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MORPHOLOGICAL ANALYSIS OF LEAF EPIDEMIS AND POLLEN IN *JATROPHA* SPECIES FROM NIGERIA

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ABSTRACT

The genus *Jatropha* was investigated through epidermal cell and pollen morphology to enhance species identification beyond conventional floral and vegetative characteristics. Four species of *Jatropha* in Nigeria was put into considerations on this study (*Jatropha curcas*, *Jatropha multifida, Jatropha. podagrica*, and *Jatropha. gossypifolia*). All species examined exhibited paracytic stomata confined to the adaxial leaf surface, with the abaxial side being devoid of stomata or trichomes. Among the studied species, *Jatropha. multifida* displayed the largest stomatal length (27.5 µm) and width (17.5 µm), whereas *Jatropha. curcas*, *Jatropha. podagrica*, and *Jatropha. gossypifolia* showed stomatal lengths ranging from 15.0 µm to widths of 12.5 µm. The anticlinal cell walls were generally straight to curve across all species. Pollen grains in the genus were predominantly large, with an exine pattern characteristic of the Croton subfamily. *Jatropha. multifida* had the smallest gemmae size (2.50 µm) and exhibited the lowest polar axis (52.1 µm) and equatorial diameter (51.6 µm) measurements. These findings suggest that *Jatropha* species may be highly susceptible to environmental influences, with morphological traits, such as epidermal and pollen structures, varying significantly depending on growth conditions. Environmental factors like temperature, humidity, and soil quality likely impact the development of these traits, leading to observable differences between species. This highlights the value of using epidermal and pollen morphology to distinguish *Jatropha* species. However, the results also underscore the need for further research on genetic expression to understand the genetic mechanisms driving species variability. A deeper understanding of these mechanisms would improve species identification and support more informed conservation and cultivation practices.

Keywords: *Jatropha*, Morphology, Abaxial, Anticlinal, Paracytic

INTRODUCTION

The genus *Jatropha* comprises approximately 175 species of succulents, shrubs, and trees, including the deciduous *Jatropha curcas L.*, belonging to the family Euphorbiaceae. These plants are native to Africa, North America, and the Caribbean (Iwu, 1993) but have now spread throughout the tropics. *Jatropha* species serve various purposes, including use in biofuel, food and fodder, medicine, hedges, landscape beautification, timber, and even superstitious practices(Shahla Abdulrazzaq Basheer *et al* 2002; Mafikeng *et al*. 2022; Rahman, *et al.* 2021; Pandey, *et al.* (2023). However, several species are also considered significant weeds in both arable farmlands and forest plantations (Akobundu and Agyakwa, 1998; Leon-Martinez, *et al*. 2023). In West Africa, particularly Nigeria, *Jatropha* species are prevalent across ecological zones (Hutchinson and Dalziel, 1963; Keay, 1989). Of the 175 species recorded globally, only nine are common in Africa: *J. podagrica L., J. curcas L., J. gossypifolia L., J. kamerunica Pax., J. chevalieri Bailie, J. neriifolia Muell., J. heudelotii Bail., J. atacorensis A. Chev.*, and *J. multifida L.*. This research focuses on four species—*J. podagrica L., J. curcas L., J. gossypifolia L.*, and *J. multifida L.*—which are abundant in Nigeria and believed to have been introduced from Portugal (Adebusuyi, *et al 2001*.; Iwu, 1999). Among these, *J. curcas* holds particular importance in traditional medicine and in the production of biodiesel (Iwu, 1993; Adebusuyi*et al*., 2021; Singh & Sharma (2021; Sarabia *etal*., 2022). The genus comprises trees, shrubs, and herbs, occasionally with milky sap, and is valued for producing non-edible oil used in candles, soap, biodiesel, cosmetics and various natural products (Pandey, V. C., *et al*. 2023; Al-Khayri *et al*., 2022; Philip, 2007; Noelly *et al 2020*).

