Leaf Epidermal And Pollen Morphological Studies Of Genus Jatropha L. (Euphorbiaceae) In Nigeria

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Abstract : The predominated plant genus of Jatropha were studied using the epidermal cell morphology and pollen morphology to express more reliable power to its identification aside from the normal flower and other features key arrangement, they all had paralytic stomata type with absence of stomata at the abaxial with no traces of trichomes, J. multifida is having the highest stomata length of 27.5µm with highest stomata with 17.5µm while J. curcas, J. podagrica and J. gossypifolia varies between 15.0 µm in stomata length to 12.5 µm in stomata width. They also have straight to curve anticlinal cell walls. They all possess large grain with exine pattern all croton while only J. multifida has the smallest gemmae size of 2.50 µm and its polar axis and equatorial diameter carries lower parameters of 52.1 µm and 51.6 µm respectively while it may be more affected by environmental factor and that is why the results is good but calls for more genetic expression.

Keywords: Jatropha, morphology, abaxial, anticlinal and paralytic

Introduction

Jatropha is a genus of approximately 175 succulents, shrubs and trees (some are deciduous, like *Jatropha curcas* L.) from the family Euphorbiaceae, they are native to Africa, North America, and the Caribbean (Iwu, 1993) and are now found throughout the tropics. Prominent uses can basically be classified into food and fodder, medicine, hedges, landscape beautification, timber, superstitious use and others; this however does not dispute the fact that some of them are still among the popularly known weed of both the arable farmlands and forest plantation (Akobundu and Agyakwa, 1998). *Jatropha* species are mostly common in Nigerian ecological zones of West Africa (Hutchison and Dalziel, 1963; Keay, 1989). Only nine species of *Jatropha* are common in Africa out of 175 species ever named – *J. podagrica* L.*, J. curcas* L.*, J. gossipifolia* L.*, J. kamerunica,* Pax., *J. chevalieri* Bailie, *J. neriifolia* Muell.*, J. heudelotti* Bail.*, J. atacorensis* A. chev.*, and J. multifida* L. while only four were investigated in the cause of my research work (*J. podagrica* L.*, J. curcas* L.*, J. gossipifolia* L. and *J. multifida* L.). *J. podagrica* L.*, J. curcas* L .and *J. gossipifolia* L. due to its availability in Nigeria and was believed to have been introduced from Portugal (Iwu, 1999). Specifically, *J. curcas* is mostly used in traditional medicine (Iwu, 1993), The genus *Jatropha* belongs to a tree, shrub or herb, occasionally with milky juice and consists of many species, which are widely used to produce the non-edible *Jatropha* oil, for making candles and soap, and as an ingredient in the production of biodiesel and various other natural products (Philip, 2007). Pollen grains have several morphological characters on the exine, which are of diagnostic importance to complement plant identification (Edeoga *et al*., 1998; Edeoga and Ikem, 2002; Mbagwu and Edeoga, 2006; Mbagwu *et al*., 2008). Pollens therefore are used to identify the source of a plant during the analysis of palynological samples in fields such as biostratigraphy, climatology, medicine-alleviation of pollinosis (hay fever-allergenic disease), forensic studies, mellisopalynology, plant evolution, taxonomy and environmental restoration activities Adeonipekun (2007) and Ige, (2009). However, due to high species diversity especially in the tropics, palynologists are unable to identify or differentiate some pollen forms, which could result in the omission of some important indicator species. At present, only few descriptions of pollen grains from some Nigeria plants exist (Ige, 2009), which are scattered over several research projects.

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Materials and method

Plant materials

Herbarium abbreviations follow Holmgren & al. (1990). Fresh leaf samples of four (4) available *Jatropha* species in Nigerian were collected from the FRIN quarters Jericho Hills, Ibadan, Nigeria and the identities were authenticated at (FRIN) and voucher specimen were deposited at the FHI Ibadan.

