

Pollen morphology and its systematic implications in *Abutilon* Mill. species from Indian Thar Desert

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Abstract

Pollen morphology is one of the important tools to solve some taxonomic problems at family, generic or species level and has become part of the multidisciplinary and collaborative approach in plant systematics and evolution. Therefore, the aim of the present study was to investigate and describe the pollen morphology of five species of genus *Abutilon* Mill. by using light microscope. The five species selected for study were *Abutilon indicum* (L.) Sweet, *A. pannosum* (Forst.f.) Schlechtl., *A. ramosum* (Cav.) Guill & Perr., *A. bidentatum* Hochst.A. Rich. and *A. fruticosum* Guill. & Perr. From Indian Thar Desert region. All studied species were stenopalynous isopolar, trizonoporate and with echinate type of exine ornamentation. Pollen grains were separated on basis of slight variation in shape which is determined by ratio of polar to equatorial diameter. Prolate spheroidal pollen grains were observed in *A. indicum*, *A. pannosum* and *A. ramosum* whereas oblate spheroidal in *A. bidentatum* and spheroidal in *A. fruticosum*. Exine is equipped with spines along with variable height and width. Spine index value was used to discriminate between species. These morphological characters of pollen grains can be used as taxonomic tool for delineation of *Abutilon* species belonging to the same genera.

Malvaceae is a large and medicinally important family among flowering plants and also called as “Mallow family”. “This family comprised approximately 243 genera and 4225 known species that are disseminated in subtropical and tropical areas of the world”¹¹. This family is characterized by 22 genera as well as 93 species in India⁴¹ It is the well-

known family of economic importance as most of its genera are the producer of cotton, fiber yielding and various medicinal utility^{5,31}.

The genus *Abutilon* Mill. commonly known as ‘Kanghi’ is one of the most heterogeneous and large genera in the family Malvaceae with a need of critical revisionary

treatment²⁶. The genus comprises approximately 200 species that are widely distributed all over the world³³. In India, 18 species have been recorded^{33,54}. This genus is differentiated from other closely related genera of Malvaceae via having tri to multi seeded mericarp, lack of an epicalyx and dorsal wing in mericarp, presence of an endoglossum, dorsal dehiscence of schizocarpic fruit and remarkably serrate to sub entire leaves²¹. From Rajasthan, approximately 8 species of *Abutilon* have been reported⁵³ while the Indian Thar desert area 6 species of *Abutilon* has been reported namely *A. indicum*, *A. pannosum*, *A. ramosum*, *A. bidentatum*, *A. fruticosum* and *A. pakistanicum*⁶.

Systematic position of *Abutilon*⁹

Kingdom: Plantae

Clade: Angiosperm

Clade: Eudicots

Clade: Rosids

Clade: Malvids

Order: Malvales

Family: Malvaceae

Genus: *Abutilon* Mill.

Taxonomic identity of *Abutilon* species can be strengthened using various taxonomic tools such as macromorphology^{3,28}, leaf micromorphology^{4,49}, pollen morphology⁵¹, cytology²⁸, phytochemistry^{29,30} and molecular markers^{36,50}. Meanwhile there is a habitat loss and fragmentation due to changes in environmental conditions of the study area from last few decades and increasing anthropogenic activities. This phenomenon resulted in destruction of the germplasm resources due to genetic erosion of wild species of Indian Thar Desert³². IUCN Red List of Threatened plants reported

two endemic species; *Abutilon bidentatum* and *A. fruticosum* from the study area⁵⁸. Therefore it is important to study both the species through various characters for correct identification and conservation of genetic resources.

There is much confusion exist in morphology among the species of the plant *Abutilon*. As the plant is important medicinally and economically, there is a need of proper identification and description of the species including all criteria.

Palynology can play an important role in the formation of natural groups and help in the assessment of taxonomical relationship between species¹⁶.

