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Original Article

Foliar epidermal anatomy and pollen morphological studies of *Mucuna pruriens* (Linn.) DC. and *Mucuna poggei* Taub. in Jos, Nigeria

OPEN ACCESS

Kehinde Adegoke ADENIJI*, Luke Temitope SOYEWO & Adewale Solomon ISHOLA

Forest Conservation and Protection, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria

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ABSTRACT

As a result of confusion in identification between *Mucuna pruriens* and *Mucuna poggei*, a study was carried out on the taxonomic characters with the aim to provide constant and reliable taxonomic character for easy identifications through foliar anatomy and palynological studies. The leaves of the two species were cut into the required sizes and put into the concentrated trioxonitrate (v) acid for 2 hours; the epidermises were removed with a forceps pass though different solutions to harden it and to remove excess stains and mounted on slides and observed under the microscope, under different magnifications. The pollen morphological characters were observed, measured and reported. The morphological component was analyzed quantitatively and qualitatively by standard methods. Anomocytic and paracytic stomatal types were found in both species studied but anisocytic stomata types was recorded in *M. pruriens* in addition to prevalent stomata type. Long Unicellular, non glandular trichomes were observed in both surfaces of the two species but longer in *Mucuna pruriens.* There was a distinctive variation in the number of stomata present on both surfaces of species studied, leading to perfect identification when dealing with fragmentation Palynologyical evidence obtained from this study shows the naturalness of the *Mucuna* species with their prolate-spheroidal grain type. It was recommended that molecular studies should be employed to separate this similar species.

K E Y W O R D S : *Anatomy, Mucuna, Species identification, Taxonomy*

INTRODUCTION

The genus Mucuna belongs to the Fabaceae family sub family Papilionaceae. It also includes approximately 150 species of annual and perennial legumes (Schrire 2005). The highest diversity of the genus occurs in Asia (68 taxa), followed by Oceania (34 taxa), the Americas (25 taxa), and Africa (19 taxa). *Mucuna sloanei* Fawc. & Rendle, occurs in America, Hawaii, and Africa*; M. gigantea* (Willd.) DC., found in Africa, Asia, and the Pacific Islands; while *M. pruriens* (L.) DC. distributed across the entire tropical region. (Garcia and Fragoso, 2003.) In Nigeria, the genus *Mucuna* is represented by six species (Soladoye and Lewis, 2003). Out of 6 species, 2 species with similar morphology have been mostly used in ethnomedicine; *Mucuna pruriens* (L.) Dc. and *Mucuna poggei* Taub. *Mucuna pruriens* commonly known as cow-age or cowitch or velvet bean in English, is also called Agbala in Igbo language, the Yoruba called it Werepe/Yerepe and it is known as Karara in Hausa.

*Corresponding author: obalomo2016@gmail.com; +2347035081157

*Mucuna poggei*is known as "horse eye bean" and other local names in respect to different tribes and ethnic groups. It is called "agbara" by Igbos; "matara" by Hausas; "igbekpe" in Benin. All parts of *M. pruriens* possess valuable medicinal properties and it has been investigated in various contexts, including for its anti-diabetic, aphrodisiac, anti-neoplastic, anti-epileptic, and anti-microbial activities (Sathiyanarayanan *et al.*, 2007). Anatomical data have been used as good tools at all levels of the taxonomic hierarchy, as well as for identification and assessment of the taxonomic relationships among taxa of the flowering plants (Stuessy, 1990.) Anatomy describes the internal structure of plants and is considered as a source of correct identification of taxa. Anatomy centers on the spatial arrangement of the dermal, ground, and vascular tissue systems (Nancy & Dengler, 2002). Similarly, foliar epidermal microscopic features like shape of epidermal cell, type of stomata, presence or absence of pubescence and cell wall thickness are also considered as useful tools for correct taxa identification and their phylogenetic relationship with other taxa (Babalola & Victoria, 2009). Pollen morphology is conducted as an aid to the morphological study and a significant tool for modern taxonomist for proper identification of species. Pollen characters are useful in solving complicated problems of interrelationships between various taxa and assessment of their status in the classification, particularly with reference to the families, subfamilies, tribes, genera, species, and subspecies. Mature pollen grain size, exine sculpturing, and number of pores are the most distinctive features (Klimko *et al.*, 2000). Palynological data has been useful at generic and specific level (Perveen and Qaiser, 2004). The aim of this study was to provide constant palynological and folia anatomical characters for easy identification of this taxa even when only fragmentary portion of either leaf or flower are available.

