

Pollen Morphology of Some Members of Nigerian *Indigofera* Linn. (*Fabaceae*) and Their Taxonomic Significance

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ABSTRACT

Pollen morphology of nine species of the genus *Indigofera* of the tribe *Indigofereae* in the family Fabaceae from Nigeria were studied with the view to evaluate the similarities and dissimilarities within and among pollen features of the nine taxa using light microscope after standard acetolysis mixture. These species were *I. capitata*, *I. congolensis*, *I. deightonii*, *I. geminata*, *I. hirsuta*, *I. macrophylla*, *I. nummulariifolia*, *I. spicata* and *I. subulata*. Results from this study revealed that the sizes of these pollen grains ranged from 22.9×20.5µm in *I. subulata* to 35.2×28.5µm in *I. nummulariifolia*. Apertures of the grains are tricolpate in *I. capitata*, *I. congolensis*, *I. deightonii*, *I. geminata*, *I. macrophylla* and tricolporate in *I. hirsuta*, *I. nummulariifolia* and *I. macrophylla*. The ornamentation of these species varied from striate in *I. capitata* to vermiculate in *I. nummulariifolia*. Photomicrographs of the pollen grains and the dendrogram of the nine taxa studied are provided to further aid the phylogeny and classification of these species.

Keywords: Pollen morphology, *Indigofera*, Similarities and Dissimilarities, Nigeria.

INTRODUCTION

The genus *Indigofera* with about 750 species is the third largest genus in the family Fabaceae [1]. The genus was formerly in the tribe Galegeae with nine genera [2], but presently in the tribe Indigofereae with six genera and in the sub-family Papilionoideae [3]. The species are mostly herbs and shrubs [4]. Most of the species are savannah plants with few rainforest species [5]. They are mainly native to the tropical and sub-tropical regions of the world [6]. Their centers of diversity are primarily in Africa and Madagascar, with about 550 species; Asia, especially the temperate Sino-Himalayan region has about 105 species; Australia, about 50 species and the New World, about 45 species [2]

Palynological characters are important for the classification of angiosperms. The publication of pollen morphology and plant taxonomy by [6] in 1952 marked the beginning of a new phase. He made available pollen characters of all families of angiosperms to taxonomists [7]. Since then, they are increasingly being used in plant taxonomic work. According to [8], Pollen characters such as number and position of apertures, size, shape and the details of sculpturing of the exines are of taxonomic values. Palynological study on eight different species of *Indigofera* was carried out by [6]. They

reported that the pollen grains of *I. hirsuta* are elliptic in shape with smooth wall sculpture and has tricolporate apertures. Other species they studied were circular and oval in shape with variations in the wall sculpture and with monoporate, tricolpate, inaperturate and polyporate apertures while some have no conspicuous colpi or pores. [9]

reported that their palynological analysis on the eight species of *Indigofera* serves as the first in literature as the probable lack of enough literatures on the palynological features of *Indigofera* species do not imply irrelevance, rather, proper investigation of the species needs to be carried out in order to clarify the taxonomic uncertainty, determine the relationship (intra and inter) and systematic value of the palynological features of the genus.

This present work seeks to evaluate pollen features of the eight species with aim of determining their similarities and dissimilarities within and among these taxa of *Indigofera* from Nigeria. The specific objectives were to study: (1) Aperture type (2) Size (3) Ornamentation and (4) Shapes of the pollen grains. The reason for the differences in the estimation of the number of taxa in this group of plants could be due to the perceived similarities in their structure and reproductive organs [6].

MATERIALS AND METHODS

SAMPLE COLLECTION

Freshly collected specimens of the nine species of *Indigofera* used for this study were collected from Ogun, Oyo and Ebonyi State of Nigeria. These species were *I. capitata* Kotschy, *I. congolensis* De Wild and T. Durand, *I. deightonii* Gillet, *I. geminata* Bak, *I. hirsuta* Linn., *I. macrophylla* Schum & Thonn, *I. nummulariifolia* (Linn.) Livera ex Alston, *I. spicata* Forssk and *I. subulata* Vahl. These species were representative taxa most common in the study area.

SAMPLE PREPARATION

Preparation of the pollen grains was done following the method of [8]. The pollen grains were crushed in plastic centrifuge tubes using glass rods and about 5ml of glacial acetic acid depending on the quantity of the samples, was added for dehydration. After this the samples were centrifuged at 5000rpm for 15 minutes why?

