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Leaf Epidermal Morphology of *Vitellaria paradoxa* Gaertn. F., Host Plant of *Cirina forda* Larva (Westwood) from Different Locations in Nigeria

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Abstract

Anatomical studies on the leaf epidermis of Vitellaria paradoxa from three different locations in Nigeria namely Bida, Irawo and Ogbomoso were undertaken with a view to providing similarities and differences in their leaf epidermal characters. All the leaves were characterized by pinnate venation, undulate margin, oblong shape and acute bases. The leaf length/width ratio was 3:1 in all, while the leaf length/petiole length ratio was 2:1 in Irawo and Bida collections and 3:1 in Ogbomoso collection. The polygonal epidermal cells observed in the leaves had wavy to slightly straight anticlinal wall patterns. The leaves from the three locations were hypostomatic with paracytic stomatal complexes. Variations in epidermal cell shapes and anticlinal wall patterns observed in the leaves were probable adaptations to environmental factors. The results of this study are suggestive of the possible occurrence of different varieties of V. paradoxa in Nigeria.

Keywords: *Vitellaria paradoxa, epidermal cells, leaves, stomata, guard cells.*

Introduction

Vitellaria paradoxa Gaertn. F., commonly known as Shea tree, Shi tree, or *Vitellaria*, is a member of the Sapotaceae family. It is the only species of the genus *Vitellaria* (Byakagaba *et al*., 2011). The traditional African food plant is indigenous to Africa which has been claimed to have potentials to improve nutrition, and boost food supply in the 'annual hungry season' (Masters *et al*., 2010). The Shea tree grows naturally in the wild in the dry savannah belt of West Africa and it occurs in 19 countries across the African continent namely Benin, Burkina Faso, Cameroun, Central African Republic, Chad, Ethiopia, Ghana, Guinea Bissau, Cote d'ivoire, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Democratic Republic of the Congo and Guinea. (National Research Council, 2006) It preferably grows on alluvial soils that are deep with free drainage and predominantly sandy-clay top soils (Hall *et al*., 1996).

On a global scale, the importance of the shea nut tree is attached to the usefulness of its seed fat in food and cosmetic industries (Umali and Nikiema, 2002). In Africa, where the species occurs, the seed fat is used for cooking, in lighting of lamps, soap and pomade preparations as well as for medicinal purposes (Awoleye, 1995; ICRAF, 2000). Other parts of the plant have been reported to possess various medicinal properties (Popoola and Tee, 2001). In addition, caterpillars of *Cirina forda* (Westwood), rich in protein and exclusive feeders on the leaves of the species, are considered a delicacy among the Yoruba, Nupe and several other Nigerian ethnic groups (Ande, 2004; Ugese *et al.,* 2010). Sale of these caterpillars is said to contribute significantly to rural household incomes (Popoola and Tee, 2001). The wood of *V. paradoxa* is hard and termite proof and is useful in constructional works and in the production of household and farm utensils. The fruit pulp has also been acknowledged to have excellent nutritional properties and is widely consumed among indigenous peoples (Maranz *et al*., 2004; Ugese *et al*., 2010).

Agroforestry species that show high potential in contributing to reduction of rural poverty, hunger and disease and enhancing environmental sustainability are considered priority species for domestication (Leakey *et al.*, 2005). Due to its various usefulness, *V. paradoxa* is threatened by over-exploitation as sources of fuel wood (Ogunkunle and Oladele, 2004) and charcoal for cooking and bread-baking activities (Oladele, 2013).

Leaf morphological characteristics are important aids for identifying plant species of agricultural and other economic values. Stomatal, epidermal cell and trichome characters of have also been useful as excellent taxonomic markers (Das *et al.,* 2004; Parveen *et al.,* 2000; Ogundipe and Akinrinlade, 1999; Edeoga and Ogbebor, 2001; Adedeji and Faluyi, 2001). As valuable as the shea nut tree is, there is lack of information on its leaf surface anatomy from different ecological locations in Nigeria.

