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POLLEN MORPHOLOGY OF SELECTED *POLYGONUM* L. SPECIES (POLYGONACEAE) FROM PAKISTAN AND ITS TAXONOMIC SIGNIFICANCE

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Abstract

Pollen morphology of 12 species belonging to the genus *Polygonum* L., (Polygonaceae) from Pakistan has been investigated by light and scanning electron microscopy. *Polygonum* L., is an eurypalynous genus and four types of surface ornamentation (granulate, granulate-coarsely reticulate, dimorphic exine and variable pattern of ornamentation in polar and equatorial view) have been observed under scanning electron microscope. The variation in exine ultrastructure makes it possible to distinguish four pollen types (*Patulum* type, *Plebijum* type, *Cognatum* type and *Avicularia* type). Pollen morphology within the genus proved to be useful for the specific delimitation.

Introduction

Polygonaceae Juss., a family of approximately 48 genera and 1,200 species (Freeman & Reveal, 2005; Sanchez & Kron, 2008), having cosmopolitan distribution is more specially concentrated in the northern temperate region (Heywood, 1978). Among the 60 species of the *Polygonum* L. distributed throughout the world, about 20 species are found in Pakistan (Qaiser, 2001). It is characterized by its prostrate habit, sometimes sub prostrate or erect, alternate leaves, presence of ochreae on nodes, axillary flowers, tepals with only one main vein, stamen in two whorls, outer smaller than the inner filaments swollen at the base and absence of nectaries (Ronse Decraene & Akeryord, 1988). Wodehouse (1931) was the first to publish a comprehensive account on pollen morphology of the family Polygonaceae. However, later on palynological characters were examined in relation to classification, to explore phylogeny and develop parallel evolutionary lines (Hedgeberg, 1946; Wang & Feng, 1994; Zhang & Zhou, 1998; Zhou *et al*., 1999; Zhou *et al*., 2002). Recently Hong *et al*., (2005) studied the genus *Polygonum s. str* with light microscope (LM) and scanning electron microscope (SEM) and discussed the systematic value of palynological characters at different taxonomic levels.

The present work reports the first detailed palynological studies of 12 *Polygonum* L., species using LM and SEM. The main aims of the work are to find out different pollen types on the basis of exine ornamentation under LM and SEM and its taxonomic importance.

Materials and Methods

Dry polleniferous material was obtained from the herbarium specimens of Quaid-i-Azam University, Islamabad, Pakistan (Table 1). Few freshly collected dried specimens were also used for palynological investigations. The pollen grains were prepared by following the acetolysis technique adopted by Erdtman (1952, 1966 and 1969). For light microscopy, pollen grains were taken out from the stamens and acetolysed by using acetolysis mixture. 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 and its sculpturing and length of colpi were examined. Details of pollen morphology were based on the measurements of 10-15 grains. The data were statistically analyzed i.e., range, mean and standard error (±) were calculated using MS excel sheet. Their photographs were taken with the Nikon FX-35 camera fixed on microscope. For SEM studies, pollen grains suspended in a drop of 40% acetic acid were transferred to clean metallic stubs and coated with gold using a JEOL JFC 1100 E ion sputtering device. SEM observations were carried out on a JEOL microscope JSM5910. The work was carried out in the Centralized Resource Laboratory, University of Peshawar (Pakistan).

