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Figures and Tables of a maximum of three pages should be clearly presented. The title of a picture is written down below the picture, while the title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; the chart is preferred to use black and white images. The author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned the source. Author is suggested referring to Wikipedia for international boundaries and Google Earth for satellite imagery. If not specifically mentioned, it is assumed to refer to these sources. **There is no appendix**, all data or data analysis is incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213.

The usage of "et al." in long author lists will also be accepted:

Smith J. Jones M Jr. Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

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Leaf anatomy of twenty *Dioscorea* cultivars from 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:

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

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).

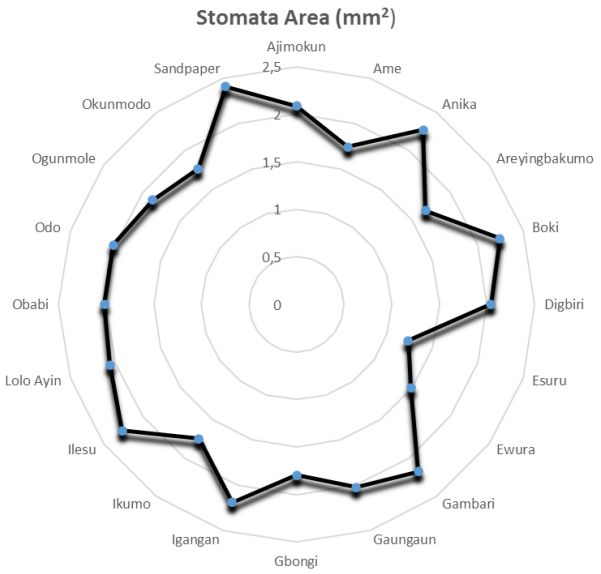

Figure 1. Stomata area of the yam cultivars studied

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

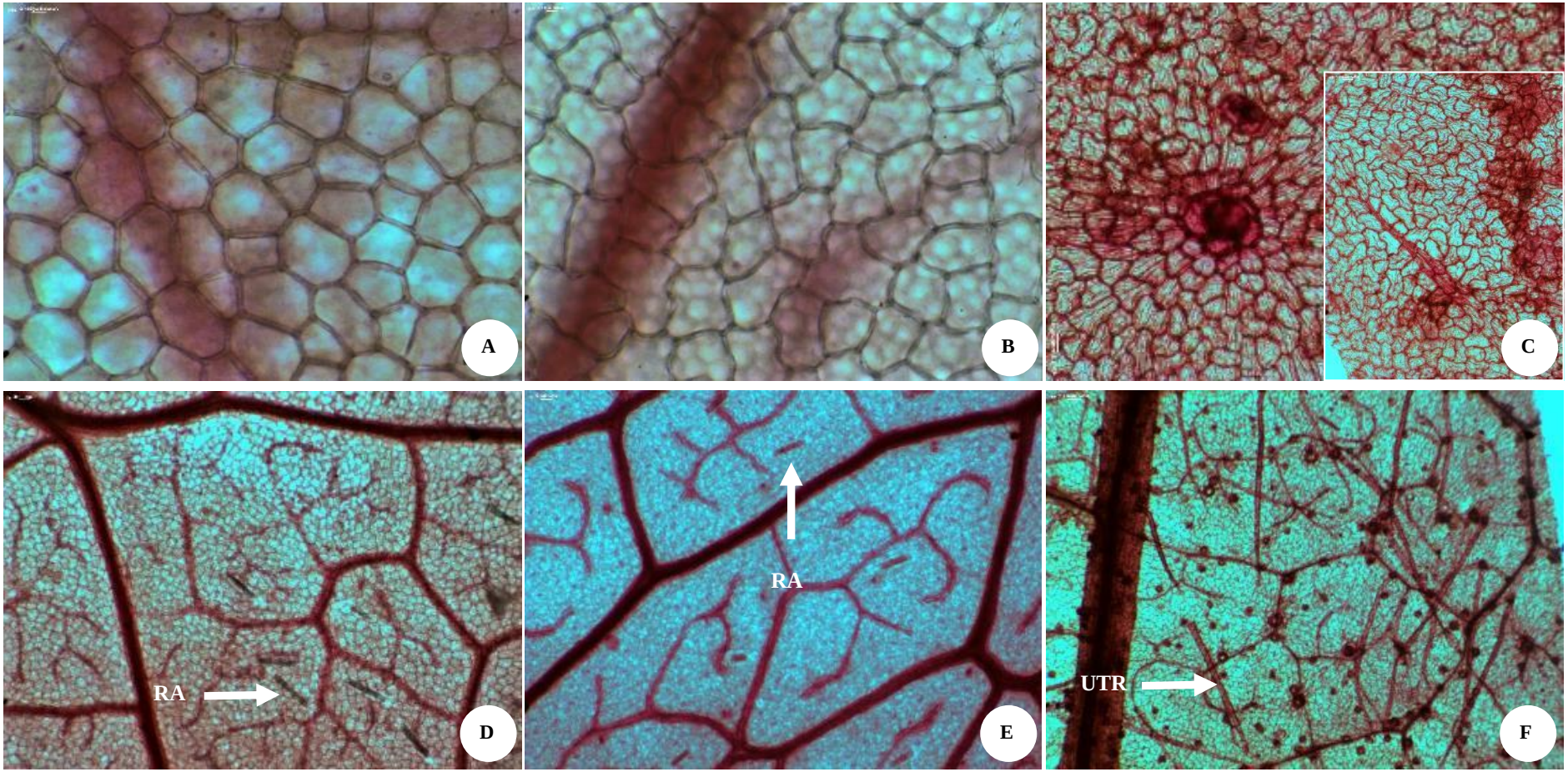

Figure 2. Epidermal features of the *Dioscorea* species studied. A. Areyingbakumo (*D. rotundata*) Adaxial Anticlinal Wall., B. Igangan (*D. cayenensis*) Adaxial Anticlinal Wall., C. Esuru (*D. dumetorum*) Adaxial Anticlinal Wall., D. Areyingbakumo (*D. rotundata*) Adaxial Venation Pattern., E. Igangan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

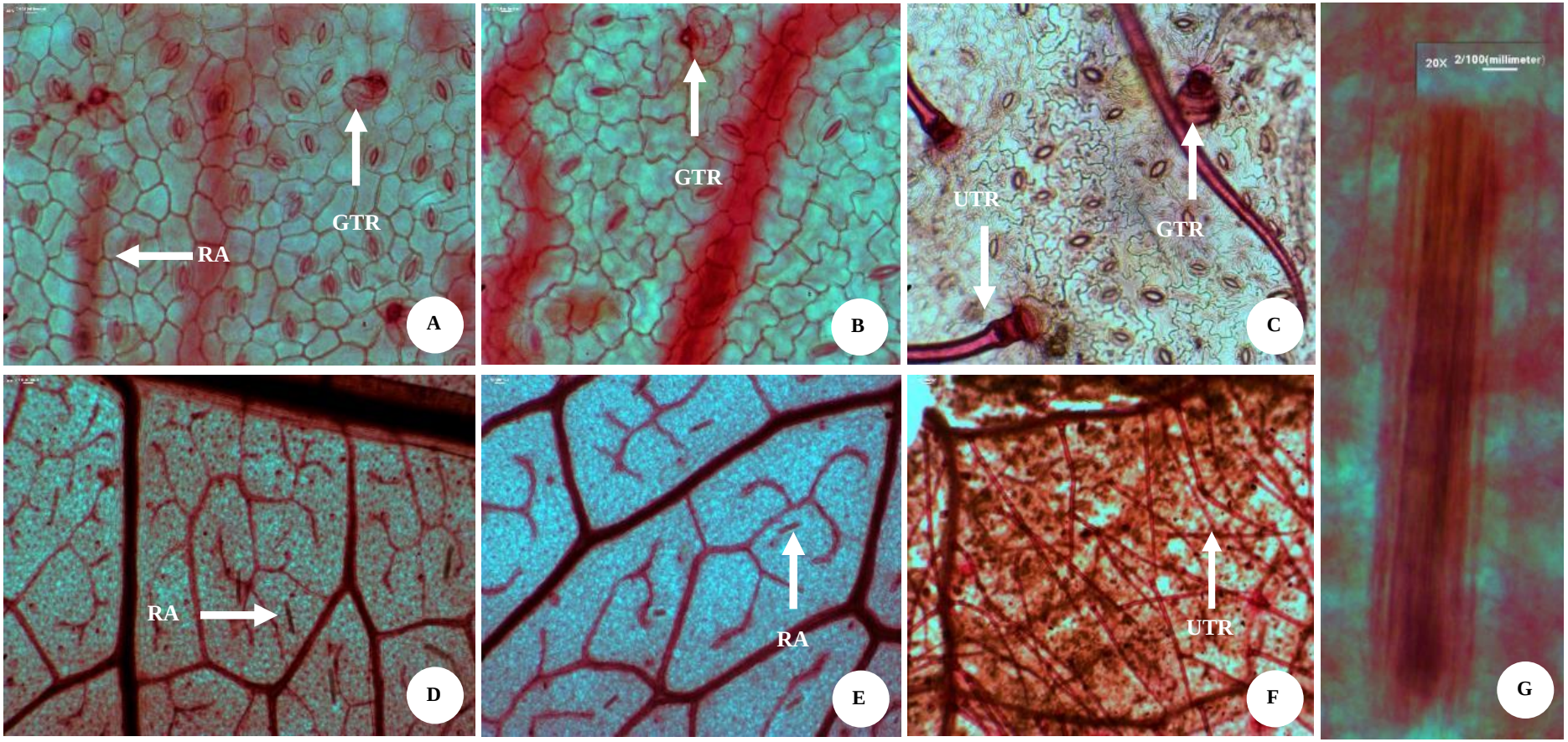

Figure 3. Epidermal features of the *Dioscorea* species studied. A. Areyingbakumo (*D. rotundata*) Abaxial Anticlinal Wall., B. Igangan (*D. cayenensis*) Abaxial Anticlinal Wall., C. Esuru (*D. dumetorum*) Abaxial Anticlinal Wall., D. Areyingbakumo (*D. rotundata*) Abaxial Venation Pattern., E. Igangan (*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 $P \leq 0.05$

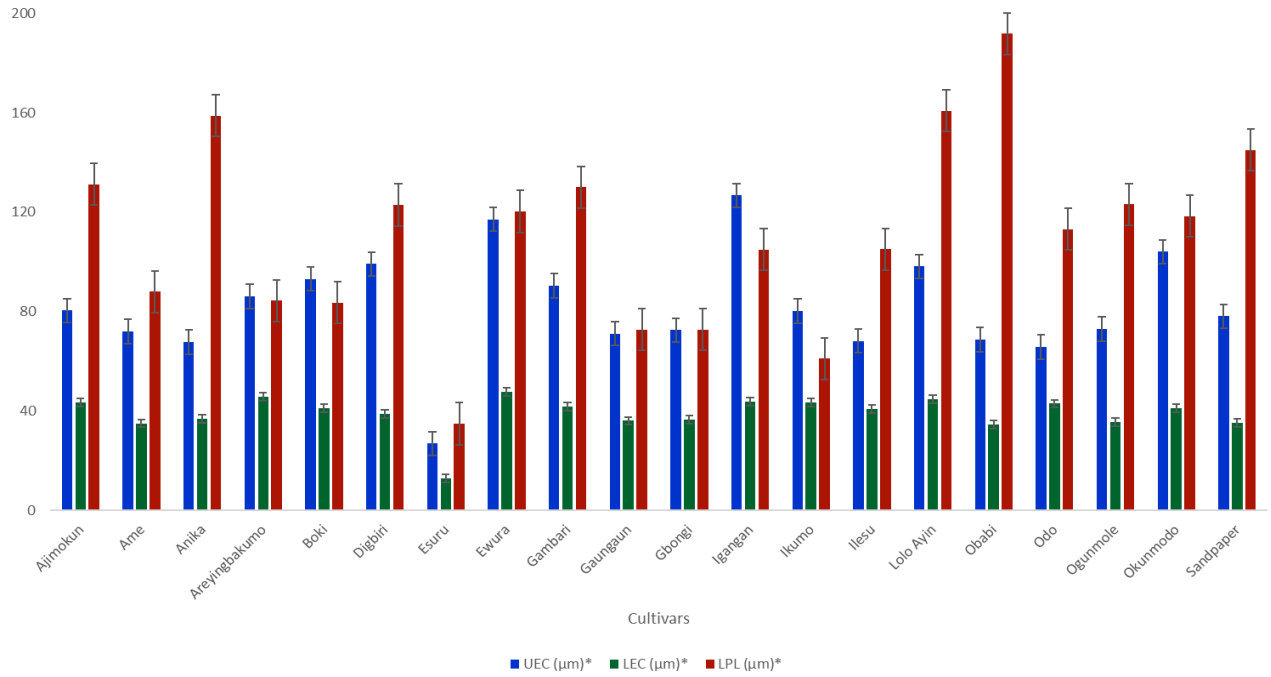

Figure 4. Quantitative characters of the leaf transverse sections of the yam cultivars studied

