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POLLEN MORPHOLOGICAL STUDIES OF SELECTED FLOWERING PLANTS IN FEDERAL UNIVERSITY OF AGRICULTURE, ABEOKUTA, NIGERI

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ABSTRACT

The taxonomic classifications of some flowering plants into families have been a subject of review in recent times. This is because many were classified in the early days of taxonomy using plant morphological characters alone. This study assessed pollen characters of ten flowering plants in Federal University of Agriculture, Abeokuta, Nigeria with a view to identifying valuable taxonomic characters which could be employed for resolving classification controversies associated with the use of morphological characters alone. Pollen study was conducted using acetolysis method. Means, standard deviations and coefficient of variations were calculated while photomicrographs of pollen grains were taken at X400 magnification. The result showed tricolporate, pantoporate, triporate, tricolpate and heterocolpate pollen types while exine patterns were granulate, reticulate, psilate and echinate. Results of pollen shape revealed oblate spheroidal, prolate and subprolate while there were variations in pollen characters among species of the same family in apertural type, sculpture, exine surface pattern, pollen shape and pollen fertility. This suggests that apertural type, sculpture, exine surface pattern, pollen shape and pollen fertility could be useful in resolving taxonomic issues related to classification associated with grouping of some flowering plants into the same family on the basis of morphological traits alone.

Key words: Pollen grains, fertility, morphology, flowering plants, plant morphology, classification

INTRODUCTION

Pollen has two male haploid cells found in a thick wall (Hind-AbelWahab, 2008). Morphologically, the pollen consists of an outer layer known as the exine and apertures as well as an inner layer known as intine. Apertures are big substances found on the surface of the pollen grain which consist of pori and colpi (Birks and Birks, 1980). In pollen grains, these apertures are not open; instead, they are covered with a thin layer of exine. Pollen grains with pori are known as porate, those with colpi are referred to as colpate, while pollen with both pori and colpi are referred to as colporate. Pollen is also classified on the basis of the number of apertures as mono- aperture, di-apertures and tri-apertures (Mignot *et al.*, 1994).

Ming *et al.* (2014) opined that pollen exine, sculpture, size and aperture type are very unique and could be used in taxonomy and in tracing evolutionary relationships among plants. Kerkhoven and Mutsaers (2003) reported the diagnostic value of most of these pollen characters, which they attributed to their uniqueness at specific level.

There are lots of unresolved taxonomic problems which could be traced to the use of morphological characters alone in classification. This could be due to the environmental influence on morphological characters. Also, there have been reported cases of cross-inconsistency or incompatibility between species due to the existence of a set of pre- and post-zygotic barriers (Hajjar and Hodgkin, 2007). These pre-zygotic barriers can either be the absence of pollen grain germination and the delay or inhibition of pollen tube growth (Hajjar and Hodgkin, 2007).

It has become necessary to use pollen characters in taxonomic classification in addition to the use of

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other systematic lines of evidence such as plant morphology, anatomy, physiology, phytochemistry, molecular systematics, genetics, ecology etc. This will eliminate the bias that may be associated with the use of only plant morphology in classification. Gretchen and Stanley (2001) reported that pollen could be used in plant classification because each plant species has specific pollen shape, size and structure peculiar to it. Also, Ming *et al.* (2014) are of the opinion that pollen exine, sculpture, size and aperture type could be considered as very unique and specific characters in taxonomy and in tracing evolutionary relationships among plants.

Pollen study is not a new concept. For instance, Hedberg (1997) reported the use of pollen morphology in taxonomy but limited his study to few pollen characters. Also, Agwu and Osibe (2005) canvassed for the use of pollen grains in taxonomy, systematics and biotechnology. Several authors have also reported the usefulness of pollen characters in taxonomy and evolutionary studies. For instance, Oyelakin and Ayodele (2011) suggested the use of germ pores in plant classification and grouping.

