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PALYNOLOGICAL FEATURES OF *Senna occidentalis L.* IN FOUR VEGETATION ZONES OF OGUN STATE, NIGERIA.

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ABSTRACT

Pollen morphology is an important taxonomic character that can provide insights into the variability and adaptation of plant species in different environments. This study aimed to analyze the pollen morphological variation of *Coffee senna* (*Senna occidentalis L*). accessions across four vegetation zones in Ogun State, Nigeria. Mature anthers were collected, and standard palynological procedures were followed to examine the pollens using light microscopy. Pollen size, shape, aperture, exine pattern, and thickness were compared across the four zones. Statistical analysis using SPSS version 27.0 revealed significant variations ($p \leq 0.05$) in pollen size, colpi size, and exine pattern across the zones. Pollen grains were oblate-spheroidal in shape, with sizes ranging from 27.63 to 36.73 μm (polar axis) and 25.7 to 33.7 μm (equator diameter). The aperture was 3-colporate in all zones, but with distinct ora in colpi in the rainforest and syncolporate in the mid-rainforest zone. Exine patterns showed slight variations, with slightly coarse reticulation in the rainforest and fine reticulation in the other three zones. The distinguishing characters of *S. occidentalis* pollen across the zones were pollen size (polar axis and equator diameter), colpi length and width, and exine pattern. These findings reveal the diagnostic features of *S. occidentalis* pollen with taxonomic significance.

Keywords: Senna, palynology, vegetation, taxonomy

INTRODUCTION

Senna occidentalis L., commonly known as *Coffee senna* belongs to the genus Senna, subfamily *Ceasalpinioideae* and family Fabaceae. The genus consists of herbs, shrubs, tree lets, tall trees and lianas. They have successfully colonized a wide range of habitats in different climates and latitudes with a large number of them been ornamental and roadside plants (Burkill, 1995). *S. occidentalis* is an important economic plant whose seeds can be roasted and used as coffee in the treatment of diverse diseases, as an expectorant or as anti-inflammatory and laxative material. Medicinally, it is used as blood cleanser and diuretic for constipation relief and skin disease medication.

Palynology is an important source of morphological information for Taxonomists in phylogenetic and evolutionary trends for angiosperm. The use of pollen morphological characters is well known as a tool for identification and classification of plants (Mander *et al.* 2021; Karna-Mallick, 2019; Mazari *et al.*, 2017; Sarkar *et al.*, 2016; Nnamani and Onu, 2014). Features such as the exine sculpturing and size, and number of apertures through which the pollen tubes grow are valuable taxonomic tools. The structure of a pollen may be so distinctive that it could be used singly to delimit species. A major limitation in the knowledge of pollen morphology of tropical plant species can be attributed to environmental variation impacts in vegetation, whether of climatic and/or anthropogenic origin (Fernandez-Pacella, 2014).

Essien and Fatoyinbo (2022) studied the pollen of seven flowering plant species belonging to *Asteraceae*, *Euphorbiaceae*, and Fabaceae sub-family *Caesalpinoideae* families to determine the morphological features that would aid in the identification and classification of the members within each family. They reported aperture measures such as number and diameter, pollen size, and spine height to be taxonomically indicative in all of the species studied. Nnamani and Onu (2012) reported pollen form, pollen shapes, pore orientation and exine ornamentations as the features of high taxonomic value in the classification of some members of Nigerian *Clusiaceae*. Borokini and Ayodele (2012) recorded an insignificant difference among the pollen grains of *Tacca leontopetaloides* collected from four different locations across Nigeria. They shared striking similarities with other Tacca species and even with members of other closely related families.

Fernandez-Pacella (2014) described the pollen morphology of 17 species of the genus Senna in Southeast Ibera, Corrientes, Argentina and recognized two groups considering *endoapertures: lalongates* and circular. Ferreira *et al.* (2010) classified pollen grains of *S. occidentalis* studied, as polar/spheroidal and 90% viability. This study seeks to analyze and give insight into the palynological variation and relation among the accessions of S. occidentalis in the four vegetation zones in Ogun State Nigeria.

MATERIALS AND METHODS

Mature flowers of *Senna occidentalis (L.)* were collected from different locations based on the vegetation zones in Ogun State (Table 1). The laboratory experiment was carried out at the Archeology Department, University of Ibadan (UI), Nigeria. Palynological procedure and descriptive terminology were done according to Faegri and Iversen (1992). Light microscopy was done under an Olympus CH 30 trinocular microscope with digital camera attached [DE 1.3 M (megapixels)] and connected to a laptop.

Table 1: Location of *Senna occidentalis* species collected based on the Vegetation zones in Ogun State.

