

## POLLEN MORPHOLOGY OF THE GENUS *RHEUM* L. (POLYGONACEAE) IN IRAN

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Pollen morphology of the genus *Rheum* (*Polygonaceae*) from Iran was studied by light microscope (LM) and scanning electron microscope (SEM). Pollen grains are usually auxiliary symmetrical, isopolar and tricolpate in equatorial view, while circular in polar outline. Exine sculpturing is variable and are sparsely faveolate to perforate, less microechinate (*Rh. turkestanicum*), perforate-favus, microechinate (*Rh. ribes*) and perforate to foveolatus, densely microechinate (*Rh. persicum*). On the basis of qualitative and quantitative characters identification keys for species were prepared.

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**Key words.** Pollen morphology, plant systematic, *Rheum*, *Polygonaceae*, Iran.

### مورفولوژی دانه گرده *Rheum* L. (*Polygonaceae*) در ایران

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مورفولوژی دانه گرده جنس *Rheum* L. (*Polygonaceae*) در ایران با استفاده از میکروسکوپ نوری (LM) و میکروسکوپ الکترونی (SEM) مطالعه شد. دانه‌های گرده در نمای قطبی معمولاً دارای تقارن محوری، در دو قطب مشابه، و سه شیار دارند که در نمای استوایی دایره‌ای شکل‌اند. تزئینات سطح آگزین متغیر است و به شکل‌های با منافذ پراکنده تا منفذدار، میخچه‌های پراکنده (*Rh. turkestanicum*)، منافذ کشیده، میخچه دار (*Rh. ribes*) و یا منافذ نوک سوزنی- با تراکم بالایی از میخچه‌ها (*Rh. persicum*) دیده می‌شوند. بر اساس صفات کمی و کیفی مطالعه شده کلید شناسایی گونه‌های ایران ارائه می‌شود.

### INTRODUCTION

The family *Polygonaceae* is a complex family (Ronse Decraene & Akeroyd 1988) consisting of nearly 48 genera and 1,200 species (Sanchez & Kron 2008). The genus *Rheum* L. of the family *Polygonaceae* comprises approximately 60 species, mainly concentrated in north temperate regions of both hemispheres. Its members are largely weeds, mostly found in disturbed areas and crop fields (McDonald 1980 Kim & Donoghue 2008). Many taxonomic characters focused on the striking differences in inflorescence and floral morphology, the inflorescence are pyramidal or spherical, consisting flowers in fasciculate or paniculate, pedicels articulate at or below the middle (Rechinger & Schiman-Czeika 1968).

The significance of palynological characters in relation to plant taxonomy needs no emphasis (Hedgeberg 1946, Nowicke & Skvarla 1979). Taxonomic position of the species within the family is justified with the help of pollen morphology (Munsif & al. 2007). Thakur & Thakur (1970) stated that the "pollen exine pattern is so genetically stable for the different species of plants that it can be used for species identification". Hebda & Chinnappa (1990) showed that exine sculpturing, aperture and aperture zone structure, grain shape, and grain size are all useful characters to distinguish genera and even species of Rosaceous pollen. Fogle (1977) used the length and width of the pollen grain, depth of exine ridges and prominence of pits in the exine to distinguish peach, nectarine, plum and cherry.

Table 1. List of *Rheum* species used in the study, with locality and herbarium numbers.

Sr. no.	Species	Locality	Collector	Herb. no.
1	<i>R. ribes</i> L.	Azerbaijan, Tabriz to Marand on the road, Mishoodagh, N slope of mountain, 1740- 2700 m	Azadi & Nikchehreh	75623
2	<i>R. persicum</i> Los.	Fars, Banu protected region- Tang-e- Chah mahaki	Mozaffarian	17752
3	<i>R. turkestanicum</i> Janish.	Khorasan, Mashhad, 80 Km N of Torbat	Rajamand & Bazargan	32029

With the advancement in microscopy especially by the discovery of electron microscope has greatly changed the study of pollen surface structures (Yasmin & al. 2009). Palynologically, *Polygonaceae* is considered to be the eurypalynous family (Wodehouse 1931, Hedgeberg 1946, Nowicke & Skvarla 1979) and its pronounced variations have great systematic potential, particularly at generic level (Nowicke & Skvarla 1977). Differences in size and surface ultrastructure of pollen grains are useful for distinguishing species of *Polygonaceae*. Exine and its sculpturing make the pollen grains highly distinguishable structures by which different genera and even species can be distinguished (Moore & Webb 1978). It also has great systematic significance in *Polygonaceae* (Hong & Hedgeberg 1990).

