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Leaf anatomy of twenty *Dioscorea* cultivars from Nigeria

JOSHUA OLUMIDE MATTHEW[1,2,♥], SEKINAT OKIKIOLA AZEEZ[1], AKINWUMI JOHNSON AKINLOYE[1], GBENGA ALEBIOWU[3], JULIUS OLAOYE FALUYI[1]

[1]Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria. ♥email: jolumatthew@gmail.com
[2]National Horticultural Research Institute. Ibadan, Nigeria
[3]Pharmaceutics Department, Obafemi Awolowo University. Ile-Ife, Nigeria

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Abstract. *Matthew JO, Azeez SO, Akinloye AJ, Alebiowu G, Faluyi JO. 2024. Leaf anatomy of twenty* Dioscorea *cultivars from Nigeria. Cell Biol Dev 8: 1-12. Dioscorea* spp. is an important food crop in Nigeria; *Dioscorea alata* L., *D. cayenensis* Lam., *D. dumetorum* (Kunth) Pax, and *D. rotundata* Poir. are the major species cultivated in this country. Previous studies on anatomical research of the genus *Dioscorea* have been concentrated mostly on quantitative morphometrics, while broad foliar anatomical studies, especially qualitative features of this crop receive little attention. Therefore, using qualitative and quantitative characteristic features in the anatomical study will be more detailed; ditto this study aimed at improving the anatomical and taxonomic knowledge to broaden evolutionary information of the cultivated *Dioscorea* spp. in Nigeria. Four major cultivated *Dioscorea* species' leaf and petiole anatomical features were evaluated, focusing on epidermis histology using transverse sections. Qualitative features were observed under a light microscope, while quantitative data were subject to mean separation using One-Way ANOVA and Principal Component Analysis (PCA). The cultivars and species studied had hypostomatic leaves with similar stoma types. Only *D. dumetorum* had distinct unicellular trichomes and lacked raphides, while the wing character of *D. alata* is a distinguishing feature for the species. PCA showed that stomata area (mm^2) is an important characteristic feature in the anatomical study of yam; also, *D. alata*, *D. cayenensis,* and *D. rotundata* were closely related. The *D. rotundata* had the highest value for stomata quantitative characteristic features.

Keywords: Leaves, petiole, raphides, stomata, trichomes, yam

INTRODUCTION

Yam (*Dioscorea* sp.) is a monocotyledonous plant that belongs to the family Dioscoreaceae, order Dioscoreales (Epping and Laibach 2020). Caddick et al. (2002) reported that the family Dioscoreaceae includes four genera, viz. *Dioscorea*, *Trichopus, Tacca,* and *Stenomeris*. The genus *Dioscorea* is the largest genus of the family, and it comprises over 90% of the species in the family. *Dioscorea* spp. are represented in the tropics, subtropics, and some temperate regions, consisting of about 600 species that are principally tuber-bearing (Smith 1937; Onwueme 1978). Yam has been cultivated for medicinal with the usage of different available species in all the cultivated regions. Kumar et al. (2017) and Zhou et al. (2022) reported over 80 *Dioscorea* species collected and conserved mainly for pharmaceuticals, traditional medicinal and nutritional uses. Despite the enormous numbers of species in the genus, about 12 species have been domesticated for food (Coursey 1967; Ayensu 1972; Mignouna et al. 2003; Adegbite et al. 2006; Quain et al. 2011), among them 8 species (*D. alata* L., *D. bulbifera* L., *D. cayenensis* Lam., *D. esculenta* (Lour.) Burkill, *D. dumetorum* (Kunth) Pax, *D. opposita* Thunb., D. *rotundata* Poir. and D. *polystachya* Turcz. are popularly cultivated for consumption (Andres et al. 2017; Zhou et al. 2022). Over the years, both the production and cultivation of yam have been domiciled in Africa; despite the enormous number of available yam species, cultivation of yam in this continent is concentrated on 6 species mainly cultivated for food (Atieno et al. 2020).

West Africa produces over 93% of the world's yam, while 66% comes from Nigeria (FAO 2018, 2019), qualifying the country to be the hub of yam production worldwide. Amusa et al. (2003) and Andres et al. (2017) reported that the most important yam species in the country are *D. alata* (water yam), *D. bulbifera* (air potato), *D. cayenensis* (yellow yam), *D. dumetorum* (bitter yam) and *D. rotundata* (white yam) but only water, yellow and white yams have been heavily cultivated over the years for food (IITA 2009; Matthew and Faluyi 2021). Although the mode of propagation and anthropogenic pressure of selection for desired cultivars has limited the number of cultivated species, a wide range of diversity still exists within and between the cultivated species (IITA 2009; Obidiegwu and Akpabio 2017). Wilkin et al. (2005) reported that *Dioscorea* poses a great challenge to systematists due to its great morphological variations, dioecy, and small flowers, which are often absent. Although studies using molecular, cytogenetics, and morphological techniques have characterized them as different species, the results of these techniques are evident or correlate with the features at the anatomical level (Atieno et al. 2020; Matthew and Faluyi 2021). Knowing that using anatomical techniques is less expensive and tasking than some of the techniques above, this technique will be useful in the relationship study of indigenous *Dioscorea* species. Moreover, the study of indigenous biosystematics often bridges between evolution and domestication for a better understanding of diversity (Worojie et al. (2021).

Moreover, using anatomical characters has proven useful in the taxonomy and delimiting of higher plants; only a few studies have been carried out on *Dioscorea* species (Abdulrahman et al. 2009; Aina and Atumeyi 2011). Likewise, the numerical taxonomical approach has been useful in classifying plant species, using more characters from sets of data (multivariate) in developing an entirely phenetic classification (Kolawole et al. 2021). These studies showed the complexity within some species of *Dioscorea*, focusing mainly on quantitative characteristics, while complimenting these reports with the broad foliar anatomical study of the major cultivated *Dioscorea* species using qualitative and quantitative characteristic features for characterization and descriptive study among and within species. Here, this study anatomically characterized the four major *Dioscorea* species being cultivated in Nigeria using leaves and petiole to broaden the taxonomical knowledge of this species, which will be useful for systematic study and breeding programs in the genus *Dioscorea*.

