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Taxonomic Investigation of Some Members of Fabaceae (Subfamily-Caesalpinioidae) With Special Reference to Pollen Morphology

Swarnaditya Mondal[1], Biswajit Roy[2] and Jnan Bikash Bhandari[3]*

[1 & 3] Pteridology and Paleobotany Laboratory, Department of Botany, University of North Bengal, Siliguri 734013.

[2] PG Department of Botany, Ramakrishhna Mission Vivekananda Centenary College, Rahara 700118

Abstract

Palynology, the study of pollen, had a big role in taxonomic identification, paleontology and forensics. Pollens from different plants had different morphology, such as in Fabaceae. This study aimed to determine the pollen grains morphology in Fabaceae (Subfamily Caesalpinioidae). Pollen morphology of 19 plant species under 7 genera Fabaceae (Subfamily Caesalpinioidae) were examined. Fresh pollen samples were collected from 4 plant species from the North 24 Pgns, 2 plant species from South 24 Pgns, 8 plant species from Kolkata, 2 plant species from Nadia and 3 plant species from Howrah. Pollens were acetolysed following standard method and observed under Compound microscope. Pollen shapes were recorded based on the P/E ratio. In general, all these pollens were small, medium and large size. Parameters measured in this study were the types of pollen sizes, pollen shape, aperture characteristic, and ornamentation type of exine and the most common aperture type was tricolporate. The most important characters included exine ornamentation (exine ornamentation type) and Apocolpium Index.

Keywords: Caesalpinioidae, morphology, palynology, pollen grains, acetolysis.

Introduction

Fabaceae a large heterogeneous family of flowering plants, occurs naturally in the tropics around the world. The Fabaceae **Adanson** or Fabaceae **Lindley** is a very large family of herb, shrub and trees with a great variety of habitat, including aquatics, xerophytes and climbers. The flowers of Fabaceae are extremely variable in size, form, colouration and pollinators. Many species are of economic importance to man. This family comprises 650 genera and 18,000 species (Dickison, 1981) with cosmopolitan distribution in tropical and temperate zones. Pollen morphology is a muchinteresting subject; it requires exact knowledge to describe pollen. This includes shape, polarity, symmetry, aperture, ornamentation, exine stratification and size of the pollen. Among the bases of angiosperm phylogeny, pollen morphology is unique in that through no other study can one obtain as great an amount of information from so little material within a short time (Walker and Doyle 1975). Palynology is the subject of modern science that deals with the study of pollen, spore and other palynomorphs (Agashe & Caulton 2007). Palynology has been recognized for years for its importance and application in different fields of sciences (Ajipe & Adebayo 2018). The pollen morphology is particularly useful in solving taxonomic problem, a specific character of pollen

***Corresponding author**

Email Id- jnanbikashbhandari@nbu.ac.in

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morphology could be designated to a certain family and sometimes even up to species level of plants (El-Amier 2015; Rabia et al. 2012). The sporopollenin layer on the pollen wall renders its inertness against acid and bacterial attack (Williams et al. 2018). Pollen grains have a unique biological characteristic, contain a large amount of genetic information, and exhibit strong genetic conservation, so they used for species identification (Almeida et al. 2018).

Materials & Methods

Pollen Morphology

All the plants were collected from South 24 Pgns, North 2 Pgns, Kolkata, Howrah and different blocks of Salt Lake City, mainly the portion of branch with inflorescence were taken. During the field trips five to ten individuals of mature plants of each species were collected and they were attached with numbered tags. The specimens were photographed, worked out, described and preserved in the form of herbarium sheets following the standard and modern herbarium techniques (Jain and Rao, 1977). The portions of plants were placed in between stacks of newspapers or blotting papers for proper dying. As a result of that, dried specimens are obtained which were mounted on herbarium sheets and properly labeled. Each species was identified in consultation with standard literature (Hooker, 1875; Prain, 1903; Anonymous, 1997; Hazra et al, 2000; Paria and Chattopadhyay, 2000). The search for literature including recent ones for determining the correct name of taxa was followed after The Plant List (web add.) and other literature including (Bennet 1987).

