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Foliar Epidermal Anatomy and Pollen Morphology of the Genera *Alcea* and *Althaea* (Malvaceae) from Pakistan

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ABSTRACT

Foliar epidermal anatomy and palynology of the four species belonging to two genera, *Alcea* L. and *Althaea* L. of family Malvaceae from Pakistan were studied and compared in detail both by light microscopy (LM) and scanning electron microscopy (SEM). Leaves were amphistomatic and amphitrichomic with higher frequency of both stomata and trichomes on the abaxial surface. Three main types of glandular and eglandular trichomes were observed. *Alcea rosea* and *A. lavateraeflora* could be delimited by the characteristic features of their stellate trichomes. Palynological studies revealed the presence of apolar, pantoporate and echinate pollen. Character of spine index was used for the first time to describe Malvaceous pollen and is found of significant taxonomic importance. Artificial keys are given for all studied taxa based on foliar epidermal anatomical and palynomorphological features. © 2010 Friends Science Publishers

Key Words: Foliar anatomy; Palynology; Malvaceae; *Alcea; Althaea*

INTRODUCTION

Both *Althaea* and *Alcea* are closely related genera and have been fused together in the past, but up to date opinion regards the two genera as clearly distinct genera (Abedin, 1979). Bates and Blanchard Jr. (1970) put *Alcea* (as *Althaea*) in the Malva alliance of the tribe Malvineae. *Alcea* L. is a genus of approximately 60 species primarily of East Mediterranean region (Abedin, 1979). Mostly erect annual, biennial, or perennial herbs, stellate pubescent to glabrate (Naqshi *et al*., 1988). The name is derived either from *alce* "remedy" in reference to its therapeutic value, or from *alke* "strength", because of its vigorous growth (Fryxell, 1997). The genus has an intricate taxonomy (Zohary, 1963). The difficulties in delimitations of *Alcea* species are primarily, because of very small number of diagnostic characters (Pakravan, 2008). *Althaea* L. is a small genus of annual or perennial herbs with about a dozen species distributed in Asia, Africa and Europe (Abedin, 1979). In Pakistan it is represented by 3 species (Abedin, 1979). The name is derived from a Greek word *altho* ("to heal") in reference to its medicinal importance (Fryxell, 1997).

The Foliar epidermis is one of the most significant taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis (Bhatia, 1984; Jones, 1986). Although the epidermal anatomy has been described in the leaves of a number of Malvaceae species (Inamdar & Chohan, 1969; Ramayya & Rao, 1976; Rao & Ramayya, 1977; Adedeji & Dloh, 2004; Celka *et al*., 2006) but surprisingly no comprehensive report exists on the quantitative characteristics of epidermal structure in *Alcea, Althaea* and foliar epidermal studies are totally missing from the taxonomic literature of Pakistan therefore one of the objectives of this study was to fill in this gap in our knowledge.

Significance of pollen morphology in plant systematics has been stressed by a number of workers, especially by Lindley (1830-1840), Fischer (1890) and Erdtman (1952) Pollen morphology of the family Malvaceae has been investigated by Master (1874-1875), Pope (1925), Erdtman (1952), Fryxell and Hashmi (1971), Perveen *et al.* (1994), El Naggar (2003) and Lakshmi (2003). In Pakistan the pollen morphology of few genera of the Malvaceae has been investigated by Siddiqui *et al*. (1984) using light microscope but Perveen *et al.* (1994) and Perveen and Qaiser (2007) 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. They reported the pollen diameter, pore diameter and spine length for each species. Besides the above mentioned characters, spine width, spine index and measurements of different layers of pollen wall are also recorded to give more comprehensive and clear picture of the pollen grain morphology of the genera concerned.

The level of confidence in taxonomic units supported by different kinds of data is likely to be much higher than

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for those supported by only one kind (Dayrat, 2005). Thus main objective of the study was to characterize the Malvaceous taxa by using multiple and complementary perspectives to supplement the traditional morphology based description with additional markers (foliar epidermal anatomy & palynology). Objective of the palynological studies was to provide additional knowledge about the pollen morphology of the taxa already investigated by the above mentioned workers as well as to include additional taxa, which were not considered in the studies of previous workers. Character of spine index was used for the first time to characterize pollen of the taxa under investigation.

