

Palynological Characterization of Four Varieties of *Vigna unguiculata* (L. WALP) and Its Taxonomic Significance

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ABSTRACT

This study examined the pollen morphology of four varieties of *Vigna unguiculata* (cowpea), an important staple legume for food security in Nigeria and Africa. Knowledge of pollen characteristics can inform efforts to conserve crop genetic variation, crucial for developing resilient food production systems. The main objective was to differentiate these *Vigna* varieties based on their pollen morphology. Pollen samples were collected from four cowpea varieties (BBT Brown, IFE BPC, BBT White, and IFE Brown) grown in a screen house and analysed using standard palynological method. Statistical analysis was done using SPSS version 27.0. Pollen characters analyzed included polar and equatorial views, pore length, muri length, exine thickness and lumina width. Results showed statistically significant differences in all quantitative pollen characters among the four varieties ($p < 0.5$). Key distinguishing features included reticulation pattern, pollen size, lumina size, presence of small columellate structures within lumina, and rounded features surrounding the pores. In conclusion, the pollen morphology of the four *Vigna unguiculata* varieties examined was quite distinct, allowing for their separation and identification. This knowledge can contribute to conservation efforts targeting the genetic variation and taxonomic classification of this important food crop, supporting sustainable development and food security.

1. Introduction

Vigna unguiculata (L.) Walp. (Cowpea) is a Dicotyledonea belonging to the order Fabales, Family Fabaceae, subfamily Faboideae, and genus *Vigna* with a unique floral pattern that makes them recognizable. Mithen and Kibblewhite (1993); Padulosi and Ng (1997) proposed in their study that *Vigna unguiculata* might have possibly originated from the southernmost part of Africa, and domesticated in West Africa (Osipitan *et al.*, 2021). Meanwhile, Herniter *et al.* (2020), posited East and West Africa regions for cowpea domestication. They are mostly distributed throughout the globe while most of the world's cowpea is cultivated in West Africa, Nigeria has been the largest producer (Singh, 2003; Wada and Abubakar, 2013; Muranaka *et al.*, 2015; Gupta *et al.*, 2016; Osipitan *et al.*, 2021). It is a drought-tolerant legume (Nkoana *et al.*, 2019; Abebe and Alemayehu, 2022).

They are economically important plant crops in developing countries as millions of people rely on cowpeas as their primary source of protein, making them a crucial crop for nutritional well-being (Nkhoma, 2020; Abebe and Alemayehu, 2022). Beyond its role in human diets, cowpea plant residues serve as premium hay for livestock, maximizing the crop's agricultural value and enriching soils through nitrogen-fixing nodules (Muranaka *et al.*, 2015; Gupta *et al.*, 2016). When combined with cereals, cowpea grains create a more complete nutritional profile by contributing high-quality proteins and essential vitamins (Gupta *et al.*, 2016). Its significant folic acid content is particularly valuable for maternal health, helping prevent birth defects such as spina bifida.

It is an important medicinal plant with a diverse pharmacological spectrum, therapeutic values, phytochemical constituents, and activities (antioxidant, antihyperglycemic, antinociceptive,

antidiabetic, antibacterial, antimicrobial, antifungal and antiviral) (Singh *et al.*, 2015; Gupta *et al.*, 2016). Self-pollination in cultivated cowpeas occurs through a controlled nocturnal process. The closed flower structure, combined with sticky, dense pollen released at night, prevents mechanical pollen dispersal. Self-fertilization becomes possible when the stigmatic surface's protective layer breaks down, releasing exudate in the night's latter half. The flower's brief morning opening and subsequent closure completes the cycle.

Pollen study, also known as palynology, can provide valuable insights that contribute to food security and sustainable development. Plant pollen grains have dissimilar morphological features that can be reliably transferred from generation to generation (Cai *et al.*, 2023). Pollen morphological characters are controlled by genes and are not easily affected by the external natural environment. Therefore, pollen morphology greatly affects plant taxonomy, evolution, and identification.

