

## Pollen Morphology of the Genus *Eremopyrum* (Poaceae) in Turkey

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**Abstract:** The genus *Eremopyrum* (Ledeb.) Jaub. and Spach belonging to tribe Triticeae Dumort. is represented by four species in Turkey, namely *E. triticeum* (Gaertner) Nevski, *E. orientale* (L.) Jaub and Spach, *E. distans* (C. Koch) Nevski and *E. boneapartis* (Sprengel) Nevski (Melderis 1985). The pollen morphology of *Eremopyrum* genus is investigated by LM and SEM. Pollen grains are monoporate, tectate, heteropolar, prolate-spheroidal and operculate-annulate. Exine sculpture is mixed scabrate type in *E. triticeum*, *E. boneapartis* subsp. *hirsutum* and *E. distans* and insular type in *E. orientale*. Furthermore, quantitative data were subjected to cluster analysis. The obtained phenogram revealed that the exclusively tetraploid *E. orientale* is strictly different from the remaining taxa as regards their quantitative pollen profile. The results of clustering is not consistent with the sectional delimitation proposed by Nevski.

**Key words:** Pollen morphology · LM · SEM · *Eremopyrum* · Turkey

### INTRODUCTION

The genus *Eremopyrum* (Ledeb.) Jaub. and Spach, is a well defined genus considered as very important in breeding experiments as a genetic source, due to its close phylogenetic relationship with *Triticum* [1].

*Eremopyrum* is represented by four species and two subspecies in Turkey: *E. boneapartis* subsp. *boneapartis* (Spreng.) Nevski, *E. boneapartis* subsp. *hirsutum* (Bertol.) Melderis, *E. distans* (K.Koch) Nevski, *E. orientale* (L.) Jaub and Spach and *E. triticeum* (Gaertner) Nevski [2]. Frederiksen [3] recognized four species in the genus *Eremopyrum* without splitting any subspecific taxa. Nevski [4] divided this genus into two sections based on disarticulation pattern of the species. Section *Micropyron* Nevski includes only one species *E. triticeum* has the tough rachis. The remaining species are included in section *Eremopyrum* has fragile rachis.

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. [5-10]. Until now no studies have been conducted on *Eremopyrum* regarding its pollen morphology.

The aim of this paper is to describe the palynological features of the genus *Eremopyrum* and also to contribute to their taxonomy showing some differences among the species regarding their palynological features.

### MATERIALS AND METHODS

The studied plant material was collected from different populations in Turkey. The list of specimens, localities are given in Table 1. The collected plants were identified according to flora of Turkey by coauthors Dogan and Cabi. Voucher specimens are deposited in the GAZI herbarium. Spikes from collected plants of each taxa were removed and brought to the laboratory at a period slightly before anther dehiscence. They were placed in small vials with water and the anthers were allowed to open. The material for LM was prepared according to Wodhouse [11] and Erdtman [12] methods and mounted in glycerin. Thirty (30) intact pollen grains from each taxon were scored under LM for the following parameters: long axis of spheroidal pollen grains (A), short axis of spheroidal pollen grains (B), long axis of elipsoidal porus (pa), short axis of elipsoidal porus (pb), annulus thickness (An), exine thickness (E) and P/E ratio and microphotographs were taken Leica DM 1000 in METU.

Table 1: Studied taxa and their sites of collection

Taxa	Abbreviation	Chromosome Number	Collection site
<i>E. bonaepartis</i> subsp. <i>hirsutum</i> (Bertol.) Melderis	hirsutum	14,28	Sivas: Boğazlıdere to Kutlukaya 1471 m
<i>E. distans</i> (K.Koch) Nevski	distans	14	Ağrı to Doğubeyazıt : Ağrı 1730 m
<i>E. orientale</i> (L.) Jaub and Spach	orientale	28	Ağrı: Ağrı to Doğubeyazıt, 21 km before Diyadin 1805 m
<i>E. triticeum</i> (Gaertner) Nevski.	triticeum	14	Erzurum: Horasan town centre 1715 m

All data were subjected to clustering analysis. A phenogram of the taxa was produced based on the Gower General Similarity coefficient using the UPGMA clustering technique [13]. For scanning electron microscopy (SEM) investigations, the pollen grains were put on stubs, sputter-coated with gold plate, and examined under a Jeol JSM-6060 in METU. The terminologies for pollen morphology were used in accordance with Wodehouse [11], Punt *et al.* [14], Faegri and Iversen [15] and Chaturvedi *et al.* [16] and Datta and Chaturvedi [17].

