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Regular Article

Morphological and anatomical studies on *Tacca leontopetaloides* (L.) Kuntze (Taccaceae) in Nigeria

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***Tacca leontopetaloides* is the only species in the genus *Tacca* naturally distributed in the Guinea savannah and dry rainforest regions in Nigeria. The study was conducted to contribute to scientific knowledge on the macro-morphological and anatomical features of the plant collected from 4 locations (Akoko, Eruwa, Bazza and Ile-Ife) across the Nigeria. Leaf epidermal peel of the 4 samples, leaf and stem Transverse Sections (T.S) and stem Tangential Longitudinal Section (T.L.S) and Radial Longitudinal Section (R.L.S) as well as stem fibres were prepared and studied. A wide range of similarities and differences were noted in the anatomical features of the 4 samples studied. The similarities include, but not limited to stomata being anomocytic, epidermis hypostomatic, cuticle single-layered and striated, vascular bundles conjointed collateral, epidermis undulating, stem rays uniserrate and procumbent, stem fibres non-septate and non-storeyed. Wide range of variations are noted in stomata density and measurements, epidermal cell density, guard cell measurements, cuticle thickness, vascular bundle arrangement, shapes of parenchyma and sclerenchyma cells and many other anatomical features. The variations observed among the samples studies were attributed to the prevailing environmental factors in each collection site.**

Keywords: *Tacca leontopetaloides*, Taccaceae, macromorphology, anatomy, Nigeria.

Tacca leontopetaloides (L.) Kuntze is a wild perennial herb belonging to the family Taccaceae. *Tacca* J.R. & G. Forst is the only genus in the family Taccaceae, a relatively newly-developed plant family carved out of the Dioscoreaceae, but both families are still closely connected taxonomically (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. Because of its wide distribution, the plant has numerous common and synonymous scientific names, but Polynesian arrowroot appears to be the most widely used. The plant is more widespread in the middle belt of Nigeria (Manek et al., 2005) and in the Southwestern states than in the northern parts of the country, thereby suggesting its

ecogeographical distribution within the tropical humid forests and Guinea savanna. It is more abundant in solitary forms, in open fields or under the shade of trees or hill tops. The plant is found mainly in rainy season (March to August), while dormant through the dry season (from September to February). It is interesting to note that this plant which produces tubers also produces fleshy sweet-tasting fruits which are dispersed by birds and mammals (Drenth, 1972). The plant still remains in the wild and is underutilized in Nigeria. The seeds have shown poor germination while vegetative propagation by tubers appears to be the most prevalent method especially in middle belt region of Nigeria. In the Pacific Islands, the plant is considered an economic food crop which is already in cultivation (Kay, 1987) and selections have been done to produce improved strains.

The leaves are erect, with long sheathing cataphylls enclosing the bases of the petioles and flowering stems. The petioles are ridged longitudinally, with a sheathing base; leaf blade is trisected at the apex of the petiole; the lobes are ovate-acuminate with smaller, orbicular lobes between them (Carter, 1962). There is just one flowering stem, also ridged longitudinally. The flowers are of the perianth type, 20 - 40, with erect perianth segments and usually green tingled with purple. However, only a few of the flowers produce fruits. The flowers are actinomorphic and hermaphroditic, umbellate, and the bracts form an involucre, which is greenish. The stamens are usually 6, the anthers are 2-celled, ovary is inferior and one-celled with three parietal placentas but the ovules are numerous (Hutchinson and Dalziel, 1968). The fruit is a berry, sub-globose and 6-ridged. The seeds are numerous with copious endosperm and a minute embryo. The seeds are ovate, longitudinally ridged, and red-brown but surrounded by a thin fleshy aril (Carter, 1962).

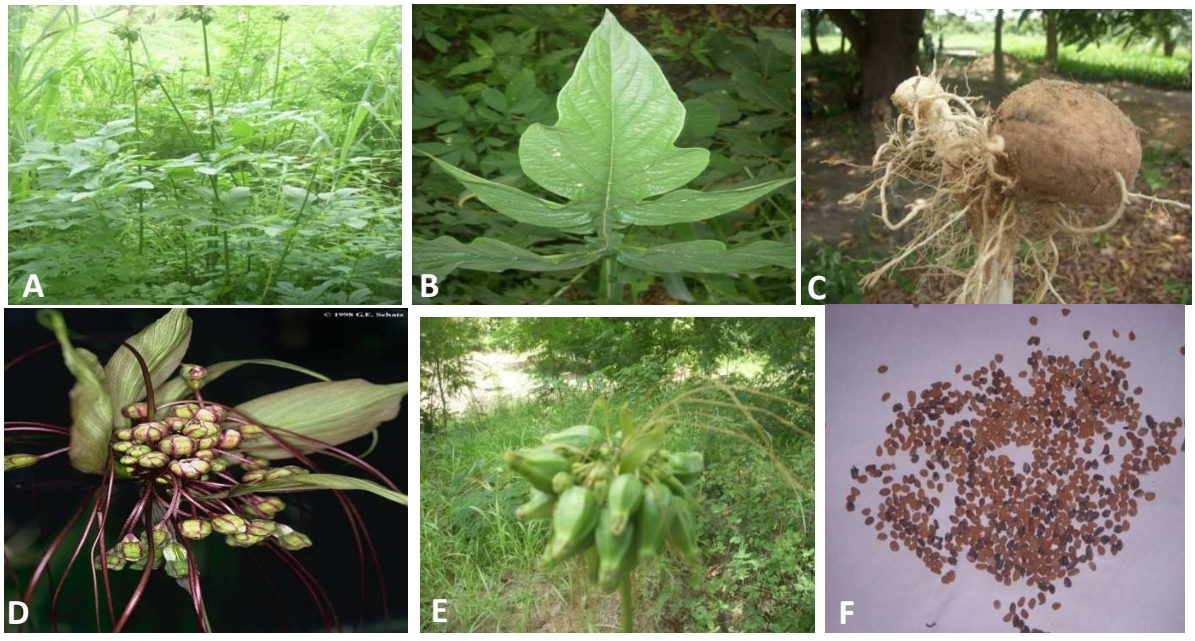

Fig. 1: Photographs showing *T. leontopetaloides* plant; A: Whole plant; B: Upper leaf surface C: Root tubers; D: Flowers; E: Fruits; F: Seeds. (All photos taken by author, except D)

In addition, the inflorescence is borne on a leafless unbranched scape arising directly from the rhizome. The flowers are made up of two whorls of three tepals, basically fused, green to dark brown or purple in colour. The fruits are indehiscent, while the seeds are prismatic with thin-walled endosperm and a distinct raphe (Caddick et al., 2002). The leaves are broad-blade, deeply lobed and divided into three sections (Ounruen and Sangyojarn, 2001). The leaf in upper surface has depressed veins and the under surface is shiny with bold yellow veins. Figure 1 illustrates the different plant parts.