The palynology of some *Rheum* species has already been investigated. Yang & al. (2001) have provided some palynological data from certain Chinese members of this genus. This paper reports detailed morphological studies using SEM and LM on *Rheum* species for the first time in Iran. We also discuss the potential contribution of pollen ornamentation to the systematic of the genus. The main objectives of the present investigation are: to give comprehensive account on the pollen morphology of three *Rheum* species available in Iran and on the basis of exine ornamentation, find out pollen diversity in the genus which would be helpful for the distinction of species.

## MATERIALS AND METHODS

Three species of *Rheum* (following Rechinger & Schiman-Czeika 1968) were investigated from Iran. Pollen samples were obtained from herbarium specimens of Ferdowsi University of Mashhad (FUHM) and Research Institute of Forests and Rangelands (TARI) (Table 1). Few freshly collected dried specimens were also used for palynological investigations.

The pollen grains were prepared for acetolysis by the modified procedure of Erdtmann (1960, 1966 and 1969). For light microscopy, acetolysed anthers were

removed from filaments of stamen with the help of dissecting needles and then crushed to release pollen grains on a clean glass slide. Anther wall material was discarded. Then pollen grains were mounted in glycerin jelly stained with 1% safranin. The slide was placed on hot plate to melt glycerin jelly and to remove bubbles from the slide. Cover slip was placed on the prepared pollen-glycerin jelly mixture. When cooled, the glass slide was labeled and edges of the cover slip were sealed with transparent nail varnish. The prepared slides were studied under the light microscope. Pollen type, its shape and diameter in polar and equatorial view, P/E ratio, exine thickness, intine thickness and length of colpi were examined. Details of pollen morphology were based on the measurements of 10-15 grains. Range and mean were calculated. Their photographs were taken with the Olympus BX-53 microscope equipped with camera photomicrograph system. For Scanning Electron Microscopic (SEM) studies, pollen grains suspended in a drop of 40% acetic acid were sifted onto separate polished aluminum disk stubs covered with double-sided transparent tape, sputter-coated with 60% gold-palladium in a polaron E 5100 coater, and viewed in a Vega II Tescan scanning electron microscope at accelerating voltages of 15.KV [10]. Shape and diameter of pollen grain in polar and equatorial view and exine sculpturing (from middle part of the pollen grains) was photographed at x20,000 and ten pollen grains were measured.

The terminology used is in accordance to Faegri & Iversen (1964) and Punt & al. (1994, 2007).

## RESULTS

The pollen morphological characters of all species examined are summarized based on observation from light (Fig. 1) and scanning electron microscopy (Table 2), the differing feature of exine sculpturing seen in SEM are mentioned in Fig. 2. Pollen morphology of the genus is noted as follows:

*Pollen class.* Tricolpate type of pollen present in all *Rheum* species.

Table 2. Pollen size, shape and aperture in *Rheum* (all measurement are in  $\mu\text{m}$ .).

	<i>Rh. turkestanicum</i>	<i>Rh. ribes</i>	<i>Rh. persicum</i>
Equatorial diameter	24.96 ( 23.76- 26.2)	17.49 (15.6- 19.38)	18.24 ( 16.53- 20.8)
Polar diameter	37.23 (35.84- 38.9)	30.78 (27.39- 3.12)	38.41 (37.12- 8.76)
P/E	1.49	1.78	2.1
Shape	prolate	prolate	prolate
Apertures type	3 colpate	3 colpate	3 colpate
Equatorial view	broad elliptic	elliptic	narrow elliptic
Polar view	circular	circular	circular
Length of colpi	34.2	28.8	36.9
Exine thickness	3	1.5	1.9
Intine thickness	1.5	0.8	0.9
Exine sculpturing	sparsely faveolate to less perforate, microechinate	perforate- microechinate favus, microechinate	perforate to foveolate, densely microechinate