ACETOLYSIS

Acetolysis mixture of acetic anhydride and H_2SO_4 in the ratio 9:1 respectively was added to the samples in the tubes for oxidation reaction (bleaching). The samples and acetolysis mixture were thoroughly mixed with Vortex Mixer. These were warmed for 13 minutes and centrifuged for 15 minutes at 5000 rpm in order to remove the supernatants.

They were rinsed three times with distilled water to remove the effect of the Acetolysis and then sieved with 120 μ m Sieve to remove the plant debris. The acetolysed samples were transferred into Vial bottles with a graded series of glycerol (50% and 100%). Temporal slides of the prepared samples in 100% glycerol were mounted on glass slides with cover-slips, sealed with nail varnish and viewed under the (the name is important) light microscope. The slides were properly labelled and proper care was taken to avoid contamination which may introduce error into the result.

PARAMETERS CONSIDERED FOR THE ANALYSIS

Statistical Package for Social Sciences (SPSS version 18) software was used to analyse the data obtained. The dendrogram (Fig. 1) below was generated by analyzing the data with the SPSS.

RESULTS

The pollen characters of the nine species of Indigofera studied are summarized in Table 1 and 2 and illustrated with photomicrographs below. The dendrogram below shows the relationships among the nine taxa studied. Sizes of the grains ranged from (22.9×20.5µm) in *I. subulata* to (35.2×28.5µm) in *I. nummulariifolia* while the mean of exine thickness is from 0.9µm in *I. congolensis* to 4.4µm in *I. capitata*. Their shape classes are subprolate and spheroidal except *I. spicata* which is prolate. The aperture is tricolpate in all the species studied except in *I. hirsuta*, *I. nummulariifolia* and *I. spicata* in which it is tricolporate. *Indigofera macrophylla* has both tricolpate and tricolporate apertures.

Table 1. Qualitative pollen features of the nine species of the Indigofera from Nigeria

S/N	Species	Aperture type	Pollen size	Shape class
1	<i>I. capitata</i>	Tricolpate	Medium	Subprolate
2	<i>I. congolensis</i>	Tricolpate	Medium	Spheroidal
3	<i>I. deightonii</i>	Tricolpate	Medium	Subprolate
4	<i>I. geminata</i>	Tricolpate	Medium	Subprolate
5	<i>I. hirsuta</i>	Tricolporate	Medium	Subprolate
6	<i>I. macrophylla</i>	Tricolpate /tricolporate	Medium	Spheroidal
7	<i>I. nummulariifolia</i>	Tricolporate	Large	Subprolate
8	<i>I. spicata</i>	Tricolporate	Medium	Prolate
9	<i>I. subulata</i>	Tricolpate	Medium	Spheroidal

Table 2: Quantitative Data on the Pollen of nine species of Indigofera from Nigeria

S/N	Species	Polar Axis (μm)	Equatorial Distance(μm)	Exine thickness(μm)
1	<i>I. capitata</i>	25(28.9 \pm 0.5)32.5	20(24.6 \pm 0.6)27.5	2.6(4.4 \pm 0.3)6.5
2	<i>I. congolensis</i>	20(28 \pm 0.7)32.5	20(26.5 \pm 0.5)30	0.7(0.9 \pm 0.1)1.3
3	<i>I. deightonii</i>	20(25.9 \pm 0.7)32.5	15(22.6 \pm 0.9)30	1.3(3.1 \pm 0.3)3.9
4	<i>I. geminata</i>	22.5(25.3 \pm 0.4)27.5	17.5(22.6 \pm 0.6)25	2.6(3.1 \pm 0.2)3.9
5	<i>I. hirsuta</i>	27.5(33.2 \pm 0.5)35	22.5(26.0 \pm 0.6)35	2.6(3.4 \pm 0.3)5.2
6	<i>I. macrophylla</i>	19.8(24.0 \pm 0.5)26.3	16.3(21.8 \pm 0.8)26.3	0.7(1.7 \pm 0.2)2.6
7	<i>I. nummularii-folia</i>	29.7(35.2 \pm 0.6)37.5	27.0(28.5 \pm 0.5)32.5	3.3(4.1 \pm 0.3)6.5
8	<i>I. spicata</i>	22.5(29.5 \pm 0.8)32.5	20(25.9 \pm 0.9)32.5	0.7(1.7 \pm 0.2)2.6
9	<i>I. subulata</i>	15(22.9 \pm 0.6)27.5	17.5(20.5 \pm 0.6)27.5	0.7(2.2 \pm 0.5)3.5

Data arranged as: minimum (Mean \pm Standard error) maximum



Fig. 1: Photographs of the species of Indigofera studied .