The influence of the environment on plant morphology has been observed over the years as one of the taxonomic challenges associated with the use of morphological traits in taxonomy, genetic improvement and breeding of plants. The use of pollen characters in resolving these challenges is imperative to serve as one of the taxonomic tools. This is because pollen characters are genetic and cannot be influenced by the environment. Thus, the basic understanding of pollen characters is required to resolve many issues associated with the use of morphological characters alone in taxonomy, genetic improvement, breeding purposes and to trace the evolutionary relationships. Therefore, this study aimed to assess pollen characters of ten flowering plants from Federal University of Agriculture, Abeokuta (FUNNAB) with a view to identifying valuable taxonomic characters that could be employed for resolving taxonomic problems associated with the use of morphological characters alone in the classification among flowering plants.

MATERIALS AND METHODS

Pollen grains were collected from five different flowers from each of the ten flowering plant species from their natural habitats in FUNAAB campus (Table 1). The identities of the flowering plants were confirmed in FUNAAB Herbarium in the Department of Pure and Applied Botany. Voucher specimens of these plants were deposited in FUNAAB Herbarium.

Table 1: List of flowering plant species used in the study

S/N	Botanical Name	Common Name	Family	Collection Number
1	*Cassia fistula* L.	Golden rain tree	Fabaceae	WO006
2	*Carica papaya* L.	Pawpaw	Caricaceae	WO007
3	*Gmelina aborea* Roxb.	Gamhar	Lamiaceae	WO005
4	*Helianthus annuus* L.	Sun flower	Asteraceae	WO001
5	*Ixora cocinea* L.	Jungle flame	Rubiaceae	WO008
6	*Mimosa pudica* L.	Sensitive plant	Fabaceae	WO010
7	*Sida acuta* Burm.f.	Wire weed	Malvaceae	WO002
8	*Senna spectabilis* DC	Yellow shower	Fabaceae	WO004
9	*Senna occidentalis* L.	Coffee senna	Fabaceae	WO009
10	*Tridax procumbens* L.	Coat buttons	Asteraceae	WO003

Preparation of Pollen Slides

Pollen grains were obtained by crushing the collected anthers and a needle was used to remove the debris. Glacial acetic acid was used to transfer the crushed anthers into micro-centrifuge tubes and centrifuged for 15 mins at 5000 rpm. The centrifuged samples were decanted and the supernatants were washed three times with distilled water. Acetolysis procedures described by Moore *et al.* (1991) were adopted in pollen sample preparation. The samples were put in acetolyzed mixture of acetic anhydride and tetraoxo-sulphate (VI) acid in the ratio of 9:1, and heated in water bath at 60^{o}C for 10 min. Thereafter, heated samples were centrifuged and washed with distilled water three times to remove the acetolysis mixture. The residues were transferred into a sterilized bottle. Glycerine jelly was added to the prepared samples in the ratio of 50:50.

Pollen Sculpture and Aperture

Pollen slides were prepared using the method of Erdtman (1971). Samples were pipetted into a clean glass slide, covered with cover slide and a transparent nail hardener. Pollen grains were properly examined under a compound microscope at X400 magnification for sculptural and apertural features. Photomicrographs of pollen grains were taken at X 400 magnification.

Pollen Shape

Pollen shape was calculated using the formula: P/E X 100

P = Measurement of the length from the "North" to the "South" pole of the pollen

E = Measurement of the equatorial portion or axis of the pollen

Pollen Size

Pollen size was determined by measuring the polar and equatorial diameters of ten deeply stained randomly selected pollen grains at X400 magnification, according to Erdtman (1971) as modified by Oyelakin and Ayodele (2011).

Means and standard deviations were calculated from the raw data. The coefficient of variation (CV) for the pollen size was also computed to compare the variation among the plants. Photomicrographs of pollen grains were taken at X400 magnification to show the pollen structure (Oyelakin and Ayodele, 2011).

Pollen Fertility

This was estimated by counting pollen grains from ten fields of observation on each of the prepared slides for each plant at X400 magnification. Pollen grains with cytoplasm stained deep-brown were considered fertile while those that were not stained or only partially stained were considered sterile (Oyelakin and Ayodele, 2011).

Pollen fertility was calculated using the formula:

$$\text{Pollen fertility} = \frac{\text{Stained pollen}}{\text{Total pollen counted}} \times 100$$

RESULTS

Detailed pollen characters of the flowering plants investigated are shown in Table 2 while pollen photomicrographs are shown in plate 1. Results of pollen fertility, size and coefficient of variation are shown in Table 3.

