Pak. J. Bot., 42(4): 2205-2214, 2010.

SYSTEMATIC IMPLICATIONS OF POLLEN MORPHOLOGY IN THE FAMILY MALVACEAE FROM NORTH WEST FRONTIER PROVINCE, PAKISTAN

NOREEN BIBI[1*], NAVEED AKHTAR[2], MANZOOR HUSSAIN[3] AND MIR AJAB KHAN[4]

[1]Nuclear Institute of Agriculture and Biology Faisalabad,
[2] Department of Botany Govt. Postgraduate College, Abbottabad,
[3]Department of Botany, University of Peshawar,
[4]Department of Biological Sciences, Quaid-e-Azam University, Islamabad, Pakistan

Abstract

Pollen morphology of 9 species belonging to 6 genera viz., *Abelmoschus, Abutilon, Alcea, Malva, Malvaviscous* and *Malvastrum* of the family Malvaceae from North West Frontier Province, Pakistan were examined by light and Scanning Electron Microscope. Pollen grains are generally radially symmetrical, apolar, mostly spherical to oblate-spheroidal and poly pantoporate. Tectum uniformly echinate, medium to finely perforated or punctuate with granules or scabrae in between spines. The results demonstrated that pollen morphological characteristics, principally spine base, spine apex, spinular morphology, aperture characters, exine sculpture and structure are taxonomically important. However some of the genera studied were found to be stenopalynous thus limiting the value of taxonomic application of their pollen characters.

Introduction

The Malvaceae are a worldwide family of herbs, shrubs and small trees with a primary concentration of genera in the tropical regions and comprises of some 110 genera and over 2000 species, divided into five or six tribes: Malopeae, Malveae, Hibiscieae, Abutilieae, Ureneae and Decaschistieae (La Duke & Doebley, 1995; Krebs, 1994, a & b). Owing to the high economic value of some genera of the Malvaceae, several studies, from different perspectives, have been carried out on this family. Especially noteworthy are those by El Hadidi *et al*., (1999) and El Naggar (2001, 2004). In Pakistan, Malvaceae is represented by 19 genera with 94 specific and intra specific taxa (Abedin, 1979).

Culhane & Blackmore (1988) divided the family into six pollen type, based on number of apertures, grains diameter and spinular morphology. Christensen (1986) conducted most comprehensive study of the Malvaceae pollen of Malvaceae using both light (LM) and scanning electron microscopy (SEM) and concluded that the generic delimitation based on pollen morphology is difficult in this family. However, Saad (1960) considered that the pollen morphology in the family Malvaceae is quite distinctive which could apparently distinguish between the genera. Hosni & Araffa (1999) used pollen characteristics in combination with other morphological characteristics to differentiate between certain taxa. In Pakistan, pollen morphology of only few genera of family Malvaceae has been studied by Sayeeduddin *et al*., (1942) Siddiqui *et al*., (1984), Tahavi (2000), Bibi *et al*., (2008) and Perveen & Qaiser (2009) using light microscope as well as Scanning Electron Microscope. Perveen *et al*., (1994) provided pollen morphology of 42 species belonging to 12 genera from Pakistan using light and scanning electron microscope. In the present studies an attempt has been made to provide complete information of pollen morphology of some selected species growing wild and cultivated as ornamental in N.W.F.P., Pakistan.

*Corresponding author: noreenahmed07@yahoo.com, noreenahmed156@gmail.com

Materials and Methods

The present study is based on the pollen morphology of 9 species belonging to 6 genera of family Malvaceae (Table 1). Pollen samples were obtained from the wild plants as well as from cultivated ornamentals at various localities of N.W.F.P., Pakistan

For light microscopy (LM) pollen were acetolysed according to Erdtman (1952) and Retisma (1969). Before acetolysis the pollen were treated with 10 % KOH for about 10 minutes to remove the oils and waxes thus causing the apertures to open and making them easier to study. Pollen grains were then mounted in safranine stained glycerin jelly and micro morphological observations were made with OLYMPUS/BX-51 light microscope under (E 40, 0.65) and oil immersion (E100, 1.25) using a 10X eye piece at National Center of Excellence in Geology, University of Peshawar, Pakistan.