Leaf epidermal

Portions of about 2–5 cm^2 were cut from the standard median part of the leaf lamina near the mid-rib, fresh leaf and subsequently soaked in con. trioxonitrate (v) acid (HNO3) in capped specimen bottles for about 8–24 hours to macerate the mesophyll, the leaves were scraped with razor blade to separate epidermis. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol, 50 % – 100 %, were added in turn to dehydrate the cells. The preparations were later stained with Safranin “O” in 50 % alcohol for about five minutes before being mounted in glycerine on glass slides. Then epidermal layers (abaxial and adaxial surfaces)were mounted on glass slides with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photomicrographs of the magnification of x400 using Olympus Biological microscope model CX31, fitted with and Olympus E – 330 digital SLR camera through E 330 – ADU 1.2 microscope adapter and measured parameters can be found on Tables 1 and 2 and on plates 1 - 4

Pollen morphology

Pollen morphology was studied using the acetolysis method (Sowunmi, 1973). Fresh pollen-bearing samples were collected and kept in glacial acetic acid vials to preserve them from wilting. The content was put in a numbered plastic centrifuge tube and centrifuged at 4,000 r.p.m for 5 minutes. The supernatant liquid was decanted in one swift movement into a special bottle labeled "Acetolysis waste". Further, about 3ml of Acetolysis mixture was added to each numbered plastic centrifuge tube containing sample (9 parts acetic anhydride to 1 part conc. Sulphuric acid) and heated in a water bath from 70^0C to boiling point, stirred occasionally. The mixture was left in boiling water for three minutes. This hot mixture was centrifuged and decanted into a special bottle, where some water was added and shaked vigorously with the whirl mixer, centrifuged and supernatant decanted. Each sample was mounted in 100% Glycerol on microscopic slides which had been properly labeled with a temporary label containing generic and specific names on the same day prepared while photomicrographs were taken using Olympus Biological microscope with camera attachment measured parameters are provided in Table 3 and on Plates 5 - 9.

Results

Leaf epidermal study and palynological investigations (Pollen analysis) carried out on four *Jatropha* species namely: *J. curcas, J. multifida, J. podagrica* and *J. gossypifolia* as mentioned above.

Table 1 *Epidermal cell features of the genus Jatropha studied.*

TAXA	**Abaxial**				**Adaxial**	
	Epidermal cell length (µm)				**Epidermal cell length (µm)**	
	Cell length	**Cell width**	**Stomata width**	**Stomata lenght**	**Cell length**	**Cell width**
J. curcas L.	32.5-47.5 38.0±1.53	15.0-32.5 26.3±1.75	15.0-17.5 19.5±1.04	7.5-12.5 9.5±0.62	32.5-62.5 51.0±3.03	27.5-40.0 35.50±1.12
J. podagrica L.	30.0-47.5 36.7±1.83	20.0-37.5 26.0±1.97	15.0-30.0 21.2±1.30	5.0-17.5 9.3±1.24	30.0-47.5 37.5±1.83	22.5-40.0 29.00±1.94
J. multifida L.	35.0-40.0 38.37±1.07	20.5-40.00 32.3±1.60	17.5-32.5 24.3±1.66	15.0-20.0 15.7±0.75	32.5-57.5 43.7 ±2.42	30.0-40.0 36.0±1.06
J. gossypifolia L.	30.0-50.0 39.3±1.83	20.0-32.5 27.0±1.96	17.5-25.0 20.3±0.78	5.0-12.5 8.7±0.67	27.5-37.5 33.0±1.22	17.5-27.5 22.05±1.32

Value of the same letter(s) are not significantly different at P<0.05.
All measurements in microns (µm) = *Range*
Mean ± Standard error

Table 2: *Stomata features of the genus Jatropha studied.*

Taxa	**Stomata length (µm)**		**Stomata width(µm)**		**Stomata type**
	Abaxial	**Adaxial**	**Abaxial**	**Adaxial**	
Jatropha curcas L.	15.0-17.5 19.53 ±0.71	Absent	5.0-12.5 9.45±0.70	Absent	Paracytic
J. podagrica L.	15.0-25.0 21.25 ± 0.90	Absent	5.0-17.5 9.25±1.20	Absent	Paracytic
J. multifida L.	25.0-27.5 24.25 ± 1.02	Absent	12.5-17.5 15.70±0.60	Absent	Paracytic