Parameters measured in this study were the types of pollen shape, pollen sizes, aperture characteristic, and ornamentation type of exine. The pollen shape classes are based on the ratio between the length of the polar axis (P) and equatorial diameter (E). P and E are measured from the equatorial view of a pollen grain and spore (Simpson 2010).

Table 1. The common shapes of the pollen grain

No.	Shape	Ration of P to E
1	Peroblate	< 0.50
2	Oblate	$0.50 \leq x < 0.75$
3	Subspheroidal	$0.75 \leq x < 1.33$
	- Suboblate	$0.75 \leq x < 0.88$
	- Oblate spheroidal	$0.88 \leq x < 1.00$
	- Prolate spheroidal	$1.00 \leq x < 1.14$
	- Subprolate	$1.14 \leq x < 1.33$
4	Prolate	$1.33 \leq x < 2$
5	Perprolate	> 2

The common shapes are presented in table 1. While the aperture morphology is an opening or thinning of the exine, physiologically it is a germination zone.

The exine ornamentation has two different types, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektexine (including sexine and nexine) characters belong to the structural features, while the sculpturing comprises external (supratectal) geometric features without reference to their internal construction (G. (Gunner) Erdtman 1986).

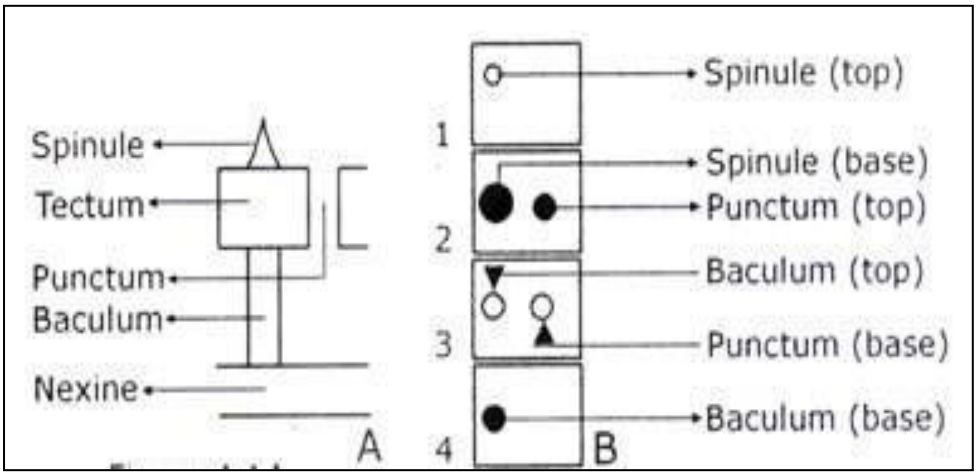

Fig. Illustrating diagram of the exine ornamentation structure. (A) Layers of exine. (B) Light and dark islands that appear from the high adjustment to low adjustment of the light microscope. 1, 2, 3, & 4 represent the first, second, third and fourth focus of microscope from the high to low adjustment.

After collection of polliniferous materials, the acetolysed methods for these material was followed as suggested by Erdtman (1952, 1969), Chanda (1966), and Bose *et al.*(2012) with slight modifications. The flowers or anthers containing pollen were fixed in glacial acetic acid, then transferred directly to the brass sieve, crushed and washed down to the centrifuge tube with 70% alcohol. The brass sieve was put into flame after each treatment until it became red hot to avoid contamination. Acetolysis mixture was prepared slowly by adding one part of concentrated sulphuric acid to nine parts of acetic acid anhydride (as alternative 5% - 10% NaOH/ KOH) in a measuring cylinder. The powdered material collected in the test tube was then suspended in the acetolysis mixture (ca. 10 ml of the mixture was added to each tube) and stirred carefully by glass rod. After thoroughly stirring, the mixture was heated to about 100˚C in a water bath, until the mixture attained light brown colour. After heating, the mixture was allowed to stand for a few minutes, then centrifuged and decanted. The process was repeated once and foam was removed by adding a few drops of 95% alcohol or acetone. The mixture was then filtered through a fine sieve and centrifuged. After decanting, distilled water was added to the sediment, half of which was kept for chlorination and 50% glycerine was added to the other half.

