex	Slightly convex
Seed tubercle shape	Elongate or polygonal cells like the jigsaw puzzle	Elongate or polygonal cells like the jigsaw puzzle	Elongate or polygonal cells like the jigsaw puzzle	Elongate or polygonal cells like the jigsaw puzzle	Distinct tuberculate arranged with elongate cells
Suture outline of testa cell	Sinuous	Sinuous	Smoothly sinuous	Undulate	Zip-shape or dentate
Testa cell size(µm)	20x80	25x75	30x65	40x80	30x60
Colour of seed	Dark reddish-brown	Dark reddish-brown	Dark reddish-brown	Golden to bright yellowish	Yellowish-brown
Number of suture points per plate	8-12	6-11	5-9	9-13	12-17
Hilum region length (µm)	328.7 (241.1-407)	310.1 (300-319.3)	320.1 (310.5-329.3)	331.2 (289.4-364)	328.4 (227-426.2)
Hilum region width(µm)	165.6 (101-286.2)	169.2 (119.2-219.1)	166.7 (112.2-221)	288.2 (212-412.5)	184.8 (105.5-267.9)
Hilum of L/W ratio	2.0	1.83	1.89	1.14	1.77

Discussion

Crow Garrett (1979) investigated seed morphology of 15 species of *Sagina* L. from North America, Europe, and eastern Asia. According to this study, seed of *Sagina* had superficial ridges and tubercles also two basic seed types were identified in the genus. The saginoid seed, characteristic of section *Sagina*, is obliquely triangular in outline, is grooved along the two dorsal ridges, and has slightly concave lateral surfaces. *Acanthophyllum* species mainly differs from these taxa with oblong or oblong-obovate seed shape.

Yıldız (2002) investigated seed macro and micromorphology of seeds of 17 species (12 genera) of Caryophyllaceae from North Anatolia (Turkey). According to this study, seed type was reniform, ovoid, reniform to orbicular, orbicular, peltate, scaled-winged, cylindrical, ovoid to reniform. Seed surface type was flat-concave, flat, convex, wringed crugosus aculeate, concavo-convex, rounded. Seed tubercle shape was tall/short conical, obtuse, rounded, flat, and aculeate-verrucate, digitate. Seed suture outline was digitate/serrate/stellate/sharply-sinuous, fine digitate and sharply serrate. Hylar zone of seed was recessed, prominent, and zip-shape facial and flat. *Acanthophyllum* species in our research had oblong or oblong–obovate seed shape, elongate or polygonal testa cells and zip-shape sometimes dentate suture outline of testa cells.

Minuto et al. (2006) carried out a study on seed morphology of 30 species of *Moehringia* L. and 12 representatives of the Caryophyllaceae were examined with the scanning electron microscope. According to this study, seed shape was subcircular, reniform or crassuloid. Seed colours were black and shiny or dark grey and dull. Smooth seed is the common condition in *Moehringia*, except for *Eastern Balkan* and *Iberian* species. Five types of strophioles were recognised. The seed shape of the investigated species in our research was oblong or oblong–obovate seed shape and absent of strophioles.

Vural (2008) investigated new species *Dianthus aytachii* C. Vural from Turkey. The seed type of *D. aytachii* was scaled-winged, 1.6–2.2 x 0.8–1.2 mm, seed back flat convex, seed surface type concavo-convex, tubercle obtuse, surface finely granulated. Seed coat cells subsquare to rectangular with suture points. Suture points per seed coat cell 21–34, suture outline sinuous to dentate and hylar zone prominent. *Acanthophyllum* species investigated in our research was oblong or oblong-obovate seed shape, shape of suture was sinuous, undulate or dentate. Number of suture points per plate of *Acanthophyllum* species were 5-17.

Yıldız and Dadandı (2009) reported the seed of *Silene cirpicii* was reniform shaped, concave, a little flat seed back, rounded or low rounded seed tubercle shaped, sinuate sometimes digitate suture outline and recessed hylar zone. *Acanthophyllum* differs from this species with oblong or oblong-obovate seed shaped and sinuous, undulate or dentate shape of suture.

Seed morphology of some *Velezia L.* species has been studied by Poyraz and Ataşlar (2010) and they reported the seed grains of *V. tunicoides* P.H.Davis, *V. hispida* Boiss. & Balansa, *V. quadridentata* Sibth. & Sm., *V. pseudorigida* Hub.-Mor. and *V. rigida* L. have bright black seed colour and cylindrical-oblong seed shape, shape of suture was sinuous or digitate. *Acanthophyllum* species mainly differs from these taxa with oblong or oblong-obovate seed shape, dark reddish-brown or yellowish-brown seed colour and undulate, zip-shape or dentate suture outline.