MATERIALS AND METHODS

Germplasm collection of cultivars of *Dioscorea*

Twenty cultivars of four *Dioscorea* species were collected and planted for this study using the On-Farm Participatory Method (OFPM). The species studied are *D. alata* (Ewura), *D. dumetorum* (Esuru), *D. cayenensis* (Igangan), *D. rotundata* (Ajimokun, Ame, Anika, Areyingbakumo, Boki, Digbiri, Gambari, Gaungaun, Gbongi, Ikumo, Ilesu, Lolo Ayin, Obabi, Odo, Ogunmole, Okunmodo and Sandpaper). The cultivars were planted for two consecutive seasons to ensure cultivars stability before they were moved to the Teaching and Research Farm, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria to validate the characters of the yam cultivars and to carry out multiplication and conservation for future usage. Morphological studies were carried out on the cultivars using the Yam descriptor (IPGRI/IITA 1997). These selected cultivars were described using leaf type, color, apex, base and presence and absence of wing and hair.

Anatomical studies

Anatomical studies were carried out on the leaf epidermis using the leaf and petiole transverse section. Tissue and cell identification and description followed using Fahn (1974), Esau (1977), Metcalfe and Chalk (1979), and Ellis et al. (1999); the stomata classification was done according to the description of Prabhakar (2004). Microscopic observation of each slide was done using a light microscope and recorded. Photomicrographs of the specimens were documented using a BK Series Phase Contrast Microscope (PW-BK 5000T) equipped with a DCM510 5 Megapixel Camera at the appropriate objective magnification. In addition, the micrometry was done using the method described by Faluyi (1992).

Leaf clearing

Matured leaves were cut into sizeable portions, boiled in absolute ethanol for 40 minutes, rinsed in water twice, and soaked in 5% sodium hydroxide for 16 hours. After rinsing in water twice, they were transferred into 30% common domestic bleach. The leaves were left in the bleach until they became completely white. The leaves were rinsed in distilled water thrice and preserved in 50% ethanol. The portions were stained in safranin O and mounted on a glass slide in 25% glycerol for microscopic examination. The leaf venation patterns, epidermal cell shapes, anticlinal cell wall patterns, free veinlet endings, and stomata (type, length, breadth, and index) were studied, and the Stomata Index was calculated using the equation below:

$$\textit{Stomata Index }(S.I) = \frac{S}{S+E} \times 100$$

Where:

S: Number of stomata per unit area,

E: Number of ordinary epidermal cells in the same area

Transverse section of leaf and petiole

Each cultivar's transverse sections of the leaf and petiole (proximal, median, and distal regions) were cut using a sledge microtome (Reichert, Austria). The sections were stained with 1% ethanolic solution of Safranin O and counterstained with Alcian Blue. The stained sections passed through a series of ethanol concentrations (50%, 70%, 80%, and 90%) for differentiation and dehydration. The specimens were mounted on a clean slide in 25% glycerol solution for microscopic examination.

Data analysis

The quantitative data such as leaf palisade layer length (µm), Upper epidermal cells length (µm), Lower epidermal cells length (µm), Stomata length (µm), Stomata breadth (µm), Stomata Area (µm^2) and Stomata Index were obtained from the *Dioscorea* anatomical studies, which were subjected to Principal Component Analysis (PCA) using PAST 4.03. Cluster analyses were carried out using Unweighted-Pair Group Method Arithmetic Averages (UPGMA) to construct a dendrogram using Gower's genetic distance while considering Eigenvalues of >0.2 in the PCA of the diversity study.

RESULTS AND OBSERVATIONS

Morphological studies

All the cultivars studied had a cordate leaf shape, except *D. dumetorum*, which had a trifoliate leaf. A particular *D. rotundata* i.e. "Areyingbakumo" had broad cordate leaf which was similar to *D. cayenensis* cultivar studied (Table 1). Leaf colors were green in all the species and cultivars studied. However, colors were darker in "Ame", "Boki", "Gambari", and "Sandpaper" *D. rotundata* cultivars, while *D. cayenensis* had light green leaf coloration (Table 1). Acuminate leaf apex was observed in *D. alata*, *D. cayenensis*, and *D. rotundata,* while acute leaf apex was observed in *D. dumetorum*. Likewise, table 1 shows that the leaf base varied among the cultivars and species studied. Only *D. dumetorum* had acute leaf base and cordate leaf base was observed in *D. alata*. The *D. cayenensis* and some *D. rotundata* (Ajimokun, Digbiri, Gambari, Gbongi, Ikumo, Ilesu, Lolo Ayin, Obabi, Ogunmole and Okunmodo). In contrast, other *D. rotundata* cultivars had sagittate leaf bases (Table 1). Hair and wings were observed only in *D. dumetorum* and *D. alata*, respectively (Table 1).

Leaf epidermal studies

The epidermal cells of leaves were polygonal with straight anticlinal wall patterns on the adaxial surfaces, while epidermal cell shapes on the abaxial surfaces were irregular with repand anticlinal wall patterns in all the cultivars studied (Figure 2 and Table 2). The areolar venation patterns observed in this study were closed, and the areolation ranges from 3-sided and above for all the yam cultivars studied (Figure 2 and Table 2). The veinlet ending for all the cultivars studied were bifurcated when present. All the yam cultivars studied had raphides on the adaxial and abaxial surfaces except the cultivar *D. dumetorum* (Esuru) (Figure 2 and Table 2). Only in the *D. dumetorum* cultivar were simple uniseriate trichomes observed on both adaxial and abaxial surfaces of the leaf (Figure 2 and Table 2).

All the *Dioscorea* cultivars studied were hypostomatous i.e., stoma were present only on the abaxial surfaces of the leaves. The stoma types observed were anisocytic, anisotricytic, isotricytic, tetracytic, staurocytic, and anomocytic (Figure 3 and Table 2). The stomata area was in the range of 1,232.00 µm^2 and 2,407.00 µm^2. The *D. dumetorum* had the least stomata area recorded for all the yam cultivars studied (Figure 1).

