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ANATOMY, PALYNOLOGY, SEED AND LEAF MICROMORPHOLOGY OF TURKISH ENDEMIC *ALLIUM BREVICAULE* BOISS. & BALANSA AND *ALLIUM SCORODOPRASUM* SSP. *ROTUNDUM* (L.) STEARN

SIBEL ULCAY

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In this study, two species belonging to the genus *Allium* and distributed in Turkey are investigated. A thick cuticle is observed on the epidermis of the scapes of the species. The epicuticular layer is not evident in *Allium scorodoprasum* ssp. *rotundum* (L.) Stearn. Secretory cavities have been formed in the pith region of the species. Vascular bundles are in the form of two rings, one above and one below the sclerenchymatic ring. The cross-section of the leaf of *Allium brevicaule* Boiss. & Balansa is circular, unlike *A. scorodoprasum* ssp. *rotundum*. In both species, the stomata are located lower than the epidermis cells. The seeds of *A. brevicaule* are smaller than in *A. scorodoprasum* ssp. *rotundum* and they are polygonal shaped. The testa cells of *A. brevicaule* seeds have scalariform and tuberculate ornamentation. *A. scorodoprasum* seeds have reticulate sculpture testa. The species have sulcate pollen types. The pollen form of *A. brevicaule* is perprolate, and that of *A. scorodoprasum* ssp. *rotundum* is subprolate. The apertures in both species are monosulcus. In *A. brevicaule*, the sulcus does not extend to the poles at the proximal end. Therefore, the differences in the scape and leaf anatomy, as well as in palynology and micromorphology, can be used to distinguish *Allium* species.

Keywords: *Allium*, anatomy, micromorphology, palynology, seed

INTRODUCTION

Allium L. (Amaryllidaceae: Allioideae) is one of the largest monocot genus, including more than 920 species (Herden et al., 2016). The taxonomic status of the *Allium* genera, which was previously in the Liliaceae family, was re-evaluated by the Angiosperm Phylogeny Group (APG). As a result of these studies, *Allium* was cited in the Amaryllidaceae family (APG III 2009). Approximately 190 taxa in 15 *Allium* sections are distributed in Turkey and one third of these taxa are endemic (Koyuncu, 2012; Ekşi et al., 2015; Özhatay and Kandemir, 2015). The section *Allium* is the richest section in terms of species (Friesen et al., 2006). Species belonging to the genus *Allium* are of medical importance (Lanzotti, 2006; Pardo et al., 2007; Mehrabi and Fazeli-Nasab, 2012).

Allium is a genus with a wide variety of morphologically diverse species (Fritschand and Abbasi, 2013). Taxonomic studies on *Allium* have taken morphometry, palynology and chronology as the criteria (Nasir, 1975; Kioug, 1998; Kwiatkowski, 1999). Later studies have shown that some anatomical characters are also crucial in identifying *Allium* species (Kioug, 1998; Gilani et al., 2002). Leaf (Webster 1983; Yousaf et al., 2008) and scape anatomy (Miryeganeh and Movafeghi, 2009) have been taxonomically vital in monocots and *Allium*. Micromorphological studies on pollen of *Allium* species have also been carried out and they have shown that pollen traits can solve specific taxonomic problems (Neshati et al., 2009; Wrońska-Pilarek et al., 2016; Hosseini, 2018). Moreover, the literature provides reports on studies on the seed micromorphology of *Allium* species. Kruse (1994)

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and Fritsch and Abbasi (2006) determined that the size of testa cells, and the shape of the anticlinal and periclinal cell walls would contribute to the revision of the genus. Choi and Cota-Sanchez (2010) and Choi and Oh (2011) revealed in their studies the seed testa structures and ornament types.

The species that are the subject of our study show natural distribution in Turkey. The endemic *Allium brevicaule* Boiss. & Balansa belongs to the section *Codonoprasum* while *Allium scorodoprasum* ssp. *rotundum* (L.) Stearn belongs to the section *Allium*. The present study aimed to determine the anatomical structure of *A. brevicaule* and *A. scorodoprasum* ssp. *rotundum*, and to choose the palynological characteristics based on seed surface micromorphology. Our study will contribute to the determination of the differences between the *Allium* species spread in the same ecological environment and the distinction between the *Allium* species.

MATERIALS AND METHODS

The samples of plants selected as the research material were collected in Kırşehir (Turkey) from May to July 2020 and they were put into alcohol. Some collected specimens were numbered and stored as herbarium specimens (Table 1). The species were identified according to Davis (1982).

ANATOMICAL METHODS

Fifteen specimens of each species were collected between April and July (Table 1). For anatomical studies, the specimens were kept in 70% ethyl alcohol. Sections were taken from the stem and leaf by hand. Anatomical measurements were based on 30 cells. The sections were made into a permanent preparation using the glycerin-gelatin method (Vardar, 1987).

In the examinations made on the superficial sections taken from the upper and lower surfaces of the leaves, 20 superficial sections were taken separately from the upper and lower surfaces of 5 randomly taken leaf samples, and the numbers of epidermis and stomata in mm^2 were determined in these sections. The following formula was used to calculate the stoma index and stoma index ratio (Meidner and Mansifield, 1968). Stomatal Index = (number of stomata in mm^2 / number of stomata in mm^2 + number of epidermis cells in mm^2) x100, Stomatal index ratio = upper stomatal index / lower stomatal index.

Hematoxylin and Giemsa stains were used at a ratio of 2 : 9 for staining the sections. One or two drops of dye mixture were applied to the preparations and left for 3-4 minutes. Then, the excess dye was removed by washing with alcohol and distilled water. The preparations were examined with a Nikon Eclipse Ni-U microscope and imaging system (Table 3).

TABLE 1. Information about the studied taxa.

Taxa	**Locality**	**Coordinates**	**Altitude (m a.s.l.)**	**Voucher date of collection**	**Alcohol stock number**	**Herbarium number**
A. brevicaule	Kervansaray Mount (Boztepe- Kırşehir)	39°19′258″N, 34°23′14″E	1684	SULCAY60, 2020.07.05	A89	404
A. scorodoprasum ssp. *rotundum*	Kervansaray Mount (Boztepe- Kırşehir)	39°19′25″N, 34°15′100″E	1684	SULCAY60, 2020.07.05	A70	405

TABLE 2. Stomata features on the upper and lower epidermis of *Allium*.

