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TAXONOMIC IMPLICATIONS OF POLLEN MORPHOLOGY OF SEVEN SPECIES OF *RUMEX* L., FROM PAKISTAN

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Abstract

Pollen morphology of 7 species belonging to the genus *Rumex* L., from Pakistan was examined by light microscope (LM) and scanning electron microscope (SEM). This investigation revealed the eurypalynous nature of the genus. Pollen grains usually tricolporate and tetracolporate, circular in polar view while equatorial outline was quite variable. On the basis of exine sculpturing under SEM three distinct pollen types were observed viz., Chalepensis type, Dentatus type and Acetosa type. Keys to the species and pollen types are also provided.

Introduction

The family Polygonaceae is a complex family (RonseDecraene & Akeroyd, 1988) consisting of nearly 48 genera and 1,200 species (Sanchez & Kron, 2008). The genus *Rumex* L., of Polygonaceae is represented by more than 200 species, dispersed in temperate regions mostly in the northern areas of the world. In Pakistan it is represented by 15 species and two hybrids (Rechinger, 2001). In the past times, Romans suck the leaves of *Rumex* to reduce their thirst (Mosyakin, 2005). The distinctive feature of the genus are the presence of racemose or paniculate inflorescence, 6 tepals arranged in two whorls, inner whorl enlarged in fruiting stage (valve) and midvein is represented as tubercles (Li *et al.*, 2003). Previously *R. dentatus*, *R.acetosa* and *R. vesicarius* were palynologically studied (Wodehouse, 1931, Perveen, 1993, Zhang & Zhou, 1998). The present work reports the first detailed pollen morphological studies of seven species of *Rumex* L., using LM and SEM as there are no palynological studies exclusively on the genus from Pakistan. The main aims of the work are to improve the understanding of palynological characters within the genus, to discuss different pollen types on the basis of exine ornamentation under SEM and their taxonomic implications.

Materials and Methods

Pollen samples were obtained from herbarium specimens of Quaid-i-Azam University, Islamabad (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 Erdtman (1952, 1960). For light microscopy, pollen grains were mounted in glycerine jelly stained with 1 % safranine. The slide was placed on hot plate to melt glycerine jelly and to remove bubbles from the slide. Cover slip was placed on the prepared pollen-glycerine jelly mixture. When cooled, the glass slide was labelted 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, intine thickness, length of

colpi and lumina size were examined. Details of pollen morphology were based on the measurements of 10-15 grains. The data were statistically analysed i.e., range; mean and standard error (±) were calculated using MS excel (Table 2). Their photographs were taken with the Nikon FX-35 microscope equipped with camera. 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 to Erdtman (1952), Faegri & Iversen (1964), Kremp (1965), Punt *et al*., (1994, 2007).

Table 1. **List of species investigated for palynological studies with locality, district, collector name and accession numbers.**

Sr. No.	Species name	Locality	District	Collector Name	Acc. No.
01.	*R. chalepensis* Mill.	Urak to Hanna Sohawa	Quetta Jhelum	Iqbal Dar & Arif Iqbal Dar *et al*.,	28607 08968
02.	*R. patientia* L.	Kargah Bakot (stony soil)	Gilgit, Hazara	Mir Ajab & M. Afzal Shahzad Iqbal & Nisar Ahmad	63829 86378
03.	*R. dentatus* L.	Chichian Fort Abbass	Mirpur, Bahawalnagar	Shahzad & Arif Mir Ajab & Manzoor	55478 45856
04.	*R. vesicarius* L.	Mach Baba Sahib to Peepal	Quetta, Bannu	Iqbal Dar & Arif Shahzad *et al.*,	26517 45887
05.	*R. nepalensis* Spreng.	Naltar Kuldana	Gilgit, Rawalpindi	S. R. Saif Shahzad *et al.*,	09131 27548
06.	*R. acetosa* L.	Nareel GolinKoh	Muzaffarabad, Chitral	Shahzad & Ayaz Muqarrab Shah & Dilawar	92930 63561
07.	*R. hastatus* D. Don	Pangargala Danin	Kotli, Chitral	Shahzad & Nisar Muqarrab Shah & Dilawar	58918 55529

Results

A summary of pollen morphological data of the genus *Rumex* is given in Table 2. LM and SEM micrographs of selected species of the genus *Rumex* are presented in Figs. 1-16. Pollen morphology of the genus is noted as follows.