Pollen Sculpture
The sculptures of the pollen types observed were reticulate in *I. coccinea* and *C. fistula* with echinate type in *H. annuus*, *S. acuta* and *T. procumbens*. Pollen sculpture was psilate in *S. spectabilis*, *S. occidentalis* and *M. pudica* while granulate type was observed in *G. aborea* and *C. papaya* (Table 2).

Pollen Aperture
The triporate pollen aperture was observed in *S. spectabilis* while *C. papaya* had tricolpate type. *H. annuus*, *S. acuta* and *T. procumbens* had pantoporate apertural type; pollen aperture was heterocolpate in *G. aborea*. Tricolporate type was observed in *C. fistula*, *I. coccinea* and *S. occidentalis* while *M. pudica* had triporate apertural type (Table 2).

Pollen Shape
Pollen shape was oblate spheroidal in *H. annuus*, *S. acuta* and *T. procumbens*, while prolate spheroidal pollen shape was observed in *S. spectabilis*, *G. aborea*, *C. fistula*, *C. papaya*, *I. coccinea* and *S. occidentalis*. *Mimosa. pudica* had subprolate pollen shape (Table 2).

Pollen Size
Small-sized pollen grains were recorded in *I. coccinea* (0.71±0.07µm), *T. procumbens* (0.74±0.1µm) and *H. annuus* (0.77±0.11µm), while medium-sized pollen grains were recorded in *M. pudica* (0.92±0.19 µm), *C. papaya* (0.93±0.15 µm), *S. spectabilis* (0.98±0.14 µm), *C. fistula* (1.0±0.20 µm) and *S. occidentalis* (1.20±0.13 µm). Relatively large pollen size was recorded in *G. aborea* (1.75±0.16 µm) while *S. acuta* had relatively very large pollen size of 2.02 µm (Tables 2 and 3).

Pollen Fertility
Pollen fertility was generally high, ranging from 96.91% in *C. fistula*, to 100% in *H. annuus*, *S. acuta*, T. *procumbens* and *M. pudica;* the lowest pollen fertility of 14.53% was recorded in *S. spectabilis* (Tab 3)