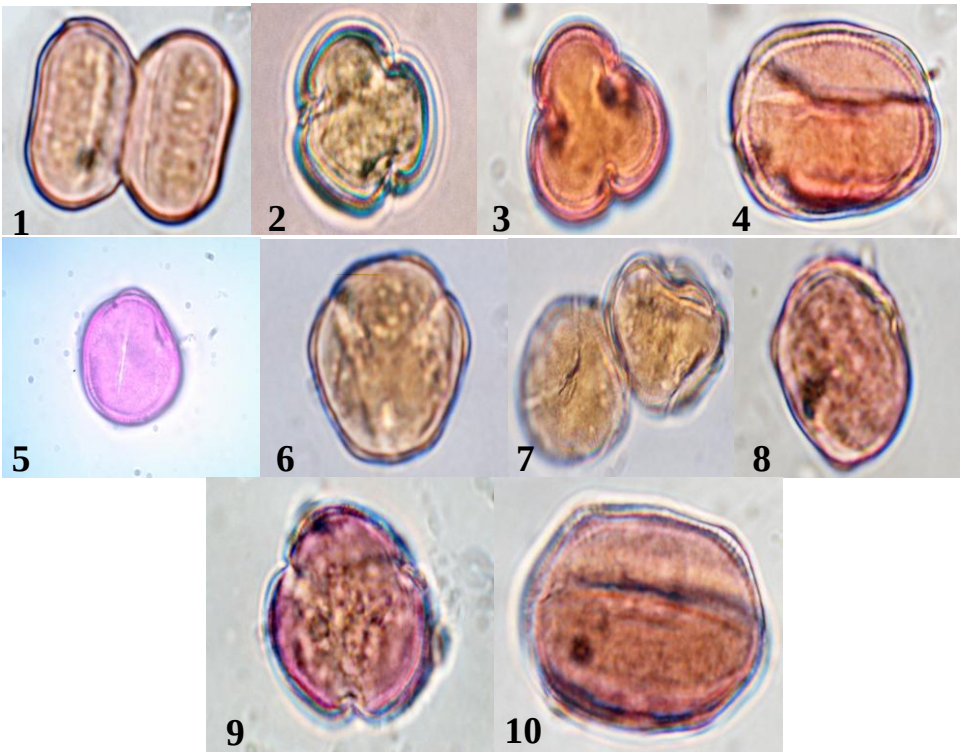
The terminology used is in accordance with Erdtman (1952), Faegri & Iversen (1964), Kremp (1965), Punt *et al*., (1994, 2007).

Results

A summarized data of pollen morphological characters of 12 selected species of *Polygonum* from Pakistan is presented in Table 2. LM and SEM micrographs of selected species of the genus are presented in Figs. 1-23. Pollen morphology of the genus is noted as follows.

Key to the species of *Polygonum* L. on the basis of pollen morphology

1a: P/E ratio 1.46 ……..……..…. 1. *P. plebijum*
1b: P/E ratio less than 1.46 …….. 2
2a: Exine 4.5 µm thick …….. 2. *P. rottboellioides*
2b: Thickness of exine less than 4.5 µm ……. 3
3a: Length of colpi 17.4 µm …… 3. *P. patulum*
3b: Length of colpi less than 17.4 µm ……. 4
4a: Exine thickness 1.00 µm ……... 4. *P. sarobiense*
4b: Exine more than 1.00 µm …….. 5
5a: P/E value 0.75 …… 5. *P. paronychioides*
5b: P/E value more than 0.75 …….. 6
6a: Length of colpi 16.8 µm …… 6. *P. molliaeforme*
6b: Colpi length less than 16.8 µm …….. 7
7a: Equatorial diameter 23.5-25.5 µm ……. 7. *P. olivascens*
7b: Equatorial diameter less than 23.5 µm …….. 8
8a: Equatorial view spheroidal to prolate-spheroidal …… 8. *P. arenastrum*
8b: Pollen prolate to sub prolate in equatorial view …… 9
9a: Polar diameter 13.5 µm …….. 9. *P. effusum*
9b: Polar diameter more than 13.5 µm …….. 10
10a: Exine dimorphic, having microspinules near the edges of pollen ……...
…… 10. *P. cognatum*
10b: No such dimorphism observed …… 11
11a: Polar outline circular ……. 11. *P. aviculare*
11b: Polar outline circular to circular lobate …… 12. *P. polycnemoides*



Figs. 1-10. LM micrographs of the pollen grains of genus *Polygonum* (1000X).
1. *P. polycnemoides:* Dyads in equatorial view, 2. *P. paronychioides*: Polar view, 3. *P. molliaeforme:* Polar view, 4. Equatorial view, 5. *P. olivascens*: Equatorial view, 6. *P. arenastrum:* Polar view, 7. Equatorial view, 8. *P. aviculare*: Equatorial view, 9. *P. cognatum:* Polar view, 10. Equatorial view.