Several researchers have used pollen characters to delimit and or differentiate species and varieties of plants at various taxonomic groups or taxa. Suratman *et al.* (2022) evaluated pollen morphological character variation and its taxonomic value among species of *Strobilanthes*—I in Sumatra. Barros *et al.* (2024) evaluated how increased temperature influences pollen viability, photosynthetic and enzymatic responses, and their consequences on the final yield of cowpea cultivars

In Nigeria, Olapade *et al.* (2002) showed significant differences among eight cowpea varieties when characterized based on their physical and functional properties. Ige *et al.* (2011) described the floral biology and pollination ecology of three varieties of *Vigna unguiculata* that are widely cultivated in Nigeria. Their results showed that the flower opening of cowpeas begins between 6:00 a.m. and 6:30 a.m. and closes between 11:30 a.m. and 12 noon, and also observed similarities in the pollen morphology of the cowpea varieties studied. Orijemie (2018) studied the pollen morphology of three Caesalpinioideae (Leguminosae) ornamental species in Nigeria. Oyelakin *et al.* (2020) carried out pollen morphological studies of selected flowering plants at the Federal University of Agriculture, Abeokuta. They found variations in pollen characters among species of the same family.

Akinyemi *et al.* (2024) explored the palynological features of *Senna occidentalis* L. in four vegetations of Ogun state. Their findings revealed diagnostic characteristics of the species' pollen with taxonomic significance. Pollen morphology on these varieties of *Vigna unguiculata* in Nigeria is very scanty. This research aims to explore the distinctive pollen characteristics of four *Vigna unguiculata* (cowpea) varieties, analyzing their morphological features to enhance botanical taxonomic understanding and support agricultural resilience. By examining the unique pollen structures, the study seeks to provide critical insights that can inform breeding strategies and genetic diversity assessment, and ultimately contribute to improving crop productivity and food security.

2. Material and Methods

A. Plant Material

Four varieties of *Vigna unguiculata* were collected from the Institute of Agricultural Research and Training (IAR&T), Ibadan Oyo State. The varieties include: BBT white, BBT brown, Ife BPC and Ife brown. The seeds were planted in 10 L planting buckets filled with soil in five replicates and grown in the screen house of the Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture Abeokuta, Ogun State Nigeria. At flowering, unopened flowers were collected and preserved in 70 % ethanol in specimen bottles.

B. Palynological Studies

The laboratory experiment was carried out at the Archeology Department, University of Ibadan (UI), Nigeria. Pollen grains for light microscope (LM) were prepared according to Faegri and Iversen's acetolysis method (Faegri and Iversen, 1992). Pollen characters were measured with a standard micrometre eye-piece under a light microscope at x400 magnification. A minimum of ten to fifteen pollen grains was measured for each variety. In addition, pollen sizes were measured for both

equatorial and polar views. Photomicrographs of pollen grains were taken by using an Olympus CH 30 trinocular microscope with digital camera attached [DE 1.3 M (megapixels)] and connected to a laptop at 100x magnification using 16x eyepieces (Plate 1 & 2). The terminology used tracks Punt *et al.* (2007).

C. Statistical Analysis

Statistical analysis was done on the collected pollen morphological data based on ten measurements for a feature per slide, using SPSS version 27.0 software. Means were separated at 5 % probability level with Duncan's Multiple Range Test (DMRT) to test the significance of variation among the cowpea varieties studied using Analysis of variance (ANOVA). Pearson correlation coefficient was analyzed to measure the strength of the linear relationship between the quantitative pollen morphological characters. The tested quantitative pollen morphological features were also subjected to Principal component analysis to determine the relative contribution of each feature to the total variation observed. A dendrogram was generated using Average linkage (between groups) to group all the tested four cowpea varieties based on quantitative pollen morphological data.

3. Results

All the pollen grains studied are morphologically similar for possessing three-porate, coarse reticulations (exine pattern) and large pollen size. The reticulations have prominent muri that are thick in BBT brown but thin in the others. Similarly, the lumina are smaller in BBT Brown and IFE Brown but larger in IFE BPC and BBT White. The lumina have small columellate structures within them; this feature is prominent and distinct in BBT Brown but less so in IFE Brown. The pores in IFE BPC contain some rounded features surrounding them; this was only observed in this pollen (Figure 1). The distinguished qualitative pollen characters of cowpea varieties studied are reticulation with muri thickness, lumina size, small columellate structure within the lumina, and rounded features surrounding the pores (Table 1).

