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Pollen Morphology of *Malvaceae* and its taxonomic significance in Yemen

Abstract

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The pollen morphology of 20 species of *Malvaceae* growing in Yemen was investigated by light (LM) and scanning electron microscope (SEM). The studied taxa belong to 9 genera and three different tribes. These taxa are: *Abelmoschus esculentus*, *Hibiscus trionum*, *H. micranthus*, *H. deflersii*, *H. palmatus*, *H. vitifolius*, *H. rosa-sinensis*, *H. ovalifolius*, *Gossypium hirsutum*, *Thespesia populnea* (L.) Solander ex Correa and *Senra incana* (Cav.) DC. (*Hibiscieae*); *Malva parviflora* and *Alcea rosea* (*Malveae*); *Abutilon fruticosum*, *A. figarianum*, *A. bidentatum*, *A. pannosum*, *Sida acuta*, *S. alba* and *S. ovata* (*Abutileae*). Pollen shape, size, aperture, exine structure and sculpturing as well as the spine characters proved that they are of high taxonomic value. Pollen characters with some other morphological characters are discussed in the light of the recent classification of the family in Yemen.

Key words: *Malvaceae*, Morphology, Yemen.

Introduction

Malvaceae Juss. (s. str.) is a large family of herbs, shrubs and trees; comprising about 110 genera and 2000 species. It is a globally distributed family with primary concentrations of genera in the tropical and subtropical regions (Hutchinson 1967; Fryxell 1975, 1988 & 1998; Heywood 1993; La Duke & Doeby 1995; Mabberley 1997). Due to the high economic value of many taxa of Malvaceae (*Gossypium*, *Hibiscus*, *Abelmoschus* and *Malva),* several studies of different perspective have been carried out, such as those are: Edlin (1935), Bates and Blanchard (1970), Krebs (1994a, 1994b), Ray (1995 & 1998), Hosni and Araffa (1999), El Naggar (1996, 2001 & 2004), Pefell & al. (2002).

The family concept as well as the classification of *Malvaceae*, particularly at the tribal and generic level, is frequently disputed (Chaudhari & al. 1965; Bates 1968; Judd & Manchester 1997; Alverson & al. 1998 & 1999; Bayer & al. 1999; Andreasen & Baldwin 2003).

Palynological studies have been frequently used in the taxonomy and phylogeny of several taxa (Ferguson 1986; Hilsebeck 1990; El Naggar 2002). Pollen morphology of *Malvaceae* or some of its representatives has been included in many studies: for example: Surova and Velieve (1984), Christensen (1986a, 1986b), Culhame and Blackmore (1988) and El Naggar (2004). The pollen of *Malvaceae* is characterized by large size, spherical shape, colporate or porporlate aperture and echinate sculpture. In spite of this, they vary greatly and their characters can be used in the classification and phylogeny within the family (Christensen 1986 a, 1986b; Culhame & Blackmore 1988; El Naggar 2004).

The *Malvaceae* s. str. are a well-represented in Yemen. It is represented by about 60 species belonging to 15 genera (Wood 1997; Al Hubashi & Muller-Hoherstein 1984).

The present study investigates the pollen morphology of some taxa of *Malvaceae* growing wild or cultivated in Yemen and the use of the palynological characters in the classification of the family as well as solving some taxonomic problems.

Material and Methods

This study is mainly based on the pollen of 20 species of wild and cultivated representatives of *Malvaceae* in Yemen. Samples of pollen of each species were collected from living material or herbarium specimens (Table 1). Plant species were identified according to Täckholm (1974); Al Hubashi & Müller-Hohenstein (1984); Wood (1997); Boulos (2000). Voucher specimens for each studied species are kept in the herbarium of Taiz University (HTU) and the herbarium of Botany Department Faculty of Science Assiut University (ASTU).

Material for LM was acetolysed according to Erdtman (1960). Before acetolysis the pollen were boiled in 10% KOH for about 8 min, causing the apertures to be open or bulge and making them easer to study (Reitsma 1969). The acetolysed pollen was mounted in glycerin jelly onto glass slides. The pollen was examined and photographed using Mic 2880 And Mic 2885 Euromex Polarising Microscope, Euromex microscope Lted. This study was carried out in the laboratory of Biology Department, Faculty of Science, Taiz University, Taiz, Republic of Yemen during 2005 – 2006.

