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Full Length Research Paper

Taxonomy, morphology and palynology of *Aegilops vavilovii* (Zhuk.) Chennav. (Poaceae: Triticeae)

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***Aegilops vavilovii* (Zhuk.) Chennav., a rare species, was collected from Southeast Anatolia, Turkey. During the field studies of the project "Taxonomic revision of Tribe Triticeae in Turkey", *Ae. vavilovii* was accidentally recollected from three localities in Şanliurfa and Mardin provinces in 2007 and 2008, respectively. The main objective of this study is to shed light on the diagnostic characteristics of this rare species including its morphological, palynological and micro morphological features. Moreover, an emended and expanded description, distribution, phenology and ecology of this rare species are also provided. *A. vavilovii* and *A. crassa* are naturally found in the Southeastern part of Turkey and they share similar morphological features that caused a confused taxonomy. Pollen grains of *A. vavilovii* are heteropolar, monoporate and spheroidal (A/B: 1,13) typically as Poaceous. However, it generally, prefers clayish loam soils that are slightly alkaline (pH 7.7) with low organic content (1.54%). Although it is a rare species with very narrow area of distribution, very few samples have been represented in *ex situ* collections and the species has not been involved in any *in situ* conservation activities to save its genetic resources in Turkey.**

Key words: *Aegilops vavilovii* (Zhuk.) Chennav., taxonomy, morphology, ecology, palynology, micromorphology, rare species, tribe Triticeae, Turkey.

INTRODUCTION

The genus *Aegilops* L. consists of more than 20 species and the largest part of them constitutes the secondary gene pool for the cultivated wheats (van Slageren, 1994). Species in this genus are mainly distributed in the Southwest and Central Asia and throughout the Mediterranean Basin. A primary center of diversity for the genus *Aegilops* is considered to be the 'fertile crescent', because more *Aegilops* species are found there than the other areas (Harlan, 1992; van Slageren, 1994).

In the Flora of Turkey, Davis (1985) recognized fifteen species among which *Aegilops vavilovii* (Zhuk.) Chennav. was not encountered. It is a taxonomical entity reported as rare in Turkey and was considered as a subspecies of *Aegilops crassa* Boiss. (van Slageren, 1994). This species is an allohexaploid (2n=6x=42) on the base of meiotic chromosome pairing in hybrids and its genomic constitution has been established as DMS (Chennaveeraiah, 1960; Waines and Barnhart, 1992). Van Slageren (1994) stated that this species is predominantly known from the western arc of 'fertile crescent': Jordan, Palestine, Syria, Lebanon and also southern part of Turkey close to Syrian border and in Iraq. *A. vavilovii* was mentioned as uncommon throughout its range and rare in Turkey (van Slageren, 1994). Maxted et al. (2008) reported that this species was represented worldwide by only 109 unique germplasm accessions, suggesting that this species was also inadequately represented in *ex situ* collections and, therefore, it should be given conservation priority.

After the publication of the Flora of Turkey and its supplements, the new grass taxa were compiled in the

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supplements (Davis et al., 1988; Güner et al., 2000). Therefore, additional taxonomical, palynological and anatomical studies have also been done on certain grass genera (Doğan, 1988, 1991, 1992, 1997, 1999; Cabi and Doğan, 2009, 2010; Cabi et al., 2009, 2010a, b; Özler et al., 2009; Baser et al., 2009).

The main objective of this study is to clarify the taxonomic status of *A. vavilovii*, as well as to give a detailed account of its morphology, palynology and micro-morphology of glumes and lemmas along an updated distribution map of the species for future conservation practices.

MATERIALS AND METHODS

Since 2006, as part of a revisional study of the tribe Triticeae Dumort. in Turkey, the authors have carried out extensive field studies and collected a large number of specimens of *Aegilops*. In addition, population size and phenological and ecological properties were observed in the field (Davis and Heywood, 1973). In 2007, during a field trip carried out in the Southeast Anatolia (C7 Urfa, C8 Mardin, C7, C8, *sensu* Davis, 1965), some interesting specimens from three unusual populations of *Aegilops* were collected. Upon closer examination and referring to the Flora of Turkey (Davis, 1985) and other relevant floras, such as Flora Orientalis (Boissier, 1884), Flora of Syria, Palestine and Sinai (Post, 1933), Flora Iranica (Bor, 1970), Flora of Iraq (Bor, 1968) and the latest monographic study of van Slageren (1994), they were identified. The specimens were compared with the material housed at various European (E, BM and K) and Turkish herbaria (ANK, GAZI, ISTE, HUB and VANF). It should be noted that the abbreviations of the herbaria are given according to Index Herbarium (Holmgren et al., 1990) and all the plant names are given by the authors according to Brummit and Powell (1992).