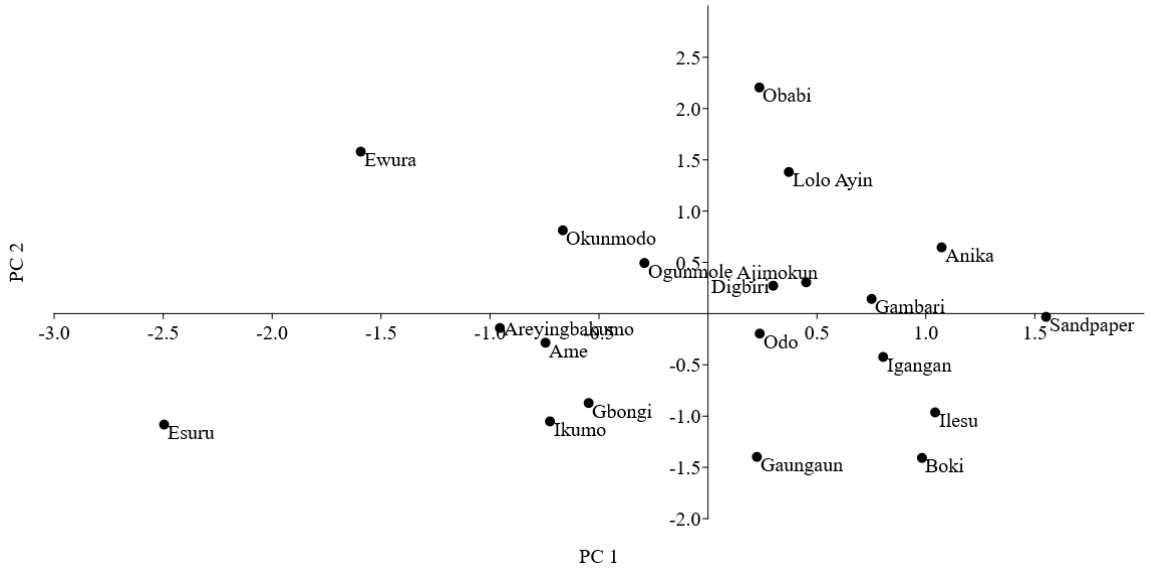

Figure 5. Anatomical diversity among the Yam cultivars using PCA

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

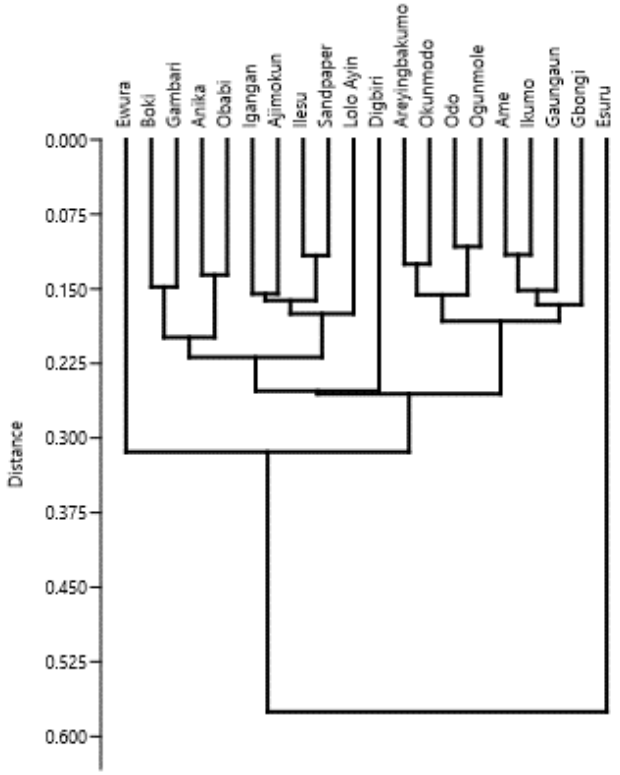

Figure 6. The dendrogram was constructed based on the quantitative characters of the 20 Yam cultivars using Gower genetic distance

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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Growth of vanilla (*Vanilla planifolia*) roots in different internodes of stem cuttings with NAA (Naphthaleneacetic Acid) treatments

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Abstract. *Mudyantini W, Huda YN, Pitoyo A. 2024. Growth of vanilla (*Vanilla planifolia*) roots in different internodes of stem cuttings with NAA (Naphthaleneacetic Acid) treatments. Cell Biol Dev 8: 13-21.* Vanilla (*Vanilla planifolia* Andrews) is a plantation commodity with high economic value. Unstable production of vanilla pods causes a decrease in vanilla pod exports. One of the obstacles is the limited availability of seeds. This study aims to determine the effect of combining two treatments on root growth in vanilla stem cuttings. The first treatment was a variation of NAA concentrations of 0, 50, 100, and 150 ppm. The second treatment was the cuttings' age on the third, fifth, and seventh nodes of vanilla stem cuttings. The planting material was soaked in a solution of NAA hormone and was carried out 3 repetitions for 60 minutes. Cuttings were planted in the growing medium for 90 days. The research parameters measured were root emergence time, first root length, number of root branches, first root diameter, root branch length, number of leaves, and shoot height. The results showed that soaking vanilla cuttings with an NAA concentration of 150 ppm accelerated root emergence and increased primary root length. The seventh node of the vanilla cuttings soaked in 150 ppm of NAA hormone solution was the best combination for the time of root emergence and root length, while the fifth node without being soaked in NAA hormone solution was the best combination for shoot height and the number of leaves. Soaking vanilla cuttings with NAA hormone solution did not affect increasing root diameter, number of roots, and length of root branches.

Keywords: Auxin, Naphthaleneacetic acid, soaking method, stem cuttings, *Vanilla planifolia*

INTRODUCTION

Indonesia has a tropical climate that is favorable for plant growth, one of which is vanilla (*Vanilla planifolia* Andrews). Vanilla is used as a mixture of food, beverages, and cosmetics industry, which makes its high economic value. Indonesia is the largest vanilla producer in the world, along with Madagascar, Papua New Guinea, and India (Frenkel and Belanger 2019). North Sumatra, Lampung, Central Java, West Java, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, North Sulawesi, and South Sulawesi are areas that cultivate vanilla plants and even become vanilla production centers (Ruhnayat 2003). According to the report of the Vanilla Plantation Statistic Directorate General of Plantations (2010), in 2004-2005, the price of dried vanilla pods was from Rp 2,000,000 to Rp 3,000,000/kg, but in 2007-2008, the price dropped from Rp 200,000 to Rp 300,000. This can happen because the quality of the vanilla fruit is low and does not meet the criteria for the international market (Baharudin et al. 2023).

Vanilla farmers in Indonesia tend to cultivate vanilla vegetatively through cuttings rather than seeds because vanilla seeds are difficult to germinate due to the immature endosperm of the plant (Umesha et al. 2011). Vegetative propagation is an alternative for farmers in the vanilla nursery process. Propagation by cuttings is easy, inexpensive, and produces the same tillers as the parent plant. According to Kartikawati and Rosman (2018), short vanilla cuttings consisting of 2 nodes with 1 node can be an alternative to limited seeds. Sources of seeds from shortcuttings take 4-6 months to be transferred to planting land (Hadipoentyanti et al. 1998). This problem hinders vanilla production, which can impact decreasing vanilla exports. In addition, the production of vanilla seeds that have been certified by the Minister of Agriculture in Indonesia is still limited due to the small number of vanilla mother farms in Indonesia (Balfas 2022). According to the Directorate General of Plantations (2021), the vanilla main gardens established in Indonesia are spread across five provinces with a total of 16 main gardens.

Vanilla planting material using short cuttings needs to pay attention to the mother tree's age, which indicates the stem's maturity on the cuttings and the vanilla stem's hardness (Sukarman 2011). According to Hartman and Kester (1975), good cutting material can be determined from the hardness of the stem. Carbohydrate distribution depends on the plant's age; young cuttings contain relatively low carbohydrates, while old cuttings contain relatively high carbohydrates (Aldrete-Herrera et al. 2019). Somantri and Evizal (1987) stated that vanilla cuttings were able to germinate due to the support of well-developed and growing roots. The hormone contained, i.e., auxin, functions in root formation by stimulating cell elongation and division in the cambium tissue.

Naphthalene-acetic Acid (NAA) is an auxin hormone consisting of a white amorphous synthetic organic compound. NAA stimulates cell division, enlargement, cell differentiation, and protoplasmic flow in the vegetative growth of plants, including root organs (Widiastoety 2014). NAA is applied for plant vegetative propagation, topically

as a paste or soaking. The concentration and duration of immersion affect the level of absorbed auxin. The longer the immersion and the higher the concentration, the greater the auxin absorbed by plant cells (Supardi and Seda 2010). Research by Yan et al. (2014) on *Hemarthria compressa* (L.f.) R.Br. plants treated with an NAA concentration of 200 ppm by immersion method for 20 minutes resulted in a root growth percentage of 97%, the number of adventitious roots per cutting, and dry weight of roots per cutting. Jamaludin's research (2019) showed that single-node vanilla cuttings treated with NAA and IBA at a concentration of 500 ppm produced a shoot height of 14 cm and a lower root length of 25 cm at 16 weeks after planting. This study used 0 ppm, 50 ppm, 100 ppm, and 150 ppm in the concentration of NAA as an exogenous auxin hormone and different stem cuttings to determine the growth of vanilla cuttings.

MATERIALS AND METHODS

Study area

The research was carried out from December 2022 to May 2023 at the Biology Laboratory Department of Biology, Universitas Sebelas Maret, Indonesia, and Universitas Sebelas Maret Integrated Laboratory Green House, Indonesia.

Tools and materials

The tools used were pruning shears for cutting cuttings, a plastic bucket for soaking cuttings, a 25 × 25 cm polybag for cuttings to grow in, a spatula for taking NAA powder, a 100 mL measuring cup for measuring water volume, a 1,000 mL beaker for preparation of hormone solutions, stir bars to stir hormone solutions, analytical balances to weigh ingredients, measuring tape to measure root length and shoot height, caliper to measure root diameter, Munsell Plant Tissue Color Chart (Munsell 2023), and plastic caps.

The materials used are Naphthaleneacetic Acid (NAA) powder, vanilla cuttings taken from the vanilla tree of the vania 2 variety with an age of ≥ 12 months that have not yet flowered from the Karavan Boyolali cooperative vanilla garden, aquadest, KOH, Dithane M45, manure, poor sand, and garden soil.