Material for SEM was prepared by mounting acetolysed pollen onto clean stubs covered with double-sided cellotape. Untreated dry pollen was mounted directly onto the cleaned stub surface. Some acetolysed pollen was mounted onto clean stubs and a clean cover slip was pressed down on to the pollen grains to fracture them, so that the wall intra-structure could be studied. Other pollens were immersed in liquid nitrogen to freeze them, and then fractured with a hammer. All stubs were coated with gold using a JEOL JFC 1100 E ion sputtering device. The pollen grains were then examined in a JEOL JSM 5400LV scanning electron microscope, operated at an accelerating voltage of 15 kV, at. the Electron Microscope Unit, Assiut University. Treated pollen grains generally increase in size, and therefore all measurements of pollen size, spine length and aperture size were taken from untreated dry pollen. . Terminology used in pollen characters follows: Erdtman 1952; Reitsma 1970 and Punt & al. 1994.

Table 1. Studied material with origin and collectors.

No.	Taxon	Collection
	Hibiscieae	
01.	*Abelmoschus esculentus* (L.) Moench.	Taiz, cultivated farm, 23.2.2006, EL Naggar, (THU, ASTU)
02	*Hibiscus trionum* L.	Taiz, Habeil Salman, 4.6.2005, El Naggar, (THU, ASTU)
03	*H. micranthus* L.	Taiz, Al Haboni, 10.6.2005, El Naggar, (THU, ASTU)
04	*H. deflersii* Schweinf.	Taiz, Al Haboni, 5.6.2005, El Naggar & Sawady, (THU, ASTU)
05	*H. palmatus* Forssk.	Taiz, Habeil Salman, 20.10.2005, El Naggar, (THU, ASTU)
06	*H. vitifolius* L.	Taiz, Habeil Salman, 20.10.2004, El Naggar, (THU, ASTU)
07	*H. rosa-sinensis* L.	Taiz, Public garden, 23.3.2005, El Naggar, (THU, ASTU)
08	*H. ovalifolius* (Forssk.) Vahl.	Naql El Ebal, on the road between Taiz and Aden, 16.12.2005, El Naggar, (THU, ASTU)
09	*Gossypium hirsutum* L.	Taiz, Al Hobani, 2.12.2005, El Naggar, Sawady, (THU, ASTU)
10	*Thespesia populnea* (L.) Solander ex Correa	Taiz, El Taawen Public Garden North of Taiz, 5.6.2005, El Naggar & Sawady, (THU, ASTU)
11	*Senra incana* (Cav.) DC.	El Anad junction, Wadi Bela, 24.2.2006, El Naggar, (THU, ASTU)
	Malveae	
12	*Malva parviflora* L.	Gebel Saber south of Taiz, 1.1.2005, El Naggar (THU, ASTU)
13	*Alcea rosea* L.	Taiz, Al Hobani, north of Taiz, 2.12.2005, El Naggar, Sawady (THU, ASTU)
	Abutilieae	
14	*Abutilon fruticosum* Guill. & Perr.	Taiz, Habeil Salman, 1.12 2005, El Naggar (THU, ASTU)
15	*A. figarianum* Webb	Taiz, Habeil Salman, , 22.11. 2004, El Naggar (THU, ASTU)
16	*A. bidentatum* A. Rich.	Taiz, Habeil Salman, 10.10 2004, El Naggar (THU, ASTU)
17	*A. pannosum* (G.Forst.) Schledt	Taiz, Habeil Salman, 10.10 2004, El Naggar (THU, ASTU)
18	*Sida acuta* Burm.	Taiz, Habeil Salman, 20.11.2004, El Naggar (THU, ASTU)
19	*S. alba* L.	Taiz, Habeil Salman, 20.11.2004, El Naggar (THU, ASTU)
20	*S. ovata* Forssk.	Taiz, Habeil Salman, 20.11.2004, El Naggar (THU, ASTU)

