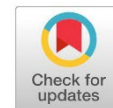


Research Article

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Investigation of Pollen Morphology of *Malva* L. (Malvaceae) in Libya

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Abstract

The *Malva* L. genus belongs to the *Malvaceae* Juss., which comprises about 40 species. This genus is represented by only six taxa in Libya, namely, *Malva aegyptia* L., *M. sylvestris* L., *M. verticillata* L., *M. nicaeensis* All., *M. parviflora* L. var. *parviflora* and *M. parviflora* L. var. *microcarpa* (Pers.) Loscos. In this study, the *Malva* L. species have been studied and investigated in terms of pollen morphology. Pollen morphology grains have been examined and studied in all taxa using a light microscope. Measurements of pollen diameter, pore diameter and arrangement, pollen shape, and spine features are recorded and comprehensively studied. Palynological results have revealed that pollen grains of *Malva* species are apolar, spheroidal, and polypantoporate. Pollen grains are characterized by spines, which are long, slender and pointed and/or short and pointed. Inter-spinal distance between apices was a remarkable feature that could be utilized with other features to demarcate species belonging to *Malva* L.

Keywords: Malva; Malvaceae; Pollen grains; Morphology; Taxonomy, Libya.

INTRODUCTION

The study of pollen morphology has gained importance owing to the crucial role it plays in the field of plant systematics. Many researchers have asserted the significance of using pollen morphology to delimit genera within different families (Bibi et al., 2010). Generally, when studying and investigating pollen grains, several characteristics must be examined and measured (Halbritter et al., 2018). These characteristics include polarity, size, shape, structure, and ornamentation. Pollen grains can be isopolar, heteropolar, or apolar. In terms of size, grains range between very small ($< 10 \mu\text{m}$), small ($10\text{-}25 \mu\text{m}$), medium ($26\text{-}50 \mu\text{m}$), large ($51\text{-}100 \mu\text{m}$) and very large ($> 100 \mu\text{m}$). The shapes of the pollen grains were determined based on the ratio between the length of the polar axis (P) and the equatorial diameter (E). Pollen grains can take several shapes such as preoblate, oblate, suboblate, oblate-spheroidal, spheroidal, prolate-spheroidal, prolate, and preprolate. The structure of pollen grains is distinguished by apertures (colpus and pores) and exine sculptures (Halbritter et al., 2018).

In plant systematics, pollen morphology has been regarded as a significant measure in plant taxonomy, as reported by systems such as Lindley, Fischer, and Erdtman (Perveen et al., 2007). The pollen morphology of different species belonging to the Malvaceae species has also been studied and investigated in several systems (El Naggari, 2004; Perveen et al., 2007; Arabameri et al., 2023;



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Abdel khalik et al., 2021). According to(Culhane & Blackmore, 1988), Malvaceae can be divided into six pollen types based on pollen grain diameter, the number of apertures, and spinular morphology (Bibi et al., 2010). According to the results obtained in (Mallick, 2020), pollen grains of *M. alcea* are periporate, circular, with echinate exine ornamentation. On the other hand, pollen grains of *M. sida* are circular, large, polyporate, and have echinate exine ornamentation. However, no exact measurements were presented in this paper for pollen grain characteristics. In this study, six *Malva* taxa represented in Libya (Jafri & Ali, 1977) were investigated in terms of their pollen morphology. This study aims to provide a thorough understanding of pollen characteristics and point out their taxonomical implications in the field of plant systematics.

MATERIALS AND METHODS

Study area

This study was conducted in the north-eastern region of Libya. Localities and their specific locations are listed in Table 1.

Collection of plants

Malva was collected during the spring seasons of 2018 and 2019. Of the 40 *Malva* species, only six taxa were present in Libya. The collected and identified *Malvas* are *M. aegyptia*, *M. sylvestris*, *M. nicaeensis*, *M. verticillata*, and *M. parviflora* (var. *parviflora* and var. *microcarpa*). All species were preserved and deposited in the Silphium herbarium, at the Department of Botany, Faculty of Science, Omar AL-Mukhtar University, Albayda, Libya. Pollen grains of all the species under investigation were obtained from the preserved dry samples.