Procedures

Obtain vanilla cuttings

Obtaining vanilla cuttings refers to the Decree of the Minister of Agriculture Number 08/KPTS/KB.020/1/2018 concerning guidelines for the production, certification, distribution, and supervision of vanilla plant seeds. The vanilla plant of the Vania 2 variety selected for cuttings is a healthy mother tree, not nutrient deficient, aged ≥ 12 months, ± 1 meter long (8-10 nodes), and vines that have not yet produced flowers. Planting material was taken from the Karavan Boyolali cooperative vanilla garden, where the vines were cut using sharp pruning shears to prevent tissue damage into one node and two node cuttings according to the treatment.

Preparation of planting media

The planting medium used is a mixture of soil, sand, and manure with a ratio of 1:1:1 (Mariska et al. 1987; Supardi and Seda 2010). The garden soil is dried for about 3 days to reduce the water content and then sieved to obtain uniform soil size. Soil, sand, and manure are mixed with a ratio of 1:1:1 until blended. The planting media mixture is filled into a polybag measuring 25 × 25 cm as much as 1,500 grams. The center of the media is given a hole with a depth of 10 cm.

Preparation of NAA hormone solution

NAA hormone powder was dissolved according to the predetermined treatment, namely 0, 50, 100, and 150 ppm. To make a NAA 50 hormone solution, 100 and 150 ppm. Next, 50, 100, and 150 mg of NAA powder were dissolved in 1000 mL of distilled water each. Before being dissolved in distilled water, the NAA powder was dissolved with a few drops of concentrated KOH. Based on several studies that have been carried out previously, the use of NAA to stimulate roots using the soaking method uses a concentration range of 20-250 ppm, so in this study, a concentration variation was taken that was still in that range (Blythe et al. 2007).

Soaking cuttings in NAA hormone solution

Vanilla cuttings were soaked in Dithane M45 fungicide solution for 15 minutes and then dried overnight to avoid pathogens that can infect vanilla cuttings due to cuttings (Holis 2017). In the next stage, cuttings were immersed in a solution of the NAA hormone according to the treatment for 60 minutes. Each treatment had 3 (three) repetitions from each segment of the stem cuttings. Soaking is done in a shady place that is not exposed to direct light.

Planting in the growing media

The soaked vanilla cuttings are dried at room temperature for overnight. Furthermore, the cuttings are planted in polybags filled with media with the node's position closed with the planting medium.

Plant maintenance

Polybags are placed in the Universitas Sebelas Maret Integrated UPT Laboratory Green House with an altitude of 106 meters above sea level, 89% humidity, and temperatures ranging from 26-29°C. The plants are covered with a bamboo and plastic frame measuring 2.5 × 0.8 × 1 meter to keep the environment moist and avoid direct sunlight. Vanilla cuttings are watered with 150-300 mL water in the morning when the planting medium is dry. After three weeks of planting, vanilla cuttings were sprayed with Dithane M-45 fungicide to prevent the cuttings from rotting. In addition, weeding plant weeds is done if there are weeds in the soil media.

Observation variable measurement

Vanilla cuttings are grown for 90 days. Vanilla roots appeared on the 10^{th}, 20^{th}, 30^{th}, 60^{th}, and 90^{th} days after planting. Measurements of the number of leaves and the height of the growing shoots were carried out once a week

for 90 days after planting. Shoot height was measured from the base of the shoot to the tip using a measuring tape. Measurement of the first root length, number of root branches, root branch length, and first root diameter was carried out 30 days after planting by dismantling the media. Next, the length of the first root and the growing root branch were measured using a measuring tape, counting the entire number of growing root branches and measuring the root diameter using a caliper.

Data analysis

Qualitative data in this study were in the form of morphological and anatomical descriptions of vanilla roots. Quantitative data for measuring parameters on the 90th day obtained in this study were analyzed using the ANOVA test with a significance value of the difference P <0.05. If there is a significant effect, continue with Duncan's Multiple Range Test (DMRT) at a 95% confidence level presented in the table. If the DMRT test shows significant results, it is followed by a different letter symbol.

RESULTS AND DISCUSSION

Morphological structure and anatomy of vanilla root

The *V. planifolia* has two types of roots: terrestrial roots in the soil and aerial roots that appear at the stem nodes. Morphologically, vanilla is classified as a monocot plant with fibrous terrestrial roots (Ruhnayat 2003). Figure 1. A shows the morphology of vanilla terrestrial roots without treatments. The texture of the vanilla root is not woody (herbaceous) and has mucilage when cut. Vanilla root has fine root hairs; elongated cylindrical round root shape with a pointed tip. Terrestrial roots consist of the first root that appears on the cutting and the root branch that grows on the first root. Based on the Munsell Book Color Chart, the terrestrial root has a yellowish-white color (2.5Y 8/6) (Figure 2. A).

The morphology of aerial roots without treatments is shown in Figure 1. B. The air root of vanilla has a slippery texture, is not woody (herbaceous), and has mucilage when cut. Aerial roots do not have root hairs. Fine root hairs appear when aerial roots grow downward and touch the ground; elongated cylindrical round root shape with a blunt end. Aerial roots do not have root branches. Vanilla aerial roots are green (2.5GY 5/8) (Figure 2. B).

Figure 1. A. Terrestrial roots, B. Aerial roots. Scale bars=100mm (Photo credit: Nurul Huda)

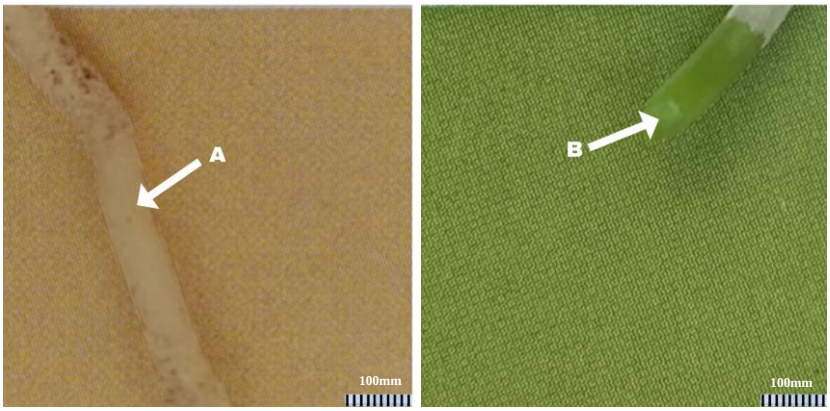

Figure 2. A. Terrestrial root color and B. Aerial root color of *Vanilla planifolia* Andrews. Scale bars= 100 mm (Photo credit: Nurul Huda)

Table 1. The average value of the first root appearance of vanilla (days) on different sections after damping with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	21.67±1.52^{a}	21.67±5.13^{a}	22.33±6.80^{a}	15.67±4.50^{abc}	20.33±5.03
R5*	15.33±3.05^{abc}	21.00±1.73^{ab}	19.67±1.52^{ab}	17.00±4.35^{abc}	18.25±3.38
R7*	14.33±4.93^{bcd}	19.33±0.57^{ab}	12.33±1.52^{cd}	8.33±2.51^{d}	13.58±4.81
Average	17.11 ± 4.56	20.67 ± 2.91	18.11 ± 5.73	13.67±5.26	17.39±5.20

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 2. The average value of the first vanilla root length (cm) on different segments of age 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	12.76±2.01^{def}	9.53±2.30^{ef}	14.66±2.65^{bcde}	18.06±3.34^{abc}	13.75±3.93
R5*	19.70±1,90^{ab}	12.03±1.35^{def}	12.06±2.05^{def}	21.70±3.38^{a}	16.37±4.97
R7*	12.36±1.26^{def}	9.10±5.31^{f}	13.53±2.37^{cdef}	17.06±3.55^{abcd}	13.01±4.19
Average	14.94±3.88	10.22±3.27	13.42±2.34	18.94±3.64	14.38±4.50

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

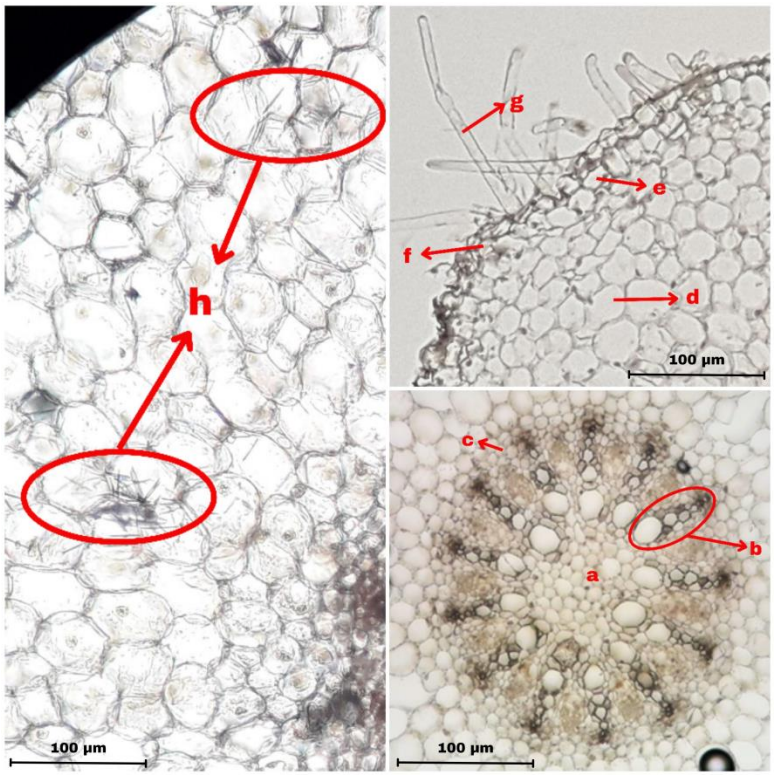

Figure 3. Anatomical structure cross-section of vanilla terrestrial root magnification 100×. a. Pith, b. Vascular bundle c. Endodermis, d. Cortex, e. Exoderm, f. Epidermis, g. Root hair, h. Rapidhes needle. Scale bars= 100 µm (Photo credit: Nurul Huda)

Anatomically, the cross-section of the vanilla terrestrial root consists of root hair, epidermis, exodermis, cortex, endodermis, vascular bundle, and pith (Figure 3). In this study, the structure of the valamen was not observed in the cross-section of the vanilla terrestrial root). The velamen structure is suspected to be only observed in cross-sections of vanilla aerial roots (Deseo et al. 2020). The structure of the epidermis terrestrial roots is thinner than the exodermis, causing the epidermis to collapse easily. The cortex structure consists of peripheral cells between the exodermis and endodermis. The cortex structure is also observed as an ergastic object that is rapid and needle-shaped. The endodermal structure consists of a single row of cells surrounding a vascular bundle consisting of the xylem and phloem. The pith was the deepest structure observed,

consisting of large, round, thick parenchyma cells (Stern and Judd 1999).

Time of first root emergence

Vanilla roots appeared on the 10th, 20th, 30th, 60th, and 90th days after planting. If roots appear before the observation day, the root emergence day is determined based on the root growth rate. ANOVA results show that variations in the concentration of the NAA hormone and the age of the cuttings, independently or in combination, significantly affect the time of appearance of the first vanilla roots.

Table 1 shows that soaking cuttings with NAA hormone was significantly different in accelerating the appearance of the first vanilla roots. The fastest roots were shown in 150 ppm NAA immersion, 13.67 days. Panjaitan et al. (2014) state that auxin stimulates root growth. This aligns with research by Agustiansyah et al. (2017) that using NAA can accelerate the emergence of roots in guava grafts by up to 73.3%. Auxin moves basipetal (from tip to base) to encourage root formation (Rashotte et al. 2000). Auxin makes it easier for cell walls to stretch so that wall pressure down the cell. Thus, cell flexing occurs so that cell elongation and enlargement occur and then encourage the formation of roots (Wijana and Lasmini 2021).

