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Epidermal morphology of Nigerian species of *Synsepalum* (Sapotaceae)

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Abstract

The leaf epidermal morphology of three Nigerian species of *Synsepalum* has been studied by light microscopy. Epidermal cells are irregular or polygonal with straight, curved, undulate or sinuate anticlinal walls. All taxa are amphistomatic although adaxial stomata are restricted to the veins and their vicinities. Stomatal type varies in the genus. Anomocytic stomata are present on both leaf surface of *S. dulcificum* and *S. stipulatum* while cyclocytic and paracytic types are recorded in *S. glycydorum* on the adaxial and abaxial surfaces respectively. The periclinal divisions in the abaxial guard cells of *S. stipulatum* is diagnostic of the species. All taxa are glabrous but numerous trichome bases are found on the abaxial surface of *S. glycydorum* sugesting an early loss of hairs in leaf development. A dichotomous key for identifying the species is presented.

Keywords: *Synsepalum*, morphology, Nigeria.

Introduction

The genus *Synsepalum* (A.Dc.) Daniell is represented by three species in Nigeria [5, 7] of which *S. dulcificum* (Schum and Thonn.) Daniell is the best known and cultivated for its high economic value which however remains to be utilized on a commercial basis. It is often referred to as the magic plant because of the reddish yellow fruits, the pulp of which contains a protein-based sweeting agent called miraculin [4, 6, 9]. Miraculin is known to have a greater effect in sweetening acidity than in countering bitterness [4, 6] and is commonly used in the southwestern Nigeria to sweeten palmwine. Miraculin is said to be widely accepted as an active sweetner [9] and it is similar to thaumatin found in *Thaumatococcus danielli* (Benn.) Benth. and molenin in *Dioscoreophyllum cumminsil* (Stapf.) Diels.[9]

The fairly hardwood of *S. dulcificum* is used as firewood by the indigenous people while the twigs are used as chewsticks [6] *S. stipulatum* (Radlk.) Engl. is known as the 'Blacksmiths' charcoal because it supplies the best charcoal to the Benin blacksmiths in area now referred to as Edo State, Nigeria [4, 7].The stems of small trees of this plant are used for axe-handles and the wood is also cut into planks [4]. *S. stipulatum* and the relatively uncommon *S. glycydorum* Wernham occur mainly in thewild. The latter is reported to be known only from Nigeria [7] where it is restricted to the forests of Umudike, Umuahia, Owerri and Calabar in the Eastern part of the country.

The morphology, ecology and sustainable utilization of the genus in the Nigerian forest is reported in Ayodele and Chukwuka [1] the present paper reports the epidermal characters of the Nigerian species of *Synsepalum* as observed by light microscopy. This is to provide, through a detailed systematic evaluation of epidermal features of the leaf, reliable taxonomic characters that could facilitate an accurate and rapid identification of the plant specimens even if they are in a fragmentary condition.

Materials and method

Specimens of the three taxa were examined at the Forestry Research Herbarium (FHI) Ibadan and the Herbarium of the Department of Botany, University of Ibadan (UIH) Ibadan.

Epidermal morphology

An area of about 2cm² was cut from a standard median position on each leaf specimen of the three species. Three to five specimens were used for each species depending on its geographical spread. Each portion was soaked in boiling water for 10-20 minutes. Each of the specimens was transferred to a Petri-dish containing 15% sodium hypochlorite (NaOcl) overnight after which it was completely bleached

and the epidermises separated from the mesophyll. This was indicated by bubbles appearing on the leaf fragments. Each sample was transferred to a Petridish containing distilled water to which two drops of acetic acid had been added to neutralize the action of the sodium hypochlorite. The abaxial membranes of each specimen were subsequently teased from the mesophyll using a pair of fine forceps and dissecting needles. The membranes were cleaned with a camel hair brush, rinsed in water and transferred to 50% ethanol for 5 minutes to harden. They were stained in aqueous safranin for 3-5 minutes before being dehydrated by passing through 50%, 70%, 90% absolute ethanol series and a mixture of equal parts of absolute ethanol and xylene (about 5 minutes in each). The membranes were cleared in xylene for 2 minutes and mounted in Canada balsam and slides were dried on a hot plate.