In Northern Nigeria, the tubers are eaten especially when other staple foods are scarce (Kay, 1987). Although the tubers are poisonous, the poison is removed by soaking or washing the starchy tubers in water and rinsing repeatedly. Thereafter they can be boiled or roasted. The tuber contains starch, ceryl alcohol, steroidal saponins and a bitter principle, Taccalin (Caddick et al., 2002). The bitter raw tubers are used to treat stomach ailments, mainly diarrhea and dysentery in many Polynesian Islands (Kay, 1987; Brand-Miller et al., 1993). The root starch is used to stiffen fabrics in some of the Islands (Ukpabi et al., 2009). Several ethnobotanical uses of the plant in Nigeria have been discussed by Ukpabi et al. (2009). Literature on *T. leontopetaloides,* especially in Nigeria is sparse. 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 observable work done on the plant is mainly on the tuber starch composition, properties and utilization (Ukpabi et al., 2009). Therefore, this study was carried out to study the anatomical features of the leaves and stem and determine morphological variations among the plant populations collected from 4 different locations in Southwest Nigeria.

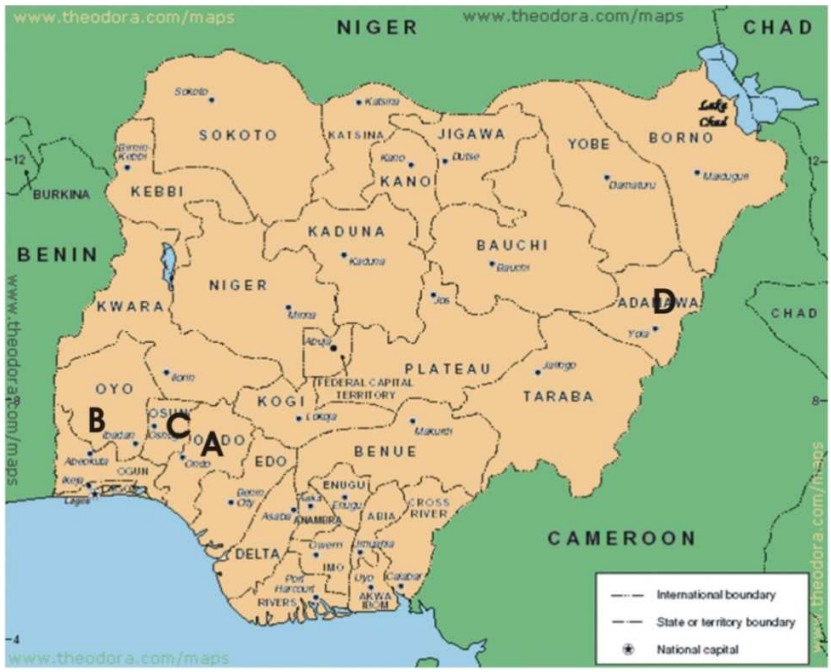

Fig. 2: Map of Nigeria showing collections sites of *T. leontopetaloides*

A: Akoko, Ondo State B: Eruwa, Oyo State

C: Bazza, Adamawa State D: Ile-Ife, Osun State

Materials and Methods

Collection of Plant Materials: Fresh samples and dried herbarium specimens of *Tacca leontopetaloides* deposited at the University of Ibadan Herbarium (UIH) were used for the study (Table 1, Fig. 2).

Leaf epidermal studies: Fresh leaf materials from Akoko, Eruwa and Ile-Ife and herbarium samples from Bazza were used for the study. About 1 cm^2 was obtained from the standard median portion of the leaf samples. These were placed in concentrated Nitric acid (HNO_3) in appropriately labeled Petri dishes placed in the sun to hasten the action of the acid. They remained in the acid until bubbles appeared in the mesophyll. Each sample was transferred to another Petri dish containing water and rinsed three times to wash off the acid. The epidermal layers were separated by carefully holding the base with fine forceps and peeling off the upper from the lower epidermal layer.

Table 1. Collection sites of the four samples of *Tacca leontopetaloides*

	Locality	Collector/No	Date collected
Akoko	Ishua/Ifira Road, Akoko, Ondo State	B.O Daramola UIH/09/245	30/07/2009
Eruwa	Olokemeji Road, Eruwa, Oyo State	T.I Borokini UIH/09/246	11/08/2009
Bazza	Bazza, Michika LGA, Adamawa State	T. Colman UIH/09/244	10/05/2009
Ile-Ife	Along hill 2, OAU, Ile-Ife, Osun State	T.I Borokini UIH/09/247	25/05/2010

The peeled epidermal layers were preserved in 50 % ethanol. They were stained in toluidene blue for about 5 minutes and excess stain washed off in water. They were thereafter mounted in 25 % glycerine on glass slides and covered with cover slip. The edges of the cover slips were sealed with nail varnish to prevent dehydration. The slides were labeled and examined under the microscope. Microscopic observations and measurements were done using micrometers (eye piece and stage micrometers). Mean and Standard Error were calculated for each ten randomly assessed quantitative characters. Photomicrographs were taken using the Olympus microscope CX31 with camera Olympus E330-ADU 1.2X attached. Terminologies were based on Metcalfe & Chalk (1988) and Guard Cell Area (GCA) was calculated using the formula described by Franco (1939):

GCA= Guard cell length × Guard cell breadth × K

Where K = Franco's constant = 0.78524

Leaf and stem anatomy: Transverse sections (TS) of the leaf and stem and tangential longitudinal section (TLS) and radial longitudinal section (RLS) of the stem samples of the four samples were made at 10 microns thickness using the sledge microtome (Reichert, Austria). The sections were preserved in 50 % ethanol in vials prior to use. The sections were stained with Safranin O for 5 minutes and dehydrated through alcohol series 50 %, 70 %, 80 %, 90 % and 100 % ethanol, mounted on clean slides in 25 % glycerol and covered with cover slips. To prevent dehydration, the edges of the cover slips were sealed with nail varnish. Photomicrographs were taken using the

Olympus microscope CX31 with camera Olympus E330-ADU 1.2X attached. Microscopic observations of each slide was made and recorded. Identification and description of tissues and cells were based on Metcalfe & Chalk (1979) and Bilgrami et al. (1982).