MATERIALS AND METHODS

Matured fresh samples of leaves and pollen were collected from Federal College Forestry, Jos and Mazah village, Jos North, Local Government Plateau State. Plateau State is located in Northern savannah and it is situated between 9° 56' 47.519" N and longitude 8° 53' 31.412" E with average elevation of about 1250m above sea level and stands at height of about 600m above the surrounding plains Jos temperature ranges between 21°C to 25°C. The climate of Jos is cool to its high altitude. The main annual rainfall is 1.26mm. It has increase relative humidity from March to October and decrease from November to April (Olowolafe, 2002 and Muhammad *et al* (2014) Field note was carried along for recording plant-related descriptions. The plants collected were identified and authenticated in Forest Herbarium Ibadan (FHI) and the specimens were deposited in Federal College of Forestry Jos Herbarium (FCFJ).

Plate 1: The leaves and fruits morphlogical characters of *Mucuna poggei*

Plate 2 The leaves and fruits morphlogical characters of *Mucuna puriens*

Anatomical Studies Light Microscopy (Lm) and Pollen Morphology Preparation

The leaves of the two species were cut to the required sizes and immersed in concentrated trioxonitrate (V) acid for 2 hours. The epidermises were then carefully removed using forceps and subjected to various solutions for hardening and excess stain removal. The samples were mounted on slides and observed under a microscope at different magnifications. The width of the epidermal cells was measured at their widest point using an eyepiece micrometer. Statistical analyses of each quantitative character were performed for all taxa (Nwankwo & Ayodele, 2017; Chukwuma *et al*, 2017; Chukwuma *et al*., 2023).

Pollen morphology was studied using acetolysis method as described by Erdtman (1969) and 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 rpm for 15 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 700C 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 shaken 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 of the magnification of x40 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 Plate 6.

$$All\ measurements\ in\ microns = Range/Mean \pm Standard\ deviation \quad (1)$$

RESULTS

Light Microscopic Study (LMS) Tables 1 and 2. Plates 3 and 4 show the results of both qualitative and quantitative anatomical characters of the leaf epidermal characters of genus studied. Leaf epidermises revealed that the shape of the epidermal cells on both surfaces of *Mucuna species* were irregular while data collected can be found in Table 2. Meanwhile, palynological studies show a distinctive result in Table 3 and Plate 3, typical pollen grains are shown in Plate 5. The leaves were amphistomatic having stomata on both surfaces. Three types of stomatal complexes were observed, they are paracytic, anomocytic and anisocytic. On the abaxial and adaxial surfaces, Paracytic, Anomocytic and Anisocytic stomatal complexes were observed in *M. pruriens*. Anomocytic and paracytic stomatal complexes were observed in both surfaces of *Mucuna poggei*. Trichome was present on both surfaces of mucuna species studied.

Table 1: Qualitative features in the epidermal morphology of *Mucuna species*

Taxa	Leaf surface	Stomatal types	Epidermal cell shape	Anticlinal wall pattern	Trichome Type
Mucuna pruriens	Abaxial	Anomocytic, paracytic Anisocytic	Irregular	Wavy/Sinuate	Long Unicellular, non Glandular
	Adaxial	Anomocytic, paracytic Anisocytic	Irregular	Wavy/sinuate	Long Unicellular, non Glandular
Mucuna poggei	Abaxial	Anomocytic Paracytic	Irregular	Wavy/sinuate	Short Unicellular, non Glandular
	Adaxial	Anomocytic Paracytic	Irregular	Wavy/sinuate	Short Unicellular, non Glandular

Anticlinal walls are sinuate in the both surfaces of *Mucuna species* (Plate 3 A -D) The number of stomata per unit area is always higher on the lower surfaces than on the upper surfaces The number of stomata ranges from 6-20 on the abaxial surface *of M. puriens*, 4 -15 on the adaxial suface while in M. poggei, it ranges from 0-15 and 0-4 in abaxial and adaxial surfaces, respectively. The paracytic and anomocytic stomata type are prominent to both species while anisocytic is in addition to M. pruriens both leaves are amphistomatic, having stomata on both surfaces. (Plate 3, B and D Table 2). *M. prurien* has a universal distribution of stomata on abaxial and adaxial surfaces; while *M. poggei* has scantily distributed stomata on adaxial surface (Plate3A).