Characters	***A. brevicaule*** **Lower/Upper**	***A. scorodoprasum*** **ssp.** ***rotundum*** **Lower/Upper**
Number of stomata (1 mm^2)	20/32	28/33
Number of epidermal cells	100/120	53/67
Stomatal index	16.66/21.05	34.56/33.00
Stomatal index ratio	1.26	0.95

TABLE 3. Pollen characteristics of *Allium* species.

Characters	***A. brevicaule***	***A. scorodoprasum***
Pollen Type	Sulcate	Sulcate
Pollen Shape	Perprolate	Subprolate
Exine thickness (μm)	1.49	1.09
Apertures	Monosulcus	Monosulcus
Sculpture (Ornamentation)	Rugulate	Perforate
P (μm)	36.58	36.03
E (μm)	18.20	20.34
Sulcus length (μm)	33.74	42.84
Sulcus width (μm)	2.45	1.311
P/E	2.01	1.77

Abbreviations: P - polar axis; E - equatorial axis

PALYNOLOGICAL AND MICROMORPHOLOGICAL METHODS

For palynological investigations, pollen samples were obtained from herbarium samples. Thirty pollen grains were measured under the light microscope and P/E ratios were calculated. Scanning electron microscopy (SEM) was used for measurements and observations. Polar (P), equatorial length (E), sulcus length and sulcus width of the pollen grains were measured. The non-acetolyzed pollen grains were first placed on double-sided carbon tape adhered to aluminum stubs. They were gold-plated with a Polaron Au / Pd sputter coater and photographed with the JEOL JSM-6060 SEM. In addition, 30 mature seeds were measured to determine the average seed sizes. As with pollen, seeds were placed on double-sided carbon stubs adhered to aluminum studs. They were gold-plated with a Polaron Au / Pd sputter coater and photographed with the JEOL JSM-6060 SEM and then the ornamentations of the seeds were determined. The nomenclature used to characterize the seed coat features was given by Juan (1998).

DATA ANALYSIS

Statistical analysis of the study was performed using Statistical Package for Social Sciences for Windows (IBM SPSS version 25.0, Armonk, NY, USA) software. The normality assumption of continuous variables was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics of the variables are given as mean ± standard deviation. Independent T test was used for univariate analyzes of the variables in the study.

RESULTS

SCAPE ANATOMY

The epidermis cells of *A. brevicaule* are rectangular and circular, and their walls are wavy and covered with a thick cuticle. The epicuticular layer is sinuate. The epidermis cells of *A. scorodoprasum* ssp. *rotundum* are primarily circular shaped, and their cell walls are slightly wavy. The epicuticular layer is not evident in *A. scorodoprasum* ssp. *rotundum*. Secretory spaces have formed between the parenchymal cells just below the epidermis cells of *A. scorodoprasum* ssp. *rotundum*, while the secretory areas of *A. brevicaule* are just around the vascular bundles. Collateral vascular bundles are double-ringed. A cortex layer consisting of parenchyma cells is observed under the epidermis cells. This parenchymatic cortex is narrowed in *A. scorodoprasum* ssp. *rotundum*. A sclerenchymatous zone separating the cortex and pith has formed in both species. The vascular bundles are neatly lined up in this sclerenchyma ring, and those in the central region are scattered. Collateral vascular bundles in the sclerenchyma ring are smaller than in the pith. In addition, in both species secretory cavities in the pith were found (Fig. 1).

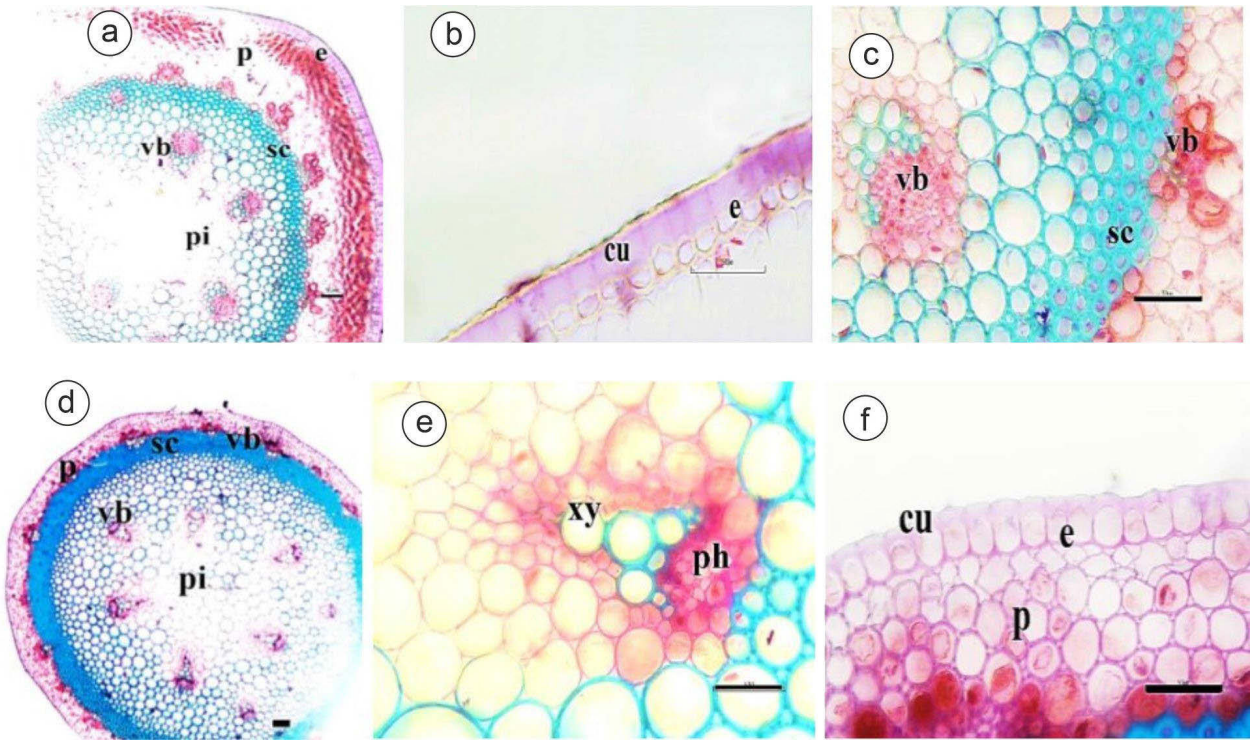

Fig. 1. Light microscope views of the anatomical structures of the scape. (**a-c**) Cross-section of scape of *A. brevicaule*, (**d-f**) Cross-section of scape of *A. scorodoprasum* ssp. *rotundum*. cu – cuticle, e – epidermis, sc – sclerenchyma, p – parenchyma, ph – phloem, pi – pith, xy - xylem, vb – vascular bundle (Scale 100 μm).