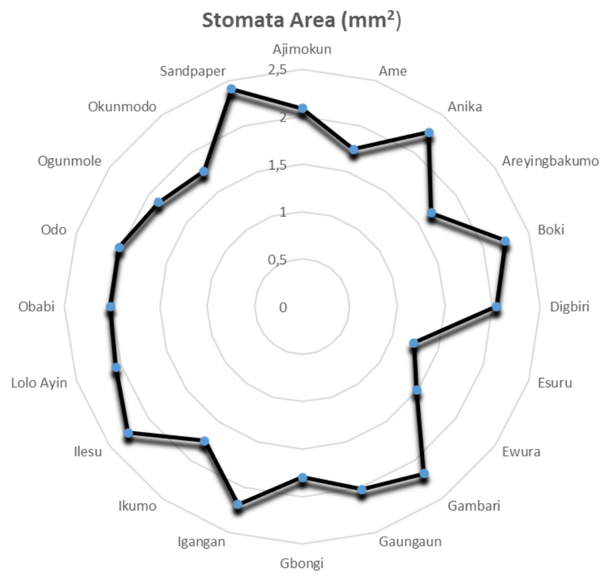

Figure 1. Stomata area of the yam cultivars studied

Table 1. Characteristics of the leaves of the yam cultivars studied

Species	Cultivar	Type	Color	Apex	Base	Hair	Wing
D. alata	Ewura	Cordate	Green	Acuminate	Cordate	+	-
D. cayenensis	Igangan	Cordate broad	Light green	Acuminate	Cordate	-	-
D. dumetorum	Esuru	Trifoliate	Light green	Acute	Acute	-	+, purple
D. rotundata	Ajimokun	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Ame	Cordate	Dark green	Acuminate	Cordate	-	-
D. rotundata	Anika	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Areyingbakumo	Cordate broad	Green	Acuminate	Cordate	-	-
D. rotundata	Boki	Cordate	Dark green	Acuminate	Sagittate	-	-
D. rotundata	Digbiri	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Gambari	Cordate	Dark green	Acuminate	Cordate	-	-
D. rotundata	Gaungaun	Cordate	Green	Acuminate	Sagittate	-	-
D. rotundata	Gbongi	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Ikumo	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Ilesu	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Lolo Ayin	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Obabi	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Odo	Cordate	Green	Acuminate	Sagittate	-	-
D. rotundata	Ogunmole	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Okunmodo	Cordate	Green	Acuminate	Cordate	-	-
D. rotundata	Sandpaper	Cordate long	Dark green	Acuminate	Sagittate	-	-

Note: *-: Absent, +: Present, ": Similar

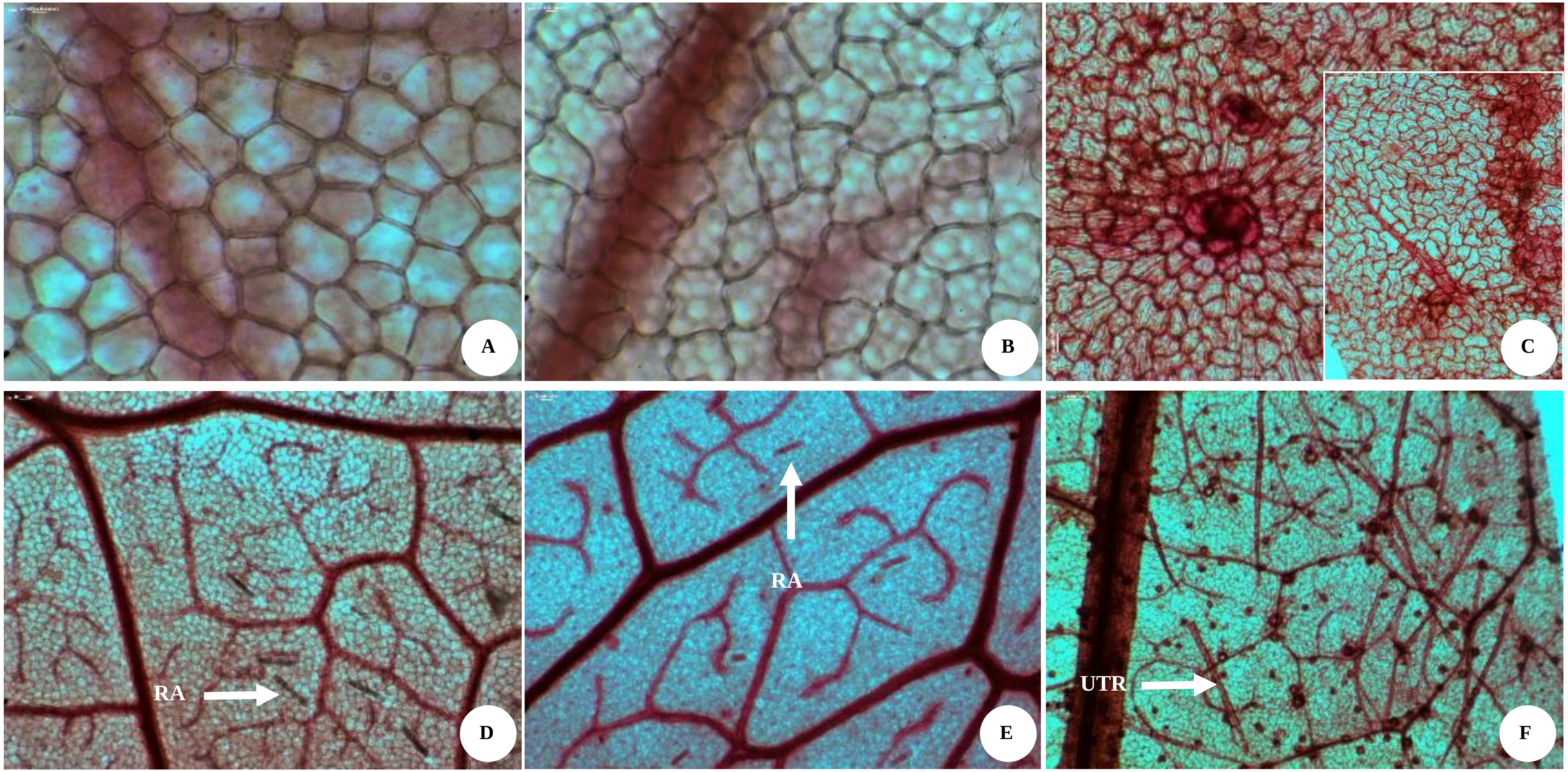

Figure 2. Epidermal features of the *Dioscorea* species studied. A. Areyingbakumo (*D. rotundata*) Adaxial Anticlinal Wall., B. Igangan (*D. cayenensis*) Adaxial Anticlinal Wall., C. Esuru (*D. dumetorum*) Adaxial Anticlinal Wall., D. Areyingbakumo (*D. rotundata*) Adaxial Venation Pattern., E. Igangan (*D. cayenensis*) Adaxial Venation Pattern., F. Esuru (*D. dumetorum*) Adaxial Venation Pattern. **RA: Raphide, UTR: Unicellular Trichome

