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POLLEN MORPHOLOGY OF *AGROPYRON* GAERTNER IN TURKEY

HÜLYA ÖZLER[1], EVREN CABI[2], EBRU US[3], MUSA DOĞAN AND SEVIL PEHLIVAN[3]

Department of Biology, Faculty of Science, Middle East Technical University, Ankara, Turkey.

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Abstract

Pollen morphology of *Agropyron cristatum* (L.) Gaertner. *s.s.* (subsp. *incanum* (Nábělek) Melderis and subsp. *pectinatum* (M. Bieb.) Tzvelev, latter including var. *pectinatum* and var. *imbricatum* (Roemer & Schultes) G. Beck) in Turkey has been studied by using light microscope and scanning electron microscope. The above-mentioned taxa are homogenous in both aperture type and exine ornamentation. Pollen grains are monoporate (rarely diporate in the case of var. *imbricatum*) having scabrate grouped exine surface. The scabra density and the height of scabrae as well as other morphological parameters such as annulus and operculum diameter are peculiar features for differentiation of taxa. Two different phenograms were created with the UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering technique using quantitative measurements of the pollen grains.

Introduction

The taxonomy of *Agropyron* Gaertner (Poaceae) was studied in different ways by various researchers. In a broad sense, it was once thought to be one of the largest genera encompassing more than 100 species in the tribe Triticeae Dumort (Dewey, 1983). Nevski (1934) treated *Agropyron* as a small genus consisting only of the species with keeled glumes. The remaining taxa were placed in *Elytrigia* Desv*, Roegneria* C. Koch and *Elymus* L. *Agropyon* has been restricted to the species with P genome composed of three ploidy levels (2x = 14, 4x = 28, 6x = 42) (Dewey and Asay, 1975; Melderis, 1978; Dewey, 1983; Assadi, 1995; Jensen *et al.*, 2006). This narrow concept of *Agropyron* has been accepted by many authorities. The major Eurasian floras have followed the generic concept of Nevski (Tzvelev, 1976; Melderis *et al.,* 1980; Melderis, 1985).

Much confusion prevailed regarding the number of species included in this genus. Dewey and Pendse (1967) considered all the crested wheatgrasses, regardless of ploidy level, as a single breeding population. Tzvelev (1976) recognized 10 species in *Agropyron* and nine subspecies in *Agropyron cristatum* (L.) Gaertner.

Agropyron cristatum is represented by two subspecies in Turkey, namely subsp. *incanum* (Nábělek) Melderis distributed in East Anatolia and subsp. *pectinatum* (M. Bieb.) Tzvelev distributed throughout Turkey. Morphologically, subsp. *incanum* is

[1]The Ministry of Environment and Forest, Forest Tree Seeds and Tree Breeding Research Directorate, P.O. Box 11, 06560 Gazi, Ankara, Turkey.
[2] Corresponding author. E-mail: ecabi@metu.edu.tr, ecabi2004@yahoo.com
[3] Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey.

distinctly different by having spikes with closely pressed together spikelets and densely pilose lemmas contrary to subsp. *pectinatum*. Subspecies *pectinatum* includes two varieties *viz.* var. *imbricatum* (Roemer & Schultes) G. Beck which has pilose spikelets and var. *pectinatum* with glabrous spikelets (Melderis, 1985).

There have been some studies regarding pollen morphology of members of tribe Triticeae, especially the economically important genera such as *Triticum* L., *Secale* L., and *Hordeum* L. (Faegri and Iversen, 1975; Köhler and Lange, 1979; Kruse, 1980; Panajiotidis *et al.*, 2000; Kalinowski *et al.*, 2005), but rarely on taxa of *Agropyron* (Smith, 2000).

The aim of this paper is to describe the palynological features of three taxa of the genus *Agropyron* available in Turkey and also to contribute to their taxonomy showing some differences between them regarding their exine sculptures.

Materials and Methods

Pollen samples from each of the three studied taxa were obtained from herbarium specimens listed in Table 1. The identifications of the specimens were made according to the *Agropyron* accounts given in Flora of Turkey (Melderis, 1985). For the light microscope study, the pollen grains were prepared following the Wodehouse (1935) and Erdtman (1952) methods. Morphological features of 30 pollen grains were measured using Leica DM 1000 and their microphotographs were taken by the Leica DFC280 camera attachment. The measurements included the following parameters: long axis of spheroidal pollen grains (A), short axis of spheroidal pollen grains (B), long axis of ellipsoidal pore, short axis of ellipsoidal pore, exine thickness, annulus diameter, A/B ratio indicating shape of a spheroidal pollen grain, operculum diameter, thickest part of intine, and intine thickness.