LEAF ANATOMY

The leaf cross-section of *A. brevicaule* is circular. The epidermis cells on the upper and lower surfaces of the leaves of the taxon are long and rectangular. The epidermis is covered with a thick cuticle. As in the stem, the epicuticle is wave-shaped. The palisade parenchyma cells are 2-3 layered. The secretory cavities are in a regular row between the palisade cells. The vascular bundles are arranged in one row. The spongy parenchyma cells surrounding the vascular bundles and located in the pith are circular or polygonal in shape. The parenchyma cells are not present in the pith region. According to the superficial sections taken from the lower and upper surfaces of the leaf, the stomatal index is 16.66 and 21.0, respectively. The stomatal index ratio is also 1.26. The stomata are located lower than the epidermis cells (Fig. 2).

According to the leaf cross-section, the leaf is not in the form of a full circle, but has a wavy shape. The epidermis cells are cylindrical and rectangular shaped. The epidermis is covered with a very thick cuticle. The epicuticular structures of *A. scorodoprasum* ssp. *rotundum* have the form of sparse papillae. The inner walls of the lower epidermis are thickened. The palisade parenchyma of the species is 1-2 layered. The spongy parenchyma of the species has 8-9 layers and the mesophyll is thin. One vascular bundle is observed in the mid-vein region of the species. The vascular bundles in the mesophyll are bi-linear (Fig. 3). According to the superficial sections taken from the lower and upper surfaces of the leaf, the stomatal index is 34.56 and 33.00, respectively, and the stomatal index ratio is 0.95 (Table 2). The stomata are located lower than the epidermis cells.

SEED MICROMORPHOLOGY

The micromorphological characteristics of seeds of the investigated *Allium* species are given in Table 3. The qualitative and quantitative aspects of the seeds of the species were determined during our study. The seeds are black, different polygonal shaped and wrinkled. The seed length of *A. brevicaule* is 2060.38 μm and its width is 957.88 μm. The testa cells of *A. brevicaule* seeds are irregularly shaped, with loose scalariform and tuberculate ornamentation, and the anticline walls are arcuate or S-shaped. The periclinal walls are concave with verrucate. The seed length of *A. scorodoprasum*

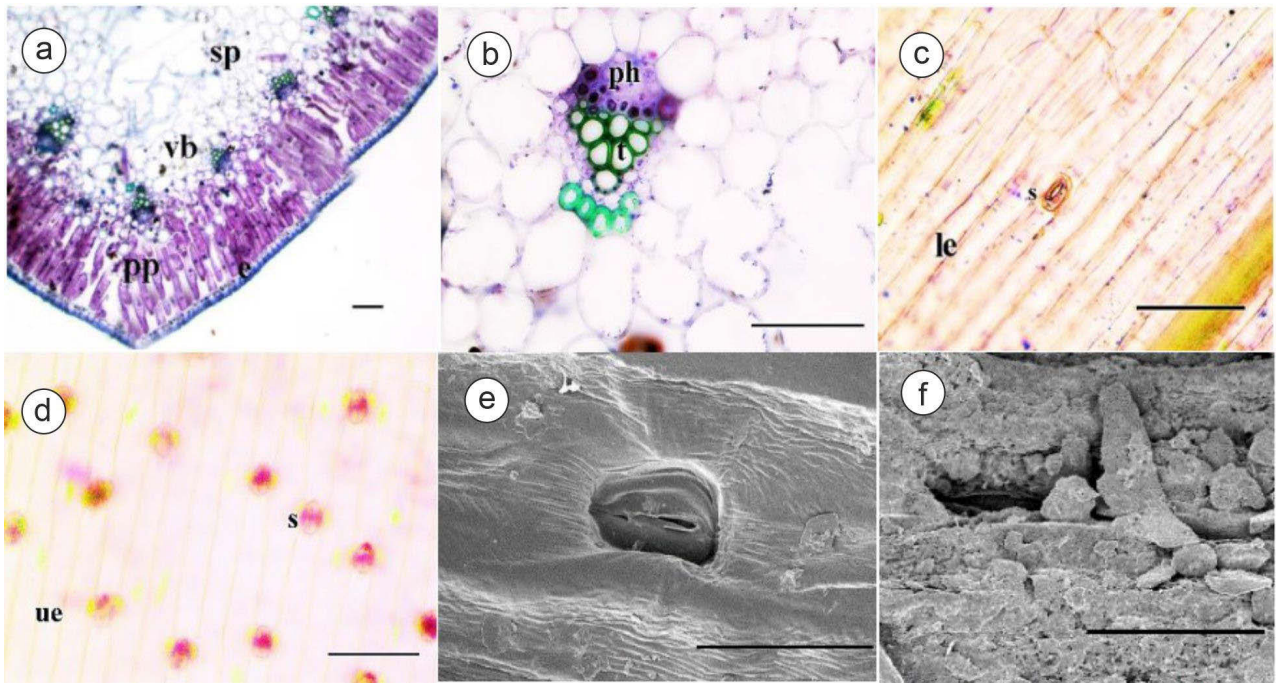

Fig. 2. Light and electron microscope images of leaf anatomical structures of *A. brevicaule*. (**a,b**) Cross-section (Scale 100 μm), (**c,d**) Superficial section (Scale 100 μm), (**e**) SEM view of leaf lower surface and stomata (Scale 10 μm), (**f**) SEM view of leaf upper surface and stomata (Scale 10 μm). le – lower epidermis, ph – phloem, pp – palisade parenchyma, s – stomata, sp – spongy parenchyma, t – trachea, ue – upper epidermis, vb – vascular bundle.