Stem fibre analysis: Samples of the stem of *T. leontopetaloides* from Akoko, Eruwa and Ile-Ife were used. These were sliced into small pieces using pen knife and macerated using Schultz's fluid obtained by mixing equal volume of 10 % Chromic Acid [1 g Potassium Nitrate (KNO_3) in 50 ml concentrated Nitric Acid (HNO_3)] and 10 % Nitric Acid. The Schultz's fluid with the sample in labeled beakers was heated on a hotplate for 20 minutes. The macerated samples were washed in five changes of water and stained in Safranin O. The Slides of the samples were prepared on microscopic slides and properly labeled. Microscopic observation of each slide was made and recorded. Measurements of fibre length, width, wall thickness and lumen; vessel element length and width were made using stage and eyepiece micrometer.

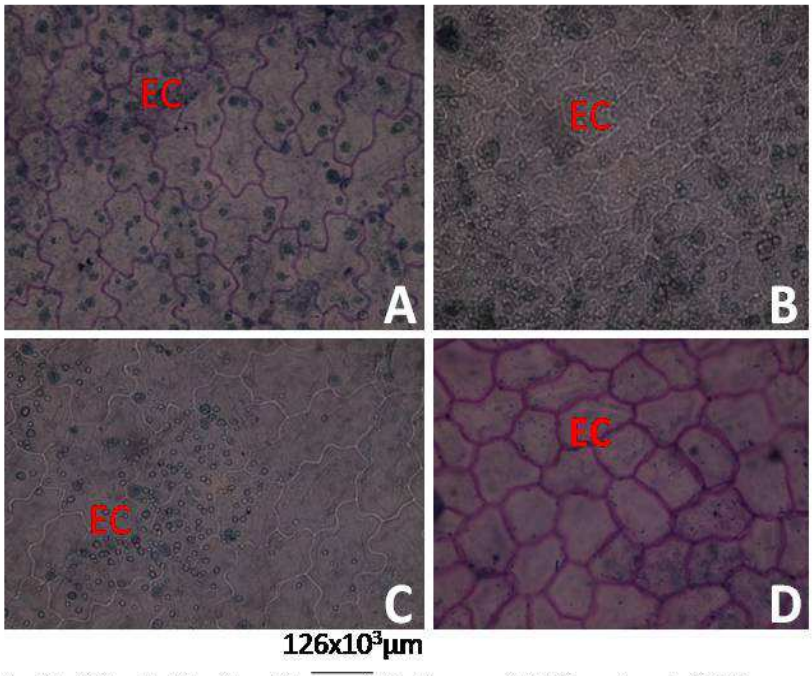

Fig. 3: Adaxial leaf epidermal features of *T.leontopetaloides* samples (126 x 10³µm)
A: *T. leontopetaloides* leaf sample from Akoko, Ondo state, B: *T. leontopetaloides* leaf sample from Eruwa, Oyo state, C: *T. leontopetaloides* leaf sample from Bazza, Adamawa state, D: *T. leontopetaloides* leaf sample from Ile-Ife, Osun state, EC : Epidermal cell

Results

Leaf epidermal studies: The epidermis exhibited wide range of differences in its features among the 4 samples studied. The number of epidermal cells per view (x400) on adaxial layer ranged from 18 in Bazza sample to 59 in Eruwa sample while it ranged from 42 in Eruwa sample to 84 in Ile-Ife sample on the abaxial epidermis (Table 2). The mean epidermal cell size on the adaxial surface ranged from 58 µm in Eruwa sample and 71.5 µm in Akoko sample to 74 µm in Ile-Ife sample and 115 µm in Bazza sample on the abaxial surface (Table 2). The anticlinal walls

were undulate in Akoko, Eruwa and Bazza samples on both surfaces, while they were straight only on the adaxial surface of Ile-Ife sample (Figures 3 and 4). Stomata were mostly anomocytic with a stoma surrounded by a number of cells that are indistinguishable in shape and size from other epidermal cells. Furthermore, the epidermal cells were observed to be hypostomatic, with the stomata found only in the abaxial leaf surfaces in all the samples studied.

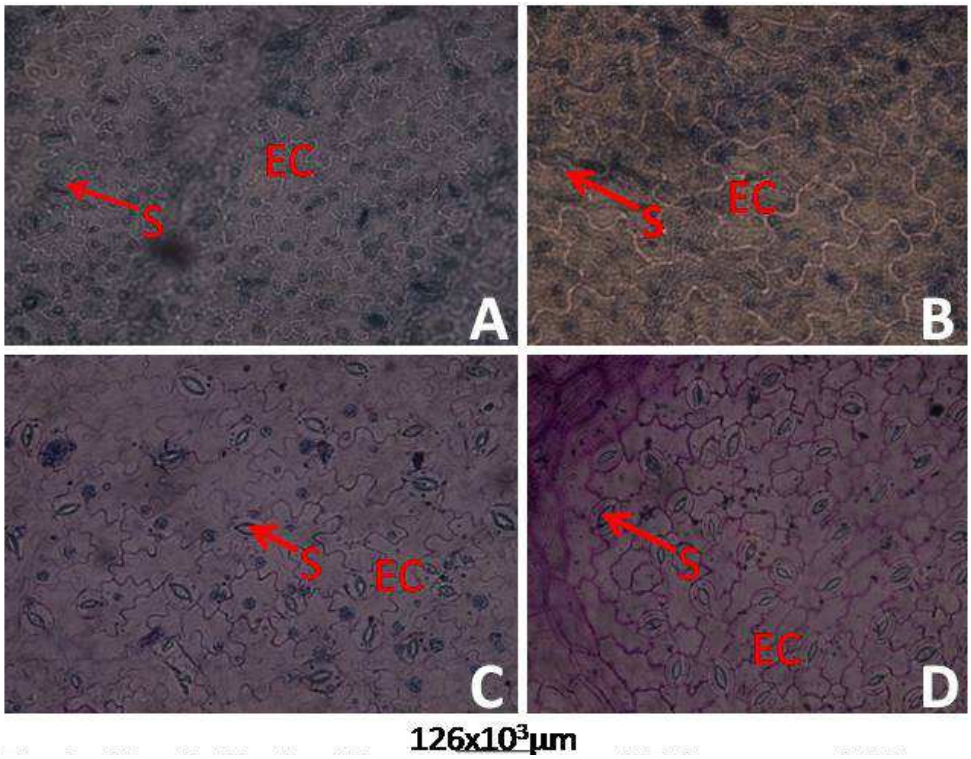

Fig. 4: Abaxial leaf epidermal features of *T.leontopetaloides* samples (126 x 10³µm)
A: *T. leontopetaloides* leaf sample from Akoko, Ondo state, B: *T. leontopetaloides* leaf sample from Eruwa, Oyo state, C: *T. leontopetaloides* leaf sample from Bazza, Adamawa state, D: *T. leontopetaloides* leaf sample from Ile-Ife, Osun state, EC : Epidermal cell S : Stomata