Micro morphological studies

The pollen grains were mounted in glycerin jelly (Wodehouse, 1935). Pollen morphological measurements, for example, size of grains, pore diameter, annulus, operculum diameter, exine and intine thickness were made on 40 fully developed pollen grains by the use of Leica DMLB2 (Leica Microsystems, Mannheim, Germany) and photographed under Leica DFC 320 (Leica Micro-systems, Mannheim, Germany) camera attachment at Forest Tree Seeds and Tree Breeding Research Directorate. The descriptive terminology mainly follows that of Faegri and Iversen (1989), Chaturvedi et al. (1994, 1998) and Punt et al. (2007). For studying pollen sculpture (ornamentation), dry and untreated pollen grains were first mounted directly on double-sided carbon tape affixed to aluminum stubs.

Grains were coated with gold-palladium that has a Polaron sputter coater (Polaron Instruments Line, Lexington, Pa) and observations were made by a JEOL JSM-6060LV (JEOL Technics Ltd., Tokyo, Japan) at the Central Laboratory of Middle East Technical University. SEM images were used to describe surface texture of the pollen. Glumes and florets were removed from voucher samples of *A. vavilovii* and cleaned with trichloromethane for 24 h, and were then mounted on stubs and coated with gold using a sputter coater. The specimens were then examined using a JEOL JSM-6060LV (JEOL Technics Ltd., Tokyo, Japan) Scanning Electron Microscope (Doğan, 1988). The terminology used for micromorphology was adopted from Ellis (1979), Acedo and Llamas (2001) and Ortunez and De la Fuente (2010).

Study area

The climate of both Şanliurfa and Mardin provinces are characterized as arid Mediterranean in which the annual precipitation is between 325 and 800 mm. In the center of Şanliurfa province, the average annual precipitation is 455 mm, while around the Syrian border of the same province, it decreased to 325 mm per annum. Distribution of the rainfall is uneven and the annual average temperature is 18.2°C. The altitude of Şanliurfa ranges between 400 and 560 m above sea level. In Mardin province, the total average annual precipitation is higher than the Şanliurfa province (689 mm). However, the annual average temperature is 15.9°C, while the altitude of Mardin ranges between 500 and 1500 m.

RESULTS AND DISCUSSION

Aegilops vavilovii (Zhuk.) Chennav.

Robust, tufted annuals

Stems that are usually erect sometimes ± geniculate at the base are 8 to 30 cm (excluding spikes). Leaf blades linear-acuminate (flat or involute) is 8 to 20 cm long and 0.3 to 0.5 cm wide, while margins of sheats hyaline have long ciliate. Spikes (5 to 10 cm) are narrowly cylindrical, gradually tapering towards the apex, and at maturity, they disarticulate from barrel type into individual spikelets with the rudimentary spikelets remaining attached to the culm. With 5 to 10 fertile spikelets, rudimentary spikelets are usually absent, but 1 - 2 are rarely developed. Moreover, spikelets are narrowly ovoid from 8 - 15 mm long, excluding awns. Glumes of lateral spikelets (8 - 10 mm long) are coriaceous, adpressed, velutinous and truncated with 2 - 3 teeth. The adaxial one that is acute and strongly developed extends up to a short setulose that awn up to 1 cm, while the other teeth that is broadly triangular and sometimes indistinct, shows depressions between teeth hyaline. However, the apical glume ovate rectangular (7 - 9 mm long), found on the apex truncate with a central awn that is up to 3.5 cm long, is flanked by 2 triangular lateral teeth. Lemmas of fertile florets exert the glumes (8 to 11 mm long) that are narrowly ovate and coriaceous, while apex of lateral lemmas has a sharp, setulose tooth at the keel that is about 5 mm long subapicaly. Sometimes, 2 - 3 indistinct lateral teeth, in the apical region of lemmas of apical spikelet flat, extend into a long, flat awn (7 to 9.5 cm long) that diverges and curves outward, with or without 2 sharp lateral teeth at the base. Paleas (8 to 8.5 mm long) are elliptical with 2 setose keels and each ending in a sharply acute apex, while caryopsis (6 to 8 mm long) are adherent to lemma and palea (Figure 1).