Pollen morphology plays a critical role in plant identification and taxonomy due to its diagnostic value, particularly the morphological features of the exine (Edeoga*et al*., 1998; Edeoga and Ikem, 2002; Mbagwu and Edeoga, 2006; Mbagwu *et al*., 2009). Pollen grains are widely used in palynological studies, including biostratigraphy, climatology, allergy mitigation, forensic science, mellisopalynology, and environmental restoration (Adeonipekun, 2007; Ige, 2009). However, high species diversity in the tropics poses challenges for palynologists in differentiating some pollen forms, potentially leading to the omission of important indicator species.(Zhang, L., *et al*.2020).Despite the importance of pollen analysis in taxonomy and environmental studies, descriptions of pollen grains from Nigerian plants remain limited and are scattered across various research works (Ige, 2009). This study aims to contribute to the existing knowledge by examining the pollen and epidermal morphology of selected *Jatropha* species, providing critical insights for plant identification and species differentiation.

MATERIALS AND METHODS

Herbarium abbreviations follow Holmgren *et al*. (1990). Fresh leaf samples of four *Jatropha* species from Nigeria were collected from the FRIN quarters, Jericho Hills, Ibadan. The species' identities were authenticated at the Forestry Research Institute of Nigeria (FRIN), and voucher specimens were deposited in the FHI herbarium, Ibadan.

Leaf Epidermal Preparation

Leaf segments measuring approximately 2–5 cm² were excised from the standard median region of the lamina near the midrib. Fresh samples were soaked in concentrated nitric acid (HNO_3) in capped specimen bottles for 8–24 hours to soften and macerate the mesophyll tissue. The softened leaves were scraped with a razor blade to isolate the epidermis. Remaining tissue debris was meticulously removed using a fine-hair brush, and the epidermal layers were rinsed in multiple changes of water.

The epidermal specimens were dehydrated by sequential immersion in ethanol concentrations ranging from 50% to 100%. The dehydrated samples were stained with Safranin O in 50% ethanol for approximately five minutes, mounted in glycerine on glass slides, and covered with cover slips. Both abaxial and adaxial epidermal layers were mounted with their uppermost surfaces facing upward. Nail varnish was applied to seal the edges of the coverslips, preventing dehydration.

The slides were appropriately labelled and examined under a light microscope. Photomicrographs were taken at 400x magnification using an Olympus Biological Microscope Model CX31, equipped with an Olympus E-330 digital SLR camera and an E-330-ADU 1.2 microscope adapter. Quantitative parameters are presented in Tables 1 and 2, while representative images are shown in Plates 1–4.

Pollen Morphology Analysis

Pollen morphology was assessed following the acetolysis (To breakdown the pollen cell) method described by Sowunmi (1973). Fresh pollen-bearing samples were collected and preserved in vials containing glacial acetic acid to prevent desiccation. The samples were transferred to numbered plastic centrifuge tubes and centrifuged at 4,000 rpm for five minutes. The supernatant was carefully decanted into an "Acetolysis Waste" container.

Approximately 3 ml of an acetolysis mixture (9 parts acetic anhydride to 1 part concentrated sulfuric acid) was added to each tube. The mixture was heated in a water bath at 70°C until boiling, with intermittent stirring. Boiling was maintained for three minutes, after which the tubes were centrifuged, and the supernatant was discarded. The pellet was washed by adding water, shaking vigorously with a whirl mixer, and centrifuging again. The supernatant was subsequently removed.

Each pollen sample was mounted in 100% glycerol on labelled microscopic slides. Temporary labels included the genus and species names. Photomicrographs were captured using an Olympus Biological Microscope equipped with a camera attachment. Measured parameters are detailed in Table 3, and representative images are presented in Plates 5–9.

RESULTS AND DISCUSSION

Leaf epidermal and palynological (pollen) analyses were carried out on four *Jatropha* species—*J. curcas*, *J. multifida*, *J. podagrica*, and *J. gossypifolia*—as outlined in the methodology.