Site of collection	Latitude	Longitude	Vegetation
Abeokuta North	7° 8' 1.9392" N	3° 18' 19.728" E	Guinea Savannah
Odeda	7° 13' 56.2476" N	3° 27' 14.8284" E	Rain forest
Obafemi Owode	6° 57' 16.9992" N	3° 31' 54.5016" E	Mid- Rain forest
Shagamu	6° 51' 15.9444" N	3° 37' 49.8864" E	Swampy area

Sample preparation and acetolysis

The fresh pollen bearing samples were treated by cutting anthers with the aid of a scapula and placed into 50 ml plastic centrifuge tubes, finely crushed using glass rods. Acetic acid (glacial) was poured into the tubes, mixed thoroughly on the vortex mixer. The tubes were centrifuged at 5,000 revolutions per minute (rpm) for 10 minutes. The supernatant liquid was poured into a special bottle labelled "Acetolysis Waste". Acetolysis mixture was prepared and poured into the residue in the tube. It was thoroughly mixed and heated in a water bath for about 10 minutes.

It was brought down and cooled, then centrifuged. The supernatant was again poured into the Acetolysis Waste bottle. Rinsing with distilled water was carried out 2 times i.e. water was poured into the tubes, they were mixed, centrifuged and the supernatant poured into the sink. The samples were treated with 50% glycerol i.e. 50% glycerol poured into the tubes, mixed thoroughly, centrifuged and decanted. A little 100% glycerol was added to the tubes, mixed and poured into appropriately labelled vials, from where slides were prepared.

Mounting of slides

Each vial was thoroughly mixed and a drop of the content taken and placed at the center of appropriately labelled commercially pre-cleaned slides. A 22×22 mm cover slip was placed on each drop and allowed to evenly spread (sometimes with slight pressure with the fingers). The slides were sealed using a colourless nail polish.

Microscopy

The slides were studied on the microscope where measurements of the different morphological features were made with the aid of a micrometer eyepiece. The slides were studied using an Olympus CH 30 Trinocular microscope with digital camera attached (DE 1.3 M (megapixels) and connected to a laptop. All measurements and photomicrographs were taken under × 1000 Magnification. The palynological data that were assessed qualitatively and quantitatively include pollen shape, pollen size, pollen aperture, exine pattern, pollen size (µm), equator diameter(µm), colpi size, exine thickness (µm) and oral size (µm).

Statistical analysis

Statistical analysis was done on the collected palynological data using SPSS version 27.0 based on ten measurements for a feature per slide. Means were separated at 5% probability level using Duncan's Multiple Range Test (DMRT), using one-way Analysis of variance (ANOVA). The mean and standard error were calculated for all the measurements.

RESULTS

Quantitative Palynological Characters: The pollen size ranged significantly from 27.63 µm to 36.73 µm at the polar axis, and 25.7 µm to 33.7 µm at the equator diameter. The study revealed significant variation in colpi size across the four vegetation zones, the colpi length ranged from 24.75 µm in the mid-forest zone to 30.95 µm in swampy area, while colpi width from 1.93 to 3.78 in swampy area. There is no significant ($p \leq 0.05$) variation in exine thickness between 1.95 µm and 2.07 µm in all the locations. Oral size (5.44 µm) was found only in the rainforest zone. There were significant differences in all the quantitative pollen characters except exine thickness across the locations (Tables 2).

Table 2: Quantitative pollen characters of *Senna occidentalis* L. in the four vegetation zones in Ogun state

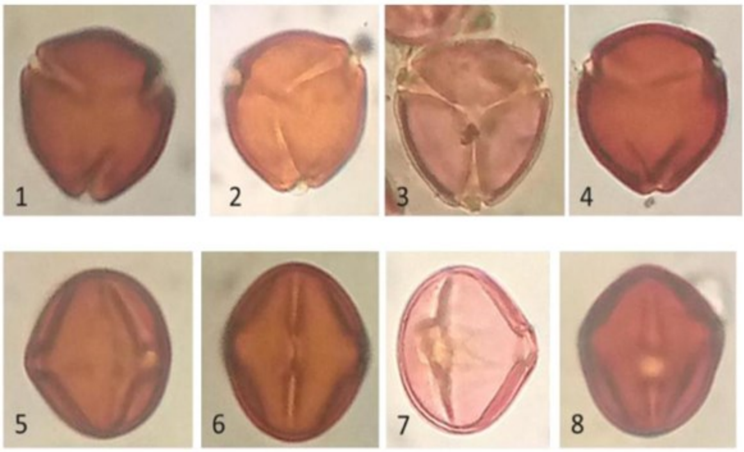
Location (Vegetation zone)/ Pollen features		Abeokuta (Guinea savanna)	Shagamu (Swampy area)	Obafemi owode (Mid-rainforest)	Odeda (Rainforest)
Pollen size (µm)	Pollen axis	32.61^{c} ± 0.12	36.73^{a} ± 0.11	27.63^{d} ± 0.154	33.10^{b} ± 0.221
	Equator diameter	33.7^{a} ± 0.124	33.69^{a}± 0.11	25.7^{c} ± 0.154	31.85^{b}± 0.221
Colpi size (µm)	Length	27.66^{b} ± 0.13	30.95^{a} ± 0.25	24.75^{c} ± 0.083	27.25^{b} ± 0.227
	Width	3.09^{b} ± 0.028	3.78^{a} ± 0.079	1.93^{d} ± 0.030	2.70^{c} ± 0.026
Exine thickness (µm)		2.03^{a} ± 0.015	2.06^{a} ± 0.016	1.95^{a} ± 0.107	2.07^{a} ± 0.015
Oral size (µm)		0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	5.44 ± 0.130

Mean ^{abc} ± Standard error with different alphabets superscript across the locations are significantly different at *P≤ 0.05*

Qualitative Palynological Characters: Pollen grains were oblate-spheroidal shape in all the zones. The aperture was 3-colporate in all the locations but with distinct ora in colpi (Which are pores at the middle of the colpi) in the rainforest and *syncolporate* (the apertures are joined at the mesocolpium) in the mid-rainforest zone (Table 3). Exine pattern showed slight variation, with slightly coarse reticulation in the rainforest and fine reticulation in the other three zones. The colpi run from north to south poles of the pollen and are 'constricted' at the middle (Figures 1-8).