RESULTS

Pollen grains are heteropolar, monoporate, tectate, annulate-operculate, prolate-spheroidal and porus shape subprolate and prolate-spheroidal. Pollen dimensions

A X B (28.77±1.11-41.38 ± 2.55 µm (W) - 30.14 ± 1.68-44, 20 ± 2.12 µm (E) X 28.06 ± 1.06-37.79 ± 2.48 µm (W)- 28.93 ± 1.49-42.56 ± 2.05 µm (E) ), Pa value (3.75 ± 0.30- 6.14 ± 0.74 µm (W)- 3.76± 0.36-4.68 ± 0.30 µm (E) ) Pb value (3.41 ± 0.36-5.31± 0.40 µm (W) - 3.48 ± 0.37-4.58 ± 0.30 µm (E) ), operculum diameter (2.14± 0.24 -2.64 ± 0.18 (W) µm) and annulus thickness (1.91 ± 0.17-2.75 ± 0.37 µm (W)- 1.80 ± 0.25 - 2.17 ± 0.20 µm (E) and exine thickness is 1.64 ± 0.20-1.86 ± 0.17 µm (W)-1.68 ± 0.21-2.10± 0.17 µm (E) (Fig. 1, Table 2). According to SEM investigations, exine sculpture is mixed scabrate (free scabra are interspaced two, three or four fused scabra) in *E. triticeum*, *E. bonaepartis* ssp. *hirsutum* and *E. distans* and insular type in *E. orientale* (Fig. 2, 3). Scabrae of *E. triticeum* and *E. bonaepartis* ssp. *hirsutum* are widely spaced and surface of *E. distans* is compact pattern.

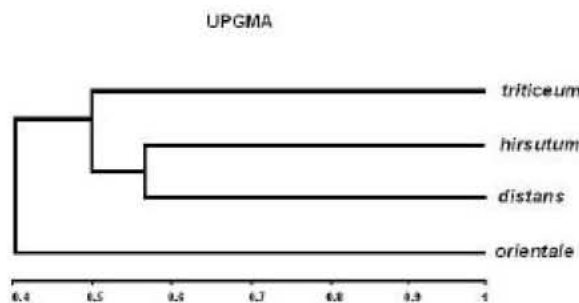


Fig. 1: UPGMA phenogram of the investigated taxa based on Gower General Similarity Coefficient

Table 2: Pollen morphological parameters of investigated taxa (µm) (Mean ± Standard Deviation)