Pollen grain is one of the most vital reproductive structure represented in all flowering plants with special structure and function¹⁷. The morphology of pollen mainly deals with the study of structural makeup and sculptural variations^{17,23,40}. The morphological characteristics of the pollen grain and spore are contained in the exine. Pollen grain such as shape, size, symmetry, type, number and position of aperture, wall architecture, exine sculpture, spine height, width and spine index are important determinants in systematic analysis to study the interrelationship of plants at various taxonomic levels^{18,59}. The surface pattern of pollen grains could be used as an important taxonomic character for the identification of taxa^{60,57}.

Furthermore, it is important to note that the most recent classification of angiosperms has made use of pollen morphological features

as one of the important taxonomical evidence^{14,35}.

The significance of pollen morphology in plant systematics has been stressed by a number of workers^{12,13,19,24,25,37,48}. Palynologically, family Malvaceae is a stenopalynous and general features of pollen are relatively uniform^{15,42}.

Earlier pollen morphological features in some plants of family Malvaceae has demonstrated the significance of palynology to understand the taxonomy of this family^{1,2,7,27,44,52,46,55}. All workers reported that pollen grain shape, size, length of the spine, interspinal distance diameter of the aperture, the distance between aperture and nature of spine were the useful taxonomic tool for identification an artificial taxonomic key was prepared based on these characters. Still, there is a gap in pollen morphology of family Malvaceae due to not much attention to the flora of Indian Thar Desert. There is no separate documentation on the pollen morphology of medicinal plants of this family such as *Abutilon* from the study site. Present study was aimed to provide a detailed knowledge about pollen morphological variations on both qualitative and quantitative parameters using light microscopy. By using this approach, the identification of complicated species of this genus can be strengthen from the study area.

Plant sampling, identification and preservation :

Five species of *Abutilon* viz. *A. indicum* (L.) Sweet, *A. pannosum* (Forst.f.) Schlechtl., *A. ramosum* (Cav.) Guill & Perr., *A. bidentatum*

Hochst. A. Rich. and *A. fruticosum* Guill. & Perr. were investigated from Indian Thar Desert (Figure 2). Periodical field survey at regular intervals in different seasons was arranged to collect the plants in flowering condition. Five population sites were selected from different localities of Jodhpur, Jaisalmer, Barmer, Bikaner and their nearby areas (Figure 1). Plant species collected were identified with the help of taxonomists from Botanical Survey of India, Jodhpur (Rajasthan). Flora of Indian Desert^{6,53}. was also reviewed to ensure the confirmation of exact species. Fresh and mature polleniferous floral buds just before anthesis were collected and preserved in 70 per cent alcohol till further use.

List of plant species, their site of the collection along with their longitude and latitude were noted (Table-1).

Herbarium specimen preparation :

Herbarium specimen for each species was prepared following the standard methods^{8,56}. and the accession number was given to each specimen. The finally prepared herbarium sheets were deposited to the Herbarium of Department Botany Jai Narain Vyas University Jodhpur.

Pollen grain analysis with light microscopy:

Anthers were plugged carefully from the collected and preserved floral buds and placed in 70 per cent ethanol for 24 hours to remove the fatty substances which were separated with the help of blotter. Slides of pollen grains were prepared using standard acetolysis method²⁰. The acetolysing mixture was made up of nine parts of acetic anhydride

and one part of concentrated sulphuric acid. The acetolyzed pollen grains were mounted in 50 per cent glycerin and the edges of the coverslip were sealed with clear nail polish.

For each plant species, separate slides of unacetolyzed pollen grain were also prepared to compare any variation in the measurement of various parameters.⁵⁹ For temporary unacetolyzed slide preparation, freshly collected pollen grain material was pretreated with 5 percent KOH for 2-3 minutes and fixed in 70 per cent ethanol for an hour and stained in 1 per cent alcoholic safranin for 2 minutes rinsed with distilled water and mounted in 50 per cent glycerin.

Prepared slides were studied under a light microscope. Various qualitative and quantitative parameters like pollen size, pollen shape, exine ornamentation, number of apertures, the height of the spine, width of the spine, interspinal distance were measured with the help of ocular and stage micrometer after calibration at 100 X and 450 X magnification. Photographs were taken with the help of Nikon FX-35A camera equipped with a light microscope.

