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Assessment of ecogeographical variation in the pollen morphology of *Tacca leontopetaloides*

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Abstract

The pollen of *Tacca leontopetaloides* collected from four different locations across Nigeria was prepared and studied under the light microscope. The exine pattern was observed to be fairly striate in all samples from the four different locations, while all the grains were monosulcate. Based on the polar axis size, the pollen sample from Akoko (sample A) was small sized, while the rest are medium sized. The pollen shape for pollen grains from Akoko (sample A) was oblate, pollen grains from Eruwa (sample B) were peroblate, and pollens from Bazza (sample C) were oblate spheroidal, while pollens from Ile-Ife (sample D) were subspheroidal. The exine thickness (2 µm) was observed to be virtually the same in all the four samples, both at polar and equatorial views, while the polar axis, however, varies among the four samples, ranging from 22 µm in sample B (Eruwa) to 44 µm in sample C (Bazza). However, the equatorial diameter of the four samples is in the range of 41.4 µm in sample D (Ile-Ife) and 46.4 µm in sample C (Bazza). P/E% varies widely from 48.8% in sample B (Eruwa) to 94.8% in sample C (Bazza), while sample A (Akoko) was 70.9% and sample D (Ile-Ife) was 77.3%. The results show that the pollen grains collected from the four different locations did not differ significantly from one another, but they share striking similarities with other *Tacca* species and even with members of other closely related families.

Keywords: *Tacca leontopetaloides,* pollen morphology, Taccaceae, Nigeria.

INTRODUCTION

Tacca leontopetaloides (L.) Kuntze (Polynesian arrowroot) is a wild perennial herb belonging to the family Taccaceae. *Tacca* is the only genus in the family Taccaceae. Taccaceae is a relatively newly-developed plant family carved out of the family Dioscoreaceae, but both families still share a close taxonomic relationship (Caddick et al., 2002). The plant is native to Malaysia and the Pacific Islands (Purseglove, 1972; Kay, 1987) and it is naturally distributed from Western Africa, through Southern Asia to northern Australia. The plant is known by other botanical names identified as synonyms such as *Tacca involucrata* Schummach and Thonn, *Tacca artocarpifolia* Seem, *Tacca pinnatifida* J.R Forst and G. Forst, *Leontice leontopetaloides* (Family Berberidaceae) (Carter, 1962). Other synonymous names include *Tacca abyssinica, Tacca brownie* among others. In Nigeria, the (plant is widespread in the middle belt of Nigeria (Manek et al., 2005) and Southwestern states. It is found in solitary forms on open fields or under the shade of trees or hill tops.

T. leontopetaloides, like other members of Taccaceae, possess hermaphroditic and perfect flowers. The inflorescence is borne on a leafless, unbranched scape arising directly from the rhizome/crown, usually cymose, but appearing umbel-like, surrounded by large, ovate involucral bracts in two whorls. The flowers are two whorls of three tepals, basally fused, green to dark brown or purple in colour, sometimes slightly fleshy (Caddick et al., 2002). The ovary is inferior, six-ribbed, unilocular, with three parietal placentas, and numerous ovules. In addition, the style is three-bilobed with broadened stigmatic branches, forming an umbrella-like structure,

Table 1. Specimens of *T. leontopetaloides* (L.) Kuntze examined.

Specimen	Locality	Collector/No	Date collected
A	Ishua/Ifira Road, Akoko, Ondo State	Mr. B.O Daramola UIH/09/245	30th July, 2009
B	Eruwa Road, Eruwa, Oyo State	Mr. T.I Borokini UIH/09/246	11th August, 2009
C	Bazza, Michika LGA, Adamawa State	Mr. T. Colman UIH/09/244	10th May, 2009
D	Along hill 2, OAU, Ile-Ife, Osun State	Mr. T.I Borokini UIH/09/247	25th May, 2010