For chlorination 5ml of glacial acetic acid, 1-2 drops of concentrated sodium chlorate solution (as alternative H_2O_2) and a few drops of concentrated hydrochloric acid were added and the mixture was stirred by a glass rod. The mixture was then centrifuged. After centrifuging and decanting 50% glycerine was added to it. Then the two parts (non-chlorinated and chlorinated) were mixed-together, centrifuged and decanted. The tubes containing acetolysed polliniferous sediments were kept in inverted condition on a filter paper for a couple of hours. For mounting on slides, a minute piece of glycerin jelly (prepared by Kisser's method) was taken on tip of a clear platinum needle (after making red hot in the flame and subsequently cooling), touched carefully the pollen precipitate in the test tube and then placed on a clean slide. Then the slide was heated gently and the after the jelly had spread evenly, by the platinum needle around the cover glass was placed on the material and sealed off with paraffin wax (melting point 60 ^{0}C to 62 ^{0}C).

The light microscopical work was done with a student microscope. Measurement was taken using high power and oil immersion objectives. The factors for measurements in high power and oil immersion objectives were 3.13µm and 1.22µm for 1 occular micro division respectively.

For taking measurements, etc. the methods of Chanda (1962, 1966) among others have been followed. In all cases, measurements and other observations were based on acetolysed grains unless otherwise mentioned. The measurements quoted in the pollen descriptions are generally based on an average of ten reading randomly choosen. In case of scanty occurrence, however, fewer grains were measured. The relevant data, measurements and other information are represented in Table 2.

Discussion

The pollen morphology of the Subfamily Caesalpinioidae of family Fabaceae representing 19 species under 7 genera have been investigated (Table 2). The pollen grains in the genera of the family are isopolar, radially symmetrical.

Pollen Size:

The size of a pollen grain are helpful for identification of species. The size of the pollen grains varies between 36.4 µm.- 72.8 µm. in case of shape-class spheroidal grains and PA/ED 10.4 µm. / 13.0 µm. to 65.0 µm. / 59.8 µm.in case of other shape. The smallest grain is found in *Cassia kleinii* (PA/ED 15.6 µm. / 10.4 µm.) and the largest grains found in *Bauhinia purpurea* (PA/ED 65.0 µm. / 72.8 µm.).

Pollen Shape:

The shape of a pollen grains are usually specific to species. Shape is determined by the ratio between the length of polar axis and the equatorial axis (P/E ratio). The pollen grains are mostly Prolate. However, other shapes such as Sub-oblate, Oblate-spheroidal, Spheroidal, and Prolate-spheroidal also encountered.

Exine Ornamentation:

It is the evident from Table 2 that the exine of the investigated taxa have variable thickness. The thickest exine is noticed in *Bauhinia purpurea (*5.72 µm.), whereas, the thinnest exine are found in several taxa *viz. Cassia alata, Cassia javanica, Cassia kleinii, Cassia occidentalis, Cassia tora, Crotalaria pallida, Crotalaria verrucosa, Desmodiumgangeticum, Melilotus alba, Melletiaovalifolia,Tephrosiapurpurea* (1.30 µm.).

Table 2. Pollen morphological data

Sl. No.	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	Colpus L/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
1	***Bauhinia acuminata***	atreme	spheroidal	globose	59.8-65.0	59.8-65.0	1	–	–	3.90	1.30	2.60	–	–	–	clavate
2	***Bauhinia purpurea***	tricolporate	sub-oblate	triangular	33.8-39.0 59.8-65.0	41.6-44.2 67.6-72.8	0.81 0.88	26.0/ 10.4	20.8 /13.0	5.72	1.30	4.42	18.2-26.0	0.4	19.5-26.0	striate
3	***Bauhinia tomentosa***	penta-colpate	spheroidal	globose	70.2-72.8	70.2-72.8	1	23.4/ 5.20	–	3.12	2.08	1.04	–	–	–	clavate
4	***Brownia coccinea***	tricolporate	prolate	globose	36.4-39.0	26.0-28.6	1.14	28.6/ 10.4	2.60 /2.60	5.20	1.30	3.90	7.80	0.3	_	reticulate

Table 2. Contd….