For Scanning Electron Microscopy (SEM) acetolysed pollen grains were directly transferred to aluminum stub covered with double sided cellotape and coated with gold, in a sputtering chamber (Ion-sputter JFC-1100), with coating restricted to $150A^0$. SEM examination was carried out on a Jeol microscope JSM-T200 at Centralized Resource Laboratory, Department of Physics, University of Peshawar, Pakistan. The measurements were based on 15-20 readings from each specimen. Various pollen characters viz. pollen class, shape, size, aperture, sporoderm stratification and exine ornamentation were investigated and the terminology used is in accordance with Erdtman (1952), Kremp (1965), Faegri & Iverson (1975) and Huang (1972).

Results

Pollen grains in family Malvaceae are usually spherical-globular in polar view and oval to elliptic in equatorial view. Pollen surface is echinate, porate with poly, panto and zono arrangement. Tectum is sub-psilate, often punctuate-granulate with scabrae in between spines/spine. Spines are evenly distributed on entire pollen surface and vary in height, apex, base and inter spinal distance. Exine is almost of the same thickness (3-5 µm) among all taxa studied except *Malvaviscus arboreus* (7.36µm).

1. *Abelmoschus esculentus* L. (Figs. 1 & 2A-B)

Pollen grains are 143(161)177 µm in size, pantoporate, isopolar, spherical to sub spherical in polar view and elliptic in equatorial view. Pores large with pore diameter 7(11)21 µm and rounded to oval in shape. Sometimes points of spine detachment are mistakenly taken as pores of large diameter. Exine 4(5)7 µm thick, tectum echinate, spine are widely spaced with inter spinal distance of 21(31)44 µm between spine apexes and 7(17)30 µm between spine bases. Spine show great variation with respect to their arrangement; central spine appear to be different from marginal ones and placed on basal cushions, apex blunt to round. Spine 12 (16)18 µm high, spine base 5(6)7 µm wide. Number of spines 34(43)54, number of pores 5(7)12. Tectum punctuates and granulates in between the spine (Tables 2, 3).

2. *Abutilon indicum* L. (Figs. 1 & 2C-D)

Zonoporate, circular in polar view with pollen diameter of about 48 (50) 69 µm. Pores small, invisible under light microscope but visible with Secondary Electron Microscope. Exine 2 (3) 4 µm thick and may be taken as overlapping spine bases. Tectum echinate, punctuate-granulate with spinules forming a ring at the base of basal cushion bearing spine (Table 2). Sometimes two spine are present on single basal cushion. Spine are provided with acute apex and wide base of about 7(8)9 µm. Spine apexes are 6(7)9 µm apart from each other. Spine height is 5(6)7 µm and number of spines is more than 60.

Table. 1. List of species studied and their origin.

S. No.	Genus	Species	Locality
1.	*Abelmoschus*	*Abelmoschus esculentus* L.	Dhodial, Mansehra
2.	*Abutilon*	*Abutilon indicum* (Cav)	University of Peshawar, Peshawar
3.		*Abutilon ramosum* L.	Khewra
4.	*Alcea*	*Alcea rosea* L.	Haripur
5.	*Malva*	*Malva mauritiana* L.	Haripur
6.		*Malva neglecta* Wallr.	Neelam Valley, AJK
7.		*Malva parviflora* L.	University of Peshawar, Peshawar
8.	*Malvaviscus*	*Malvaviscus arboreus* var. *penduliferous* (Mocino and Sesse ex DC.)	Islamia College, Peshawar
9.	*Malvastrum*	*Malvastrum coromendelianum* L.	Quaid-e-Azam University, Islamabad

Table. 2. Tectum sculpture.

