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Anatomical and Palynological Studies on Napoleona imperialis P. Beauv. (Lecythidaceae)

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Abstract

Napoleona *imperialis* P. Beauv is the most widespread Napoleona in Nigeria. It belongs to the family Lecythidaceae, a small tropical family that grows in all regions of Nigeria and other parts of West Africa. However, scientific data concerning this species are scarce. Therefore, the aim of the present study was to conduct an anatomical and palynological assessment of this plant species. For the anatomical evaluation, the leaves and stems were fixed and subjected to common plant anatomy techniques. The acetolysis method was used for Palynology study. Result for palynological study showed that N. *imperialis* is characterized by tricolpate pollen, oval in shape,with microspinulose type of exine ornamentationand Pollen fertility and viabiligy is 84.66%. Anatomical characters such as periderm cylinder, phellem cells and primary and secondary vascular bundles of leaf and stem explains typical features of dicotyledonous plants that have undergone secondary growth. This study provides valuable information for reference and correct identification of this species.

Keywords: Anatomical; Palynological; Napoleona imperialis.

1. Introduction

Lecythidaceae is a small tropical family that grows in all regions of Nigeria and other parts of West Africa [1, 2]. There are about 12 species found in West Tropical Africa and are widely distributed in the forest and savanna regions. In Nigeria, there are a total of nine species of *Napoleona*, with one occuring in both the northern and southern parts and eight including *N. imperialis P. Beauv* and *N. vogelli. Hook* occurring only in the Southern part of Nigeria.

Napoleona imperialis P., Beauv is the most widespread *Napoleona* in Nigeria. They are trees or shrubs, seldom more than 6m high, branching low down and with a very dense crown. The leaves are large, sometimes reaching nearly 30cm length and 8 cm in breadth. The showy flowers often occur on the main branches and trunk as well as among the leaves. The bark is grey, smooth, slash whitish, shallow, and fibrous. Branches are more or less whorled. Leaves are mostly broadly elliptic, often elongated, abruptly acuminate, mostly broadly cuneate or rounded at the base., glossy, the glands at the base of the acumen usually conspicuous and sometimes standing out as small teeth, often also with 1 to 2 pairs of glands above the leaf- stalk, margin sometimes vaguely wavy, with 6 – 8 pairs of upcurving lateral nerves. Flowers (Nov – Jan) variable in colour but usually cream at the circumference, red at the centre (varying to apricot and purple respectively), staminodes white, calyx leathery, valvate, glabrous, with 5 triangular teeth. Stigma is pink. Fruits (Apr. – June) are brownish to reddish spotted white about 5cm in diameter, flattened globose with a depressed centre.

Napoleona imperialis known as irosun – igbo in Yoruba, Ukpa konrisa in Edo, mabungi in Hausa and Akbodo in Igbo is important economically. Different parts of the plants are used for different purposes in the region including mulching and fodder (leaves and twigs), and firewood, chewing stick and ethno – medicine (stem and root) [3]. Humans consume the juice from the fruits as desserts and pods. The seed form an alternative feed ingredient for livestock production. The leaf infusion is used to dissolve clotted blood in freshly delivered women, also used as a vermifuge for children. The stem is used to cure gonorrhea while the roots are used to cure fever [4].

Anatomical and micromorphological characteristics of leaves have played an important role in plant taxonomy, especially at generic and specific levels. Studies in this field have attracted the attention of plant morphologists and systematists to resolve taxonomic conflicts in different groups of plant [5, 6]. Many studies have been conducted in this area in plants for the purpose of correct identification of plant [7-11].

Pollen morphology is conducted as an aid to the morphological study and a significant tool for modern taxonomist for the delimitation 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 [12]. Palynological data has been useful at generic and specific level [13]. This analysis also helps in qualitative analysis of drug powder and the correct identification of drug. It plays an important role in our daily life as well. Aerobiology has received much attention due to its wide application in allergology, forestry, agriculture, horticulture, archaeology, and plant geography [14, 15].

The application of pollen morphology to plant systematic is comparatively a recent trend. It can provide a wealth of taxonomically useful information and would be useful in providing new information on infrageneric

relationships. Bolick [16] reported that the taxonomic and evolutionary importance of pollen morphology may be at specific, generic or higher level. The first palynological study in Pedaliaceae was that described by Erdtman [17], who studied the pollen grains of 15 species belonging to 11 genera from this family. He showed that they are usually 5-13 colpate, oblate – subprolate, united in tetrads in *Sesamothamnus* and *Sigmatosiphon* species. Maria, *et al.* [18] described the pollen morphology of *Sesamothamnus lugardii*. Ramakrishna and Bushan [19] studied the pollen morphology of different genera including the genus *Sesamum*. Anjum and Qaiser [20] reported that some of the pollen grains of the family Acanthaceae are similar to those of the family Pedaliaceae. In Nigeria, the pollen morphology of Pedaliaceae has not been investigated at length.