Distributed in Southeast Anatolia, C7 Şanliurfa

Dry meadows, 471 m, 36° 51.904' N 039° 34.410' E 22 May 2007, E. Cabi 2228b (with *A. crassa*), were gotten from Ceylanpinar State Farm, South of Horozmiran area,

Figure 1. Habit of *Ae. vavilovii* (Zhuk.) Chennav.

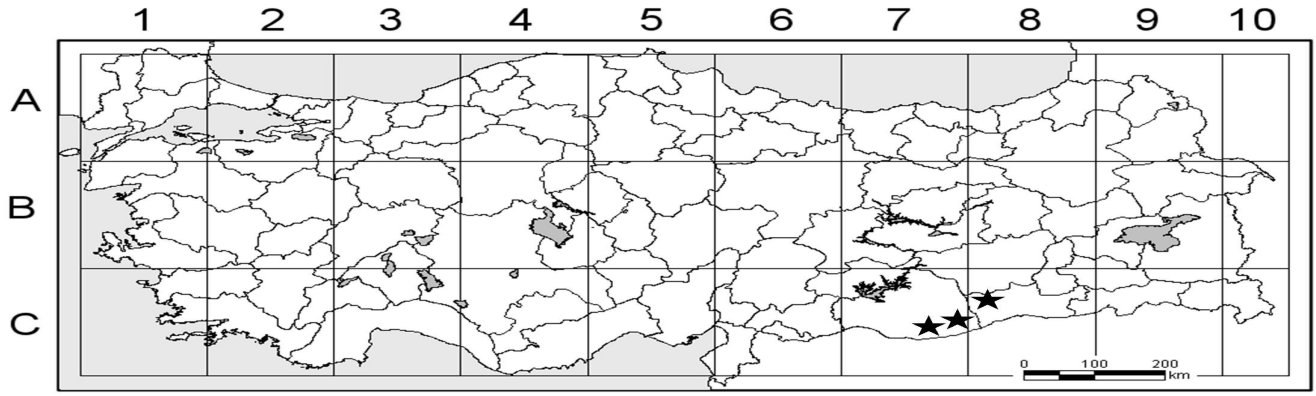

Figure 2. Distribution map of *Aegilops vavilovii* (Zhuk.) Chennav. in Turkey.

Ceylanpinar; C7 Şanliurfa: Calcareous soils and meadows, 381 m, 36° 49.163' N 039° 57.238' E 23 April 2007, E. Cabi 1839 (soil sample taken), were gotten from Ceylanpinar State Farm, Kaziktepe area, Ceylanpinar; C8 Mardin: Mazidağı to Derik, 9 km to Derik, 1055 m, 37° 25' 17" N 040° 19' 30" E, roadsides, 23 May 2007, E. Cabi 2322, E. Cabi 1839 (Figure 2). The specimens are deposited at the Department of Biological Sciences, METU.

Distribution

Western arc of the 'fertile crescent' is found in Jordan, Palestine, Syria and Lebanon and is uncommon throughout its range and rare in Turkey and Iraq.

Ecology and phenology

A. vavilovii grows in the steppes of Southeast Anatolia, where it is found at the edges of cultivated fields and roadsides at altitudes between 381 and 1055 m. As such, it flowers in April and May. The other grass species growing in the area are as follows: *Taeniatherum caput medusae* (L.) Nevski, *Hordeum bulbosum* L., *Hetaranthelium piliferum* (Banks and Sol.) Hochst., *Crithopsis delileana* (Schultes) Roshev and *A. crassa* Boiss. According to the results of analyses of the soil samples collected from the locality of *A. vavilovii*, it generally prefers clayish loam soils that are slightly alkaline (pH 7.7) with low organic content (1.54%). Levels of phosphorous and potassium present in the soil were found to be 3.67 and 182.99 ppm, respectively, while $CaCO_3$ was 36.24%. The habitat of *A. vavilovii* has low rainfall, which varies between 109.2 and 333.4 mm per year.

A. vavilovii (Figure 3A) can be confused with *A. crassa* (Figure 3B), but it seems quite different from it, in that the morphological differences between *A. vavilovii* and *A. crassa* are given in Table 1.