Pollen class: Tricolporate pollen grains are noted in all species of *Polygonum*.

Size: The size of pollen grains (polar axis × equatorial diameter) is in the range of 13.5×14.0 - 26.3×24.8 µm. *P. effusum* (13.5×16 µm) appear to be smallest in size while *P. olivascens* (26.3×24.8 µm), *P. arenastrum* (23.4×22.1 µm), *P. cognatum* (24.4×21.6 µm) and *P. patulum* (23.5×24 µm) are among the members with comparatively large sized pollen grains. In *P. sarobiense* polar and equatorial diameter are nearly equal i.e., 16×15.8 µm (Table 2).

Symmetry and shape: The pollen grains are usually radially symmetrical and isopolar. Shape of pollen in polar view is circular followed by circular-lobate. In equatorial view pollen grains are prolate, sub-prolate, prolate-spheroidal, sub-oblate, oblate-spheroidal to spheroidal showing variation in pollen shape and size (Figs. 1-23, Table 2). The P/E (polar axis/equatorial diameter) ratios are in the range of 0.75 (*P. paronychioides*) to 1.46 (*P. plebijum*). In *P. arenastrum* and *P. olivascens* P/E value is same (Table 2). Dyads are very frequent in *P. polycnemoides*. In most of the taxa of *Polygonum*, columella is well developed and evenly distributed. It gives stripped appearance in *P. effusum*, *P. molliaeforme* and *P. olivascens*.