Key to the species of *Rumex*

1a. Exine 3.0 µm thick ……..…… 1. *R. chalepensis*
1b. Thickness of exine less than 3.0 µm …….…….. 2

2a. P/E ratio 1.24 ……... 2. *R. patientia*
2b. P/E ratio less than 1.24 ……...…….. 3

3a. Length of colpi 18.6 µm ……... 3. *R. dentatus*
3b. Colpi length less than 18.6 µm …….…… 4

4a. Intine 1.5 µm thick …… 4. *R. vesicarius*
4b. Intine thickness less than 1.5 µm ……. 5

5a. Tectum perforate-punctate under SEM ……..… 5. *R. nepalensis*
5b. Tectum coarsely reticulate …….…….. 6

6a. Spheroidal pollen in equatorial view ……. 6. *R. acetosa*
6b. Spheroidal to prolate-spheroidal in equatorial view ……..…… 7. *R. hastatus*

Table 2. Summary of Pollen measurements, shape and sculpturing features in the genus *Rumex* L. species.
(All measurements are in µm)

Sr. No.	Species	Pollen class	Aperture type	Shape in equatorial view	Shape in polar view	Equatorial diameter µm	Polar diameter µm	P/E ratio	Length of colpi µm	Exine thickness µm	Intine thickness µm	Sculpturing	
												Under LM	Under SEM
01	*R. chalepensis*	Tricolporate, tetracolporate	Non-lacunate	Prolate, subprolate to slightly rhomboidal	Circular	*25.4±0.24 (25-26)	27±1.15 (25-30)	1.06	18.4±0.20 (17-20)	3.00	1.2	Granulate	Perforate-punctate
02	*R. patientia*	Tricolporate, tetracolporate	Non-lacunate	Subprolate, prolate	Circular	21±0.61 (20-22.5)	26.2±0.56 (25-27.5)	1.24	17±0.44 (16-18)	2.3	0.75	Areolate-foveolate	Perforate-punctate
03	*R. dentatus*	Tricolporate, tetracolporate	Non-lacunate	Spheroidal, prolate-spheroidal to subprolate	Circular	27.6±0.94 (25-30)	28.4±0.48 (27.5-30)	1.02	18.6±0.68 (17-20)	2.2	1.0	Granulate	Granulate
04	*R. vesicarius*	Tricolporate, tetracolporate	Non-lacunate	Spheroidal, prolate-spheroidal	Circular	21±0.61 (19.5-22)	21.4±1.4 (18.5-25)	1.01	13.4±0.44 (12-14.5)	2.8	1.5	Indistinct	Perforate-punctate
05	*R. nepalensis*	Tricolporate, tetracolporate	Non-lacunate	Spheroidal	Circular	22±1.22 (20-25)	24±1.50 (20-27.5)	1.09	17.9±0.58 (17-19.4)	1.25	0.75	Indistinct	Perforate-punctate
06	*R. acetosa*	Tricolporate, tetracolporate	Non-lacunate	Spheroidal	Circular	22±1.22 (20-25)	23.5±0.61 (22.5-25)	1.06	15.4±0.26 (15-16)	2.5	0.75	Areolate-foveolate	Coarsely reticulate
07	*R. hastatus*	Tricolporate, tetracolporate	Non-lacunate	Spheroidal, prolate-spheroidal	Circular	16±0.54 (15-18)	18.6±0.47 (17.5-20)	1.16	16.08±0.4 (15-17.2)	1.7	1.25	Areolate-foveolate	Coarsely reticulate,

* Mean values followed by min-max in parentheses. P=Polar, E=Equatorial, ± Standard error

Pollen class: Tricolporate and tetracolporate type of pollen present together in *Rumex* species (Table 2).

Size: The size of pollen grain (polar axis × equatorial diameter) varies from 18.6×16 µm to 28.4×27.6 µm. *R. hastatus* (18.6×16 µm) appears to be smallest in size in the genus while pollen of *R. dentatus* (28.4×27.6 µm) have maximum size. In *R. vesicarius* polar and equatorial diameter is nearly same i.e., 21.4×21 µm (Table 2).

