

**ANATOMY AND POLLEN MORPHOLOGY OF *LEYMUS RACEMOSUS* (LAM.)
TZVELEV SUBSP. *SABULOSUS* (BIEB.) TZVELEV AND *LEYMUS*
CAPPADOCICUS (BOISS. & BAL.) MELDERIS**

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Keywords: Leymus; Anatomy; Leaf; Pollen; Taxonomy.

Abstract

The present study aims to evaluate the anatomy of leaf and stem as well as pollen morphology in two taxa, namely *L. racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev and *L. cappadocicus* (Boiss. & Bal.) Melderis. Also, it is targeted to contribute to the morphology of these two taxa. The results have revealed varying anatomical characters in the types of stomata, wall appearance of the long cells, indumentum densities, dispositions of sclerenchyma around the vascular bundles, girders and strand shapes of the sclerenchymatic cells in the leaves, epidermal cell arrangements and epidermal cell sizes in the attachment points with sclerenchyma in the stems. Moreover, alternation in the pollen morphology concerning pollen size, operculum, undulation and the number of scabrae has also been demonstrated.

Introduction

Leymus (Poaceae) is one of the 16 genera of the tribe *Triticeae* Dumort. in the *Flora of Turkey* (Davis, 1985) and represented by two taxa, one of which is a Euro-Siberian element, *L. racemosus* subsp. *sabulosus*, and the other is an Irano-Turanian element, *L. cappadocicus*.

The anatomy of leaves within the Gramineae was first used for systematic reasons by Duval-Jouve (1875), who stated that the position, presence or absence and type of the bulliform cells could be considered as important diagnostic characters.

Schwendener (1890) emphasized on the presence of sclerenchyma between the vascular bundles and the upper or lower epidermis to be of systematic importance. Moreover, Vukolov (1929) showed the arrangement of sclerenchyma around the vascular bundles diagrammatically. After these remarkable studies, some researchers separated the family into the subgroups as such festucoid, panicoid, bambusoid, chloridoid, arundinoid and aristidoid grasses based on their leaf anatomy (Prat, 1932). Furthermore, according to the previous studies on stem anatomy of the grasses most of the hollow stemmed grasses were those with only one or two cycles of vascular bundles around a large pith (Stover, 1934). Metcalfe (1960) examined about 345 genera of Poaceae and found the

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diagnostic microscopical characters as the shape of girders, strands and the stoma types based on the subsidiary cells. Girders and strands on the sclerenchymatic cells around the vascular bundles and stomata of the family were also classified according to the shapes of their subsidiary cells (Metcalf, 1960). In the more recent studies, shape of leaf blades in cross-sections, epidermal cell types, floral morphology including glume, awn and caryopsis cross-sections were examined and useful anatomical features in characterizing the major taxa within the family were demonstrated (Doğan, 1985, 1988, 1991a, b, c, 1997, 1999; Doğan and Tosunoğlu, 1992). Several studies have been carried out about the pollen morphologies of the family (Liu *et al.*, 2004; Özler *et al.*, 2009). Perveen (2006) indicated that the taxonomic value of numerous pollen characters, such as size, aperture, shape and exines could not be dependable. However, palynology was found to be helpful to discriminate the genera and species within the tribe. According to Perveen (2006), Poaceae is a stenopalynous family including monoporate pollens.

Although there have been several studies on the tribe Triticeae (Xu and Zhou, 2008; Islam *et al.*, 2009), there is a limited number of studies based on the taxonomic significance of the anatomy or palynology in the genus *Leymus* (Li *et al.*, 2005; Chen and Wang, 2009). The present study aims to illustrate anatomical and palynological properties of *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev and *L. cappadocicus* (Boiss. & Bal.) Melderis on the basis of their leaf and stem anatomy and also pollen morphology.