Stomata densities on the abaxial surfaces of the leaves of the four samples vary widely, ranging from 18 in Eruwa sample to 37 in Ile-Ife sample. The mean stomata length and width as well as guard cell width for the four samples did not show significant wide variation unlike for guard cell length and guard cell area (GCA) with wider variations. The stomata length was 31 µm for both Bazza and Ile-Ife samples and 29.5 µm for Akoko sample, while it was 30.5 µm for Eruwa sample (Table 2). Likewise for the stomata width, both samples from Ile Ife and Bazza had the least width of 25 µm followed by Eruwa sample with 26 µm, while Akoko sample had widest width of 27.5 µm, (Table 2). Guard cell length was in the range of 18 µm in Ile-Ife sample and 24 µm in Eruwa sample. Guard cell width followed similar pattern, with Ile-Ife sample having the lowest guard cell width of 9.5 µm, while Eruwa sample had the widest guard cell of 11.5 µm (Table 2). Guard cell area calculated for Ile-Ife sample was the lowest at 134.3 µm² and highest for Eruwa sample at 216.7 µm² (Table 2). Calcium oxalate crystals were

observed in both the adaxial and abaxial surfaces of the epidermal cells in all the samples while trichomes were completely absent in all the samples studied.

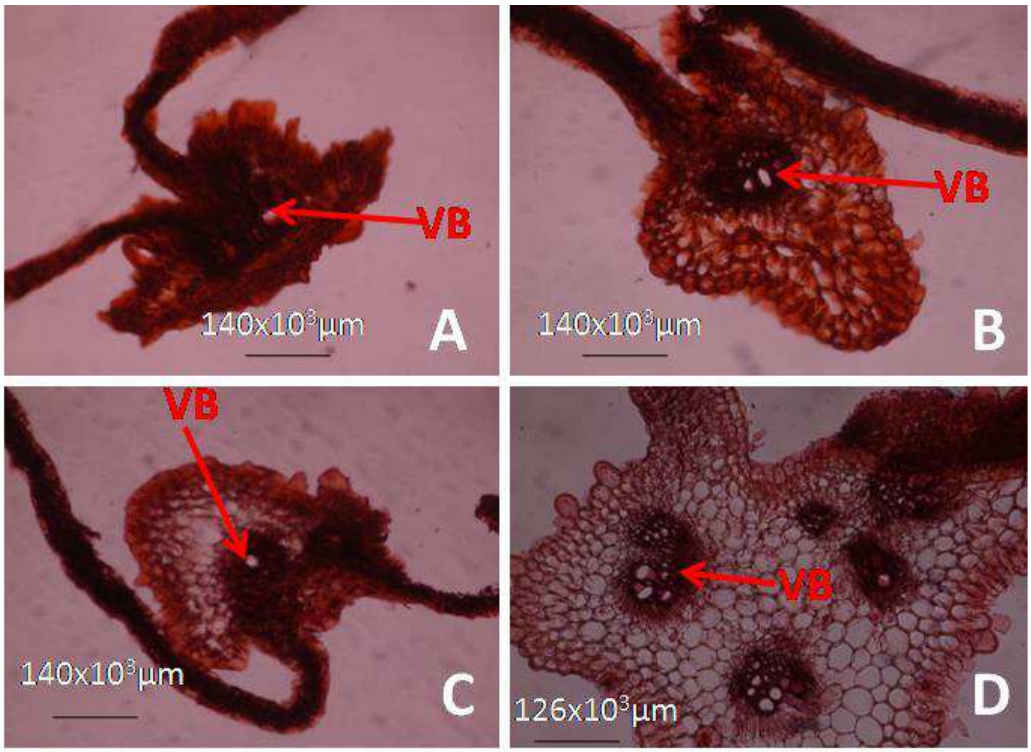

Fig. 5A – D: Transverse section (TS) of the leaf of *T. leontopetaloides*samples
A: *T. leontopetaloides* leaf sample from Akoko, Ondo state (140 x 10^3µm), B: *T. leontopetaloides* leaf sample from Eruwa, Oyo State (140 x 10^3µm), C: *T. leontopetaloides* leaf sample from Bazza, Adamawa State(140 x 10^3µm), D: *T. leontopetaloides* leaf sample from Ile-Ife, Osun State(126 x 10^3µm), VB : Vascular Bundle

Taking the significant mean difference to be 1.00, the mean stomata length of Akoko sample was significantly shorter than that of Eruwa (Table 2), indicating a significant difference between stomata length of Akoko and Eruwa samples. In the same vein, there is significant difference between Akoko and Ile-Ife samples, as well as Akoko and Bazza samples. However, there is no significant difference between Eruwa and Bazza samples, and between Eruwa and Ile-Ife and Bazza samples (Table 2). From the principle of taking significant mean difference to be 1.0 for stomata width, it could be observed that stomata width was significantly different across the 4 locations, therefore any null hypothesis formulated among the locations was rejected (Table 2). In the same vein, comparison of guard cell width across different locations indicated significant difference across the 4 locations except between samples from Akoko and Eruwa.

Furthermore, guard cell length of Bazza sample was significantly longer than the guard cell from other locations, while guard cell length of Ile-Ife sample was significantly shorter than guard cell from other locations (Table 2). However, there is no significant difference between guard cell length of Akoko and Eruwa samples, and between Eruwa and Ile-Ife samples. Furthermore, significant variations were

observed in the mean epidermal cell width in leaf adaxial region across the four locations, with Akoko sample having broadest width while Bazza sample had the smallest width. Similarly, significant variations were recorded in epidermal cell width in abaxial region across the 4 locations.

Table 2: Leaf macro morphological characters of the four samples of *Tacca leontopetaloides* studied

Characters/samples	Akoko	Eruwa	Bazza	Ile-Ife
Epidermal cell density (adaxial)	36	59	18	47
Epidermal cell density (abaxial)	55	42	51	84
Stomata density (Abaxial)	20	18	25	37
Stomata density(Adaxial)	Absent	Absent	Absent	Absent
Epidermal cell width (Adaxial)	57.5 – 90	55 – 65	57.5 – 82.5	50 – 77.5
	71.5±5.39	58±2.0	64±4.8	60±4.9
Epidermal cell width (Abaxial)	80 – 105	67.5 –100	77.5 – 140	62.5– 92.5
	92.5±4.2	87.5±5.5	115±10.5	74±5.3
Stomata length	27.5– 32.5	25 – 35	30 – 32.5	27.5 - 35
	29.5±1.04	30.5±1.66	31±0.61	31±1.27
Stomata width	22.5– 32.5	20 – 27.5	22.5 – 30	20 - 30
	27.5±1.77	26±1.5	25±1.37	25±1.77
Guard Cell length	17.5 - 20	22.5-27.5	17.5 – 20	15 – 27.5
	19±0.61	24±1.0	18.5±0.61	18±1.46
Guard cell width	10 – 12.5	10 – 12.5	7.5 – 15	7.5 – 10
	10.5±0.5	11.5±0.61	10.5±1.22	9.5±0.5
Guard Cell Area (GCA) (μm²)	156.7	216.7	152.5	134.3
Stomata type (Adaxial)	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Stomata type (Abaxial)	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Anticlinal cell wall pattern (Adaxial)	Undulating	Undulating	Undulating	Straight
Anticlinal cell wall pattern (Abaxial)	Undulating	Undulating	Undulating	Undulating