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

Taxa	Abaxial Epidermal cell length (µm)				Adaxial Epidermal cell length (µm)	
	Cell length	**Cell Width**	**Stomata Width**	**Stomata Length**	**Cell length**	**Cell Width**
J. curcas L.	32.50-47.50 38.00±1.53	15.00-32.50 26.30±1.75	15.00-17.50 19.50±1.04	7.50-12.50 9.50±0.62	32.50-62.50 51.00±3.03	27.50-40.00 35.50±1.12
J. podagrica L.	30.00-47.50 36.70±1.830	20.00-37.50 26.0±1.970	15.00-30.00 21.20±1.30	5.00-17.50 9.30±1.24	30.00-47.50 37.50±1.83	22.50-40.00 29.00±1.94
J. multifida L.	35.00-40.00 38.37±1.07	20.50-40.00 32.30±1.60	17.50-32.50 24.30±1.66	15.00-20.00 15.70±0.75	32.50-57.50 43.70 ±2.42	30.00-40.00 36.00±1.06
J. gossypifolia L.	30.00-50.00 39.30±1.830	20.00-32.50 27.0S0±1.96	17.50-25.00 20.30±0.78	5.00-12.50 8.70±0.67	27.50-37.50 33.00±1.22	17.50-27.50 22.05±1.32

Value of the same letter(s) are not significantly different at P<0.05
All measurement in micron = Range ÷ Mean ± Standard deviation

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

Taxa	Stomata length (µm)		Stomata width(µm)		Stomata type
	Abaxial	**Adaxial**	**Abaxial**	**Adaxial**	
J. 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

All measurement in micron = Range ÷ Mean ± Standard deviation

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

Taxa	Polar Axis (µm)	Equitorial Diameter (µm)	Exine Pattern	Exine Sculpture Gemmae	Tectum (µm)	Aperture	Shape	Grain size
J. curcas L.	68.75 90.0±65.0	58.75 65.0±50.0	Croton	3.58 2.5±4.5	0.80 0.5±1.0	Non	Subprolate	Large
J. podagrica L.	55.00 57.5±52.5	55.00 52.0±52.0	Croton	1.60 1.25±2.0	0.45 0.25±0.75	Non	Oblate spheroidal	Large
J. multifida L.	52.10 45.0±57.5	51.60 45.0±55.0	Croton	2.50 2.0±2.75	0.50 0.27±0.92	Non	Oblate spheroidal	Large
J. gossypifolia L.	56.80 47.5±65.0	53.40 43.8±57.5	Croton	2.25 2.0±2.75	0.54 0.25±0.75	Non	Prolate spheroidal	Large

All measurement in micron = Range ÷ Mean ± Standard deviation

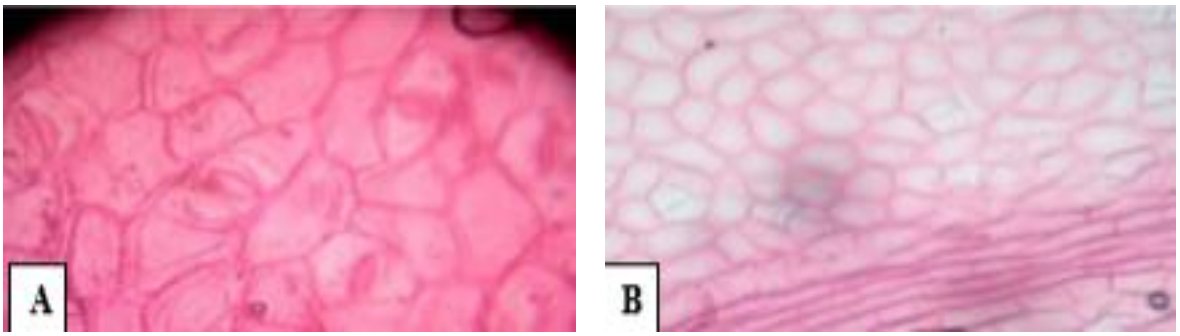

Plate 1: Photomicrographs showing (A) the paracytic stomata type and polygonal-shaped leaf epidermal cells, and (B) the polygonal cell type with irregular cell shapes on the abaxial and adaxial surfaces, featuring straight anticlinal walls of *Jatropha curcas* L. Magnification: X400 for both.

