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Palynological Studies on Certain Members of the Malvaceae Family in Rivers State, Nigeria

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Abstract—Palynological study was undertaken in five species Hibiscus rosa- sinensis L., Abelmoschus esculentus (L) Moench, Abelmoschus caillei (A.Chev.) Stevels, Sida acuta Burm. F. and Sida rhombifolia L. belonging to three genera of the family Malvaceae. The family Malvaceae, commonly known as the hibiscus, or mallow contain about 85 genera and 1500 species of herbaceous, shrubby, and tree plants. Representatives of this family can be found in all except the coldest parts of the world but are most abundant in the tropics. The aim was to establish some useful diagnostic pollen morphological features that may be employed in combination with other characters as inter- specific or generic tools for identification. Pollen samples were collected manually from mature closed anthers. Mature anthers were teased out in water in a petri dish for 5 minutes. Fixation of the pollen grain in 70% alcohol was undertaken, followed by decantation and rinsing to wash off the alcohol. The samples were placed on clean microscope slides. Another slide was used to tease out the content of the anther after which it was mounted in glcerol. The results revealed oblate to spheriodal pollen shape and pantoporate pollen class in all the studied species. Pollen diameter ranged from 139.01 μ m – 359.10 μ m. The largest pollen diameter was recorded in A. esculentus (359.10 μ m). All the species possessed spinous pollen and the longest spine was observed in A. esculentus (359.10 μ m) while spine index was highest in A. caillei (3.65 μ m) and lowest in S. rhombifolia (1.62 μ m). The information provided in this study may be useful in delimiting the family alongside other taxonomic markers and also in the preparation of the pollen flora of Nigeria.

Keywords-Malvaceae, pollen morphology, Nigeria, Spine index, Pollen diameter, Hibiscus, Abelmoschus, Sida

I. INTRODUCTION

The family Malvaceae, commonly known as the mallow family is estimated to contain about 85 genera with 1500 species [1]. The family is cosmopolitan in distribution with most of the species confined to the tropics and subtropics. 13 genera and 78 species have been recorded in West Africa [2]. *Hibiscus* is the largest genus in terms of number with about 300 species [3] and *Hibiscus rosa-sinensis* is the most popular while *Abelmoschus sp* is a common vegetable in Nigeria.

The Malvaceae are herbs, shrubs or lianas although there are some trees [4]. They can easily be recognized in the field by their funnel shaped flower with five separate petals and a distinct column of stamens surrounding the pistil. Their leaves are alternate with well developed stipules, petiolate with palmate venation [5]. A diagnostic feature of the family is the presence of a natural gum (mucilage, pectin and asparagines) which gives them a slimy texture when crushed. This feature is pronounced even in the dry desert species. The investigated species *Abelmoschus esculentus, Abelmoschus caillei, Hibiscus rosa-sinensis, Sida acuta* and *Sida rhombifolia* belong to the division Magnoliophyta, class Magnoliopsida, subclass Dilleniidae, order Mavales and family Malvaceae.

The family has been investigated severally due to the great economic importance of some of its genera. Owing to the high fiber content, the Malvaceae family is of great economic importance throughout the world. Nearly all genera can produce some kind of fibres. The genus Gossypium is the source of the commercial cotton. After the removal of fiber, cotton seeds are used as fodder to feed cattle and the seed oil used for edible purposes while the oil cake is useful as good organic manure. Abelmoschus esculentus, A. manihot, A. callei, Hibiscus sabderiffa are eaten as vegetable in all the parts of the country. Urena lobata, Hibiscus tiliaceus, Thespesia populnea are good sources of wood and fiber. The roots of Sida cordata and Hibiscus hispidissimus are used in the treatment of urinary tract diseases [6, 7] due to their cooling, diuretic and anti-inflammatory properties. Many species are cultivated as ornamentals including the popular Hibiscus rosa sinensis, H. mutabilis, H. radiates, Abutilon striatum. Several species of the genera Sida and Abutilon are common weeds along road sides and equally serve as fodder for sheep and cattle.

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Pollen morphology has in recent times played an important role in plant systematics and experimental taxonomy as documented by various researchers [8, 9, 10, 11]. Structural characters of pollen are of great taxonomic investigative importance [12] and are important in species identification in areas of evolution and often times in environmental restoration activities [13]. Pollen characters are more or less uniform [14]. [15] divided the family into six pollen types based on the number of apertures, pollen diameter and spinular morphology. However, the most comprehensive study conducted on pollen characters of the malvaceae family was carried out by [16]. Generic delimitation based on pollen morphology was termed difficult according to the findings. Prior to that, [17] had in his study concluded that pollen morphology can be used in the malvaceae family to distinguish species at the generic level owing to their distinctive characteristics. Emphasis were laid on the importance of the aperture and spine morphology as well as exine stratification to distinguish taxa.