All measurements are in microns (μm) = Range / Mean ± Standard Error (SE)

Leaf Anatomy: Samples from Akoko, Eruwa, Bazza and Ile-Ife were used for the leaf anatomy studies. The cuticle was striated in all the samples, thin in samples from Akoko, Eruwa and Bazza and thick in sample from Ile-Ife (Figures 5A-D). The palisade and spongy mesophyll were clearly differentiated. The palisade mesophyll consisted of a layer of rectangular-shaped cells, compact, with the absence of airspaces while the spongy cells were circular, with airspaces (Figs. 5A-D). The vascular bundles were concentric with 1 – 6 xylem vessels surrounded by the phloem. The vascular bundles were located close to the centre of the midrib in Akoko, Eruwa and Bazza samples while the vascular bundles were scattered in Ile-Ife sample (Figs. 5A-D).

Stem Anatomy: Samples collected from Akoko, Eruwa and Ile-Ife were used for the stem anatomical studies. It could be noted from the results that the cuticle is single layered, striated with undulating epidermal layer in all the 3 samples, while the epidermis is characterized by undulations (Figs. 6A-C). The shape of the cells in the epidermis and the cortex vary among the 3 samples. Samples from Eruwa and Ile-Ife were observed to have distinct hypodermal layer, which was not observed in the sample from

Akoko (Figs. 6A-C). Table 3 shows the details of the results.

The presence or absence of sclerenchyma in the cortex and the arrangement of the sclerenchyma cells in the vascular bundles of the samples studied is of taxonomic importance (Table 3). In addition, the Tangential Longitudinal Section (TLS) and Radial Longitudinal Section (RLS) of stem of the 3 samples show rays, which are predominantly uniserrate and procumbent (Figures 7 and 8).

Table 3: Stem anatomy of the three samples of *Tacca leontopetaloides* studied

Tissue	*T. leontopetaloides* samples		
	Akoko sample	**Eruwa sample**	**Ile-Ife samples**
Cuticle	Single layer, striated	Single layer, striated	Single layer, striated
Epidermis	Undulating with V-shaped groves	Undulating with V-shaped groves	Undulating with hills
Hypodermis	Absent	Present	Present
Parenchyma cell shape	Oval, circular and few polygonal	Polygonal	Polygonal
Sclerenchyma	Absent in the cortex	Present, forming a ring around the pith in the cortex	Present, forming a ring around the pith in the cortex
Vascular bundles	All are conjoint collateral and arranged on 2 radii within the cortex.	All are conjoint collateral and arranged on 2 radii within the cortex. Vascular bundles associated with sclerenchyma cells	All are conjoint collateral and arranged on 2 radii within the cortex. Vascular bundles associated with sclerenchyma cells
Sclerenchyma cells on vascular bundles	The sclerenchyma cells form cap-shape in interior and cup-shape in exterior end of vascular bundles	The sclerenchyma cells form cap-shape in both interior and exterior ends of vascular bundles	The sclerenchyma cells form cap-shape in both interior and exterior ends of vascular bundles
Pith	Made up of circular to polygonal shaped cells with large spaces	Made up of polygonal shaped cells with large spaces	Made up of polygonal shaped cells with large spaces

Stem fibre analysis: Samples from Akoko, Eruwa and Ile-Ife were available for use for this study. The fibres in all the samples were observed to be non-septate and non-storeyed (Figs. 9A-C). The average fibre length among the samples varied from 1170 µm in Akoko sample, 1573 µm in Eruwa sample to 1553 µm in Ile-Ife sample (Table 4). Furthermore, average fibre width showed similar trends of variations of 15.75 µm, 27.88 µm and 22.5 µm in samples from Akoko, Eruwa and Ile-Ife respectively. Wall thickness was fairly similar in all samples studied. The lumen size was widely different. While samples from Akoko and Ile-Ife had 3.75 µm and 8.50 µm respectively, the lumen size in Eruwa sample was 13.13 µm (Table 4). Vessel members had scalariform perforation plate and simple pitting in all the samples studied. The average vessel length was 3595 µm in Akoko sample, 3675 µm in Eruwa sample and 5345 µm in Ile-Ife sample; while vessel width was 130 µm in Akoko sample, 145 µm in Eruwa sample and 120 µm in Ile-Ife sample (Table 4). Eruwa sample has the longest fibre length while the Akoko sample has the smallest. Also Eruwa has the broadest fibre width while Akoko sample has the narrowest fibre

width. Again Eruwa sample has the thickest fibre wall followed by Ile-Ife and Akoko has the least. Considering the lumen of the three samples, broadest lumen was found in the Eruwa sample while the least was found in Akoko sample. Furthermore, the longest vessel was also found in Ile-Ife sample while the shortest was found in Akoko sample. Experiment has also shows that Eruwa has the broadest vessel width while the sample from Ile-Ife has the narrowest. Therefore, considering the mean measurement of all the parameters in the table above, it shows that there is significant different in the fibre characteristics across the three samples. As a corollary, Eruwa sample has the highest value in all the parameters except in vessel length, Akoko has the lowest value in all the parameters except in vessel length and width while Ile-ife sample has the medium size in all the parameter observed except in vessel length which it has highest value.