Taxa	A (µm)	B (µm)	A/B	E (µm)	I (µm)	i (µm)	Pa (µm)	Pb (µm)	Pa/Pb	An. (µm)	Operculum (µm)
<i>E. orientale</i> (W)	41.38±2.55	37.79±2.48	1.10	1.68±0.18	3.06±0.20	0.61±0.12	6.14±0.74	5.31±0.40	1.16	2.21±0.20	2.52±0.31
<i>E. orientale</i> (E)	39.49±2.25	37.65±2.19	1.05	1.70±0.21	-	-	4.68±0.30	4.58±0.30	Subprolate	2.14±0.24	-
			Prolate spheroidal						1.02		
									Prolate spheroidal		
<i>E. distans</i> (W)	35.10±1.53	34.37±1.19	1.02	1.86±0.17	3.21±0.49	0.51±0.15	4.58±0.35	4.26±0.33	1.08	1.91±0.17	2.64±0.18
<i>E. distans</i> (E)	30.14±1.68	28.93±1.49	1.04	1.76±0.17	-	-	3.76±0.36	3.48±0.37	1.08	1.74±0.19	-
			Prolate spheroidal						Prolate spheroidal		
<i>E. triticeum</i> (W)	28.77±1.11	28.06±1.06	1.03	1.64±0.20	3.99±0.58	0.41±0.18	3.75±0.30	3.41±0.36	1.10	1.80±0.36	2.14±0.20
<i>E. E. triticeum</i> (E)	44.20±2.12	42.56±2.05	1.04	2.10±0.17	-	-	4.09±0.55	3.82±0.41	1.07	2.17±0.20	-
			Prolate spheroidal						Prolate spheroidal		
<i>E. bonaepartis</i> ssp. <i>hirsutum</i> (W)	37.38±1.36	36.29±1.48	1.03	1.72±0.25	5.05±1.20	0.36±0.16	4.88±0.48	4.72±0.43	1.03	2.75±0.37	2.41±0.31
<i>E. bonaepartis</i> ssp. <i>hirsutum</i> (E)	31.78±1.58	30.37±1.50	1.05	1.68±0.21	-	-	3.85±0.34	3.56±0.29	1.08	1.80±0.25	-
			Prolate spheroidal						Prolate spheroidal		

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 E: Exine thickness pa: Long axis of ellipsoidal porus pb: Short axis of ellipsoidal porus I: Thickest part of the intine i: Intine thickness Op: Operculum diameter An.: Annulus thickness

