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Taxonomic Implication of Palynological Characters in the Genus *Malva* L., Family Malvaceae from Pakistan

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Abstract: Pollen morphology of six species of the genus *Malva* L., *Malva microcarpa* Pers. *M. neglecta* Wallr., *M. nicaeensis* All., *M. parviflora* L., *M. sylvestris* L. and *M. verticellata* L. was studied and documented in detail using L.M (light microscopy) and SEM (scanning electron microscopy). The genus is characterized by apoplar, pantoporate and spheroidal pollen. The wall of a mature pollen was composed of unremitting intine and exine overspread with spines of variable width and height. The character of spine index was used for the first time to describe pollen of the genus *Malva* L. and was found to be of significant taxonomic importance. In most of the studied characters, the genus was found stenopalynous thus limiting the value of taxonomic application of these pollen characters.

Key words: *Malva* · Malvaceae · Pollen morphology

INTRODUCTION

Malva L. is a large genus of annual or perennial herbs with over 100 species, native of Europe, Asia and Africa [1]. The natural origin of species within the genus *Malva* is not certain, as many species have become widespread weeds [2]. Bates and Blanchard [3] placed *Malva* along with *Alcea* (as *Althaea*) and *Lavatera* in *Malva* alliance of tribe Malveae. The genus is closely related to *Lavatera* [4]. Carolus Linnaeus was the first to separate the two genera on the basis of fusion and non-fusion of epicalyx lobes (monophyllum in *Lavatera* and triphyllum in *Malva*), but several authors implied that the use of epicalyx character to discriminate between *Malva* and *Lavatera* is not reliable [5, 2]. García *et al*., [6] by using five molecular markers also concluded that there is extensive homoplasy and reticulate evolution in *Malva* alliance, thus the relationships among the taxa as well as the evolution of morphological traits have remained indescribable and the traditional classifications are highly artificial.

The significance of pollen morphology in plant systematics has been stressed by a number of workers, especially by Lindley [7], Mohl [8], Fritzsche [9], Fischer [10], Selling [11], Cranwell [12] and Erdtman [13, 14]. Palynologically, family Malvaceae is a stenopalynous and general features of pollen are relatively uniform [15-17]. In Pakistan the pollen morphology of few genera of the Malvaceae has been investigated by Siddiqui *et al*., [18,19], using light microscope but Perveen *et al*., [17] and Perveen and Qaiser [20] tried to give a more comprehensive picture of Malvaceous pollen morphology by studying pollen of 42 species representing 12 genera and 12 species representing 3 genera, respectively of the family Malvaceae. Pollen morphology of the genus *Malva* L. from Pakistan has been investigated by Perveen *et al*., [17]. They reported pollen diameter, pore diameter, spine length and exine thickness for five species. Besides the above mentioned characters, spine width, spine index and measurements of different layers of pollen wall are also recorded to give a more comprehensive and clear picture of the pollen grain morphology of the genera concerned. Character of spine index is used for the first time to characterize pollen of the genus *Malva* and is found to be of significant taxonomic importance. Therefore, the main objectives of this paper are; to provide additional knowledge about the pollen morphology of the taxa already investigated as well as to include additional taxa which were not considered in the previous studies and to determine the extent to which the data can be used as a taxonomic character in the genus.

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MATERIALS AND METHODS

Prepration of Glycerine Jelly: Glycerin jelly was prepared according to the modified method of Ahmad *et al*., [21]. A500 mL of distilled water was taken in a beaker and heated on a hot plate (model UELP Scientifica, Germany). 35 gm of gelatin was added when temperature reached to 70-80°C. After increase in temperature it became a viscous liquid of glycerin jelly. Whole solution was kept on heating for one hour. 35 gm of glycerol was mixed in it with few crystals of phenol. Then 0.1% safranine was added with 1/8th volume of glycerin jelly. It was stirred till uniform pink color appeared. Jelly was stabilized at room temperature.