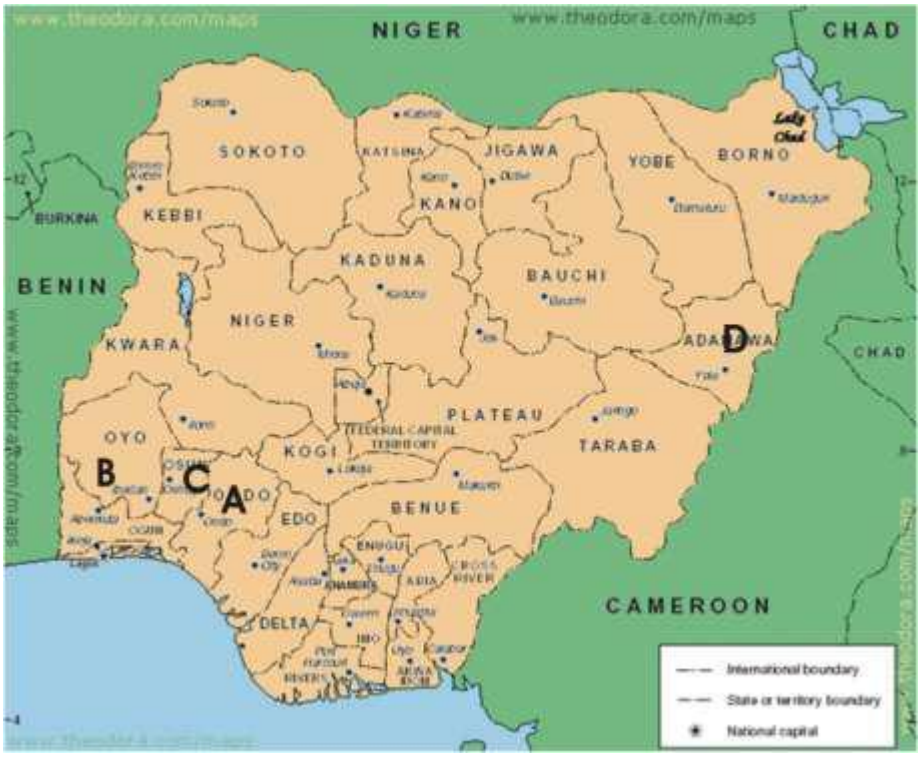

Figure 1. Map of Nigeria showing the sites of Plant collections.

papillose on the underside. The base of style is often broadened and covered with multicellular glandular hairs (Caddick et al., 2002).

There is dearth of knowledge on the pollen of *T. leontopetaloides* from the tropical Africa. It is regarded as one of the underutilized plants that appear abandoned by both the local people and the scientists, especially in this part of the world. The only work done on the plant is mainly on the tuber starch composition, properties and utilization. Therefore, this study was carried out to investigate the ecogeographical variation in the pollen morphology of *T. leontopetaloides*.

MATERIALS AND METHODS

Collection of plant materials

Fresh samples from wild collections in four different locations across the country were used for the study. Three of them were collected within May and August 2009, while one was collected in May 2010 (Table 1, Figure 1). The four samples were labeled A – D (Table 1, Figure 1), and voucher specimens were deposited in the Herbarium of the Department of Botany, University of Ibadan (UIH) and Herbarium of National Centre for Genetic Resources and Biotechnology (NACGRAB).

Pollen preparation

Fresh samples of A, B, and D and herbarium sample of C (Table 1) were used for the study. Erdtman's (1952) acetolysis method was used for the preparation of the pollen grains from the specimens. The flower buds were crushed with a glass rod in centrifuge tube. 9 part of acetic anhydride and 1 part of concentrated H_2SO_4 acid were prepared to make the acetolysis mixture. Three milliliter of the acetolysis mixture was added to the crushed flower buds of each of the four plant samples in separate test tubes, labeled A, B, C and D. The content was heated in a water bath to boil for 10 min and stirred occasionally with a glass stiring rod. Thereafter, the hot contents were centrifuged at 4,000 revolutions per minute (rpm) for

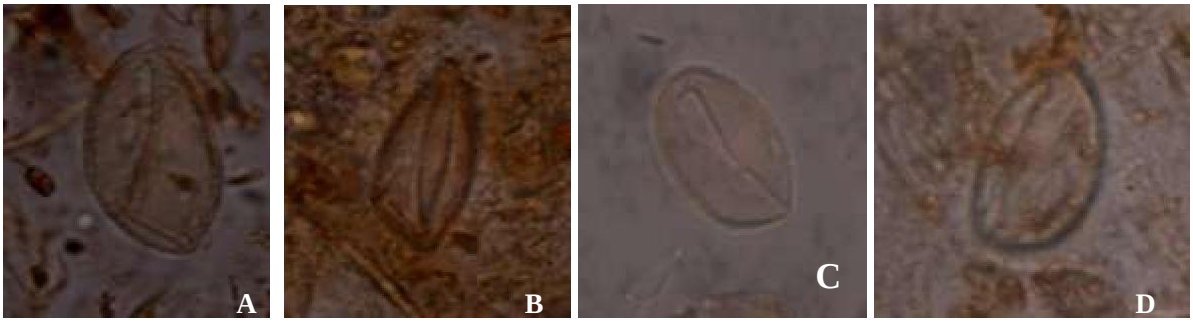

Figure 2. Equatorial views of the four collections of *T. leontopetaloides* Pollen grains (Scale bars = 5 µm) . (A) Akoko sample, (B) Eruwa sample, (C) Bazza sample, (D) Ile-Ife sample.