J. gossypifolia L.	17.5-25.0 20.25 ± 1.50	Absent	5.0-12.5 8.75±0.70	Absent	Paracytic

ll measurements in microns (µm) = Range
Mean ± Standard error

Table 3: *Pollen morphological features of the Jatropha species studied*

Taxa	**Polar Axis (µm)**	**Equitorial Diameter (µm)**	**Exine Pattern**	**Exine Sculpture**	**Tectum (µm)**	**Aperture**	**Shape**	**Grain size**
Jatropha curcas	68.75 [90.0±65.0]	58.75 [65.0±50.0]	Croton	Gemmae 3.58 [2.5±4.5]	0.80 [0.5±1.0]	Non-aperturate	Sub-prolate	Large grain
Jatropha Podagrica	55.00 [57.5±52.5]	55.00 [52.0±52.0]	Croton	Gemmae 1.60 [1.25±2.0]	0.45 [0.25±0.75]	Non-aperturate	Oblate spheroidal	Large grain
Jatropha Multifida	52.10 [45.0±57.5]	51.60 [45.0±55.0]	Croton	Gemmae 2.50 [2.0±2.75]	0.50 [0.27±0.92]	Non-aperturate	Oblate spheroidal	Large grain
Jatropha gossypifolia	56.80 [47.5±65.0]	53.40 [43.8±57.5]	Croton	Gemmae 2.25 [2.0±2.75]	0.54 [0.25±0.75]	Non-aperturate	Prolate spheroidal	Large grain

All measurements in microns (µm) = Range
Mean ± Standard error

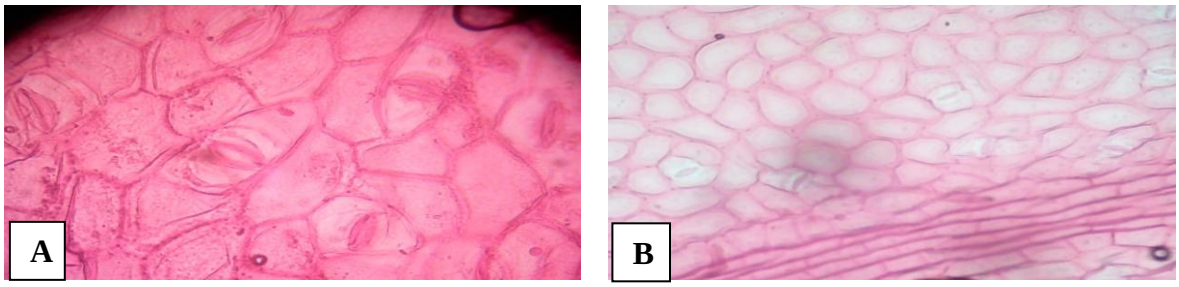

PLATE 1: *Photomicrograph showing (A) the paracytic stomata type and polygonal shape Leaf epidermal cell and (B) the polygonal cell type and irregular cell shape on the abaxial and adaxial surfaces with straight anticlinal wall of Jatropha curcas L. Mag. X400 and Mag. X400 respectively.*