SL No	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	ColpusL/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
5	***Caesalpinia pulcherrima(RED)***	trisyn-colporate	sub-oblate	triangular	41.6-46.8	36.4-39.0	1.14	33.8/10.4	3.90/2.60	3.12	1.30	1.82	5.20	0.14	20.8-26.0	reticulate
6	***Caesalpinia pulcherrima(YELLOW)***	trisyn-colporate	sub-oblate	triangular	41.6-46.8	33.8-39.0	1.20	33.8/10.4	5.20/3.90	3.90	1.30	2.60	7.80	0.23	26.0-31.20	reticulate
7	***Cassia alata***	tricolporate	prolate	triangular	20.8-23.4	10.4-13.0	2	18.2/5.20	3.90/3.90	1.30	0.65	0.65	7.80-10.40	0.44	10.4-13.0	reticulate
8	***Cassia fistula***	trisyn-colporate	prolate	triangular	18.2-20.8	15.6-16.9	1.66	18.2/6.50	1.30/1.30	2.60	1.30	1.30	3.90-5.20	0.25	10.4-13.0	rugulate
9	***Cassia javanica***	trisyn-colporate	prolate	triangular	18.2-20.8	13.0-15.6	1.38	15.6/5.20	1.30/1.30	1.30	0.65	0.65	2.60	0.2	13.0	rugulate

Table 2. Contd….

Sl. No.	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	Colpus L/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
10	***Cassia kleinii***	trisyn-colporate	prolate	triangular	15.6-18.2	10.4-13.0	1.50	15.6/5.20	3.90/2.60	1.30	0.65	0.65	2.60	0.25	7.80-9.10	psilate
11	***Cassia laevigata***	tricolporate	prolate	triangular	20.8-23.4	10.4-13.0	2.00	13.0/5.20	3.90/2.60	2.60	0.78	1.82	3.90-5.20	0.37	10.4-13.0	reticulate
12	***Cassia occidentalis***	tricolporate	sub-prolate	triangular	23.4-26.0	18.2-20.8	1.28	23.4/7.80	3.90/3.38	1.30	0.65	0.65	13.0-15.6	0.71	13.0-15.6	regulate-fossulate
13	***Cassia siamea***	tricolporate	prolate	triangular	26.0-28.6	18.2-20.8	1.42	26.0/7.80	3.90/2.60	2.60	1.30	1.30	5.20-6.50	0.28	13.0-15.6	reticulate
14	***Cassia sophera***	tricolporate	sub-prolate	triangular	36.4-39.0	28.6-31.2	1.27	26.0/10.4	3.90/2.60	2.60	0.78	1.82	3.90-5.20	0.13	–	reticulate

Table 2. Contd….

SL No	Taxa	Aperture	Shape	Equitorial outline	PA	ED	PA/ ED	ColpusL/B	Pore diam.(L/B)	Exine	Sexine	Nexine	Apocolpium/ Apoporium	Apocolpium Index	Mesocolpium/ Mesoporium	Exine Ornamentation
15	***Cassia tora***	trisyn-colporate	prolate	triangular	18.2-20.8	10.4-13.0	1.75	26.0/10.4	3.90/2.60	1.30	0.65	0.65	2.60-3.90	0.25	7.8-10.4	reticulate
17	***Delonix regia***	tricolporate	prolate-spheroidal	triangular	36.4-39.0	31.2-36.4	1.16	28.6/7.80	7.80/6.50	5.20	3.12	2.08	23.4-26.0	0.75	18.2-20.8	reticulate
18	***Peltophorum pterocarpum***	tricolporate	oblate-spheroidal	triangular	39.0-41.6	36.4-40.3	0.98	28.6/10.4	3.90/3.90	3.90	1.30	2.60	23.4-26.0	0.71	20.8-23.4	reticulate
19	***Saraca asoca***	trisyn-colporate	prolate	triangular	36.4-39.0	20.8-23.4	1.75	26.0/7.80	3.12/2.60	2.60	1.30	1.30	2.60-3.90	0.11	10.4-13.0	reticulate