Mean quantitative pollen characters as shown in Table 2 reveal BBT brown has the highest polar axis of the equatorial view (59.3 ± 0.1) μm and equatorial diameter (75.6 ± 0.1) μm while IFE brown has the lowest polar axis of the equatorial view (44.3 ± 0.2) μm and equatorial diameter (58.4 ± 0.5) μm . The polar axis of the polar view ranges from 95.6 ± 0.1 μm in IFE BPC to 57.6 ± 0.2 μm in BBT white; the equatorial diameter of the polar view ranges from 95.6 ± 0.1 μm in IFE BPC to 57.6 ± 0.2 μm in BBT white. Pore length in IFE BPC (13.8 ± 0.3) μm is the longest and smallest in IFE brown (9.1 ± 0.3) μm . Exine thickness ranges from 4.3 ± 0.1 to 2.7 ± 0.1 μm in BBTwhite, and (BBT brown and IFE BPC) respectively. Muri length is prominent in IFE BPC with 16.3 ± 0.2 μm and small in IFE brown (10.8 ± 0.5) μm . BBT brown has the largest lumina width of 2.8 ± 0.0 μm and BBT white ($0.6 \pm 0.0 \mu\text{m}$) the smallest (Table 1). They are all significantly different at $p < 0.5$. The distinguished quantitative pollen characters among the four varieties of cowpea studied are equatorial view (polar axis and equatorial diameter), polar view (polar axis and equatorial diameter), pore length, exine thickness, muri, and lumina length.

The Pearson correlation coefficient (Table 3) shows the association(s) between and among the quantitative pollen characters of the four cowpea varieties studied. Equatorial view equatorial diameter has a positive association with the equatorial view polar axis at $r = 0.977$. Polar view equatorial diameter associated positively with the polar view polar axis at $r = 0.992$. Pore length has positive associations with equatorial view polar axis, equatorial view equatorial diameter, polar view polar axis, and polar view equatorial diameter at $r = 0.698, 0.683, 0.787$, and 0.778 respectively. Meanwhile, exine thickness has opposing associations with equatorial view polar axis, equatorial view equatorial diameter, polar view polar axis, and polar view equatorial diameter at $r = 0.715, 0.759, 0.585$, and 0.569 respectively. Muri length has a progressive association with polar view polar axis and polar view equatorial diameter at $r = 0.595$ and 0.592 correspondingly. Lumina width has positive associations with equatorial view polar axis and equatorial view equatorial diameter at $r = 0.578$ and 0.655 respectively. More so, Lumina width has negative associations with exine thickness and muri length at $r = 0.579$ and 0.516

individually. They all are significantly different at a 99 % confidence level.

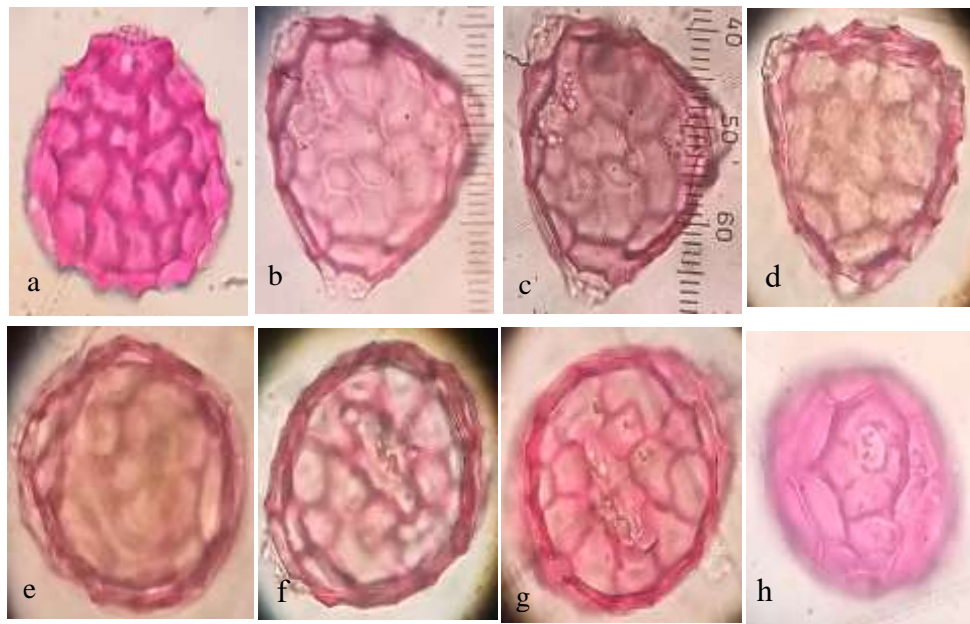


Figure 1: Polar views: a. BBT brown, b. Ife BPC, c. BBT white, d. IFE brown

Equatorial views: e. BBT brown, f. Ife BPC, g. BBT white, h. IFE brown

Table I: Qualitative pollen characters of four varieties of *Vigna unguiculata* studied