The terminology used in the present study for pollen grain was followed by standard glossary^{22,45}.

Statistical analysis :

Measurements were taken for 15 pollen grains per plant species and the values are represented as Mean \pm SE (Standard error).

In the present study pollen grains of five species of *Abutilon* were studied using light microscopy. All the detailed pollen morpho-

logical features were summarized, both qualitative and quantitative palynological features were listed (Table-2) and all the representative species were illustrated (Figure 1). Pollen morphological variants of taxonomic significance were represented graphically (Figure 3). Pollen grains of *Abutilon* are monad, isopolar type, three zonoporate with circular pore and the excrescence type is spinate.

The pollen shape was prolate spheroidal in *A. indicum*, *A. pannosum* and *A. ramosum*, oblate spheroidal in *A. bidentatum* while spheroidal in *A. fruticosum* (Figure 1.) Earlier oblate spheroidal shape of pollen grain in *A. indicum* was reported from Punjab¹⁰ while sub oblate 3-zonoporate pollen grain was reported in same species from Pakistan⁵¹.

Sub oblate shape pollen grain were recorded in *A. pannosum* and *A. ramosum*³⁹. Although shape of pollen grain is not considered as a reliable taxonomic marker to delineate species in angiosperms^{18,59} because pollen grain shape is an unfixed character and can be influenced by existing media³⁴.

Trizonoporate is observed as a constant feature for the pollen grain of all studied *Abutilon* species while earlier studies supported existence of 8-12 aperture in some *Abutilon* species³⁹.

The species of subtribe Abutilinae mostly possess trizonocolporate pollen grains^{15,44,38} but in current study pollen grains with only pores were observed without any colpi. Earlier it was confirmed that the aperture in tribe Abutilinae is porate but due to verrucate nature of pollen surface the aperture appears

as colpus of different shape⁴⁴.

Pollen grains shows considerable variations in size in both polar and equatorial axis. The polar axis length ranges from 56.16-71.24 μm while equatorial diameter ranges from 55.12-68.64 μm . Smallest pollen size was observed in *A. ramosum* while biggest pollen grains were of *A. pannosum* in both polar and equatorial diameter.

The result of the pollen size does not fall within the prescribed range for the genus that is 37-45 μm reported in Egypt³⁸ but supports the findings from *Abutilon* species of Pakistan⁵¹ Although pollen size does not show much phylogenetic significance and generally considered as a tertiary character in phylogenetic studies.

Pollen grains of Malvaceae characterized by having echinations on the exine surface³⁸. The spines show reliable variations in size, shape and surface distribution. 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. These excrescences were of diagnostic value in the identification and delineation of genera and species. The plant genera show variations in number, height and position of these spines and constitute some of the most significant characters for identification purpose^{38,43}. Longest (7.63 μm) and narrowest spines(2.6 μm) were observed in *A.indicum* these were further distinguished from other species of pollen grains by having blunt apices against acute apices of other species under study. Presence of blunt apices echination was first time reported in *Abutilon indicum* earlier

blunt apices spines were regarded as constant feature of *Hibiscus* species^{12,51}. Furthermore highest value for spine index was calculated for *A. indicum* similarly lowest spine index were reported from *A. ramosum*. From the previous study in *A. indicum* height of the spine and spine index reported were shorter *i.e.* 2.5 μm and 1.2 μm respectively⁵¹. The interspinal distance was recorded maximum in *A. bidentatum* followed by *A. indicum* other three species showed little variation in these characters. Earlier *Abutilon* was recognized under a group with *Sida* on basis of spine index value less than 1.5 in Pakistan⁵¹ whereas present study records highest spine index value 2.93 in *A. indicum* which was more than the value 1.5 hence the species can be delineated from *Sida* on basis of this feature. Interspinal distance is variable character in species^{38,43,44,47}. The above statement was justified in the present study and can be used for the identification of *A. bidentatum* and *Abutilon indicum* from the rest of the species. In both species interspinal distance was recorded as almost double from other three species.