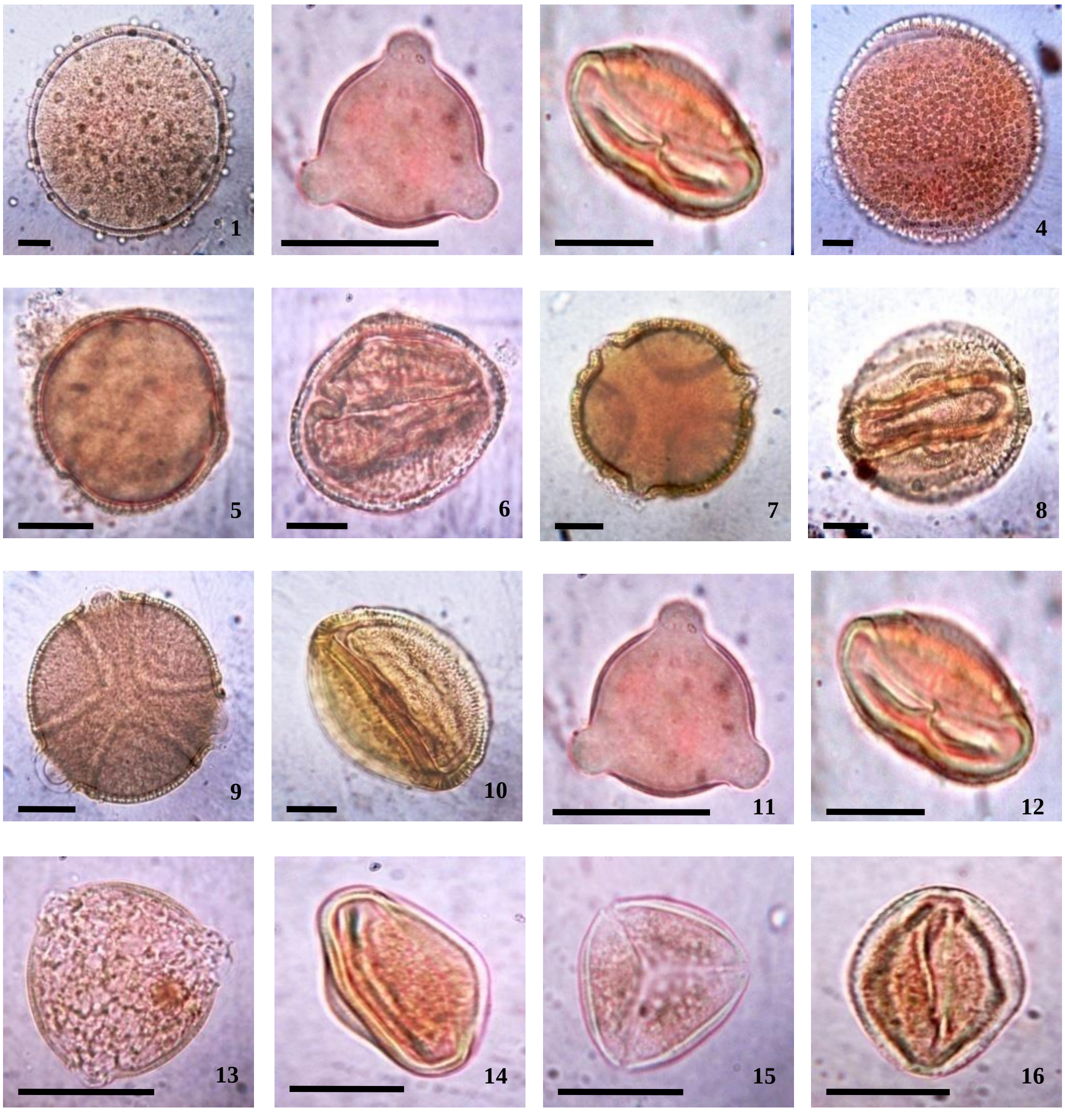

PLATE I. Pollen morphology of investigated taxa (Scale Bar -10µm.).