Table 1. Investigated taxa with their site of collections.

#	Coll. No.	Taxon	Locality	Altitude
1	E. Cabi 2545	*Agropyron cristatum* (L.) Gaertner subsp. *incanum* (Nábělek) Melderis	A8 Erzurum: Aşkale to Bayburt, Kop Mount, Kop pass, calcerous slopes (40°01'38"N, 40°31'20"E)	2401 m
2	E. Cabi 2258	*Agropyron cristatum* subsp. *pectinatum* (M. Bieb.) Tzvelev var. *imbricatum* (Roemer & Schultes) G. Beck	A9 Kars: Kuyucak village, Kuyucak Lake environments, dry pastures (40°43'41"N, 43°25'30"E)	1642 m
3	E. Cabi 2244	*Agropyron cristatum* subsp. *pectinatum* var. *pectinatum*	B6 Sivas: Sivas Cumhuriyet University Campus, roadside and under forest (39°42'28"N, 37°01'10"E)	1275 m

Using the average values of pollen measurements, phenograms of the investigated taxa were produced for fresh (W, Wodehouse) and acetolysed (E, Erdtman) pollen grains

based on the Gower General Similarity Coefficient. Gower's (1971) coefficient was chosen to generate a distance matrix. This distance matrix was used for cluster analysis with the help of UPGMA algorithm (Sneath and Sokal, 1973). Correlation values were obtained using SPSS version 11.0 (SPSS, 1999).

For scanning electron microscope (SEM) studies, pollen grains were put on stubs, sputter coated with gold and examined under Jeol JSM-6060LV SEM at the central laboratory of Middle East Technical University. The terminologies for pollen morphology were used in accordance with Wodehouse (1935), Faegri and Iversen (1989), and Chaturvedi *et al.* (1998).

Results and Discussion

The means and standard deviations of the measured pollen parameters of taxa are given in Table 2. All investigated taxa had heteropolar, monoporate (rarely diporate in the case of var. *imbricatum*) and spheroidal pollen grains. The pore was surrounded by an annulus and it was partly covered by an operculum. Pollen grains of Gramineae were classified as annulate or nonannulate and operculate or nonoperculate by Perveen (2000, 2006), Chaturvedi *et al.* (1998) and Salgado-Labouriau and Rinaldi (1990). They also rarely observed diporate pollen grains. In this study, we observed that all taxa were annulate and operculate (Figs 1-3).

The exine ornamentation types in Gramineae were defined as insular, granulose, spinulose, verrucose, brevicerebro ornate (Chaturvedi *et al.,* 1994, 1998; Liu *et al.*, 2004). Erdtman (1969), Moore and Webb (1978) and Moore *et al*. (1991) used the term scabrate for exine sculpture covered with small (<1 μm) elements, equivalent to granulate exine ornamentations. According to our SEM investigations, exine sculpture is scabrate in all examined taxa (Figs 1-3).

Köhler and Lange (1979) proposed that the number of spinules may be used for identification. In present study, we found that the investigated taxa could be differentiated based on exine parameters such as the number and height of scabrae and the distance between the scabrae. In all investigated taxa, 2 to 4 scabrae were observed in each group. The distance between scabrae was greater in *Agropyron cristatum* subsp. *incanum* than other two taxa. Based on scabra number and height, var. *pectinatum* can be differentiated from other two taxa (Figs 1-3). Scabrae density was 19-20 no./μm^2 in subsp. *pectinatum* var. *pectinatum*, 8-9 no./μm^2 in subsp. *pectinatum* var. *imbricatum,* and 5-7 no./μm^2 in subsp. *incanum*. The highest scabra was 0.29 μm in subsp. *pectinatum* var. *pectinatum*, 0.18 μm in subsp. *pectinatum* var. *imbricatum*, and 0.17 μm in subsp. *incanum*. The scabrae were wider in subsp. *incanum* (0.32 μm) than in subsp. *pectinatum* var. *imbricatum* (0.26 μm) and in var. *pectinatum* (0.21 μm).