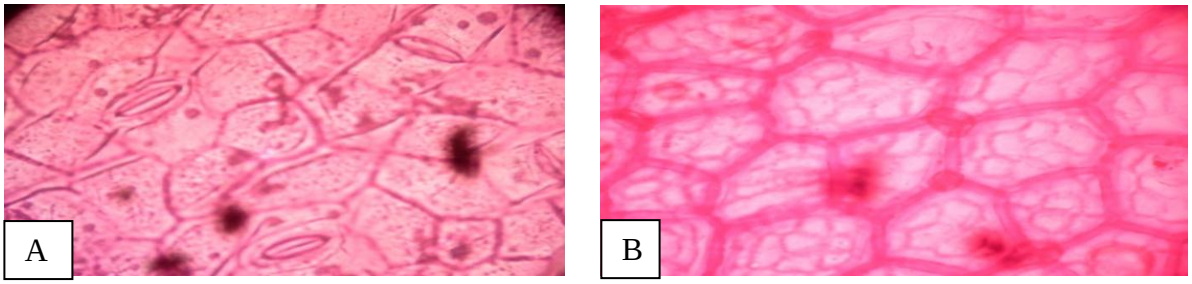

PLATE 2: *Photomicrograph showing (A) the paracytic stomata cell type and (B) polygonal cell type of Jatropha multifida L. on abaxial and adaxial surfaces with straight anticlinal wall Mag. X400 and Mag. X400 respectively.*

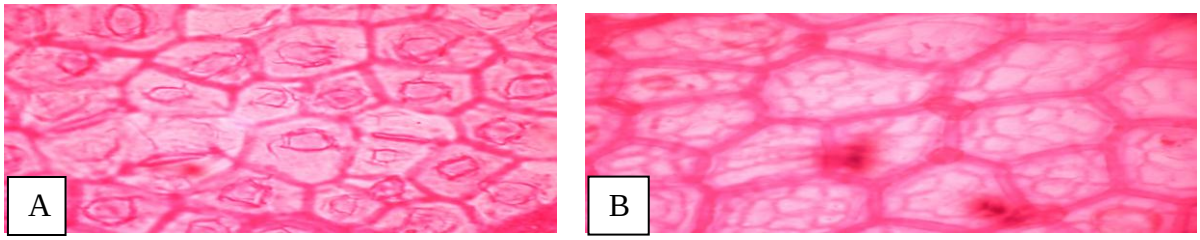

PLATE 3: *Photomicrograph showing (A) paracytic stomata type with polygonal cell shape and (B) the polygonal cell shape of Jatropha podagrica L. on abaxial and adaxial surfaces with straight anticlinal wall Mag. X400 and X400 respectively.*

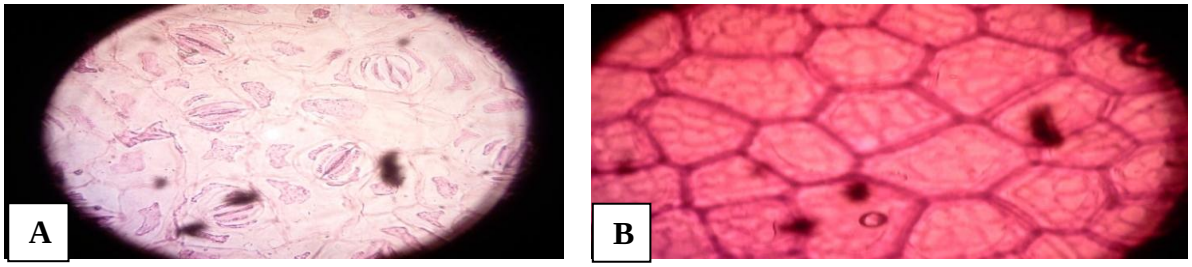

PLATE 4: *Photomicrograph showing (A) paracytic stomata type with curve/irregular cell shape and (B) the polygonal cell shape Jatropha gossypifolia L. on abaxial and adaxial surfaces with straight anticlinal wall Mag. X400 and Mag. X400 respectively.*