Table 2: Pollen characters of the studied taxa

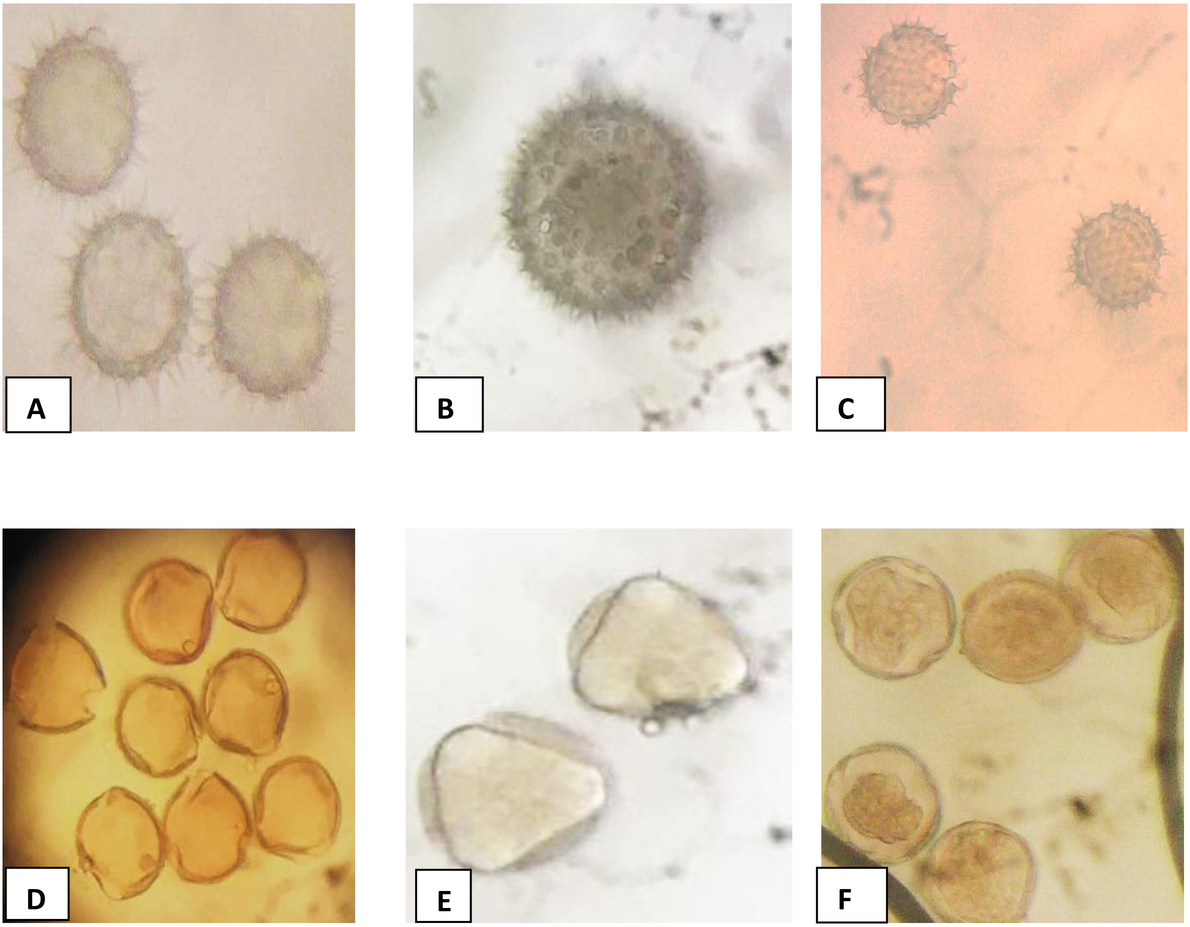
S/N	Botanical Name	Common Name	Family	Collection Number	Aperture	Sculpture	Size	Shape
1	*C. fistula* L.	Golden rain tree	Fabaceae	WO006	Tricolporate	Reticulate	Medium	Prolate sphe
2	*C. papaya* L.	Pawpaw	Caricaceae	WO007	Tricolpate	Granulate	Medium	Prolate sphe
3	*G. aborea* Roxb.	Gamhar	Lamiaceae	WO005	Heterocolpate	Granulate	Large	Prolate sphe
4	*H. annuus* L.	Sun flower	Asteraceae	WO001	Pantoporate	Echinate	Small	Oblate spher
5	*I. cocinea* L.	Jungle flame	Rubiaceae	WO008	Tricolporate	Reticulate	Small	Prolate sphe
6	*M. pudica* L.	Sensitive plant	Fabaceae	WO010	Triporate	Psilate	Medium	Subprolate
7	*S. acuta* Burm.f.	Wire weed	Malvaceae	WO002	Pantoporate	Echinate	Very large	Oblate spher
8	*S. spectabilis* DC	Yellow shower	Fabaceae	WO004	Triporate	Psilate	Medium	Prolate sphe
9	*S. occidentalis* L.	Coffee senna	Fabaceae	WO009	Tricolporate	Psilate	Medium	Prolate sphe
10	*T. procumbens* L.	Coat buttons	Asteraceae	WO003	Pantoporate	Echinate	Small	Oblate spher

Keys: Small (below 0.8 µm), Medium (0.8-1.4 µm), Large (1.5-1.99 µm), Very large (2.0 µm and above)

Pollen grain less than 50 % represents peroblate, 50-75 % oblate, 75-88 % suboblate, 88-100 % oblate spheroidal, 100-114 % prolate spheroidal, 114-133 % subprolate, 133-200 % prolate, over 200 % perprolate