Fig: 1. Pollen of *Bauhinia acuminata*; 2. Polar view of *Bauhinia purpurea*; 3. Equatorial view of *Bauhinia purpurea*; 4. Pollen of *Bauhinia tomentosa*; 5. Polar view of *Brownia coccinia*; 6. Equatorial view of *Brownia coccinia*; 7. Polar view of *Caesalpinia pulcherrima*; 8. Equatorial view of *Caesalpinia pulcherrima*; 9. Polar view of *Caesalpinia pulcherrima* var. *flava*; 10. Equatorial view of *Caesalpinia pulcherrima* var. *flava*; 11. Polar view of *Cassia alata*; 12. Equatorial view of *Cassia alata*; 13. Polar view of *Cassia fistula*; 14. Equatorial view of *Cassia fistula*; 15. Polar view of *Cassia javanica*; 16. Equatorial view of *Cassia javanica*;

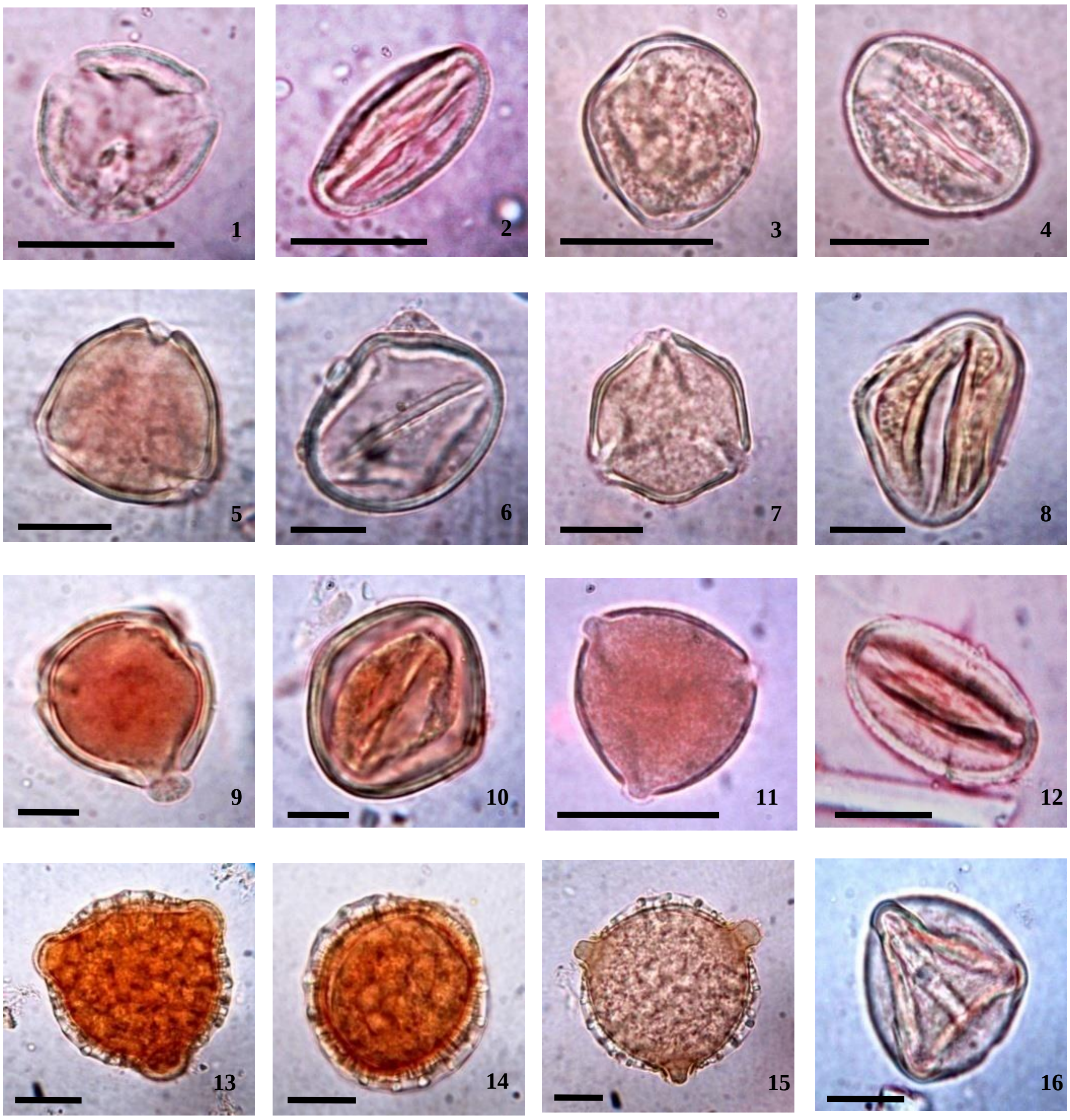

PLATE II. Pollen morphology of investigated taxa (Scale Bar -10µm.).

Fig: 1. Polar view of *Cassia kleinii*; 2. Equatorial view of *Cassia kleinii*; 3. Polar view of *Cassia laevigata*; 4. Equatorial view of *Cassia laevigata*; 5. Polar view of *Cassia occidentalis*; 6. Equatorial view of *Cassia occidentalis*; 7. Polar view of *Cassia siamea*; 8. Equatorial view of *Cassia siamea*; 9. Polar view of *Cassia sophera*; 10. Equatorial view of *Cassia sophera*; 11. Polar view of *Cassia tora*; 12. Equatorial view of *Cassia tora*; 13. Polar view of *Delonix regia*; 14. Equatorial view of *Delonix regia*; 15. Polar view of of *Peltophorum pterocarpum*; 16. Polar view of *Saraca asoca*;

Exine ornamentation of investigated pollen grains include psilate, verrulate, reticulate, reticulate-fossulate, regulate, clavate, striate (following Traverse 1988). Ornamentation like psilate and reticulate are common among the taxa studied (Table 2)

Apertures