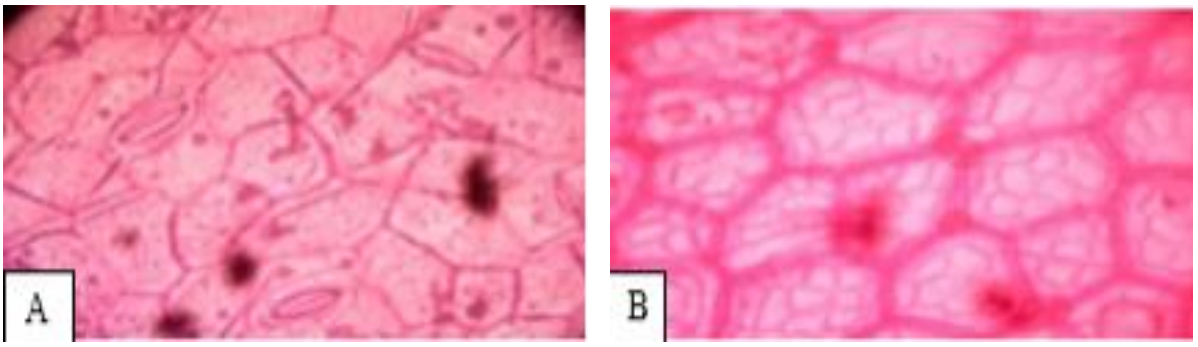

Plate 2: Photomicrographs showing (A) the paracytic stomata type, and (B) the polygonal cell type of *Jatropha multifida* L. on the abaxial and adaxial surfaces, with straight anticlinal walls. Magnification: X400 for both

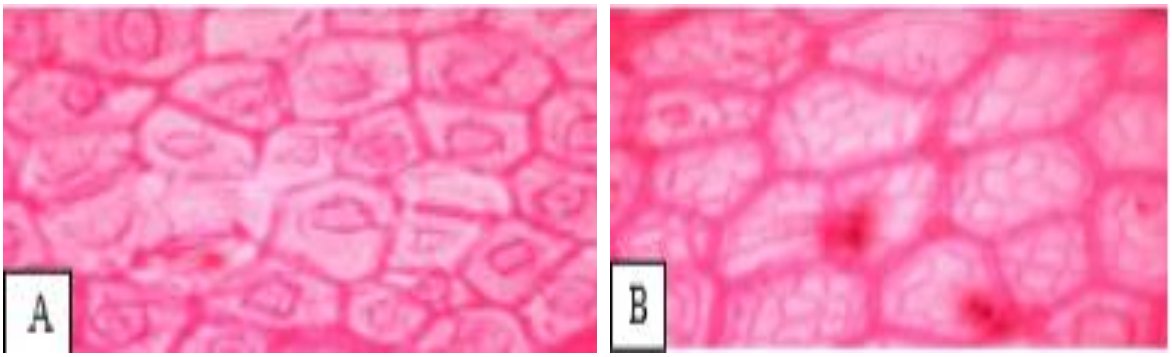

Plate 3: Photomicrographs showing (A) paracytic stomata type with polygonal cell shapes, and (B) polygonal cell shapes of *Jatropha podagrica* L. on the abaxial and adaxial surfaces, with straight anticlinal walls. Magnification: X400 for both