Table 2: Quantitative parameter of mucuna species studied

Species	Epidermal cell length (µm)	Epidermal cell width (µm)	Stomata length (µm)	Stomata width (µm)	No. of stomata per view	No. of epidermal cell per view	No. of Trichome	Trichome length (µm)	Trichome width (µm)
M. poggei (AB)	22.2-33.1 27.19 ±4.11	10.4-17.0 14.22±2.7	11.6-16.6 12.69±5.05	8.0-10.41 9.6±0.8	8-25 13.4±5.8	28-60 41.1±12.59	1-10 7.3±3.7	101-181 129.4±29	7.2-11.4 9.59±1.7
AD	32.2-49.52 43.1± 6.53	14.86-31 24.5±6.0	11.6-16.6 14.52± 1.99	8.0-10.44 9.6±0.8	0-2 1.1±	28-60 41.1±12.59	0-7 2±2.26	140-155 149.5±7.1	11.44-17.614.52
M.Pruriens	50-75.5 43.1±6.5	21.2-61.5 24.5±6.0	20-29 14.5±1.99	18-21 9.6±0.8	4-15 1.1±1.19	35-70 41.1±12.5	2-7 2±2.26	85.5-193 149.5±7.14	7.0812.56 14.6±2.6
AD AB	36.5-65 60.5±10.1	26.5-38.50 43.29±135	14-26 25.2±3.2	11.5-2.5 19.3±1.3	3-15 9.4±3.90	24-6 50.80±12.3	3-9 5.80±1.6	82.90-206.26 140.2±432	10-11.44 8.97±1.99

Table 3: Pollen features of the mucuna species studied

Taxa	Polar axis (µm)	Equiatorial diameter (µ)	Exine Thickness (µm)	Length of colpus (µm)	Width of colpus(µm)	Exine pattern	Pollen Shape	P/E
M.pruriens	52.5-60 56.6±0.86	50 -72.5 60.28±2.68	2 - 2.5 2.30±0.09	47.5-57.5 50.1 ±0.78	7.5- 12.5 9.38±12.75	Granulate reticulate	Prolate Spheroidal	0.93
M.poggei	60-65 63.63±0.53	57.5-61.00 60.29±0.76	2.2-2.5 2.44±0.04	40- 45 43.42±0.60	Small 12.50-15.50)	Granulate reticulate	Prolate Spheroidal	1.05

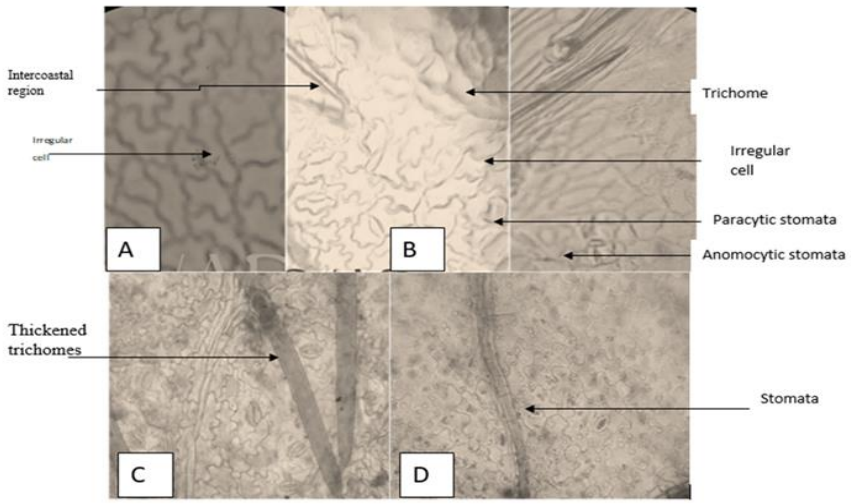

Plate 3. Photomicrographs (x400) of leaf surfaces of the Mucuna

A. Adaxial surface of Mucuna poggei showing Irregular and sinuate anticlinal wall, B. Abaxial surface of Mucuna poggei showing irregular cell shape and sinuate anticlinal wall, C. Adaxial surface of Mucuna pruriens showing paracytic stomata and sinuate anticlinal wall, D. Abaxial surface of Mucuna pruriens showing paracytic stomata and irregular cells. Mag x 400

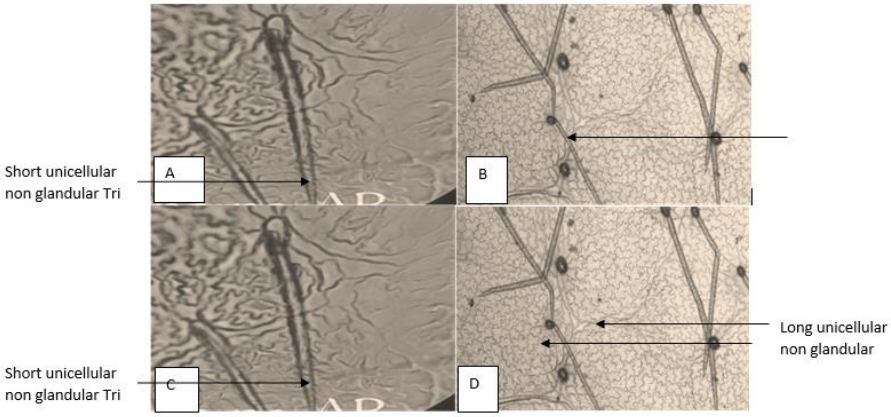

Plate 4. A &B Adaxial surfaces of *Mucuna pogggei* showing short unicellular non-glandular trichome C&D.
Abaxial surfaces of M. pruriens showing long Trichome Mag X400.