Fig 2 A – D: Equatorial views of the four collections of *Tacca leontopetaloides* Pollen grains (Scale bars = 5 µm)

A: Akoko sample B: Eruwa sample C: Bazza sample D: Ile-Ife sample

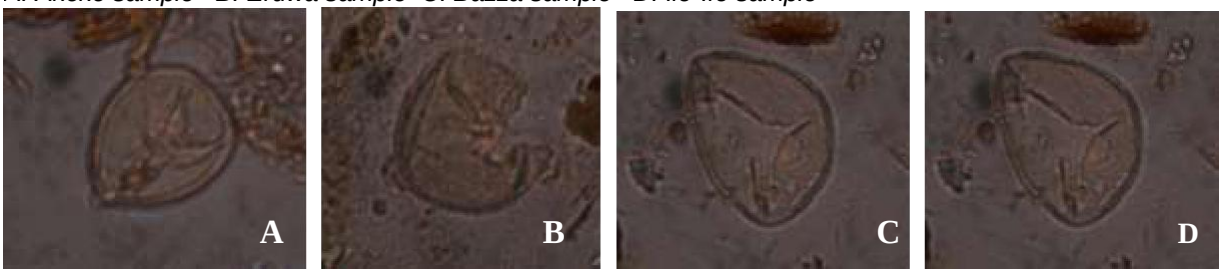

Figure3 3A. Polar**–D:** views**Polar**of **views**thefour **of**collections**thefour**of *T***collections**.*leontopetaloides***of*Tacca***leontopetaloides***(Scalebars=3µm)**Pollen**.(A)Akoko**grains**sample,**(Scale bars = 3 µm)** (B) Eruwa sample, (C) Bazza sample, (D) Ile-Ife sample.

A: Akoko sample B: Eruwa sample C: Bazza sample D: Ile-Ife sample

5 min and the supernatant decanted into acetolysis waste bottle. Some quantity of water was added to the precipitate and the sediments in each of the four test tubes and shaken vigorously; centrifuged and decanted so as to remove excess acetolysis mixture. Washing with water, centrifuging and decanting were repeated four times and to the resulting residue, 50% glycerol was added and left standing for two hours. Thereafter, the contents were shaken and centrifuged at 4,000 rpm for 10 min, decanted off finally, inverted over paper and left overnight. 100% glycerol was added to the contents of each test tube and later poured into storage vials and labeled. Pollen of the four samples in the vials were made on clean slides, labeled A – D and covered with a cover slip and the edges sealed with nail varnish.

Data collection

Parameters studied include polar axis, exine pattern, pollen shape, exine thickness, and equatorial diameter, while the P/E% was calculated. A total of 10 grains were studied for each of the four samples collected. The mean and standard error were calculated for all measurements. Photomicrographs were taken using NIKON AFX-DX microscope with NIKON FX-35DX camera attached. The exine pattern and pollen shape of the pollen grains for the four samples were determined using the terminologies of Erdtman (1952), Moore et al. (1991) and Hyde and Adams (1958).

RESULTS AND DISCUSSION

The pollens from the four different locations did not show any significant variation from one another. The exine pattern was observed to the fairly striate in all the four samples, while all the grains were monosulcate (Figures 2A-D and 3A-D). In the same vein, the pollen shape of the four pollen samples differs. Samples collected from Akoko (sample A) were oblate, sample from Eruwa (sample B) were peroblate, while samples collected from Bazza (sample C) were oblate-spheroidal, and those from Ile-Ife (sample D) were subspheroidal (Table 2). The exine thickness of 2 µm was the same in all the four samples both at polar and equatorial views (Table 2). The polar axis varies among the four samples, ranging from 22 µm in sample B (Eruwa samples) to 44 µm in sample C (Bazza samples) (Table 2). And based on their polar axis, the pollen samples were classified into pollen sizes. The pollen samples from Eruwa (sample B) were small sized, while the rest were medium sized (Table 2). However, the equatorial diameter of the four samples did not show any significant variation, ranging between 41.4 µm in sample D (Ile-Ife collections) and 46.4µm in sample C (Bazza samples) (Table 2). The P/E% varies widely from 48.8% in sample B (Eruwa) to 94.8% in sample C (Bazza), while sample A (Akoko) was 70.9% and sample D (Ile-Ife) was 77.3% (Table 2).