In Nigeria, the pollen morphology of only a few genera of the family has been studied. This study is therefore aimed at bridging this gap by providing general knowledge of the palynology of these species as well as a comparison of the pollen morphology of different species of these three genera within the same family.

II. RELATED WORK

The authors [18, 19, 20] have worked on species of Malvaceae and also reported Spheroidal, oblate or globular shaped pollen grains as revealed in this study. Malvaceous pollen are characteristic for the extension of their exine into definite spines [19].

The author, [21], carried out a research on Palynological studies on some medicinal mallows from Punjab, India. He worked on the pollen grains of seven species of the family Malvaceae. From his results, it was recorded that the pollen grains are oblate spheroidal, porate with reticulate exine and are echinate. His study showed that palynological markers are useful in the identification and classification of the plant taxa in this family.

A study was also carried out by [22]. The research was based on Palyno-taxonomic study of nine members of Malvaceae in Purba Medinipur, West Bengal, India. The results showed that the pollen shape of the species were Oblate, Oblate-spheroidal, Subprolate and Per-oblate. He concluded that pollen morphology is one of the most significant tool for the taxonomic study as well as systematics of plant taxa in this family.

III. METHODOLOGY

Plant specimens were collected from different areas of Rivers state. Pollen samples were collected manually from mature closed anthers. Collected plant specimens were identified at the department of Plant Science and Biotechnology herbarium of Rivers state University, Rivers state, Nigeria using field reference materials and relevant taxonomic literature. Voucher specimens were deposited in the herbarium of the Department.

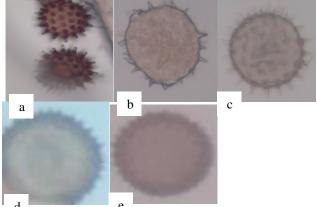
The specimens were prepared following [23] simplified method. Mature anthers were teased out in water in a petri dish for 5minutes. Fixation of the pollen grain was followed by placing it in 70% alcohol for another 5minutes. This were decanted and rinsed several times in distilled water to wash off the alcohol. The pollen grains were stained with 1% safranine and mounted in glycerol on microscopic slide. A cover slip was placed on the pollen glycerol mixture and left to cool; the microscopic slide was labeled and edges of the cover slip sealed with nail polish. 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 observed. Phytomicrographs of the pollen grain were taken. Pollen shape, class, spine description and aperture type were noted and pollen diameter, spine height and width were accurately measured.

IV. RESULTS AND DISCUSSION

Findings from the study of palynological characters in the five species are presented in Plate 1 and summarized in Table 1 below:

Hibiscus rosa sinensis

Pollen grains of *H. rosa sinensis* was globulose, pantoporate and colporate. The pollen diameter measured 173.54(211.43 \pm 10.66) 244.16µm. The spines were short and straight with blunt apices. Spine height measured 19.86(28.11 \pm 3.68) 42.82 µm and the width measured 5.97(8.16 \pm 0.62)9.63 µm. The spine index recorded was 3.45.



d gure 1. Pollen ^e phology of: *a - H. rosa sinensis*; b -Abelmoschus caillei; c - A. esculentus; d - S. acuta; and e - Sida rhombifolia L. X10

Abelmoschus caillei

Pollen grain of *A. caillei* was oblate-spheroidal, pantoporate and colporate. Pollen diameter measured $300.78(351.07\pm11.29)370.82$ µm. Spines varied from straight to slightly bent with a height of

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 $15.57(32.97\pm5.09)51.15$ µm and a width of $5.97(9.04\pm0.89)11.94$ µm. Spine index recorded 3.65.

Abelmoschus esculentus

Pollen of *A. esculentus* was spheroidal, pantoporate and colporate. The pollen diameter measured $307.04(359.10\pm22.66)$ 406.62 µm which was the largest pollen in all the studied species. The spines measured $26.71(44.13\pm3.11)56.66$ µm in height and $11.01(11.16\pm1.52)21.33$ µm in basal width. Spine index measured 2.74.

Sida acuta

Pollen grain of *S. acuta* was oblate-spheroidal, pantoporate and colpate. The Pollen diameter measured

 $126.22(152.22\pm8.02)175.53\mu$ m. Spines were very short with height of $3.76(5.15\pm0.33)5.97\mu$ m and width of $2.67(2.67\pm0)2.67\mu$ m. Spine index recorded 1.92.