A: *I. capitata*

B: *I. congolensis*

C: *I. deightonii*

D: *I. geminata*



Fig. 2: Photographs of the species of Indigofera studied

- E: *I. hirsuta*
- F: *I. macrophylla*
- G: *I. nummulariifolia*
- I: *I. spicata*
- J: *I. subulata*

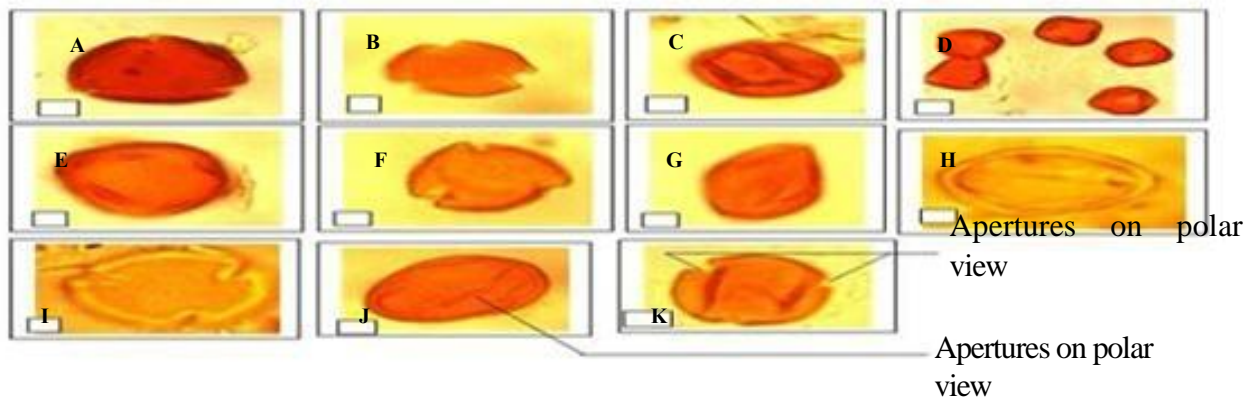


Fig 3: Photomicrographs of pollen grains of the species of Indigofera studied.

- A: Pollen grain of *I. capitata* showing tricolpate apertures on polar view with thick exine pattern.
- B: Pollen grain of *I. congolensis* showing tricolpate apertures on polar view.
- C: Pollen grain of *I. deightonii* showing tricolpate apertures on equatorial view.
- D: Pollen grains of *I. geminata* showing tricolpate apertures on polar and equatorial views.
- E: Pollen grain of *I. hirsuta* showing tricolpate apertures on equatorial view.
- F: Pollen grain of *I. macrophylla* showing tricolpate apertures on polar view.
- G: Pollen grain of *I. macrophylla* showing tricolpate apertures on equatorial view.
- H: Pollen grain of *I. nummulariifolia* showing tricolpate apertures on equatorial view.
- I: Pollen grain of *I. nummulariifolia* showing tricolpate apertures on polar view.
- J: Pollen grain of *I. spicata* showing tricolpate apertures on equatorial view.
- K: Pollen grain of *I. subulata* showing tricolpate apertures on polar view

DISCUSSION

The pollen analysis revealed two pollen classes (tricolpate and tricolporate) among the nine species of *Indigofera* analysed based on their apertures as proposed by [10]. The tricolporate aperture of *I. hirsuta* agrees with the report of [6], although they inadvertently wrote tricolpate to describe the presence of colpi and pores. Figure 1 above shows the photomicrographs of pollen grains of the nine taxa of *Indigofera* studied. From the dendrogram above, at 99.9% greater affinity exists between 3 (*I. deightonii*) and 4 (*I. geminata*) and this indicates that the two species cannot be easily separated using their pollen characters. At 0.00% of the dendrogram, *I. macrophylla* is totally different from other species studied. The variation in the size of the pollen grains and apertures of *I. macrophylla* collected from two different locations could result to variations in their stigma bearing areas and depth and these variations may hinder effective fertilization between the two specimens [11]. Further studies should be carried out on the species of the genus *Indigofera*, especially, *I. macrophylla*, *I. nummulariifolia* and *I. subulata* in order to obtain their status in the taxon.

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