The spine index, spine height and interspinal distances are of diagnostic value in the identification and delineation species. *A. bidentatum* could be identified from rest of *Abutilon* species on the basis of maximum interspinal distance in the pollen grain wall while *A. indicum* could be delineated from other species on basis of maximum spine index, spine height and blunt apices of spines. *A. fruticosum* can be differentiated from other species of *Abutilon* on basis of triangular and short spines without basal cushion while in *A. ramosum* spines on the tectum were present on well developed basal cushion. The present

Table-1. List of *Abutilon* species and their site of collection with coordinates

No.	Species	Localities	Longitude	Latitude	No. of samples for each location
1.	<i>A. indicum</i> (Linn.) Sweet	New campus JNVU	73.0170	24.1063	3
2.	<i>A. indicum</i> (Linn.) Sweet	Mandore garden, Jodhpur	73.0353	26.3525	3
3.	<i>A. indicum</i> (Linn.) Sweet	Ashok Udhyan, Jodhpur	72.9751	26.2514	3
4.	<i>A. indicum</i> (Linn.) Sweet	Circuit house, Jaisalmer	70.9055	26.9197	3
5.	<i>A. indicum</i> (Linn.) Sweet	Museum circle, Bikaner	73.3286	28.0177	3
6.	<i>A. pannosum</i> (Forst.f.) Schlectdl.	Baiji Talab, Jodhpur	73.0189	26.2866	3
7.	<i>A. pannosum</i> (Forst.f.) Schlectdl.	Gadisar lake area, Jaisalmer	70.9231	26.9085	3
8.	<i>A. pannosum</i> (Forst.f.) Schlectdl.	Amarsagar, Jaisalmer	70.8060	26.9137	3
9.	<i>A. pannosum</i> (Forst.f.) Schlectdl.	Industrial area, ITI college Bikaner	73.3402	28.0048	3
10.	<i>A. pannosum</i> (Forst.f.) Schlectdl.	Gudamalani, Barmer.			3
11.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	Mehrangarh, Jodhpur	73.0188	26.2979	3
12.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	New campus JNVU.			3
13.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	Near Bhootnath temple, Jodhpur	73.0048	26.2925	3
14.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	Outside Circuit house (Barmer),	71.4159	25.7649	3
15.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	Ghattu village Bikaner	73.5240	27.6392	3
16.	<i>A. bidentatum</i> Hochst.	Rao Jodha Desert Rock Park and Mehrangarh	73.0167	26.3043	3
17.	<i>A. bidentatum</i> Hochst.	Kiradu hillock, Barmer	71.0977	25.7528	3
18.	<i>A. bidentatum</i> Hochst.	RIICO industrial area, Bikaner	73.3402	28.0048	3
19.	<i>A. bidentatum</i> Hochst.	Bada Bagh, Jaisalmer)	70.8874	26.9553	3
20.	<i>A. bidentatum</i> Hochst.	Desert National Park, Jaisalmer	70.8085	26.9191	3
21.	<i>A. fruticosum</i> Guill. & Perr.	Mandore Garden, Jodhpur	73.0353	26.3525	3
22.	<i>A. fruticosum</i> Guill. & Perr.	Machia Safari Park, Kaylana road, Jodhpur,	72.9763	26.3020	3
23.	<i>A. fruticosum</i> Guill. & Perr.	Desert national Park, Jaisalmer	70.8085	26.9191	3
24.	<i>A. fruticosum</i> Guill. & Perr.	Wood fossil Park, Akal, Jaisalmer	71.0423	26.8263	3
25.	<i>A. fruticosum</i> Guill. & Perr.	Kuldhara village Jaisalmer	70.8030	26.8086	3

Table-2. Qualitative and quantitative features of pollen grains of *Abutilon* species