Processing of Pollen: Specimens collected from different localities in Pakistan and representative specimens in the Herbarium of Quaid-i-Azam University, Islamabad Pakistan (ISL), listed in Table 1 were used as a source for polliniferous material. Vouchers of fresh collection were deposited in the Herbarium of Quaid-i-Azam University (ISL). For processing of pollen, for light and scanning microscopy, the slides were prepared by the modified procedure of Erdtman [13, 22]. For light microscopy, the pollen grains were mounted in glycerine jelly stained with 1% safranine, on a glass slide. A glass cover slip was placed on the prepared pollen glycerine jelly mixture. When cooled, the glass slide was labeled and edges of the cover slip were sealed with transparent nail vanish. The prepared slides were studied under the light microscope. Pollen shape, pollen diameter, exine thickness, exine sculpturing, height of the spine, width of the spine at its base, spine index, inter-spinal distance and pore diameter were examined. Details of pollen morphology were based on the measurements of 10-15 grains. 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 was that of Erdtman [13], Moore *et al*., [23] and Punt *et al*., [24].

Table 1: List of Specimen used in palynological investigations

S. No.	Taxa	Locality	District	Collector Name	Acc. No.
1.	*Malva Microcarpa Pers.*	Poonch, Peharpur to Dhela	Poonch	Shahzad and Dilawar	58513 (ISL!)
2.	*M. neglecta Wallr.*	Gujar Khan	Rawalpindi	M. Arif and Maqsood;	55185 (ISL!)
		Kurram Agency	Parachinar	Hifizullah and Nisar	63700 (ISL!)
3.	*M. nicaeensis All.*	Poonch	Poonch	Shahzad and Nisar	58583 (ISL!)
		Muzaffarabad	Muzaffarabad	Jan Mohammad	17988 (ISL!)
4.	*M. parviflora L.*	Muzaffarabad,	Muzaffarabad	Jan Mohammad	21828 (ISL!)
		Shuja-abad	Multan	Mir Ajab and Manzoor Hussain	46776 (ISL!)
5.	*M. sylvestris L.*	Rawal Dam	Rawalpindi	Iqbal Dar, Nisar and Maqsood	21834 (ISL!)
		Kuchlar, Ziarat Road	Quetta	M.A. Siddiqi and Nisar ahmad	07675 (ISL!)
		Kurram Agency	Parachinar	Hafizullah and Nisar	63701(ISL!)
6.	*M. verticellata L.*	Mansehra	Mansehra	Muqarrab Shah and Dilawar Khan	82171(ISL!)
		Dhamtor	Abbottabad	Muqarrab Shah and Dilawar Khan	83613 (ISL!)

Table 2: Qualitative and quantitative palynological features in investigated in the genus Malva L

Taxa investigated	Shape	Min. (Mean±S.E) Ma. Pollen diameter µm	Pollen class	Min. (Mean±S.E) Ma. Spine height µm	Min. (Mean±S.E) Ma. Spine width µm	Spine index
Malva sylvestris	Spheroidal	80(86.4±1.7)90	Pantoporate	8(9.7±0.4)11.2	3(3.1±0.07)3.5	3.1
M. neglecta	Spheroidal	70(83.54±5.2)100	Pantoporate	5(5.85±0.3)7.5	2(2.5±0.1)3	2.34
M. microcarpa	Spheroidal	50(53.05±0.8)55	Pantoporate	3.5(4.5±0.2)5	2.5(2.6±0.07)3	1.73
M. nicaeensis	Spheroidal	62.5(67.08±1.6)72.5	Pantoporate	4.4(4.7±0.1)5	2.5(2.25±0.1)3	2.08
M. Verticellata	Spheroidal	67.5(83.75±6.5)120	Pantoporate	3.5(4.2±0.2)5	2.5(2.6±0.09)3	1.6
M. parviflora	Spheroidal	50(64.3±1.6)72.5	Pantoporate	2.5(5.3±0.6)7.5	2.5(2.75±0.09)3	1.92