MATERIALS AND METHODS

Foliar epidermal analysis: Dried leaves of representative specimens of genus *Althaea* and *Alcea* in Herbarium of Quaid-e-Azam University Islamabad Pakistan , listed in Table I were used for anatomical studies. Dried leaves were placed in boiling water for few minutes to soften until they became unfolded and were ready for epidermal scrapping. Leaf samples were prepared according to the modified method of Cotton (1974). The leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath (Model, Memmert-91126-FRG, Germany) for about 30 to 40 min. Lactic acid softens the leaf due to which it was possible to scrap the leaf surface with sharp scalpel. Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88% Lactic acid. Both qualitative and quantitative micromorphological characteristics of foliar epidermis were observed using LM. Microhistological photographs of both surfaces were taken by Nikon (FX-35) camera equipped light microscope. Basic terminology used in trichome classification and description is that suggested by Harris and Harris (2001). However simple self explanatory terms are added to identify the specific types of trichomes.

Palynological studies: For these studies the glycerin jelly was prepared according to the modified method of Ahmad *et al.* (2008). A 500 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 $1/8^{th}$ volume of glycerin jelly. It was stirred till uniform pink color appeared. Jelly was stabilized at room temperature.

Specimens used for foliar epidermal analysis were also used as a source for polliniferous material. For processing of pollen for light and scanning microscopy, the slides were prepared by the modified procedure of Erdtman (1952 & 1969). 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 (1952), Moore *et al.* (1991) and Punt *et al*. (2007).

RESULTS AND DISCUSSION

Foliar epidermal analysis: The data for quantitative micromorphological features of foliar epidermis of *Alcea* and *althaea*. is presented in Table II. The Foliar epidermis is one of the most significant taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis (Bhatia, 1984; Jones, 1986). Scotland *et al.* (2003) hold the opinion that rigorous and critical anatomical studied of fewer morphological characters in the context of molecular phylogenies is fruitful to integrate the strength of morphological data with those of sequence data (Hayat *et al*., 2009).

Epidermal cells in both species (*Alcea rosea* & *Alcea lavateraeflora*) of the genus *Alcea* were smooth walled with almost similar anatomical measurements. Leaves amphistomatic and amphitrichomic but generally the stomata and trichomes were more concentrated on the abaxial surface. Anisocytic and diacytic stomata were found frequently on both leaf surfaces of *A. lavateraeflora*, whereas in *Alcea rosea* the most frequent were the diacytic ones. Leaves are thin but appeared thick due to the presence of a thick cover of eglandular trichomes. Solereder (1908), Metcalfe and Chalk (1950), Ramayya (1962), Inamdar (1967), Ramayya and Rao (1976), Rao and Ramayya (1977), Yan-Ming and Ru-Wen (1993) and Celka *et al.* (2006) emphasized the taxonomic implication of trichomes. Inamdar *et al.* (1983) described stellate trichomes as the common type of foliar epidermal appendages in *Alcea rosea* (described as *Althaea rosea*). *Alcea rosea* and *A. lavateraeflora* in the current study could be delimited by the characteristic features of their stellate trichomes. Stellate trichomes in *A. lavateraeflora* were dimorphic, one with thicker and longer ray cells and other with shorter and thinner ray cells, whereas in *A. rosea* the ray cells were comparatively longer and thinner (Fig. 1). The maximum length of single ray cell observed in *A. rosea* was up to 700 µm whereas in *A. lavateraeflora* the observed length was

Table I: List of Specimen used in foliar epidermal anatomoical and palynological investigations

Taxa investigated	Locality	District	Collector Name	Acc. No.
Alcea rosea L.	Fort Sandeman	Zhob	M.Shaukat and Nisar	24630 (ISL!)
	Govt College	Quetta	Manzoor and Maqsood	72349 (ISL!)
A. lavvateraeflora L.	Munnawar Hill	Chitral	Muqarrab Shah and Dilawar Ayaz.	33716 (ISL!)
	Muzaffarabad	Muzaffarabad	Jan Mohammad	21850 (ISL!)
Altheae ludwigii L.	Hanna	Quetta	Manzoor and Maqsood	103350(ISL!)
	Bandazai	Zhob	Manzoor Hussain and Muhammad Arif	82192(ISL!)
A. officinalis L.	Muzaffarabad	Muzaffarabad	Shahzad Iqbal and Nisar	82196 (ISL!)
	Parachinar	Parachinar	Bashir Ahmad and Javaid	11406 (ISL!)