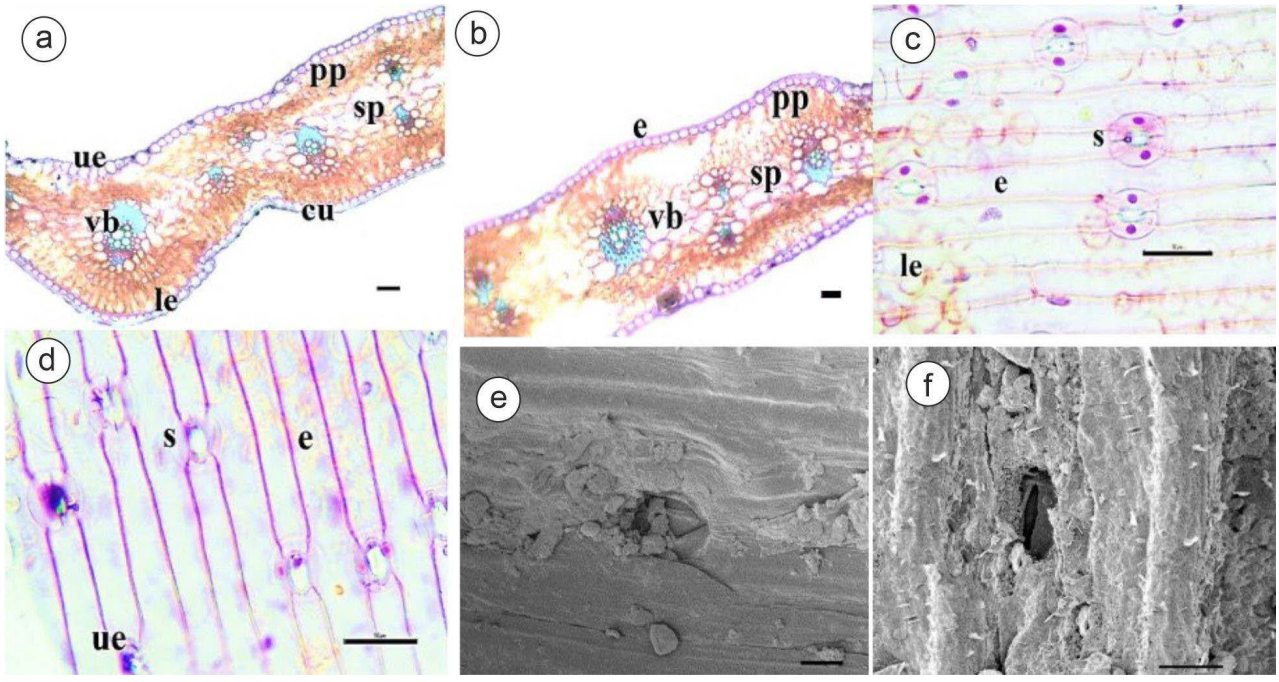

Fig. 3. Light and electron microscope images of leaf anatomical structures of *A. scorodoprasum* ssp. *rotundum*. (**a,b**) Cross-section (Scale 100 μm), (**c,d**) Superfacial section (Scale 100 μm), (**e**) Lower surface SEM and stomata view (Scale 10 μm), (**f**) Upper surface SEM and stomata view (Scale 10 μm). cu - cuticle, e - epidermal cells, le - lower epidermis, pp – palisade parenchyma, s – stomata, sp – spongy parenchyma, ue – upper epidermis, vb – vascular bundle.

ssp. *rotundum* is 3554.16 μm and its width is 2416.9 μm. The shape and arrangement of the testa epidermal cells of the seeds, and the micromorphological features of the anticlinal and periclinal walls differ from each other. The testa cells of *A. scorodoprasum* ssp. *rotundum* are irregularly shaped, and relatively tightly arranged. The anticlinal walls of testa cells are zigzag or U-shaped. The periclinal walls are granulated and the ornamentation is reticulate (Fig. 4).

POLLEN MORPHOLOGY

The palynological characteristics of the investigated *Allium* species are given in Table 4. The pollen type of the *A. brevicaule* is sulcate. The sulcus does not extend to the poles at the proximal end. According to the aperture type, pollen grains are monosulcus. The shapes of pollen (P/E = 2.01) are perprolate. The ornamentation concerning the tectum surface is regulated (Fig. 5).

The pollen type of the *A. scorodoprasum* ssp. *rotundum* is sulcate. The sulcus extends to the poles at the proximal end. The aperture is monosulcus, in the form of a very thin groove. The shapes of pollen (P/E = 1.77) are subprolate. The ornamentation concerning the tectum surface is perforated. Young pollen grains are unperforated. The average number of pores per 1 μm^2 in pollen is 4-5. The average diameter of the pore is 0.6 μm (Fig. 5).