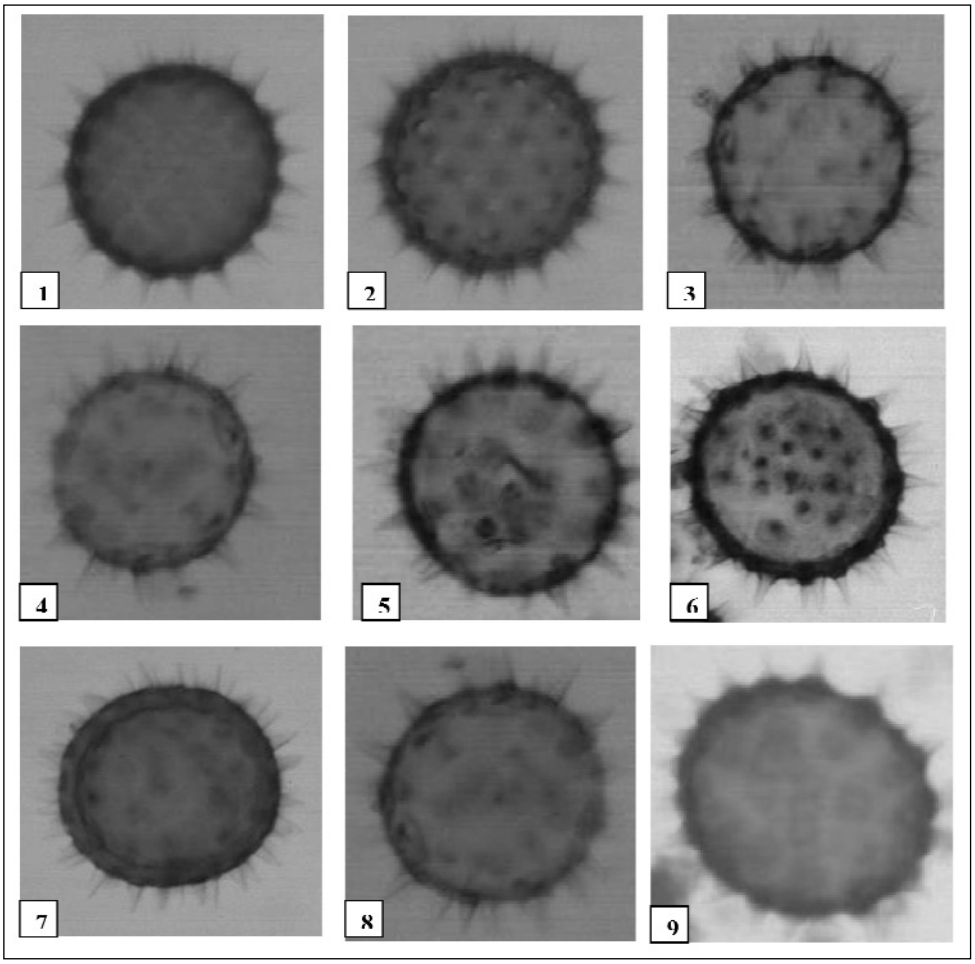
Results and Discussion

Pollen grains of *Malvaceae* are usually spheroid or globular in its outline, colporate or porate with echinate sculpture. Pollen exine always consists of sexine and nexine; the latter is usually thicker than the former (Christensen 1986, a). Spines are always evenly distributed over the surface of the grain and vary in their length, shape and density (Figs. 1-21).

In spite of the Malvaceae being a stenoplynous family (Erdtman 1952) there are high variations in pollen morphological characters such as: size, aperture, spine characters as well as exine stratification and sculpture, which can be used in the taxonomy of *Malvaceae* as follows.

Pollen size:

Pollen grains of the *Malvaceae* are considered to be among the largest among Angiosperms (Christensen 1986a). Pollen size varied greatly among the studied taxa: it ranges from 42 µm to 130 µm. These results are useful as a taxonomic character, particularly at tribal level: for example, it is about 130 µm in some studied taxa of tribe *Hibiscieae* (*Abelmoschus*