Preparation of Glycerin Jelly

The glycerin jelly technique is one of the most common techniques used for pollen investigation. Glycerin jelly was obtained following procedures described in the literature (Erdtman, 1952; Shaheen *et al.*, 2009). It consisted of 10 g of gelatin, distilled water (35 ml) placed taken into a beaker and heated up to 70-80° C at which point 10 g of gelatin was added. When the temperature increased, the solution became viscous, and 30 ml of glycerol was added and mixed with the solution. Finally, 0.1% safranin stain was added and it was approximately 1/8th of the volume of the glycerin jelly solution. The whole solution was stirred up until a uniform dark pink color was obtained.

Mounting of pollens

To make it possible to examine pollen grains of the collected species, pollen grains were embedded in glycerin jelly and mounted on microscopic slides. Firstly, the pollen grains of each species were placed on a clean slide by gently tapping the anthers. Drops of the prepared glycerin jelly with 0.1% safranin were placed on pollen grains, which were then mixed together. A cover slip was carefully placed to avoid the formation of air bubbles. The slides were then left to cool to room temperature. After cooling, the cover slip edges were sealed with nail polish.

An OPTECH (Optical Technology) light microscope, (Model B4, EXACTA+ by EX-ACT+OPTECH Germany), was used to investigate the prepared pollen grains. All measurements were taken for 10-15 pollen grains for each species. Images of pollen grains were captured using a Samsung Galaxy A6+ smartphone camera. Microscope magnification of 40x and smartphone camera magnifications of 4x, 5x, and 6x were used according to pollen grain sizes and clarity of samples.