Detailed anatomical and palynological investigation of *N. imperialis* have not been carried out. Therefore, the purpose of this article is to investigate the anatomical and palynological features of this plant species which forms part of the research work on this plant by the author. Morphology and epidermal characters of this taxa has already been published. This study will contribute to the proper identification of the species in addition to providing more information about this species.

2. Materials and Methods

2.1. Plant Collection and Authentication

Fresh plant samples were collected from various parts of Imo, Abia and Rivers State of Nigeria (Table 1). The various useful parts like leaves, stem and mature flowers were separated and preserved for the study. Identification of the species was done by comparing with authenticated herbarium specimens, later confirmed with the help of diagnostic keys and morphological description given in various floras. Voucher specimens were deposited in Rivers State University Herbarium.

Table-1. Sources of *Napoeleona imperialis* Studied.

Species	Collection Number	Locality
Napoleona imperialis	Ajuru 001	Forest opposite university of Port Harcourt Botanic garden
	Ajuru 002	Federal University of Science and Technology, Owerri, Imo State
	Ajuru 003	Naze vollage farm land, near Owerri town, Imo State
	Ajuru 004 Ajuru 005	Forestry Research Institute, (FRIN), Ahia Eke village, Umuohia, Abia State Forest behind a residential area, Rumuosi, Port Harcourt

3. Anatomical Method

3.1. Transverse Section

The stem and leaf of *N. imperialis* were fixed in F.A.A. (*e*. Formalin acetic acid-alcohol, 1:1:18) after trimming them to correct dimensions. Leaf and stem transverse sections were made with free hand sectioning using surgical scapel. Briefly, scalpel was used to cut 3 cm×4 cm of the plants through the midrib region. The small portion was inserted into a 3 cm×4 cm section of unripe pawpaw to enhance easy cut. The transverse sections obtained were cleared using sodium hypochlorite 3.85% M/V and stained with Safranin O reagent and rinsed with 70% alcohol. Glycerol was added as mountant. Special identifying features of the plant part(s) were studied and identified and was examined under low power magnification and was photographed with compound microscope fitted with a digital camera.

4. Palynological Method

4.1. Pollen Structure

In the extraction of pollen grains for palynological study, the acetolysis method was used. The technique employed was the Punt-Hou method of Punt [21, 22]. This is a micro – method for preparing pollen slides from single anther or even single pollen grain. The flower together with the anther was heated with a few drops of distilled water in a watch glass on a hot plate maintained at 95 – 100^{0}C. When fully softened, the flower was teased open with fine forceps and a mounted needle under a dissecting microscope. The anthers were dissected out and the remaining unwanted parts drawn to the side of the watch glass leaving the anthers in the middle. The pollens was then teased out by crushing the anthers with a glass rod taking great care so as to avoid crushing the pollen. The anther fragments were drawn to the side of the watch glass leaving the pollen in the middle. The watch glass was later returned to the hot plate and evaporated to complete dryness. The acetolysis mixture was made by mixing up nine parts of acetic anhydride with one part of concentrated sulphuric acid. Few drops of this mixture was added with a bulb pipette to the dry pollen in the watch glass on the hot plate. This was warmed until the pollen darkened and the solution became brown in colour. This lasted for about 30 seconds to 5 minutes.

The watch glass was allowed to cool for few minutes. With a bulb pipette, methylated spirit was added drop by drop to the center of the remaining acetolysis mixture. This displaced the acetolysis mixture to a ring round the rim of the watch glass leaving the pollen at the center of the watch glass. The acetolysis mixture was wiped away with box Kleenex tissue, taking care not to get it on fingers nor to disturb the pollen. More methylated spirit was added. The point of a clean mounted needle was dipped into 1% alcoholic safranin solution and then using bulb pipette a drop or two of methylated spirit was allowed to run down the needle and drip from the point onto the pollen in the watch glass. This provided a sufficiently small quantity of stain for a few pollen grains. A clean slide and coverslip were warmed on the hot plate at a temperature of about 60^{0}C. A small block of glycerine jelly was cut with a fine

scapel and rolled over the pollen in the watch glass until most of the grains have adhered to it. The block of glycerine jelly was transferred to the slide and as soon as it has completely melted any bubbles were carefully drawn to the edge with a clear mounted needle. The coverslip was carefully lowered onto the glycerine jelly. The slide was then labelled and sealed with balsam. The Pollen samples were examined using the microscope. Photomicrographys were taken from the slides using a leitz laborlux – 12 microscope fitted with WILD – MPS camera.