For statistical analysis, 50 epidermal cells and stomata were measured using a micrometer eyepiece from each surface. Descriptive statistics of mean, standard deviation and standard error were calculated for all variables. The stomatal index (SI) was calculated using the formular of Salisbury (1927): $\frac{S}{S+E} \times 1000$, where S denotes the number of stomata per unit area, and E the number of epidermal cells of the same area. Photomicrographs were made using Reichert Microstar IV micoscope to which a camera is attached.

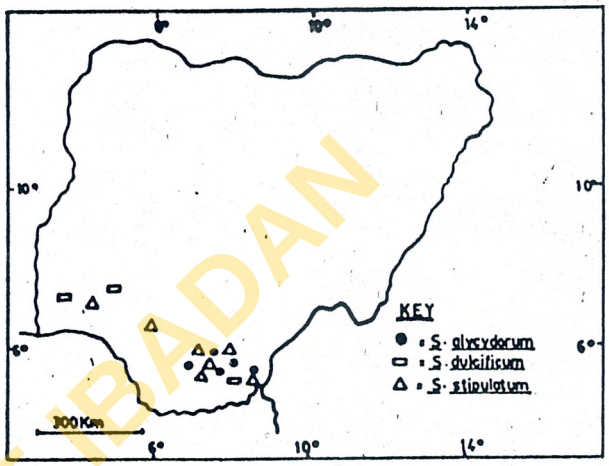


Fig. 1: Map showing the distribution of *Synsepalum* species in Nigeria

Results

Light microscope features of the epidermis given below are summarised in Tables 2 and 3 and Figure 2. Table 1 lists the sources and materials used for the epidermal morphology while Figure 1 shows the distribution of the taxa examined in Nigeria.

Table 1: Sources of *Synsepalum* specimens used for the study

	Taxa	Collector(s)	Locality	Herbarium
1	*S. dulcificum*	Prof. Alasoadura	Polytechnic Campus, Ibadan	UIH 16192
		B. O. Daramola	Uyo, Cross River State	FHI 55315
		C. Onochie	Abeokuta, Ogun State	FHI 13517
		Goerge, E. Pilz	University of Ibadan Campus	FHI 98890
		Onyeachusim, Binuyo and	Eleyele, Ibadan	FHI 57970
		Enwioghon Ladipo and Oni	Oyo Road, Ibadan	FHI 34565
2.	S. stipulatum	Ariwaodo, J.	Oguta, Imo State	FHI 85369
		J. Kennedy	Sapoba, Benin	FHI 9552
		A.P.D. Jones and C. F. Onochie	Omo Forest	FHI 17377
		Donald Ekong	Uyo, Akwa Ibom State	FHI 55950
		Brenam J. P. M.	Shasha Forest Reserve	FHI 17522
		J. Olorunfemi	Owerri, Imo State	FHI 34206
3.	*S. glycydorum*	Onwaubani & Ariwaodo	Umuagon-Ibeku, Umudike Forest	FHI 17679
		Latilo M. G.	Calabar, Cross River	FHI 41350
		Okeke and Macauley	Umuahia, Abia State	FHI 72193
		Mallam	Owerri, Imo State	FHI 5801

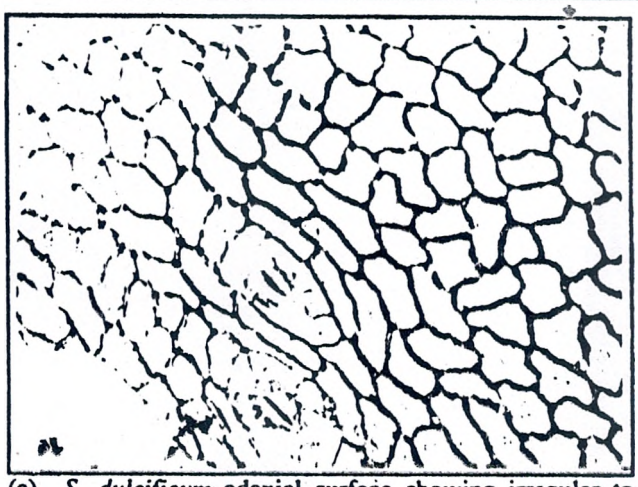
The adaxial epidermal cells are either irregular or polygonal. This however varies both within and among the three species (Fig 2). On the abaxial surface, the epidermal cells are irregular in all taxa (Fig. 2b. d, & f).The anticlinal cell wall pattern is straight, curved, undulate or sinuate in the three species (Table 2. Fig. 2). There are also considerable intra-and inter-specific variations in the size of the adaxial and abaxial epidermal cells. The abaxial cells are generally larger than the adaxial ones, the former ranging from 26.9 µm in *S. stipulatum* to 29.6 µm in *S. dulcificum* while the latter vary between 20.5 um in *S. glycydorum* and 23.5 µm in *S. stupulatum* (Table 2). The number of epidermal cells per unit area is usually more on the adaxial surface than on the abaxial surface. It ranges between 606 in *S. duilcificum* to 889 in *S. glycydorum* on the former and between 476 in both *S. dulcificum* and *S. glycydorum* and 517 in *S. stipulatum* (Table 2).

