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Research Article

Anatomical, Palynological and Seed Surface Characteristics of *Aethionema sancakense* Yıld. & Kılıc (Brassicaceae)

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Abstract

In this study, *Aethionema sancakense* Yıld. & Kılıç (Brassicaceae) anatomical, palynological and seed surface features were determined. This study aims to reveal the structural features of this species. Root, stem cross-sections, leaf epidermis features were investigated from the plant and anatomical features were determined by taking images. Superficial and transverse sections were taken from the plant by hand, stained with alcian blue and safranin at a ratio of 3:2, examined under a light microscope and photographed. Multi layered cortex structure was detected under the outermost epidermis of the trunk. Peridermis layer is torn in some points and removed from the root. The cortex layer is composed of parenchymal cells and is in 7-8 rows. The sclerenchymal tissue in the cortex layer consists of 2-4 rows of cells arranged intermittently. For palynological examinations, pollen of *A. sancakense* were examined under the light microscope by Woodhouse method. The type of pollen, whose electron microscope images were taken, was determined as monad and aperture type tricolpat. It was observed that the pollen grains were radially symmetrical and isopolar and their measurements were taken. The seed surface was dark brown and oval shaped, and the surface ornamentation was reticulate-verrucate. In our study, we tried to determine the taxonomic characters of *A. sancakense* in terms of both anatomical characters and palynological features.

Keywords: *Aethionema,* Anatomy, Palynology, Seed surface, Cruciferae.

Aethionema sancakense Yıld. & Kılıç'ın (Brassicaceae) Anatomik, Palinolojik ve Tohum Yüzeyi Özellikleri

Öz

Bu çalışma ile Bingöl'de yayılış gösteren, yeni tür olarak tespit edilen *Aethionema sancakense* Yıld. & Kılıç (Brassicaceae) türünün anatomik, palinolojik ve tohum yüzey özellikleri tespit edilmiştir. Bu çalışma, bu türün yapısal özelliklerini ortaya çıkarmayı amaçlamaktadır. Bitkiden kök, gövde enine kesitleri, yaprak epidermis özellikleri araştırılmış ve görüntüleri alınarak anatomik özellikleri tespit edilmiştir. Yüzeysel ve enine kesitler bitkiden el ile alınarak alcian mavisi ve safranin ile 3:2 ile oranında boyandıktan ve ışık mikroskobu altında incelenip fotoğrafları çekilmiştir. Gövde, en dışta epidermisinin altında çok katmanlı korteks yapısı tespit edilmiştir. Periderm tabakası bazı yerlerde yırtılmış ve kökten uzaklaştırılmıştır. Korteks tabakası parankimatik hücrelerden meydana gelmiştir ve 7-8 sıralıdır. Korteks tabakasındaki sklerenkimal doku 2-4 sıra hücreden oluşur. Palinolojik incelemeler için *A. sancakense* polenleri ışık mikroskobu altında Woodhouse yöntemiyle incelenmiştir. Elektron mikroskobu görüntüleri alınan polenlerin tipi monad ve apertür tipi trikolpat olarak belirlenmiştir. Polen tanelerinin radyal simetrik ve izopolar olduğu gözlemlenmiş ve ölçümleri alınmıştır. Tohum yüzeyi, koyu kahverengi ve oval şekilli, yüzey ornemantasyonu ise retikulat-verrukat olarak belirlenmiştir. Çalışmamızda *A. sancakense* türünün hem anatomik karakterler hemde palinolojik özelllikler açısından, taksonomik karakterleri belirlenmeye çalışılmıştır.

Anahtar Kelimeler: *Aethionema*, Anatomi, Palinoloji, Tohum yüzeyi, Cruciferae.

1. Introduction

The monophylectic Brassicaceae family, to which *Aethionema sancakense* Yıld. & Kılıç species is a member, is a large family consisting of 345 genera and 4020 species distributed all around the world except Antarctica (Ertuğrul et al., 2021). The main spreading area of Brassicaceae are the Mediterrranean, Iranian-Turanian and Saharo-Sindian regions (Hedge, 1976). *Aethionema* is an Iranian-Turanian genus belonging to Aethionemae tribe and has around 60-70 species worlwide (Yılmazoğlu, 2022). The main gene centers of the genus *Aethionema* are Anatolia and Iran (Hedge, 1965; Mohammedin et al., 2017; Moazzeni et al., 2018).