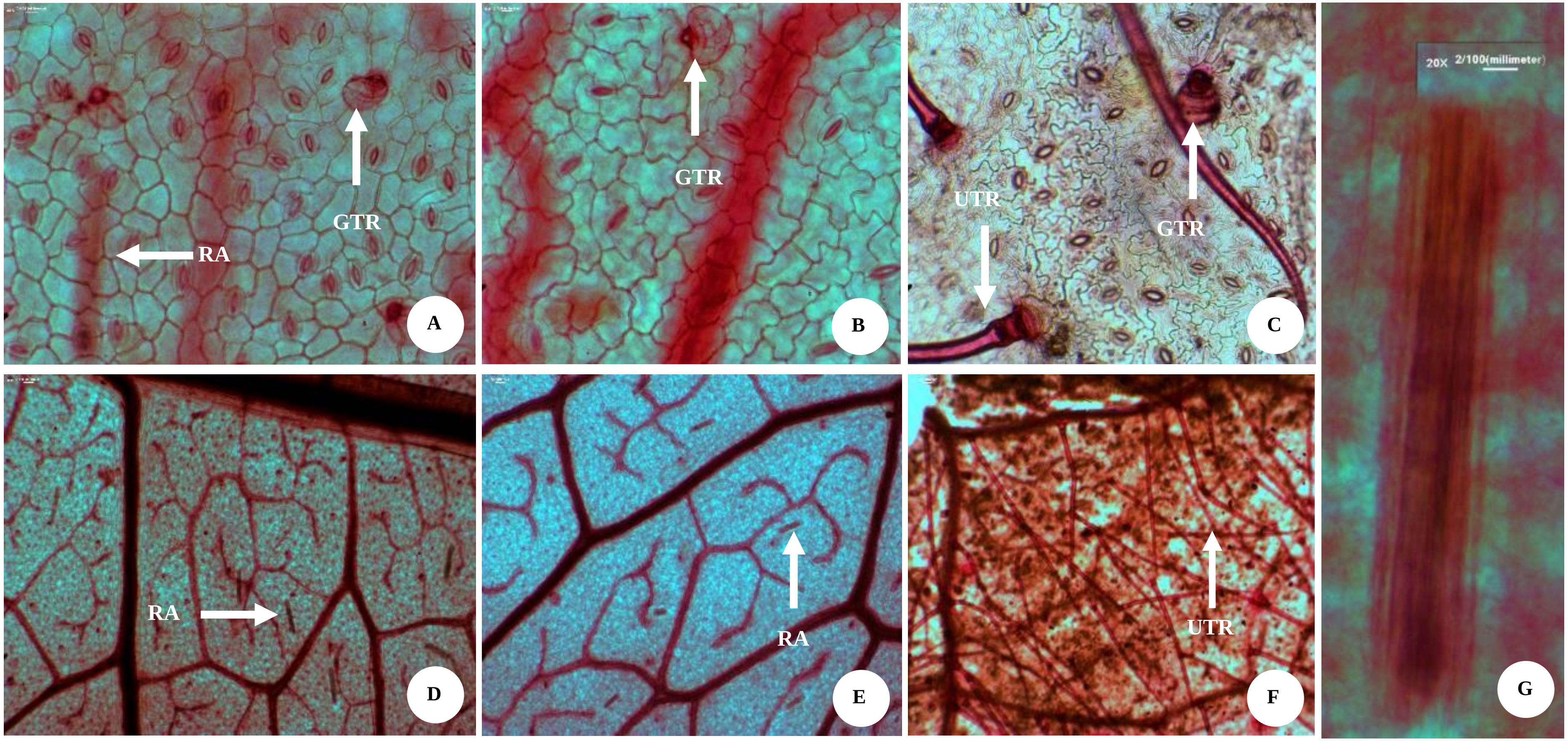

Figure 3. Epidermal features of the *Dioscorea* species studied. A. Areyingbakumo (*D. rotundata*) Abaxial Anticlinal Wall., B. Igangan (*D. cayenensis*) Abaxial Anticlinal Wall., C. Esuru (*D. dumetorum*) Abaxial Anticlinal Wall., D. Areyingbakumo (*D. rotundata*) Abaxial Venation Pattern., E. Igangan (*D. cayenensis*) Abaxial Venation Pattern., F. Esuru (*D. dumetorum*) Abaxial Venation Pattern, G. Raphide. **RA: Raphide, UTR: Unicellular Trichome, GTR: Glandular Trichome

Table 2. Summary of the leaf epidermal characteristics of the yam cultivars studied

Species	Cultivars	Pattern	Cell shape	Anticlinal Wall Pattern	Areolar Venation Pattern	Venation Ending*				Areolation	Stomata Type*	Egastic Substances
						Abs	Un	Fu	Bi			
D. alata	Ewura	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. cayenensis	Igangan	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. dumetorum	Esuru	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	-
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Unicellular and Glandular Trichomes
D. rotundata	Ajimokun	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Ame	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Anika	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Areyingbakumo	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Boki	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Digbiri	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Gambari	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Gaungaun	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Gbongi	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Igangan	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Ikumo	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Ilesu	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Lolo Ayin	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Obabi	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Odo	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Ogunmole	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Okunmodo	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes
D. rotundata	Sandpaper	Adaxial	Polygonal	Straight	Closed	+	+	+	+	3-more	-	Raphides
		Abaxial	Irregular	Repand	Closed	+	+	+	+	3-more	ANI, AIT, ISO, TET, STR, ANO	Raphides, Glandular Trichomes