	BBTBrown	Ife BPC	BBT white	Ife BROWN
Exine pattern	Coarse reticulation	Coarse reticulation	Coarse reticulation	Coarse reticulation
Polar size	Large	Large	Large	Large
Muri	Prominent thick muri	Thin muri	Thin muri	Thin muri
Lumina size	Small with prominent and distinct small columellate structures	Large	Large	Small
Pore number	Three-porate	Three-porate	Three-porate	Three-porate

Table 2: Mean quantitative pollen characters of four varieties of *Vigna unguiculata* studied

	BBT BROWN	IFE BPC	BBT WHITE	IFE BROWN
EQ (PA)(μm)	59.3 \pm 0.1	56.4 \pm 0.2	49.8 \pm 0.1	44.3 \pm 0.2
EQ(ED)(μm)	75.6 \pm 0.1	71.1 \pm 0.3	62.8 \pm 0.1	58.4 \pm 0.5
PV (PA)(μm)	62.8 \pm 2.3	95.6 \pm 0.1	57.6 \pm 0.2	63.4 \pm 0.2
PV (ED)(μm)	61.6 \pm 2.2	95.6 \pm 0.1	57.6 \pm 0.2	63.5 \pm 0.2
PL(μm)	11.4 \pm 0.0	13.8 \pm 0.3	10.3 \pm 0.1	9.1 \pm 0.3
EXT(μm)	2.7 \pm 0.1	2.7 \pm 0.1	4.3 \pm 0.1	3.6 \pm 0.0
Muri (L)(μm)	11.1 \pm 0.3	16.3 \pm 0.2	14.3 \pm 0.7	10.8 \pm 0.5
Lumina W(μm)	2.8 \pm 0.0	0.9 \pm 0.0	0.6 \pm 0.0	1.2 \pm 0.0

Mean \pm Standard error p<0.5

Key: EQ (PA): Equatorial view (Polar axis), EQ(ED): Equatorial view (equatorial diameter), PV(PA): polar view (polar axis), PV(ED):polar view (equatorial diameter), EXT: exine thickness, MURI(L): muri length, LUMINA W: lumina width.

Table 3: Pearson correlation coefficient on quantitative pollen characters of four varieties of *Vigna unguiculata* studied

Accessions	EQ (PA)	EQ(ED)	PV (PA)	PV (ED)	PL	EXT	Muri (L)	Lumina W
EQ (PA)	1							
EQ(ED)	.977**	1						
PV (PA)	.384*	.361*	1					
PV (ED)	.356*	.333*	.992**	1				
PL	.698**	.683**	.787**	.778**	1			
EXT	-.715**	-.759**	-.585**	-.569**	-.601	1		
Muri (L)	.239	.149	.595**	.592**	.582	.012	1	
Lumina W	.578**	.655**	-.225	-.261	.015	-.579**	-.516**	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Key: EQ (PA): Equatorial view (Polar axis), EQ(ED): Equatorial view (equatorial diameter), PV(PA): polar view (polar axis), PV(ED): polar view (equatorial diameter), EXT: exine thickness, MURI(L): muri length Lumina W: lumina width.

Table 4: Principal Component analysis based on quantitative pollen characters of four varieties of *Vigna unguiculata* studied

	Principal Component	
	1	2
EQ (PA)	0.917	-0.288
EQ(ED)	0.917	-0.361
PV (PA)	0.691	0.653
PV (ED)	0.669	0.674
PL	0.866	0.369
EXT	-0.857	0.181
Muri (L)	0.332	0.774
Lumina W	0.416	-0.867
Eigenvalues	5.124	2.795
Variation %	56.934	31.051
Cumulative %	56.934	87.985

Eigen factor>0.6 was considered

Key: EQ (PA): Equatorial view (Polar axis), EQ(ED): Equatorial view (equatorial diameter), PV(PA): polar view (polar axis), PV(ED):polar view (equatorial diameter), EXT: exine thickness, MURI(L): muri length , Lumina W: lumina width.