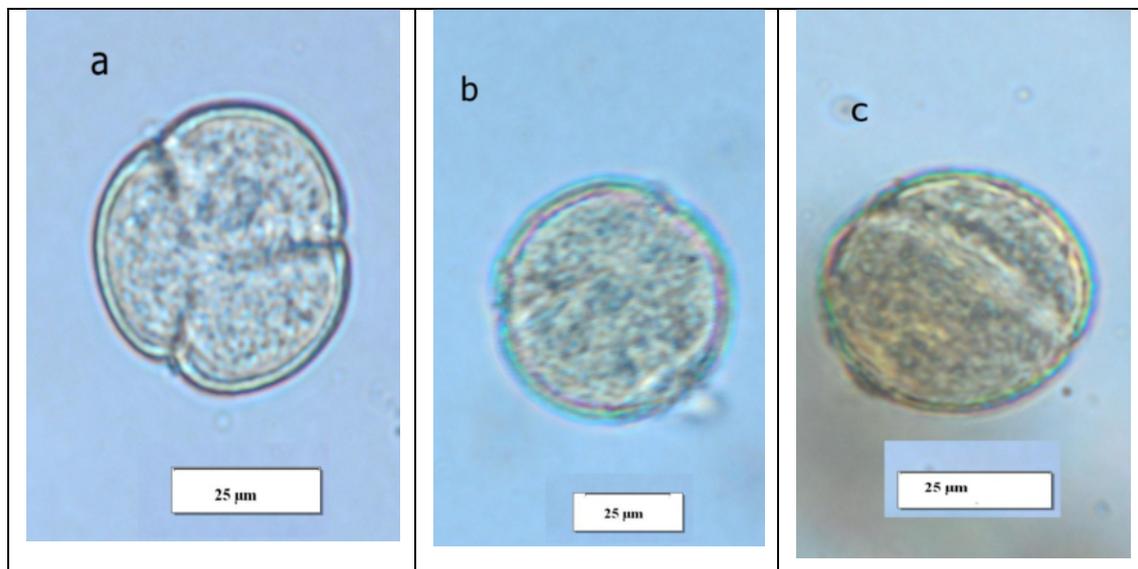


Fig. 1. Light microscopic features of pollen grains, a: *Rheum turkestanicum*, b: *Rh. persicum* c: *Rh. ribes*.

**Size.** Pollen medium to large- sized, P (polar axis length) 30.78- 38.41 $\mu\text{m}$   $\times$  E (equatorial diameter) 17.49-24.16  $\mu\text{m}$ . *R. persicum* (18.24 $\times$ 38.41  $\mu\text{m}$ ) appears to be narrowest in the genus while pollen of *R. ribes* (17.49 $\times$ 30.78  $\mu\text{m}$ ) have broad diameter (Table 2).

**Symmetry and shape.** The pollen grains are axially symmetrical and isopolar. Outline in polar view is circular in all taxa of the *Rheum* (Fig. 1). P/E (polar axis/equatorial diameter) ratio is in the range of 1.49 in *Rh. turkestanicum* to 1.78 in *Rh. ribes* and 2.1 in *Rh. persicum*(Table 2).

**Aperture.** Apertures are non-lacunate type while colpi are long and narrow. Length of colpi varies from 28.8  $\mu\text{m}$  in *Rh. ribes* to 34.2  $\mu\text{m}$  in *Rh. turkestanicum* and 36.9  $\mu\text{m}$  in *Rh. Persium* (Table 2).

**Exine and intine:** Exine thickness is a variable character and it varies from 1.5  $\mu\text{m}$  (*Rh. ribes*) to 3  $\mu\text{m}$  (*Rh. turkestanicum*). Intine is clearly visible in all species of *Rheum* and it varies from 0.8  $\mu\text{m}$  in *Rh. ribes* to 1.5  $\mu\text{m}$  in *Rh. turkestanicum*.