This study's treatment combinations significantly differed in the vanilla roots' emergence time. The fastest roots were shown in the combined treatment of the seventh segment of 150 ppm NAA immersion, 8.33 days. Table 1 shows that the roots of the seventh internode of vanilla cuttings appeared faster than the third and fifth internodes. It was shown that the roots appeared in 13.58 days. The seventh cutting segment is thought to have older cuttings, so they have sufficient food reserves. Root formation on cuttings requires energy in the form of carbohydrates and protein stored in the origin of the planting material (Suryanti et al. 2022).

First root length

The parameters measured were vanilla terrestrial roots. The ANOVA results showed that variations in the concentration of the NAA hormone and the age of the cuttings, independently or in combination, significantly affected the length of the first vanilla root.

Table 2 shows that soaking cuttings with different NAA hormones significantly increased the length of the first vanilla root. The NAA concentration of 150 ppm shows the longest root, 18.94 cm. Root elongation results from the enlargement of new cells in the apical meristem area due to continuous cell division (Khadr et al. 2020). Auxin induces cell division by regulating the cell cycle with the help of glucose. Auxin and glucose signals are needed to regulate cell proliferation, especially preparation for cell replication from the G1 phase to the S phase. Auxin and glucose induce the expression of the Cyclin D3;1 (CYCD3;1) gene in plants to increase the cell cycle (Sablowski and Dornales 2014). Activated cyclin D can still be blocked by the CDK inhibitor KRP. Auxin can reduce the expression of several KRP genes so that Cyclin D can still trigger transcriptional suppressor RBR phosphorylation and release complex transcription regulators E2FA/B and DPA. Auxin stabilizes the E2FA/B and DPA complexes, which trigger gene expression transcription for the initial S phase. Furthermore, after the S phase is complete, glucose plays a role again in starting the transition from the G2 phase to the M phase (Wang and Ruan 2013).

Table 2 shows that the combination treatment of the fifth node of vanilla cuttings and an NAA concentration of 150 ppm produced the longest root, 21.70 cm. The fifth segment of vanilla cuttings is suspected to be physiologically young (juvenile), so the meristem cells are still active (Suryanti et al. 2022). The root meristem is located at the distal part of the root, which continues to grow to produce new cells supported by the expression of the NAC1 gene (Xie and Ding 2022). Continuous cell division causes cell elongation, thereby promoting vanilla root elongation.

First root diameter

Root diameter is an important parameter to observe. Roots have carrier bundles for transporting nutrients and photosynthetic products. The first root diameter measurement using a caliper was measured on the 90th day after planting. ANOVA results show that variations in the concentration of NAA hormone and the age of the cuttings independently or in combination have no significant effect on the diameter of the first vanilla root.

Table 3 shows that soaking cuttings with NAA hormone did not show any significant difference in increasing the diameter of the first root. The treatment with the NAA hormone concentration of 50 ppm, which was 2.86 mm, showed the largest diameter of the vanilla root. Auxin plays a role in cell expansion. Auxin will stimulate H+-ATPase in the plasma membrane through upregulation of phosphorylation to pump H+ ions into the cell wall. H+ ions cause acidification of the apoplast in the plant cell wall. Expansion proteins work optimally at low pH to break the hydrogen bonds between the polymer walls. Plant cell walls contain cellulose and hemicellulose fibrils embedded in a pectin and protein matrix (Wolf et al. 2012). This allows slippage between cellulose microfibrils, resulting in cell wall elongation (Sablowski and Dornales 2014). Plant cell walls elongate, resulting in water entering by osmosis and active cell walls loosening proteins. This process causes enlargement of the cell wall. Cells continue to grow by re-synthesizing cell walls and cytoplasmic materials (Putra and Shofi 2015; Barbez et al. 2017; Majda and Robert 2018).

Number of root branches

Root branches that appeared on the first vanilla root were counted on the 90th day after planting. In general, from all treatments, root branches grew on the first root of the vanilla plant. The results of ANOVA showed that variations in the concentration of the NAA hormone and the age of the vanilla cuttings, either independently or in combination, had no significant effect on the number of root branches.

Table 3. The average value of the first root diameter of vanilla (mm) on different segments 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	2.86±0.16	2.96±0.30	2.83±0.15	2.66±0.15	2.83±0.20
R5*	2.60±0.50	2.83±0.57	2.53±0.36	2.63±0.25	2.66±0.40
R7*	2.50±0.50	2.73±0.12	2.56±0.25	3.26±0.10	2.76±0.40
Average	2.65±0.39	2.86±0.34	2.64±0.27	2.85±0.34	2.75±0.34

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 4. The average value of the number of vanilla root branches (strands) at different ages 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	3.67±1.52	3.33±2.51	6.67±2.08	5.00±4.35	4.67±2.77
R5*	6.67±3.05	7.00±4.58	8.33±2.08	3.67±3.21	6.42±3.37
R7*	5.33±1.52	3.67±3.21	4.33±3.05	9.67±4.50	5.75±3.69
Average	5.22±2.27	4.67±3.53	6.44±2.74	6.11±4.45	5.61±3.28

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 5. The average value of the length of the vanilla root branch (cm) at different ages of 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	1.87±0.22	2.28±1.87	2.31±0.83	2.51±2.17	2.24±1.30
R5*	3.96±1.74	2.23±0.56	3.64±0.76	2.96±1.12	3.20±1.19
R7*	3.22±1.28	1.86±1.64	2.90±1.00	3.88±1.36	2.97±1.37
Average	3.01±1.42	2.12±1.29	2.95±0.95	3.12±1.52	2.80±1.32

Note: R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 4 shows that soaking cuttings with NAA hormone did not significantly differ from the number of vanilla root branches. Soaking NAA at a concentration of 100 ppm resulted in the highest average number of root branches, namely 6.44 strands. Root branches originate from pericycle cells that have differentiated during the root branch initiation process (Alarcon et al. 2016). Sufficient auxin levels can increase the number of root branches. In some plants, auxin does not induce root branching. The independent meristem initiation and branching mechanisms influence this; the structural differences in the roots cause no auxin-induced branching mechanisms (Fang et al. 2019). In this study, the combination of the seventh segment treatment and 150 ppm NAA immersion concentration had the highest average number of root branches, namely 9.67 strands. Exogenous auxin resulted in the number of root branches growing on the first root. The growth of root branches is affected by the length of the first root. The first roots grow well, and then the root branches have room to develop (Alarcon et al. 2019).

Research on the growth of corn root branches conducted by Alarcon et al. (2019) showed that applying NAA doses of 0.01µM and 0.05µM significantly increased the average number of root branches. The effect of stimulating root branch growth is lost when high doses are applied. This is not the case with vanilla root. NAA immersion at concentrations of 100 ppm and 150 ppm still showed the growth of root branches. It is suspected that there is a raphide needle structure in the cortex of the vanilla root. The presence of raphide needles causes cells to produce clear, homogeneous mucus. The mucus has a role in protecting the protoplasm from raphide spiky needles. It also functions to regulate osmotic pressure. The roots do not lose water when there is a high solution concentration (Smith 1923; Seker et al. 2016).

Long branch root

Root branch length was measured on the 90th day after planting the cuttings. The measured root branches are the root branches that appear on the first vanilla root. Root branches that grow are measured using a measuring tape. The results of ANOVA showed that variations in the concentration of NAA hormone and the age of vanilla cuttings, either independently or in combination, had no significant effect on the length of the root branches.

Root branches generally originate from the pericycle of the vascular cylinder. If the first root has developed well, the emergence of root branches will develop well, too. Based on Table 5, soaking cuttings with NAA hormone did not show a significant difference in the length of the vanilla root branches. NAA concentration of 150 ppm produced the best average root branch length of 3.12 cm. This shows that auxin can encourage root elongation through cell division.

Table 6. The average value of vanilla shoot height (cm) on different segments 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	8.90±6.03^{abc}	5.56±6.49^{abc}	4.03±6.05^{bc}	15.53±14.09^{ab}	8.50±8.85
R5*	18.00±5.89^{a}	11.23±1.76^{abc}	1.10±1.15^{c}	10.16±9.22^{abc}	10.12±7.87
R7*	10.96±5.08^{abc}	0.00±0.00^{c}	0.43±0.75^{c}	6.50±8.04^{abc}	4.47±6.25
Average	12.62±6.42^{x}	5.60±5.91^{yz}	1.85±3.52^{z}	10.73±10.12^{xy}	7.70±7.88

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

Table 7. The average value of the number of vanilla leaves on different segments of age 90 days after planting after soaking with NAA

Cutting Segment	Concentration of NAA				Average
	0 ppm	50 ppm	100 ppm	150 ppm	
R3*	5.67±0.57^{ab}	1.33±2.30^{bc}	2.00±3.46^{abc}	4.67±4.16^{ab}	3.42±3.14
R5*	6.33±1.15^{a}	4.67±0.57^{ab}	0.00±0.00^{c}	3.33±2.87^{abc}	3.58±2.77
R7*	4.67±1.15^{ab}	0.00±0.00^{c}	0.00±0.00^{c}	2.33±4.04^{abc}	1.75±2.70
Average	5.56±1.13	2.00±2.39	0.67±2.00	3.44±3.39	2.92±2.92

Note: Means with the same letter along columns are not significantly different based on the DMRT test at a 95% confidence level. *R3: Third segment, R5: Fifth segment, R7: Seventh segment

The addition of exogenous auxin can stimulate existing endogenous auxin for cell division. In this study, combining the fifth node of vanilla cuttings and 0 ppm NAA concentration resulted in the best average root branch length of 3.96 cm. This is presumably because the fifth segment of vanilla cuttings has high meristem activity, so the endogenous auxin is still sufficient for cell division. If endogenous auxin is sufficient, plant cells do not need exogenous auxin (Tamba et al. 2019).

High shoots

New vanilla shoots start growing from the cutting nodes. The height of the growing vanilla shoots was measured on the 90th day. Shoots are measured from the base of the emergence of shoots to the tip using a measuring tape. ANOVA results show that the age of vanilla cuttings has no significant effect on shoot height, but variations in the concentration of NAA hormone independently and the combination of the two have a significant effect on shoot height.

The growth of shoots is an organogenesis carried out by meristem cells. The response is dependent on the phase of the G1 phase of the cell cycle. The formation of shoots requires the hormones auxin and cytokinins (Tyas et al. 2016). Table 6 shows that soaking cuttings with different NAA hormones significantly increased the height of vanilla shoots. Although auxin increased the height of vanilla shoots, optimal results were obtained by treating NAA at a concentration of 0 ppm, namely 12.62 cm. Dinarti et al. (2010) stated that if the concentration of cytokinins is higher than auxin, shoots are formed faster.

Number of leaves

Vanilla leaves are located alternately on each stem node. The number of leaves was counted on the 90th day after planting. The leaves that are counted are leaves that have opened or rolled. The results of ANOVA showed that the age of the vanilla cuttings did not significantly affect the number of leaves, but variations in the concentration of the NAA hormone independently and the combination of the two had a significant effect on the number of leaves.

Good root growth causes more nutrients to be absorbed, and it can increase plant growth. Good plant growth causes leaf growth so that photosynthesis is more optimal. The results of photosynthesis are used by plants for growth and development (Arat et al. 2021). Based on Table 7, soaking cuttings with different NAA hormones significantly increased the number of vanilla leaves. The highest number of leaves was shown in soaking NAA cuttings with a concentration of 0 ppm, namely 5.56 leaves. Wibowo et al. (2023) on vanilla plants stated that the highest number of leaves was shown in the treatment of natural auxin (Indole Acetic Acid) compared to synthetic auxin (Indole Butyric Acid) with an average value of 12 leaves. The hormone cytokinin influences the growth of shoots and leaves, while auxin inhibits shoots at high concentrations (Wulandari and Darwati 2015). In this study, it was suspected that plant tissue already contained sufficient cytokinins to help leaf growth.