Apertures are simple having colpus or pore only as well as compound consisting of an ectocolpus and an endocolpus, which make the pollen grains as colporate. The number of aperture are 3 in most of the taxa investigated. The colpus are long and narrow.

Apocolpium, mesocolpium and apocolpium index are some of the quantitative measurements of pollen grains, which sometimes help to distinguish closely related species. In the present investigation, apocolpium ranges from 2.60 µm.- 26.0 µm. among the investigated taxa. The mesocolpium varies from 7.80 µm.- 31.2 µm. among the investigated taxa. Apocolpium index ranges from 0.11 µm.- 0.80 µm. and may be used in distinguishing some taxa (Table 2).

Based on number, position and type of apertures of pollen grains, six pollen types can be recognized among the investigated taxa in order to summarize the pollen morphological features (Table 2). These pollen types include in the following sequence: trizonicolporate type, trisyncolporate type, penta-colpate type, atreme type, based on the numerical strength of taxa.

Conclusion

The morphological study of pollen of Subfamily Caesalpinioidae (Fabaceae) have revealed variation in pollen size, shape, aperture and exine ornamentation. The pollen observed were small, medium and large size, Prolate, Sub-oblate, Oblate-spheroidal, Spheroidal, in shape. In Caesalpinioideae tricolporate, trisyncolporate pollen grains are found More works need to be carried out to investigate the effects of climate change on pollen production and its morphology as these factors ultimately lead to success of pollination and more works need to get a successful result on pollen allergies. The results obtained indicate the need to continue palynological investigations on the Subfamily Caesalpinioidae of Fabaceae family.

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