Table 2: Epidermal characters of the genus *Synsepalum* in Nigeria

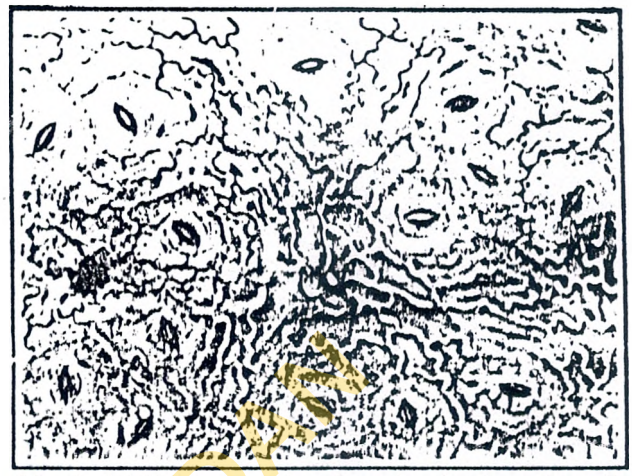
Taxa	Leaf Surface cells/mm²	No. of thickness	Cell wall	Cell width	Epidermal cell shape cell	Anticlinal wall pattern wall
S. dulcificum	Adaxial	506 - 754 606 ± 12.5	2.3 - 3.0 2.5 ± 0.1	12.5 - 27.5 20.9 ± 0.8	Irregular/ Polygonal	Curved
	Abaxial	399 - 575 476 ± 11.8	0.8 - 1.5 1.1 ± 0.1	17.5 - 45.0 29.6 ± 1.5	Irregular	Sinuate
S. stupulatum	Adaxial	616 - 992 747 ± 17.3	2.5 - 3.0 2.7 ± 0.1	17.5 - 27.5 23.5 ± 0.6	Polygonal Irregular	Straight/ Slightly curved
	Abaxial	435 - 594 517 ± 11.7	1.3 -2.3 2.0 ± 0.1	17.5 - 35.0 26.9 ± 1.1	Irregular	Sinuate
S. glycydorum	Adaxial	759 - 1050 889 ± 17.0	2.0 - 5.0 2.2 ± 0.5	12.5 - 25.0 20.5 ± 0.8	Irregular/ Polygonal	Curved/ Undulate
	Abaxial	352 - 550 476 ± 13.5	2.0 - 5.0 2.6 ± 0.1	17.5 - 35.0 27.1 ± 1.2	Irregular	Undulate

All measurement in microns = $\frac{\text{Range}}{\text{Mean} \pm \text{standard error}}$