Table (1). Localities and coordinates of *Malva* samples used in this study

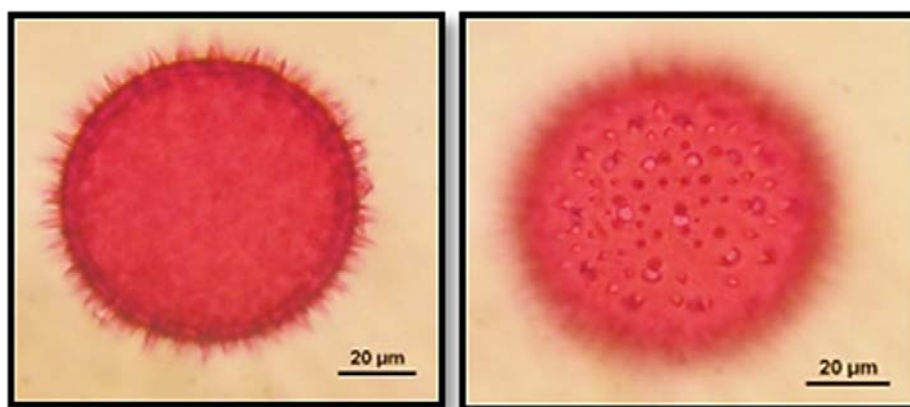
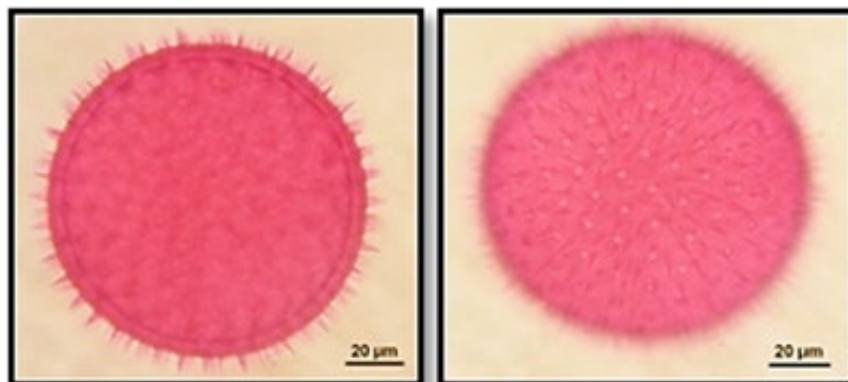
Locality	Latitude	Longitude	<i>M. aegyptia</i>	<i>M. sylvestris</i>	<i>M. verticillata</i>	<i>M. nicaeensis</i>	<i>M. parviflora</i> var. <i>parviflora</i>	<i>M. parviflora</i> var. <i>microcarpa</i>
Tobruk	32° 4' 29.982" N	23° 53' 6.9498" E		X			X	X
Al-Tamimi	32° 20' 0.528" N	23° 3' 42.1308" E					X	X
	32° 23' 26.7324" N	23° 3' 24.1380" E		X				
Martoba	32° 36' 10.0008" N	22° 45' 53.4954" E		X			X	
Shahat	32° 49' 23.2386" N	21° 51' 12.8376" E		X	X			
	32° 49' 57.4176" N	21° 51' 47.8326" E	X					
Alfaydiah	32° 42' 0.561" N	21° 54' 40.3092" E					X	
	32° 37' 19.2822" N	21° 54' 58.7658" E					X	X
	32° 34' 11.0814" N	21° 54' 31.2042" E	X	X		X		
Solonta	32° 35' 29.4462" N	21° 43' 5.6274" E		X				X
Gandola	32° 32' 22.6026" N	21° 34' 36.8724" E		X			X	X
Albayda	32° 46' 7.8888" N	21° 44' 57.1668" E			X	X	X	X
	32° 45' 54.162" N	21° 45' 7.6032" E			X			
	32° 46' 13.4724" N	21° 47' 3.8214" E					X	
	32° 46' 28.4586" N	21° 44' 23.2686" E			X	X		
	32° 45' 48.1104" N	21° 43' 49.2666" E				X	X	X
	32° 45' 34.5636" N	21° 42' 38.2314" E		X				
Massa	32° 44' 55.1466" N	21° 37' 34.1394" E				X	X	X
	32° 44' 55.8162" N	21° 37' 34.8846" E					X	X
	32° 45' 8.3052" N	21° 37' 39.2298" E					X	X
Wadi Alkuf	32° 41' 41.6286" N	21° 33' 44.1498" E			X	X	X	
Marawa	32° 29' 48.069" N	21° 25' 21.1866" E	X	X				
Taknis	32° 27' 51.069" N	21° 8' 36.0312" E					X	
	32° 28' 47.7546" N	21° 7' 25.7988" E		X		X	X	X
Algarieb	32° 34' 11.2296" N	21° 10' 24.693" E		X		X	X	X
Almarj	32° 29' 34.5726" N	20° 49' 58.0038" E			X			
	32° 29' 36.6648" N	20° 49' 19.2432" E					X	X
	32° 29' 55.323" N	20° 49' 36.4728" E			X			
Tocara	32° 31' 55.9446" N	20° 35' 24.1872" E					X	X
	32° 29' 4.0878" N	20° 30' 40.8666" E		X			X	
Deriyana	32° 21' 38.6424" N	20° 18' 56.4078" E					X	X
Benghazi	32° 5' 8.9838" N	20° 3' 56.0046" E					X	X
	32° 3' 50.8458" N	20° 5' 45.7074" E					X	
Qaminis	31° 39' 18.7956" N	20° 1' 6.7152" E					X	X
	31° 39' 39.4128" N	20° 1' 8.9106" E					X	X

RESULTS

Light microscopy investigations of pollen grains of all studied species have shown similar structures with some differences in size and arrangement, as shown in Figures 1 to 6. According to quantitative and qualitative measurements summarized in Table 2, pollen grains are 58.7-133.4 µm in diameter, spheroidal, and polyaperturate. Pores are 1.4-4 µm in diameter and are sometimes in a spiral pattern. The exine thickness is 4.3-6.4 µm, whereas nexine is about two times thicker than sexine. Spines of all studied species were long, slender and pointed (6.8-11.2 µm) and/or short and pointed (2.5-4.1 µm), regularly and densely distributed. Under a light microscope, the pollen sexine sculpture was difficult to distinguish. However, in some pollen grains the sculpture appeared rugulose to perforate.