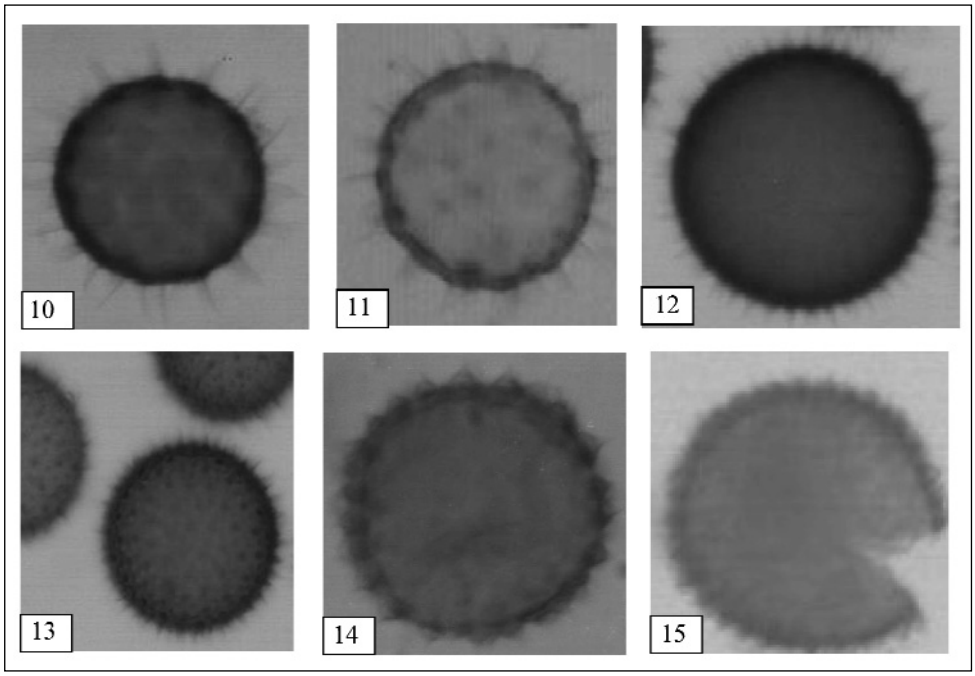


Figs. 1-9. Light micrographs of pollen grains of the studied taxa: **1,** *Abelmoschus esculentus*, × 280; **2,** *Hibescus trionum*, × 420; **3,** *H. micranthus*, × 500; **4,** *H. deflarsii*, ×490; **5,** *H. palmatus*, × 420; **6,** *H. vitifolius*, × 450; **7,** *H. rosa-sinensis*, × 390; **8,** *H. ovalifolius*, × 430; **9,** *Thespesia populnea* ×780.

esculentus), while it is about 42 µm in taxa of tribe *Abutilieae* (*Abutilon*) (Table 2; Figs. 1-15). S'aad (1960) reported that there is a correlation between pollen size and chromosome number. This fact was confirmed by Christensen (1986a) and El Naggar (2004). The present results agree with the above authors and emphasise the high taxonomic value of pollen size in the family. (Figs. 1-15; Table 2).

Aperture characters:

Apertures number is also found to be useful as a taxonomic character at tribal level. This number is 6-15 in all studied taxa of Abutilieae, 22 - 45 in studied taxa of Hibiscieae while it is numerous in all studied taxa of *Malveae*. The present results agree with those of S'aad



Figs 10-15. Light macrographs of pollen of the studied taxa: **10,** *Gossypium hirsutum*, × 660; **11,** *Senra incana*, × 800; **12,** *Malva parviflo*ra, × 800; **13,** *Alcea rosea*, × 320; **14,** *Abutilon fargianum*, × 960; **15,** *Sida acuta*,× 750.