Note: ***-**: Absent, +: Present, Un: Unbranched, Fu: Furcate, Bi: Bifurcated, ANI: Anisocytic, AIT: Anisotricytic, ISO: Isotricytic, TET: Tetracytic, STR: Staurocytic, ANO: Anomocytic

Table 3. Quantitative characters of the stomata of the yam cultivars studied

Species	Cultivars	Stomata length (µm)	Stomata breadth (µm)	Stomata area (µm^2)	Stomata index	UEC (µm)*	LEC (µm)*	LPL (µm)*
D. alata	Ewura	43.80±1.1136^{b}	33.70±0.9434^{abcde}	1488.00±71.0682^{b}	17.22±0.8607^{def}	117.10±4.6237^{i}	47.60±1.7924^{g}	120.30±5.1078^{fg}
D. cayenensis	Igangan	60.00±1.0761^{ijk}	36.40±0.7341^{efg}	2190.20±69.0589^{fg}	12.63±0.7662^{abc}	126.80±3.1133^{j}	43.70±1.1542^{efg}	104.90±2.6417^{e}
D. rotundata	Ajimokun	57.00±1.3416^{ghi}	36.50±0.7090^{efg}	2085.80±70.4437^{ef}	9.60±1.1329^{a}	80.40±2.9222^{de}	43.30±1.7000^{efg}	131.20±1.9216^{h}
D. rotundata	Ame	53.00±2.2172^{defg}	32.60±1.0063^{abc}	1739.00±100.7984^{c}	15.46±0.5051^{cde}	72.00±4.1701^{bcd}	35.00±1.7681^{b}	88.00±3.2249^{d}
D. rotundata	Anika	62.00±1.7136^{lm}	35.90±0.7030^{cd}	2266.00±86.5082^{fg}	21.18±0.4827^{fg}	67.70±2.8183^{b}	36.90±1.9330^{bcd}	158.80±3.1065^{j}
D. rotundata	Areyingbakumo	48.40±1.3887^{c}	34.40±0.9358^{bcdef}	1677.80±82.5200^{bc}	19.84±0.4429^{fg}	86.10±2.3641^{ef}	45.60±1.9117^{fg}	84.40±2.5073^{d}
D. rotundata	Boki	61.30±1.4088^{jk}	36.60±0.9188^{efg}	2244.40±75.8524^{fg}	22.76±0.7432^{g}	93.10±2.8265^{fg}	41.20±1.6118^{cdef}	83.60±1.8388^{d}
D. rotundata	Digbiri	49.40±1.5967^{cd}	40.70±1.6175^{h}	2042.00±133.5042^{ef}	13.99±0.3808^{bcd}	99.20±3.8427^{gh}	38.90±2.0493^{bcde}	122.90±3.0830^{gh}
D. rotundata	Gambari	55.60±1.3734^{fgh}	38.90±0.7605^{gh}	2173.60±82.8373^{fg}	20.40±0.6306^{fg}	90.40±3.9672^{fg}	41.60±1.7082^{def}	130.10±5.7003^{h}
D. rotundata	Gaungaun	56.50±1.0942^{ghi}	35.70±0.7578^{def}	2024.00±69.6064^{def}	12.88±0.5365^{abc}	71.10±1.9220^{bcd}	36.00±1.9735^{bc}	72.70±2.1387^{c}
D. rotundata	Gbongi	57.10±0.9676^{ghi}	31.50±0.3517^{a}	1798.00±34.5942^{cd}	20.50±0.7771^{fg}	72.50±3.0912^{abc}	36.40±1.8728^{bcd}	72.70±2.2538^{c}
D. rotundata	Igangan	60.00±1.0761^{ijk}	36.40±0.7341^{efg}	2190.20±69.0589^{fg}	12.63±0.7662^{abc}	126.80±3.1133^{j}	43.70±1.1542^{efg}	104.90±2.6417^{e}
D. rotundata	Ikumo	51.50±1.0551^{cde}	33.90±0.6065^{abcdef}	1746.80±49.8749^{c}	13.99±0.3808^{bcd}	80.20±3.2700^{de}	43.40±1.2061^{efg}	61.00±2.1051^{b}
D. rotundata	Ilesu	62.30±1.0689^{lm}	36.20±0.6943^{defg}	2261.00±69.5416^{fg}	13.16±0.5621^{abc}	68.10±2.4256^{b}	40.90±1.1741^{cdef}	105.10±2.2779^{e}
D. rotundata	Lolo Ayin	62.30±0.9947^{hij}	35.40±0.8221^{cdef}	2060.00±68.9946^{ef}	15.56±0.947^{cde}	98.20±2.4662^{gh}	44.60±1.0770^{fg}	160.80±2.8225^{j}
D. rotundata	Obabi	58.00±1.3016^{efgh}	36.70±1.0591^{fg}	2019.00±73.2855^{def}	18.32±1.0402^{ef}	68.70±2.1535^{bc}	34.50±1.6599^{b}	191.80±5.7380^{k}
D. rotundata	Odo	55.10±0.9567^{ghi}	35.40±0.8092^{cdef}	2025.00±66.2600^{def}	19.89±1.6664^{fg}	65.70±2.4204^{b}	42.90±0.9676^{efg}	113.10±3.7514^{ef}
D. rotundata	Ogunmole	52.30±0.8495^{def}	35.70±0.5671^{def}	1869.40±47.3164^{cde}	18.08±0.5665^{ef}	73.00±3.2879^{bcd}	35.60±1.5735^{b}	123.10±1.9546^{gh}
D. rotundata	Okunmodo	52.50±1.2215^{def}	33.40±1.2986^{abc}	1759.60±82.1064^{c}	19.58±3.3921^{fg}	104.10±3.9403^{h}	41.10±2.5109^{cdef}	118.40±2.1745^{fg}
D. rotundata	Sandpaper	65.20±1.6569^{m}	36.70±0.9870^{fg}	2407.00±105.4637^{g}	13.05±0.6158^{abc}	78.10±2.5730^{cde}	35.10±1.2853^{b}	145.00±3.7906^{i}