This paper therefore describes the leaf epidermal characters of *V. paradoxa* from three different locations in Nigeria: Bida ($3^0$20'E and $8^0$11.3'N, 41^0C, 40% humidity,

Table 1: Some qualitative leaf morphological features of *Vitellaria paradoxa* from three different locations in Nigeria

LOCATION	VENATION	MARGIN	SHAPE	APEX	BASE
OGBOMOSO (OGB)	Pinnate	Undulate	Oblong	Emarginate	Acute / Rounded
IRAWO (IRW)	Pinnate	Undulate	Oblong	Obtuse	Acute / Rounded
BIDA (BDA)	Pinnate	Undulate	Oblong	Emarginate/Obtuse	Acute

Table2: Some quantitative features of *V. paradoxa* leaves from three different locations in Nigeria

LOCATION	LAMINA LENGTH	LAMINA WIDTH	PETIOLE WIDTH	LEAF L/PETIOLE RATIO	LEAF L / WIDTH RATIO
OGB	12.5 (19.4±0.31) 23.0	4.8 (6.4±0.13) 8.5	5.0 (7.2±0.18) 11	3:01	3:01
IRW	10.9 (16.9±0.35) 20.3	4.7 (6.3±0.11) 7.7	5.2 (7.8±0.15) 9.5	2:01	3:01
BDA	11.7 (16.6±0.29) 20.7	4.6 (6.1±0.10) 7.8	5.1 (6.7±0.12) 9.0	2:01	3:01
O/I	0.000^{b}	0.357^{a}	0.008^{b}		
O/B	0.000^{b}	0.032^{b}	0.035^{b}		
I/B	0.478^{a}	0.189^{a}	0.000^{b}		

All measurements in cm [min (mean ± SE) max] O/I – Ogbomoso and Irawo, O/B – Ogbomoso and Bida, I/B – Irawo and Bida; a- Not significantly different (P>0.05), b- Significantly different (P<0.05).

Table 4: Quantitative stomata character of *V. paradoxa* in the three locations

TAXA	STOMATA DENSITY (items/mm2)	STOMATA LENGTH (µm)	WIDTH (µm)	INDEX %
	Abaxial	Abaxial	Abaxial	Abaxial
OGB	417(482.2±9.15)567	10.2(14.0±0.40)17.9	2.6(4.6±0.19)5.1	3.337
IRW	500(667.8±19.69)850	10.2(14.2±0.45)17.9	2.6(4.6±0.19)5.12	3.331
BDA	500(625.6±11.09)750	10.2(15.5±0.68)23.0	2.6(2.6±0.00)2.6	3.334
O/I	0.000^{b}	0.780^{a}	1.000^{b}	
O/B	0.000^{b}	0.057^{a}	0.000^{a}	
I/B	0.067^{a}	0.101^{a}	0.000^{a}	

All measurements in cm [min (mean ± SE) max], '-' denotes 'absent'. **Note:** O/I – Ogbomoso and Irawo, O/B – Ogbomoso and Bida, I/B – Irawo and Bida; **a**- Not significantly different (P>0.05), **b**- Significantly different (P<0.05).

4.2mm precipitation and silt-clayey soil), Irawo (8^{0}33'0'N and 3^{0}20'0'E, 30^{0}C, 54% humidity, 4.2mm precipitation and silt-loamy soil) and Ogbomoso (8^{0}15'S and 4^{0}15'E, 36^{0}C, 61% humidity, 5.4mm precipitation and silt-loamy soil) for the purpose of documentation and in order to assess the variation of these features in relation to different ecological locations.

Materials and Methods

About twenty mature leaves of *V. paradoxa* were collected in the field from each of the three locations. Two leaves closest to the point of emergence of the branch from the main tree trunk were collected. These leaves were observed and characterized morphologically. Samples of the fresh leaf specimens were fixed in formal-acetic alcohol (FAA) for the purpose of keeping them anatomically intact. Epidermal peels and paradermal sections were obtained from a standard middle region of each leaf, stained in 1% safranin for about three minutes, washed of excess stain and mounted in glycerine. Photomicrographs were taken using a microscope fitted with a Bresser micro ocular II VGA camera.

Guard Cell Area was calculated using the method of Franco (1939) as follows:

Guard cell area = Length × Width × k

(where k = Franco's constant = 0.78524).