In this study, it was observed that the aperture type of the pollens of *T. leontopetaloides* are monosulcate, with fairly striate exine pattern; while there were insignificant

Table 2. Quantitative characters on *Tacca leontopetaloides* pollen grain samples from the four locations.

Character	Sample A	Sample B	Sample C	Sample D
Polar Axis (µm)	30 - 34.4	20-24	42.1 - 46	30.5- 33.1
Range/Mean ± S.E	32.5± 0.46	22 ± 0.45	44 ± 0.41	32 ± 0.26
Equatorial diameter (µm)	43.8 - 48	43 - 46.7	44.5 - 48	39.8 - 43.4
Range/Mean ± S.E	45.8± 0.38	45 ± 0.4	46.4± 0.35	41.4± 0.38
P/E%	70.9	48.8	94.8	77.3
Exine thickness (Polar view) (µm)	2± 0	2 ± 0	2± 0	2± 0
Exine thickness (Equatorial view) (µm)	2± 0	2 ± 0	2± 0	2± 0
Pollen shape	Oblate	Peroblate	Oblate spheroidal	Subspheroidal
Pollen size	Medium	Small	Medium	Medium
Aperture type	Monosulcate	Monosulcate	Monosulcate	Monosulcate

differences, especially in the pollens shape and size among the pollen samples collected from the four locations within Nigeria. The observation of monosulcate pollens in *T. leontopetaloides* confirms the hypothesis by Schols et al. (2001) that monosulcate pollens are plesiomorphic in monocots generally. This agrees with earlier established fact that pollens are important plant parts that do not change easily with changes in the environment. These results also suggest a common ancestral origin for the plants and an insignificant evolutionary change in the taxa over the years. However, it has been noted that of all the morphological characters of the pollen grain, the shape is less useful taxonomically (Davis and Heywood, 1963; Moore et al., 1991) because it can vary considerably within one grain type or even within one species and the variation can be caused by the choice of extraction methods and embedding media (Moore et al., 1991). Therefore, the differences in the pollen shape and class in *T. leontopetaloides* studied may not be considered as a variation among the four pollen samples.

The results from this study corroborated previous work done of Caddick et al. (1998, 2002) on Taccaceae where it was discovered that the pollen grains were monosulcate and spheroidal, while the sexine sculpturing was finely reticulate, similar to that of some species of *Dioscorea* (*e.g. D. lanata*), while the intine was not thickened. Furthermore, Caddick et al. (1998) observed monosulcate pollen grains in *Tacca artocarpifolia* and *T. chantrieri* indicating that *T. leontopetaloides, T. artocarpifolia* and *T. chantrieri* all share similar pollen morphology. In addition, Caddick et al. (1998), studying other representative members of several families in the Order Dioscoreales, observed monosulcate pollen grains for Corsiaceae, Petrosaviaceae, Japonoliriaceae, Nartheciaceae, Stenomeridaceae and some members of Discoreaceae, Trichopodaceae and Stemonaceae. However, in another study by Schols et al. (2003), on 61 *Dioscorea* species, about 10% of the taxa studied were monosulcate, which has been established by Furness and Rudall (1999) as the most common aperture type in monocots. Furthermore, Schols et al. (2003) observed that the mean polar axis for all taxa studied ranged from 20 to 58 µm, while the equatorial diameter was in the range of 13 µm (in *Dioscorea nako, Dioscorea ovinala, Dioscorea sinuata*) and 45 µm (in *Dioscorea rupicola*). Like *T. leontopetaloides*, the pollen exine pattern of many of the *Dioscorea* species studied by Schols et al. (2003) was striate. While *T. leontopetaloides* pollen exine pattern was observed to be striate, only 28% of the total 61 *Dioscorea* species studied were striate; majority (54%) of them were perforate (Schols et al., 2003).

Several current taxonomic hypotheses are tested and supported by pollen data, and the increasing amount of palynological data makes it possible to propose some hypotheses about the relationships among plant taxa and families. Since Palynology is the science of pollen and spore morphology (Khan, 2005); this study is relevant as an instrument of multiple research for systematic botany, paleobotany, paleoecology, pollen analysis, areopalynology, criminology, allergy stratigraphic correlation of oil bearing rocks and coal fields, as well as improvement of honey (Khan, 2005).

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