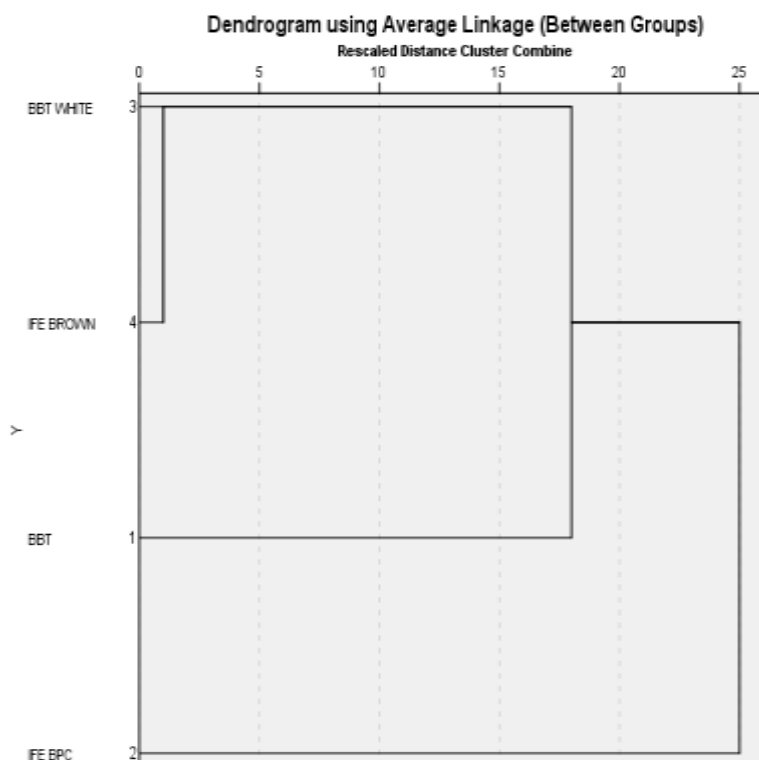


Figure 2: Dendrogram based on pollen quantitative characters of four varieties of *Vigna unguiculata* studied

4. Discussion

The morphological similarity observed in all the qualitative pollen characters studied includes the exine pattern (coarse reticulation), pollen size (large), and the number of pores (three-porate), which are critical features for identifying these varieties within the same taxonomic category. The overall morphological consistency across the varieties suggests a close evolutionary relationship, which is essential for understanding their taxonomy despite their differences. Essien and Fatoyinbo (2022) discovered in their study that there are some similarities among species within the same family and distinct variations among species within other families.

The distinguished qualitative pollen characters of cowpea varieties studied are reticulation with prominent thick muri in BBT brown, lumina size (large in BBT white and Ife BPC; and small in Ife brown and BBT brown), small columellate structure within the lumina is prominent in BBT brown, and rounded features surrounding the pores in Ife BPC, which is not seen in other varieties. They are distinctive features for differentiating between the cowpea varieties. They are unique traits that could help as indicators of genetic diversity within cowpea populations. Different patterns may reflect underlying genetic differences, suggesting that preserving these morphological traits is essential for maintaining the overall genetic variability of the species.

There is a significant variation across all quantitative parameters at a confidence level of $p < 0.5$. This high level of significance indicates that the observed differences are unlikely to be due to random variation, reinforcing the reliability of these findings for breeding decisions. The significant variations across parameters like equatorial/polar view measurements, pore length, exine thickness, muri length, and lamina width provide clear differentiation between the cowpea varieties. The combination of large overall size in equatorial view with wide lumina makes BBT Brown distinctively identifiable suggesting that this variety may have larger pollen grains compared to others. Wang *et al.* (2022) found out that the pollen polar length is the decisive characteristic in distinguishing the two subsections in section Moutan belonging to Paeaniaceae.

The combination of large polar view measurements with the longest pore and prominent muri lengths makes Ife BPC easily distinguishable. The thick exine combined with small lumina and polar view measurements creates a unique identification profile in BBT white. The consistently small measurements across multiple parameters make Ife Brown distinctly identifiable indicating smaller pollen grains which could impact its reproductive success under certain conditions. The variation in exine thickness could influence pollen durability and dispersal mechanism. The disparity in polar axis and equatorial diameter measurements indicates variations in the overall pollen size. Umdale *et al.* (2017) observed polymorphism in the pollen sizes, equatorial views, lumina shapes, and muri widths of the Asian *Vigna species* studied.

The $p < 0.5$ indicates that there is less than a 50% probability that these differences occurred by chance. This suggests that the observed variations between varieties are real and reliable. The differences are consistent enough to be considered taxonomically meaningful. The significant variations confirm these varieties are morphologically distinct, validate the use of pollen characteristics for variety identification, suggest these traits are genetically controlled rather than random variation, and provide reliable markers for taxonomic classification. Thus, the statistically significant variations in the pollen parameters can be used as diagnostic taxonomic characters.