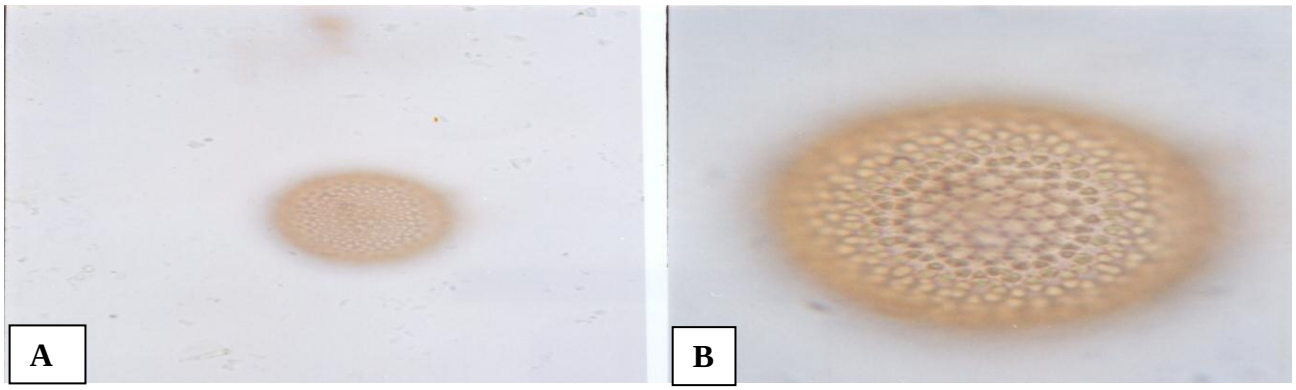

PLATE 5: *Photomicrograph showing (A) the apocolpium view of pollen morphology and (B) dark gemmae arrangement in Hexagonal pattern of Jatropha gossypifolia at Mag. X400 and Mag. X1300 respectively.*

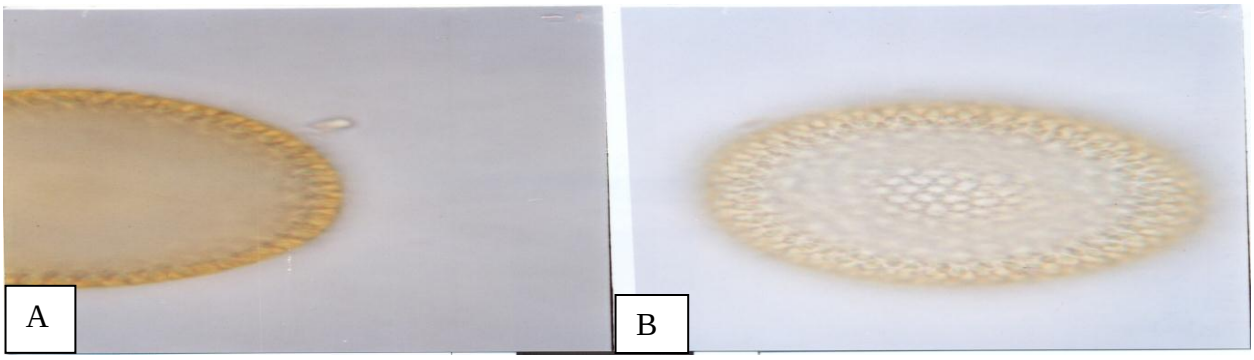

PLATE 6: *Photomicrograph (A)showing a well displayed exine stratigraphy of pollen morphology and (B) well displayed exine pattern at apocolpium of Jatropha podagrica L. at Mag. X1300 and Mag. X1300 respectively.*

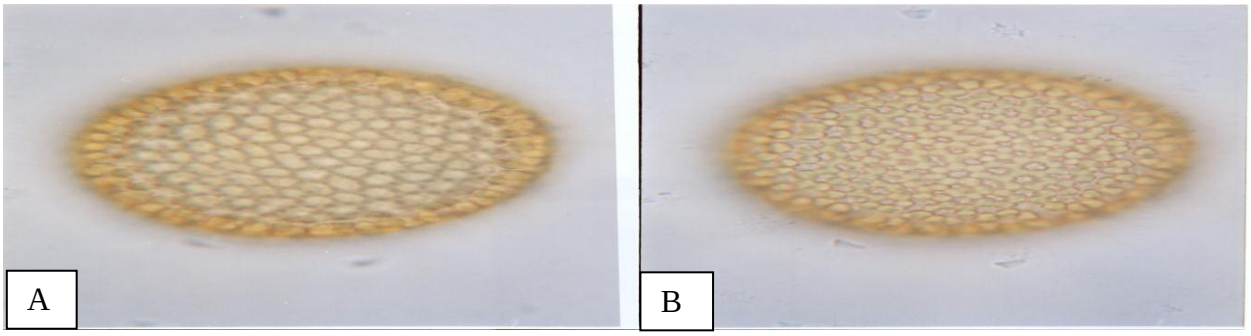

PLATE 7: *Photomicrograph (A) showing a dark croton pattern view of pollen morphology and (B) croton pattern appears light of Jatropha multifida L. at Mag. X1300 and Mag. X1300 respectively.*