In the first volume of Flora of Turkey, the number of *Aethionema* taxa in Turkey according to Hedge was 30 species, then Davis et al. (1965) added 9 taxa and Adıgüzel (2000) added 5 taxa, the total number of taxa has reached up to 44. It is known that the number of taxa of *Aethionema*, which is named as "Rock Rose" in Turkey, has increased to 55 together with the newly defined species in taxonomic arrangement based on morphological observations only (Ertuğrul, 2012, Karabacak et al., 2013; Kandemir et al., 2017; Yıldırımlı & Kılıç, 2016-2018; Ertuğrul et al., 2021).

Some recent studies have revealed both stem-leaf anatomy and palynology of *Aethionema* taxa. Atçeken et al. (2016) determined the morphology, anatomy and pollen morphology of four taxa (*A. arabicum, A. armenum, A. cordatum* and *A. karamanicum*) belonging to the genus *Aethionema* and emphasized that the sculpture structure plays a major role to distinguish taxa according to pollen morphology. Birgi & Sezer (2019) examined the morphology and anatomy of endemic *A. turcicum* H.Duman & Aytaç. Karaismailoğlu (2018) examined some taxa belonging to the genus *Aethionema* in a study based on pollen morphology and stated that these taxa differ from each other by features such as pollen and colpus sizes. Pınar et al. (2007) conducted a sead coat mikrosculpturing study in some Turkish *Aethionema* species. Recently, the anatomical features of another endemic species, *A.dumanii* Vural & Adıgüzel, were studied (Dural & Çıtak, 2020). The seed structures of 12 taxa of the genus *Aethionema* distributed in Turkey were examined. Also, the anatomical structure of testa and endosperm of the species were studied and it has been reported that it is of great importance to show the interrelationships between species (Karaismailoglu, 2019).

Aethionema sancakense was proposed as a new species (Yıldırımlı ve Kılıç, 2019). *Aethionema sancakense* is morphologically close to *Aethionema membranaceum* DC. However, because of its characteristics such as flowering stem c. 5-8 cm (not c. 10-17 cm); flat leaf (not incurved); siliculae 5 x 4-5 mm (not 6.5-10 x 6-11 mm) cymbiform (not flat), it has been placed in a new species category (Yıldırımlı ve Kılıç, 2019). Therefore, the present study aims to determine the unknown anatomical and seed-micromorphological features of *A.sancakense*. The genus *Aethionema* is one of the most difficult taxonomic species in Turkey. Many herbarium materials are not sufficient for the determinaton as both flowering and fruiting samples are required for the determination. The main aim is the taxonomy of the genus by fully examining the anatomical features of *A. sancakense*.

2. Material and Method

2.1. Plant Material

The plant sample was collected from Bingöl district and turned into a herbarium sample (Bingöl: Center, 7 km towards Aşağıköy, oak forest clearing, slope, 1450-1550 m, 19.05.2018 AD 687). Fresh specimens of this new species, defined according to the flora of Turkey, were stored in 70% alcohol for anatomy studies. For palynology studies, with flowering specimens of *A. sancakense* were kept and used.

2.2. Anatomical Study

For the anatomical study, cross sections from the stem and leaf of the samples prepared with 70% alcohol and superficial sections from the upper and lower surfaces of the leaf were made by hand. Safranin-fast green (Bozdağ et al., 2016) was used for painting anatomical sections (Table 1). Anatomical photography and measurement of the specimens were performed with the help of a digital camera with a Euromex CMEX-10PRO trinocular microscope (Figure 1, 3).

2.3. Palynology Study

The pollen morphology of the taxon in the study was examined with light microscopy and Scanning Electron Microscopy. The pollens obtained from the samples in light microscopy examinations were prepared according to the method of Wodehouse (1935). Measurements were performed and the photographs were taken with Euromex CMEX-10PRO trinocular microscope (100x). Polar axis (P), equatorial axis (E), and exine thickness (Ex). The terminology used is mainly from Faegri & Iversen (1975) and the study of Kılıç et al. (2020). These measurements are reported in Table 2 and micrographs in Figure 3.