Positive associations were found among the quantitative pollen characters measured in this study. In the equatorial view measurements, the strong positive correlation ($r = 0.977$) between the equatorial diameter and polar axis suggests that as the equatorial diameter increases, the polar axis also tends to increase proportionally. Thus, selecting for one desired trait could lead to improvements in others. For instance, focusing on increasing the equatorial diameter may also enhance the polar axis, leading to overall larger pollen grains. This could be beneficial for pollination efficiency and seed set in breeding programs. In polar view measurements, an even stronger correlation ($r = 0.992$) indicates a very close relationship between the equatorial diameter and polar axis measurements, implying that they may be influenced by similar genetic or environmental factors. The moderate positive correlations showed by pore length with several dimensions suggest that pore length is positively associated with overall pollen size and shape. The positive associations of pore length with pollen size suggest that longer pores might facilitate better pollen tube growth and successful fertilization. Breeding for increased pore length could enhance reproductive success. Lumina width has positive correlations with equatorial view polar axis ($r = 0.578$) and equatorial view equatorial diameter ($r = 0.655$), indicating that wider lumina are associated with larger pollen dimensions.

Meanwhile, negative associations were observed in exine thickness which exhibits negative correlations with all measured dimensions: equatorial view polar axis ($r = 0.715$), equatorial view equatorial diameter ($r = 0.759$), polar view polar axis ($r = 0.585$), and polar view equatorial diameter ($r = 0.569$). These suggest that thicker exines are associated with smaller pollen dimensions, which could indicate a trade-off between structural robustness and size. There may be a consideration of whether thicker exines (which can provide protection and enhance pollen resilience against environmental stressors) are worth the potential reduction in pollen size, which could impact fertilization rates.

Lumina width shows negative associations with exine thickness ($r = 0.579$) and muri length ($r = 0.516$). This indicates that as lumina width increases, exine thickness and muri length tend to decrease, suggesting a complex interaction between these structural components and the traits are interrelated. Hence, Breeders might focus on optimizing lumina width while managing exine thickness to ensure robust pollen grains that maintain viability without compromising size. This balance could be crucial for developing cowpea varieties that thrive in varying environmental conditions.

More so, muri length has positive associations with polar view polar axis ($r = 0.595$) and polar view equatorial diameter ($r = 0.592$). This indicates that larger muri lengths are correlated with increased dimensions in other pollen characteristics. All correlations are reported to be significantly different at

a 99 % confidence level, indicating that the observed associations are highly unlikely to be due to random chance. This high level of significance reinforces the liability of the findings and suggests potential biological relevance in understanding pollen morphology among cowpea varieties.

The principal component analysis reveals diagnostic characters in PC1 as primary taxonomic markers (polar and equatorial measurements (both views) and exine thickness with 56.9 % variance) and secondary taxonomic markers (PC2 - 31.05% variance) which were muri length and lumina width. The high combined variance (87.95%) from PC1 and PC2 indicates and confirms that these pollen characters are reliable taxonomic markers, can be used confidently for the cowpea varieties identification, and reflect genuine evolutionary divergence among the varieties of cowpea studied. This was in line with the findings of Suratman *et al.* (2022). They found out in their study that the observed variation among the pollen morphology of *Strobilanthes species* was confirmed by PC analysis. Thus, pollen morphological characters are important for differentiating taxa.

The clear separation into two distinct groups based on quantitative pollen characters suggests that Group 1 (BBT white, Ife brown, BBT brown) represents varieties with closer taxonomic relationships while Group 2 (Ife BPC) represents a more distinct taxonomic lineage due to its unique pollen characteristics. This clustering pattern provides insights into the evolutionary relationships among these cowpea varieties. Pore length and surrounding rounded features in Ife BPC suggest a unique diagnostic feature. The distinct pollen morphology of Ife BPC suggests a possible different evolutionary history, potential adaptation to different environmental conditions, and or unique genetic basis for pollen development.

Conclusively, the pollen grains of these varieties are quite distinct and can be separated. The variations in pollen grain structure significantly contribute to the taxonomic identification of cowpea varieties by providing distinct morphological traits that aid in differentiation, reflecting genetic variability, supporting phylogenetic analysis, and enabling the establishment of standardized classification systems. The statistically significant variations in the multiple pollen parameters can be used as diagnostic taxonomic characters in the cowpea varieties studied. Breeders, Taxonomists, and seed analysts can reliably distinguish between different cowpea varieties based on these pollen morphological markers. Thus, improving the accuracy of germplasm characterization and cataloging of these varieties.

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