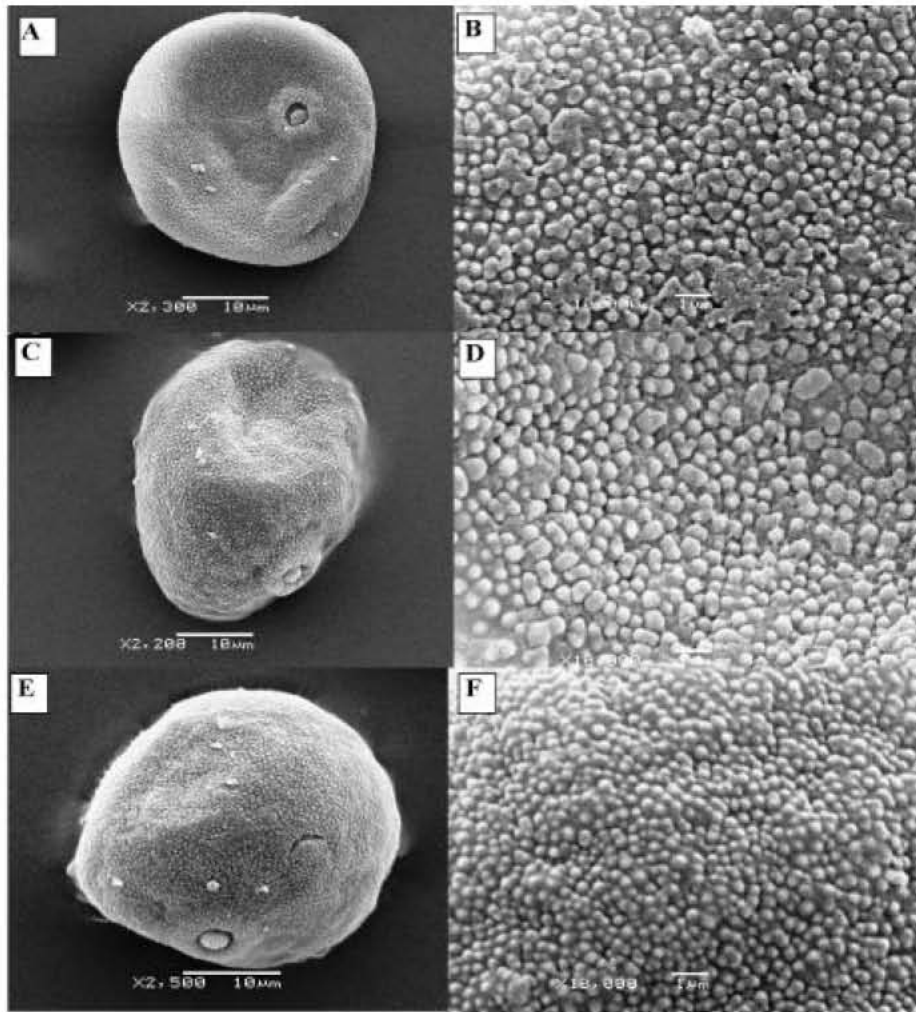


Fig. 2: SEM photographs of the studied taxa. (A-B) *E. triticeum* A. Slightly oblique polar view with annulate-operculate pore (x 23000) B. Mixed scabrate exine surface (x 10 000) (C-D) *E. bonaepartis* ssp. *hirsutum* C. Equatorial view with annulate-operculate aperture (x2200) D. Mixed scabrate exine surface (x 10000) (E-F) *E. distans* E. Equatorial view with annulate-operculate aperture (X 2500) F. Mixed scabrate exine surface (Scale bar: A, C, E: 10  $\mu$ m; B, D, F: 1  $\mu$ m)

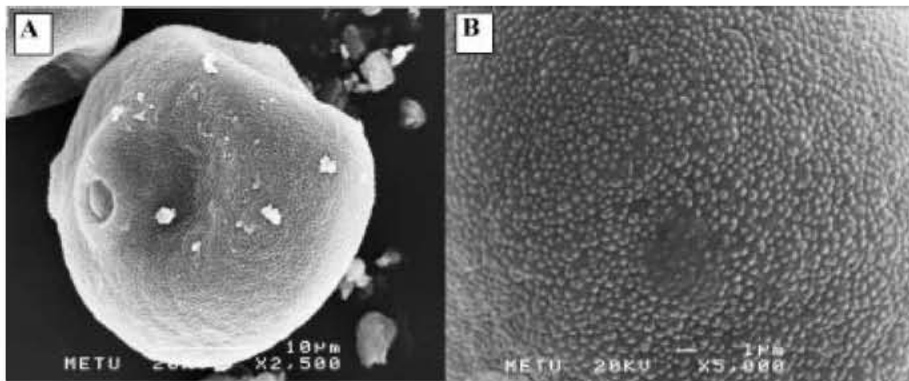


Fig. 3: *E. orientale* A. Polar view with annulate-operculate aperture (x 2500) B. Insular exine surface (X 5000) ( A: 10  $\mu$ m; B: 1  $\mu$ m)

Scabra number in 1  $\mu\text{m}^2$  is 5-7 in *E. Bonaepartis* ssp. *hirsutum* and *E. orientale*, 5-7 in *E. triticeum* and 10-12 in *E. distans* (Fig. 2 B, D, F, 3B)<sup>a</sup>

## DISCUSSION

The pollen grains in Gramineae generally are spheroidal, ovoidal, elipsoidal subelipsoid, subprolate and suboblate [11, 18-23]. Pollen dimensions were measured A X B ( $28.77 \pm 1.11 - 41.38 \pm 2.55 \mu\text{m}$  (W) -  $30.14 \pm 1.68 - 44.20 \pm 2.12 \mu\text{m}$  (E) X  $28.06 \pm 1.06 - 37.79 \pm 2.48 \mu\text{m}$  (W) -  $28.93 \pm 1.49 - 42.56 \pm 2.05 \mu\text{m}$  (E)). Pollen dimension AXB  $44.20 \pm 2.12 - 42.56 \pm 2.05 \mu\text{m}$  (E) is the most in *E. triticeum*,  $30.14 \pm 1.68 - 28.93 \pm 1.49 \mu\text{m}$  (E) is the lowest in *E. distans* (Table 2). Smith [24] measured pollen dimension 31-52  $\mu\text{m}$  for *Agropyron* pollen grains and 62-64  $\mu\text{m}$  for *Triticum aestivum* pollen grains. Annulus thickness was measured at most in *E. triticeum* and the lowest in *E. distans* (Table 2). Chaturvedi *et al.* [16] and Liu *et al.* [22] were found that annulus diameter were 5-16  $\mu\text{m}$  and 0-9.4  $\mu\text{m}$ . Moore *et al.* [25] explained that wild Gramineae taxa had annulus diameter small than 8  $\mu\text{m}$ , annulus diameter for *Avena-Triticum* had bigger than 10  $\mu\text{m}$  for *Secale* and *Hordeum* had 8-10  $\mu\text{m}$ . Pa/Pb value was between 1.03-1.16 (W) and 1.02-1.08 (E). Porus shape is subprolate and prolate-spheroidal. Some authors classified Gramineae pollens as annulate-nonannulate and operculate-nonoperculate [7, 20, 22]. *E. distans* and *E. orientale* had most operculum diameter value and *E. triticeum* had the lowest value. Operculum is elipsoidal in *E. triticeum* and circular in *E. bonaepartis* ssp. *hirsutum*, *E. distans* and *E. orientale* (Table 2, Fig. 2 A,C, E & Fig. 3 A). According to Thanikamoni [26], Gramineae pollens were evolved from monosulcate-monoporate to operculate.