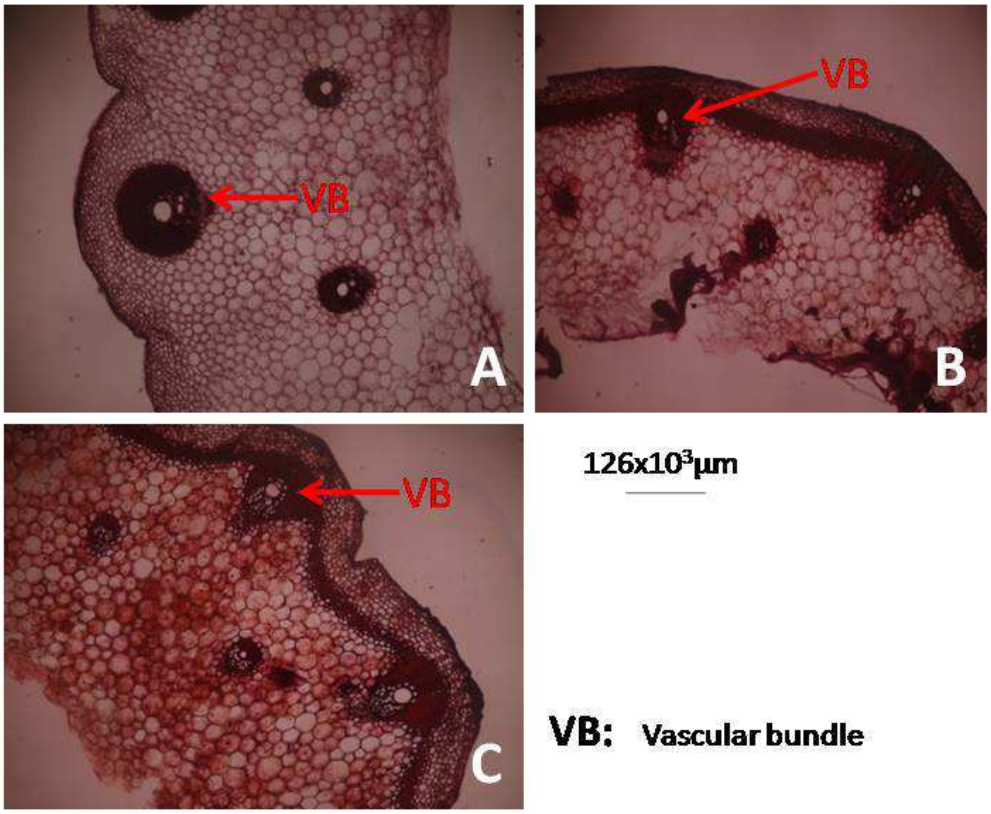

Fig. 6A-C: Transverse section (TS) of the stem of *T.leontopetaloides* samples (126 x 10³µm)
A: *T. leontopetaloides* stem sample from Akoko, Ondo State, B: *T. leontopetaloides* stem sample from Eruwa, Oyo State, C: *T. leontopetaloides* stem sample from Ile-Ife, Osun State

Table 4: Stem fibre analysis of the three samples of *Tacca leontopetaloides* studied

Sample	Fibre length	Fibre width	Wall thickness	Lumen	Vessel length	Vessel width
Akoko	1170	15.75	6.0	3.75	3595	130
Eruwa	1573	27.88	7.4	13.13	3675	145
Ile-Ife	1553	22.50	7.0	8.50	5345	120

*All measurements in *µm*

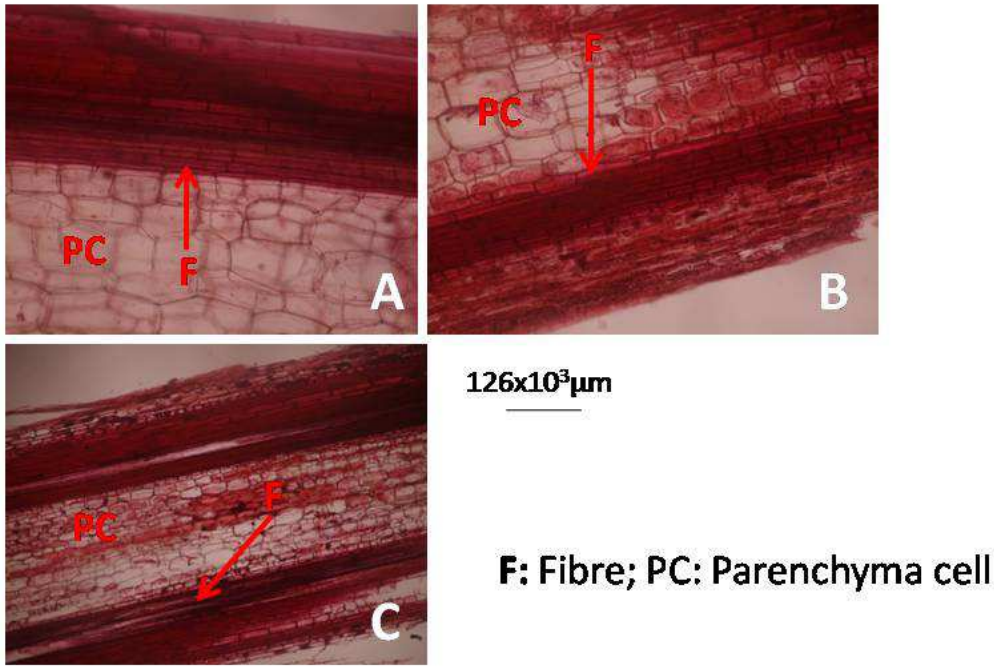

Fig. 7A-C: Stem tangential longitudinal section (TLS) of *T.leontopetaloides* samples (126 x 10^3µm) A: *T. leontopetaloides* stem sample from Akoko, B: *T. leontopetaloides* stem sample from Eruwa; C: *T. leontopetaloides* stem sample from Ile-Ife

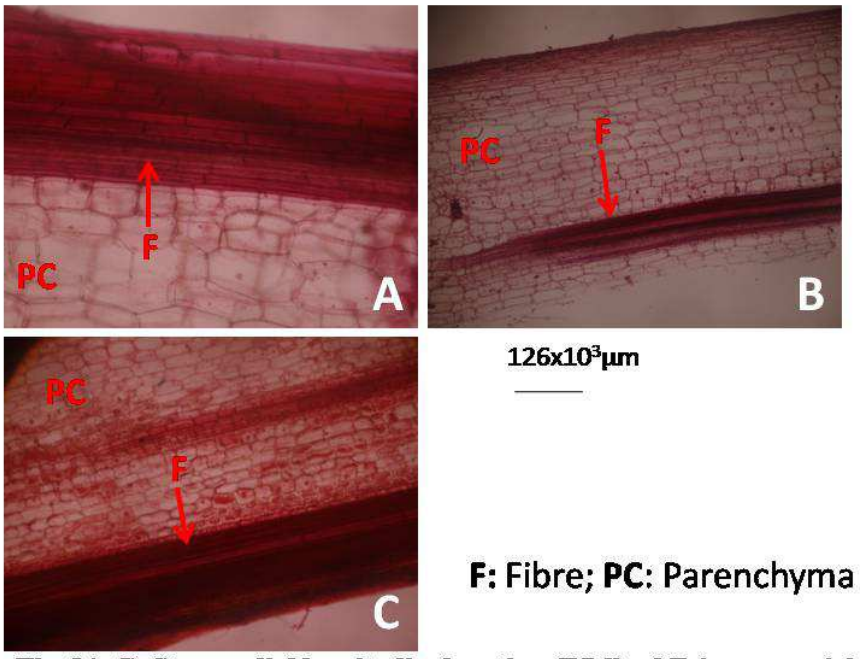

Fig. 8A-C: Stem radial longitudinal section (RLS) of *T. leontopetaloides* samples (126 x 10^3µm)
A: *T. leontopetaloides* stem sample from Akoko, B: *T. leontopetaloides* stem sample from Eruwa
C: *T. leontopetaloides* stem sample from Ile-Ife