Table 3: Pollen characters of the taxa studied

S/N	Botanical Name	Common Name	Family	Collection Number	Number of pollen counted	Pollen fertility (%)	Pollen size Mean ± S.E (µm)	Coefficient variation (%
1	*C. fistula* L.	Golden rain tree	Fabaceae	WO006	486	96.91	1.0 ± 0.20^{bc}	20.00
2	*C. papaya* L.	Pawpaw	Caricaceae	WO007	156	98.72	0.93 ± 0.15^{c}	16.13
3	*G. aborea* Roxb.	Gamhar	Lamiaceae	WO005	44	100	1.75 ± 0.16^{d}	9.14
4	*H. annuus* L.	Sun flower	Asteraceae	WO001	271	100	0.77 ± 0.11^{d}	14.29
5	*I. cocinea* L.	Jungle flame	Rubiaceae	WO008	140	99.29	0.71 ± 0.07^{d}	9.86
6	*M. pudica* L.	Sensitive plant	Fabaceae	WO010	81	100	0.92 ± 0.19^{c}	20.65
7	*S. acuta* Burm.f.	Wire weed	Malvaceae	WO002	16	100	2.02 ± 0.20^{a}	9.90
8	*S. spectabilis* DC	Yellow shower	Fabaceae	WO004	296	14.53	0.98 ± 0.14^{c}	14.29
9	*S. occidentalis* L.	Coffee senna	Fabaceae	WO009	273	98.54	1.20 ± 0.13^{b}	10.83
10	*T. procumbens* L.	Coat buttons	Asteraceae	WO003	168	100	0.74 ± 0.10^{d}	13.51


A
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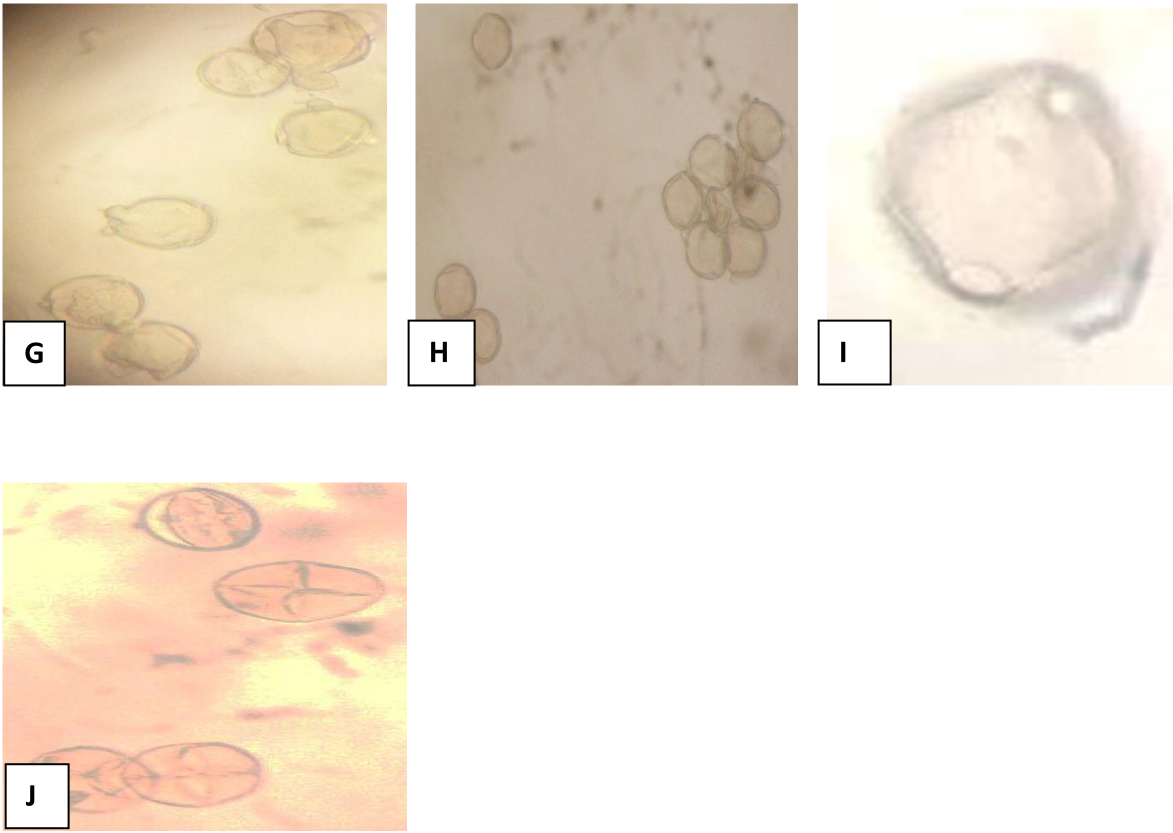