S. No.	Sample name	Shape	Tectum sculpture in between spines	Flask shape	Spine apex	Spine base	Spine type	Class
1.	*Abelmoschus esculentus*	Spherical	Micro reticulate-punctuate, Granulate	Absent	Blunt & Bifurcated	Bulbous	Monomorphic	Pantoporate
2.	*Abutilon indicum*	Globose	Punctuate	Present	Blunt	Bulbous	Monomorphic	Zonoporate, Spinules form ring at Bulbous base
3.	*Abutilon ramosum*	Circular	Granulate	Present	Acute	Bulbous	Monomorphic	Zonoporate
4.	*Alcea rosea*	Spherical	Sub Psilate, Smooth	Present	Acute/Blunt	Bulbous	Dimorphic	Polyporate
5.	*Malva mauritian*	Spherical	Granulate	Absent	Acute	Bulbous	Monomorphic	Pantoporate
6.	*Malva neglecta*	Spherical	Sub echinate, Punctuate	Absent	Acute	Bulbous	Dimorphic	Pantoporate
7.	*Malva parviflora*	Spherical	Sub psilate, Granulated to punctuate	Absent	Acute	Bulbous	Monomorphic	Pantoporate
8.	*Malvaviscous arboreus*	Globose	Verrucate, Granulate	Absent	Acute	Bulbous	Monomorphic	Polyporate,
9.	*Malvastrum coromendelianum*	Spheroidal	Verrucate	Absent	Acute	Flat	Monomorphic	Pantoporate

Table. 3. Quantitative measurements of Malvaceous pollen.

S. No.	Species	Pollen size (µm)	Exine thickness (µm)	No. of Spine	Spine height (µm)	Spine base (µm)	Distance b/w spine bases (µm)	Distance b/w spine apexes (µm)	No. of pores	Pore diameter (µm)
1.	*Abelmoschus esculentus*	160×140	5	43	16	6	17	31	7	11
2.	*Abutilon indicum*	69	4	35	6	7	Joined	6	Many	Not distinct
3.	*Abutilon ramosum*	58×57	4	≥ 60	4	6	Not distinct	7	Many	Not distinct
4.	*Alcea rosea*	132×129	3-5	145	12	4	11	20	Many	4
5.	*Malva mauritiana*	117	5	≥ 80	9	3	9	14	15	4
6.	*Malva neglecta*	125×104	5	Many	7	4	8	9	33	3
7.	*Malva parviflora*	94×92	3	43	5	≤1	5.3	8	Many	4
8.	*Malvaviscous arboreus*	179× 172	7	58	14	6	19	30	14	6
9.	*Malvastrum coromendelianum*	78×77	3	97	8	7	Joined	9	Not distinct	Not distinct