Note: *UEC: Upper Epidermal Cells, LEC: Lower Epidermal Cells, LPL: Leaf Palisade Layer. **Means with the same letter along columns are not significantly different at $P \leq 0.05$

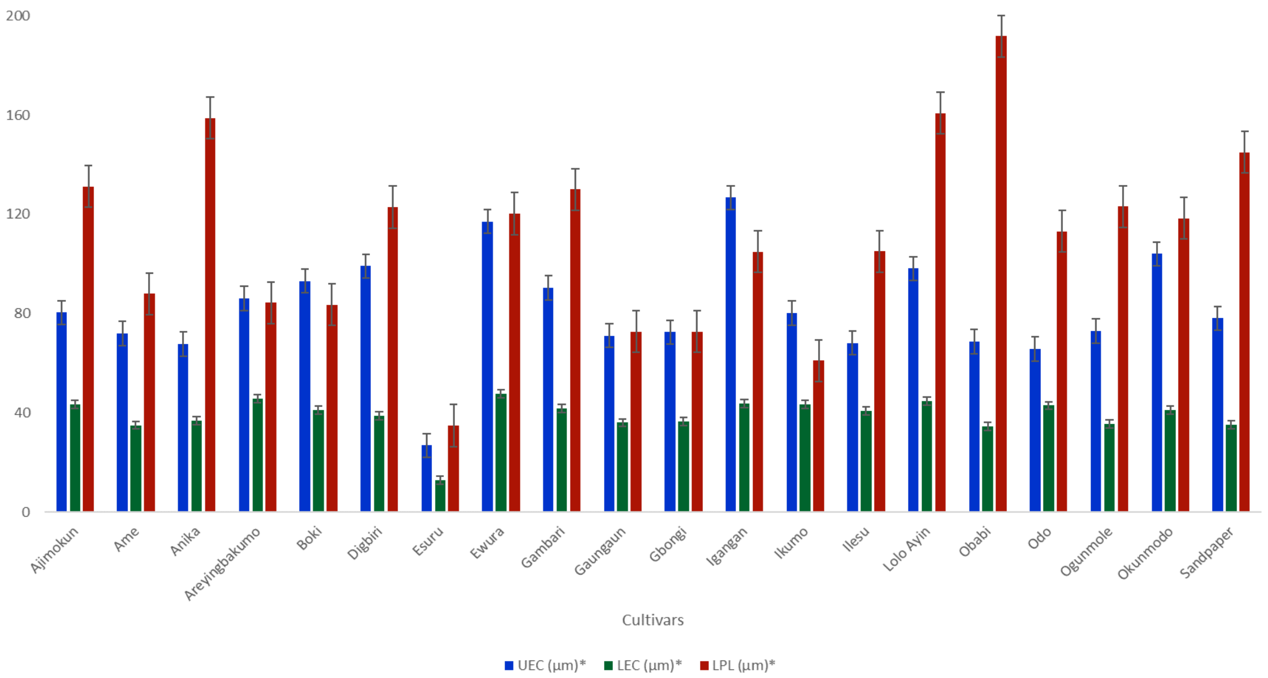

Figure 4. Quantitative characters of the leaf transverse sections of the yam cultivars studied

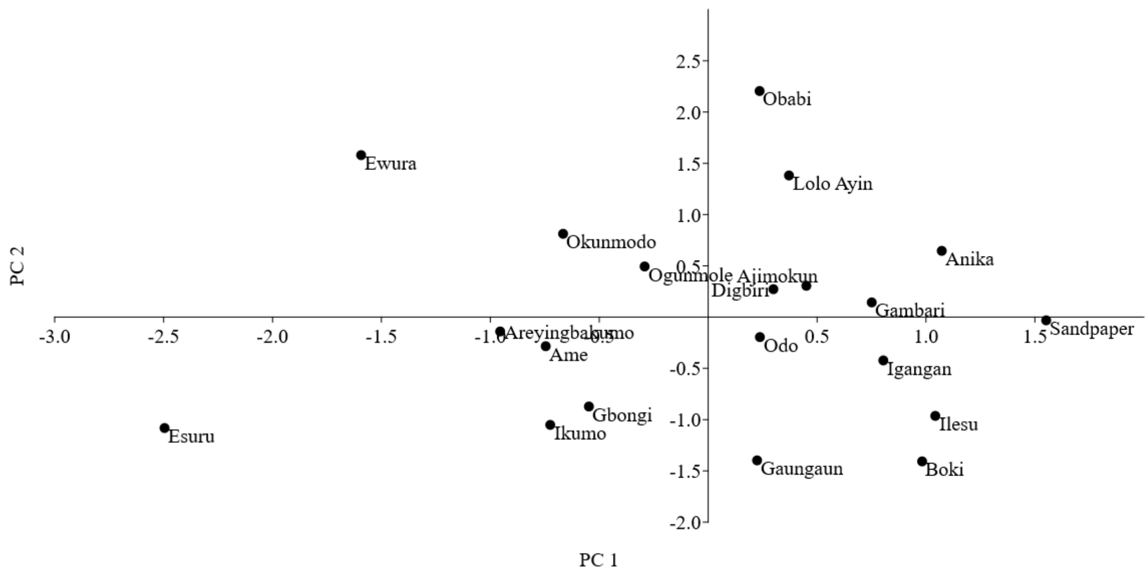

Figure 5. Anatomical diversity among the Yam cultivars using PCA

Table 4. Principal components, eigenvalues, and proportion of variation of the anatomical characters of yam cultivars

Parameters	PC 1	PC 2	PC 3	PC 4
Stomata Length (µm)	0.0209	0.0004	-0.0126	0.1903
Stomata Breadth (µm)	0.0053	0.0124	0.0091	-0.1236
Stomata Area (µm^2)	0.9971	-0.073	-0.0035	-0.0067
Stomata Index	0.0010	0.0220	0.0142	0.4831
Upper epidermal cells (µm)	0.0177	0.2091	0.9456	-0.2144
Lower epidermal cells (µm)	0.0092	0.0566	0.2408	0.8179
Leaf palisade layer (µm)	0.0702	0.9732	-0.2179	-0.0114
Eigenvalue	85160.70	1033.10	436.02	21.57
% Variance	98.26	1.19	0.50	0.02
Cumulative % variance	98.26	99.45	99.95	99.97