Stomata index was obtained using the formula:

I= S/S+E

(I= Stomata index; S= Number of Stomata per unit area; E= Number of ordinary epidermal cells in the same unit area)

Results

Leaf morphological characters obtained from selected features of the leaf surfaces are presented in Tables 1 and 2 while the anatomical characters are presented in Tables 3 to 6.

There was variation in size of the leaves, the smallest being in Bida collection and the largest in Ogbomosho

Table 5: Variation in number of epidermal cells per unit area and cell wall thickness

TAXA	NO. OF CELLS PER VIEW		CELL WALL THICKNESS (µm)	
	Adaxial	Abaxial	Adaxial	Abaxial
OGB	37(49.8±0.93)59	35(38.7±0.49)45	5.1(6.1±0.23)7.7	2.6(4.6±0.33)7.7
IRW	35(46.5±1.27)60	27(35.8±1.09)47	2.6(4.4±0.25)7.7	2.6(3.6±0.23)5.1
BDA	62(73.9±1.25)85	21(24.7±0.36)29	5.1(7.2±0.33)10.2	2.6(6.2±0.27)7.7
O/I	0.042^b	0.017^b	0.000^b	0.015^b
O/B	0.000^b	0.000^b	0.008^b	0.666^a
I/B	0.000^b	0.000^b	0.000^b	0.000^b

NOTE: All measurements in cm [min (mean±SE) max] O/I – Ogbomoso and Irawo, O/B – Ogbomoso and Bida, I/B – Irawo and Bida; **a**- Not significantly different (P>0.05), **b**- Significantly different (P<0.05).

Table 6: Quantitative Features of the Guard cells of *V. paradoxa* leaves

LOCATION	GUARD CELL LENGTH (µm)	GUARD CELL WIDTH (µm)	GCA (μm^2)
	Abaxial	Abaxial	Abaxial
OGB	17.9(21.7±0.49)28.2	12.8(14.5±0.28)17.9	248.04
IRW	17.9(22.4±0.44)25.6	10.2(14.1±0.47)17.9	250.98
BDA	12.8(20.8±0.52)25.6	7.70(12.3±0.41)15.4	202.07
O/I	0.245^a	0.441^a	0.842^a
O/B	0.234^a	0.000^b	0.001^b
I/B	0.020^b	0.006^b	0.002^b

NOTE: All measurements in cm [min (mean ± SE) max]'-' denotes 'absent'. O/I – Ogbomoso and Irawo, O/B – Ogbomoso and Bida, I/B – Irawo and Bida, a- Not significantly different (P>0.05), b- Significantly different (P<0.05).

collection. Petiole length ranged from 5.0 - 11.0 in Ogbomosho specimen. The smallest leaf length/petiole length ratio value of 2:1 was recorded in Bida and Irawo specimens while the highest of ratio 3:1 was recorded in Ogbomosho specimen. Also, the leaf length/width ratio is 3:1 for Ogbomosho, Irawo and Bida specimen (Table 2).

No considerable variation occurs in the distribution and type of stomata among the studied species. They are all hypostomatic i.e. stomata are present on the abaxial surface only. Using the classification terms proposed by Metcalfe and Chalk (1979), the entire leaf specimens collected from various sources possessed paracytic type of stomatal complex where each stoma is surrounded by a limited number of cells (2 subsidiary cells) that are indistinguishable in size and shape on the abaxial surface only.

The results obtained revealed that there are no variations in the qualitative anatomical features of leaves from the three locations. While there were no stomata on the adaxial surface of the leaves, the abaxial surfaces all had paracytic type of stomata. Polygonal cell shape was the observed type in all abaxial and adaxial surfaces of the leaves. All the anticlinal walls on both adaxial and abaxial surfaces were observed to be Wavy/straight.

The distributions of stomata varied within the specimen on the abaxial surfaces of each specimen. The highest stomata length was recorded in Bida sample. The Irawo and Ogbomoso sample recorded the highest stomatal width. Stomata index of 3.3% was however obtained in all the three samples (Table 4).