Sida rhombifolia

Pollen of *Sida rhombifolia* L. was spheroidal, pantoporate and porate. The pollen diameter measured 124.91(139.01±1.33) 142.93 μ m making it the smallest pollen grain in all the studied taxa. Spines were almost inconspicuous and measured 2.00(2.35±0.11)2.67 μ m in height and 1.00(1.45±0.14) 1.83 μ m in width. Spine index was 1.62.

Species	Pollen diameter (μm) Min(mean ±S.E)Ma	Spine length (µm) Min(mean ±S.E)Ma	Spine width(µm) Min(mean ±S.E)Ma	Spine index (µm)
H. rosa sinesis	173.54(211.43±10.66)244.16	19.86(28.11±3.68)42.82	5.97(8.16±0.62)9.63	3.45
A. caillei	300.78(351.07±11.29)370.82	15.57(32.97±5.09)51.15	5.97(9.04±0.89)11.94	3.65
A. esculentus	307.04(359.10±22.66)406.62	26.71(44.13±3.11)56.66	11.01(16.08±1.52)21.23	2.74
S. acuta	126.22(152.22±8.02)175.53	3.76(5.15±0.33)5.97	2.67(2.67±0)2.67	1.92
S. rhombifolia	134.91(139.01±1.33)142.93	2.00(2.35±0.11)2.67	1.00(1.45±0.14)1.83	1.62

Table 1 Pollen Structures of the Studied Species

		Table 2.		
Species	Pollen shape	Pollen Class	Spine description	Aperture shape
			short Spines with blunt	
H. rosa sinesis	Oblate-spherical	Pantoporate	apice	Colporate
			Long Spines with	
A. caillei	Oblate	Pantoporate	pointed apice	Colporate
			Long Spines with	
A. esculentus	Spheroidal	Pantoporate	pointed apice	Colporate
S. acuta	Spheroidal	Pantoporate	short Spines	Porate
S. rhombifolia	Spheroidal	Pantoporate	short Spines	Porate

Min= Minimum, S.E= Standard error, Ma= Maximum

Discussion

Pollen morphology of the studied species showed considerable variations indicating that the parameter can be used in distinguishing the species. Generally, pollen shape was observed to be spheroidal or oblate and spinous, possessing spines of different heights and description. This agrees with the findings of [16, 18, 19], that pollen grains are more or less uniform in the Malvaceae family. However, the variations in the pollen diameter proves that it can serve as a useful tool in distinguishing species at the generic level [14].

Pollen diameter varied between $126.22\mu m - 406.62 \mu m$. The maximum diameter was observed in *A. esculentus*(359.10 µm) followed by *A. caillei*, *H. rosa sinensis*, *S. acuta* and the minimum size in *S. rhombifolia*. This result contradicts that reported by [14] that *Hibiscus* pollen are the largest in the family whereas *A. eculentus* is reported here to be larger. [20] did not agree with [14] findings also. However, his other results are in line with those confirmed in this study. The variations in pollen size is considered to be an index to variation in the number of chromosomes and proven to be of value in cytopalynological studies [24]. Spheroidal and oblate shapes were observed in the study. This findings are in accordance with [18, 19, 20] that pollen of the family are generally round, spheriodal or globular. [25, 26] have opined that pollen shape is taxonomically less useful due to it variation even within species. They attributed this fact to the method of extraction or the media used. Malvaceous pollen are characteristic for the extension of their exine into definite spines [19]. Spine types were monomorphic for all the studied species indicating only one form. A. esculentus recorded the longest spine of $26.71(44.13 \pm 3.11)$ 56.66 and a width of $11.01(16.08 \pm)$, while the S.rhombifolia had the shortest spine of length $2.00(2.35 \pm 0.11)$ 2.67 and width $1.00(1.45 \pm 0.14)$ 1.83. The size of the spine appears to be directly proportional to the pollen size such that larger pollen had longer spines and vice versa. [6] reported that spine index was significant in clearly differentiating the genera Alceae and Althaea in Pakistan especially at the intrageneric level. Spine index was highest in A.caillei (3.62) and lowest in S. rhombifolia

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V. CONCLUSION AND FUTURE SCOPE

Pollen morphology is one of the most significant tool for taxonomic study as well as systematics of plant taxa in this family, as seen from this study.

The extension of the exines into spines in all the studied species justifies their placement in the same family. However variations in pollen diameter observed are of taxonomic importance and can be useful in distinguishing the species. Spine height and width are also diagnostic at the generic level. This study showed that palynological markers are useful in the identification and classification of the plant species in this family. The information provided in this study may be useful in delimiting the family alongside other taxonomic markers such as phytochemistry, anatomy, cytology and morphology and also in the preparation of the pollen flora of Nigeria.

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