Taxa	Pollen grain diameter Mean \pm S.E. μ m		P/E ratio	Pollen shape	Pollen class	Spine height Mean \pm S.E. μ m	Spine width Mean μ m	Spine index	Interspi- nal distance Mean \pm S.E. μ m
<i>A. indicum</i>	65 \pm 0.8	61.88 \pm 0.96	1.05	Prolate spheroidal	Trizonoporate	7.63 \pm 0.23	2.6 \pm 0	2.93	4.94 \pm 0.26
<i>A. pannosum</i>	71.24 \pm 1.04	68.64 \pm 1.04	1.03	Prolate spheroidal	Trizonoporate	5.46 \pm 0.26	5.2 \pm 0	1.05	2.8 \pm 0.2
<i>A. ramosum</i>	56.16 \pm 0.64	55.12 \pm 1.5	1.02	Prolate spheroidal	Trizonoporate	5.72 \pm 0.5	7.28 \pm 0.5	0.78	2.6 \pm 0.3
<i>A. bidentatum</i>	59.8 \pm 1.64	61.36 \pm 1.76	0.90	Oblate spheroidal	Trizonoporate	5.2 \pm 0	2.6 \pm 0	2.0	8.84 \pm 0.64
<i>A. fruticosum</i>	63.44 \pm 5.55	64.48 \pm 5.55	1.00	spheroidal	Trizonoporate	2.6 \pm 0	2.6 \pm 0	1.0	2.6 \pm 0