Table (2). Summary of pollen grain, shapes, and sizes

	<i>M. aegyptia</i>	<i>M. sylvestris</i>	<i>M. verticillata</i>	<i>M. nicaeensis</i>	<i>M. parviflora</i> var. <i>parviflora</i>	<i>M. parviflora</i> var. <i>microcarpa</i>
Shape	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal
Pollen Diameter (μm)	74.2(82.8)93.3	98.4(108.3)111.6	93.6(101.6)133.4	78.5(99.2)116.6	66.8(71.3)78.1	58.7(67.9)73.9
Pollen Class	Pantoporate	Pantoporate	Pantoporate	Pantoporate	Pantoporate	Pantoporate
Spine height (μm)	2.8(5.5)8.6	2.5(6.3)11.2	3.2(7.2)9.5	4.1(6.8)9.1	3.7(6)7.5	3.1(5.1)6.8
Spine width (μm)	2(3.1)4.2	2.4(3.3)4.6	2.2(3.2)4	2(3)4.1	1.8(2.4)3.1	1.8(2.4)3.4
Interspinal distance between apices (μm)	5.1(7.5)10.9	5.7(9.2)13.3	6.3(9.7)12.5	6.2(10.4)16	4.3(6.5)9.3	5(6.6)8.1
Pore diameter (μm)	2(2.9)3.8	1.4(2.4)3.3	2(3.1)4	2.1(2.8)4	2(2.6)3.4	1.5(2.3)3.2
Sexine thickness (μm)	1.5	2.0	2.0	2.1	1.8	1.7
Nexine thickness (μm)	3.0	4.4	3.5	2.5	2.5	3.1
Intine thickness (μm)	1.4	2.1	1.7	2.1	1.6	1.7