Ae. vavilovii (Zhuk.) Chennav., Acta Horti Gotoborg. 23:167 (1960, "Vavilovii"); Migushova and Chakimova, Bull. WIR 119:76 (1982); Chaudary, Grasses of Saudi Arabia 179 (1989, as Ae. "aff. Crassa"; see at Distribution).
Basionym: Ae. crassa Boiss. Ssp. vavilovii Zhuk., Bull. Appl. Bot., Gen. & Pl. Breeding 18(1): 554 (1928, "Vavilovii"); Hammer, Feddes Repert. 91: 234 (1980b); Feibrun-Dothan, Fl. Pal. 4: 173 (1986). Syn: Triticum syriacum Bowden Canadian Journal of Genetics and Cytology 8: 135. 1966.
Lectotype: Syria, Salamie ("Salamich") near the road, 30.IX.1926, Vavilov 29028 (WIR 747!).

Conservation

The genus *Aegilops* is likely to play a fundamental role as a valuable source of genetic diversity for breeding activities of wheat cultivars (Maxted et al., 2007) and it is critical that *Aegilops* is effectively conserved and made available to breeders for utilization. Maxted et al. (2008) used the available *ex-situ* data and proposed a list of species including *A. vavilovii* with the highest conservational priority.

The southwestern part of Turkey is very important on the basis of its genetic reserves for many economically important plant species. Moreover, Agriculture emerged about 10000 years ago in the 'fertile crescent' near East Gene Center (Karagöz, 1998), which includes the Southeast Anatolia.

A project called "*In-situ* Conservation of Genetic Diversity Project" was performed in Turkey between 1994 and 1998. The project aimed to maintain wild crop genetic resources in their natural habitats. For this purpose, natural and seminatural areas were selected for only 5 target crop species namely *T. monococum* L., *T. dicoccoides* (Koern.) Koern, *Aegilops speltoides* var.

Figure 3. (A) Spikes of *A. vavilovii* (Zhuk.) Chennav, (B) *A. crassa* Boiss.

Table 1. Morphological differences between *A. vavilovii* and *A. Crassa.*

Characters	*A. vavilovii*	*A. crassa*
Spike	Erect, narrowly cylindrical	Erect, distinctly moniliform
Glume apex	Apex of lateral glumes truncate with two-three teeth.	Apex of lateral glumes truncate with two unequally wide teeth, and sometimes, one of these teeth may develop into a short awn
Glume indumentum	Glumes covered densely with silky adpressed pubescence and longer hairs	Glumes covered densely with short silky adpressed pubescence
Lemmas of lateral spikelets	Apex of lateral lemmas with a sharp tooth up to 5 mm long	Apex of lateral lemmas with 1 short tooth increasing and developing towards the apex of spike
Lemmas of apical spikelets	Apex of apical spikelet lemmas extending into a long, flat awn, 5-8 (-10) cm long, diverging curving outward with or without teeth at the base	A single well-developed spikelet in the apical part may thus have up to 5 large lemma awns, dominating the aspect of spike

speltoides and var. *ligustica* and *A. tauschii*. Although it was not possible to allocate a gene management zone (GMZ) for every target species, the distribution and rarity of taxa should be taken into consideration in future conservation efforts. Until now, no special area has been reserved for *A. vavilovii* in Turkey. Kaya et al. (1998) indicated that recognition and initiation of GMZ concept is an important task in effective conservation activities. However, lack of information on taxonomy, biology, ecology and population genetics of potential target species, such as *A. vavilovii*, may have caused the oversight of some wild crop plant species. Besides those indicated as target species, new target species and conservation area(s) should be established as soon as possible by utilizing the distribution and commonness of the taxa.

Micro morphology

Epidermal features of the florets have been used initially to unravel the relationships in the grasses (Ellis, 1979; Doğan, 1988; Barkworth, 1990; Acedo and Llamas, 2001; Ortunez and De la Fuente, 2010). A study of glume and lemma epidermal features was ignited in an attempt to give detailed information about micro morphological structures of some parts of spikelets in *Ae. vavilovii* (Figure 4).