S.E.=Standard error

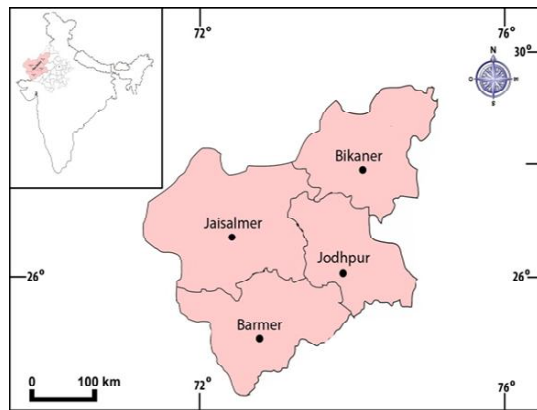


Figure 1: Map of the Study area of Indian Thar Desert

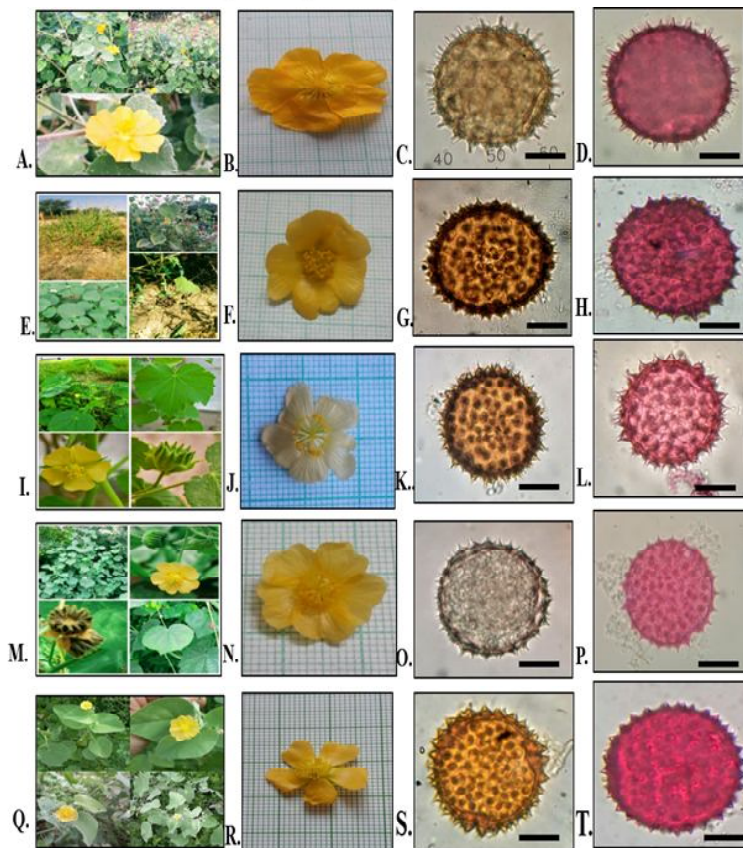


Figure 2. Field view, floral morphology and light microscopic images of acetolyzed and unacetolyzed pollen grains of *Abutilon* species: A-D . *A. indicum* E-H. *A. pannosum* I-L. *A. ramosum* M-P. *A. bidentatum* Q-T. *A. fruticosum* (Scale bar= 50 μ m)

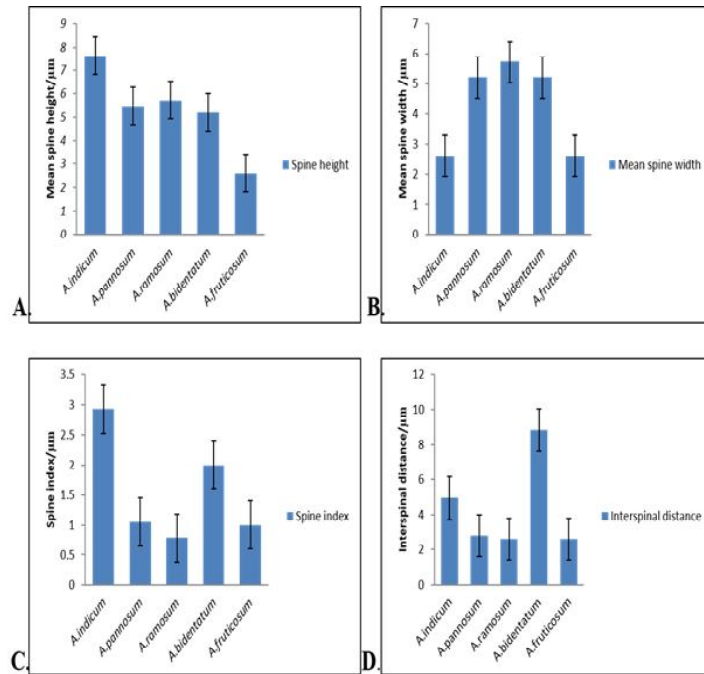


Figure 3. Pollen morphological variations in *Abutilon* species: A. Spine height B. Spine width
C. Spine index D. Interspal distance

study is first report of pollen data from the study area that can provide supportive tool for identification of plant species along with other criteria.

Detailed pollen morphology has been of considerable help in preparing key to the identification of the species as follows:

Key to the species of *Abutilon* on basis of pollen morphological variations :

- 1a. Pollen grain shape spheroidal, mean value for spine index 1*A. fruticosum*
- 1b. Pollen grain shape sub spheroidal2.
- 2a. Pollen grain oblate spheroidal, mean value for interspal distance 8.84 µm*A. bidentatum*
- 2b. Pollen grain prolate spheroidal, interspal distance less than 8.84 µm.....3.
- 3a. Mean value for spine height 7.63 µm, spine with blunt apices.....*A. indicum*
- 3b. Mean value for spine height less than 7.63 µm, spine with acute apices and basal cushion.....4.
- 4a. Pollen diameter 68.64 ± 1.04 µm, spine index more than 1 *A. pannosum*
- 4b. Pollen diameter less than 68.64 ± 1.04 µm, spine index less than 1. Maximum spine width 7.28 ± 0.5 *A. ramosum*

The current research on pollen morphological features revealed the importance of light microscopic investigation in identification and authentication of *Abutilon* species of Indian Thar Desert. However, palynological features should only be treated as supportive taxonomic tool for species classification yet the data from pollen morphological features are of substantial taxonomic importance and must be combined with traditional morphology-based classification for delineation of taxa at species level with certainty.

Further studies should be carried out under Scanning electron microscopy and transmission electron microscopy for improved knowledge of exine ornamentation and highlighting on other traits.

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