Pollens belonging to the *A. sancakense* were placed on metal pollen carriers (stap) with double-sided adhesive tape under a binocular microscope to be examined by SEM and covered with gold to provide the appearance of the pollen in the electron microscope. The pollen appearances and detailed surface ornamentations of the studied pollen were obtained from Bingöl University Central Laboratory. Microphotographs at 1500x – 10000x magnification were taken for each taxon.

2.4. Seed Surface Study

Seeds belonging to the *A. sancakense* were placed on metal seed carriers (stap) with double-sided adhesive tape under a binocular microscope to be examined by SEM and covered with gold to provide the appearance of the pollen in the electron microscope. The pollen appearances and detailed surface ornamentations of the studied pollen were obtained from Bingöl University Central Laboratory (Figure 2).

3. Results and Discussion

In cross-sections taken from the *A. sancakense* roots, it consists of peridermis, cortex sclerenchyma, phloem and xylem cells, from the outside in. Peridermis is the outermost layer of the root. Peridermis layer is torn in some points and removed from the root. The cortex layer is composed of parenchymal cells and is in 7-8 rows. The sclerenchymal tissue in the cortex layer consists of 2-4 rows of cells arranged intermittently.

Sclerenchymal cells can be pentagonal or in different shapes. Conduction tissue consists of phloem and xylem elements. Phloem is located on the outside and xylem towards the center. The pith region is narrowed and filled with xylem cells (Table 1 and Figure 1).

In the transverse sections taken from the stem, there are epidermis, cortex, sclerenchyma, vascular bundles and pith region from the outside to in. The outermost layer, the epidermis, is in 2-3 rows. Epidermal cells are oblong-oval shaped. There is no epidermal hair in the epidermis. The cortex region, which is located in the continuation of the epidermal layer, consists of 7-12 rows of parenchymal cells. Cortex parenchymal cells are round or oval in shape. The sclerenchymal tissue cells in the cortex layer consist of clusters of 1-2 rows of cells. Sclerenchymal cells can be pentagonal in shape. Just below the cortex parenchyma, vascular bundles are arranged parallel to the body axis. Phloem and xylem are well developed. Vascular bundles are of collateral type. The pith region contains circular, oval parenchymal pith cells (Table 1 and Figure 1).

In the cross-sections taken from the leaves, it was determined that the stomata are amaryllis type and according to the arrangement of the stomatal cells and auxiliary cells, they are anisocytic type. The leaf is amphistomatic. The stoma index is 22.5% for the upper epidermis and 26.1% for the lower epidermis. So, the number of stomata in the lower epidermis was found to be higher than upper epidermis (Figure 1).

The pollen grains of the examined samples belonging to the *A. sancakense* species are symmetrical with respect to the equatorial diameter and are isopolar. Also, pollen grains are monad and tricolpate. Pollens in polar view are subtriangular or oval. In equatorial view, pollens are elliptical, not flattened at the poles. Equatorial axis (E) is 16.65-26.84 µm, polar axis (P) is 16.80-25.52 µm, P/E ratio is 1.01 µm pollen shape is prolate-spheroidal. Average colpus length is 11.41 µm and width is 3.5 µm. The mean exine thickness is 1.24 µm and exine sculpture is reticulate (Table 2 and Figure 3).

The seed shape of the examined taxa *A. sancakense* species is broadly ovate, and the seed color changes from brown to dark brown. The micropillar pole is acute and the calazal pole is orbicular. Seed sizes vary between 1.81-2.23 x 1.15-1.89 mm. In the SEM micrograph of the seed samples, the seed surface ornamentation is determined as reticulate-verrucate. The shapes of the cells on the seed surface vary as terragonal and polygonal cells. Cell ridges are observed as striat (Figure 2).