**Exine sculpturing.** The general pattern of the exine differs from species to species (Fig. 2). Under scanning electron microscope exine is sparsely faveolate to perforate, less microechinate in *Rh. turkestanicum*, perforate-favus, microechinate in *Rh. ribes* and perforate to faveolate, densely microechinate in *Rh. persicum* (Table 2).

## DISCUSSION

*Rheum* (*Polygonaceae*), a highly diversified genus with about 60 species, is mainly distributed on the

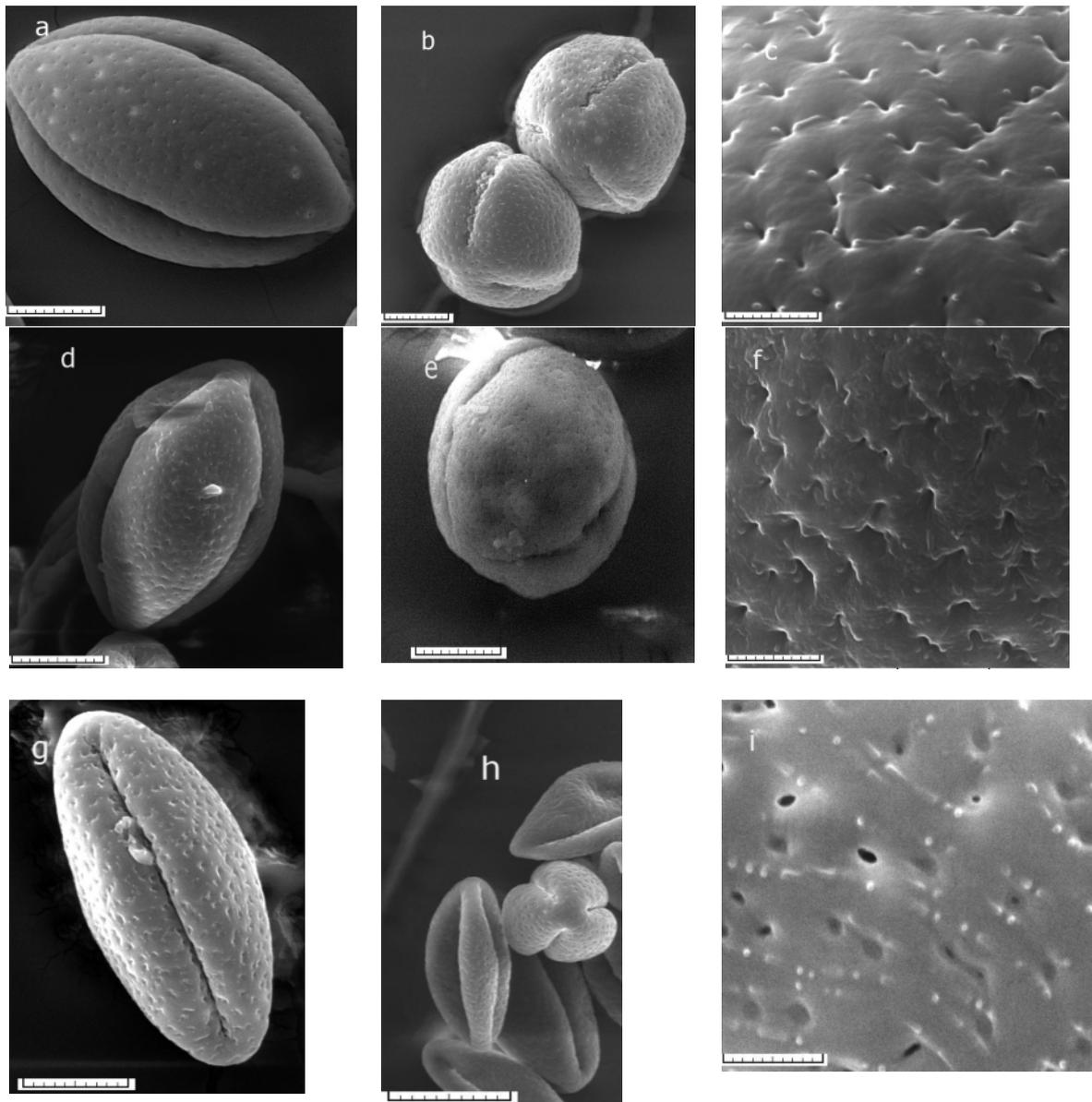


Fig. 2. Characteristic features of pollen grains. a, b, c: *Rh. turkestanicum*. d, e, f: *Rh. ribes*. g, h, i: *Rh. persicum*. Scale bare 2  $\mu$ m.