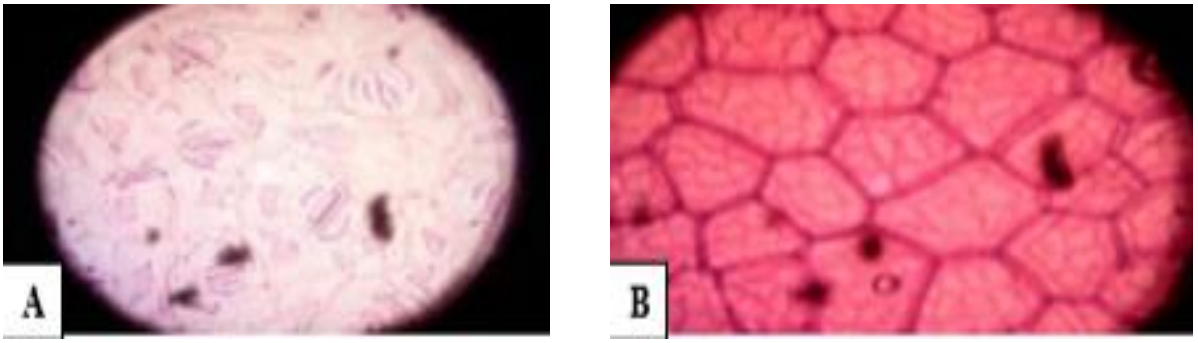

Plate 4: Photomicrographs showing (A) paracytic stomata type with curved/irregular cell shapes, and (B) polygonal cell shapes of *Jatropha gossypifolia* L. on the abaxial and adaxial surfaces, with straight anticlinal walls. Magnification: X400 for both

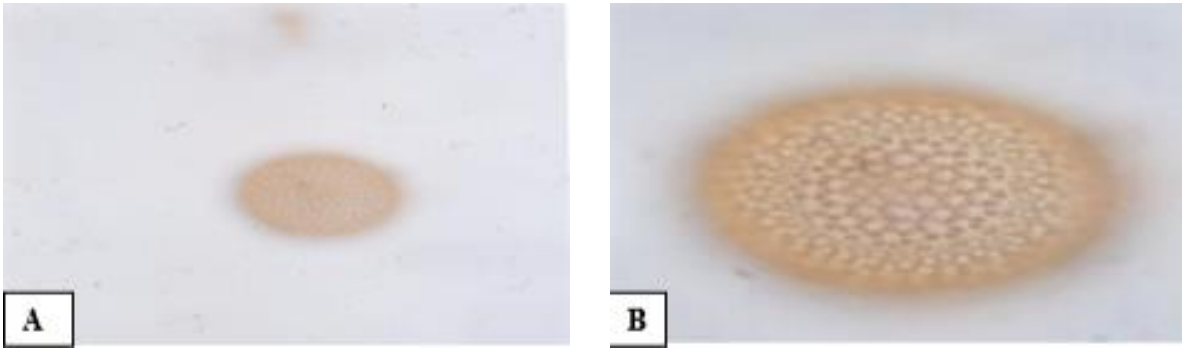

Plate 5: Photomicrographs showing (A) the apocolpium view of pollen morphology, and (B) the dark gemmae arrangement in a hexagonal pattern of *Jatropha gossypifolia*, at magnifications of X400 and X1300, respectively

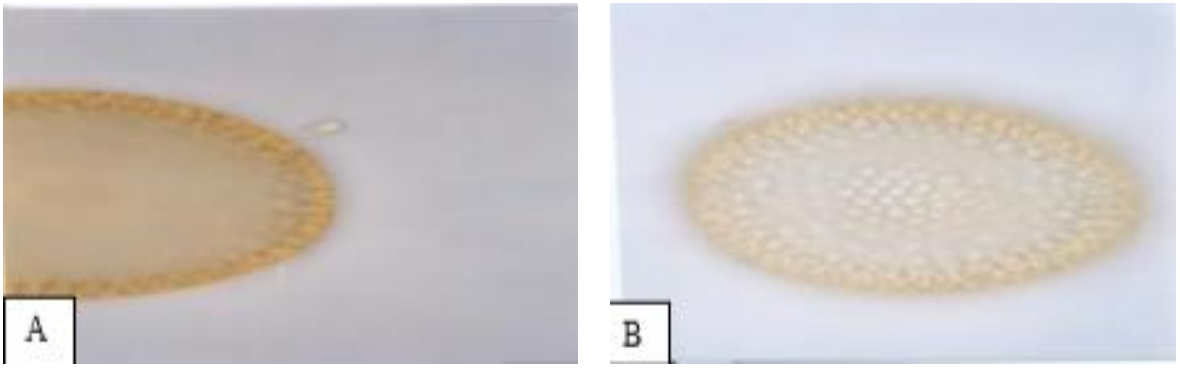

Plate 6: Photomicrographs showing (A) the well-displayed exine stratigraphy of pollen morphology, and (B) the well-displayed exine pattern at the apocolpium of *Jatropha podagrica* L., at magnifications of X1300 for both