4. Conclusions and Recommendations

Tekin (2022) in his anatomical *Aethionema lepidioides* Hub.-Purple study, determined that the pith region of the root was filled with xylem, and that there was a multilayered cortex under the outermost single-layered epidermis. In the root sections of the *A. sancakense* species we studied, the results were similar because the pith area was covered with xylem tissue. Tekin determined that, stomatal characteristic of leaf is amphistomatic, stoma type is anisocytic and the stoma index of the upper epidermis and lower epidermis are 26% and 28.4% respectively (Tekin, 2022). However, stoma index of *A.sancakense* species was found to be 22.5% for the upper epidermis and 26.1% for the lower epidermis, while the stoma type was found to be anisocytic. Our study is parallel to these results. In the study examining the anatomical features of *A. dumanii*, a single layer of epidermis, parenchymal cortex, and vascular bundles were detected in the transverse sections of the trunk (Dural & Çıtak, 2020). This study is parallel to our study, except for the number of layers found in the cross-section of the trunk. Birgi and Sezer (2019) examined the root, stem and leaf anatomy of *Aethionema* species that are endemic to Turkey and obtained results consistent with our study. In the study examining the anatomical features of four *Aethionema* species (*A. arabicum, A. armenum, A. cordatum* and *A. karamanicum*) endemic to Turkey, only *A. arabicum* is annual, while the other three are perennial. In the study, which is said to reflect the anatomical features of this situation, it has been observed that the number of layers and size of *A. arabicum* is less in all sections compared to other species. It was stated that pith cells were not found in all of the studied species (Atçeken et al., 2016). In the root sections of the *A. sancakense* species we studied, the results were similar because the pith was covered with xylem tissue.

The morphological features of pollen of the genus *Aethionema* were evaluated by different researchers and the pollen type with monad, isopolar and generally tricolpate aperture was determined (Atçeken et al., 2016; Çeter et al., 2018). Çeter et al. (2018), studied the pollen morphology of *A. membranicum* which is morphologically close to *A. sancakense* and found the results as (P) 17.1 µm, (E) 10.4 µm, P/E ratio 1.64 µm, pollen shape subprolate, exine 0.9 µm, clg (length) 13.3 µm, aperture type tricolpate. In our study, the mean of the polar axis (P) was 22.62 µm, the mean of the equatorial axis (E) was 22.23 µm, and the mean of the exine was 1.24 µm. *A. sancakense* species, which is quite different from *A. membranicum* in terms of pollen shape and dimensions is similar in that the aperture type is tricolpate. Phylogenetic relationships of taxa were investigared by Batur (2014) according to the sequence of bases in the tmL-F region and rbcL-a region of the chloroplast DNA of some species belonging to the genus *Aethionema. A. membranicum* which is morphologically close to *A. sancakense* species, was included in the same clade with *A. spicatum* and *A. grandiflorum* species in Batur's study. *A. grandiflorum* pollen measurements are (E) 8.63-16.42 µm, (P) 10.28-21.9 µm, pollen shape is given as prolate, subprolate and prolate-spheroidal, colpus length 11.26-16.86 µm, colpus width 1.75-5.61 µm (Yılmazoğlu, 2022). The pollen size of *A. sancakense* species included in our study is quite large compared to *A. grandiflorum* species. On the other hand, pollen morphology of *A. spicatum* was studied and its measurements were determined as P 18.7 µm, E 18.7 µm, P/E ratio 1 µm, pollen shape spheroidal, exine 0.8 µm, clg (length) 14.1 µm, aperture type tricolpate and tetracolpate (Çeter et al., 2018). When evaluated in terms of pollen sizes and shapes, differences emerge in pollen shapes of taxa.

Seed micromorphology in the Brassicaeae family includes seed characteristics that can be used to distinguish species (Brochmann, 1992; Koul et al., 2000; Pınar et al., 2007; Karaismailoğlu, 2019). When the seed of the genus *Aethionema* was evaluated in terms of surface ornametations, the importance of seed surface details in solving taxonomic problems was seen (Buth & Roshan, 1983; Brochmann, 1992; Koul et al., 2000). Pınar et al. (2007) determined that there are four basic seed surface ornamentations in the scanning electron microscopy study of the seed surface of 17 species belonging to the genus