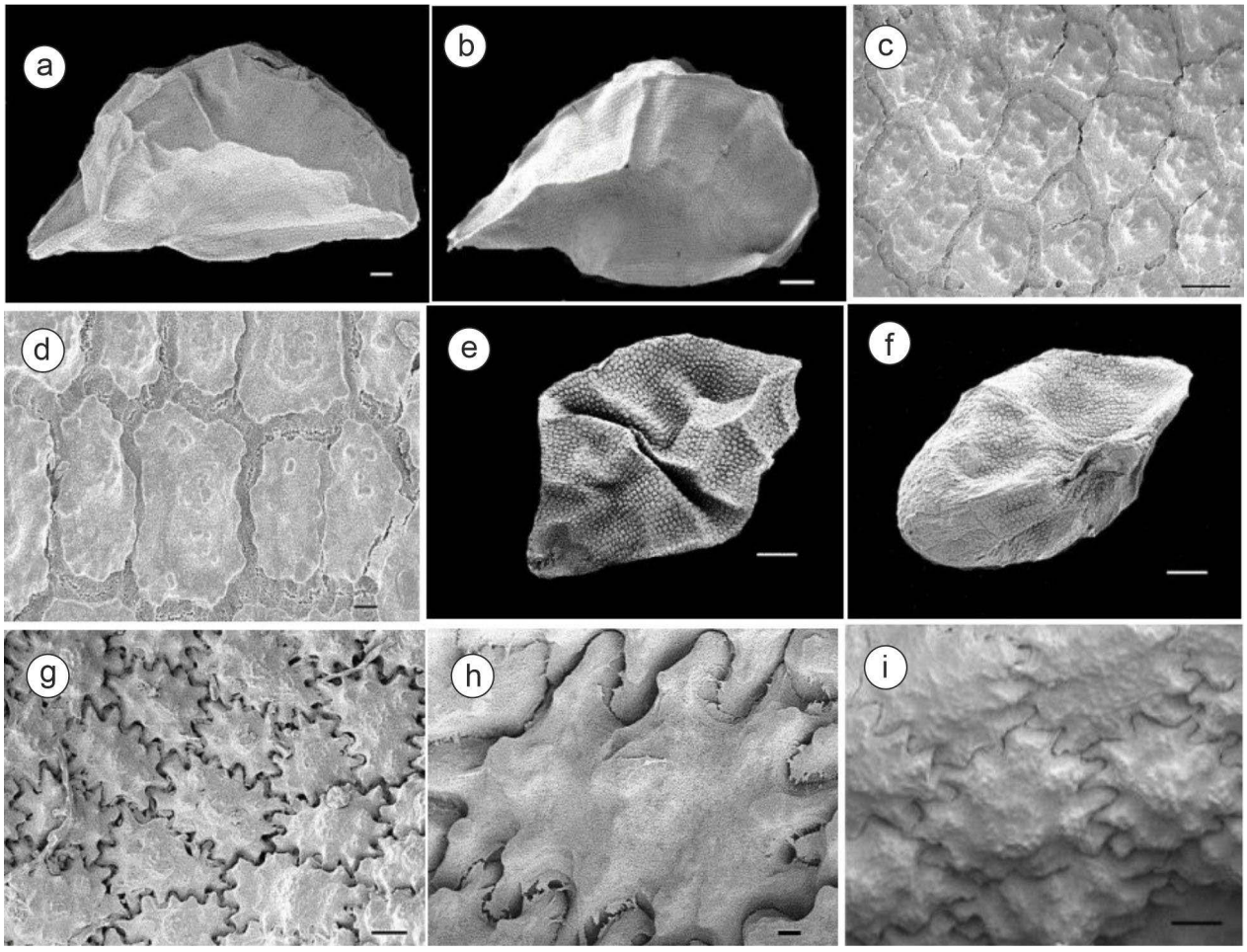

Fig. 4. SEM micrographs of the seed coat of *Allium*. (**a,b**) General view of the seed *A. brevicaule*, (**a**) Seed of *A. brevicaule* (Scale 100 μm), (**b**) Seed of *A. brevicaule* (Scale 200 μm), (**c**) View of testa ornamentation of *A. brevicaule* (Scale 10 μm), (**d**) View of testa cell of *A. brevicaule* (Scale 2 μm), (**e,f**) General view of seed of *A. scorodoprasum* ssp. *rotundum* (Scale 200 μm), (**g**) View of testa ornamentation of *A. scorodoprasum* ssp. *rotundum* (Scale 10 μm), (**h**) View of testa cell of *A. scorodoprasum* ssp. *rotundum* (Scale 2 μm), (**i**) Sculpture of seed of *A. scorodoprasum* ssp. *rotundum* (Scale 10 μm).

TABLE 4. Descriptive statistics of variables measured for the scape.

Variables (μm)	***A. brevicaule***	***A. scorodoprasum* ssp. *rotundum***	**P**
Width of epidermis	17.23 ± 4.49	16.92 ± 1.87	0.779
Length of epidermis	23.04 ± 6.26	23.40 ± 2.61	0.815
Diameter of xylem	18.73 ± 2.20	20.64 ± 2.28	0.420
Diameter of pith parenchyma cells	50.56 ± 17.09	56.11 ± 9.99	0.221
Diameter of phloem	4.24 ± 0.33	4.23 ± 0.33	0.948
Diameter of cortex parenchyma cells	23.79 ± 2.46	19.52 ± 3.10	0.000
Diameter of sclerenchyma cells	24.58 ± 1.72	26.85 ± 3.36	0.012
Thickness of cuticle	25.21 ± 7.75	7.63 ± 0.55	0.000
Diameter of central cylinder	644.24 ± 106.86	3132.69 ± 71.07	0.000
Diameter of cortex	349.66 ± 82.27	207.91 ± 29.46	0.000
Diameter of scape	981.76 ± 11.26	3262.07 ± 63.44	0.000

STATISTICAL ANALYSIS

In the study, measurements of different variables were made for the scapes and leaves of two different species. Descriptive statistics of the variables and comparisons between the species are summarized in Table 4 and Table 5. The difference between the species in the cortex parenchyma diameter is statistically significant (P = 0.000). The diameter of the cortex parenchyma of *A. brevicaule* (23.79 ± 2.46) is more important than that of *A. scorodoprasum* ssp. *rotundum* (19.52 ± 3.10). The sclerenchyma diameter of *A. scodoroprasum* (26.85 ± 3.36) is more significant than that of *A. brevicaule* (24.58 ± 1.72). This difference between the sclerenchyma diameters is statistically substantial (P = 0.012). The difference between the species in the cuticle values is statistically significant (P = 0.000). The cuticle values of *A. brevicaule* are significantly higher than those of *A. scorodoprasum* ssp. *rotundum*. The difference between the two species in the central cylinder diameter is statistically significant (P = 0.000). The central cylinder diameter of *A. scorodoprasum* ssp. *rotundum* is significantly larger than in *A. brevicaule*. The general cortex diameter of *A. brevicaule* (349.66 ± 82.27) is more significant than the available cortex diameter of *A. scorodoprasum* ssp. *rotundum* (207.91 ± 29.46). In terms of general cortex diameter, this difference between the species is statistically very significant (P = 0.000). The differences of the species in terms of the available diameter of the scape are statistically substantial (P = 0.000). *A. scorodoprasum* ssp. *rotundum* has a larger scape diameter than *A. brevicaule* (Fig. 6). The difference between the species in terms of the epidermis width, epidermis length, xylem diameter, pith parenchyma diameter and phloem diameter is not statistically significant (P > 0.05).