4.2. Pollen Fertility and Size

Mature flowers of *Napoleona imperialis* were collected and pollen from anthers teased out on a slide stained with cotton – blue in lactophernol and covered with a cover slip and viewed under the microscope. The number of fertile and non-fertile grains was counted to estimate the pollen fertility. Measurement of pollen diameter was done at X400 magnification using an eye – piece graticule. Deeply stained and rounded pollen grains were considered to be fertile. The variance of pollen grain size in each entity appears to vary with the mean pollen grain size. The coefficient of variation (CV) which was calculated from the formula:

$$\mathbf{CV} = \frac{S.D}{\bar{\times}} \times \frac{100}{1}$$

Where S.D = standard deviation and

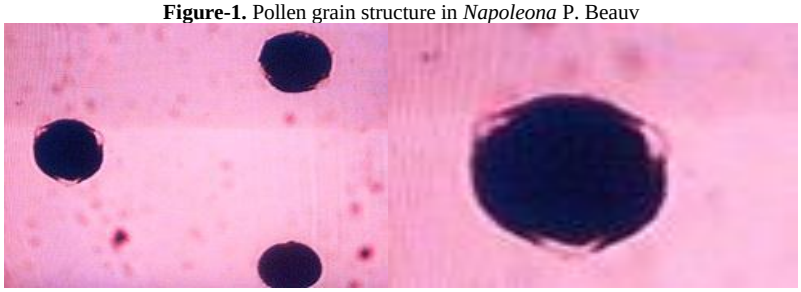
$\bar{\times}$ = the mean pollen size, was therefore used as measure of variability [23].

5. Results

5.1. Floral Micro Morphology (Pollen Morphology)

The class of the pollen is tricolpate with the pollen grain size (polar axis x equatorial diameter) of 23x22.8 µm. The average diameter in polar view was 23 µm. The pollen are radially symmetrical and isopolar. The shape is circular, though semiangular in polar view. The polar diameter/equatorial diameter ratio is 1.09. The thickness of the exine is 3.2 µm and appears granular (Fig. 1).

Figure-1. Pollen grain structure in *Napoleona* P. Beauv

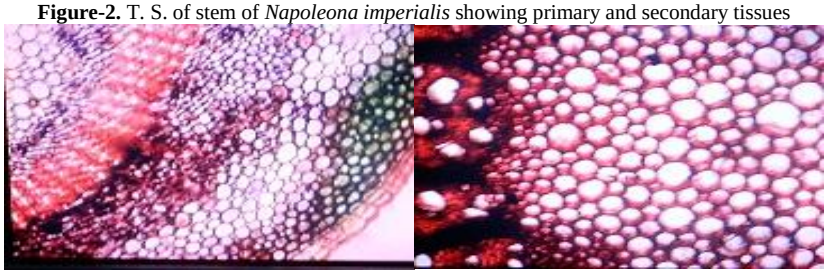


6. Anatomical Results

6.1. Stem

There are two rows of cork cells compactly arranged, covered by a thin cuticle. The collenchyma cells are well developed, about 7 to 8 layers of cells, followed by 5-6 layers of parenchyma cells with intercellular air spaces between them. The primary parenchyma cells are followed by the secondary cortex composed of collenchyma and parenchyma cells. A layer of sclerenchyma cell surrounds the primary and secondary phloem and xylem tissues. Sandwiched in between the secondary phloem and xylem is the cambium. The primary xylem and phloem is towards the center of the stem. The pith cavity is filled with parenchyma cells with intercellular spaces. The parenchyma cells in the peripheral portion are compactly arranged and are smaller than those in the central portion of the pith (Fig. 2).

Figure-2. T. S. of stem of *Napoleona imperialis* showing primary and secondary tissues

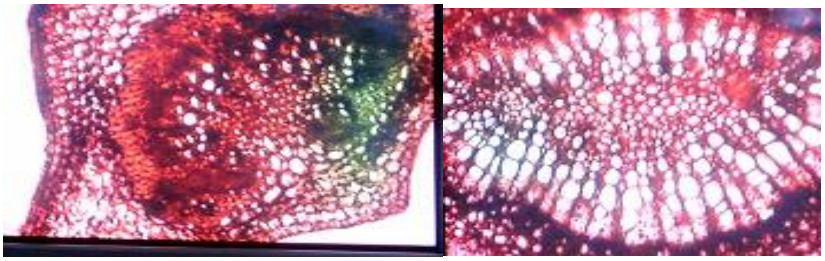


6.2. Midrib

The abaxial surface is convex and the adaxial concave in the species. The midrib is covered on both surfaces by a single layer of epidermal cells. Immediately below the upper epidermal cells are elongated palisade mesophyll cells with intercellular spaces between them. Immediately above the spongy mesophyll are the spongy mesophyll with intercellular spaces between them. There are 3-4 layers of collenchyma cells at the upper and lower portions of the midrib which are thickened at the corners. The parenchyma cells between the collenchyma cells and the central portion of the midrib are 5 – 6 layers.