Aethionema: reticulate, ruminate, reticulate-verrucate, and verrucate. Our research is in accordance with the studies carried out. Seed color, seed size and ornamentation are more prominent among these characteristics. Pınar et al. (2007) reported that, *A. grandiflorum* taxa had the largest seeds among 17 species in the genus *Aethionema*. Rectangular, elliptical and sickle-shaped, broadly ovate and ovate seeds have been reported in previous studies in *Aethionema* genus (Pınar et al., 2007; Karaismailoğlu, 2019). When the examined species were evaluated in terms of seed color, light brown or brown seeds were detected. Pınar et al. (2007) also found dark green seeds. Karaismailoğlu (2019) reported that he observed brown-gray colored seeds. In terms of seed surface ornametation, Pınar et al. (2007) identified four different seed surface types. *A. sancakense* species examined in our study with reticulate-verrucate seed surface ornamentation, fits Pınar et al's "Type III: the ones with reticulate-verrucate seed surface ornamentation". In the reticulate ornamentation structure of the species, it is noteworthy that the walls are prominent or not.

In this study, the anatomical, palynological and seed surface characteristics of *A. sancakense*, which was accepted as a new species, were determined. In anatomical studies, it is compatible within the genus *Aethoinema* with features such as the pith being filled with xylem in the root, the presence of sclerenchyma layer in the stem and root, the stomata in leaves being amphistomatic and the stomatal type being anisocytic. However, *A. sancakense* species differs within the genus in terms of number of layers and size. In terms of pollen shape and dimensions, *A. sancakense* species is similar to *A. membranicum* species, to which the pollen shape is morphologically close, and it has been determined that it is a different species due to its large pollen size. *A. sancakense* species have reticulate-verrucate seed surface ornamentation within the genus *Aethionema*. Anatomy of this endemic species, which was determined to be a new species morphologically, was supported by the pollen and seed surface studies, and its differences were revealed within the genus.

Table 1. Anatomical measurement results of A.sancakense (µm)(SD – standard deviation).

	Min–Max	Mean ± SD	Min–Max	Mean ± SD
Root				
Cortex cells	10.23–46.62	24.18 ± 9.21	5.54–20.13	16.90 ± 5.43
Trachea diameter	6.23–24.86	13.21 ± 4.83	–	–
Stem				
Cuticle thickness	0.12–2.11	0.52 ± 0.22	–	–
Epidermis cells	11.28–24.21	17.80 ± 5.96	4.13–23.55	13.76 ± 5.32
Cortex cells	5.45–27.34	14.89 ± 6.52	4.56–15.75	13.56 ± 3.70
Leaf				
Upper epidermis cell	5.14–24.13	16.04 ± 5.33	12.17–29.01	22.36 ± 4.55
Lower epidermis cell	15.23–35.65	25.12 ± 5.65	8.22–28.32	17.31 ± 4.36
Leaf thickness	476.2–718.05	618.37 ± 49.01	–	–

Table 2. Palynological measurement results of A.sancakense (mm) (SD – standard deviation)

	P				**E**				**Ekzin**	**Shape**	**clg**	**clt**	**Ornamentation**
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Ort.				
A.sancakense	16.80	25.52	22.62	2.37	16.65	26.84	22.23	2.52	1.24	1.01 Prolatae-Sipheroidal	11.41	3.5	Reticulate-perforate