Three types of pointed structures, hooks, prickles and macrohairs were found on the glume and lemma epidermises of specimens. Hooks are generally less than 30 µm (often 20 to 30 µm or less) in any dimension that has rounded bases (Figures 4 E and F). The density of hooks in 1 mm^2 is 30 to 40 and have also been called barbs, crochets or crown cells (Ortunez and De la Fuente, 2010), or papillae in the literature (Liu et al., 2009).

Prickles are one-celled, rigid and have pointed structures that vary greatly in size and shape. They are one of the most abundant and widespread epidermal features of glume and lemma in the *Aegilops.* Prickles are visible with the naked eye and are always larger than hooks. However, their length is up to 200 µm and their densities are higher towards the apex part of glume and lemma.

Silica bodies are elliptic or circular in shape and are also arranged longitudially between the long cells. Their lengths are between 15 and 20 µm and in general, between 15 and 20 in 1 mm^2.

In this descriptive study, we examined the glume and lemma micromorphology of *Ae. vavilovii.* To be able to discusss phylogenetic implications for the taxonomy of *A. vavilovii*, it should be needed to study the micromorphological parts of the other taxa in the genus, especially having a genome containing D, M and S genomes or combinations of them. So, we can now understand how much correlation exist between micromorphology of glumes and lemmas and genomic constitution.

Palynological features

Typically, pollen grains of *Ae. vavilovii* are heteropolar, monoporate and spheroidal (A/B: 1, 13) as Poaceous and the outline is circular in polar view. The spheroidal pore (pa/pb: 1, 20) is surrounded with a distinct annulus and is covered by an operculum (Figures 5A, B, C). However, the pollen morphological parameters are presented in Table 2.

Pollen grains of Gramineae were classified in previous studies as annulate or nonannulate and operculate or nonoperculate by Salgado-Labouriau and Rinaldi (1990), Chaturvedi et al. (1998) and Perveen (2006). The exine ornamentation types in Gramineae were defined as insular, granulose, spinulose, scabrate, verrucose and brevicerebro ornate by most authors (Chaturvedi et al., 1994, 1998; Liu et al., 2004; Özler et al., 2009). Erdtman (1969) and Moore et al. (1978, 1991) used the term scabrate for exine sculpture covered with small (<1 µm) elements, equivalent to granulate exine ornamentations. According to the present study, SEM photomicrographs show that exine sculpture is an insular (formed by the fusion of scabrae, dividing the surface into small areas) type in *A. vavilovii* (Figure 5D). Each of the insulae have various number of scabra that shows a compact pattern and the scabra number changed from 2 to 11 in each insula and 22 to 31 in 4 μm^2 (Figure 5D). The scabrae are characterised closely as spaced, while the distance between scabra varies from 0.10 to 0.30 µm. The height and base of scabrae varies from 0.14 to 0.25 µm and 0.25 to 0.37 µm, respectively. Exine sculpture on the operculum is similar to that on the pollen surface. Some authors reveal that pollen exine surface show differences at generic or higher taxonomic levels in the family Poaceae (Köhler and Lange, 1979; Chaturvedi et al., 1994). Similarly, exine thickness of Gramineae was found to be 1 to 1.5, 1 to 1.53, 0.90 to 1.10, 0.5 to 1, 0.85 to 1.11 and 1.46 to 1.58 (Erdtman, 1943; Lewis et al., 1983; Salgado-Labouriau et al., 1990; Liu et al., 2004; Pehlivan et al., 2004; Özler et al., 2009).