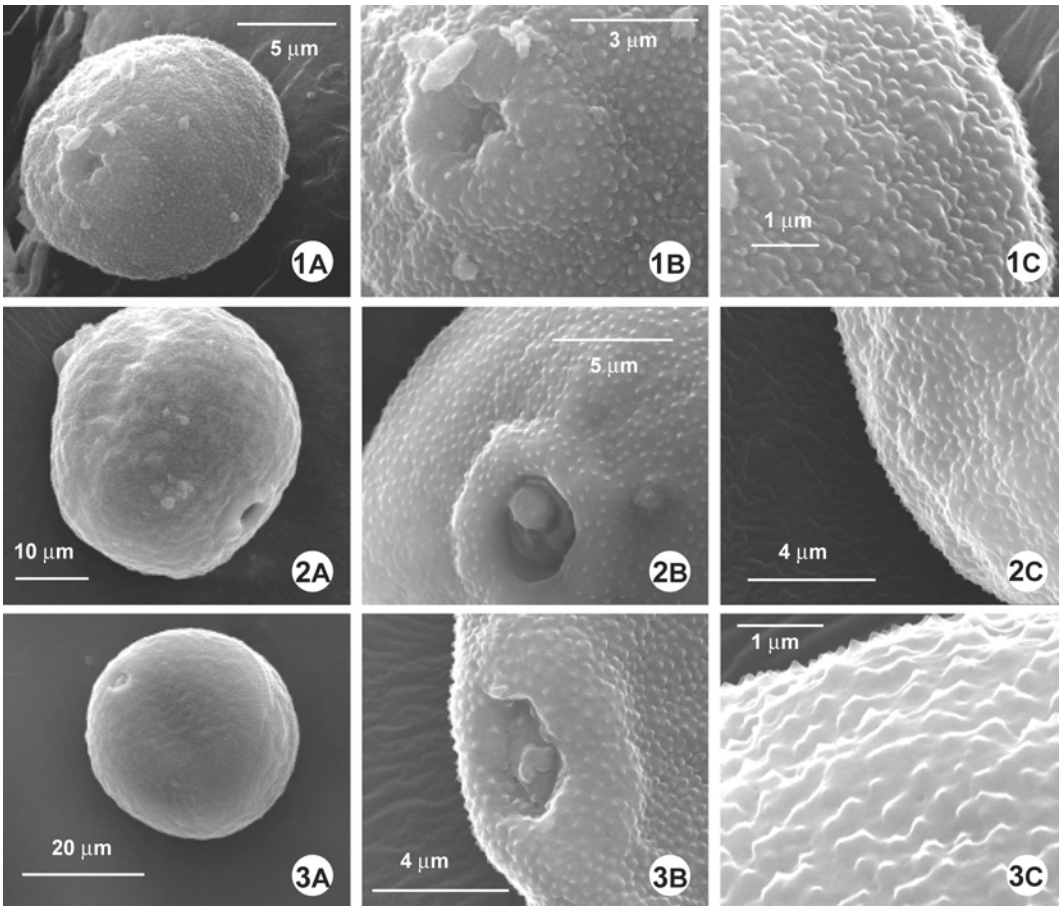
Exine was thicker in subsp. *pectinatum* var. *imbricatum* and subsp. *incanum* than subsp. *pectinatum* var. *pectinatum* (Table 2). Typical measurements for exine thickness

Table 2. Pollen morphological parameters (µm) of three investigated taxa of *Agropyron* (mean ± standard deviation) (n = 30).

Taxon	Preparation	A	B	A/B	Pa	Pb	I	i	Exine	Op	An
A.cristatum subsp. *pectinatum* var. *pectinatum*	W	34.84 ± 2.27	31.46 ± 2.77	1.11	3.73 ± 0.45	3.26 ± 0.36	2.84 ± 0.39	0.77 ± 0.17	1.46 ± 0.15	2.40 ± 0.36	8.63 ± 0.78
	E	33.05 ± 2.08	28.91 ± 1.88	1.14	3.31 ± 0.29	2.84 ± 0.26	-	-	1.46 ± 0.15	-	8.11 ± 0.41
A. cristatum subsp. *pectinatum* var. *imbricatum*	W	33.31 ± 2.31	31.02 ± 2.88	1.08	4.29 ± 0.49	3.77 ± 0.44	2.90 ± 1.49	0.71 ± 0.16	1.66 ± 0.15	2.55 ± 0.49	9.63 ± 1.00
	E	40.74 ± 1.88	37.91 ± 2.00	1.05	4.79 ± 0.36	4.22 ± 0.45	-	-	1.46 ± 0.15	-	11.55 ± 0.81
A. cristatum subsp. *incanum*	W	32.01 ± 2.76	29.69 ± 2.85	1.08	3.34 ± 0.40	2.82 ± 0.38	3.20 ± 1.15	0.74 ± 0.16	1.58 ± 0.19	1.84 ± 0.41	8.31 ± 0.75
	E	38.38 ± 1.73	36.38 ± 1.88	1.05	3.75 ± 0.35	3.43 ± 0.44	-	-	2.05 ± 0.18	-	10.22 ± 0.83