According to Table 5, which summarizes the descriptive statistics of the variables measured for the leaf and the comparisons between the species, the difference between the species in terms of the palisade width is statistically significant (P = 0.004). The palisade parenchyma cells width values of *A. brevicaule* (36.89 ± 11.56) are significantly lower than in *A. scorodoprasum* ssp. *rotundum* (48.23 ± 11.25). The differences in the length of the palisade parenchyma cells between the species are statistically very significant (P = 0.000). The palisade parenchyma cell length values of *A. brevicaule* (132.15 ± 31.31) are larger than in *A. scorodoprasum* ssp. *rotundum* (86.81 ± 16.55). The spongy parenchyma cell diameter differences between the species are statistically significant (P = 0.000). The spongy parenchyma diameter value of *A. brevicaule* (93.00 ± 22.14) is larger than in *A. scorodoprasum* ssp. *rotundum*. The difference between the species in the cuticle values is statistically significant (P = 0.000). The cuticle values of *A. brevicaule* (20.03 ± 3.43) are significantly higher than the cuticle values of *A. scorodoprasum* ssp. *rotundum* (12.39 ± 0.89). The upper surface stoma length

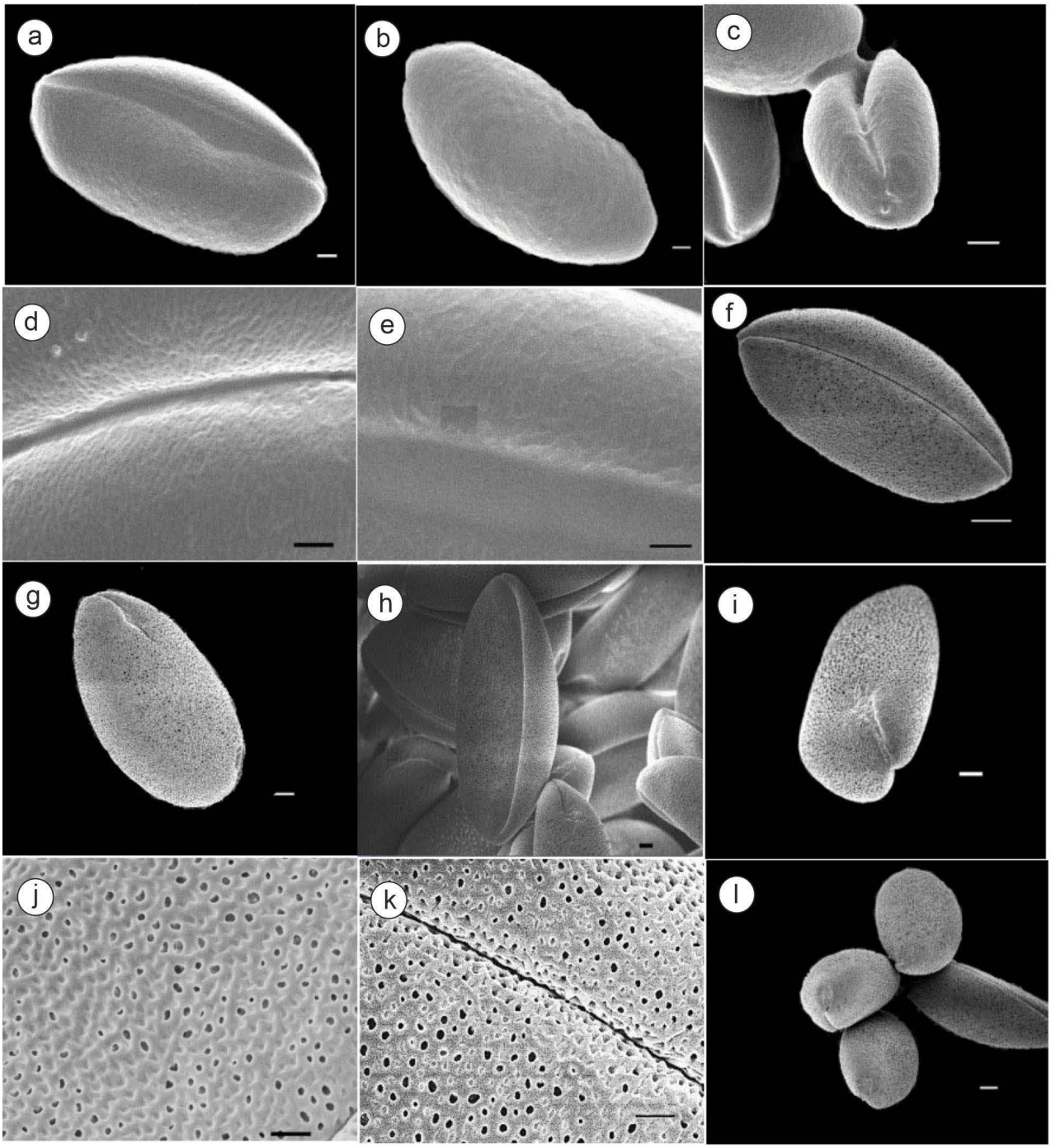

Fig. 5. Micromorphological characteristics of *Allium* seeds according to SEM. (**a**) *A. brevicaule* pollen distal view (Scale 2 μm). (**b**) *A. brevicaule* proximal view (Scale 2 μm), (**c**) *A. brevicaule* polar axis view (Scale 2 μm), (**d**) *A. brevicaule* excin surface and sulcus (Scale 1μm), (**e**,**f**) *A. brevicaule* sulcus membrane and pollen ornamentation (Scale 1 μm). (**g**) *A. scorodoprasum* ssp. *rotundum* proximal view (Scale 2 μm), (**h**) *A. scorodoprasum* ssp. *rotundum* pollen distal view, (**i**) *A. scorodoprasum* ssp. *rotundum* polar axis view (Scale 2 μm), (**j**) *A. scorodoprasum* ssp. *rotundum* perforate pollen ornamentation and pore view (Scale 1 μm), (**k**) *A. scorodoprasum* ssp. *rotundum* sulcus view (Scale 1 μm), (**l**) *A. scorodoprasum* ssp. *rotundum* pollen grains (Scale 2 μm).