Materials and Methods

For both anatomical and palynological investigations, fresh samples were collected from their natural habitats during the field trips in 2006 and 2008 (Table 1). For anatomical studies, the samples were placed in 70% ethyl alcohol solution. The specimens, consisting of leaf and stem tissues were fixed in formalin-acetic-alcohol (F.A.A.) solution for 48 hours (Metcalf, 1960). After removing the fixative by distilled water, they were dehydrated with ethyl alcohol solution of increasing strength. Then, dehydrated specimens were embedded into paraffin and sectioned following paraffin sectioning method (Johansen, 1944). The transverse sections were stained with safranin. However, the tangential sections were not stained. After fixing with Entellan, the slices were observed under 'Euromex FE 2025' microscope and photographed by using a 'Euromex CMEX DC.1300' camera.

The upper and lower sides of leaves of each taxon were examined by 30 slides prepared from each side and the number of stomata with the number of prickles were counted. The average of the stomata and the prickle numbers per 234 X 186 μm^2 area of the leaf surfaces of each taxon were given in Table 2.

Table 1. Collectors and collection areas of the plant samples investigated.

Taxon	Samples	Collectors	Collection number	Locality
<i>Leymus racemosus</i> subsp. <i>sabulosus</i>	Anatomical	E. Cabi E. Karabacak G. Akaydin	E. Cabi 767	A1: Tekirdağ to Silivri, 15 km from Tekirdağ, 28.05.2006, N: 41°00.269' E: 27°41.029', Alt. 15 m
	Palynological	E. Cabi E. Karabacak G. Akaydin	E. Cabi 761	A1: Tekirdağ. Between Gazikoy and Kumbağ, 5 km to Kumbağ, 28.05.2006, N: 40°50.472'; E: 27° 26.101', Alt. 238 m
<i>Leymus cappadocicus</i>	Anatomical	E. Cabi E. Karabacak	E. Cabi 3313	A6: Ankara Polatlı to Sivrihisar, 32 km to Sivrihisar, 16.06.2008, N: 39°33.843' E: 31°48.627', Alt. 870 m
	Palynological	E. Cabi B. Başer	E.Cabi 3662	A6: Ankara Gölbaşı to Koçhisar, 10 km to Vezirhane, 23.07. 2008, N: 39°34.610'; E: 32°51.317', Alt. 1066 m

Pollen samples from the taxa were studied both using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). Pollens were obtained from herbarium specimens at Middle East Technical University (METU). Totally 30 pollen samples were investigated for each taxa. From these samples, 15 of them were used for LM and the remaining samples were used for SEM observations. The samples for LM were prepared following the procedure of Wodehouse (1935) and also acetolysed according to Erdtman (1960) method. Morphological measurements of the pollen grains, which had been mounted in glycerine jelly on glass slides, were obtained by using a 'MICROLUX-11' trinocular light microscope and photographed with a 'Leica DM1000' light microscope. Pollens were fixed on metallic stubs using double sided cellotape and covered with gold in a 'POLARON CA508 Evaporation psv' model of sputtering chamber for morphological measurements of ornamentations. Following this step, the samples were microphotographed by using 'JSM JEOL 6060 LV' SEM at the Department of Biology, Gazi University. The terminology used is in accordance with Faegri and Iversen (1975), Chaturvedi *et al.* (1994, 1998) and also Punt *et al.* (1999, 2007).

Results and Discussion

Anatomy of leaves:

L. racemosus subsp. *sabulosus* has horizontally arranged abaxial epidermal cells which seem to have different sizes, including stomatal apertures and one short cell between two long cells, which have moderately thick and sinuous walls (Fig. 1A). The tangential sections of the leaves of *L. cappadocicus* demonstrate that the epidermal long cells have clearly thick and markedly sinuous walls (Fig. 1B).

L. racemosus subsp. *sabulosus* has stomata having parallel-sided subsidiary cells as in the tangential section of the leaf (Fig. 1A).