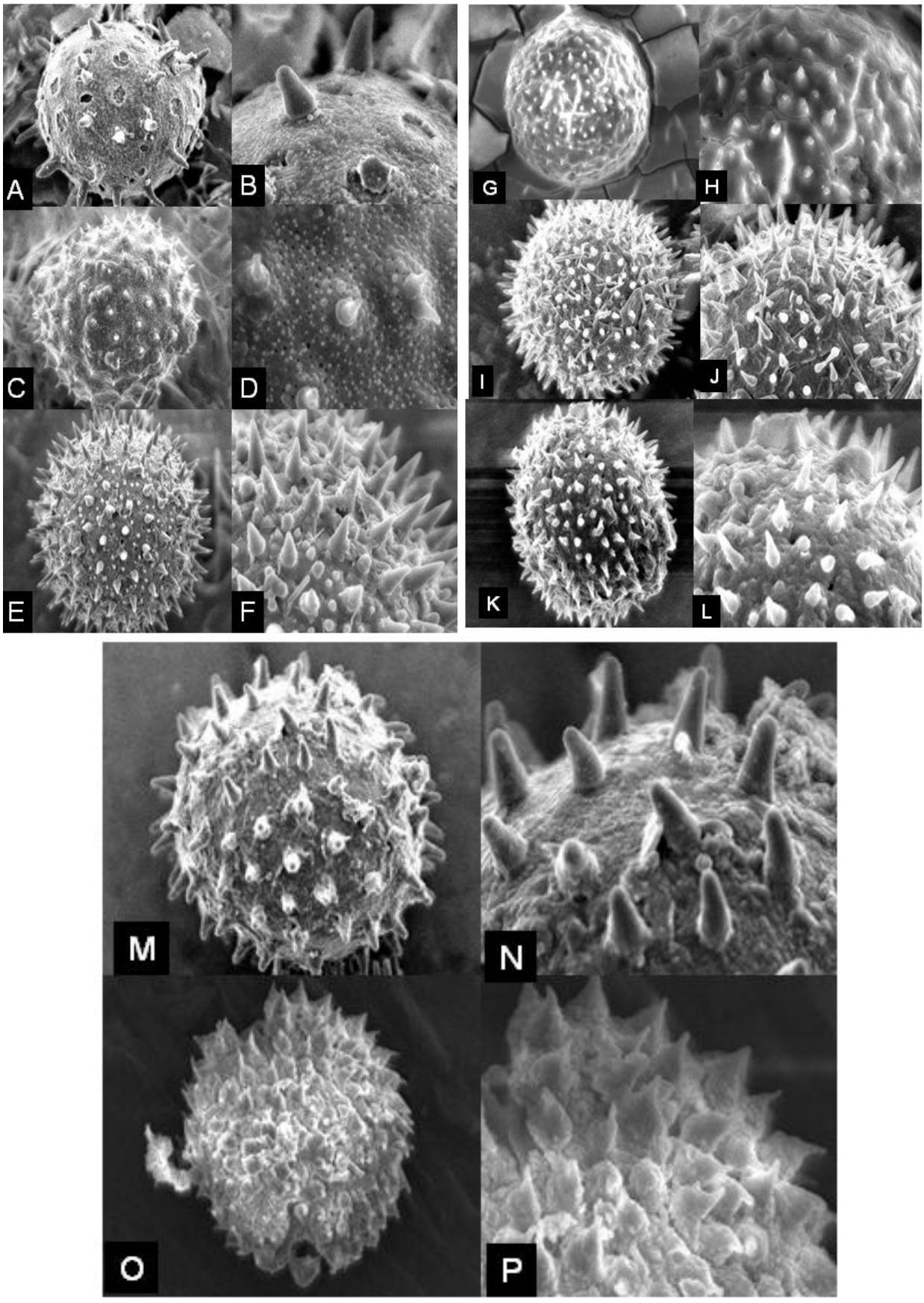

Fig. 1. Scanning electron micrograph: A-B. *Abelmoschus esculentus*, C-D. *Abutilon indicum*, E-F. *Alcea rosea*, G-H. *Malva mauritiana*, I-J. *Malva neglecta*, K-L. *Malva parviflora*, M-N. *Malvaviscus arboreus*, O-P. *Malvastrum coromendelianum*.