Exine is the thickest in *E. triticeum* and the thinnest in *E. orientale* (Table 2). Most researcher measured exine in Gramineae as 1  $\mu\text{m}$ , 1-1.5  $\mu\text{m}$ , 0.90-1.10  $\mu\text{m}$ , 1.53  $\mu\text{m}$ , 1.20  $\mu\text{m}$  and 0.5-1  $\mu\text{m}$ , 1.20  $\mu\text{m}$  [9, 18-22]. Two types exine sculpture were determined according to SEM microphotographs. *E. triticeum*, *E. bonaepartis* ssp. *hirsutum* and *E. distans* is characterized as mixed scabrate. *E. orientale* is insular type exine sculpture. Scabrae of *E. triticeum* and *E. bonaepartis* ssp. *hirsutum* are widely spaced and surface of *E. distans* is compact pattern.

Most authors have grouped cereal and wild grass pollen grains according to exine sculptures. Our findings are conformity with the results of Peltre *et al.* [27], Liu *et al.* [22], Chaturvedi *et al.* [7] and Datta and Chaturvedi [17].

Nevski [4] divided the genus into two sections: section *Micropyrum* and section *Eremopyrum* on the bases of fragility of spikes. According to him section *Micropyrum* includes only *E. triticeum* and section *Eremopyrum* includes the remaining taxa in the genus. However the present palynological investigation showed that there are no significant differences among the taxa belonging two different sections. The obtained UPGMA phenogram (Fig. 1) based on Gower General Similarity Index supplements the species delimitation based on morphology but does not show no consistency with the sectional delimitation of Nevski [4]. The results indicate that the genus *Eremopyrum* has stenopalynous pollen grains thus the value of pollen characters for taxonomic implications is limited. Faegri and Iversen [5], and Perveen [23] also mentined about the uniformity of grass pollen causes one of the greatest diffuculties in pollen analysis.

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## REFERENCES

1. Zhang, G.F., J.W. Liu, Y.Z. Huang, M. Ding, S.X. Tang and X. Jia, 1999. Cytogenetic studies on the cross generations between *Triticum aestivum* and *Eremopyrum orientale*. *Acta Botanica Sinica* 41(11): 1150-1160.
2. Melderis, A., 1985. *Eremopyrum* (Ledeb.). *Jaup.andSpach* 227-231 in *Flora of Turkey and the East Aegean Islands*, Vol. 9 (P.H. Davis, ed.). University Press, Edinburgh, Scotland.
3. Frederiksen, S., 1991. Taxonomic studies in *Eremopyrum* (Poaceae). *Nordic Journal of Botany*, 11: 271-285.
4. Nevski, S.A., 1936. *Conspectus Loliearum, Nardearum, Leptuearum hordeocarumque florum Unionis Rerum Publicarum Sovieticarum Socialisticarum*. -Trudy Bot. Inst. Akad. Nauk SSSR. Ser. 1, 2: 33-90.
5. Faegri, K. and J. Iversen, 1975. *Textbook of Pollen Analysis*. Hafner Press. New York.
6. Köhler, E. and E. Lange, 1979. A contribution to distinguishing cereal from wild grass pollen grains by LM and SEM. *Grana*, 18: 133-140.