The studied species possess no trichomes but wax was observed on their surfaces. The leaf epidermal cells are polygonal in shape and are more often irregular. Anticlinal walls are wavy to slightly straight. The epidermal cell walls are thick ranging from 2.6µm in Irawo specimen to 10.2µm in Bida on the adaxial surface while it ranged from 2.6µm in all the specimens to 7.7µm in Ogbomosho and Bida specimen on the abaxial surface (Table 5). Variations noted also include the number of epidermal cells on the adaxial and abaxial surfaces of each species and within the studied species in general. More cells occur on the adaxial surface than on the abaxial surfaces of the species studied. On the adaxial surface the highest mean number (73.9) was recorded in Bida specimen while Irawo specimen has the lowest mean number (46.5). Bida specimen has the lowest mean number of epidermal cells on the abaxial surface with 24.7 while the highest number 38.7 was recorded in Ogbomosho specimen (Table 5).

No clear difference was noted between the lengths and widths of the guard cells possessed by the samples from all three locations, the guard cell area ranged between 202.07µm ^{2} in Bida samples to 256.45μm^2 in Irawo samples (Table 6).

Apart from all reports presented, trichome-like structures were also observed on the leaves collected from all the locations. No reasonable data of the trichomes could be taken because they were not attached to the leaf surfaces, but were just hanging on the surfaces as dust particles which easily wiped off when rinsed in water.

Plates 1-6 below reveal the leaf epidermal cells of the samples as they appear on a camera-fitted microscope.