Symmetry and shape: The pollen are radially symmetrical and isopolar. Outline in polar view is circular in all taxa of the *Rumex* while it is spheroidal, prolate-spheroidal, subprolate, prolate to slightly rhomboidal in equatorial view indicating great range of variation in equatorial shape (Figs. 1-16). P/E (polar axis/equatorial diameter) ratio is in the range of 1.01 (*R. vesicarius*) to 1.24 (*R. patientia*) and P/E value is same in *R. chalepensis* and *R. acetosa* (Table 2). Evenly distributed granular type columella is noted in *R. patientia*, *R. dentatus*, *R. chalepensis* and *R. patientia*.

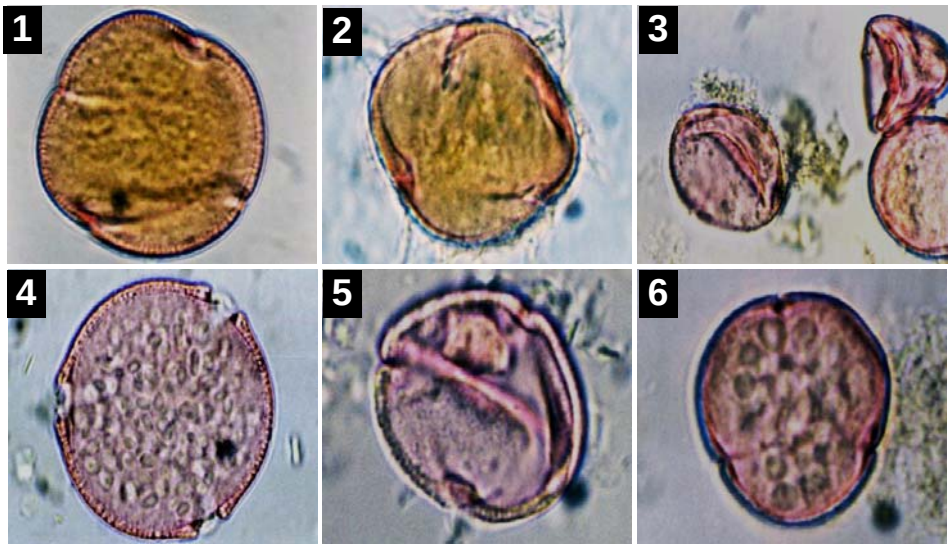
Aperture: Apertures are non-lacunate type while colpi are long and narrow. Length of colpi varies from 13.4 µm in *R. vesicarius* to 18.6 µm in *R. dentatus*and 18.4 µm in *R. chalepensis* (Table 2).

Exine and intine: Exine thickness is a variable character and it varies from 1.25 µm (*R. nepalensis*) to 3 µm (*R. chalepensis*). Intine is clearly visible in all species of *Rumex* but very thin in *R. patientia*, *R. acetosa* and *R. nepalensis* (0.75 µm).

Exine sculpturing: Under light microscope exine is areolate-foveolate in *R. patientia*, *R. acetosa* and *R. hastatus*, granular in *R. chalepensis* and *R. dentatus* while it remains indistinct in *R. vesicarius* and *R. nepalensis*. When observations are made under SEM, tectum is granular in *R. dentatus*, perforate-punctate in *R. chalepensis*, *R. vesicarius*, *R. patientia* and *R. nepalensis* and coarsely reticulate pattern is visible in *R. hastatus* and *R. acetosa* (Table 2).

Discussion

A comparative investigation was conducted on the pollen morphology of seven species (*R. chalepensis*, *R. patientia*, *R. dentatus*, *R. vesicarius*, *R. nepalensis*, *R. acetosa* and *R. hastatus*). In this study important pollen morphological characters of *Rumex* species (polar and equatorial outline and their diameter, length of colpi, exine and intine thickness and sculpturing under LM and SEM) were compared. Pollen shape, aperture type and exine sculpturing are strikingly significant characters (Perveen & Qaiser, 2005). Palynologically, Polygonaceae was found to be eurypalynous family (Wodehouse, 1931; Hedgeberg, 1946; Nowicke & Skvarla, 1979, Perveen, 1993) and it was also confirmed from the presently studied genus *Rumex*. Three pollen types were investigated in the present material, only one (Acetosa type) which is similar to the type recognized earlier by Zhang & Zhou (1998) while two other type shave not previously been recorded.