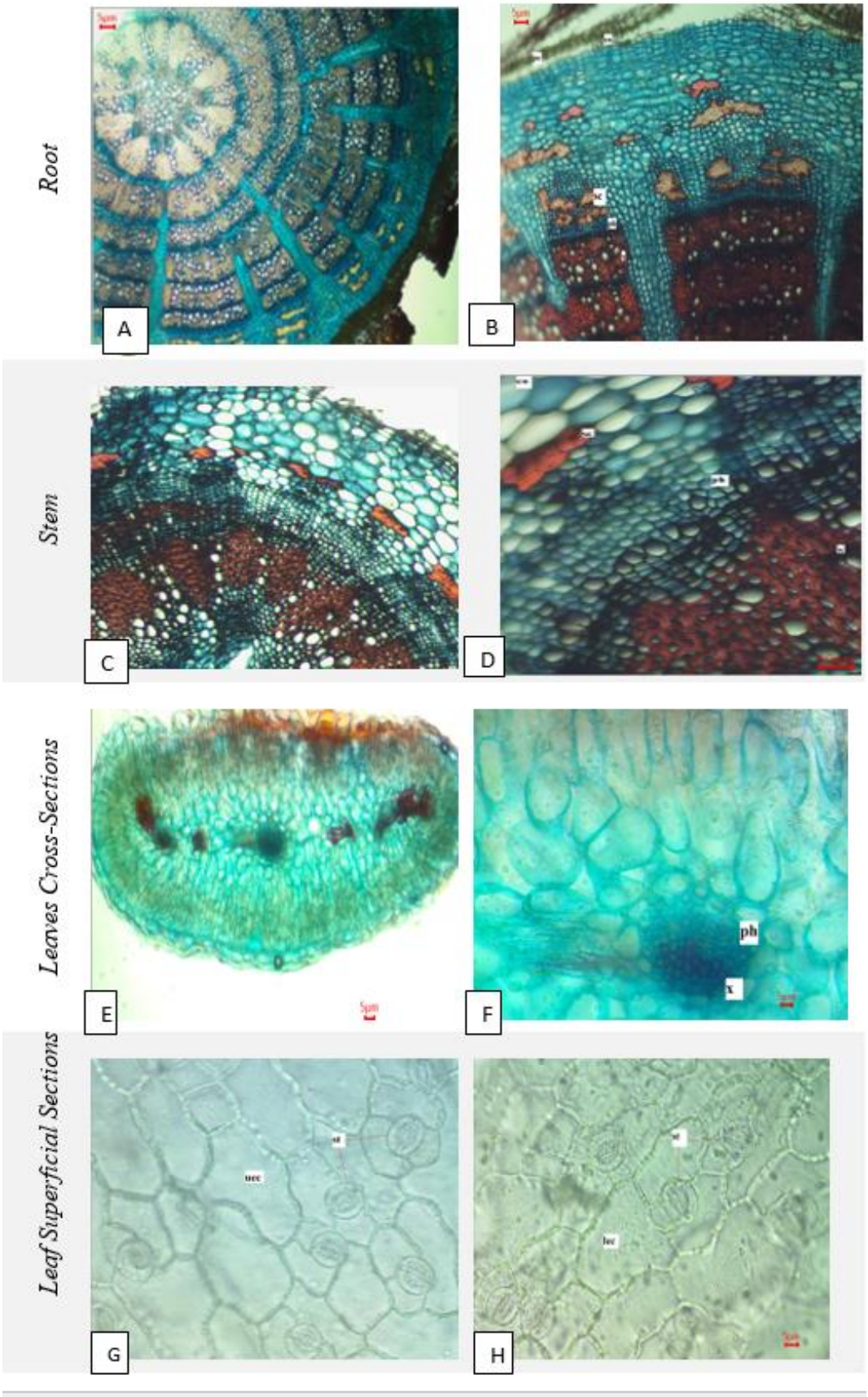

Fig. 1. Photomicrographs of root (A–B) and stem (C-D) cross sections, leaf-cross section (E-F), leaf superficial sections (G–H) of *A.sancakense*. Abbreviations: co – cortex, pe – periderm, ph – phloem, st – stoma, sc – sclerenchyma, xy – xylem, lec – lower epidermis cell, st – stoma, uec – upper epidermis cell.