Plate 1**:** Pollen external structure (exine) of the plant species collected

(A) Echinate in *H. annuus*

(B) Echinate in *S. acuta*

(C) Echinate in *T. procumbens*

(D) Psilate in *S. spectabilis*

(E) Granulate in *G. aborea*

(F) Reticulate in *C. fistula*

(G) Granulate in *C. papaya*

(H) Reticulate in *I. cocinea*

(I) Psilate in *S. occidentalis*

(J) Psilate in *M. Pudica*

DISCUSSION

Pollen characters have been a subject of discussion over the years as taxonomic tools in taxa delimitation in angiosperms. The results from this study showed variations in pollen characters (apertural type, sculpture, exine surface pattern, pollen shape and pollen fertility) among species of the family Fabaceae. This suggests that apertural type, sculpture, exine surface pattern, pollen shape and pollen fertility could be useful in resolving taxonomic classification associated with grouping of some plants into the same family on the basis of morphological traits alone. Most orthodox family classifications were based on few morphological characters which have been found to be easily influenced by environmental factors. This finding corroborates the reports of Egbe *et al.* (2018), who reported that differences in pollen shape, aperture type, grain arrangement and exine surface pattern are useful in the delimitation of taxa.

Results of pollen shape showed similarities and differences in pollen morphology at the family level. For instance, prolate spheroidal and subprolate shapes were observed among species in the family Fabaceae. The discovery of subprolate shape in the Fabaceae family in this study is contrary to the findings of Jensen (2000) and Egbe *et al.* (2018),who reported only prolate pollen shape for the family Fabaceae. Findings from this study indicate that pollen shape is a good taxonomic tool in delimiting the flowering plants.

Furthermore, oblate spheroidal shapes were observed among the members of the families Asteraceae and Malvaceae. This has been reported earlier by Johan *et al*. (1997). The type of apertural characters observed is synonymous with the findings of El Naggar and Sawady (2008), who reported that evolutionary trend of pollen in flowering plants showed that the primitive angiosperms had acolpate aperture but later advanced to monocolpate, multicolpate and multiporate types. The findings from this study documented triporate (*S. spectabilis*), tricolpate (*C. papaya*), pantoporate (*H. annuus*, *S. acuta* and *T. procumbens*), heterocolpate (*G. aborea*), tricolporate (*C. fistula*, *I. cocinea* and *S. occidentalis*) and tetrad (*M. pudica*) as six (6) apertural types among the ten flowering plants investigated.

Multiple apertural pollen type observed among the studied taxa in the family Fabaceae, *S. spectabilis* (triporate), *C. fistula* (tricolporate), *S. occidentalis* (tricolporate) and *M. pudica* (triporate) is instructive, suggesting that it could be used in taxa delimitation. These findings corroborated the reports of Jensen (2000), who noted that members of Fabaceae were stenopalynous. This study showed that pollen traits could be employed to distinguish members of a family that are difficult to separate morphologically.

The results of exine variation recorded in this study even among members of the same family corroborated the reports of Richard and Josh (2011). They reported the use of external structure of pollen grains in classification. This suggests that exine diversity is a useful tool in resolving taxonomic issues since it varies among species in the same family. There was a high variation in the pollen size of the investigated species. Pollen fertility was very high in all species studied except in *S. spectabilis*. This is probably the reason for the high occurrence of these species in FUNAAB campus. Oyelakin and Ayodele (2011) reported that high pollen fertility could result in high frequency of the species in an environment.

CONCLUSION

Wide variations of pollen characters in this study suggest that pollen characters could be used in resolving taxonomic controversies arising from the use of plant morphological characters alone. This is because plant morphological characters are under the influence of the environment, which affects the phenotypic expression of the plants, and could lead to wrong classification.

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