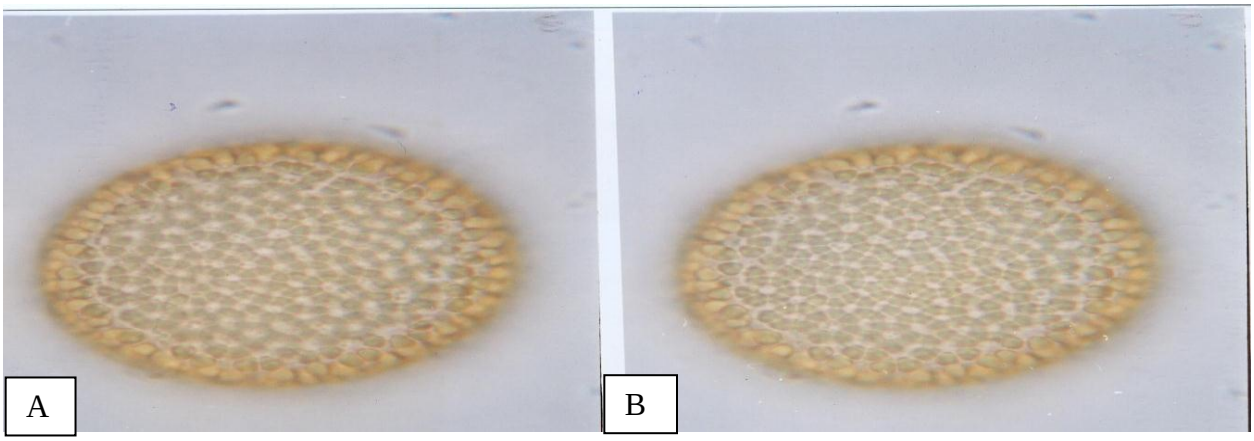

PLATE 8: *Photomicrograph showing (A) the croton pattern connected to each gemmae of pollen morphology and (B) hexagon-heptagon-shaped gemmae of Jatropha multifida L. at Mag. X1300 and Mag. X1300 respectively.*