Table 2: Continued

Taxa investigated	I. d. from apex (µm) Min. (Mean±S.E) Ma.	Pore diameter (µm)Min. (Mean±S.E) Ma.	Sexine thickness (µm)	Nexine thickness (µm)	Intine thickness (µm)
Malva sylvestris	10(10.7±0.2)11.2	3(3±0)	0.5-1.25	6.25-7.5	0.5-1.25
M. neglecta	8(8.6±0.3)10	2(2.4±0.1)3	0.5	2.5	0.5
M. microcarpa	4(4.8±0.2)6	2(2±0)	0.5	2.5-3	0.5
M. nicaeensis	4.4(4.6±0.07)5	2.4(2.5±0.08)2.7	0.5	2.5-5	0.5
M. verticellata	7.5(9.6±0.8)12.5	2.5(2.5±0)	1.25	2.5	1.25
M. parviflora	7.5(9.4±0.8)12.5	2.5(2.5±0)	1.25	2.5	1.25

Min. Minimum; Ma. Maximum; S.E. Standeard Error; I.d. Interspinal distance

RESULTS

Pollen grains are 50-120 µm in diameter, apolar, pantoporate and spheroidal. The wall of a mature pollen grain is composed of unremitting intine and exine overspread with echini of variable height and width. Sexine is thinner than nexine or rarely thicker and is approximately as thick as intine (Table 2). Spines are variable in length with sharp pointed or blunt apices. Spine index ranges from 1.6 (*M. verticellata*) to 3.1 *(M. sylvestris*). (Table 2).

Key to the Species of *Malva* L:

1a : Spine height 8(9.7±0.4) 11.2µm, spine index 3.1…… *M. sylvestris*
1b : Spine height less than 8µm,Value of spine index less than 3.1..
……2
2a : Spine index 2.34……..…… *M. neglecta*
2b : Spine index less than 2.34……...…… 3
3a : Pore diameter 2 µm ……
M. microcarpa
3b . Pore diameter larger than 2 µm……4
4a . Interspinal distance 4.4 (4.6±0.07)5 µm……
M. nicaeensis
4b . Interspinal distance more than 5 µm……..5
5a. : spine index 1.6, maximum pollen diameter 120 µm……
M. verticellata
5b : Spine index 1.92, maximum pollen diameter 72.5 µm……..
M. parviflora

DISCUSSION

The data for palynomorphological studies is provided in Table 2. Representative pollen grains are illustrated in Figures 3-13 whereas palynological variants of taxonomic significance are represented graphically in Figures 1 and 2.

Pollen morphological studies revealed that the genus *Malva* is stenopalynous and all the pollen grains studied are apolar, pantoporate and more or less spheroidal. Being stenopalynous the genus is represented by a single morphological type thus making the demarcation of different species quite difficult based on pollen morphology. Pollen are usually classified on the basis of their shape, size, symmetry, polarity, apertural types and exine sculpturing [25].

Pollen size varies greatly among the different species as well as among different pollen of the same species; it ranges from 50 - 100 µm in the studied taxa (Table 2). Present findings disagree with the conclusions made by Bibi *et al*., [26] that pollen size is a reliable taxonomic tool to separate species. Walker [27] regarded pollen size as a tertiary character in phylogenetic studies which does not possess much phylogenetic significance. El Naggar [28] describes pollen size as a useful taxonomic tool at tribal level.

One of the most prominent and interesting features of malvaceous pollen is the echinations or prolongations of the exine into definite spines [28]. External marking of the pollen grains is described as the best, most constant and distinct character by which grains may be delimited at different taxonomic levels in case of stenopalynous families [29,30]. Distinctly dimorphic spines characterize the genus *Malva* i-e within the same grain both large pointed and bluntly rounded end spines are found. Spines dimorphism in the genus *Malva* has also been reported by Nair [31], Erdtman *et al*., [32], Culhane and Blackmore [16] and Perveen *et al*., [17]. Spines are quite variable in their height and width. Range of spine height measured for studied taxa of *Malva* is 2.5-11.2 µm. El Naggar [28] reported a range of 3-8 µm for spine height in selected *Malva* species whereas all the *Malva* species studied by Perveen *et al*., [17] had spine height of less than 7 µm. Perveen *et al*., [17] included all the studied taxa of *Malva* in *Malva parviflora* type and spine height of less than 7 µm was described as the distinguishing feature of this pollen type and the genus *Malva*. Present investigations showed that *M. sylvestris* can not be included in this type as the spine height of 8(9.7±0.4)11.2 µm has been recorded for the said taxa which does not fall within the prescribed range by Parveen *et al*., [17] and by. El Naggar [28] for the genus.