In conclusion, treatment of varying concentrations of the NAA hormone increased the first root length, shoot height, number of leaves, and accelerated root emergence on each tested cutting segment. The higher the NAA concentration, the average root length, shoot height, and the number of leaves, also increased the appearance of the first roots of vanilla cuttings on each tested cutting, faster. Treatment of variations in cuttings increased the length of the first root and accelerated the appearance of roots on each segment of the cuttings tested. Medium age of stem cuttings increased the average value of first root length, while older stem cuttings accelerated the emergence of first roots of vanilla stem cuttings. The combination of treatments with variations in NAA concentrations and the age of vanilla cuttings increased first root length, shoot

height, number of leaves, and accelerated root emergence on each tested cutting segment. The most optimal length of the first root was obtained by combining the treatment of the fifth internode with an NAA concentration of 150 ppm. The optimal shoot height and number of leaves were obtained by combining the fifth node treatment with an NAA concentration of 0 ppm. The most optimal time for roots to appear was obtained by combining the treatment of the seventh segment with an NAA concentration of 150 ppm.

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Allelopathic effects of leachates of African peach (*Nauclea diderrichii*) and white afara (*Terminalia superba*) on germination and growth of maize

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Abstract. *Onefeli AO, Oluranti OI, Uthman KT, Aderibigbe AA, Mustapha MB. 2024. Allelopathic effects of leachates of African peach (*Nauclea diderrichii*) and white afara (*Terminalia superba*) on germination and growth of maize. Cell Biol Dev 7: 22-27.* This study was done to identify the allelopathic interference and nature of *Nauclea diderrichii* (De Wild.) Merr. and *Terminalia superba* Engl. & Diels on seed germination, the Seedling Vigour Index (SVI), and the growth of the seed of maize (*Zea mays* L.), to determine the best selection of tree species for agroforestry. The study was done at the University of Ibadan, Ibadan, Nigeria, both in the laboratory and on an agroforestry site. The freshly dried leaves of *N. diderrichii* and *T. superba* were collected and ground into powder to make leachate. The 3%, 6%, and 12% treatment leachate were made by soaking the powder for 24 hours, and the maize seeds were watered with the leachate. Statistical analysis was done with the data collected from the growth parameters for the two species using ANOVA, T-test, and Correlation. The *N. diderrichii* showed a slightly more effect on the maize than *T. superba*; the inhibitory effect of *N. diderrichii* was high with the increase in concentration of 3% (1.52), 6% (0.35), and 12% (0.81)) treatments, comparing mean values with the control. The two species have an inhibitory effect on most of the growth parameters. It was evident that *N. diderrichii* has more inhibitory effect on maize at high concentrations than *T. superba*. Seedling shoot dry weight had the strongest correlation with the SVI among the growth parameters. The result shows that *T. superba* is more suitable for agroforestry systems than *N. diderrichii*.

Keywords: Agroforestry, allelopathy, growth parameters, inhibitory effect, *Nauclea diderrichii*, *Terminalia superba*

INTRODUCTION

Agroforestry is one of the aspects of forestry that accommodates various specialized knowledge for the development of sustainable rural production. Agroforestry is a recognized land use method, where trees provide environmental services and products. It is widely known that agroforestry activities have environmental benefits. However, the decrease in agricultural crop yield in agroforestry practices has recently been traced to the allelopathic effect of the trees on the crops (Kunzelmann 2021). Kumar and Guru (2014) agree that agroforestry has many benefits for crops, as well as promoting a good livelihood for rural communities. However, these benefits have been threatened due to some negative factors through chemicals released by the trees used for agroforestry practices.

Nauclea diderrichii (De Wild.) Merr. is an indigenous tree species that is used in agroforestry practices to promote land use and improve the yield of farmers in Nigeria. This species is known as an evergreen species, and belongs to the family Rubiaceae. It is mostly found in the Tropical Rainforest zone (Abdulrahman and Adamu 2019). The leaf of *N. diderrichii* is rich in phytochemicals such as tannins, hexane, ethyl acetate, methanol, alkaloids, glycosides, phenolic compounds, phytosterols, saponins etc. that justify the tree as a traditional medicinal tree (Mustofa et al. 2000; Neuwinger 2000; Obute and Ekiye 2008; Addo-Danso et al. 2012). *Terminalia superba* Engl. & Diels belongs to the families of Combretaceae. It is also an indigenous tree species that is used in agroforestry practices to promote land use and improve the farmers yield (Onefeli and Stanys, 2019; Onefeli et al. 2019). Much research has not been done on the effect of *T. superba* allelochemicals on crops like other species. However, indigenous tree species have been found to contain some phytochemicals that can either stimulate or inhibit the growth of crops (Onefeli et al. 2021). This allelopathic chemical could be further investigated to know the differential effects of *T. superba* on various crops and probably if the allelopathic nature of the tree species can be used in weed management to enhance the productivity of the agroforestry systems further.

Allelopathy is known as the interference process in which chemical substances released from living or dead plants, can either have a positive or negative effect on the germination or growth of plants associated with it (Lalmuanpuii 2012). Allelopathy is said to have an involvement in many natural and manipulated ecosystems, which can have an impact on the transformation or evolution of various plant communities, plant failure or exotic plant invasion (Ridenour and Callaway 2001; Inderjit and Nilsen 2003). The chemicals that cause allelopathic interference are present in many plants and in

various organs or parts of the plant, which include the bark, fruits, leaves and exudate (Sazada et al. 2009). Allelopathic chemicals are known as unwanted byproducts, products of metabolic reactions in plants or secondary plant products (Turk and Tawaha 2003; Yokotani et al. 2003; Iqbal et al. 2006). Victor et al. (2006) reported that the chemicals can be transferred, transported, or diffused through the soil and can be metabolized, transformed, formed into compounds, or attached to organic matter, which may have effect on the seeds. The span or time of field autotoxicity differs with the geographic location and with the environment. The rain leachate extract is a more ecological method to check for the allelopathic potentials of tree species because it represents the natural field conditions better than the other methods (Lorenzo et al. 2011; Song et al. 2018). The symptoms of allelopathy interactions should be specific, because these interactions are essential to knowing how to eliminate the causes of interference in chromatographic studies (Oyun 2006).

Garrity (2012) reported that in the future, land use may face pressing challenges across countries; however, these challenges can be managed well with the proper knowledge of agroforestry systems. One of the main setbacks that can be associated with allelopathic research is the single usage (application) of leachate in most of the culture studies or segmented treatments, this culture excludes the biotic and abiotic factors that have the potential to interfere with the allelopathic reactions in the field (Ens et al. 2009). There are several environmental factors (such as soil, light, temperature, and precipitation), the availability of water resources, understory vegetation, and nutrients that are essential to determining or estimating the allelopathy interference as this relies on them (Catherine et al. 2006; Ahmed et al. 2008; Catherine et al. 2008; Ahmed et al. 2017). It is important that we check or estimate the compatibility of allelopathic trees and crops before choosing the crops for an agroforestry system (Parvin et al. 2011).

Therefore, this study was carried out to evaluate and estimate the interferences of leachate from *N. diderrichii* and *T. superba* on seed germination, growth, and seedling vigor index of *Zea mays* L. with a view of determining the compatibility of African peach (*N. diderrichii* and white afara (*T. superba*) on maize, as well as the morphological traits for allelopathic interference assessment of tree species on the maize seedlings.

MATERIALS AND METHODS

Study area

The study was conducted at the David Okali Laboratory of the Department of Forest Production and Products (Figure 2), and the agroforestry site of the University of Ibadan, Ibadan, Nigeria (Figure 1). The University of Ibadan is located between the latitude of $7^0$28`N and longitude of $3^0$52`E at an altitude of 277m. The land is characterized by dry and wet seasons and tropical forests. From April to October is the wet season in the country, and the other season (dry) runs from November to March, likewise, the temperature ranges from 22^0C to 31^0C (Onefeli and Agwu 2015).

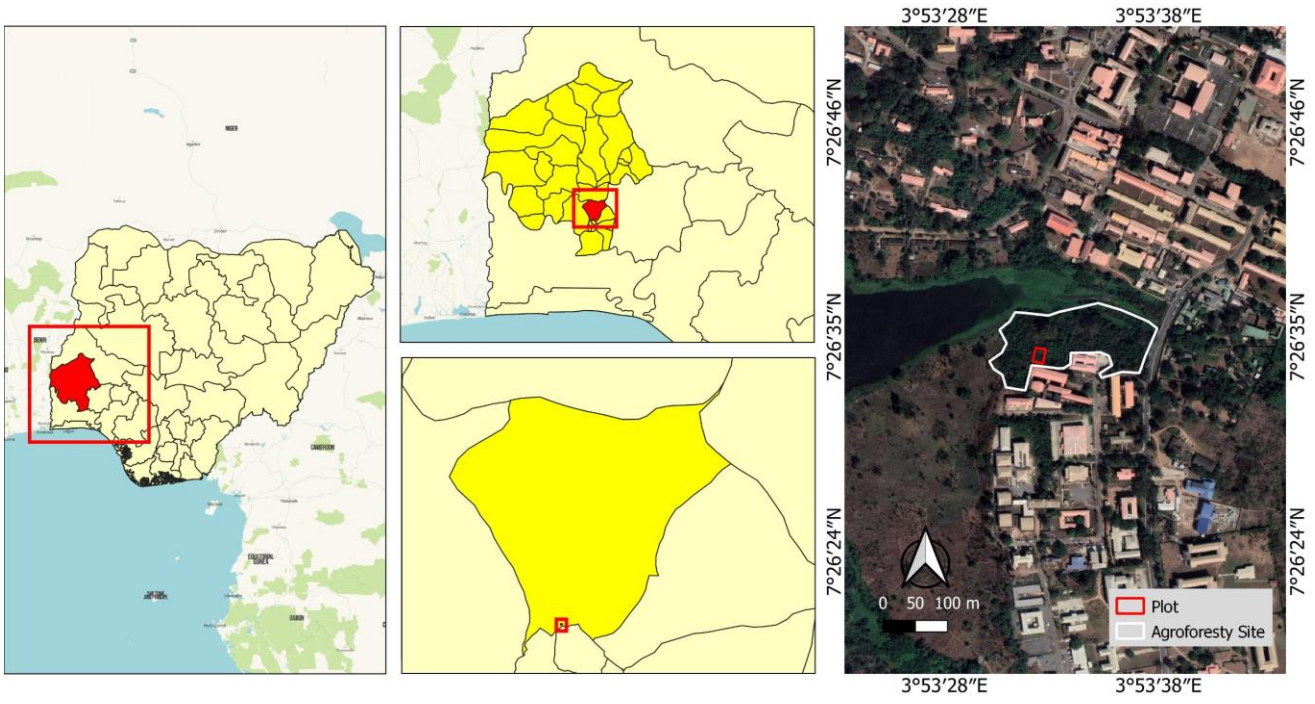

Figure 1. Map of the agroforestry plot, University of Ibadan, Nigeria

Experiment procedure

The sterilized grains of *Z. mays* were collected from the Department of Forest Production and Products at the University of Ibadan, Oyo State. The collected sterilized grains were to be used for planting on the agroforestry plot and also for an allelopathic experiment to determine the impact of the trees species leachates on growth variables and the germination of maize. There was a total of 18 replicates for the experiment, with 6 maize grains planted in each polythene pot using a loamy topsoil as the planting media.