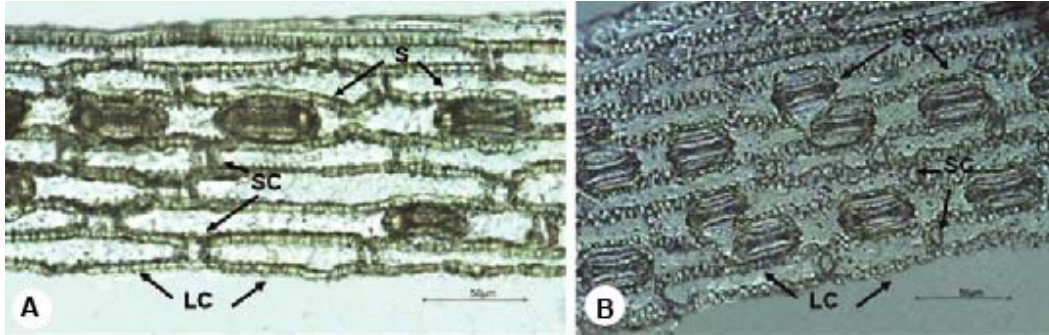


Fig. 1. Tangential sections of abaxial epidermal sides of the leaves: A) *Leymus racemosus* subsp. *sabulosus*; B) *Leymus cappadocicus*; S = Stoma, SC = Short cell, LC = Long cell.

Stomata are confined to the intercostal zones, each of which is composed of two or three stomatal rows at the abaxial surface, while there are three rows at the adaxial surface. *L. cappadocicus* has mostly three rows in the intercostal zones of both abaxial and adaxial epidermis. Stomata of the species have two low-dome-shaped subsidiary cells (Fig. 1B). Stomata are more dense in lower side of leaves than upper side of leaves for both taxa (Table 2).

Table 2. Stoma and prickle densities of leaves in *L. racemosus* subsp. *sabulosus* and *L. cappadocicus* (per $234 \times 186 \mu\text{m}^2$ of 30 slides prepared from each side of the leaves).

Taxon	Upper surface of leaves		Lower surface of leaves	
	Number of stoma	Number of prickle	Number of stoma	Number of prickle
<i>Leymus racemosus</i> subsp. <i>sabulosus</i>	4 ± 0.8	2.07 ± 0.8	6.4 ± 0.9	1 ± 0
<i>Leymus cappadocicus</i>	4.2 ± 0.8	3.3 ± 1.18	10.5 ± 0.72	Absent

The measurements using 20 slices of tangential sections of leaves show that the length of stomata is vertically $15.0 \pm 0.9 \mu\text{m}$ and horizontally $39.0 \pm 0.6 \mu\text{m}$ in *L. racemosus* subsp. *sabulosus* and vertically $18.5 \pm 1.03 \mu\text{m}$ and horizontally $29.8 \pm 0.2 \mu\text{m}$ in *L. cappadocicus*. Short cells are fairly solitary but moderately in pairs in the intercostal zones of the leaves of *L. racemosus* subsp. *sabulosus*. However, there are also triple short cells in the costal zones of abaxial surface. The adaxial surface has short cells mostly in pairs in the intercostal zones and has also triple short cells on the costal zones as well. In *L. cappadocicus*, the costal zones in both sides of leaf blade cover short cells, majority of which are solitary and the remaining ones are in pairs.

In *L. racemosus* subsp. *sabulosus* abaxial side of leaf appears to be comprising slightly of monotypic hairs with an average length of $40.35 \pm 1.1 \mu\text{m}$ and an average width of $15.15 \pm 2.0 \mu\text{m}$. The adaxial side includes more prickles than the lower side and also includes bulliform cells (Fig. 2A). Despite its glabrous abaxial side, *L. cappadocicus* has prickles and longer hairs on the adaxial side of the leaf blade (Fig. 2B). The base width of these prickles seems to be longer than the length of them (Table 3).