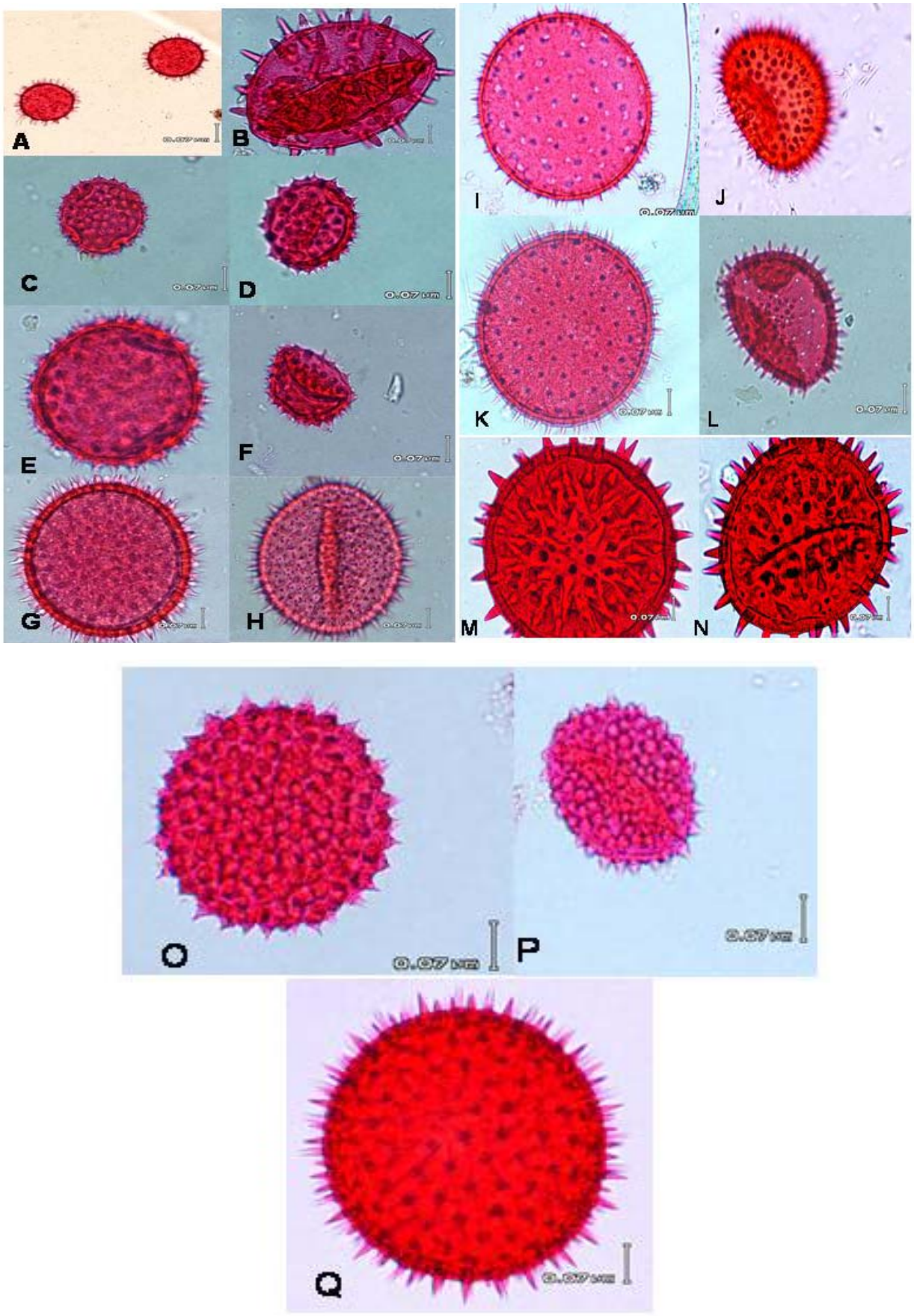

Fig. 2. Pollen view through light microscope: A-B. *Abelmoschus esculentus* (10X, 40X), C-D. *Abutilon indicum* (40X), E-F. *Abutilon ramosum*, G-H. *Alcea rosea* (40X), I-J. *Molva neglecta* (40X), K-L. *Molva parviflora* (40X), M-N. *Molvaviscus arboreus* (40X), O-P. *Malvastrum coromendelianum* (40X), Q. *Malva mauritiana* (40X).

3. *Abutilon ramosum* Cav (Fig. 2E-F)

Zonoporate, circular in polar and oval to reniform in equatorial view. Exine thickness is about 2.3(4)4.6 µm. Tectum echinate, punctuate-granulate with spine height of 3.45(4)5.7 µm and spine base 4.6(6)8.05 µm wide. Spine are more than 60 in number with spine apexes 5.75(7)9.2 µm apart and are arranged in a similar fashion to that of *Abutilon indicum*. Marginal spines are distinct with broad base and acute apex hence can be counted easily while surface spines are very closely arranged (Table 2). Pollen diameter is about 50.6(58)62 µm.