**Figure (1).** Pollen grains of *M. aegyptia*.,**Figure (2).** Pollen grains of *M. sylvestris*.,

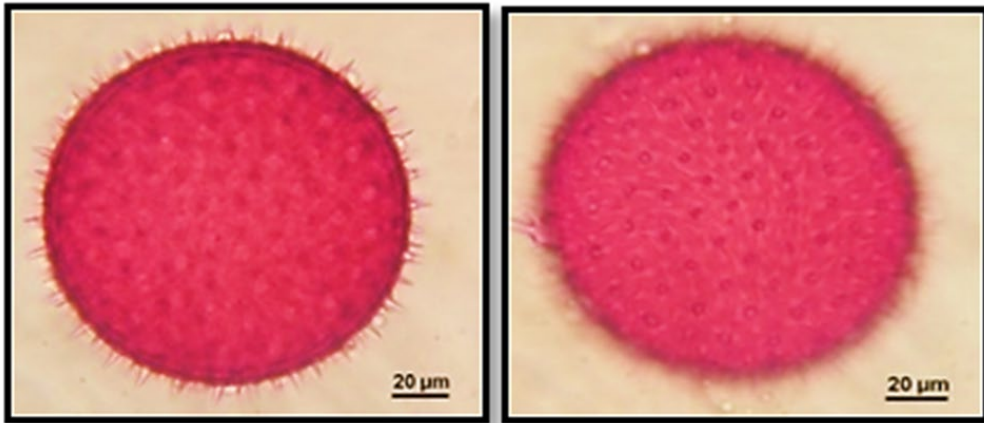


Figure (3). Pollen grains of *M. verticillate*.,

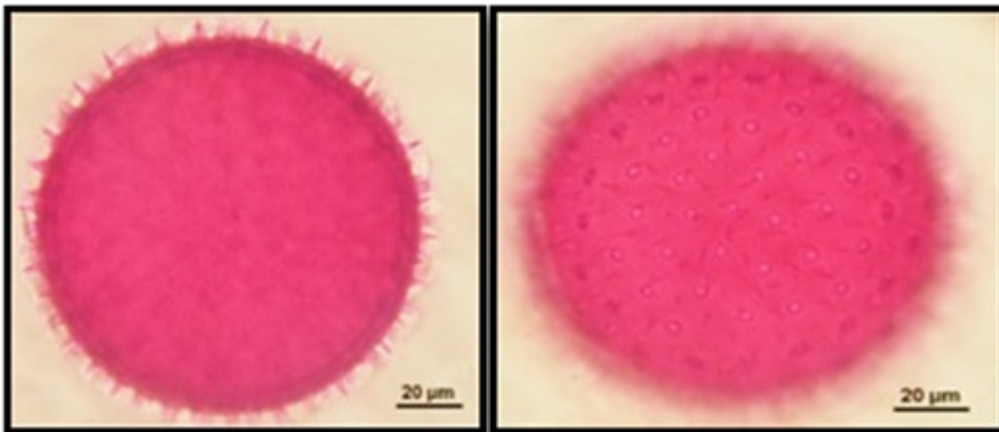


Figure (4). Pollen grains of *M. nicaeensis*.,

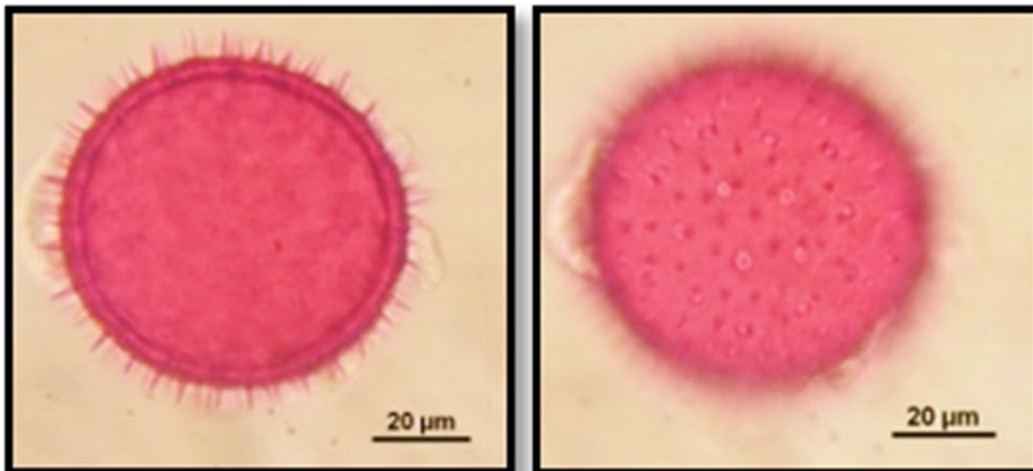


Figure (5). Pollen grains of *M. parviflora* var. *parviflora*.,

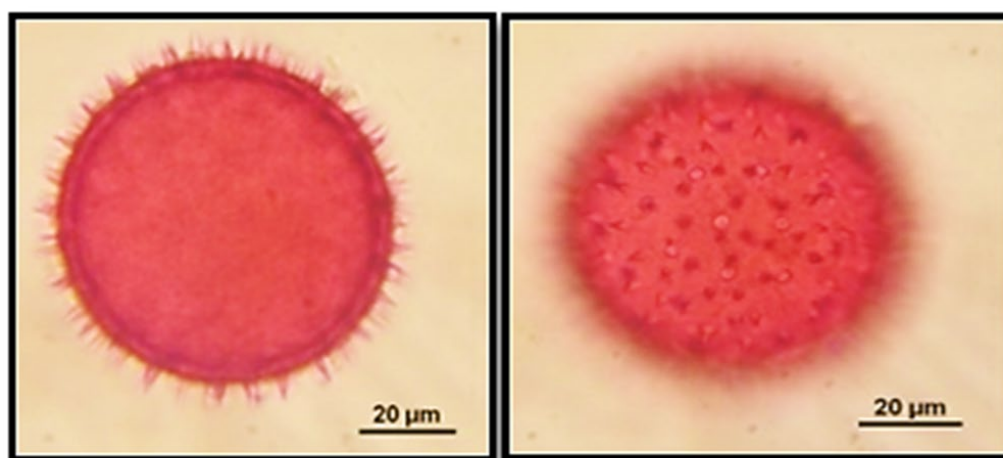


Figure (6). Pollen grains of *M. parviflora* var. *microcarpa*.,

DISCUSSION

The qualitative and quantitative data obtained for pollen morphology studies of all studied species are summarized in Table 2. Representative pollen grains are shown in Figures 1-6. These palynomorphological studies have confirmed that *Malva* is stenopalynous making the delimitation of different species quite difficult. The pollen grains are shown to be apolar, polyaperturate, and spheroidal. The size of the pollen varies considerably among pollen grains of the same species, as well as among pollen grains of different species. The smallest pollen size was reported in *M. microcarpa* of approximately 58.6 µm in diameter, whereas the largest size was reported in *M. verticillata* of approximately 133.4 µm in diameter. In agreement with (Shaheen et al., 2009 ; Abdel khalik et al., 2021), and despite the variations in pollen sizes, the present findings disagree with (Bibi et al., 2010) who state that pollen size is a reliable taxonomic tool for delimiting species. However, pollen size characteristics can be of taxonomic importance at the tribal level as reported by (El Nagggar, 2004). Disagreement is extended to the study presented in (Arabameri et al., 2023), where the pollen size of *M. verticellata* was reported to be the smallest among the studied *Malva* taxa.

Spines of pollen grains are regarded as remarkable characteristics of malvaceous pollens (El Nagggar, 2004; Shaheen et al., 2009). The spines show reliable variations in size, shape, and surface distribution (El Nagggar, 2004). Spine height and width in the present study showed variations among pollens of the studied species. Spine height ranges from 2.5-11.2 µm in species of interest, which agrees with measurements reported in (Arabameri *et al.