Spine index, the proportion between the height and width of the spine at its base defines the spine configuration and is used as a taxonomic characteristic to delimit different species of the genus *Malve* for the first time in the present work. Two main groups are identified. *M. microcarpa, M. parviflora and M. verticellata* are placed in Group-I with spine index of less than 2, whereas *M. sylvestris, M. nicaeensis and M. neglecta* are included in Group-II with value of spine index more than two.

Pollen exine varies considerably in the studied taxa and this variation in thickness is related to both nexine and sexine thus disagreeing with Christensen [15] that sexine is usually of constant thickness in Malvaceae whereas the nexine is variable. Present work supports El Naggar [28] that variation in exine

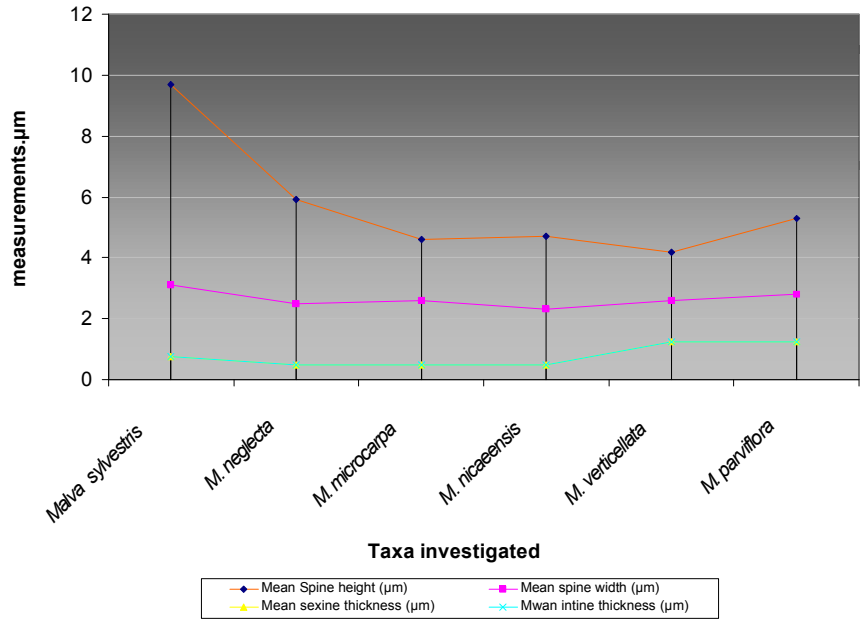

Fig. 1: Quantitative variation in different palynological features investigated in the genus *Malva* L.