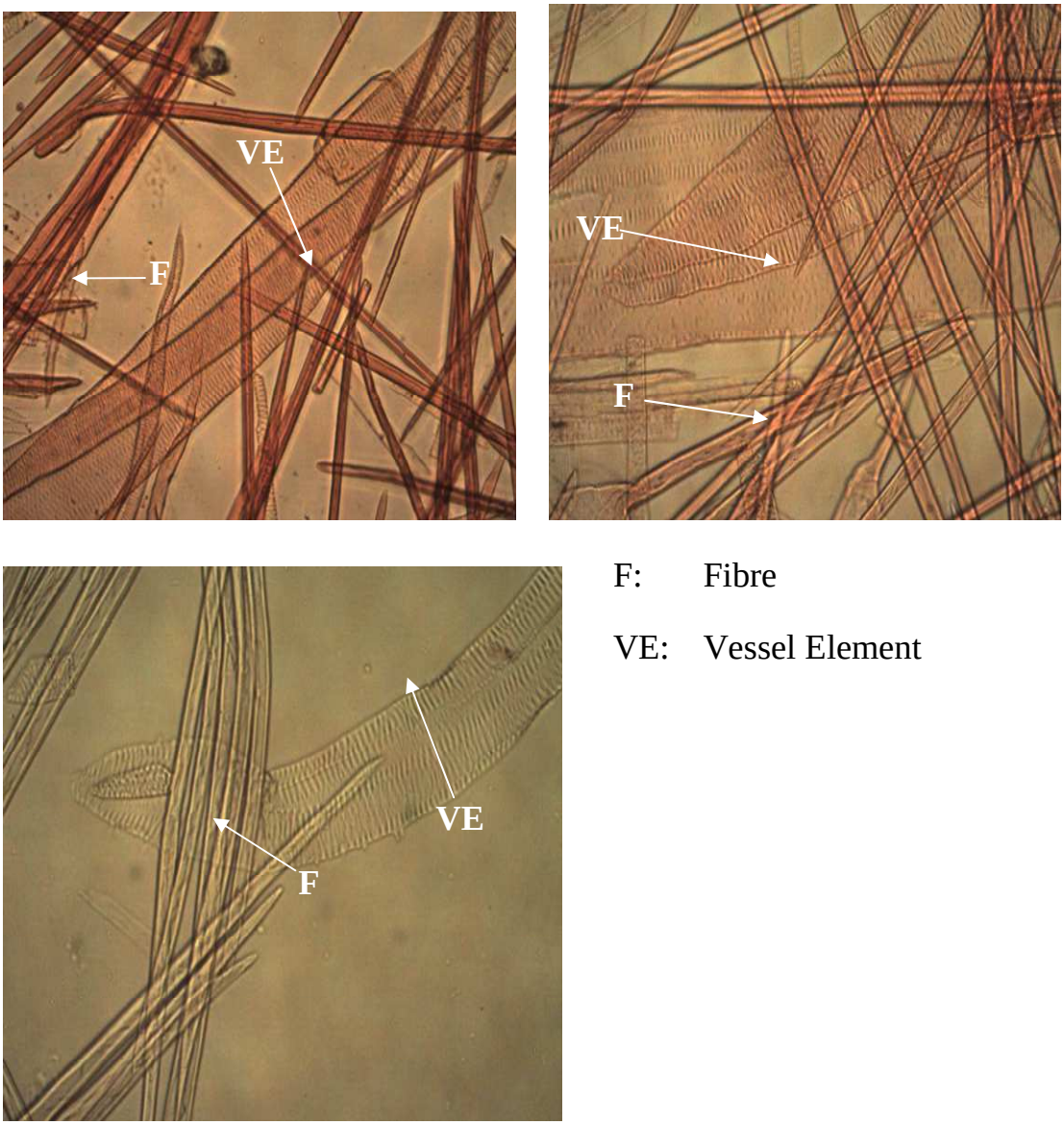

Fig. 9A-C: Stem fibres and vessels of *T. leontopetaloides* samples (126 x 10³µm)
A: *T. leontopetaloides*from Akoko, Ondo State, B: *T. leontopetaloides*from Eruwa, Oyo State
C: *T. leontopetaloides*Ile-Ife, Osun State

Discussion

Leaf epidermal characteristics are of taxonomical importance in many plants species (Stace, 1965, 1969; Wilkinson, 1979; Baranova, 1972, 1987, 1992a,b, 2004; Barthlott et al., 1998; Barthlott, 1981, 1990; Kong, 2001; Carpenter, 2005). Stomata diversity (size, shape, types and orientation) in leaf epidermis has great values in plant systematics (Tripathi and Mondal, 2012). The stomata length, stomata breadth and stomata shape, did not show striking variations

among the 4 samples studies. However, the guard cell length, guard cell breadth and Guard Cell Area (GCA) widely differed among the four samples. Since GCA is a function of the size of the guard cell, it affects the size of the stoma. The observed changes in the guard cell area, length and breadth of the 4 samples could be traced to varying environmental factors prevalent in the 4 collection sites. Several previous studies have been carried out to establish the effects of environmental factors on stomata development and density in plants. Such reported environmental factors include light intensity (Lake et al., 2001), light quality (Schoch et al., 1980), humidity (Serna and Fenoli, 2000), UV-B radiation (Kakani et al., 2003), drought signals (Franks and Farquhar, 2001), ozone (Warren et al., 2006), atmospheric CO_2 (Woodward, 1998) and elevated CO_2 concentrations (Woodward and Kelly, 1995).

Pompelli et al. (2010) noted that stomata density is "an important eco-physiological parameter that affects gas exchange and photosynthesis". Leaf stomata are the principal means of gas exchange in vascular plants, and it should be noted that the more stomata per unit area (stomata density) the more CO_2 can be taken up and the more water can be released. Thus, a higher stomata density can greatly amplify the potential for behavioral control over water loss rate and CO_2 uptake. The variation in the stomata number per view could be attributed to an adaptive feature of the plant in response to the climatic conditions prevalent in the location where they were collected.