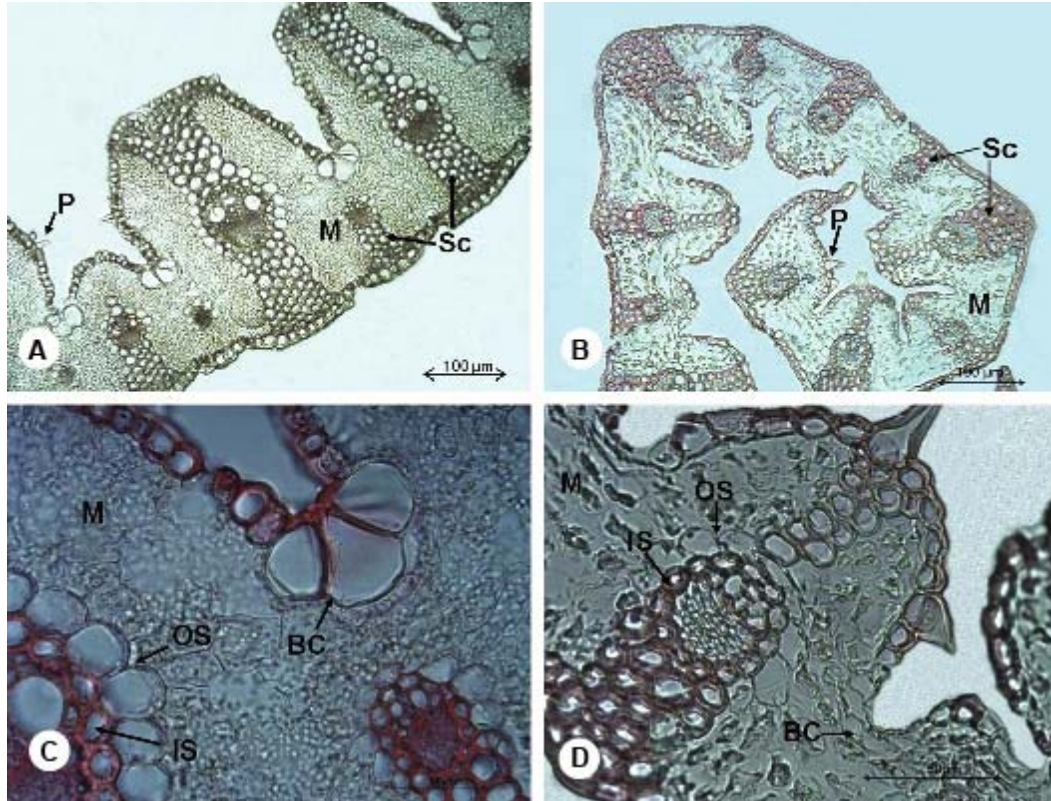


Fig. 2. Transverse sections of leaves of *Leymus*: A) *Leymus racemosus* subsp. *sabulosus*, B) *Leymus cappadocicus*, C) *Leymus racemosus* subsp. *sabulosus*, D) *Leymus cappadocicus*; BC = Bulliform cells, M = Mesophyll, IS = Inner sheath, OS = Outer sheath, P = Prickle, Sc = Sclerenchyma.

Table 3. The range of the length and width measurements of hairs of the taxa (μm).

Taxon	Leaf surface	Length of prickle	Width of the prickles at the base	Length of long hairs	Width of the long hairs at the base
<i>L. racemosus</i> subsp. <i>sabulosus</i>	Upper surface	30.58 - 97.95	11.16 - 73.87	absent	absent
	Lower surface	29.48 - 47.67	11.15 - 19.05	absent	absent
<i>L. cappadocicus</i>	Upper surface	13.63 - 57.89	32.04 - 60.55	92.9 - 74.7	17.22 - 32.49
	Lower surface	absent	absent	absent	absent

Leaves of *L. racemosus* subsp. *sabulosus* has bulliform cells, inflated towards the homogenous mesophyll. They are regularly fan-shaped in pairs or triple cells (Fig. 2A & 2C). These colourless cells have thinner cell walls but larger dimensions than the adjacent ordinary epidermal cells. Fig. 2D shows a part of the transverse section of leaf of *L. cappadocicus*. According to this observation, the bulliform cells of the species appear to be uncertain, instead they form groups of shapeless cells which have thinner cell walls than the adjacent ordinary epidermal cells.