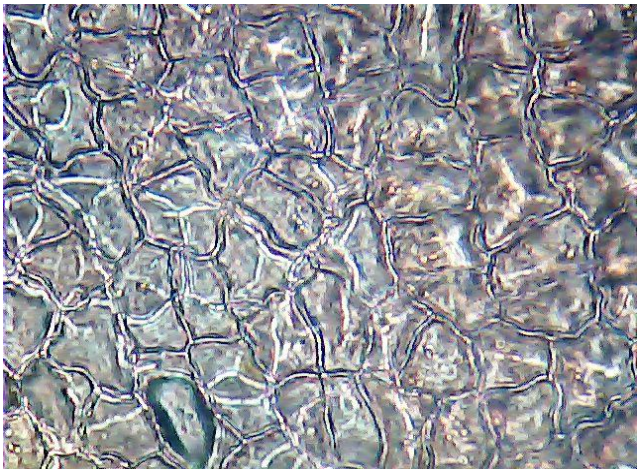

Plate1: Photomicrograph of the Adaxial surface of Ogbomoso Specimen

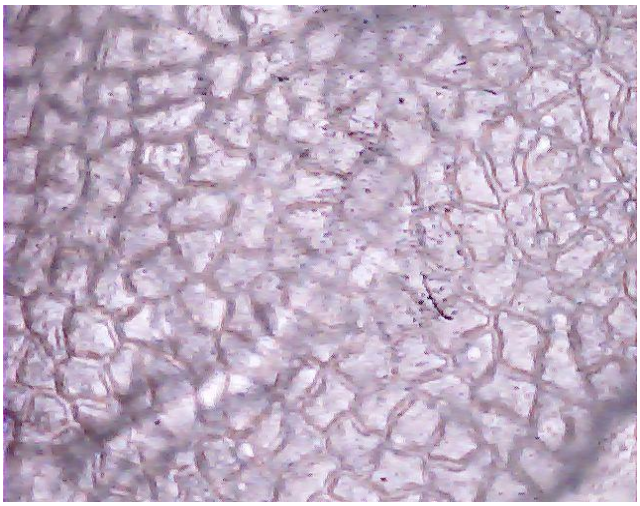

Plate 2: Photomicrograph of the Adaxial surface of Irawo Specimen

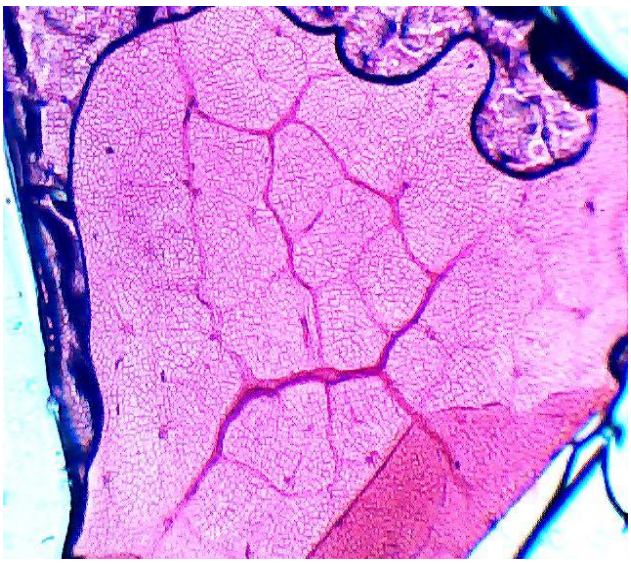

Plate 3: Photomicrograph of the Adaxial surface of Bida Specimen

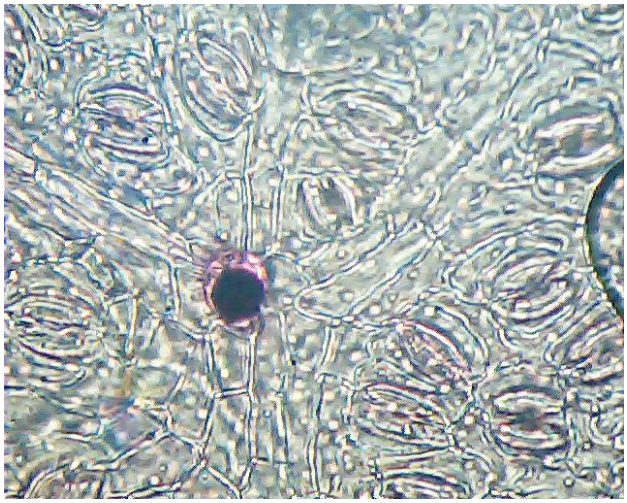

Plate 4: Photomicrograph of the Abaxial surface of Ogbomoso Specimen

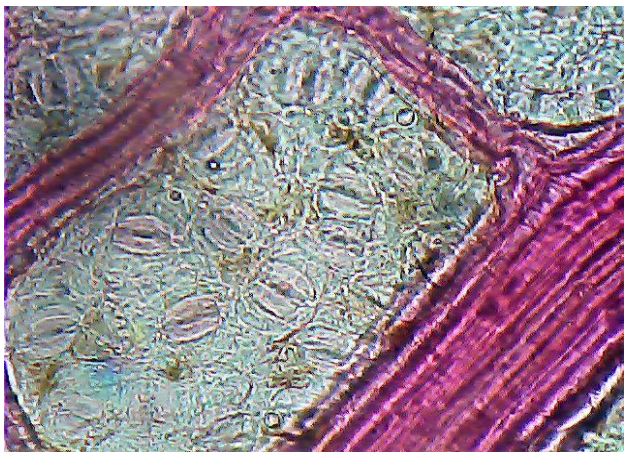

Plate 5: Photomicrograph of the Abaxial surface of Irawo Specimen

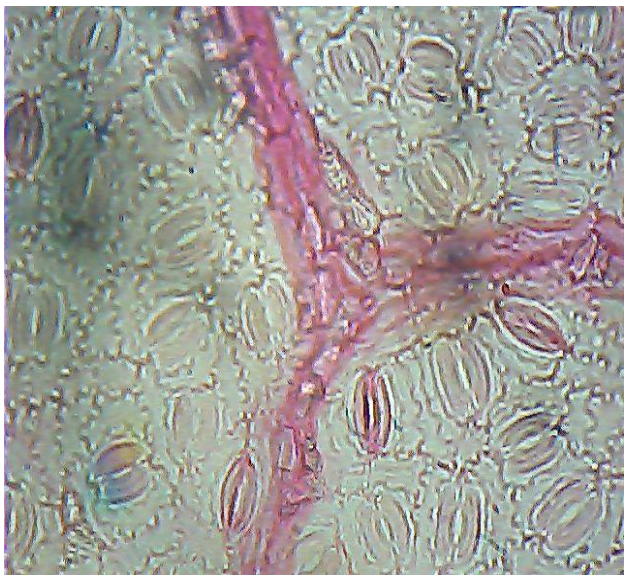

Plate 6: Photomicrograph of the Abaxial surface of Bida Specimen

Discussion

According to Okolo *et al* (2011), variation study is an essential step in characterizing Shea resources. This study will aid the evaluation of the genetic diversities of *v. paradoxa* in the stated location of collection in Nigeria.