TABLE 5. Descriptive statistics of variables measured for the leaf.

Variables (µm)	***A. brevicaule***	***A. scorodoprasum* ssp. *rotundum***	**P**
Width of palisade parenchyma cells	36.89 ± 11.56	48.23 ± 11.25	0.004
Length of palisade parenchyma cells	132.15 ± 31.31	86.81 ± 16.55	0.000
Diameter of spongy parenchyma cells	93.00 ± 22.14	51.65 ± 13.51	0.000
Thickness of cuticle	20.03 ± 3.43	12.39 ± 0.89	0.000
Length of upper surface stomata	41.94 ± 5.98	30.56 ± 5.11	0.000
Width of upper surface stomata	28.33 ± 5.57	26.55 ± 7.03	0.385
Width of lower surface stomata	24.47 ± 5.57	36.89 ± 1.71	0.000
Length of lower surface stomata	49.45 ± 11.87	39.69 ± 2.18	0.001
Width of epidermis	24.15 ± 2.22	25.03 ± 6.39	0.567
Length of epidermis	30.59 ± 4.31	27.39 ± 3.33	0.014

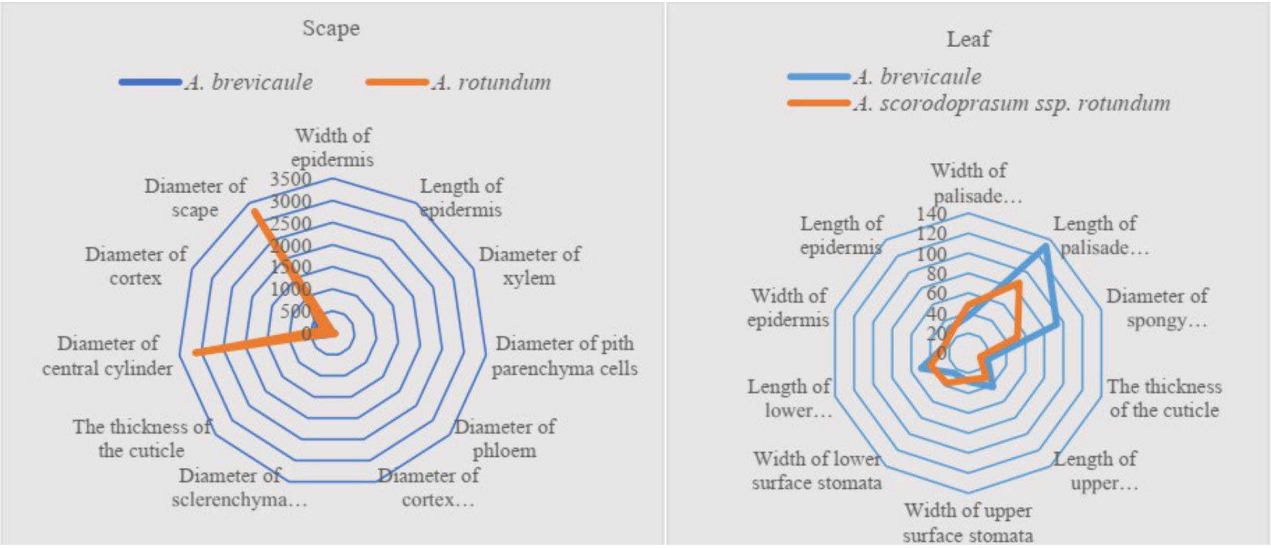

Fig. 6. Averages of characters that are important in Independent T test.

values of *A. brevicaule* (41.94 ± 5.98) are significantly bigger than the upper surface stomata length values of *A. scorodoprasum* ssp. *rotundum* (30.56 ± 5.11) (P = 0.000). The lower surface stomata width of *A. scorodoprasum* ssp. *rotundum* is higher than in *A. brevicaule*. The lower surface stomata length of *A. brevicaule* (49.45 ± 11.87) is larger than in *A. scorodoprasum* ssp. *rotundum* (39.69 ± 2.18). The differences between the species in terms of the epidermis width values are not statistically significant (P > 0.05). On the other hand, the difference between the species in terms of the epidermis length is statistically significant (P = 0.014). The epidermis length of *A. brevicaule* (30.59 ± 4.31) is greater than that of *A. scorodoprasum* ssp. *rotundum* (27.39 ± 3.33) (Fig. 6).

DISCUSSION

Due to its geographical location, geomorphological structure, and the influence of different climate types, Turkey has a vibrant flora. The number of plant taxa in Turkey is increasing day by day, with new taxa being defined due to studies. *Allium* is one of the largest genera in the flora of Turkey, with 146 species. (Güner et al., 2000). The genus *Allium* is

quite complex in nomenclature and taxonomy (Hanelt et al., 1992). Gregory et al. (1998) claim that the names of many species are now the same as the names of their subgenera.

The fact that the genus is complex has led to comprehension of the basic morphology as well as the unique anatomy, palynology, cytotaxonomy, and seed micromorphology in order to solve the existing taxonomic problems and better understand its phylogeny (Miryeganeh and Movafeghi, 2009; Özler and Pehlivan, 2010; Choi and Oh, 2011).

Both globally and in Turkey, there are scarce studies on the *Codonoprasum* and *Allium* sections of the *Allium* genus. The section most affluent in species of this genus is the *Allium*. Due to the high variability of the features of individual species and the occurrence in similar habitats, it is necessary to continue research on the variability of various groups of features (Friesen et al., 2006). This will allow to determine the level of differentiation of the analyzed features, which, as a consequence, will make it possible to decide on the actual taxonomic status of the studied taxa. Due to the high variability of individual characteristics of *Allium* species and their occurrence in similar habitats, it is necessary to continue research on the variability of various groups of features. Our study is essential to reveal the characteristics of species belonging to the same genus with the same ecological factors. Anatomical features, pollen and seed microbiology, and other evidence from the literature show that the *Allium* genus is very important in determining its characteristics (Namin et al., 2009; Neshati et al., Lin and Tan, 2015). An extensive literature study has not resulted in finding a report on the anatomical, palynological, and micromorphological features of the Turkish endemic *A. brevicaule*. In our analysis, *A. brevicaule* was examined for the first time in the anatomical, palynological, and micromorphological aspects. Moreover, there are limited studies in the literature on *A. scorodoprasum* ssp. *rotundum* (Şelem et al., 2020).