Memon et al. (2010) carried out a study on seed morphology of *Spergularia marina* (L.) Griseb., occurring in wheat fields of Khairpur district. According to this study, four types of seeds were detected in each capsule as unwinged ovate with tuberculate, unwinged ovate with tuberculiformis projections, unwinged ovate with striate pitted and winged ovate with papilliformis protuberances. Species in our research was unwinged and were not have projections.

Amini and et al (2011) investigated seed morphology of 22 species of *Gypsophila* L., five species of *Saponaria* L., two species of *Allochrusa* Bunge ex Boiss. and one species of *Ankyropetalum* Fenzl were examined by scanning electron microscope. According to this study, seed shape was generally reniform, seed colour was generally black

and shiny or grey, dull black and shiny, surface of the seed had elongate, oblong, irregular or rounded testa cell and zip-shaped, digitating, reticulate, puzzle-shaped, straight, undulate or polygonal margin of testa cell. The seeds size of examined species between 0.5-2.1 mm in length and 0.3-2.4 mm in diameter. The seed shape of the investigated species in our research was oblong or oblong–obovate, seed colour was dark reddish-brown or yellowish-brown.

Mahdavi et al (2012) examined seed micromorphology of eight species of *Stellaria* L. and two of its relatives by scanning electron microscope. They emphasised that seed shape was reniform, pyriform or cubical-spheroidal. The shape of testa cells was rounded, interlaced, elongated or irregular. Ornamentation of testa cells were secondary micro-sculpture. *Acanthophyllum* species in our research was oblong or oblong–obovate seed shape and elongate or polygonal testa cells.

Kanwal et al (2012) studied seed micromorphological characters of 59 species, distributed in 21 genera of the family Caryophyllaceae using light and scanning electron microscopy. According to this study, investigated species in Caryophyllaceae had large variety of seed characters such as seeds reniform, subreniform and rarely elliptic pyriform, cuneate, transversely cuneate, ovate, oblong, orbicular or suborbicular. Seed size ranges from 0.4-3 x 0.3-3 mm. The seed shape of the investigated species in our research was oblong or oblong–obovate.

Seed morphology of some Iranian endemic Silene species has been studied by Arman and Gholipour (2013). They reported the seeds shape of the most species is reniform. The size of seeds ranges from 0.85 × 0.62 mm in case of *S. elymaitica* to 2.28 × 1.4 mm in *S. nizvana*. The ornamentation of seed coat is papillate in *S. elymaitica* and *S. oligophylla*, tuberculate in *S. daenesis* and the seed surface of the rest is smooth. Three types of the edge of testa cells; V form, U form and entire were observed. Hilum region length ranges from (216-596) μm and hilum region width (109-322) μm. *Acanthophyllum* species mainly differs from these taxa with oblong or oblong-obovate seed shape. The larger seeds were found in *A.acerosum* (2.45mm in length and 1.16mm in width). The smallest seeds were found in *A.verticilatum* (1.61mm in length and 0.84 mm in width). *Acanthophyllum* species have tuberculate seed surface patterns. The shape of suture was sinuous, undulate or dentate. Hilum region length ranges from (227-426) μm and hilum region width (101-412.5) μm.

Koç et al (2014) investigated new species *Arenaria microcalyx* from Turkey. *Arenaria microcalyx* had 0.4-0.7 x 0.2-0.5 mm, reniform, blackish seeds. Shape of suture of this species was ribbed fingerless. *Acanthophyllum* species in our research was oblong or oblong–obovate seed shape and sinuous, undulate or dentate shape of suture.

Dadandı and Yıldız (2015) worked on seed micro and macromorphology of 39 *Silene* taxa by stereomicroscope and scanning electron microscope. They reported that seed shape was dominantly reniform. Tubercles were mostly rounded or conical. Hilum of seed was generally depressed. The seed shape of the investigated species in our research differ from these taxa with oblong or oblong–obovate seed shape.

Conclusions

Seed morphology of *Acanthophyllum* shows some noteworthy features within the Caryophyllaceae. The most striking aspects are shape and colour. The aims of the present paper are a comprehensive study of the seed micromorphology of *Acanthophyllum* species in Turkey.

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