However, the cell shape and cell wall pattern in the samples were slightly different, with the features in the samples collected from Ile-Ife different from the other three. It could be observed that the shape of a cell is highly dependent on the cell wall pattern. The way in which new structures mature as they are produced may be affected by the point in the plants' life when they begin to develop, as well as by the environment to which the structures are exposed. Several models, including the finite element model (Bolduc et al., 2006; Geitmann, 2010; Fayant et al., 2010) and anisotropic-viscoplastic model (Dumais et al., 2006), have been developed to explain the morphogenesis pathway for cell wall pattern and cell shape. Therefore, the shape of the cell wall and its patterns depends on the pattern of the morphogenesis of new cells, plant growth pattern, the position of sister cells, internal hydrostatic pressure and the type, composition and quantity of polysaccharide materials deposited in the cell wall.

Furthermore, sharp differences were observed in several of the anatomical features studied, such as cell number per view (cell density) at adaxial and abaxial surfaces, cell diameter, GCA, fibre length, fibre width, lumen, vessel length, stomata density and sclerenchyma shapes and formations in the stem among the samples used for the study. Samples from Akoko and Ile-Ife had V-shaped groves on the epidermis, which is clearly different in the sample from Eruwa with hills on the epidermal surface. Furthermore, there are no sclerenchyma cells in the cortex of samples from Akoko, while samples from Eruwa and Ile-Ife had sclerenchyma cells forming a ring in the cortex. Furthermore, the formation of the sclerenchyma cells at the exterior and interior ends of the vascular bundles differ among the 3 samples studied. These anatomic differences are of taxonomic significance which, if they become hereditary, could lead to evolutionary processes among the populations of the same species producing morphotypes, ecotypes, metapopulations and even new species.

From the studies, a wide range of similarities and differences were noted in the features studied among *T. leontopetaloides* stem and leaf samples collected in 4 different sites. For instance in the leaf epidermis of the

4 samples, stomata was anomocytic, epidermis is hypostomatic, presence of calcium oxalate crystals and undulating anticlinal cell wall pattern in the abaxial layer. However, variations were noted in the stomata density, epidermal cell density, stomata length, stomata breadth, guard cell length, guard cell width, guard cell area and the anticlinal cell wall pattern in the adaxial layer. In the same vein in leaf TS, cuticle is striated, mesophyll layer is distinct and vascular bundles divided into xylem and phloem for all the 4 samples; but the thickness of the cuticle and vascular bundle arrangement in the cortex vary.

Likewise, in the stem TS of the four samples, cuticle is single-layered and striated, epidermis was undulating and the vascular bundles were conjoint collateral. However, differences occur in the type of undulations in the epidermis, parenchyma cell shape, sclerenchyma cell shape in the interior and exterior end of vascular bundles and pith parenchyma cell shapes while hypodermis was not present in all the samples, neither was sclerenchyma present in the cortex of all the samples. In addition, sclerenchyma was observed in the vascular bundles of only 2 samples. The stem RLS and TLS showed uniformity in the rays of all the samples studied, while the stem fibre analysis showed that the fibres are non-septate and non-storeyed in all the samples; however the fibre length, fibre width, wall thickness, lumen, vessel length and vessel width differ considerably among the samples studied.

The variations observed in these plants collected from different locations were suggested to be environmentally-induced, and this agrees with previous studies. Phytochemical content studies conducted on the leaf and tuber of the same samples showed variations in the phytochemicals in them (Borokini and Ayodele, 2012a), while the pollen analysis of the same samples showed variations in the pollen polar axis, equatorial diameter, pollen size, pollen shape and polar axis-equatorial diameter ratio (Borokini and Ayodele, 2012b). Stace (1965) has noted that undulation of the cell wall is a mesomorphic character and it has been found that environmental conditions such as humidity play a significant role in determining the pattern of anticlinal cell wall. He further stated that straight or curved walls are characteristic of species growing in drier conditions, while undulate walls are found mostly in species growing in areas of high humidity. Ayodele and Olowokudejo (1997) noted the environmental influence on epidermal cell size, number and anticlinal undulation in *Eugenia obanensis* Bak. f. Yapp (1912), Salisbury (1927) and Watson (1942) further elucidated that variations in epidermal cell characters may be attributed to ecogeographical differences, such as light intensity, atmospheric humidity and pressure. They agreed that cells are generally known to be larger in size in humid or more shaded environment than in the less humid areas. Rea (1921) noted that sun-exposed plants of *Campanula rotundifolia* L. had more stomata per unit area than those growing in normal or shade conditions. This was corroborated by a study carried out by Penfound (1931). Stevovic et al. (2010) noted that different environmental conditions always have effects on the plants, while observing the differences in leaf thickness, chlorophyll ***a*** and ***b*** content and mesophyll thickness in *Tanacetum vulgare* L. collected from two sites in Serbia.

The leaf section revealed a number of similarities in the four samples collected, such as thin cuticle and one layer of epidermal cells common to them. But in sharp contrast, the shape of the vascular bundles differs considerably among the four samples, and this was determined by the abundance and distribution of the phloem cells surrounding the xylem. The density and distribution of the phloem cells is determined by the rate and amount of photosynthetic products made by the leaf to be transported

to other plant parts. And this is determined by the prevailing climatic conditions of the area where the plants were collected, access to the sunlight and day length being the important factors. Furthermore, Rocas et al. (2001) noted that *Alchornea triplinervia* (Spreng.) Muell. Arg.populations developed different response levels to the environmental factors studied, namely light and soil water regime. He noted that light accounted for the variations found in palisade and spongy parenchyma while the combination of light and soil water determined the variations found regarding the outer epidermal cell wall of the abaxial surface, the percentage of sclerenchymatous area in relation to the total midrib area and the compaction of the spongy parenchyma.

Furthermore, Stevovic et al. (2010) noted that well developed and thick cuticle is an effective peripheral protection and adaptation to environmental conditions such as increased insolation and temperature and life in the open habitat. Together with the scelerenchyma fibres, the cuticle offers mechanical support for the plant stem against damage from wind action. This could mean that changes in environmental conditions can affect the formation of the epidermal layers and cause variations in the anatomical features among populations of the same species. Pith with parenchyma cells containing large air spaces is common to all the samples studied and this is a common feature in many monocotyledonous plants.

Several researchers have worked on the environmental effects on the morphology, anatomy and physiology of different plants over a considerable long period of time, all producing positive results. Variations in plants are due to either hereditary or environmental factors, or a combination of both. These variations form basis for selective breeding of a plant to improve its vigour, yield or a particular trait. It could be concluded that environmental factors have a great influence on the anatomy, physiology and morphological features of *Tacca leontopetaloides*. Therefore, this study has further confirmed the impact of environmental factors on plant anatomy, morphology and physiology. As a plant responds to changes in its environment, such changes could be inherited by the next generations and thus could lead to permanent evolutionary change in the plant, which could lead to speciation. Though environmental factors contribute largely to the variations observed among the four samples of *Tacca leontopetaloides* collected from the different locations, they were not strong enough to delimit them and establish them as different morphotypes or forms.

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