W: Non-acetolysed pollen grains, E: Acetolysed pollen grains, A: Long axis of spheroidal pollen grains, B: Short axis of spheroidal pollen grains, A/B: Shape of pollen grains, Pa: Long axis of ellipsoidal pore, Pb: Short axis of ellipsoidal pore, I: Thickest part of the intine, i: Intine thickness, Op: Operculum diameter, An: Annulus diameter.

of Gramineae are (0.5-)0.85-1.10(-1.53) μm (W) and 1.02-1.61 μm (E) (Erdtman, 1943; Lewis *et al.,* 1983; Salgado-Labourian and Rinaldi, 1990; Liu *et al.,* 2004; Pehlivan *et al.,* 2004).



Figs 1-3. 1. *Agropyron cristatum* subsp. *pectinatum* var. *pectinatum*. A. Slightly oblique polar view with distinct annulus; B. Aperture view with operculum; C. Scabrate grouped exine surface. 2. *A. cristatum* subsp. *pectinatum* var. *imbricatum*. A. Pollen grain in equatorial view; B. Aperture view with distinct annulus; C. Scabrate with grouped exine surface. 3. *A. cristatum* subsp. *incanum*. A. Pollen grain in equatorial view; B. Aperture view with distinct annulus; C. Scabrate with grouped exine surface.

Liu *et al.* (2004) found a positive correlation between pollen and aperture size and between aperture and annulus diameter. In our study, both acetolysed (E) and fresh pollen (W) measurements also showed that there are strong positive correlations between the long axis of pollen grain and pore, and short axis of pollen grain and pore (Table 3).

Table 3. Correlation coefficient for different pollen morphological parameters at $P < 0.001$.

	Wodehouse (W)			Erdtman (E)		
	Pb	A	B	Pb	A	B
Pa	0.999	0.365		0.989	0.901	
Pb			0.687			0.875
A			0.946			0.946

W: Non-acetolysed pollen grains, E: Acetolysed pollen grains, 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.

Differences between measurements of fresh and acetolysed pollen grains resulted in the formation of different clusters. Although the two subspecies differentiated distinctively and the varieties of subsp. *pectinatum* formed a tight cluster with respect to their quantitative pollen data obtained from fresh pollen grains, var. *pectinatum* formed a different cluster due to their acetolysed pollen grains (Fig. 4). The phenogram obtained from measurements of fresh pollen grains is much more suitable for separating the taxa regarding their morphological pollen features in the genus *Agropyron*. Although acetolysed pollen grains give excellent topographic information, but due to the very process of acetolysis they get modified, thus do not represent actual size.

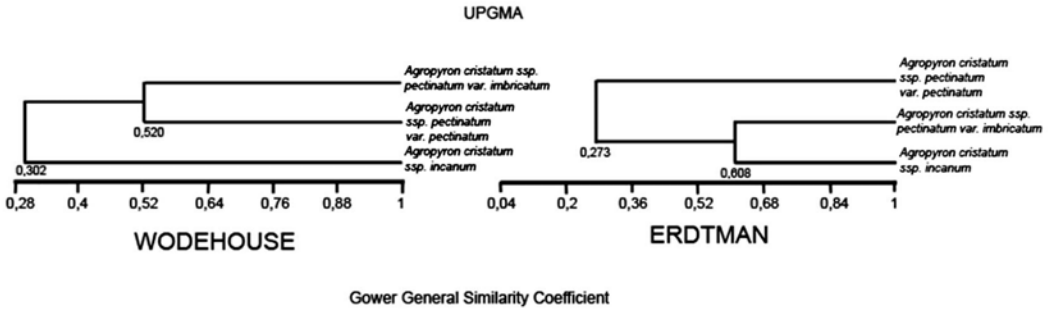


Fig. 4. UPGMA phenograms of the investigated taxa based on Gower General Similarity Coefficient.

The results indicate that the genus *Agropyron* has stenopalynous pollen grains, thus the value of pollen characters for taxonomic applications is limited. Faegri and Iversen (1975), Andersen (1978), and Perveen (2006) also indicated similar uniformity in pollen grains of Poaceae. The application of cluster analysis showed the possibility of using quantitative data based on fresh pollen grains for differentiating the taxa. The density of scabrae and the distance between the scabrae can be used as the most functional differentiating characters.

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