4. *Alcea rosea* L. (Fig. 1E-F, Fig. 2G-H)

Pollen grains are spherical in polar view with pollen diameter of about 115(132)142 µm, oval to elliptic in equatorial view, monocolpate, polyporate. Pores are distinct, rounded in shape with pore diameter of about 2(4)5 µm and scattered on entire pollen surface. Exine 3.4–5 µm thick and differentiated into sexine and nexine. Tectum perforated, echinate and sub psilate. Spine 140 (145)150 in number, dimorphic, larger one with acute apex while shorter one with blunt apex, arranged regularly and are resistant to acetolysis (Tables 2, 3). Spines of similar morphology are widely spaced with spine bases 7(11)21µm and spine apexes 12 (20)28 µm apart. Basal cushion is present beneath the central spines. Spine height is 9.2(12)14 µm and spine base is 3.4(4)5µm wide.

5. *Malva mauritiana* L. (Fig. 1G-H, Fig. 2Q)

Spherical to sub spherical in outline with 117 µm pollen diameter. Pantoporate, pores large and circular to oval, exine is about 5 µm thick and differentiated into sexine and nexine. Tectum echinate with monomorphic spine (having acute apex and bulbous base, granulate with no spinules (Tables 2, 3). Spine arrangement is regular being directed outward (away from pollen centre), inter spinal distance is larger (14 µm between spine apexes) than other species of *Malva.* Spine height is almost 9 µm while spine base is 3.3 µm wide. No of spines are more than 80.

6. *Malva neglecta* Wallr. (Fig. 1I-J, Fig. 2G-H)

Pollen of *Malva neglecta* are spherical in polar view and oval to elliptic in equatorial view with pollen diameter of about 81(125)131µm. Pantoporate, with 28 (33) 41 pores. Pores large with diameter of 2.3 (3) 6 µm, distinct and circular to oval in outline. Exine 3(5)9 µm thick and differentiated into sexine and nexine. Tectum punctuates and echinate with many, dimorphic spine having blunt and curved apexes, sensitive to acetolysis, with spine height of 7(8)9 µm and spine base 2.3(4)9 µm wide (Tables 2, 3). Spine apexes are 7(9)16 µm apart while spine bases are 5(8)14µm apart. Central spines are smaller in size, many in number and arranged regularly with their apexes directed towards the centre of pollen. However marginal spines are large and directed away from the centre.

7. *Malva parviflora* L. (Fig. 1 & 2K-L)

Pantoporate, monocolpate, spherical to sub spherical in polar view and reniform in equatorial view with pollen diameter 69(94)117 µm. Pores are small with pore diameter of about 2.3(4)5 µm, many in number, circular in shape and are located usually at the base of spine in spiral pattern (Tables 2, 3). Exine thickness is about 2.3(3)5µm and

differentiated into sexine and nexine. Tectum echinate, finely perforated, punctuate and often micro reticulate–micro verrucate. Spine dimorphic, 27(43)57 in number, vary in size, larger one with spine height of 3(5)7 µm, pointed apex and with spine basis of ≤1µm. Smaller spine have blunt apex and occupy the centre of pollen. Distance between spine apexes is 5(8)12µm and between spine bases is 2.3(5.3)8 µm.

8. *Malvaviscus arboreus* var. *Penduliferous* (Mocino and Sesse ex DC.) (Fig. 1 & 2 M-N)

Spherical in polar view while oval to elliptic in equatorial view, pollen diameter of about 151(179)218 µm. Polyporate, pores 11(14)21 in number, distinct and clear with pore diameter of 2.3 (6)9 µm. Exine 5(7)9 µm thick, differentiated into sexine and nexine and provided with distinct rod like columella near or beneath the basis of spine. Tectum echinate, spine appear to be monomorphic with respect to size but are dimorphic with blunt rounded and bifurcated apex. Spine are 43(58)78 in number with spine height of about 9(12)14 µm and spine base 5(6)9 µm wide. Arrangement of the spines on pollen surface varies. Central spines are directed with their apexes towards the centre and are sparsely distributed over entire pollen surface. Tectum granulates and verrucate–punctuate. Spine are widely spaced with spine apexes 21 (30)41 µm and spine bases are 14(19)35 µm apart. Basal cushion was observed under central spines (Tables 2, 3).