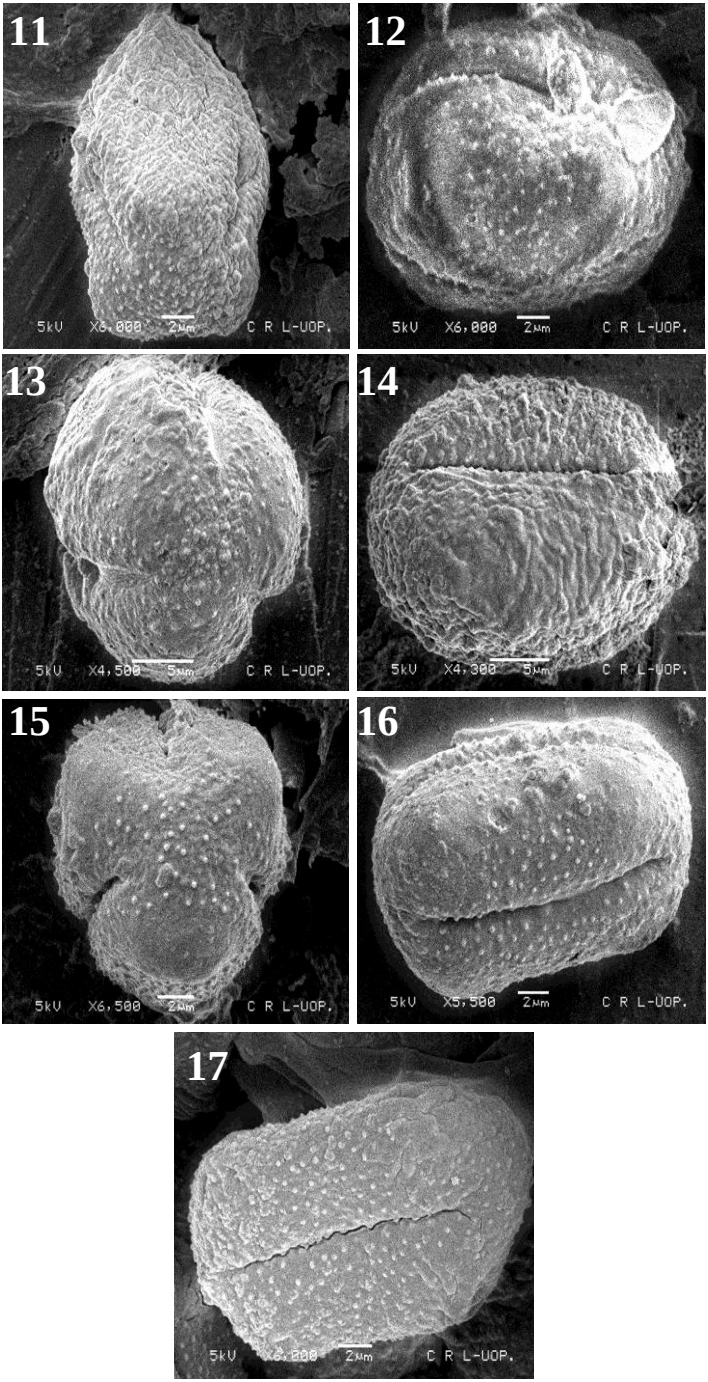
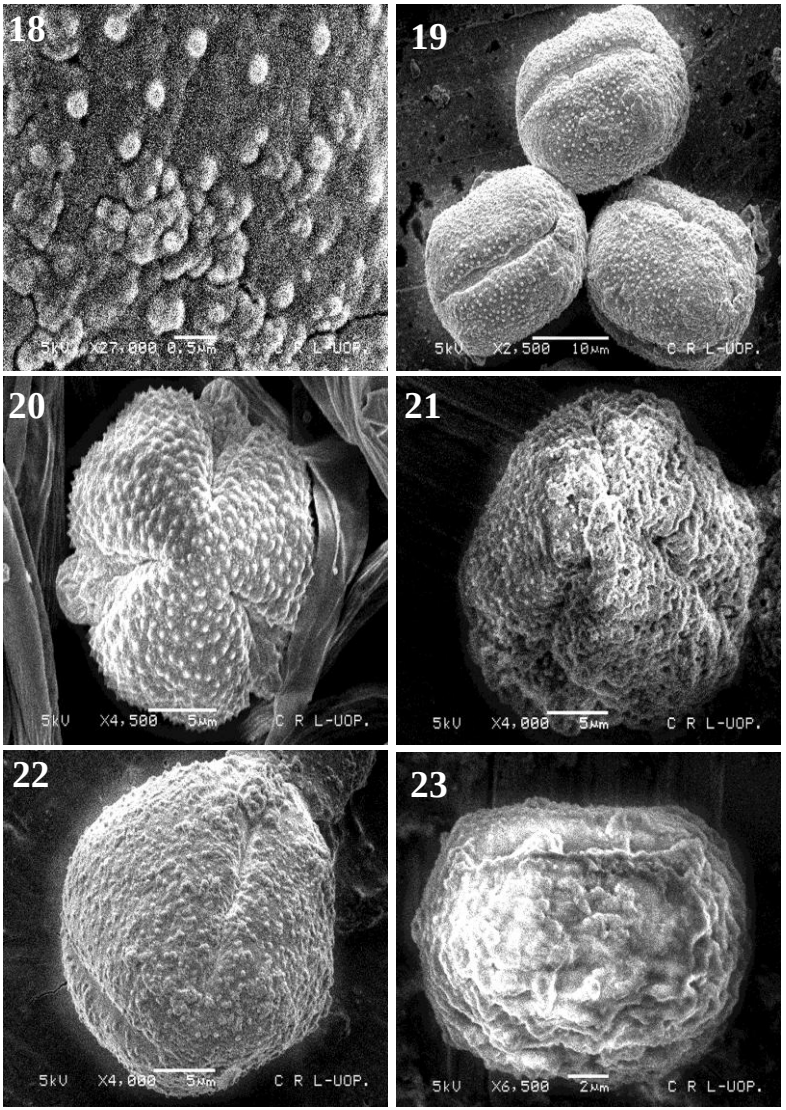


Fig. 11-17. SEM micrographs of the pollen grains of genus *Polygonum.*
11. *P. plebijum:* equatorial view, 12. *P. rottboellioides*: Equatorial view, 13. *P. patulum:* Polar view, 14. Equatorial view, 15. *P. sarobiense*: Polar view, 16. Equatorial view, 17. *P. polycnemoides*: Equatorial view



Figs. 18-23. SEM micrographs of the pollen grains of genus *Polygonum*.
18. Exine ornamentation pattern. 19. *P. paronychioides:* Equatorial view, 20. Polar view. 21. *P. olivascens*: Polar view. 22. *P. arenastrum*: Polar view. 23. *P. effusum:* Equatorial view.