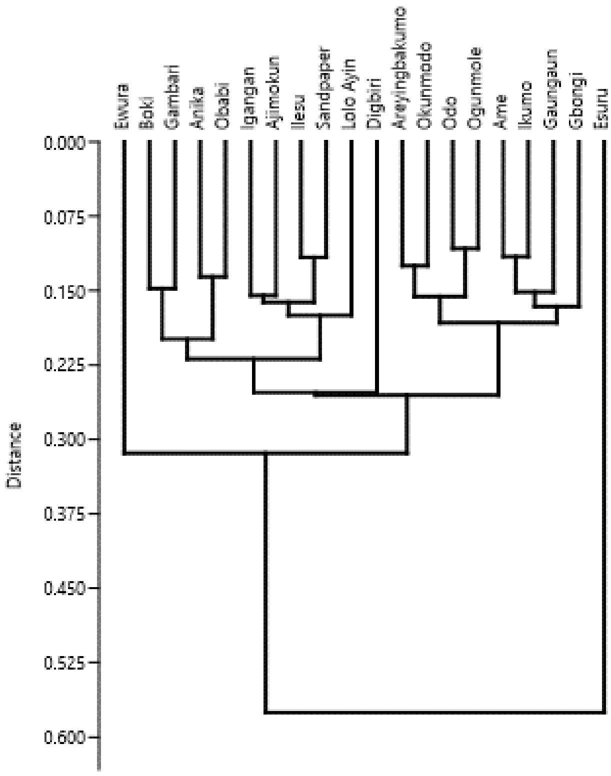

Figure 6. The dendrogram was constructed based on the quantitative characters of the 20 Yam cultivars using Gower genetic distance

Leaf transverse sections

The leaf transverse sections of the yam cultivars studied were outlined by thin non-striated, straight, groovy curticle, while *D. dumetorum* (Esuru) stomata and epidermal layer could not be studied because it is obscure by the trichomes. (Table 3). The single-layer epidermal cells were parallel to the cuticle, and were rectangular or polygonal in all the yam cultivars studied (Table 2). The adaxial epidermal cells (26.80±0.62 µm - 117.10±4.6237 µm) observed were thicker than the abaxial epidermal cells (12.80±0.69 µm-47.60±1.7924 µm) in all the cultivars studied (Tables 3; Figure 4). The shortest epidermal cell sizes (26.80±0.62 µm and 12.80±0.69 µm) were observed in the Esuru cultivar adaxial epidermal cells (Figure 4). Unicellular trichomes were observed on the adaxial and abaxial epidermal cells of the Esuru cultivar. The distinct, closely packed single palisade mesophyll parallel to each other was elongated to cylindrical shape in all the cultivars. Spongy mesophylls observed in all the cultivars were loosely packed with starch granules, raphides (Esuru lacks raphides), and crystals (Table 2). The midribs were fixed cortically positioned with concentric amphivasal vascular bundles in all the cultivars (Table 2).

Petiole transverse sections

The outlines of the petiole transverse sections were cup-shaped for the median and distal transverse sections but varied from saucer- to cup-shaped for the proximal sections of the yam cultivars except in Ewura that had star shape due to the wings present on its petiole (Table 2). The adaxial and abaxial surfaces of the transverse sections of the petiole were concave and convex, respectively, in all the cultivars. All the cultivars had thin cuticles that were striated and undulating. The epidermal layer was thin and single, with parallel epidermal cells that housed starch granules (Table 2). The cortical cells observed in the cultivars were oval collenchyma and parenchyma cells in which druses, crystal sands, and raphides were present, except in Esuru, which lacked raphide but had unicellular and glandular trichomes. The vascular bundles observed in all the cultivars were cortical and concentric amphivasal radially arranged with distinct piths (Table 2).

Data analysis

The study took into account the first four PC, which influenced 99.97% of the cumulative variance (Table 4) in this study. PC 1 contributed 98.26% of the total anatomical variation among the *Dioscorea* cultivars evaluated, which was influenced mainly by the Stomata Area (µm^2). The leaf palisade layer was the major contributor to the 1.19% variation observed in the PC2; the PC3 contributed 0.50% of the cumulative variance; this variation was influenced mainly by Upper epidermal cells (µm). In addition, 0.02% of the variation observed in PC4 was influenced by Stomata Index and Lower epidermal cells.

The correlation among the *Dioscorea* cultivars and the separation of PC 1 and PC 2 showed dispersion in all the quarters for the anatomical characters (Figure 5). Figure 6 shows the agglomerative hierarchical clustering dendrogram, which illustrates the relationship among the cultivars using the unweighted pair group method with arithmetic mean (UPGMA). The cluster analysis classified the 20 *Dioscorea* cultivars into different clades using the Gower similarity index of 0 to 0.60; at a genetic distance of 0.25, the dendrogram was divided into four branches. This similarity coefficient put only Ewura in Cluster I; Boki, Gambari, Anika, Obabi, Igangan, Ajimokun, Ilesu, Sanpaper, Lolo Ayin, and Digbiri were grouped into Cluster II. While Areyingbakumo, Okunmodo, Odo, Ogunmole, Ame, Ikumo, Gaungaun, and Gbongi were grouped into Cluster III, also, Cluster IV was comprised of only Esuru cultivar.

Discussion

The morphological description remains a major technique in plant description, especially among indigenous farmers, due to its ease and cheap (Matthew and Oziegbe 2016; Arogundade et al. 2022). However, crops like *Dioscorea* with taxonomic challenges often require the assistance of experts for proper identification, a skill that indigenous farmers have acquired over the years of cultivation (Ude et al. 2019; Worojie et al. 2021; Matthew and Faluyi 2021); hence, On-Farm Participatory Method (OFPM) is important which assisted in improving the indigenous identification of *Dioscorea* cultivars with scientific descriptions. Trifoliate leaf type and presence of hair observed in *D. dumetorum* corroborate with previous studies, ditto wing observed on leaf petiole of *D. alata*, which inspired their names as trifoliate and winged yams respectively (Andres et al. 2017; Siadjeu et al. 2018). Although there were relatively singular diagnostic features in delineating *D. rotundata* cultivars; however, this study showed ample variations in leaf base, color, and shape. Ude et al. (2019) and Faluyi et al. (2022) reported using multiple features and techniques to identify *Dioscorea* species.