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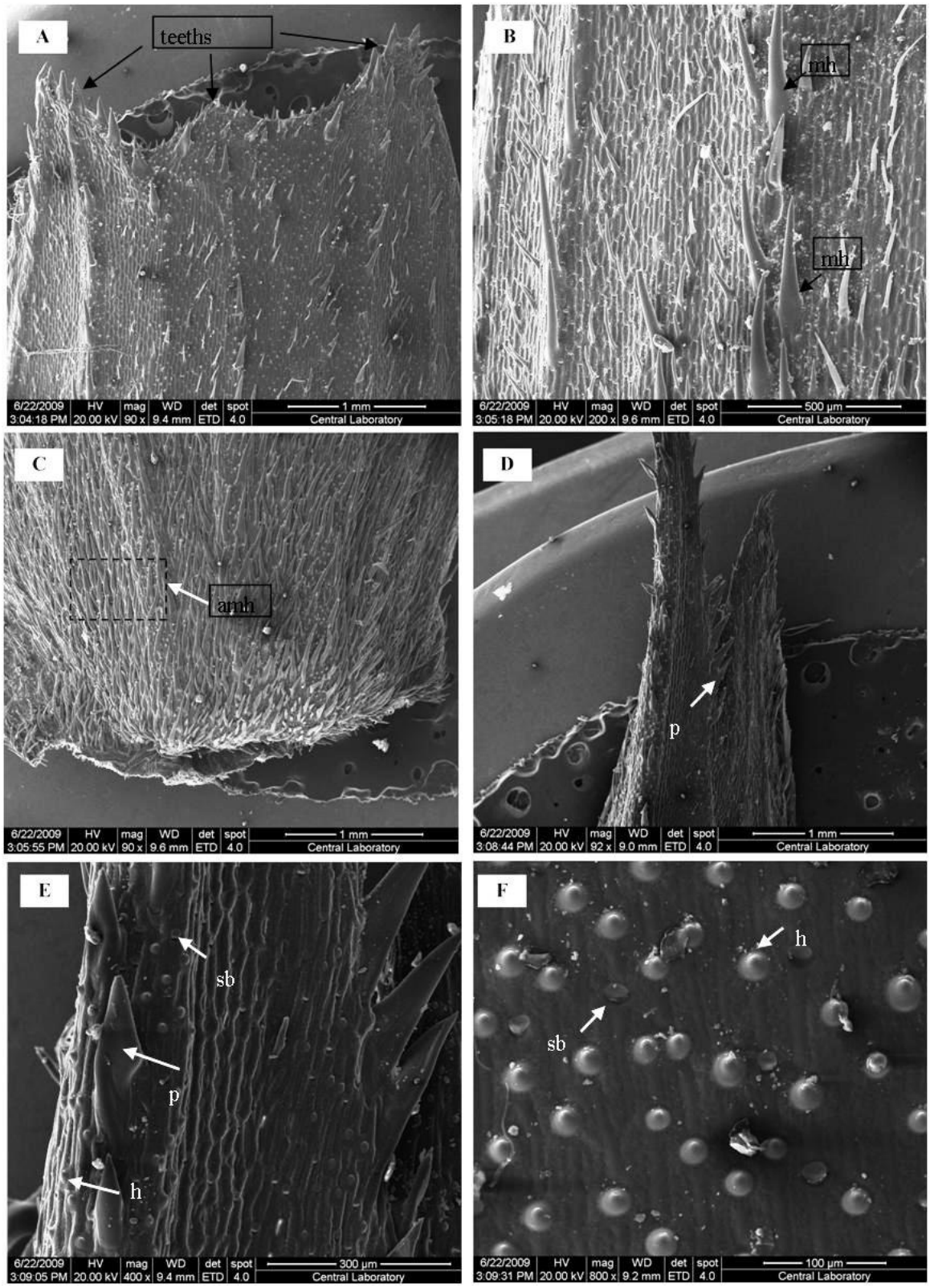

Figure 4. Micromorphology of abaxial glume and lemma epidermises of *A. vavilovii.* A - Apex of glumes and truncate glume apex with three teeth; B - Surface of glumes and macrohairs (arrows); C - Lower part of glumes (adpressed macro hairs); D-F – Lemmas of *A. vavilovii*; D- Apex of lemmas, prickles (arrows); E - Surface of lemmas towards the awn, prickles, hooks and silica bodies; F - Surface of lemmas, hooks and silica bodies. Abbreviations: sb, silica body; mh, macrohairs; amh, addpressed macro hairs; p, prickles; h, hooks.