Fig. 1: Peltate glands (type-II) on abaxial surface of *Alcea rosea* (200X)

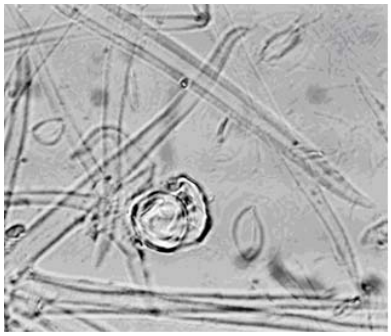


Fig. 2: Epidermal cells with undulating walls on abaxial surface of *Althaea ludwigii* (400X)

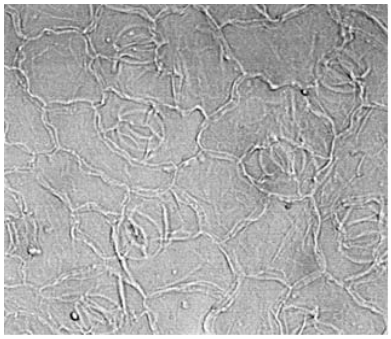


Fig. 3: Seven-rayed stellate trichomes on adaxial surface of *Althaea ludwigii* (200X)

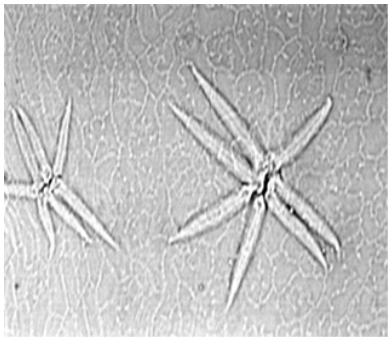
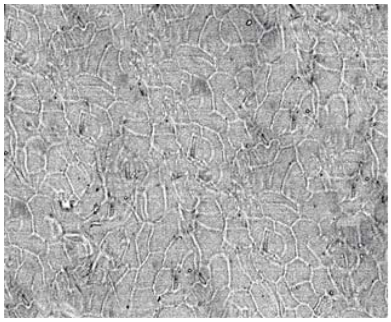


Fig. 4: Smooth walled epidermal cells on adaxial surface of *Althaea officinalis* (200×)



110 (192.14±32.87) 300 µm (Table II). Ramayya and Rao (1976) described the stellate trichomes *of A. rosea* as "tufted hair" as their rays are raised slightly above the epidermis but they were of the view that ontogeny is essentially similar to that of stellate hair. Both tufted and stellate hair originates from single protodermal initial.

Epidermal cells in *Althaea officinalis* were smooth-walled (Fig. 4) but that of *Althaea ludwigii* were with undulating walls (Fig. 2). *A. ludwigii* was also distinct in having a characteristic arrangement of ray cells of its stellate trichomes (Fig. 3). Size of the epidermal cells is of taxonomic significance and can be helpful in delimiting *Althaea* and *Alcea*, the closely related genera. Epidermal cells as observed in *Althaea* species were comparatively longer 50 (67±5.38) 80 x µm than those in *Alcea* species where maximum observed length was 55 µm in *A. lavateraeflora* (Table II). Three main types of trichomes were observed, eglandular stellate trichomes, glandular capitate trichome and peltate glands (Type-II) as described by Shaheen *et al.* (2009), while describing the diversity of foliar trichomes within the genus *Hibiscus* L. of family Malvaceae. Capitate trichomes were either with unicellular head or with multicellular and uniseriate head. Anatomical measurements for capitate trichomes were rather uniform and are of little taxonomic importance but stellate trichomes unlike capitate trichomes were found of significant taxonomic interest.

Key to the species of *Alcea*

1a: Stomata mostly diacytic, stellate trichomes monomorphic, peltate glands (Type-II) and capitate trichomes present ……*Alcea rosea*.