Figs. 1-6. LM micrographs of the pollen grains of genus *Rumex*.
R. chalepensis: 1. Polar view *R. dentatus*: 2. Polar view 3. Equatorial view *R. patientia*: 4. Polar view 5. Equatorial view. *R. acetosa*: 6. Polar view.

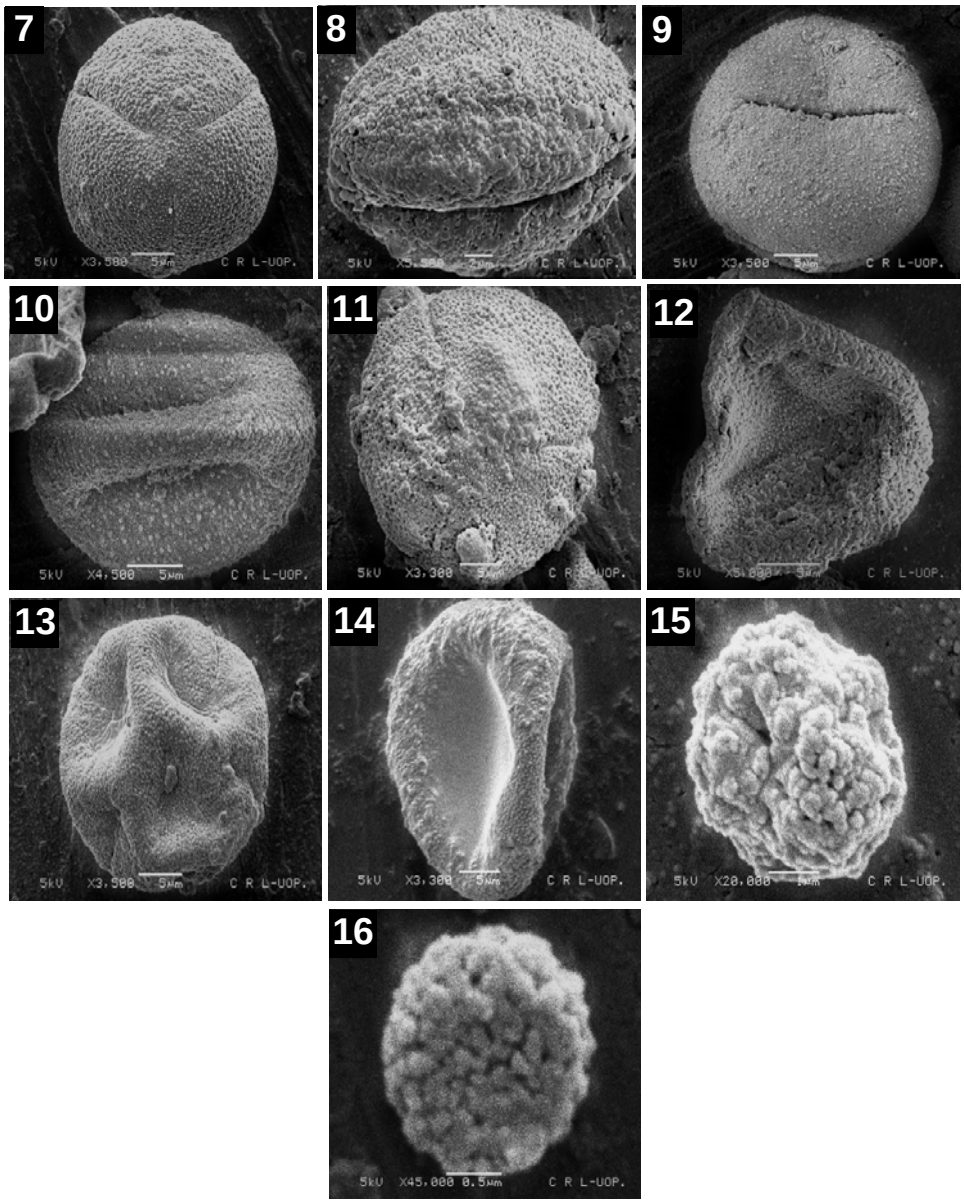
Key to the different pollen types

1a. Perforate-punctate tectum ……..….. Chalepensis type
(*R. chalepensis*, *R. nepalensis*,*R. vesicarius*, *R. patientia*)
1b. Granulate or coarsely reticulate tectum …… 2