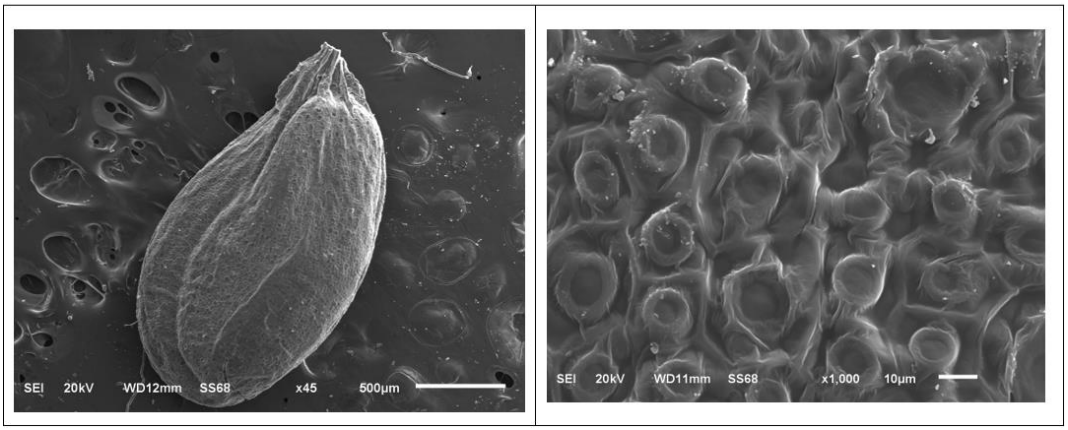

Fig. 2. Micrographs of *A. sancakense* seed. General view of the seed and ornamentation of the seed-coat surface.

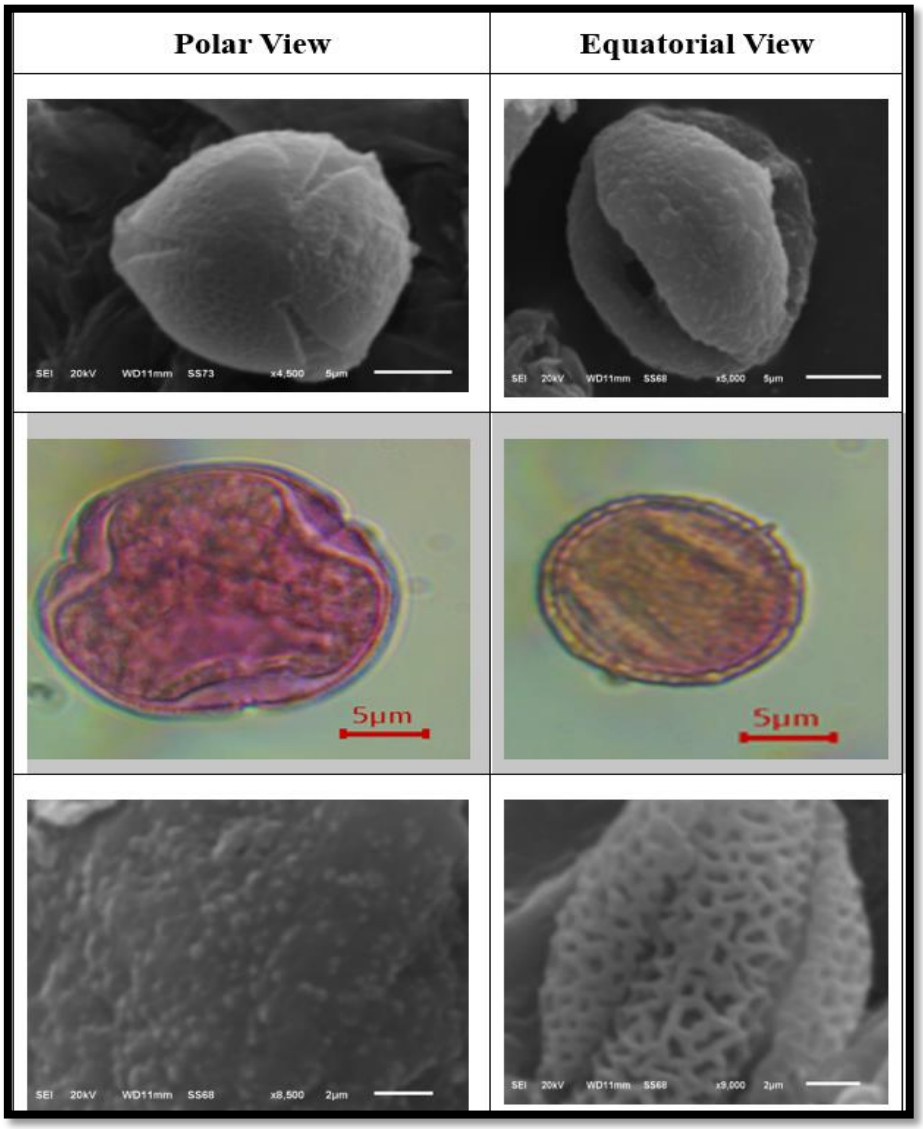

Fig. 3. Pollen grains photomicrographs of *A. sancakense*

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