mountains of desert regions of the Qinghai–Tibetan Plateau area and Asian interior (Losina-Losinskaya 1936). Infrageneric classification of this genus has been numerous, but none covers both the entire distribution and all currently known species. Based on macromorphological characters, *Rheum* was subdivided by Losina-Losinskaya (1936) in nine sections, viz. *Rh. sect. Rapontica* Los., *sect. Deserticola* (Maxim.) Los., *Sect. Glabrifolia* Los., *Sect. Spiciformia* Los., *Sect. Acaulia* Los., *Sect. Ribesiformia* Los., *Sect. Palmata* Los., *Sect. Rheum* and *Sect. Nobila* Los. She further

suggested that *Sect. Palmata* is closely related to *Sect. Rheum*, and that both sections are primitive groups of the genus. Kao & Cheng (1975) acknowledged only five of Losina-Losinskaya's sections and proposed two new sections: *Sect. Acuminata* based on the cordiform leaves of several species that originally placed in *Sect. Rheum*; and the monotypic *Sect. Globulosa*, which has spherical inflorescence, but lacks distinct stems. To date, eight sections have been established and acknowledged under *Rheum*, according to Li (1998). He further accepted Losina-Losinskaya's phylogenetic

hypothesis of *Rheum*, although no new data were provided to support it (Wang et al. 2005). In this study, we have used the groups representing the *Rheum* in Flora of USSR classification, but for *Rh. persicum*, an endemic species in Iran, we grouped it in *Rh. Sect. Ribesiformia* based on its macro and micro-morphological characters. *Polygonaceae* is an eurypalynous family. The pollen of members of the *Polygonoideae* subfamily, to which *Rheum* belongs, has been studied previously by Nijs & al. (1980), Hee (2001) and Yasmin & al. (2010). Yaung & al. (2001) found that the pollen of *Rheum* species had diverse exine ornamentation and the variations in ornamentation are not consistent with the morphological classification. Some species with distinctly different morphology in different sections share similar types of pollen ornamentation while some species with very similar morphology has contrasting pollen ornamentation. For example, microechinate pollen ornamentation has been found in species of both Sect. *Spiciforma* and Sect. *Palmata*, while two species of Sect. *Nobilis* have very different ornamentation, pollen being distinctly and densely microechinate, sparsely perforate in *R. globulosum*, but distinctly rugulate, high relief, verrucate in *R. nobile*. Based on our studies, the exine ornamentation in *Rh. turkestanicum*, belong in *Rh. sect. Deserticola*, show similar type of sculpturing with other member of this section, *Rh. sublanceolatus*, that reported previously by Yaung & al. (2001).

The significant variation found on pollen grains of *Rheum* suggests the potential taxonomic value of pollen characteristics. In all of the species examined, tricolpate pollen is characteristic without any exceptions. Exine sculpturing is not homogenous. Variation in exine sculpture was observed in the pollen of all taxa.

Our results from only a very small sample of the total number of species of *Rheum*, and from a limited part of geographical range of the genus, support this and suggest that there is much scope for further research on the pollen morphology of *Rheum* in relation to the systematic of the genus.

### Key to the species of *Rheum*

1. Pollen grains in equatorial view narrow elliptic  
*Rh. persicum*
- Pollen grains in equatorial view late elliptic 2
2. Exine Perforate- favus, microechinate *Rh. ribes*
- Exine sparsely faveolate to perforate, less microechinate *Rh. turkestanicum*

### Conclusion

Pollen morphological characters particularly exine sculpturing is considered to be an impressive feature of taxonomic significance at specific level in the genus *Rheum*. However, in order to reach any taxonomic conclusion, micromorphological features of the pollen grains should be used in combination with other characters of morphology, anatomy, cytology and geographical distribution.

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