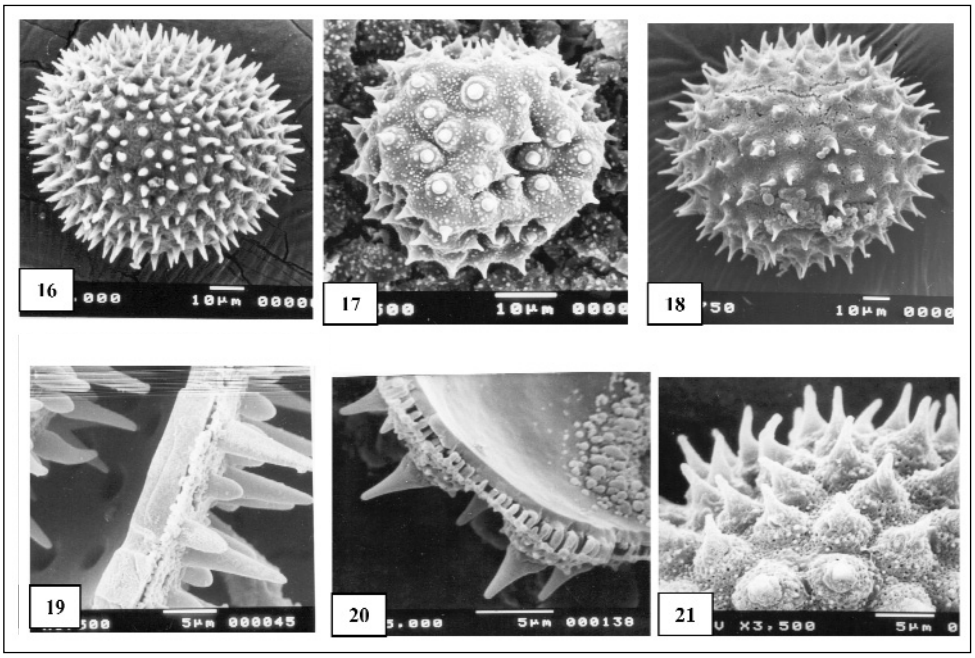
(1960); Erdtman (1962), Christensen (1986, a) and El Naggar (2004) in this respect.

b- Aperture shape: The present results revealed that aperture shape in the studied taxa is in two categories: pollen of Malveae is usually porate; while colpate or colporate in *Hibiscieae* and *Abutiliea* (Figs. 1-21; Table 2).

The present results revealed that the appearance of aperture from the inner surface of the acetolyzed pollen, has proved that it is a reliable taxonomic character. These apertures have a distinct costate ring surrounding the pore in *Abutilon, Hibiscus* and *Sida*, while they are without a ring as in *Malva* and *Alcea*. On the other hand, these pores are smooth and featureless in the inner side as in *Alcea rosea,* while they are verrucate in *Sida alba*, and *Abutilon pannosum* (Figs 19, 20). The present results confirm those of El Naggar (2004).

EXINE CHARACTERS:

Exine in *Malvaceae* usually consists of sexine and nexine. Exine stratification is of high taxonomic value in Malvaceae. Both sexine and nexine have variable thickness. (Table 2). El Naggar (2004) reported that sexine is variable in thickness in studied taxa, thus disagreeing with the conclusion of Christensen (1986b) who stated that sexine is usually of constant thickness in Malvaceae, while nexine is variable.The present results revealed that nexine is usually thicker than sexine in the tribe *Malveae*. Nexine in *Abutilieae* and *Hibiscieae* may be thinner or thicker or the same thickness as sexine. The variation of



Figs 16-21. SEM micrographs of the pollen of the studied taxa: **16,** *Malva parviflora* × 800; **17,** *Abutilon pannosum*, × 1200; **18,** *Gossypium hirsutum* × 600; **19,** Exine structure of *Alcea rosea* × 2800; **20,** Exine structure of *Abutilon pannosum*, × 4000; **21,** spine morphology of *Sida alba* × 2800.

exine thickness is obviously related to both nexine and sexine thickness not to nexine thickness as Christensen (1986a) stated. Columellae in *Malvaceae* are always similar, rod-shaped or with a broad lower part; they vary in length in the studied taxa, which reflects the difference in sexine thickness. However, columellae length differs in the same grain because the length of the columellae increases near or at the bases of spines and this increase is responsible for the cushion-shape in the pollen spines of *Sida*, *Abutilon*, *Abelmoschus* and *Gossypium*. Pollen exine in Malvaceae is usually tectate, which is more or less in the same thickness among all studied taxa. The tectum is the upper layer of the sexine. This surface pattern of the tectum varies from microreticulate or punctate, as in *Alcea rosea, Abutilon pannosum* and *Gossypium hirsutum* to rugulate, granulate or verrucate in other studied taxa.