As in most grasses (Watson and Dallwitz, 1992), midribs of the taxa are not readily distinguishable. The vascular bundles are of two orders throughout the chlorenchyma from one apex to the other. These circular bundles have different sizes with their 'double-type', conspicuous bundle sheaths around them. The bigger bundles have translucent parenchymatic outer sheath cells that have nearly the same size of the neighbouring mesophyll cells. The sclerenchymatous inner sheath cells are smaller in diameter and have thicker walls than those of the outer sheaths (Fig. 2C & 2D).

In *L. racemosus* subsp. *sabulosus* disposition of sclerenchyma comprises both the abaxial girders and abaxial with adaxial girders. With reference to Metcalfe (1960), it seems that the taxon includes I-shaped abaxial girders and T-shaped adaxial girders. As observed in Fig. 2A, bundle sheath interruptions by sclerenchyma can be classified into 3 groups, first of which includes outer sheath interruption both adaxially and abaxially with complete inner sheath. Abaxially interruption of outer sheath with complete inner sheath may be the second group of this classification. In the last group, the outer sheath has neither abaxial nor adaxial interruption by sclerenchyma and does not appear to completely surround the inner-sheath. In *L. cappadocicus* the arrangement of sclerenchyma of the leaves comprises the abaxial and adaxial girders and also strands. Both adaxial and abaxial girders are I-shaped (Fig. 2B) and also the bundle sheath interruptions by sclerenchyma have complete inner sheaths with abaxially and adaxially interrupted uncompleted outer sheaths.

Anatomy of stems:

Stem sections of *L. racemosus* subsp. *sabulosus* (Fig. 3A) demonstrate that the vascular bundles are arranged in two circular rings, composed of circular small bundles and elliptical larger bundles with no connection between each other. Small vascular bundles, connecting each others with sclerenchyma, are also attached to the epidermis with 4-5 layers of sclerenchymatic cells, which seem to make epidermis to form domes outwardly. Moreover, at this attachment point the epidermal cells tend to get larger. Stem transverse sections of *L. cappadocicus* represent that there are two types of vascular bundles, both of which are connected to each other and to the epidermis with 4-5 layers of sclerenchymatic cells (Fig. 3B). In this stem, regularly arranged large bundles and the circular small bundles seem to be in the same line.

In *L. racemosus* subsp. *sabulosus* the assimilatory tissue, forming about 3-4 cells wide, thin and flattened layers, is covered by sclerenchyma, not only subtending the epidermis but also surrounding the small bundles. Irregularly arranged large bundles of inner ground tissue have no relation with sclerenchyma. Each large bundle has protoxylem vessels between large metaxylem vessels. The ground tissue is made of