From the results obtained in this work, it could be deduced that for leaf laminar length, there existed significant difference between samples collected from Ogbomoso and Irawo; and also between those from Ogbomoso and Bida. For leaf width, significant differences were only observed in the case of Ogbomoso and Bida. Petiole length values all had significant differences between samples from each location and those from the other ones. Similar results were reported in Cote d'Ivoire (Delome 1947). On the basis of the morphology of the leaf, five forms were identified (Oblong, narrowly elliptical, broadly elliptical and elliptical).These characteristics were very discriminative and easily observable on a large scale. The distinction of Shea population using these characteristic is easy. All the qualitative variables considered in the study were observed at all the sampling sites. Though these parameters varied significantly between sampling sites, the qualitative parameters seemed to have been little influenced by environmental factors. The variations of the proportions of the different parameters observed between the sites could be attributable to anthropogenic activities given the fact that the species grows mostly in areas close to human settlements.

There was no significant difference between the stomatal densities of Irawo and Bida samples while significant differences was observed between Ogbomoso samples and both of Irawo and Bida samples. No significant differences in all stomatal length values from all samples from the three locations. In the case of stomatal width, only values obtained from Ogbomosho and Irawo samples had significant differences.

Values of numbers of epidermal cells per view and cell wall thickness all had significant differences across all samples (one with another), except for cell wall thickness values obtained between Ogbomoso and Bida samples. Considering guard cell length, only values between Irawo and Bida had significant difference. However, for Guard cell width and area, values from all the locations had significant differences with one another except for Ogbomosho and Irawo values in both cases.

Ogbomoso specimen had highest value of laminar length, leaf width, number of epidermal cells per view on abaxial surface and guard cell width; Irawo specimen had highest value of petiole length, stomata density, guard cell length and guard cell area while Bida specimen has highest value of number of cells on the adaxial surface and cell wall thickness on both surfaces. This finding corroborate the findings of Odebiyi *et al.*, (2000) that the leaves produced by Shea trees in the woodland savannah were significantly longer than those produced in the northern guinea savannah. This was attributed to differences in genetic variability of the parent trees, competition for nutrient with other trees present as well as environmental factors, which led to differences in leaf production as well as other traits measured.

Ogbomoso specimen had the lowest value of stomata density; Irawo specimen had lowest value of number of cells on adaxial surface, cell wall thickness on both surfaces while Bida specimen had the lowest value of laminar length, leaf width, petiole length, number of cells on abaxial surface, guard cell length, guard cell width and guard cell area.

Considering all data obtained in this study, similarities and differences between samples from the three locations could be deduced as follows:

OI- Ogbomoso and Irawo are 35.7% similar and 32% different.

OB- Ogbomoso and Bida are 28.6% similar and 36% different.

IB- Irawo and Bida are 35.7% similar and 32% different.

No research work could be traced out on the anatomy of *V. paradoxa* in Nigeria. Studies carried out in some West African countries have shown the existence of a high intra-specific variation among shea trees (Chevalier 1984; Ruyssen 1957). A phenotypic variation among variation of Shea trees and a correlation between its different physical properties have been reported in Ghana (Lovett and Haq, 2004), Mali (Sanou *et al*., 2005) and in Cote d' Ivoire (Diarrasouba *et al*., 2007).

The comparison of *V*. *paradoxa* from the three locations had revealed some significant similarities and some significant differences in leaf anatomical characters and leaf morphological characters with reference to their various locations. Going by the postulate of Barthlott (2008), that epidermal characters are only slightly influenced by environmental conditions and the results obtained in this work, it could be concluded that the results of this work could be a pointer to the fact that *V. paradoxa* in Nigeria may be represented by more than one variety. This moreover will require further investigations beyond anatomical or morphological analyses. However, molecular analyses may be required to verify if the samples are of different varieties or not.

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