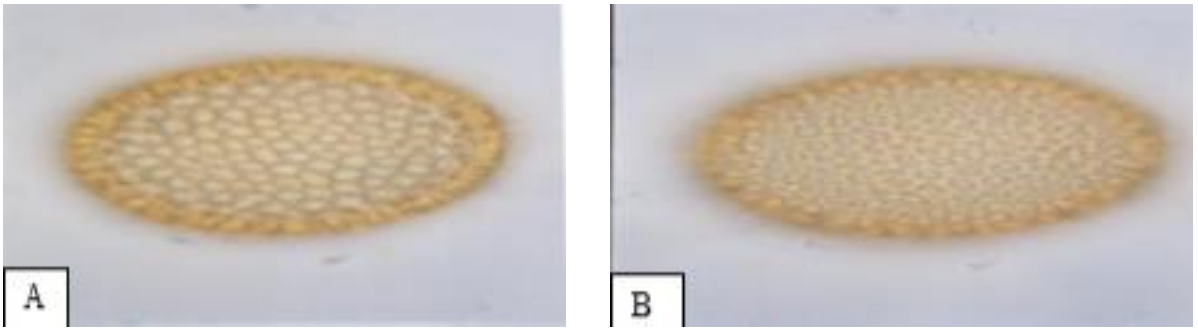

Plate 7: Photomicrographs showing (A) a dark croton pattern view of pollen morphology, and (B) a light croton pattern of *Jatropha multifida* L., at magnifications of X1300 for both

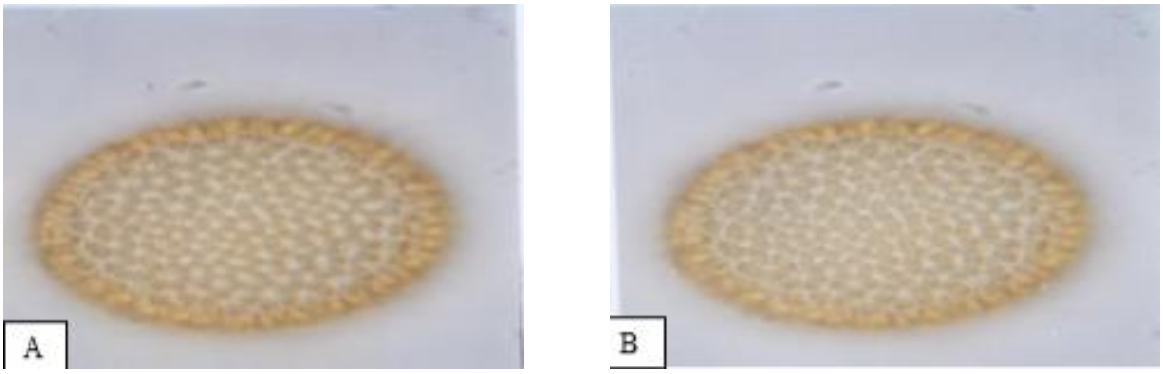

Plate 8: Photomicrographs showing (A) the croton pattern connected to each gemmae of pollen morphology, and (B) hexagonal-heptagonal-shaped gemmae of *Jatropha multifida* L., at magnifications of X1300 for both