A plot of 5 m by 10 m was allotted for the practical activity of the agroforestry planting. Four ridges were made on the plot with a depth of 0.3m, a width of 0.6m, and an enspacement of 0.6 m. While the dimension of each ridge was also 0.6 m by 10 m. The freshly dried leaf samples of *N. diderrichii* and *T. superba* were collected from the leaf litters in front of the Department of Forest Production and Products and the freshly dried leaves that were picked were all sun-dried, ground into powder by mechanical grinder. The 12% leachate of *N. diderrichii* and *T. superba* was obtained by mixing 30 g of the ground fine powder into 960 mL of distilled water. The soaked ground powder of the prepared leaves was shaken thoroughly and then kept for 24 hours. Also, the 6% leachate of *N. diderrichii* and *T. superba* was obtained by mixing 30 g of the ground fine powder into 480 mL of distilled water, and 30 g into 240 mL, then shaking the mixture well and leaving it for 24 hours.

The leachate was sieved out of the water using a mesh sieve after 24 hours, and 100 mL of the leachate water was used to water the 6 planted maize grains in the treatment polythene pots, while the distilled water was used for the control experiment for 7 days starting from day 1 of planting. The polythene pots of the treatments were arranged in a Completely Randomized Design (CRD) on the agroforestry plot. The day of first germination of the seed was after four days of planting, and the final germination of the seeds was obtained before the eleventh day of planting, according to procedure by Oyun (2006). The interference of the leachates on seed germination, shoot growth, and root growth was determined and evaluated by harvesting three plants randomly from the treatment polythene pots after 21 days of planting. The data relating to germination, yield and growth were analyzed. The mean germination time of the seeds was calculated using:

$$\sum = \frac{\text{nxd}}{N}$$

Where, n: no of seeds that germinated in day 1, d: number of days, N: Total number of seeds that germinated at the end of the experiment.

After 21 days, the seedlings growth/yield was evaluated by harvesting randomly, three plants from the treatments were collected and various growth variables were assessed Number of leaves was determined by visual counting. The dry root weight, dry shoot weight fresh root weight, and fresh shoot weight were measured using an electronic weighing balance while the fresh shoot length and fresh root length weight were measured by a ruler. The dry plants were dried in an oven for more than 48 hours to get a constant dry weight to be recorded. The SVI was evaluated as Seedling Vigor Index = dry weight per seedling/MGT X 100, and the data collected were subjected to Analysis of variance (ANOVA) and T-Test analysis using Microsoft Excel and SPSS. The Correlation co-efficient of the growth parameters were also evaluated, to know the relationship between SVI and the other growth parameters for all the treatments.

RESULTS AND DISCUSSION

Table 1 shows the interaction of the phytochemicals through the mean values of the treatment variables from the ANOVA table of *N. diderrichii* leachate on *Z. mays*. The table deduced that the leachate treatment significantly ($p<0.05$) influenced – Mean Germination Percentage (MGP) and Root Fresh Weight (RFW) of the maize, unlike the other variables that are not significantly ($p>0.05$) affected by the leachate concentrations. From the table, variables DFSG, RFW, NL, RL, RDW and SVI have their 3% treatment mean values greater than the control mean values, while the DFSG and NL variables have their 6% treatment mean values greater than the control mean values, and the variables RL, RFW, SDW, RDW, and SVI have their 12% mean values greater than the control mean values. MGT, MGP, SL, and SFW are the only variables with control means greater than the mean of the treatments.

Table 2 shows the mean values of the treatment variables from the ANOVA table of *T. superba* leachate on *Z. mays*. The table deduces that all the maize growth parameters present in the table are not significantly ($p<0.05$) influenced by the variation in *T. superba*'s leachate concentration except for the Root Fresh Weight (RFW), unlike the ANOVA table for species 1: *N. diderrichii*. From the table, variables SL, RL, RDW, RFW, SDW and SVI have their 3% treatment mean values greater than the control mean values, while the DFSG, NL, SL, RL, SFW, SDW and SVI variables have their 6% treatment mean values greater than the control mean values, and the variables DFSG, NL, RL, RDW, SFW, RFW, SDW, SL, and SVI have their 12% mean values greater than the control mean values. MGT and MGP are the only variables with control means greater than the mean of the treatments.

The p values of values in Table 3 below are not significant, showing no relationship between the two species, *N. diderrichii* and *T. superba* in tems of their leachate effect on the germination and growth of *Zea mays*. However, in terms MGT, DFSG, SL, RL, SFW, RFW, SDW, and RDW, *T. superba* had higher average values of 5.19, 3.87, 57.74, 23.87, 14.43, 3.03, 1.91 and 0.83 respectively as compared to the *N. diderrichii* with 4.91, 3.84, 57.10, 21.78, 11.88, 2.62, 1.64 and 0.70 average respectively. Mean Germination Time, DFSG: Day of First Seed Germination, MGP: Mean Germination Percentage, NL: Number of Leaves, SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry

Weight, SVI: Seedling Vigour Index.

From Table 4, it can be deduced that all the growth parameters significantly correlated with the Seedling Vigour Index (SVI). The shoot dry weight appeared to have the highest value closer to 1 (0.57), followed by the root dry weight (0.47), and then the Shoot Fresh Weight (0.27).

Table 1. Mean values of the variables from the ANOVA table, showing the effect of *Nauclea diderrichii* treatments on *Zea mays*

ND Treatment Conc.	MGT	DFSG	MGP (%)	NL	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	SVI
Control	5.07	3.76	95.75^{a}	6.25	60.46	21.93	13.39	2.29^{b}	1.59	0.55	52.08
3%	4.95	3.88	89.61^{ab}	6.96	46.31	25.38	7.35	4.77^{a}	1.28	1.52	52.93
6%	4.81	3.8	88.93^{ab}	6.42	54.31	17.89	9.47	1.27^{b}	1.34	0.35	42.69
12%	4.56	4.1	82.6^{b}	6.04	58.62	22.28	13.39	3.16^{ab}	2.33	0.81	71.88
P value	0.460^{ns}	0.460^{ns}	0.007*	0.489^{ns}	0.104^{ns}	0.469^{ns}	0.316^{ns}	0.042*	0.073^{ns}	0.121^{ns}	0.530^{ns}

Note: ns: not significant at 5% probability level, *: significant at 5% probability level.

Table 2. Mean values of the variables from the ANOVA table, showing the effect of *Terminalia superba* treatments on *Zea mays*

TS Treatment Conc.	MGT	DFSG	MGP (%)	NL	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	SVI
Control	5.3	3.85	93.73	6.27	55.21	22.52	13.7	2.55^{b}	1.66	0.73	38.68
3%	4.93	3.9	86.43	6.02	58.78	25.01	12.6	2.59^{b}	2.58	1.02	62.72
6%	5.08	3.89	83.33	6.44	61.42	22.71	15.03	1.82^{b}	1.98	0.38	40.3
12%	5.3	3.88	91.41	6.64	60.81	28.29	18.51	6.54^{a}	1.81	1.45	39.27
P value	0.760^{ns}	0.998^{ns}	0.213^{ns}	0.830^{ns}	0.799^{ns}	0.601^{ns}	0.801	0.026*	0.269^{ns}	0.273^{ns}	0.257^{ns}

Note: ns: not significant at 5% probability level, *: significant at 5% probability level.

Table 3. The T-Test table of the mean values with the P value of the parameters showing the relationship between the two tree species

	Species	Mean	P value	Std. Deviation	Std. Error Mean
MGT	ND	4.91	0.17^{ns}	1.15	0.15
	TS	5.19		1.00	0.14
DFSG	ND	3.84	0.84^{ns}	0.59	0.08
	TS	3.87		0.75	0.10
MGP	ND	91.23	0.70^{ns}	11.46	1.51
	TS	90.30		14.23	1.94
NL	ND	6.34	0.90^{ns}	1.33	0.18
	TS	6.31		1.44	0.20
SL	ND	57.10	0.84^{ns}	14.94	1.961
	TS	57.74		19.07	2.60
RL	ND	21.78	0.29^{ns}	9.98	1.31
	TS	23.87		10.95	1.49
SFW	ND	11.88	0.24^{ns}	9.36	1.23
	TS	14.43		13.29	1.81
RFW	ND	2.62	0.51^{ns}	2.78	0.36
	TS	3.03		3.68	0.50
SDW	ND	1.64	0.22^{ns}	1.03	0.13
	TS	1.91		1.25	0.17
RDW	ND	0.70	0.54^{ns}	1.13	0.15
	TS	0.83		1.18	0.16
SVI	ND	54.33	0.16^{ns}	46.53	6.11
	TS	43.49		33.41	4.55

Note:ns: not significant, ND: Species 1- *Nauclea diderrichii*, TS: Species 2 - *Terminalia superba*, MGT: Mean Germination Time, DFSG: Day of First Seed Germination, MGP: Mean Germination Percentage, NL: Number of Leaves, SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, SVI: Seedling Vigour Index.

Table 4. The correction coefficient (r) showing the association between the SVI and the growth parameters

Seedlings growth parameters	Correction Coefficient (r)	p-value
Shoot length (cm)	0.205	0.300*
Root length (cm)	0.196	0.390*
Shoot fresh weight (g)	0.268	0.004*
Root fresh weight (g)	0.249	0.008*
Shoot dry weight (g)	0.571	0.000*
Root dry weight (g)	0.468	0.000*

Note: *: significant at 5% probability level

Discussion

From Tables 1 and 2, the allelopathic effects of *N. diderrichii* and *T. superba* leachates on the growth of maize, and the germination were shown according to the mean values of the growth parameters. The data showed that the two tree species leachates inhibited the growth of maize at different concentration.

At ($P<0.05$), the total days taken for the first germination of seed were delayed due to the reaction of the leachates of *N. diderrichii* and *T. superba* significantly higher with 12% concentration in *N. diderrichii* and 3% concentration in *T. superba*. Also, the germination percentage of the seeds decreased with increased concentration for *N. diderrichii* while there wasa significant decrease in germination percentage with increase in concentration except for 12% for *T. superba* compare to the control.

Also, there was a slight significant difference in the leachate effect of *N. diderrichii* only on the seedling root dry weight among the growth, and there was no significant difference among all the growth parameters . Therefore, it can beinferred that *N. diderrichii* has a more negative effect on the germination and growth of maize than *T. superba*.

The result of this study is in line with those reported by Abdulrahman and Adamu (2019), which estimated that *N. diderrichii* has a high potential negative effects on the germination of agricultural crops due to the presence of many phytochemicals in the plant that make it useful for medicinal purposes. From these findings, it was concluded that the inhibitory effect of *N. diderrichii* on the growth parameters on the growth parameters is more significant at a concentration of 12% than the germination as compared with *T. superba*. That was more pronounced in germination than the growth parameters.

The negative interference (inhibitory) of the leachates on *Z. mays* may be due to the interference in the physiological process of maize that leads to growth by the effect of the leachate. However, this was not directly evaluated, the inhibited effect of the leachates on the germination may be due to some reactions that might have occurred that affected the water absorption of the seed before the seed germination was initiated. Likewise, the seedling growth might have been affected by the leachates through the hindrance in the absorption of water and nutrients from the soil, which is essential for the growth of the plant, depending on the concentration of the leachates.

The two tree species, *N. diderrichii* and *T. superba* 3 are not significantly different according to the findings obtained from the current study. The species has a negative effect on germination and the growth of *Z. mays*. The seedling growth parameters showed positive correlation, with the Seedling Vigour Index (SVI). And the shoot dry weight happened to have exhibited the highest trait for allelopathic interference evaluation of maize.

In conclusion, the study provided essential information about the interference of these species allelopathic nature, which is highly important in understanding their potentials and an insight into the best species suitable for agroforestry practices. According to this study, *N. diderrichii* and *T. superba* have an inhibiting effect on the germination and growth of *Z. mays* at different concentrations.

Nevertheless, we have found out that the allelopathic nature of *N. diderrichii* is more effective in inhibiting maize than *T. superba* (*T. superba* stimulates growth more than the other species). This suggests that *T. superba* can be proposed for agroforestry using maize as the agricultural crop. This study revealed that more research should be done on the allelopathic nature or effect of *T. superba* on other agricultural crops aside from maize to determine its potential to inhibit the growth of other plants and to have a better understanding of how to use the tree species in agroforestry systems.