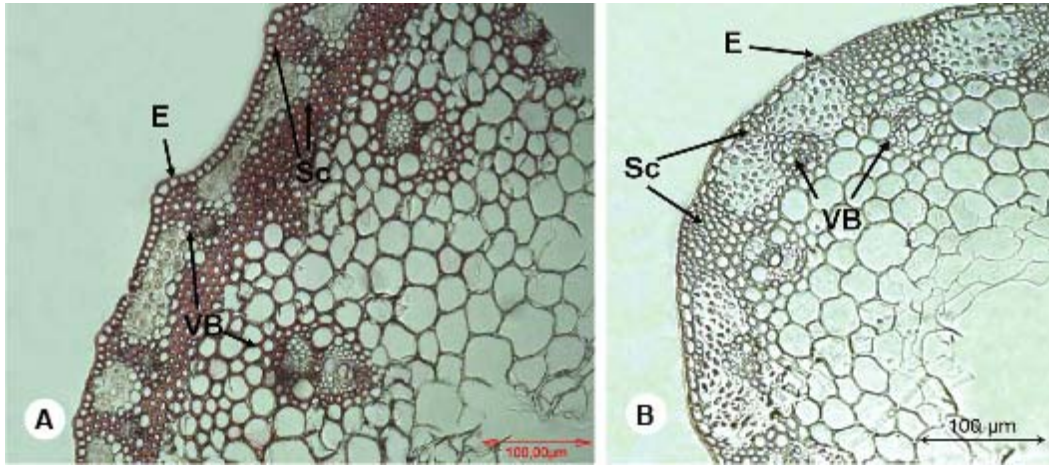


Fig. 3. Transverse sections of stems: A) *Leymus racemosus* subsp. *sabulosus*; B) *Leymus cappadocicus*; E = Epidermis, Sc = Sclerenchyma, VB = Vascular bundle.

parenchymatic cells. The central part of this tissue seems to be free of cells. At the contact regions of epidermis and sclerenchyma of stems of *L. cappadocicus*, the epidermal cells tend to become smaller. The thickness of the sclerenchymatous tissue, separating the near columns of assimilatory tissue from one another, is based on the size of the bundles. The protoxylem and metaxylem elements are clearly seen in both large and small bundles. The middle region of the stem appears to be hollowed.

Pollen morphology:

As shown in Fig. 4A-D non-acetolyzed pollen grains (W) of *L. racemosus* subsp. *sabulosus* are prolate-spheroidal and acetolyzed pollen grains (E) are subprolate. The inner and the outer edges of the annulus of each pollen are not clearly protruding (Fig. 5A-C). The operculum, which has the same size of the pore, has pentagon shape.

Using the LM observations of *L. racemosus* subsp. *sabulosus* (Fig. 4A-D), the average length of the annulus was measured from the non-acetolyzed pollen grains as 2.52 μm and from the acetolyzed pollen grains as 3.61 μm . Table 4 shows pollen morphological parameters of the investigated taxa. Some ratios of these parameters were calculated using both W and E. According to these calculations, the ratio of long axis of pore (pa) to long axis of pollen (A) is 9 % using both W and E; long axis of pore to annulus diameter is 35 % using W and 41 % using E; length of annulus to exine is 247 %

using W and 171 % using E; and lastly, annulus diameter to long axis of pollen is 27% using W and 23 % using E. Table 4 was constructed according to the LM observations (Fig. 4E-H) of *L. cappadocicus*. Using the measurements in Table 4, pa/A ratio was calculated as 11 % with both W and E. Moreover, the ratio of pa/annulus diameter was calculated as 38 % (W) and 48 % (E), the annulus diameter/A ratio is 28 % (W) and 23 % (E) and the height of annulus/exine thickness ratio is 189 % (W) 143 % (E).