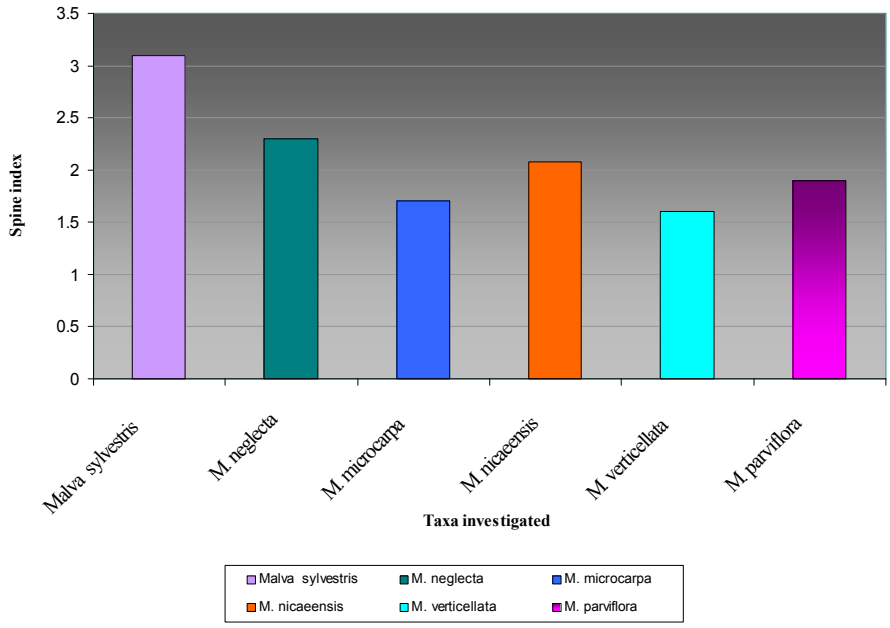

Fig. 2: Variation in Spine index in the genus *Malva* L.

thickness of Malvaceous pollen is related to both nexine and sexine thickness (Table 2, Fig. 1). Sexine is of the same thickness as intine in all studied taxa of the genus *Malva* (Fig. 1).

In conclusion, *Malva* is a stenopalynous genus with only slight disparities (e.g, variation in pollen size and spine index) in pollen features. The similarity in pollen shape, exine structure and ornamentations, as well as inconsistency of the various parameters investigated at interspecific level makes it hard to establish taxonomic boundaries with certainty but these features are significant enough to supplement the traditional morphology based description.