7. Chaturvedi, M., K. Datta and P.K.K. Nair, 1998. Pollen morphology of *Oryza* Poaceae. Grana, 37: 79-86.
8. Özler, H., E. Cabi, E. Us, M. Doğan and S. Pehlivan, 2009. Pollen morphology of *Agropyron* Gaertner in Turkey. Bangladesh J. Plant Taxon., 16(1): 21-28
9. Panajiotidis, S., N. Athanasiadis, L. Symeonidis and S. Karataglis, 2000. Pollen morphology in relation to the taxonomy and phlogeny of some native Greek *Aegilops* species. Grana, 39: 126-132.
10. Kruse, J., 1980. Skulpturuntersuchungen an Pollen der Gattung *Triticum* L. and *Aegilops* L. Kulturpflanze, 28: 341-359.
11. Wodehouse, R.P., 1935. Pollen grains, Hafner, NewYork.
12. Erdtman, G., 1952. Pollen morphology and plant taxonomy, Angiosperms. The Chronica Botanica Co., Waltham, Mass., Printed by Almquist Wiksell, Stockholm, Sweden.
13. Sneath P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. Freeman, San Francisco.
14. Punt, W., S. Blackmore, S. Nilsson and A. Le Thomas, 1994. Glossary of Pollen and Spore Terminology LPP Contributions Series No. 1. 71P.
15. Faegri, K. and Iversen, 1989. Textbook of Pollen Analysis (IV. Edition by K. Faegri, P.E. Kaland and Kryzinwinski). John Wiley and Sons, New York.
16. Chaturvedi, M., D. Yunus and K. Data, 1994. Pollen morphology of *Sorghum* Moench-sections *Eusorghum* and *Para-sorghum*, Grana, 33: 117-123.
17. Datta, K. and M. Chaturvedi, 2004. Pollen morphology of Basmati cultivars (*Oryza sativa* race Indica)- exine surface ultrastructure. Grana, 43: 89-93.
18. Erdtman, G., 1943. An Introduction to Pollen Analysis", Chronica Botanica, Waltham, MA, 239.
19. Lewis, W.H., P. Vinay and V.E. Zenger, 1983. Airborne and Allergenic Pollen of North America", The John Hopkins University Press, pp: 105-127.
20. Salgado-Labouriau, M.L. and M. Rinaldi, 1990. Palynology of Gramineae of the Venezuelan Mountains. Grana, 29: 119-128.
21. Kaya, Z., 1990. "Nişantaşı yöresinin havasında tespit edilen Gramineae polenleri ve polen morfolojileri", Marmara Üniversitesi Der., 6(1): 1-15.
22. Liu, Q., N-X, Zhao and G. Hao, 2004. Pollen morphology of the Chloridoideae (Gramineae). Grana, 43: 238-248.
23. Perveen, A., 2006. A Contribution of the pollen morphology of family Gramineae. World Applied Sciences Journal, 2: 60-65.
24. Smith, E.G., 2000. Sampling and identifying allergenic pollens and molds, Blewstone Press, San Antonio, pp: 97-105.
25. Moore, P.D., J.A. Webb and M.E. Collinson, 1991. Pollen Analysis. Second Edition, Blackwell Scientific Publications, Osney Mead, Oxford, pp: 62-101.
26. Thanikamoni, G., 1985. Pollen apertures: form and function. In: Pollen and spores (Edt. by Blackmore and Ferguson) Academic Press, London, pp: 119-136.
27. Peltre, G., M.T.H. Cerceau-Larrival, M. Hideux, M. Abadie and B. David, 1987. Scanning and Transmission electron microscopy related to immunochemical analyses of grass pollen. Grana, 26: 158-170.