Table 3: Qualitative pollen characters of *Senna occidentalis* L. in the four vegetation zones in Ogun state

Location (Vegetation zone)/ Pollen features	Abeokuta (Guinea savanna)	Shagamu (Swampy area)	Obafemi owode (Mid-rainforest)	Odeda (Rainforest)
Pollen shape	Oblate-spheroidal	Oblate-spheroidal	Oblate-spheroidal	Oblate-spheroidal
Pollen size	Medium	Medium	Small	Medium
Pollen aperture	3-colporate	3-colporate	3-colporate and syncolporate	3-colporate with distinct ora
Exine pattern	Fine reticulate	Fine reticulate	Fine reticulate	Slightly coarse reticulate



Figures 1- 8: Palynological characters of *Senna occidentalis* L. in the four vegetation zones of Ogun state
1-4. *S. occidentalis'* Pollen in polar views: 1. Abeokuta (Guinea savanna) 2. Shagamu (swampy area) 3. Obafemi owode (mid-rainforest) 4. Odeda (rainforest)
5-8. *S. occidentalis'* Pollen in equatorial views: 5. Abeokuta (Guinea savanna) 6. Shagamu (swampy area) 7. Obafemi owode (mid-rainforest) 8. Odeda (rainforest)

The main distinguishing characters of *S. occidentalis* pollen across the locations are the pollen size (polar axis and equator diameter), colpi length and width, and exine pattern. Pollen aperture, aperture type and exine thickness are consistent across the four zones.

DISCUSSION

The main distinguishing characters of *S. occidentalis* pollens studied are the pollen size (polar axis and equator diameter), colpi length and width, and exine pattern which expressed them as distinct individuals in different environments. These variations propose that the environments may certainly have an impact on the observed variances in the data. Environmental factors such as temperature, humidity, and nutrient availability can affect the growth and development of plants, which in turn can influence the characteristics of their pollens. In contrast, Borokini and Ayodele (2012) observed no significant variation in the pollens characters of *Tacca leontopetaloides* collected from four different locations in Nigeria. Sowunmi (1973) noted intraspecific variability in 30 % of the Nigerian species investigated.

The slight variation observed in the structure of the pollen wall (exine thickness) of the *S. occidentalis* studied was statistically similar in all the locations, implying that environmental factors did not affect them. This confirms that exine protects the pollen grains from environmental damage and dehydration. This result is in line with Nnamani and Onu (2014) who observed no significant difference ($p< 0.5\%$) in the exine thickness and polar diameter for the species of Senna studied. Ejsmund *et al.* (2011) reported that pollen grains under more intense desiccation during flowering periods tend to be larger but do not change shape. Orijemie (2018) reported exine and aperture characteristics as the most significant pollen features useful in distinguishing among the pollen of three species of *Caesalpinoideae* studied.

In addition to the three apertures in the accession of *S. occidentalis* collected from Obafemi owode, a mid-forest zone, the apertures were joined at the mesocolpium. Being able to produce polymorphic pollen with variation in aperture linkage may provide adaptive advantages in different environments. It demonstrates developmental plasticity in response to pollination or dispersal selective pressures. Sultan (2000) posited that a single genotype can produce different phenotypes in different environments. This fundamental property of organisms is known as phenotypic plasticity. The linked colpi may aid hydration while the additional colpi facilitate greater pollen tube emergence. So, this morphology could optimize both hydration and germination capacity. More so, accessions from Odeda (rainforest zone) have colpi with distinct ora (pores at the middle of the colpi). This may provide structural support for oriented pollen tube emergence. It may facilitate tube penetration through the stigmatic surface. An oral ring can influence hydration dynamics, directing early influx via the pore area and thus orienting metabolic activity toward targeted germination pore sites.

CONCLUSION

The observed variation of the pollen characters in this study may be due to environmental variation and adaptation of the species to different environments. These findings show the diagnostic features of S. *occidentalis* with taxonomic significance. These environment-specific differences revealed in pollen size, aperture size, and exine pattern provide new insights into intraspecific variation in *S. occidentalis*, and have implications for taxonomic identification of pollen from this species. By analyzing pollen from multiple locations, this study expands the understanding of how environment shapes micromorphological traits in *S. occidentalis*. Overall, this work demonstrates the utility of pollen characteristics for elucidating taxonomic boundaries and environmental relationships within *S. occidentalis*.

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