Fig. 3

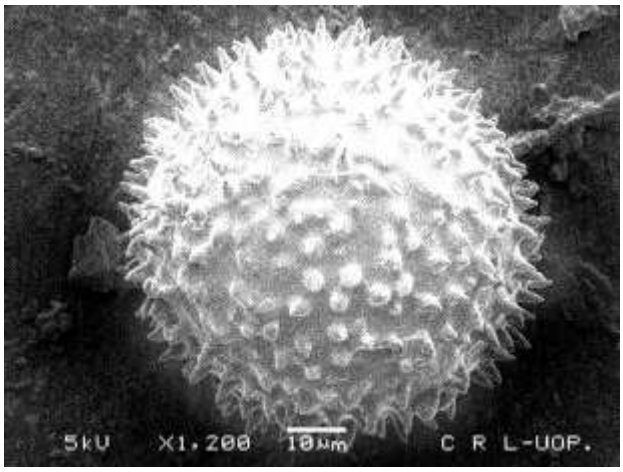

Fig. 6

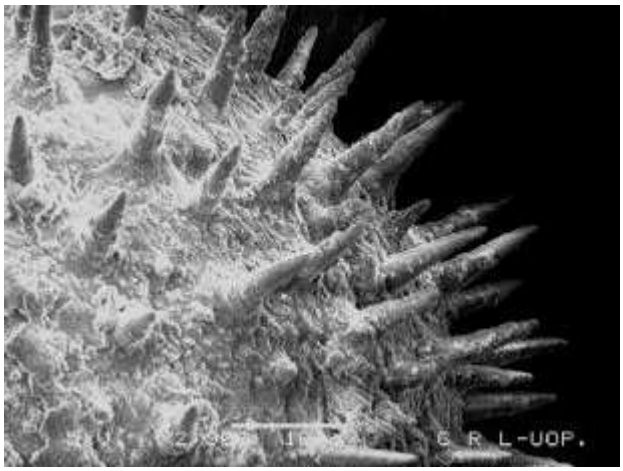

Fig. 4

Fig. 7

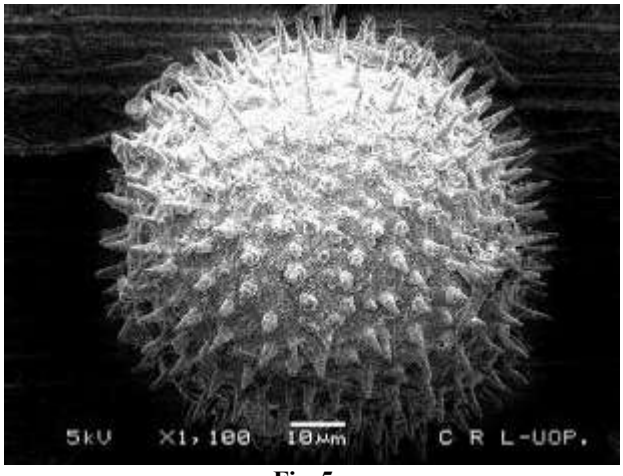

Fig. 5

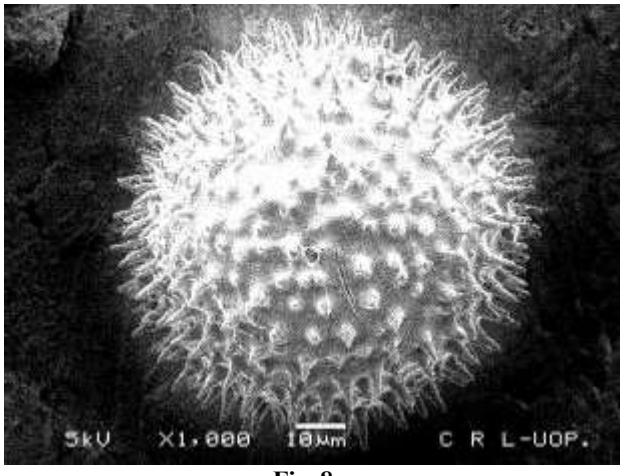

Fig. 8

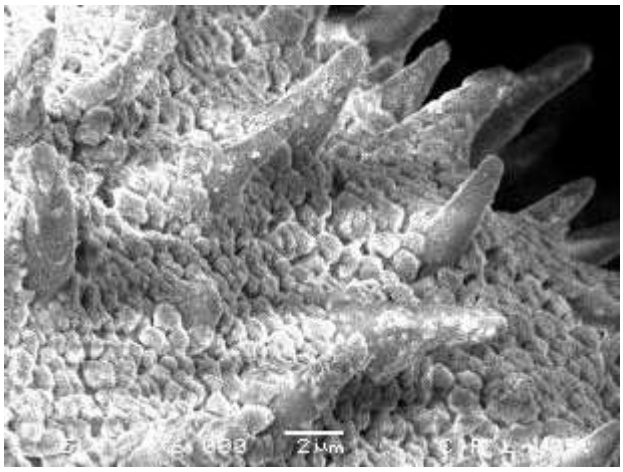

Fig. 9

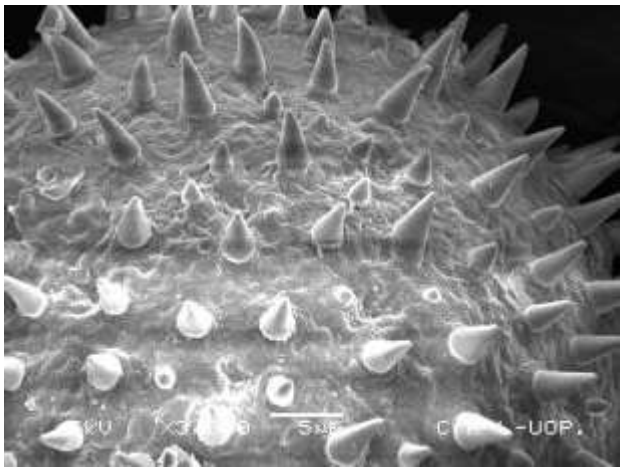

Fig. 11

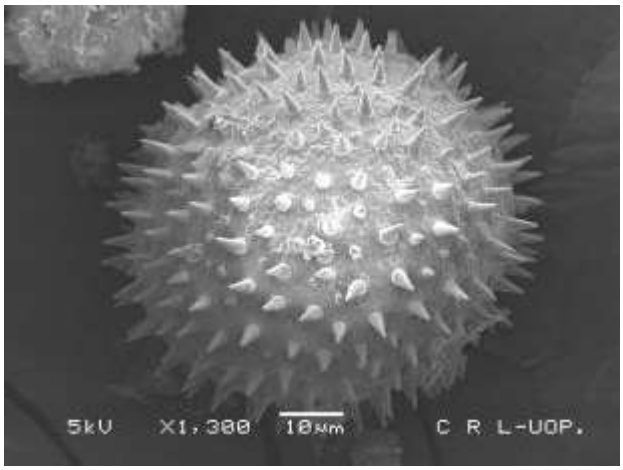

Fig. 10

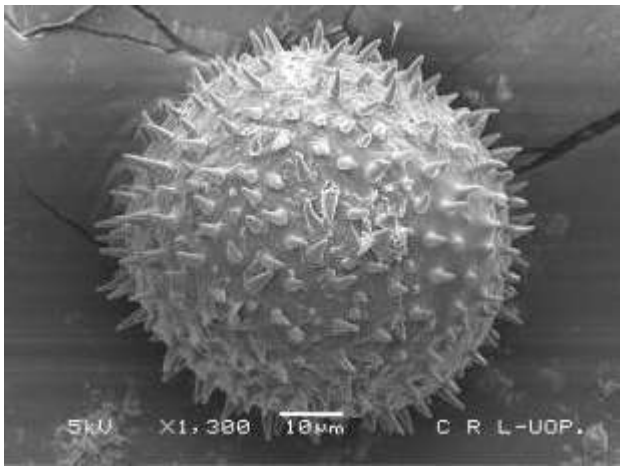

Fig. 12

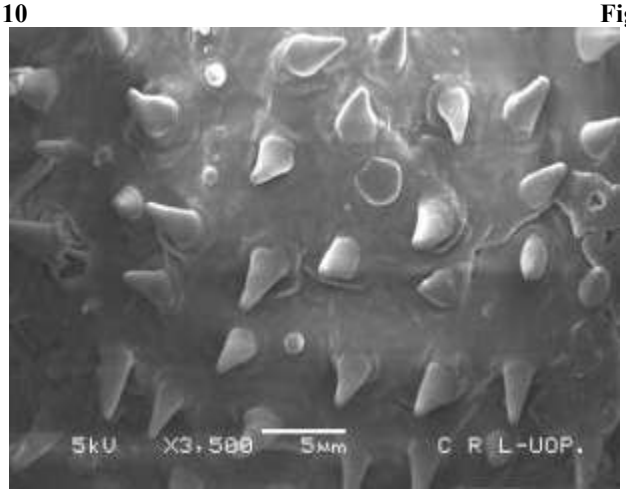

Fig. 13

Figs. 3-13: SEM micrographs of pollen grains of *Malva* L.
Figs.3,4. Exine pattern. *M. sylvestris*, Fig. 5. Pollen grain. *M. neglecta*, Fig. 6. Pollen grain. *M. microcarpa*, Fig. 7. Exine pattern. *M. microcarpa* , Fig. 8. Pollen grain. *M. nicaeensis*, Fig. 9. Exine pattern. *M. nicaeensis* , Fig. 10. Pollen grain. *M. verticellata*, Fig. 11. Exine pattern. *M. verticellata,* Fig. 12. Pollen grain *M. parviflora*, Fig. 13. Exine pattern. *M. parviflora*.