9. ***Malvastrum coromendelianum* L. (Fig. 1 & 2O-P)**

Spherical to sub spherical in polar view with pollen diameter of about 62(78)87µm, pantoporate with pores invisible under Light Microscope but visible under Electron Microscope. Exine is not visible as distinct outline rather taken as overlapping spine apexes with exine thickness of about 2(3)5 µm. Tectum echinate, perforated and punctuate between spines (Tables 2, 3). Spine height is about 7(8)9 µm, monomorphic with only acute apex. Spine bases are 6(7)8µm wide and placed closely almost united to one another. However spine apexes are 6(9)12 µm apart. Sometimes spine height is so short making it almost sessile with curved apex.

Key to species on the basis of pollen morphology

1 a. Pollen zonoporate …….. *Abutilon* species
b. Pollen Poly pantoporate ……..……... 2

2 a. Pollen with spinules at the base of spine ……..……. *Abelmoschus esculentus*
b. Pollen without basal spinules ……. 3

3 a. Pollen with flat spine base …….. *Malvastrum coromendelianum*
b. Pollen with bulbous spine bases …… 4

4 a. Pollen with flask shape structure …….. *Alcea rosea*
b. Pollen without flask shape structure …….. 5

5 a. Pollen with pantoporate, sub psilate Tectum ……..... 6
b. Pollen with polyporate, verrucate Tectum ……..... *Malvaviscus arboreus*

6 a. Pollen with distance between spine apexes ≥ 10 µm …….. *Malva mauritiana*
b. Pollen with spine distance ≤ 10 µm ……... 7

7 a. Pollen with monomorphic spine …….. *Malva neglecta*
b. Pollen with dimorphic spine apex …… *Malva parviflora*

Discussion

Palynologically Malvaceae is a stenopalynous family and pollen characters of this family are more or less uniform (Tahavi, 2000). Saad (1960) studied the pollen morphology of 35 species of Malvaceae. He emphasized the importance of the aperture and spine characteristics, as well as exine stratification, to distinguish between different taxa.

Pollen grains are generally radially symmetrical, apolar, poly pantoporate and zono aperturate. The present findings are in accordance with Perveen *et al.*, (1994) and EI Naggar (2004) that pollen grain in Malvaceae are usually spheroidal or globular in outline and are colporate or porate with an echinate sculpture. Tectum uniformly echinate with monomorphic spine in most of the cases except *Alcea rosea* and *Malva neglecta* where spine are of different sizes hence dimorphic (Fig. 1. E, F, I and J).

The surface pattern of the tectum varies from sub psilate-smooth as in *Alcea rosea* (Fig. 1E&F) to micro reticulate, Verrucate and punctuate-granulate with scabrae in between spines. Spine are evenly distributed over the entire surface of pollen grain and vary in length, shape, density and apex which vary as pointed, rounded blunt and bulbous. Exine is almost of the same thickness (3-5 μm) among all taxa except *Malvaviscous arboreus* (7.36 μm).

Microscope slide mounting media differentially affect pollen diameter by as much as 10 % enough to confound differentiation among closely related taxa (Andrew, 1997). In present study pollen measurements were made from treated pollen that normally grow in size. That's why the pollen diameter of *Malvaviscus arboreus*, *Malva parviflora*, *Malva neglecta*, *Abelmoschus esculentus* and *Alcea rosea* does not fall within the prescribed range (100-128 μm) of EI Naggar (2004) who made use of untreated dry pollen. However results of pore diameter, exine thickness, columella structure and spine type are highly in accordance to him.

The appearance of pores on the inner surface of the acetolysed pollen has proved to have reliable taxonomic characteristics (EI Naggar, 2004). The spine show considerable variations in base, apex, inter spinal distance and their distribution on pollen surface. The variations are of value at different taxonomic levels, because they may occur not only between genera but also between species of the same genus.

Spine height on individual pollen may be uniform (monomorphic spines) or vary thus giving a dimorphic pattern (*Alcea rosea and Malva parviflora* Figs. 3 & 4). Results exhibited the smallest spine in *Malva parviflora* (3-5 μm).