Spines (echinae): Pollen grains of the Malvaceae are characterized by having spiny tecta. The spines show reliable variation in size, shape and surface distribution. These variations are of taxonomic value at different taxonomic levels, because they may occur not only between genera but also between species of the same genus. Spine length may be similar on a pollen grain (monomorphic) as in most species of tribe *Hibiscieae*, *Abutilieae* and or it may vary on the same grain giving a dimorphic pattern as in species of tribe Malveae. The present results show that the longest spines are recorded in *Abelmoschus esculentus* (15 µm) and the smallest ones are in *Malva parviflora* (3 µm) (Table 2, Figs. 1-21).

Table 2. Palynological data of the studied taxa.

No.	Taxon	Pollen size (µm)	Aperture characters			Spine characters		Exine character	
			No.	Arrangement	Shape	Base	Length (µm)	Sexine (µm)	Nexine (µm)
	Hibiscieae								
01	*Abelmoschus esculentus* (L.) Moench	130	22-45	Panto	porate	raised	15	2.5	1.75
02	*Hibiscus trionum* L.	92	22-45	Panto	porate	Raised	9	3	3
03	*H. micranthus*L.	70	22-45	Panto	porate	Raised	14	0.6	0.7
04	*H. deflersii* Schweinf.	80	22-45	Panto	porate	Raised	12	1.3	1.4
05	*H. palmatus* Forssk.	92.3	22-45	Panto	porate	Raised	9	2.0	2.1
06	*H. vitifolius* L.	88.78	22-45	Panto	Porate	Raised	10	1.5	1.7
07	*H. rosa-sinensis* L.	100	22-45	Panto	colpoate	Raised	12	1.3	2.2
08	*H. ovalifolius* (Forssk.) Vahl.	97	22-45	Panto	colporate	Raised	13	1.5	2.3
09	*Gossypium hirsutum* L.	61	22-45	Panto	colporate	Raised	8	1.9	1.9
10	*Thespesia populnea* (L.) Solander ex Correa	66.27	22-45	Panto	Porate	Raised	8	2.3	1.9
11	*Senra incana* (Cav.) DC	50	22-45	Panto	porate	Raised	9	1.4	1.6
	Malveae								
12	*Malva parviflora* L.	52	Numerous	Panto	porate	-	3	1..2	2.0
13	*Alcea rosa* L.	92	Numerous	Panto	porate	-	7	0.8	2.8
	Abutilieae								
14	*Abutilon fruticosum* Guill. & Perr.	50	6-15	Zono	porate	Raised	6	0.9	0.9
15	*A. figarianum* Webb	42.25	6-15	Zono	porate	Raised	4	0.7	0.7
16	*A. bidentatum* A. Rich.	42.25	6-15	Zono	porate	Raised	5	0.95	0.95
17	*A. pannosum* (G.Forst.) Schledt	45	6-15	Zono	porate	Raised	5	0.8	0.8
18	*Sida acuta* Burm.	53.76	6-15	Zono	Colpo-porate	Raised	3	1.2	1.2
19	*S. alba* L.	57.5	6-15	Zono	porate	Raised	3	1.4	1.4
20	*S. ovata* Forssk.	50	6-15	Zono	porate	Raised	4	1.6	1.6

Spine shape: The spines are conical with straight sides in most of the investigated taxa. Hocked or curved ones may be recorded in some different taxa in different tribes such as: *Abutilon* and *Sida alba* (*Abutilieae*), *Malva parviflora* (*Malveae*) and *Abelmoschus esculentus* (*Hibiscieae*). Spines are either conical and taper into an acuminate or pointed tip as those in the pollen of *Alcea rosea*, and *Malva parviflora.*. Spines with blunt or rounded apices are recorded only in pollen of some species of *Hibiscieae* or, together with spines which have tapering apices, in pollen of some species of Malveae. Globular or flask-shaped spines are found only in *Alcea rosea*. In *Sida alba*, *Abutilon* and *Gossypium* the spines are raised on rounded bases which are formed by an increase in the length of the columellae. In *Hibiscus rosa- sinensis,* spines may be branched from the bases or from above. The distribution of spines on the surface of the grain is fairly constant in the same species but differs between different species and genera. They are always evenly distributed. The spine distances, i.e. the distance between two neighbouring spines, differs among species. In pollen of *Hibiscus, Gossypium* and *Abelmoschus,* it is relatively large (10-15 µm), while it is small and narrow in pollen of *Malva and Alcea* (5-7 µm).

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