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Morphological and foliar epidermal studies of fronds of *Platycerium bifurcatum* and *P. superbum* in Rivers State, Nigeria

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Abstract. *Ajuru MG, Joshua JA, Chikere LC, Ibiye A. 2024. Morphological and foliar epidermal studies of fronds of* Platycerium bifurcatum *and* P. superbum *in Rivers State, Nigeria. Cell Biol Dev 8: 28-35.* This work investigated the morphological and foliar epidermal characters of the sterile and fertile fronds of *Platycerium bifurcatum* (Cav.) C. Chr. and *Platycerium superbum* de Jonch. & Hennipman in Rivers State University, Nigeria. The two species of ferns, *P. bifurcatum* and *P. superbum*, belong to the family Polypodiaceae. They are unique epiphytes and have intrigued botanists, horticulturists, and plant enthusiasts; investigating plants' morphological and foliar anatomical structures has been crucial for plant classification. Qualitative and quantitative analytical methods were used for morphological study, while light microscopy was used for foliar anatomical study. Results showed that *P. bifurcatum* is a perennial, evergreen, broadly terrestrial, epiphytic fern. The rhizome is clingy, brown, or copper-colored, short, coarse, hidden, and enclosed in more than two peltate scales, and the frond is dimorphic (sterile and fertile), while *P. superbum* is a perennial, broad, and multi-branching terrestrial and epiphytic fern. The rhizome is a short-creeping, hidden, brown, or copper-colored structure, covered with chaffy, lanceolate scales and embedded in a mass of fronds and roots, and the frond is dimorphic. Epidermal cell shapes were found to be irregular, with thick and straight to wavy anticlinal walls in the fertile fronds, and irregular, with wavy to sinuous, thick anticlinal walls in the sterile fronds. The stomata types observed were diacytic, amphidiacytic, and anomocytic, except for the abaxial and adaxial surfaces of the sterile fronds of *P. bifurcatum*, which were paracytic and hemiparasitic. This study focuses on recognizing morpho-anatomical characters of the fronds of these species, which will help in taxonomy, species identification, future comparisons, and quality control of the botanicals.

Keywords: Dimorphic frond, epiphytic ferns, *Platycerium*, Polypodiaceae, stomata

INTRODUCTION

In tropical and subtropical regions of the world, *Platycerium* ferns primarily inhabit the canopies of trees, mostly growing as epiphytes. They thrive very well in the restricted space of tree branches, due to their unique growth form, and this helps them make efficient use of available light and nutrients. The lifestyle of this epiphytes play a vital role in forest ecosystems, and this contributes to canopy structure, thereby providing microhabitats for various organisms, and it enables them to participate in nutrient cycling (Koller 2005).

Horticulturists and gardeners are awed and captivated by the species of *Platycerium* because of their aesthetic beauty and ability to adapt to any environmental conditions during cultivation (Koller 2005). Several species of *Platycerium*, including *P. bifurcatum* and *P. superbum*, are popularly used for ornamental purposes in gardens; they are also used in indoor spaces and as greenhouse plants. They are seen as unique plants due to their intriguing frond shapes, addition to landscape environments, and growth habits that beautify the environment (Hennion et al. 2019). *Platycerium* serves as microhabitats for different organisms and helps recycle nutrients (Koller 2005).

Platycerium bifurcatum (Cav.) C. Chr., belonging to the family Polypodiaceae, is commonly called Elkhorn fern. It is an attractive and distinctive epiphyte, and it is known and recognized for its unique frond appearance. The fertile fronds resemble the antlers of a stag, while the sterile fronds form a shield-like basal structure. The shield-like structure of the sterile frond protects and supports the fern, while the antler-like structure of the fertile fronds is important for reproduction and spore production (Hennion et al. 2019). The *P. bifurcatum* is economically important for horticultural and ornamental purposes. The uniqueness of its and its adaptability to cultivation make it a very desirable ornamental houseplant. It is usually placed on wooden plaques or bark to facilitate its natural epiphytic growth habit, giving a touch of tropical elegance to indoor greenery and gardens (Hennion et al. 2019).

Platycerium superbum de Jonch. & Hennipman, commonly called Staghorn, is an epiphytic fern or sometimes lithophytic. The fern resembles a tangle of antlers, but on closer look, it possesses an impressive 'nest' frond, 1m wide when it matures. Falling leaves and insects are easily collected inside the 'nest' frond, serving as a valuable source of potassium and calcium. These are nutrients required for the production of their large fronds, and this frond enables the fern to attach itself to the host tree. The 'nest' frond wraps around the rhizomes and short roots to protect them. It is clasped using its root-like features onto the furrows in the bark (Kreier and Schneider 2006). The fern produces a mass of spores during the summer, which coupled with its general size, is a

distinguishing feature for the species (Hennipman and Roos 1982).

Young leaves of *P. superbum* are used to treat ulcers in Nigeria (Pemberton 2003). The leaf extract is also taken orally, two months after conception, to prevent miscarriage in women (Flora and Ubah 2006). It is also used to treat the following ailments: edema, coughs, and hypertension (Okoli et al. 2007). It has been reported that polysaccharides have been isolated and characterized in this species (Omeje et al. 2007). Morphological and anatomical plant features are seen as valuable tools for classification, and this has enabled plant scientists to organize and categorize plants into distinct groups based on shared characteristics (Kissinger 2015).

Moreover, little attention has been paid to species of *Platycerium*, including *P. bifurcatum* and *P. superbum*, especially regarding morphological and anatomical studies. Also, owing to the importance of morpho-anatomical characters as an additional tool to taxonomic and phylogenetic studies, the present study aimed at investigating the morphological and foliar anatomical characters of the leaves of *P. bifurcatum* and *P. superbum* in Rivers State University, Nigeria.

MATERIALS AND METHODS

Study area

The study area was Rivers State University (RSU) campus within Port Harcourt metropolis, Rivers State, Nigeria. Port Harcourt is an industrialized cosmopolitan city located in the heart of the Niger Delta. The study area, RSU, lies South-South of the Niger Delta within latitudes 4°31'-4°40' N and longitudes 70°'-7°10'E (Figure 1). It has an elevation of about 10-15 m above sea level (Ubong et al. 2015).

Sample collection, identification, and preparation

The research study took place from July to September 2023. The samples of the two fern species were freshly collected from Pine trees found in Rivers State University, Nkpolu-Oroworukwo, Rivers State (Table 1). They were taken to the Department of Plant Science and Biotechnology Laboratory in polythene bags with tags. They were identified and authenticated by Dr. M. G. Ajuru, a Plant Taxonomist in the Department. The samples were rinsed in distilled water several times and used for various taxonomic studies.

Table 1. Sources of collection of plant materials used for the study

Plant Samples	Accession Number	Date of Collection	Location of Collection (Lat 4°31'-4°40' N, Lon 70°'-7°10' E)
Platycerium bifurcatum	RSUPb015	7-8-2023, 8-8-2023, 9-8-2023	EF, FSCP, RSC
Platycerium superbum	RSUPb016	7-8-2023, 8-8-2023, 9-8-2023	RMG, RCC, RRA

Note: RCC: RSU Catholic Church, RMG: RSU Main Gate, RRA: RSU Roundabout, EF: Engineering Faculty, FSCP: Faculty of Science Carpark, RSC: RSU Staff Club

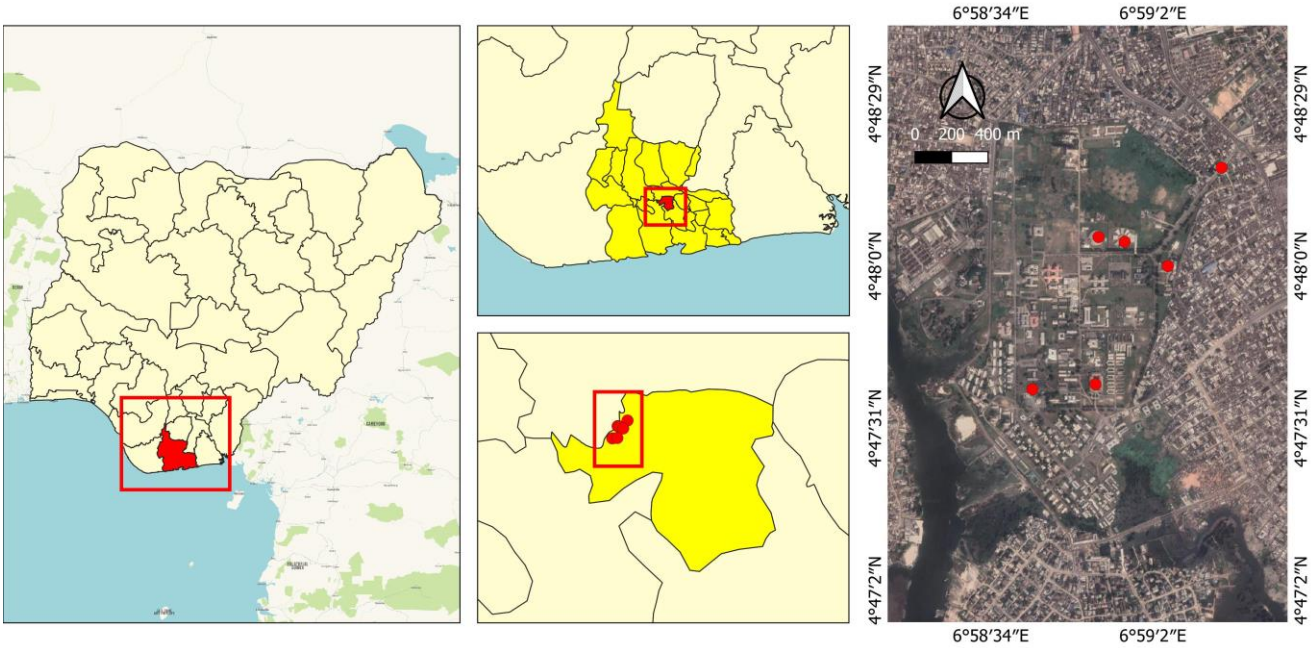

Figure 1. The map of Rivers State University (RSU), Nigeria shows the sampling stations

Procedures

Morphological study

The morphological features of the selected fern species, were recorded on matured living plant samples. References were also made to Cryptogrammic Gallery: A Reference Field Guide (Edwin-Wosu 2019). Quantitative morphological studies included measuring the frond length, width, petiole length, and plant height using a meter rule and measuring tape. Qualitative morphological plant features studied included the frond nature, color, texture, shape, surface, presence or absence of spore, plant habit, etc.

Foliar epidermal study

Fresh matured fronds were prepared according to the simplified method described by Okoli (1992). The fresh fronds were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues and make it easy to scrap. The fronds were placed on a flat surface, and the adaxial surface was scraped off gently with a razor blade until the abaxial surface was reached. Equally, the abaxial surface was scraped off to reveal the adaxial surface. The transparent epidermal peels were soaked in distilled water to rehydrate the cells, which were stained with 1% safranin for 3 minutes and rinsed again in distilled water. The specimens were mounted with 3 drops of glycerine and a cover slip placed correctly. Slides of both abaxial and adaxial surfaces of fertile and sterile fronds were prepared. These were examined using a light microscope, and photographs were taken.

The qualitative characteristics of the foliar epidermis were observed and taken with a light microscope. The number of epidermal cells and stomatal per field view were noted and recorded.

The Stomatal Index for both species was calculated using the formula below:

The calculation for the Stomatal Index (I)

$$I = \frac{S}{E+S} \times 100\%$$

Where:

I : Stomatal Index

S : Number of stomata per unit area

E : Number of epidermal cells plus the subsidiary cells in the unit area.