Apertures: The pollen grains are of non-lacunate type. Apertures are circular or elongated while colpi are very long and deep. The length of colpi is in the range of 9.0 µm in *P. rottboellioides* to 17.4 µm in *P. patulum* and 17.2 µm in *P. paronychioides.* In *P. molliaeforme* and *P. olivascens*, colpi are of the same length (Table 2).

Exine thickness and its sculpturing: Thickness of exine varies from 1.00 µm to 4.5 µm. *P. sarobiense* possesses thinner exine about 1.00 µm while it is thick in *P. rottboellioides* I-e., 4.5 µm (Table 2). Exine pattern under light microscope is invisible in some species

of the genus *Polygonum*, however it appears granular in *P. plebijum*, *P. rottboellioides*, *P. aviculare*, *P. effusum*, *P. arenastrum*, *P. molliaeforme*, *P. patulum* and *P. olivascens* (Figs. 1-10). SEM studies generally show granulate ornamentation in most of taxa, few microspinules are visible near the edges of colpi and pores in *P. cognatum* and *P. rottboellioides*. Somewhat granulate-coarsely reticulate pattern is observed in *P. aviculare*, *P. effusum* and *P. olivascens*. In *P. patulum*, ornamentation pattern is granulate in polar outline while roughly reticulate in equatorial view (Figs. 13-14, Table 2).

Discussion

As far as palynolgical characters of Polygonaceae are concerned it has been found to be one of the diverse eurypalynous families and its extensive variations have great systematic potential at all levels, especially for generic delimitation (Nowicke & Skvarla, 1977). Using the light and scanning electron microscopy, the palynological study of 12 available species belonging to the genus *Polygonum* L. was conducted. Present study revealed the utility of both qualitative and quantitative characters in taxonomic studies; also the potential influence of pollen morphology in the delimitation of species cannot be ignored. Palynological characters such as shape in polar and equatorial view, aperture type and number and exine ornamentation are markedly imperative characters (Perveen & Qaiser, 2005).This study particularly showed distinct variation in exine ornamentation under the SEM. On the basis of exine ornamentation 4 pollen types could be recognized in the taxa of *Polygonum* L., viz., *Patulum* type, *Plebijum* type, *Cognatum* type and *Avicularia* type.

Key to the different pollen types in *Polygonum* L.

1a: Exine ornamnetation granular in polar view while roughly reticulate in equatorial view ……... *Patulum* type (*P. patulum*)
1b: Exine ornamentation same in polar and equatorial view ……... 2
2a: Pollen surface appear granular ……... *Plebijum* type (*P. plebijum*, *P. sarobiense*, *P. polycnemoides*, (*P. paronychioides*, *P. molliaeforme*, *P. arenastrum*)
2b: Pollen surface granulate-coarsely reticulate, or dimorphic in a single view …….. 3
3a: Granular exine with microspinules present near the edges of colpi …….. …… *Cognatum* type (*P. cognatum*, *P. rottboellioides*)
3b: Exine granulate-coarsely reticulate ……...…. …….. *Avicularia* type (*P. aviculare*, *P. effusum* and *P. olivascens*)

1. *Patulum* type pollen: Patulum type was represented in *P. patulum* and recognized by the different exine sculpturing pattern in polar and equatorial view (Figs. 13-14). The pollen size varied from 20-32.5×20-25 µm (polar × equatorial diameter) with long colpi and 2.5 µm exine. Zhang & Zhou (1998) recorded 23.8-30.6×18.7-23.8 µm pollen with 2.5 µm exine. They observed granulate-foveolate pollen surface. Present SEM studies revealed granulate pattern in polar view and roughly reticulate in equatorial view.