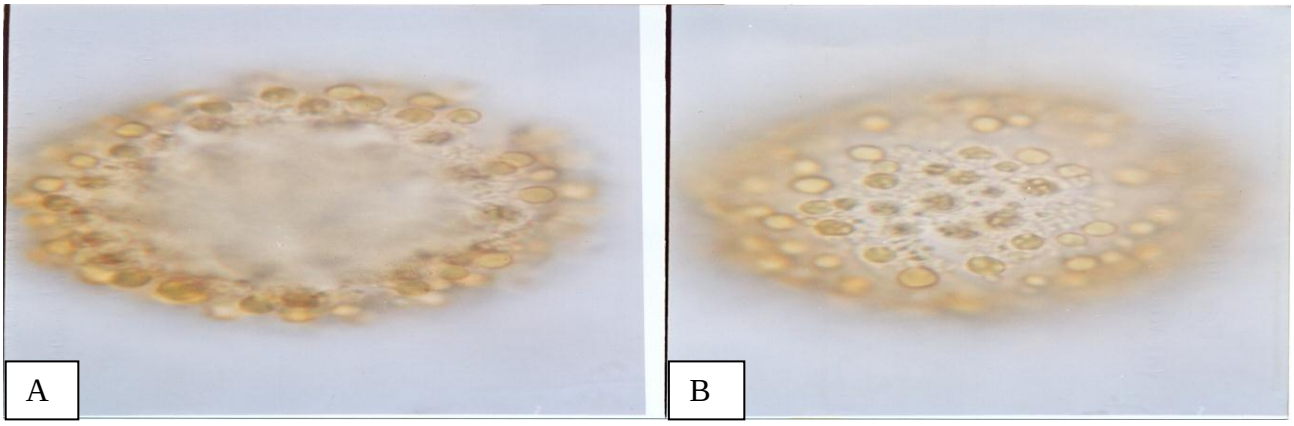

PLATE 9: *Photomicrograph showing (A) the whole grain; not well spaced gemmae on tectum of pollen morphology and (B) exine stratigraphy of Jatropha curcas L. at Mag. X1300 and Mag. X1300 respectively.*

Conclusion

This present study shows that there are remarkable similarities and fair differences among the four species. This present study shows that there are remarkable similarities and the differences among the four species are negligible. The result of quantitative studied on the stomata parameters (Table 2) indicates that *J. curcas*, *J. podorgrica* and *J. gossypifolia* are close with evidence in the stomata length mean values based on the abaxial surface. While *J. multifida* has different stomata width and mean values on the adaxial surfaces with very close values of the mean values of their stomata length. In spite of the fact that, vegetative and floral characters are markedly differentiated in relation to the habitat and pollination mechanisms (Kakkar and Paliwal, 1974), the preceding observations and the summaries of character variation in tables 1 and 2 indicates that the taxonomic application of the diversity of epidermal morphology within the cannot be overemphasized. The cell shape and cell wall pattern are considerably similar within the genera. Taxonomic use of epidermal characters has been reviewed by some workers (Winkinson, 1979, Metcalfe and Chalk, 1979., and Stace, 1980) while the merit and demerit of using these features as taxonomic markers have been discussed by many authors (Olowokudejo, 1993., Ayodele *et al* and Soladoye, 1982). According to Winkinson (1979) the taxonomic significance of the similarity of stomata type in a mature leaf often provides a reliable diagnostic character, especially when the ontogeny of the stomata is unknown or different. Although, absence of trichomes in some species is of little importance in distinguishing them Metcalfe and Chalk (1979) stated that trichome frequency and size are environmentally controlled, straight or curved walls are characteristic of species growing in drier conditions. The epidermal cell size, stomata sizes are variable and overlapping between these species and paracytic stomata types are seen tallied with Olowokudejo (1993). Palynological evidence obtained from this study in Table 3 shows the sizes of *Jatropha* pollen to range from 52.1/51.6µm to 68.75/58.75µm (PA/ED) the grains are large grains. The largest and smallest are *Jatropha curcas* and *J. multifida* respectively. All the pollen has gemmae (sculpturing elements) on the tectum; the exine pattern in all the pollen is croton pattern. A closer examination of the exine pattern shows that the croton pattern is made up of a set of gemmae (six or seven) which are arranged to produce a regular polygon (hexagon or heptagon) pattern. This regular pattern is not well displayed in *J. curcas*. In other words, the gemmae are not arranged in any regular pattern in J. *curcas*. In all the *Jatropha* species studied, the shape of each gemma in *J. curcas* is round or spherical shape; in *J. multifida* and *J. gossipifolia*, the shape of the gemma is triangular with rounded ends and in *J. podagrica*, the shape is mostly rectangular. The gemmae are clustered on the tectum of two of the four species: *Jatropha multifida* and *J. podagrica*. Those on *J. curcas* and *J. gossypifolia* are spaced with some gemmae standing alone. Numerous round-headed bacules are clearly seen in the center of each hexagon-shaped pattern, and under each gemmae. The bacules are more numerous in *J. curcas* and fewer in *J. gossypifolia*. Usually, there are at least three bacules under each gemmae with no aperture observed among the four species**.**

In conclusion, based on the palynological studies, the four *Jatropha* species have some features that allows for their fair differentiation which could be better to implore biotechnological application to solve the genetic differences.

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