The bundle sheath sclerenchyma cells completely envelops the vascular bundles and protrudes into the area of the minor rib. The phloem tissue is towards the abaxial surface while the xylem is towards the adaxial portion (Fig.3).

Figure-3. T. S. of the midrib of *Napoleona imperialis* showing the cuticle, epidermal cells, mesophlls, collenchyma, sclerenchyma cells and vascular bundles



7. Discussion

Not only general morphology of plant but also pollen morphology is of great taxonomic significance [24]. Pollen morphological characters have been used for the identification of taxa [25] and also for number of phylogenetic studies [26].

The pollen grains of the examined taxa of the genus were tricolpate, circular and semiangular in polar view. The average diameter in polar view was 23 µm. The shape was found to be circular.

Colpi were found to be deep, long and narrow with small variation in length. Exine thickness was 3.2 µm and it proved to be helpful at specific level. When sculpturing of exine was examined with light microscope (LM) it appeared granular.

Anatomical features of *N. imperialis* have not been investigated until now. Anatomical features are widely used in systematics for identification, for placing anomalous groups in satisfactory position, in classification and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features [27]. Anatomical study of the stem of *N. imperialis* species corresponds to the general anatomy of stem as reported by Esau [28], Metcalfe and Chalk [29]. The presence of the cork cells indicated that the plant has undergone secondary growth where the cork cells (Periderm) replaces the epidermal cell. The periderm is normally present in older N. imperialis stems and adds extra protection to the stem as it grows. The suberized phellem would be of great benefit to the *Napoleona* plant growing in the hot, dry climate as it helps to seal o ff water loss. Very young shoots do not have mature periderm but rather an epidermal layer characterized by primary cell walls. Just inside this epidermis, angular collenchyma (characteristic of stems) are found which aid in the support of the rapidly elongating stem and then parenchyma containing chloroplasts, signifying the photosynthetic activity of the young stem. The thin layer of fibres found just outside the vascular bundles exists to protect the outer most layer of phloem. Moving further inwards, a second fibre band with sclerified parenchyma appears. This thick fibre band surrounds the innermost vascular bundles. As secondary growth proceeds, some of the parenchyma cells found within and outside of this band will become meristematic and contribute to the formation of new vascular cambia and a new layer of fibres, providing them with extra strength and flexibility as the stem continues to grow and expand. A second ring of cambium is formed from the axial parenchyma cells, which underwent periclinal divisions to form a wide band of cells referred as secondary cortex, which served as site for formation of next cambial ring by their repeated divisions, reported by [30, 31].

Each year, parenchyma cells in the stem dedifferentiates and become meristematic again forming new phloem and xylem tissue where phloem is always to the periphery and xylem to the middle of the stem [32]. This was closely followed by the pericycle, made up of sclerenchyma tissue to provide mechanical support to the plant. The secondary xylem and phloem produced by the vascular cambium which is a secondary meristematic tissue enables the plant conduct and translocate much water/nutrients and food respectively to meet the increasing need of the plant. As growth continues, the vasculature appear to be randomly dispersed due to the activity of each independent cambium. The organization of the vascular bundles themselves appear to exhibit a collateral arrangement with fibres, sclerenchmya and possible angular collenchyma found surrounding these bundles. These cell types provide extra protection and add support to the growing stem. The pith cavity was filled with parenchyma cells to enable the plant store more starch.

The leaves of *N. imperialis* plant have adapted to a hot, dry climate in several ways. Their flat surface makes it ideal for maximum light interception while the wide surface allows for a denser distribution of stomata complexes. The stomata complexes, all found on the abaxial side of the leaf, allows the leaf to carry out gas exchange during the hot day without the direct heat of the sun drying out the leaf. The anatomical study of the leaf of *Napoleona* reveals that they have highly evolved characters of dicotyledons. This agrees with the findings of Metcalfe and Chalk [29]. The common anatomical features included upper and lower epidermises, covered by a thin cuticle. The cuticle layer allows the leaf to prevent water loss in the hot environment [33]. This is followed by the mesophyll cells (Palisade and spongy mesophylls). Other cells found in the midrib included collenchyma, parenchyma, bundle sheath made of sclerenchyma cells, xylem towards the adaxial surface and phloem towards the abaxial portion.

8. Conclusion

Modern taxonomy leans very heavily on multiple characters rather than on single character difference. This study, in addition to the earlier published research work on morphology and epidermal characters has provided important data for identification as well as a contribution towards a better knowledge of this species. More work should be undertaken, in the area of cytology, phytochemistry, etc to provide more data for taxonomic purposes.

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