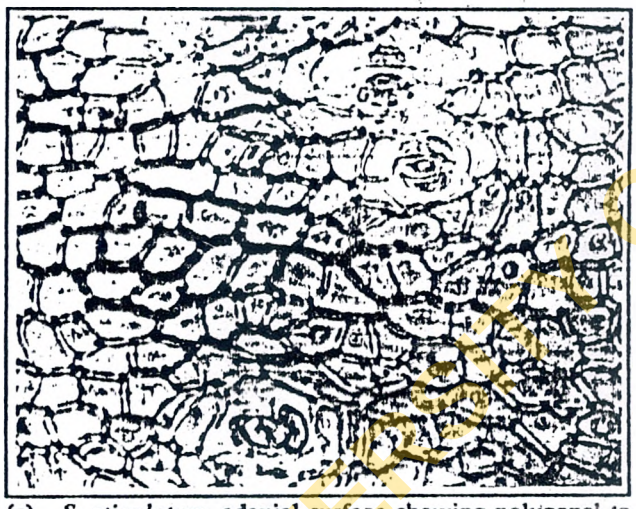
Adaxial cell walls are thicker in *S. dulcificum* and *S. stipulatum* (2.5 µm and 2.7 µm respectively) while in *S. glycydorum* the abaxial cell walls are thicker than those on the adaxial surface (Table 2). All taxa are amphistomatic although it must be emphasised that stomata are restricted to the veins and their vicinities on the adaxial surface of all taxa except for the few scantily distributed on the lamina of *S. glycydorum* (Fig. 2) Anomocytic type of stomata is found on the adaxial and abaxial surfaces of *S. dulcificum* and *S. stipulatum*, each stomata being surrounded by cells that are not distinguishable from other epidermal cells (Fig. 2) while in *S. glycydorum*, the stomata type is cyclocytic on the adaxial surface, each stoma being encircled by a variable number of cells and paracytic on the abaxial surface, each stoma being surrounded by two subsidiary cells parallel to the guard cells. More stomata occur on the abaxial surfaces of all taxa ranging from 62 in *S. glycydorum* to 82 in *S. dulcificum*. Adaxial stomata are generally larger than the abaxial ones, the former varying from 15.5 x 8.6 µm in *S. stipulatum* to 16.8 x 14.4 µm . in *S. glycydorum* and the latter from 12.1 x 12.3 µm to 13.6 x 14.1 µm in *S. glycydorum* and *S. dulcificum* respectively. The mean stomatal index is highest in *S. dulcificum* (14.7%) and lowest in *S. glycydorum* (11.5%) on the abaxial surface while on the adaxial surface, it is highest in *S. stipulatum* (0.7%) and lowest in *S. glycydorum* (0.3%).The guard cells on the abaxial surface of *S. stipulatum* are characteristics of the species. They appear as having divided by a perclinal wall (Fig. 2d). All taxa are glabrous on both surfaces. However, numerous trichome bases are found on the abaxial surface of *S. glycydorum* (Fig. 2f).



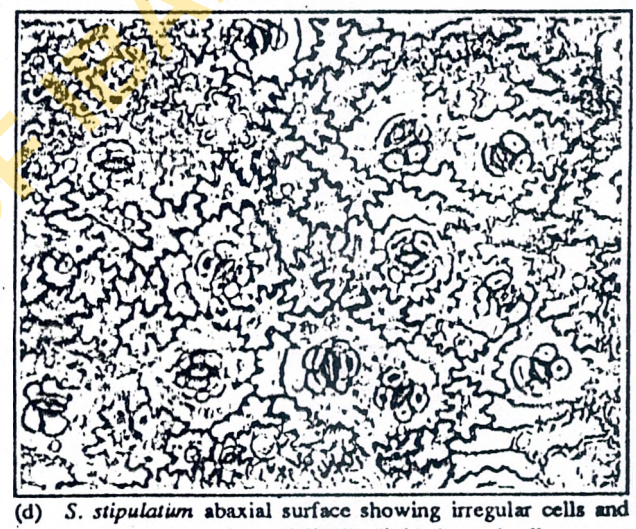
(a) *S. dulcificum* adaxial surface showing irregular to polygonal cells and stomata along the main vein.



(b) *S. dulcificum* abaxial surface showing irregular cells and anomocytic stomata.



(c) *S. stipulatum*, adaxial surface showing polygonal to irregular cells and stomata on the main vein.



(d) *S. stipulatum* abaxial surface showing irregular cells and anomocytic stomata with periclinally divided guard cells.

(e) *S.glycydorum*, abaxial surface showing irregular cells and cyclocytic stomata.

(f) *S. glycydorum*, abaxial surface showing irregular cells, paracytic stomata and numerous trichome bases.

All same scale = 25μm

Fig. 2: Photomicrographs of adaxial and abaxial epidermal surfaces of *Synsepalum* species.