2. *Plebijum* type pollen: This tricolporate pollen type was represented in 6 species of *Polygonum* L., viz., *P. plebijum*, *P. sarobiense*, *P. polycnemoides*, *P. paronychioides*, *P. molliaeforme* and *P. arenastrum*. It is comparable to Zhang & Zhou's (1998) Plebijum type pollen with dimorphic exine. However, in the present study granulate pollen surface was found under SEM. The pollen grains were mostly circular to circular-lobate in polar

outline and the dimensions of the polar axis varied from 16 µm (*P. sarobiense*) and 23.4 µm (*P. arenastrum*). The equatorial shapes were quite variable and their diameters ranged from 14 µm (*P. plebijum*) to 22.4 µm (*P. arenastrum*). Perveen (1993) observed *P. plebijum* as a tricolporate small sized grain with an average size of 16.21×11.73 µm. Wang & Feng (1994) and Zhang & Zhou (1998) reported prolate pollen of *P. plebijum* with more or less same dimensions. Hong *et al*., (2005) investigated the pollen morphology of *P. paronychioides*, *P. plebijum* and *P. molliaeforme* and gave their sizes as 31.7×23.3 µm, 28.3×24.4 µm and 19.5×14.7 µm, respectively. Besides pollen size, P/E value proved to be useful character of systematic value (Table 2).

Long and sunken colpi characterized the genus (Table 2). Perveen (1993) recorded 12.90 µm long colpi in *P. plebijum*. *Hong et al*., (2005) measured the colpi length varied between 13.4 µm (*P. molliaeforme*) to 24.5 µm (*P. paronychioides*). Although during the present study, longest colpi were observed in *P. paronychioides* (17.2 µm) but less than the value recorded by Hong *et al.,* (2005). Exine ornamentation provided impressive variation for taxonomic distinction. LM and SEM studies were conducted in order to study the pollen surface ornamentation in detail (Table 2). Under LM, sculpturing remained indistinct in *P. sarobiense*, *P. polycnemoides* and *P. paronychioides* (Figs. 1-2) while SEM showed exine granular exine ornamentations (Fig. 15-17, 20-21). The pollen surface of *P. plebijum* is generally termed as scabrate, microechinate-foveolate, dimorphic and smooth with spinules, respectively by previous workers (Perveen, 1993; Wang & Feng, 1994; Zhang & Zhou, 1998; Hong *et al*., 2005).

3. *Cognatum* type pollen: It is distinguished by the presence of dimorphic exine and observed only in *P. rottboelliodes* and *P. cognatum* (Figs. 9-10). The pollen grains were circular to circular-lobate in polar view and prolate and prolate-spheroidal equatorial view. In addition, pollen grains of *P. cognatum* were comparatively larger in size with much longer colpi (16.2 µm) than that of *P. rottboellioides* where colpi were only 9 µm. The significance of dimorphic exine is not known (Nowicke & Skvarla, 1979) but it may have some relationship with harmomegathic extension (Hong *et al*., 2005).

4. *Avicularia* type pollen: This pollen type is comparable to *Avicularia* type proposed by Wang & Feng (1994) and Zhang & Zhou (1998). This characteristic type of pollen was recorded in *P. olivascens, P. effusum* and *P. aviculare*. Among the three species, *P. olivascens* (26.3×24.8 µm) showed largest size range followed by *P. aviculare* (23×21.2 µm) and *P. effusum* (13.5×16 µm). All the three species exhibit sub-prolate to prolate pollen grains in equatorial view (Table 2). *Avicularia* type was distinguished on the basis of granulate-coarsely reticulate pattern in SEM (Figs. 21, 23). However, pollen grains of *P. aviculare* are variously suggested to have microechinate-foveolate, granulate-perforate and smooth surface with spinules, respectively (Wang & Feng, 1994; Zhang & Zhou, 1998; Hong *et al*., 2005).

Conclusion

The investigation of pollen micromorphological characters suggests diversity in pollen types in the genus *Polygonum* L., especially with reference to exine ornamentation pattern. It is clear from the present findings that qualitative and quantitative micromorphological features of the pollen can be utilized to delimit the taxa at specific level.

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