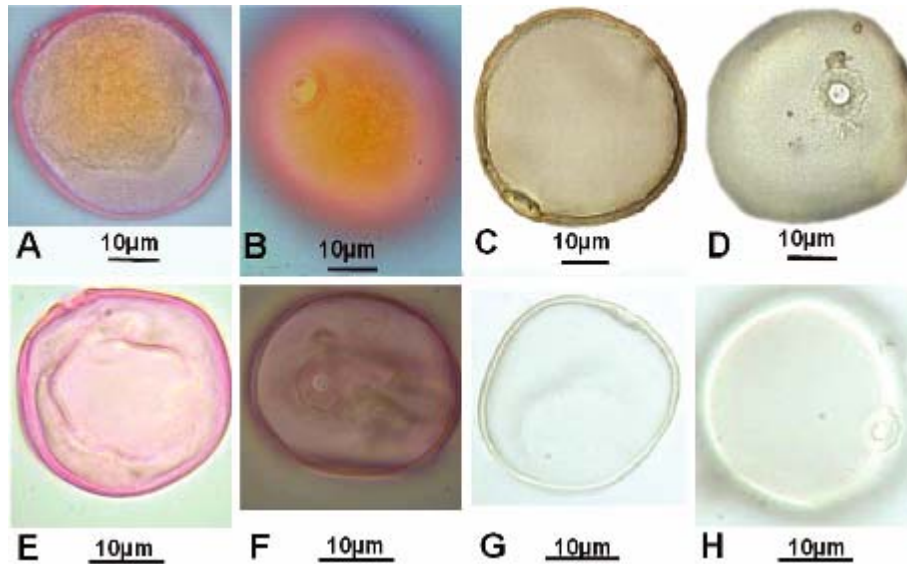


Fig. 4. LM observations of pollen grains; A-D. *Leymus racemosus* subsp. *sabulosus*: A) Equatorial view (W), B) Polar view (W), C) Equatorial view (E), D) Polar view (E); E-H. *Leymus cappadocicus*: E) Equatorial view (W), F) Polar view (W), G) Equatorial view (E), H) Polar view (E).

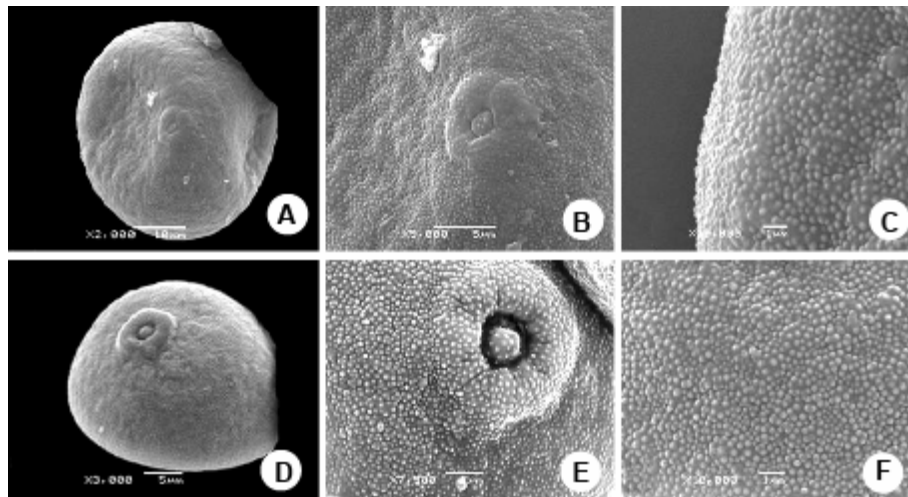


Fig. 5. SEM micrographs of pollen grains; A-C. *Leymus racemosus* subsp. *sabulosus*: A) General appearance, B) Aperture, C) Surface view; D-F. *Leymus cappadocicus*: D) General appearance, E) Aperture, F) Surface view.

Table 4. The measurements of pollen parameters of the taxa using LM observations (μm). The values are given as mean \pm standard deviation for 15 samples for each taxon.

Taxon	A	B	A/B ratio	Exine thickness	i	I	Pa	Pb	Pa/Pb ratio	Operculum diameter	Annulus diameter	Highness of Annulus
<i>L. racemosus</i> subsp. <i>subulosus</i> (W)	43.87 ± 3.37	40.19 ± 2.69	1.09 prolate- spheroidal	1.02 ± 0.19	0.65 ± 0.22	6.08 ± 1.32	4.09 ± 0.43	4.08 ± 0.36	1.00 prolate- spheroidal	2.45 \pm 0.17	11.71 ± 0.23	2.52 ± 0.13
<i>L. racemosus</i> subsp. <i>sabulosus</i> (E)	42.49 ± 3.68	34.62 ± 3.67	1.23 sub- prolate	1.95 ± 0.17	-	-	4.30 ± 0.27	3.95 ± 0.22	1.09 prolate- spheroidal	-	10.48 ± 0.33	3.61 ± 0.16
<i>L. cappadocicus</i> (W)	30.91 ± 2.28	33.28 ± 2.50	0.93 oblate- spheroidal	1.19 ± 0.15	0.43 ± 0.16	4.60 \pm 0.54	3.35 ± 0.41	3.14 ± 0.15	1.07 prolate- spheroidal	1.54 \pm 0.25	8.75 ± 0.25	2.25 ± 0.08
<i>L. cappadocicus</i> (E)	44.7 ± 4.30	41.12 ± 3.70	1.09 prolate- spheroidal	1.98 ± 0.17	-	-	5.07 ± 0.19	4.92 ± 0.16	1.03 prolate- spheroidal	-	10.51 ± 0.89	2.83 ± 0.05