Moreover, the epidermal cell shapes of the *Dioscorea* cultivars studied were uniform. Lema et al. (2019) reported that the genes that condition for diversity in domesticated cultivars retain low variabilities due to domestication; however, the foliar taxonomic study is still an important diagnostic feature in plant taxonomy (Akinsulire et al. 2018). It can be said that the crop epidermal architecture aids in photosynthesis by trapping light, which is important for *Dioscorea* tuberization. However, *Dioscorea* is classified under monocot; the epidermal cell shape assumed by the cultivars studied was dicot-like. Such epidermal cell shape is essential in trapping light for plant photosynthesis, which in turn aids rapid diffusion of carbon dioxide for photosynthesis and mechanical support of foliar structures, especially the leaves (Glover 2000). However, the indigenous belief in yam cultivation laid more emphasis on water as the major requirement for tuberization, while the importance of light is underplayed. Further studies are recommended to correlate water and light's significance in Dioscorea tuberization.

Likewise, the cultivars studied had hypostomatic leaves, i.e., the stomata were only found on the abaxial surface of the leaves. This disagrees with the study of Abdulrahaman et al. (2009) that reported epistomatic leaves (i.e., stomata occurring on the adaxial surface of the leaves only) for *D. rotundata*. Tajuddin et al. (2013) and Sheikh and Kumar (2017) reported that hypostomatic is a common feature in the genus. Hypostomatic leaves are important for efficient photosynthesis and plant transpiration in a climber. Driscoll et al. (2006) and Song et al. (2020) reported that photosynthesis and transpiration are more efficient in hypostomatic leaves compared to epistomatic leaves. Prabhakar (2004) stated that the stomata classification used in this study might result in discrepancies of this present and the previous studies; therefore, characterizing each stoma is paramount in plant taxonomy. This recommends the Prabhakar (2004) stomata for further studies on plant anatomy.

This study also contradicts the report of Edeoga and Okoli (1995) that reported the presence of raphides in the anatomical structure of *D. dumetorum* which was found only in *D. alata, D. cayenensis* and *D. rotundata* according to the observation in the study. However, the absence of raphides in the leaves of *D. dumetorum* might be compensated by the presence of unicellular trichomes on the leaf surfaces, which is also a distinguishing characteristic of *D. dumetorum*. Nevertheless, raphides, trichomes, starch, and tannins, etc., which are classified as egastic substances (calcium oxalate crystals), although inorganic, are useful in plant defense and for plant taxonomic classification (Molano-Flores 2001; Nwachukwu and Edeoga 2006). Thus, the presence of trichomes in *D. dumetorum* could make up for the absence of raphides in this plant, which might be for defense and a taxonomic feature of the species. However, glandular trichomes are a common taxonomic feature in all the *Dioscorea* species studied.

The importance of the combination of both quantitative and qualitative anatomical features in plant taxonomical study cannot be overemphasized. The Transverse Section (TS) result showed that *D. dumetorum* (Esuru) had characteristic unicellular trichomes on the TS of the leaf and petiole. In comparison, unicellular trichomes, spines, and protrusion of glandular trichomes were observed on the TS of the leaf. The *D. alata* (Ewura) cultivar was distinguished by its wing, which showed on both the transverse sections of the leaf and petiole. The species' leaf and petiole transverse sections (TS) showed vast distinction except between and within *D. cayenensis* and *D. rotundata*. There were no variations in the studied cultivars' cuticle, epidermal cell, midrib bundle, and palisade mesophyll layer. Although, previous anatomical classifications reported on *Dioscorea* have been more of quantitative classification (Edeoga and Okoli 1995; Abdulrahaman et al. 2009). The highest stomata area calculated was recorded for *D. rotundata* (Sandpaper and Anika cultivars) which could have contributed to the high tuber yield from these cultivars (Faluyi et al. 2022). Stomata sizes have been reported to have a relationship with CO_2 diffusion efficiency, photosynthesis, and food production, which often translates into tuberization in *Dioscorea* (Saadu et al. 2009; Chathlingathe et al. 2017; Bertolino et al. 2019).

Principal Component Analysis (PCA) showed that the stomata area had a major contribution to the result of this study. The dendrogram showed great diversity, separating *D. dumetorum* (Esuru) and *D. alata* (Ewura) from *D. cayenensis* (Igangan) and *D. rotundata* cultivars. However, at the coefficient of 0.31, the dendrogram showed that both *D. cayenensis* (Igangan) and *D. rotundata* cultivars studied had similar progenitors. Norman et al. (2011) reported that *D. alata* and *D. rotundata* originated from the section Enanthiophylum. However, *D. rotundata* has been reported to have a close relationship with other species. Ude et al. (2019) reported the lowest phylogenetic diversity in studying different yam species and accessions using molecular markers. This could also corroborate the studies of Atieno et al. (2020) and Yue et al. (2022) that reported a close sibship relationship between *D. alata*, *D. cayenensis,* and *D. rotundata*.

These features are not taxonomically diagnostic; however, *D. dumentorum* could be differentiated from other studied species with the absence of raphides and druses in its spongy mesophyll. The thin characteristic features of leaf TS recorded in *D. dumentorum* could be a trade-off for the presence of unicellular trichomes, which could serve a similar purpose in the transpiration rate reduction (Galdon-Armero et al. 2018). The similarities between *D. cayenensis* and *D. rotundata* and their distinction from other yam species studied agree with the findings of Lowe and Soladoye (1990) and Edeoga and Okoli (1995) using leaf characteristics in their classifications.

Moreover, using qualitative and quantitative anatomical studies of the four *Dioscorea* species study showed distinguishable variation. This study showed that quantitative and qualitative variables complement each other in taxonomic classification, especially for the genus *Dioscorea*. The four species studied were anatomically distinguishable except *D. cayenensis* and *D. rotundata*; therefore, this study recommends further studies to this effect.

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