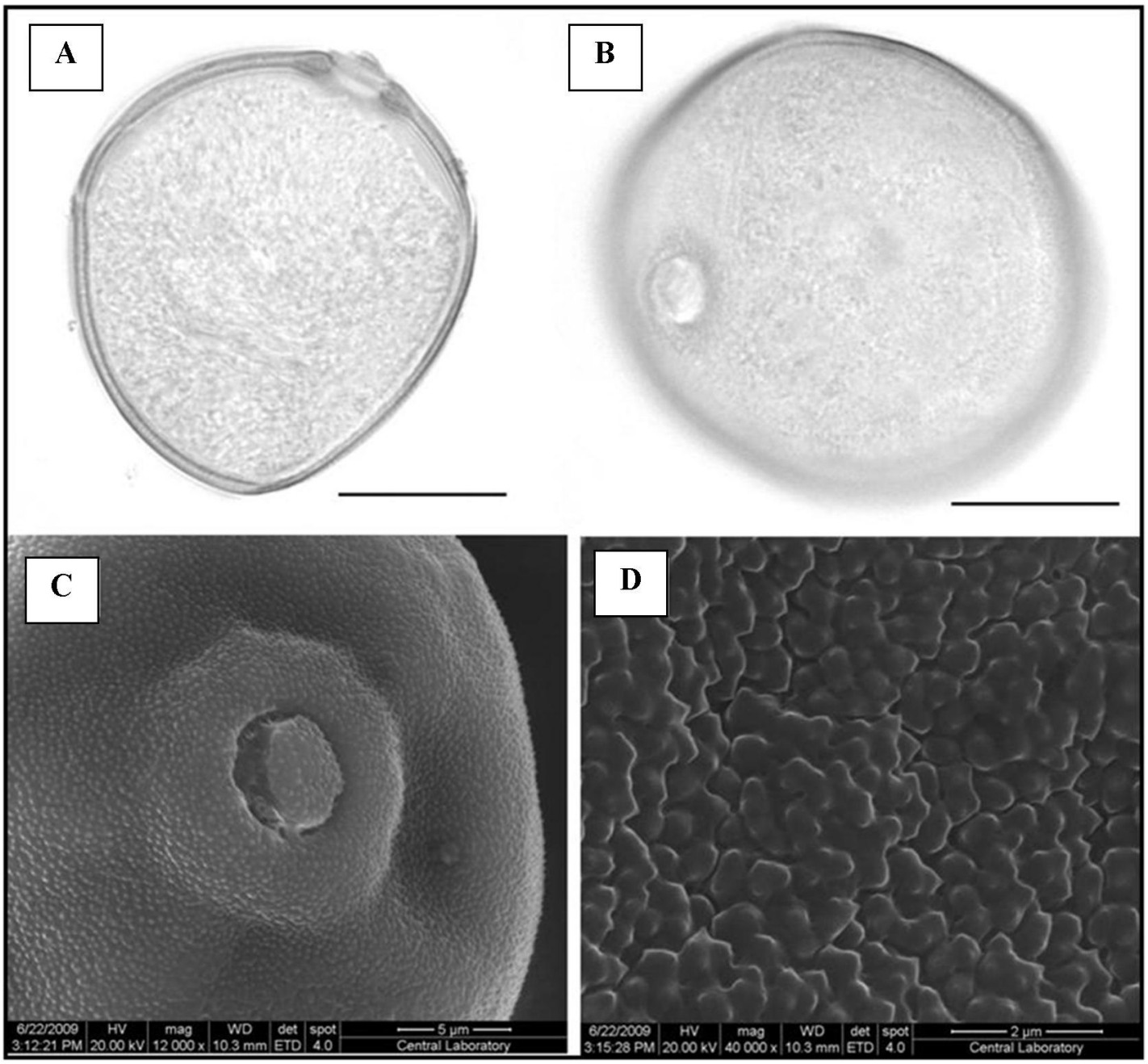

Figure 5. Pollen grains of *A. vavilovii* in LM (A-B) and SEM (C-D). A - Equatorial view; B - Polar view; C - Operculate-annulate pore; D - Insular type exine surface (Scale bar: A, B: 20 µm; C: 5 µm D: 2 µm).

Table 2. Palynological characters (µm) of *A. vavilovii* (mean ± standard deviation).

	A	B	pa	pb	Anndiam	Annheigh	Opercu	E	i	I
A. vavilovii	51.52±2.55	45.40±2.42	6.66±0.78	5.52±0.78	4.76±0.63	2.64±0.38	4.10±0.60	1.44±0.27	1.09±0.23	3.05±0.83
Minimum	44.97	41.52	5.22	3.86	3.82	1.94	2.80	0.92	0.50	2.12
Maximum	57.52	51.12	8.21	7.75	6.80	3.75	5.39	1.99	1.62	5.85

A: long axis of spheroidal pollen grains; B: short axis of spheroidal pollen grains; pa: long axis of ellipsoidal pore; pb: short axis of ellipsoidal pore; Anndiam: annulus diameter; Annheigh: annulus height; Opercu: operculum diameter; E: exine; i: intine thickness; I: the thickest part of the intine (*n* =40).

examinations.

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