Tahavi (2000) arguments that *Hibiscus* pollen are the largest among all the genera of family, contradict our results where the pollen grains of *Malvaviscus arboreus* have the largest pollen size (179 μm) however results about tectum, which is uniformly echinate, medium to finely perforate sparsely to densely granulate between spines, confirm the present findings.

References

Abedin, S. 1979. Malvaceae. In: *Flora of Pakistan*. (Eds.) E. Nasir & S.I. Ali, 130: 1-98.

Andrew, S. 1997. Analysis of Maize (*Zea mays* subsp. *mays*) Pollen: Normalizing the Effects of microscope-slide mounting media on diameter determinations. *Palynology*, 21: 35-39.

Christensen, P.B. 1986. Pollen morphological studies in the Malvaceae. *Grana,* 25: 95-117.

Culhane, K.J. and S. Blackmore. 1988. Malvaceae. In: *The North West European Pollen Flora,* (Eds.): W. Punt, S. Blackmore & G.C.S. Blackmore & G.C.S. Clarke, V.41: 45-79.

El Hadidi, M.N., H.A. Hosni., A.M.H. El Hadidy and S. Araffa. 1999. Malvaceae in the flora of Egypt. 1- Systematic revision of the indigenous taxa. *Taeckholmia*. 19: 127-146.

El Naggar, S.M. 2004. Pollen morphology of Egyptian Malvaceae: An assessment of taxonomic value. *Turk. J. Bot*., 28: 227-240.

Erdtman, G. 1952. *Pollen morphology and plant taxonomy: (Angiosperms. An introduction to Palynology-I)* Almqvist and Wiksell, Stockholm. pp. 539.

Faegri, K. and J. Iverson. 1975. *Text Book of Pollen Analysis. 3rd revised edition by K. Faegri*. Munksgaard, Copenhagen and Denmark. pp. 295.

Hosni, H.A and S. Araffa. 1999. Malvaceae in the flora of Egypt. 2- Pollen morphology and its taxonomic significance. *Taeckholmia*, 19: 147-156.

Huang, T.C. 1972. *Pollen flora of Taiwan.* National Taiwan University Botany Department Press. pp. 297; Pl: 177.

Krebs, G. 1994a. Taxonomische Untersuchungen in der Subtribus Malvinae. *Feddes Report*. 105: 7-18.

Krebs, G. 1994b. Taxonomische Untersuchungen in der Subtribus Malvinae II. Dinacrusa. *Feddes Report*, 105: 299-315.

Kremp. G.O.W. 1965. *Morphologic Encyclopaedia of Palynology*. The University of Arizona Press. Tucson. pp. 263.

La Duke, J. C. and J. Doebley. 1995. A chloroplast DNA based phylogeny of the Malvaceae. *Syst. Bot*., 20: 259-271.

Perveen, A. and M. Qaiser. 2009. Pollen flora of Pakistan-Malvaceae, Dombeyoideae-LXII . *Pak. J. Bot*., 41(2): 491-494.

Perveen, A., S. Siddiqui, A. Fatima and M. Qaiser. 1994. Pollen Flora of Pakistan- I. Malvaceae. *Pak. J. Bot*., 26(2): 421-440.

Reitsma, T. 1969. Size modification of recent pollen grains under different treatments. *Rev. Palaeobot* Palyno, l9: 175-202.

Saad, S.I. 1960. The sporoderm stratification in Malvaceae. *Pollen and spore,* 2: 13-41.

Sayeeduddin, M., M. Saleem and M.R. Suxena. 1942. A comparative study of the structure of pollen grain in some of the families of angiosperms. *J. Osmania Univ.,* 10: 12-15.

Siddiqui, S., K.M. Khan and S. Abedin. 1984. Pollen morphology of *Abutilon* Mill., from Pakistan. *J. Pharm. Kar. Univ*., 2: 105-119.

Tahavi, M. 2000. *Palynological studies of arboreal plants growing in Lahore and their impact on aeropalynology*. Ph. D. Thesis, Punjab University Lahore.

(Received for publication 12 April 2009)