*, 2023; Abdel khalik et al., 2021; Shaheen et al., 2009). These values of spine height in our results disagree with those of (Perveen et al. 1994), who emphasized that spine height is strictly less than 7µm, which is considered as a significant delimiting feature of pollens of *Malva* species. In addition to spine height, the interspinal distance between apices is found to be a distinguishing feature of pollen grains of *Malva* species. It ranges between 4.3 µm and 16 µm. According to the data in Table 2 the studied species can be categorized into two main groups. Group I: species with an interspinal distance more than 12 µm, and Group II: species with an interspinal distance less than 12 µm. Group I contained *M. nicaensis*, *M. sylvestris*, and *M. verticillata*, whereas Group II comprised *M. aegyptia*, *M. parviflora*, and *M. microcarpa*. Thus, the interspinal distance between apices features combined with spine height characteristics can be useful in delimiting species of *Malva*.

Pollen exine thickness varies greatly in the studied taxa and ranges between 4.3µm and 6.4 µm. This is due to variations in both sexine and nexine, which disagrees with (Christensen, 1986) who

states that sexine is usually of constant thickness in Malvaceae whereas nexine is of variable thickness. Based on present results, sexine is variable in thickness and ranges between 1.5 μm and 2.1 μm and nexine ranges between 2.5 μm and 4.4 μm . The present findings support (El Nagggar, 2004; Shaheen et al., 2009) who both reported the variations in exine thickness owing to variations in sexine and nexine thicknesses. Furthermore, sexine thickness was almost the same as the intine thickness for all studied species.

CONCLUSION

The pollen morphology of the studied taxa was thoroughly investigated, and the study concluded that these taxa were stenopalynous. This makes the demarcation of species belonging to this genus difficult. Based on palynological studies, pollen grains of the studied taxa are apolar, spheroidal in shape, and polyaperturate in arrangement. Pollen sizes vary within the same species as well as among different species. Spine height and interspine distance between apices are of the most importance according to the conducted study. They can be used to delimit between *Malva* species. Pollen exine thickness varied remarkably in the studied plants. This variation is related to the variations in sexine and nexine thicknesses.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

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