Table 3: Stomatal characters of the genus synsepalum in Nigeria

Taxa	Leaf Surface frequency/ mm²	Somata	Stomatal length	Stomatal width	Stomatal type	Index %
S. dulcificum	Adaxial	0 - 12 3 ± 0.8	12.5 - 17.5 15.5 ± 0.4	10.0 - 12.5 11.9 ± 0.3	Anomocytic	0.5
	Abaxial	74 - 89 82 ± 1.1	10.0 - 17.5 13.6 ± 0.4	12.5 - 17.5 14.1 ± 0.3	Anomocytic	14.7
S. stipulatum	Adaxial	0 - 10 5 ± 0.7	12.5 - 17.5 15.5 ± 0.3	7.5 - 10.0 8.6 ± 0.3	Anomocytic	0.7
	Abaxial	64 - 75 70 ± 0.8	12.5 - 17.5 14.1 ± 0.3	12.5 - 15.0 13.3 ± 0.3	Anomocytic	11.9
S. glycydorum	Adaxial	0 - 8 3 ± 0.7	15.0 - 17.5 16.8 ± 0.3	12.5 - 17.5 14.4 ± 0.5	Cyclocytic	0.3
	Abaxial	49 - 69 62 ± 1.1	10.0 - 12.5 12.1 ± 0.2	10.0 - 15.0 12.3 ± 0.4	Paracytic	11.5

All measurements in microns = $\frac{\text{Range}}{\text{Mean} \pm \text{standard error}}$

Discussion

The results obtained from this study clearly indicate that the micromorphological characters are useful for the identification of *Synsepalum* species even if only their leaf fragments are available. The irregular epidermal cells with curved to undulate anticlinal walls on the adaxial surface of *S. glycydorum* distinguish it from the other two taxa with both irregular or polygonal cells and curved to straight anticlinal walls. The cycloytic and paracytic stomata types found on the adaxial and abaxial surfaces of *S. glycydorum* respectively further distinguish it from the other two taxa with anomocytic stomata on both surfaces of their leaves. The bistratose guard-cells [12] which have divided by a periclinal wall as observed in the abaxial surface of *S. stipulatum* is of diagnostic value in the genus. The view of Solereader [11] that the upper epidermis is generally composed of larger cells than the lower is in contrast with the results of the present study in which the abaxial epidermal cells are relatively larger than the adaxial ones. The numerous trichome bases observed on the abaxial surface of *S. glycydorum* is a reflection of some sort of hairness of the surface, which may have been lost with the ageing of the leaf. Of the three taxa examined, *S. glycydorum* is the only species described as having silky hairs completely covering the lower surface of the leaves [7] but this character is hardly visible on herbarium specimens. *S. dulcificum* with the largest epidermal cells on the abaxial surface (29.6 µm) and the highest stomatal number (82) and index (14.7%) on the same surface can be easily separated from the two other species.

The leaf surface patterns have been shown to be of great taxonomic significance because they are under strong genetic control (8). Although some authors [10,12,13] have noted that the variations in them may be under different environmental pressures in different habitats, Cutler [2] and Cutler and Brandham [3] have shown that the environment plays very little, if any part, in the determination of the appearance of the leaf. The dichotomous key presented below, based on the observed leaf epidermal features allows a separation of the taxa even if only the leaf fragments are available for study.

Key to species of *Synsepalum* in Nigeria

1. Adaxial epidermal cells irregular with curved to undulate anticlinal walls; stomata cyclocytic and paracytic on adaxial and abaxial surfaces respectively; stomata size more than 16 x 14 um on adaxial surface; adaxial epidermal cells more than 800 ... *S. glycydorum.*

1. Adaxial epidermal cells irregular or polygonal with straight to curved anticlinal walls, stomata anomocytic on both surfaces, stomatal size less than 16 x 14 µm on

adaxial surface; adaxial epidermal cells less than 800 ... 2

2. Stomatal index less than 14% on abaxial surface; abaxial epidermal cells less than 29µm;... *S. stipulatum*

2. Stomatal index more than 14% on abaxial surface; abaxial epidermal cells more than 29µm ... *S. dulcificum.*

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