2a. Pollen surface granular ……...…….. Dentatus type
(*R. dentatus*)
2b. Pollen surface coarsely reticulate ……..…… Acetosa type
(*R. acetosa*, *R. hastatus*)

Chalepensis type pollen: Tricolporate and tetracolporate Chalepensis type pollen characterizes *R. chalepensis*, *R. nepalensis*, *R. patientia* and *R. vesicarius*. It is postulated that variation in colpi number is related with the level of ploidy (Van Leeuwen *et al.*, 1988) but it is early to declare that colpi number is related to ploidy level (Hong *et al.*, 2005). Considerable variation was noted in the equatorial shape of pollen while polar outline was circular. Slight variation in pollen size was also observed, for instance minimum polar diameter in *R. vesicarius* (21.4 µm) while maximum in *R. chalepensis* (27 µm). Likewise, the minimum equatorial diameter was 21 µm in *R. vesicarius* and *R. patientia* and maximum was 25.4 µm in *R. chalepensis* (Table 2). Perveen (1993) examined 3-6 colporate *R. vesicarius* grains and their size ranged from 21.51-25.12×17.91-26.91 µm.

Relatively long and deep colpi were noted in *R. chalepensis* (18.4 µm) while somewhat smaller in *R. vesicarius* (13.4 µm). Exine was rather thick in *R. chalepensis* (3.0 µm) and *R. vesicarius* (2.8 µm) while intine was found to be very thin but quite distinct in appearance. Exine sculpturing when observed through LM and SEM, showed



Figs. 7-16. SEM micrographs of the pollen grains of genus *Rumex*.
R. chalepensis: 7. Polar view. *R. vesicarius*: 8. Equatorial view. *R. dentatus*: 9-10. Equatorial view. *R. patientia*: 11. Polar view 12. Equatorial view. *R. nepalensis*: 13. Polar view 14. Equatorial view. *R. acetosa*: 15. An overall view of the pollen grain. *R. hastatus*: 16. An overall view of the pollen grain.

great range of variation. Wodehouse (1931) suggested distinctly pitted exine in *Rumex* species. SEM studies have revolutionized palynological characters of pollen grains (Tahir, 2005). SEM studies of the pollen in *R. vesicarius* indicated punctate-scabrate sculpturing (Perveen, 1993). Present LM studies showed granulate and areolate-foveolate

surface (Figs. 1-2, 4) while in SEM pollen surface appeared perforate-punctate (Figs. 7-8, 11-14) on the basis of which Chalepensis type of pollen was suggested.

Dentatus type pollen: 3-4 colporate pollen of R. *dentatus* with granular exine represents this pollen type (Figs. 3, 9-10, Table 2). The pollen grains were larger in size (27.5-30×25-30 µm) with long colpi (18.6µm) and 2.2µm thick exine. Perveen (1993) found these grains to be the pantocolporate, 21.54-28.72×21.41-29.12 µm in size and densely punctate to finely scabrate tectum. Similarly, Zhang & Zhou (1998) reported subspheroidal 3-4 colporate pollen with equatorial diameter 20-27 µm and microechinate-perforate surface.

Acetosa type pollen: This type is the characteristic feature of *R.acetosa* & *R. hastatus* (corresponding to Zhang and Zhou 1998, Acetosa type). The size of spheroidal and prolate-spheroidal grains ranged from 18.6-23.5×16-22 µm (polar × equatorial axis) Wodehouse (1931) reported tricolporate and less frequently tetracolporate pollen in *R. acetosa* with equatorial diameter in the range of 18.2-21.6 µm. Zhang & Zhou (1998) investigated 3-4 colporate pollen in *R. acetosa* with 17-20 µm of equatorial diameter and microechinate-perforate tectum. Current LM studies revealed areolate-foveolate exine but pattern was coarsely reticulate when observed with SEM (Figs. 6, 15).

Based on the present study, it would appear that there is quite considerable variation in pollen morphology especially; exine and its sculpturing under SEM is of real systematic value and make the pollen grains decidedly distinct structures which can be employed for the delimitation of closely related species.

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