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REFERENCES

1. Abedin, S., 1979. No. 130. Malvaceae. In: E. Nasir and S.I. Ali, (eds). Flora of West Pakistan. University of Karachi, Pakistan, 130: 1-107.
2. Ray, M.F., 1995. Systematics of *Lavatera* and *Malva* (*Malvaceae, Malveae*) - a new perspective. Pl. Syst. Evol., 198: 29-53.
3. Bates, D.M. and O.J. Blanchard Jr., 1970. Chromosome numbers in the Malvales. II. New or otherwise noteworthy counts relevant to classification in the Malvaceae, tribe Malveae. American J. Botany, 57: 927-934.
4. Fryxell, P.A., 1997. The American genera of Malvaceae-II. Brittonia, 49: 204-269.
5. Bates, D.M., 1968. Generic relationships in the Malvaceae, tribe Malveae. Gentes Herbarum, 10: 117-135.
6. García, P.E., P. Schönswetter, J.F. Aguilar, G.N. Feliner and G.M. Schneeweiss, 2009. Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae). Molecular Phylogenetics and Evolution, 50(2): 226-239.
7. Lindley, J., 1830-1840. The genera and species of *Orchidaceous* plants. Ridgways London.
8. Mohl, H., 1835. Sur la Structure et les formes des graines de pollen. Ann. Sci. Nat. Ser., 2(3): 148-346.
9. Fritzsche, C.J., 1832. Beitrage zur kenntniss des pollen. Berlin., pp: 48.
10. Fischer, H., 1890. Beitrage zur vergleichende Morphologie der pollen-korenen , kern's Verlag, Bresula.
11. Selling, O.H., 1946-47. Studies in Hawaiian Pollen Statistics, Part I and II. Bishop Museum Publ. Honolulu, Hawaii.
12. Cranwell, L.M., 1952. New Zealand pollen studies. The monocotyledons. Bull. Auck. Inst. Mus., 3: 1-91.
13. Erdtman, G., 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist and Wiksell, Stockholm, pp: 539.
14. Erdtman, G., 1957. Pollen and Spore Morphology. Plant Taxonomy. Gymnospermae, Pteridophyta, Bryophyta. Almquist and Wiksell, Stockholm, pp: 151.
15. Christensen, P.B., 1986. Pollen morphological studies in the Malvaceae. Grana, 25: 95-117.
16. Culhane, K.J. and S. Blackmore, 1988. Malvaceae. In: W. Punt, S. Blackmore and G.C.S. Clarke, (eds), The North West European Pollen Flora, 41: 45-79.
17. Perveen, A., S. Siddiqui, A. Fatima and M. Qaiser, 1994. Pollen flora of Pakistan- 1. Malvaceae. Pakistan J. Bot., 26(2): 421-440.
18. Siddiqui, S., K.M. Khan and S. Abedin, 1982. Pollen morphology of *Lavatera* from Pakistan. J.Pharm. Kar. Univ., 1: 67-70.
19. Siddiqui, S., K.M. Khan and S. Abedin, 1984. Pollen morphology of *Abutilon* Mill., from Pakistan. J. Pharm. Kar. Univ., 2: 105-119.
20. Perveen, A. and M. Qaiser, 2007. Pollen Flora of Pakistan - Malvaceae - Grewioideae - LII. Pak. J. Bot., 39(1): 1-7.
21. Ahmad, M., M.A. Khan, A. Hassan, M. Zafar and S. Sultana, 2008. Chemotaxonomic standardization of herbal drugs Milk Thistle and Globe Thistle. Asian J. Chem., 20(6): 4443-4459.
22. Erdtman, G., 1969. Handbook of Palynology - An Introduction to the Study of Polllen Grains and Spores. Munksgaard, Copenhagen, pp: 486.
23. Moore, P.D., J.A. Webb and M.E. Collinson, 1991. Pollen analysis. Blackwell Scientific Publication. London, pp: 216.
24. Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas, 2007. Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol., 143(1-2): 1-81.
25. Perveen, A., 1993. A preliminary study of the pollen flora of Karachi.Ph.D. Thesis. Department of Botany. University of Karach. Karachi.
26. Bibi, N., M. Hussain and N. Akhtar, 2008. Palynological studies of some cultivated species of genus *Hibiscus* from North west Frontier Province (N.W.F.P.) of Pakistan Pak. J. Bot., 40(4): 1561-1569.
27. Walker, J.W., 1976. Evolutionary significance of the exine in the pollen of primitive angiosperms. In: I.K. Ferguson and J. Muller (eds), The evolutionary significance of Exine. Acadamic Press, London, pp: 251-308.
28. El Naggar, S.M.I., 2003. Pollen morphology of Egypyian Malvaceae: an assessment of taxonomic value. Turk. J. Bot., 28: 227-240.
29. Pope, M.A., 1925. Pollen morphology as an index to plant relationship. I. Morphology of pollen. Botanical Gazette, 80(1): 63-73.
30. Nair, P.K.K. and M. Sharma, 1965. Pollen morphology Liliaceae. J. Palynol., 1: 38-61.
31. Nair, P.K.K., 1958. Dimorphic spines in the pollen of *Malva parviflora*. J. Sci. Indust, 17: 35-36.
32. Erdtman, G., B. Berglund and J. Praglowski, 1961. An introduction to Scandinavian pollen flora. Grana Palynol., 2: 3-92.