1b: Stomata diacytic and anisocytic stellate trichomes dimorphic, only capitate trichomes present……..*A. lavateraeflora*.

Key to the species of *Althaea*

1a: Epidermal cells irregular in shape with undulating walls, few capitate trichomes with unicellular head present…….. *A. ludwigii.*

1b: Epidermal cells rectangular and polygonal in outline, capitate trichomes with multicellular and uniseriate head……*A. officinalis*.

Fig. 5: SEM micrographs of pollen grains of *Alcea* L.
A. Pollen grain. *Alcea rosea*, B. Exine pattern. *A. rosea* and C. pollen grain *A. Lavateraflora*

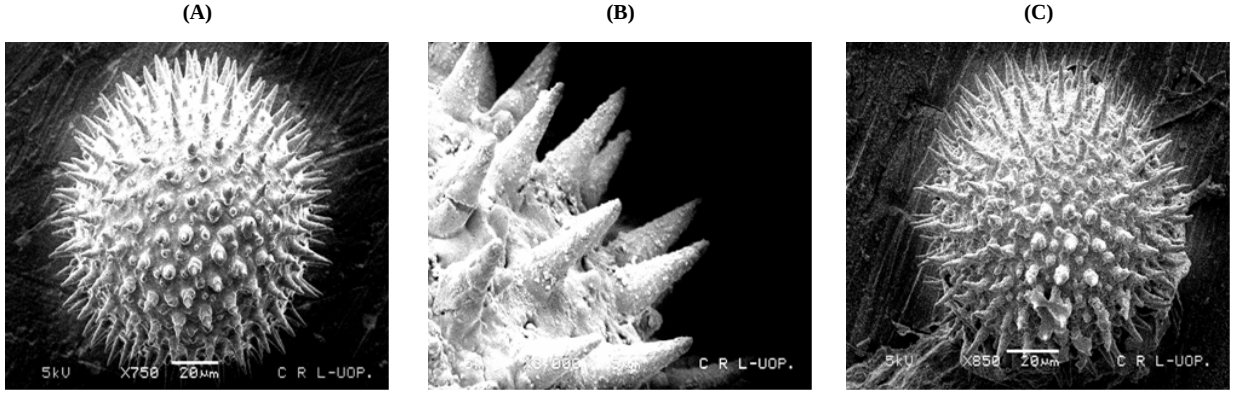
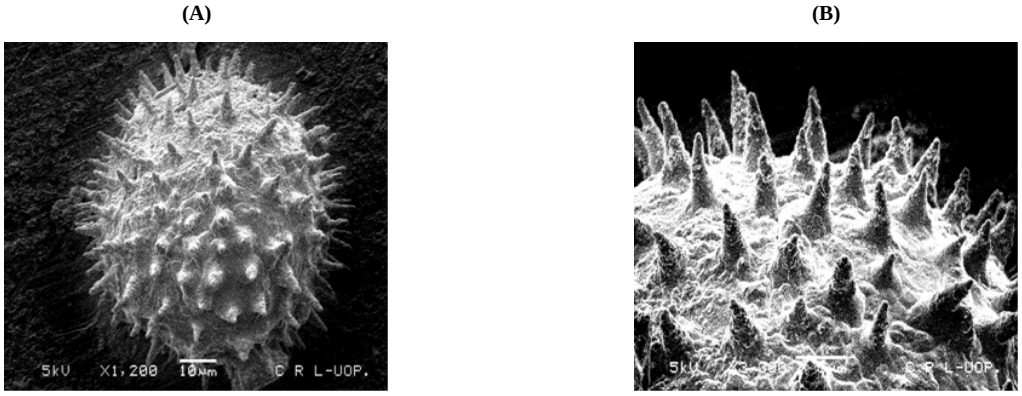


Fig. 6. SEM micrographs of pollen grains of *Althaea* L.
A. Pollen grain *Althaea ludwigii* and B. Exine pattern. *A. ludwigii*



Palynological studies: Detailed pollen morphological features of the investigated taxa are summarized in Table III and representative pollen grains are illustrated in Fig. 5-6. Pollen are usually classified on the basis of their shape, size, symmetry, polarity, apertural types and exine sculpturing (Perveen, 1993). Variations in pollen size, aperture and spine features as well as exine stratification are all of taxonomic significance of which the aperture character is considered to be of primary importance, whereas exine sculpturing secondary and the others as tertiary (Nair, 1965; Lakshmi, 2003).