W: Non acetolyzed pollen grains, E: Acetolyzed pollen grains, A: Long axis of spheroidal pollen grains, B: Short axis of spheroidal pollen grains, A/B Shape of pollen grains, i: Intine thickness, I: Thickest part of the intine, Pa: Long axis of ellipsoidal porus, Pb: Short axis of ellipsoidal porus, Pa/Pb: Shape of the pore.

SEM micrographs of *L. racemosus* subsp. *sabulosus* demonstrate that the spinulose scabrae are mix grouped and undulations are distinctive in the regions that are close to aperture (Fig. 5A-C). According to the measurements based on these observations, the average number of scabrae is 7.40 per 1 μm^2 , and scabrae are 0.32 μm in width and 0.18 μm in length (Table 5).

Non-acetolyzed and acetolyzed pollen grains of *L. cappadocicus* are oblate-spheroidal and prolate-spheroidal respectively (Fig. 4E-H). The annulus has protruding edges (Fig. 5D-F). In *L. cappadocicus* pollen grains do not have any undulations. The spinulose scabrae are observed in mixed groups, each of which may include one, two, three or rarely four scabrae (Fig. 5F). In 1 μm^2 region of pollen, there are 11.30 scabrae having the width of 0.25 μm and the length of 0.15 μm , on average.

Table 5. Ornamentations of the taxa according to SEM observations (The values are given as mean \pm standart deviation for 15 pollen samples for each taxa).

Taxon	Scabrae number per 1 μm^2	Width of scabrae	Length of scabrae	Group	Undulation
<i>L. racemosus</i> subsp. <i>sabulosus</i>	7.40 \pm 2.14	0.32 \pm 0.09	0.18 \pm 0.04	Mixed including 1 or 2 scabrae	+ (close to aperture)
<i>L. cappadocicus</i>	11.30 \pm 1.70	0.25 \pm 0.06	0.15 \pm 0.07	Mixed including 1, 2, 3 or rarely 4 scabrae	-

In conclusion, tangential sections including the surface view of the leaves contain diagnostic features such as stoma type, wall appearance of long cells, prickle density, the presence of sclerenchyma and its arrangement around the vascular bundles. Moreover, it is evident that at the attachment point of the epidermis and sclerenchymatic cells of the stems, *L. racemosus* subsp. *sabulosus* tend to have large epidermal cells, whereas *L. cappadocicus* have smaller ones. In addition, these larger epidermal cells of the former taxon form domes outwardly. There are limited number of studies about the stem or culm structures of the family. Therefore, it would be more clear with further studies whether characters such as epidermal arrangements and cell sizes of the attachment points with sclerenchymatic cells can be used as distinguishable characters. Furthermore, the pollen of *L. racemosus* subsp. *sabulosus* with undulations near the aperture which has the same size of the operculum is larger than the pollen of *L. cappadocicus* without undulations. Also the aperture of the latter pollen is larger than the operculum.

The alternations in characters of anatomy and pollen morphology of the investigated taxa may be correlated with certain environmental conditions that are typical of the growth habitats or natural constituents. Therefore, the number of studies concerning grasses should be increased in order to relate the anatomical and palynological characteristics in large groups.

Acknowledgement

We are very grateful to the Scientific and Technical Research Council of Turkey (TUBITAK TBAG-105 T 171) for their financial assistance.

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(Manuscript received on 8 October 2009; revised on 30 December 2010)