The scape and leaf anatomical features of *A. brevicaule* and *A. scorodoprasum* ssp. *rotundum* were investigated. The outermost layer of the circular-shaped stems has single-layered epidermis cells. As in the species that are the subject of our study, stomata located lower than the epidermis cells have been reported in *A. reuterianum* Boiss (Özdemir et al., 2008). The vascular bundles of the species are collateral and double-ringed. Miryeganeh and Movafeghi (2009) mention the presence of double-ringed collateral bundles in *A. subvineale* Wendelbo, *A. iranicum* Wendelbo, *A. qaradaghense* Feinber, *A. dictyoscordum* Vved, *A. phanerantherum* Boiss & Hausskn, *A. atroviolaceum* Boiss. Also double ringed vascular bundles have been reported in *A. rupestre* Steven and *A. kunthianum* Vved. (Özdemir and Altan, 2011). The sclerenchyma is in a continuous ring structure in the species constituting the subject of our study. This ring narrows the cortex area considerably in *A. scorodoprasum* ssp. *rotundum*. The sclerenchyma in *Allium caspium* (pall.) M. Bieb. is in the form of a continuous ring and separates the central cylinder from the parenchymal cortex (Abdullaeva et al., 2020). The sclerenchyma is absent in *A. paradoxum* (M. Bieb.) G. (Namin et al., 2009). In *A. leave* Regel and *A. phanerantherum* 3-layered, in *A. atroviolaceum*, *A. longicuspis* and *A. subvineale* 4-layered, in *A. qaradaghense* Wendelbo and Von Bothmer 7-layered sclerenchyma were determined (Miryeganeh and Movafeghi, 2009).

The leaf cross-section shapes of the species are quite different from each other. The *A. brevicaule* leaf cross-section is circular. The midrib is prominent in the leaf of *A. scorodoprasum* ssp. *rotundum*. In the studied species, the leaf epidermis cells are quite elongated, cylindrical or rectangular. Lin and Tan (2015) reported that the epidermis cells of 30 *Allium* species are long and rectangular. While the cross-section of the leaf of *A. brevicaule* was circular, the parenchyma cells in the pith area were lost. No hair was detected on the epidermis of either species. The epicuticle of the leaf cuticle in both species is wavy. The cuticle of *A. reuterianum* has papilla (Uysal, 1999). No glandular and non-glandular hair was found in the epidermis of any of the species in our study. The number of stomata in 1 mm^2 was established as 663.3 in *A. akaka* S. G. Gmelin and 726 in *A. scorodoprasum* ssp. *routundum* (Şelem et al., 2020). We recorded 28 stomata on the lower leaf surface and 33 stomata on the upper surface. In the leaf of *A. scorodoprasum* ssp. *rotundum*, a mid-vein region containing one vascular bundle was formed. The stomatal size and cuticle thickness of the *A. brevicaule* leaf are greater than those of *A. scorodoprasum* ssp. *rotundum*. Considering that they are taken from the same ecological environment and altitude the stomatal diameters and cuticle thickness can be the distinguishing features. The palisade parenchyma cell sizes, the diameter of the spongy parenchyma cells, epidermis cell lengths, stoma sizes and cuticle thickness of the species were statistically significant.

In the seed surface cells of *A. brevicaule*, the periclinal walls are verrucate, and the anticlinal walls are arcuate or S-shaped. In the seed surface cells of *A. scorodoprasum* ssp. *rotundum*, the periclinal walls are granulated, and the anticlinal walls are zigzag or U-shaped. The cells of *A. paniculatum* L. are periclinal walls verruca, and anticlinal walls are striate (Şelem et al., 2020). The anticline walls of *A. aucheri* Boiss. and *A. gayi* Boiss are more or less S-shaped as in *A. brevicaule* (Şelem et al., 2020). The testa ornamentation in *A. brevicaule* is scalariform-tuberculate and in *A. scorodoprasum* ssp. *rotundum* it is granulate- reticulate.

In our study, the properties of pollen grains were investigated. The pollen sizes of the species are almost the same. The aperture of the species is monosulcus, which is usually the case in the genus *Allium* (Harley and Zavada, 2000; Özler and Pehlivan, 2010). The sulcus does not extend to the poles at the proximal end in *A. brevicaule*. In the *A. scorodoprasum* ssp. *rotundum*, the sulcus extends proximally. The same was reported for *A. anacoleum* Hand.-Mazz., *A. arlgirdense* Blakelock and *A. pervariensis* F rat & Koyuncu (Başer et al., 2019) and *A. calyptratum* Boiss (Özler and Pehlivan, 2010). Some researchers claim that the sulcus features are taxonomically crucial in some families (Kosenko, 1991; Neshati et al., 2009). The sulcus membrane ornamentation of *A. brevicaule* is rugulate and the ornamentation of *A. scorodoprasum* ssp. *rotundum* is perforate. In the present study, we established the pollen length of *A. scorodoprasum* ssp. *rotundum* as 36.03 μm and its width as 20.34 μm. In the literature, pollen length of the same species was reported as 32 μm and width as 25 μm. (Şelem et al., 2020). This is the first study to reveal micromorphological features of the pollen of these two species.

CONCLUSION

Allium has many morphologically different species and is a taxonomically problematic genus. For this reason, the scape and leaf anatomy findings we obtained and the micromorphological characteristics of the seed and pollen may help to distinguish the species. Particularly rich in species of this genus is the *Allium* section, where 1/3 represent species with an endemic status (Friesen et al., 2006). Due to the high variability of the features of individual species and the occurrence in similar habitats, it is necessary to continue research on the variability of various groups of attributes. This will allow for determination of the level of differentiation of the analyzed features, which, as a consequence, will make it possible to decide on the actual taxonomic status of the studied taxa.

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