Pollen grains were fairly large and data of pollen size showed wide range of variations among the genera and species as well as among different pollen of the same species; it ranged from 62.5-120 µm in studied taxa of *Althaea* and 90-162.5 µm in the genus *Alcea*, which is in contrast with the observations of El Naggar (2003) for Egyptian species. He recorded the maximum value of 90 µm for pollen size of *Alcea*, which is the smallest value for the said genus in the current studies. The smallest mean pollen size is recorded for *Althaea ludwigii* (37.5 µm), which is also quite different from that described by Perveen *et al.* (1994), they recorded the value of 68.2 µm for mean pollen diameter of of *A. ludwigii*. In general polymorphism in pollen size has been to be an index to chromosome numerical variations thus quite useful in cytopalynological studies (Lakshmi, 2003). Saad (1960) also held the opinion that there is a correlation between pollen size and chromosome number. Christensen (1986) is in agreement with Saad and believes small size to be relatively underived in comparison to large size in terms of pollen Evolution in family Malvaceae. The maximum value for pollen size is obtained for *Alcea rosea* that is 125(143.2±4.2)162.5 µm.

Pore diameter and the appearance of pores on the inner surface of the acetolysed pollen are also of taxonomic significance (El Naggar, 2003). Pore diameter in the studied taxa was in the range of 2-4.5 µm and serves as a distinguishing feature at infrageneric level in the genus *Alcea* as *Alcea rosea* could be delimited from *A. lavateraeflora* on the basis of pore diameter (Table III).

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 (Pope, 1925; Nair & Sharma, 1965). One of the most prominent and interesting features of malvaceous pollen is the echinations or prolongations of the exine into definite spines (El Naggar 2003). Malvaceae is fairly advanced because of the echinate sculpturing and

Table II: Quantitative data for foliar trichomes in *Alcea* L. *and Althaea* L.

Taxa investigated	Foliar trichomes			
	Peltate H. x W. µm Min. (Mean±S.E) Ma.	Capitate H. x W. µm Min. (Mean±S.E) Ma.		Stellate L x W µm Min. (Mean±S.E) Ma.
	Type-II		N.r.c	S.r.c.
Alcea lavateraeflora	-	35 (35+/-0) x25 (25+/-0)	5-10 rarely 3	110 (192.14+/- 32.87)300 x 10 (30.71 +/- 7.35) 60
A. .rosea	15 (17.86+/- 1.01)20 x 20 (22.5 +/-0.94)25	35 (38.33+/- 2.108) 45 x 22.5 (25.92 +/- 1.08)30	5-10	150 (387.5 +/- 75.76)700 x 5 (15 +/- 2.18) 20
Althaea ludwigii	-	15 (25 +/- 2.74)30 x 20 (22.5 +/-0.94)25	3-7	75(160.83 +/- 23.64) 225 x 20 (22.14 +/- 1.01015)25
A. officinalis	-	30 (33.33 +/- 1.67)40 x 20 (20.83 +/- 0.83)25	4-10	275 (379.17 +/- 43.50)550 x 15 (21.66667 +/- 1.66667)25