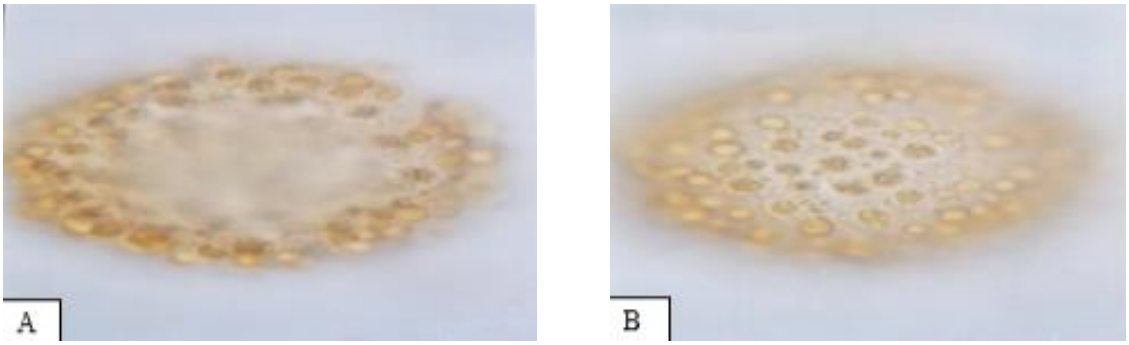

Plate 9: Photomicrographs showing (A) the entire grain with poorly spaced gemmae on the tectum of pollen morphology, and (B) the exine stratigraphy of *Jatropha curcas* L., at magnifications of X1300 for both

This study reveals notable similarities and some differences among the four *Jatropha* species examined. Quantitative analysis of stomatal parameters (Table 2) indicates that *J. curcas*, *J. podagrica*, and *J. gossypifolia* share similar stomatal length values on the abaxial surface, while *J. multifida* differs in stomatal width on the adaxial surface, although its stomatal length values are comparable to those of the other species.

Despite distinct differences in vegetative and floral traits, which are influenced by habitat and pollination mechanisms (Kakkar & Paliwal, 1974), the observed patterns of epidermal variation (summarized in Tables 1 and 2) highlight the taxonomic importance of epidermal morphology. The similarities in cell shape and cell wall patterns within the genus are noteworthy, underscoring the value of epidermal features in systematic studies. Previous research (Wilkinson, 1979; Metcalfe & Chalk, 1979; Stace, 1980) has documented the role of epidermal characters in taxonomy, emphasizing their utility and limitations as diagnostic markers (Olowokudejo, 1993; Ayodele *et al*., 1982). According to Wilkinson (1979), the consistency of stomatal type in mature leaves serves as a reliable diagnostic characteristic, particularly when stomatal ontogeny is unknown or variable. While the absence of trichomes in some species is not a major distinguishing feature, Metcalfe and Chalk (1979) observed that trichome size and frequency are influenced by environmental conditions, and that straight or curved cell walls are often associated with species in drier habitats. The variability in epidermal cell size and stomatal dimensions observed in this study, coupled with the predominance of paracytic stomata types, aligns with the findings of Olowokudejo (1993) based on environmental factors.

Palynological analysis (Table 3) reveals that pollen grain sizes in *Jatropha* range from 52.1/51.6 µm to 68.75/58.75 µm (polar axis/equatorial diameter), with *J. curcas* having the largest grains and *J. multifida* the smallest. All four species exhibit croton-patterned exines with gemmae (sculpturing elements) on the tectum. The croton pattern consists of six or seven gemmae arranged in a regular hexagonal or heptagonal configuration, although this pattern is less defined in *J. curcas*, where the gemmae appear more randomly distributed. Gemmae shape also varies; round or spherical in *J. curcas*, triangular with rounded edges in *J. multifida* and *J. gossypifolia*, and predominantly rectangular in *J. podagrica*. The gemmae are clustered on the tectum in *J. multifida* and *J. podagrica*, while in *J. curcas* and *J. gossypifolia*, they are more spaced, with isolated gemmae observed in some areas. Additionally, round-headed bacules are found at the center of each hexagonal pattern, beneath each gemma. Bacule counts are highest in *J. curcas* and lowest in *J. gossypifolia*, with at least three bacules observed under each gemma across all species. Notably, no apertures were identified in the pollen grains of any of the species.

CONCLUSION

In conclusion, the palynological and epidermal characteristics of the four *Jatropha* species present distinct morphological markers that facilitate their differentiation. These results highlight the potential for leveraging biotechnological methods to further investigate and address the genetic variations among these species. Such approaches could play a crucial role in optimizing their utilization in various applications, as well as in promoting their conservation and sustainable management for future research applications.

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