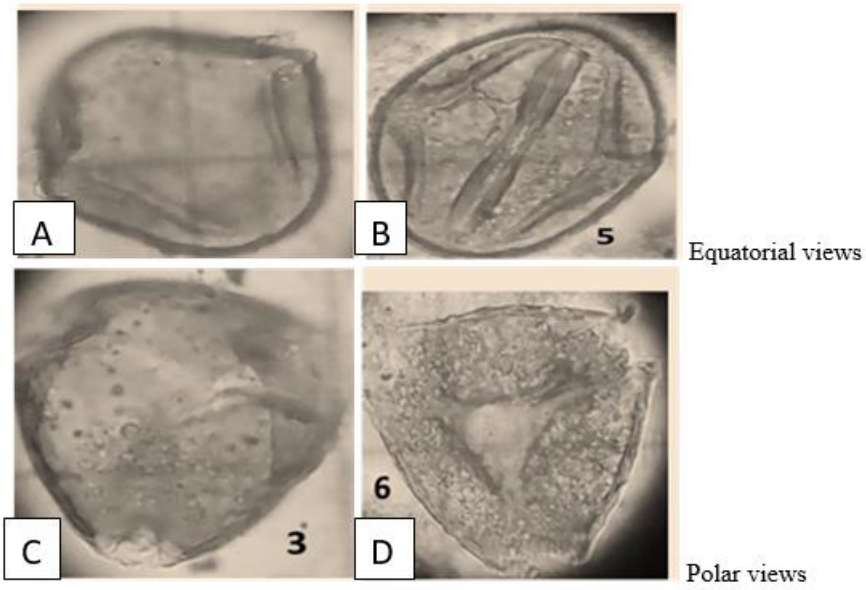

Plate 5: Pollen Morphology of the *Mucuna*.
A. Equatorial view of Mucuna pruriens (x400) and B. Equatorial view of Mucuna poggei C. Polar view of Mucuna pruriens (x 400) and D. Polar view of Mucuna poggei

DISCUSSION

The taxonomic significance of epidermal features is well-established, with studies demonstrating their utility in delineating various plant taxa (Chukwuma *et al*. 2017). Adeniji and Ariwaodo (2012) found that the epidermal morphology of *Pericopsis* allows for identification even in fragmented leaf samples, which is valuable for pharmacognostic research. Wilkinson (1979) noted that similarities in stomatal structure often serve as reliable diagnostic traits. This study confirms that both species exhibit amphistomatic leaves, but stomatal distribution varies*: M. poggei* has sparse stomata on the adaxial surface, while *M. pruriens* shows an even distribution. Stomata are primarily located in the intercostal regions of the adaxial surfaces, consistent with previous findings (Sonibare *et al*., 2005; Dhale *et al*., 2010).

Both species possess anomocytic and paracytic stomatal types, with *M. pruriens* also displaying anisocytic stomata, which helps differentiate it from *M. poggei*. This aligns with Owolabi and Adedeji (2018), who identified multiple stomatal types in *M. pruriens*. Variations in trichome types have long been used in the comparative systematics of angiosperms (Ilkay *et al*. 2014), with trichome morphology providing taxonomic insights (Stace 1980). Studies have shown that glandular trichomes and calcium oxalate crystals can distinguish between genera (Metcalfe & Chalk 1950), while Kiran *et al*. (2011) and Frehat *et al*. (2011) emphasized the importance of trichome characteristics in plant systematics. In this study, *M. pruriens* exhibited longer trichomes compared to the shorter ones found in *M. poggei*, which can aid in species delimitation. Non-glandular trichomes were more abundant on the abaxial surfaces in both species. Palynological analysis revealed prolate-spheroidal pollen grains, reinforcing the natural relationships among *Mucuna* species. Pollen characteristics, such as size, equatorial diameter, colpus length, and the ratio of polar to equatorial diameter, were identified as useful parameters for distinguishing *M. pruriens* from *M. poggei*, illustrating the importance of pollen data at various taxonomic levels (Stuessy, 1990).

CONCLUSION AND RECOMMENDATIONS

The study highlights that while *Mucuna* species have overlapping anatomical features, they can be distinguished by specific foliar and pollen micro characters. Key differences include stomatal type, trichome size, stomatal distribution, and the ratio of polar to equatorial diameter, all of which are useful for species identification even in fragmented or sterile specimens. The study recommends incorporating additional taxonomic tools, such as phytochemical analysis, morphometrics, and molecular techniques, to further clarify their relationships.

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Authors' Contributions

All authors significantly contributed to species collection, manuscript development, and data analysis for this publication.

Ethics Statement

Not applicable.

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