	Ordinary Epidermal cells L. x W. µm Min. (Mean±S.E) Ma.		Stomata L. x W. µm Min. (Mean±S.E) Ma.		L. Stomatal opening µm Min. (Mean±S.E) Ma.		Stomatal Complex L. x W. µm Min. (Mean±S.E) Ma.	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Alcea rosea	30 (40 ± 2.89) 50 x 20 (22 ±1) 25	30 (36 ± 2.91) 45 x 15 (322.3±2.11) 30	20 (23 ± 1.22) 25 x 15 (15.42 ±0.27) 16.5	25 (25± 0) x 15(15± 0)	10 (10.9 ±0.56)12.5	10(12.5±1.12) 15	50 (61 ± 4.58) 70 x 35 (39.17 ± 1.62) 45	40(48.33 ±3.3)60 x 40 (42.4 ±1.12) 45
A. lavateraeflora	45 (49 ± 1.87) 55 x 15 (21.67 ± 2.79) 30	45 (49 ±1.87) 55 x 10 (20 ± 2.89) 30	25 (25 ±-0) x 15(17 ± 1.22) 20	25 (25± 0) x 15 (15± 0)	15 (15± 0)	20 (20± 0)	35 (44 ± 4.58) 60 x 40 (57.57 ± 5.06) 75	30 (41.67 ±3.57) 55 x 45 (56.67 ± 4.59) 70
Althaea ludwigii	50 (64.17 ± 3.74) 75 x 25 (26.67± 1.05) 30,	50 (67 ± 5.38) 80 x 25 (26.67 ± 1.05) 30	20 (20± 0) x 15 (15± 0)	20 (20± 0) x 15 (15± 0)	15 (16.3 ±0.54) 17.5	15 (15± 0)	60 (74 ± 4.30) 85 x 35 (44.83± 2.69) 50	50 (64 ±4.30) 75 x 40 (52.6 ± 3.57) 60
A. officinalis	50 (62 ± 4.83046) 75 x 20 (23.33 ±1.05) 25	50 (60 ±5.24) 75 x 20 (22.7 ± 1.74) 28.5	25 (25± 0) x 15 (15± 0)	(25±0) x 15 (15± 0)	10 (13 ±1.22) 15	15 (15± 0)	50 (66.67 ± 4.41) 80 x 30 (38.33 ± 2.11) 45	60 (71.43± 3.57) 85 x 35 (42.17 ±2.65)50

L. length; W. width; Min. minimum; Ma. Maximum; S.E. standard error; N.r.c. number of ray cells; L. sto. opening length of stomatal opening

Table III: Qualitative and quantitative palynological features in *Alcea* L. and *Althaea* 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
Alcea rosea	globose	125(143.2±4.2)162.5	pantoporate	10(11.2±0.3)12.5	4.5(4.9±0.07)5	2.28
A. lavateraflora	spheroidal	90(97.8±2)107.5	pantoporate	10(11.25±0.5)12.5	2.5(2.8±0.1)3	4.01
Althaea ludwigii	spheroidal	62.5(73.4±2.2)80	pantoporate	5(6.75±0.5)8	2.5(2.6±0.06)2.8	2.59
A. officinalis	spheroidal	107(113±2.2)120	pantoporate	10(11.07±0.5)12.5	2.5(2.5±0)	4.6

	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)
Alcea rosea	10(13.4±1.2)17.5	4.2(4.2±0.05)4.5	1.25-2.5	5	2.5
A. lavateraflora	7.5(10.3±0.7)12.5	2(2.25±0.09)2.5	1.25	5	1.25
Althaea ludwigii	5.8(6.8±0.5)10	2.5(2.7±0.1)3.75	0.5-1.25	4.5-5	0.5-1.25
A. officinalis	7.5(10.5±0.5)12.5	2.5(2.5±0)	1.25	2.5-3.75	1.25-2.5

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

pantoporate character of the pollen grains (Perveen, 1993). 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 Malvaceous taxa for the first time in the present work. It was found quite significant to clearly differentiate all the studied taxa at infrageneric level (Table III).

Pollen exine varies considerably in studied taxa of Malvaceae and this variation in thickness is related to both nexine and sexine thus disagreeing with Christensen (1986) that sexine is usually of constant thickness in Malvaceae, whereas the nexine is variable. Present work supports El Naggar (2003) that variation in exine thickness of Malvaceous pollen is related to both nexine and sexine thickness (Table III).

Key to the species of *Alcea* L.

1a: Pollen diameter 125(143.2±4.2)162.5 µm, spine index 2.28……..…*A. rosea.*

1b: Pollen diameter 90(97.8±2)107.5 µm, spine index 4.01……..…..……*A. lavateraflora.*

Key to the species of *Althaea* L.

1a: Pollen diameter 62.5(73.4±2.2)80 µm, spine index 2.59…..……..……*A. ludwigii.*

1b: Pollen diameter 107(113±2.2)120 µm, spine index 4.6…...…….*A. officinalis.*

It is concluded that both anatomical and palynological markers can be used as important supportive taxonomic tool to delimit the Malvaceous taxa at different taxonomic levels.

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