RESULTS AND DISCUSSION

Morphological features of *Platycerium bifurcatum*

The *P. bifurcatum*, commonly called staghorn or elkhorn fern, belongs to the family Polypodiaceae. It is a perennial, evergreen, broadly terrestrial, epiphytic fern growing up to 1 m tall and 90 cm wide (Figure 2). It grows on trees, trunks, and branches. The stem or rhizome is clingy, brown, copper-colored, short, coarse, hidden, and enclosed in more than two peltate scales. The roots are tufted solely for anchoring the plant to the tree trunk. The fronds are evergreen, gray or silver colored, leathery or velvety, pinnately compound, complex, oblong, lobed, and glabrous.

The frond is dimorphic in two rows: The sterile fronds are few, persistent, appressed to substrate, sessile, heart-shaped, shield-like, papery, and grow close or cover the root crown. The sterile frond is 34 cm long and 24 cm wide. The fertile fronds are deciduous, short-stipitate, erect to arching or pendant, dichotomously branched, antler-like, and covered with a grayish-white felt. The fertile frond is 50 cm long and 24 cm wide. The spores are borne in sori on the lower part of the frond at the tips of the fertile fronds, as shown in Figure 2 and Table 2.

Morphological features of *Platycerium superbum*

The *P. superbum*, commonly called giant staghorn fern and of the family Polypodiaceae, is a perennial, broad, multi-branching terrestrial and epiphytic fern, growing up to 1.1 m tall and 95 cm wide (Figure 3). It has short roots that are not prolific and solely for anchoring the fern to the host tree trunk. The rhizomes are short-creeping, hidden, brown, or copper-colored structures, covered with chaffy, lanceolate scales and embedded in a mass of fronds and roots. The fronds are bright greenish, evergreen, leathery, simple pinnate, rosettely arranged, wedge-shaped, lobed, and entire, glabrous, though sometimes covered with stellate hairs.

The frond is dimorphic in two rows: The nest frond or sterile frond is rounded or reniform, appressed to the substrate, and densely covered with fawn stellate hairs, irregularly divided into elongated dichotomous spreading lobes, measuring 34 cm long and 25 cm wide. The fertile or foliage fronds are paired, pendulous, up to 5 times forked, base broadly cuneate, measuring up to 60 cm long and 30 cm wide. The sporangia are in a single patch, up to 20-40 cm wide, occurring at the sinus of a first fork of foliage frond on the lower surface of the frond, as shown in Table 2 and Figure 3.

Foliar epidermal features of *Platycerium bifurcatum* and *P. superbum*.

Foliar epidermal characteristics of *P. bifurcatum* and *P. superbum* are summarized in Figures 4-7:

Foliar epidermal features of fertile fronds of *Platycerium bifurcatum*

Abaxial surface or lower epidermal features

The epidermal cell is irregular, with thick and straight to wavy anticlinal walls, 85-102 per field. The stomata are diacytic and anomocytic with elliptical guard cells. Trichomes were absent. There were numerous crystal sands. Stomata were 56-63 per field.

Adaxial surface or upper epidermal features

Epidermal cells are irregular or polygonal in shape with thick and straight to waxy anticlinal walls, 116-126 per field. There are crystal sands numerous on the epidermal cell. There are no trichomes but very few stomata, 12-16 per field. They are diacytic and anomocytic. The guard cells are elliptical, surrounded by subsidiary cells.

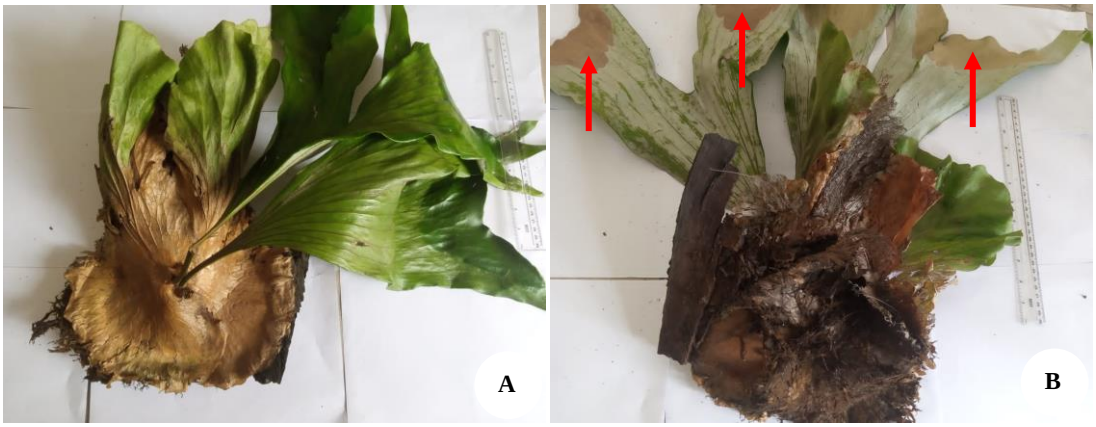

Figure 2. *Platycerium bifurcatum*: A. Habit, B. Sori (*arrow*)

Figure 3. *Platycerium superbum*: A. Habit, B. Sori (*arrow*)

Foliar epidermal features of sterile fronds of *Platycerium bifurcatum*

Abaxial surface or lower epidermal features

The epidermal cells are irregular, with wavy to sinuous, thick anticlinal walls. There are from 136 to 147 epidermal cells per field. The stomata are paracytic and hemiparasitic and ranging 121 to 134 per field. The guard cells are widely elliptic. There are no trichomes. There are numerous crystal sands present in the epidermal and subsidiary cells.

Adaxial surface or upper epidermal features

The epidermal cells are also irregular, with wavy to sinuous anticlinal walls that are thick. There are 111-122 epidermal cells per field, few stomata, and no trichomes. There are numerous crystals of sand in the epidermal cells. The stomatal type is paracytic and hemiparasitic, ranging between 52-67.

Foliar epidermal features of fertile fronds of *Platycerium superbum*

Abaxial surface or lower epidermal features

The epidermal cell is irregular, with thick, straight-to-wavy anticlinal walls. There are between 79-101 per field. The stomata were diacytic and amphidiacytic, with elliptical guard cells. Trichomes were present, stellate and non-glandular trichomes. Stomata were 75-84 per field with numerous crystal sands.

Adaxial surface or upper epidermal features

Epidermal cells are irregular, with thick and straight to wavy anticlinal walls. They are pentagonal, numerous, between 79-88 per field. There are no stomata and trichomes, with numerous crystal sands.

Foliar epidermal features of sterile fronds of *Platycerium superbum*

Abaxial surface or lower epidermal features

The epidermal cells were irregular, with thick, straight-to-wavy-to-sinuous anticlinal walls. They were hexagonal in shape, numerous, between 82-95 per field. The stomata were diacytic and amphidiacytic, with elliptical to oblong guard cells. Stomata were between 29-34 per field, with numerous crystal sand and no trichomes.

Adaxial surface or upper epidermal features

The epidermal cells were irregularly shaped, specifically pentagonal, with thick, straight-to-wavy anticlinal walls. The epidermal cells are between 76-84 per field. The stomata were diacytic and amphidiacytic, with elliptical to oblong guard cells. Stomata were between 39-48 per field with numerous crystal sands and no trichomes.

Table 2. Morphological features of *Platycerium bifurcatum* and *P. superbum*

Parameters	***Platycerium bifurcatum***	***P. superbum***
Plant lifespan	Perennial	Perennial
Habit/form	Cascading/Mounding	Broad and multi-branching
Habitat	Terrestrial	Terrestrial
Plant type	Epiphytic fern	Epiphytic fern
Root system	Tufted for anchoring	Short roots, not prolific
Plant height	1 m tall and 90cm wide	1.1m tall and 95cm wide
Climbing method	Clinging	Drooping
Stem/rhizomes color	Brown/Copper	Brown/copper
Texture	Coarse	Coarse
Stem bud scales	Enclosed in more than two scales.	Covered with chaffy lanceolate scales.
Frond nature	Evergreen broad-leaf plant	Greenish and evergreen
Frond colour	Gray/Silver	Bright green
Frond texture	Leathery and velvety	Leathery
Leaf type	Compound (Pinnate compound)	Simple pinnate
Frond arrangement	Complex	Complex (rosette)
Frond shape	Oblong	Wedge-shaped
Frond margin	Lobed	Lobed and entire
Frond surface	Glabrous	Glabrous, sometimes covered with stellate hairs
Frond length and width	Sterile frond = 34 cm long and 24 cm wide. Fertile frond = 50 cm long and 28 cm wide	Sterile frond = 34 cm long by 25 cm wide. Fertile frond = 60 cm long by 30 cm wide.
Frond appearance	Dimorphic in two rows 1. Sterile fronds, few persistent, appressed to the substrate, sessile, heart-shaped, shield-like, and papery. Grow close to the crown. 2. Fertile fronds are deciduous, short-stipitate, erect to arching or pendant, dichotomously branched (antler-like) covered with a grayish-white felt.	Dimorphic in two rows 1. Sterile or nest fronds rounded or reniform, appressed to the substrate, and densely covered with fawn stellate hairs, irregularly divided into elongated dichotomous spreading lobes. 2. Fertile or foliage fronds paired, pendulous, up to 5 times forked, base broadly cuneate.
Stipe length and width	11 cm long and 4.7 cm wide	15 cm long by 5.7 cm wide
spores		
Rhizomes	In sori on the lower part of the frond, at the tips of the fertile fronds Short, hidden, with peltate scales	Sporangia were in a single patch, 20-40 cm wide, occurring at the sinus of a first fork of foliage frond on the lower surface of the frond. Short-creeping, hidden, covered with chaffy, lanceolate scales, embedded in a mass of fronds and roots.

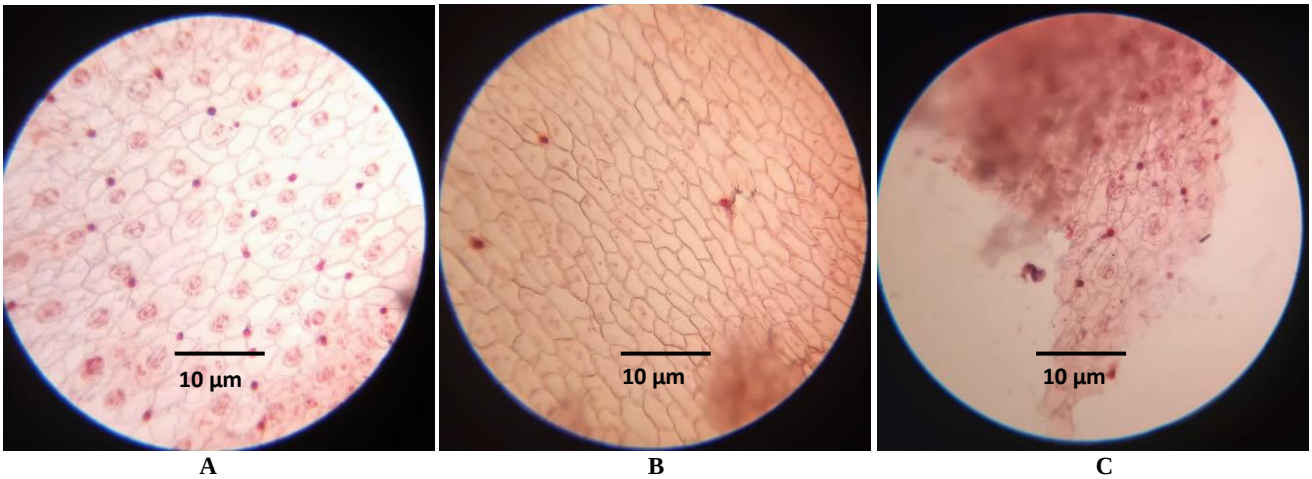

Figure 4. Foliar epidermal features of fertile frond of *Platycerium bifurcatum.* A. Abaxial Surface, B. Adaxial Surface without stomata, C. Adaxial Surface with stomata (x100)

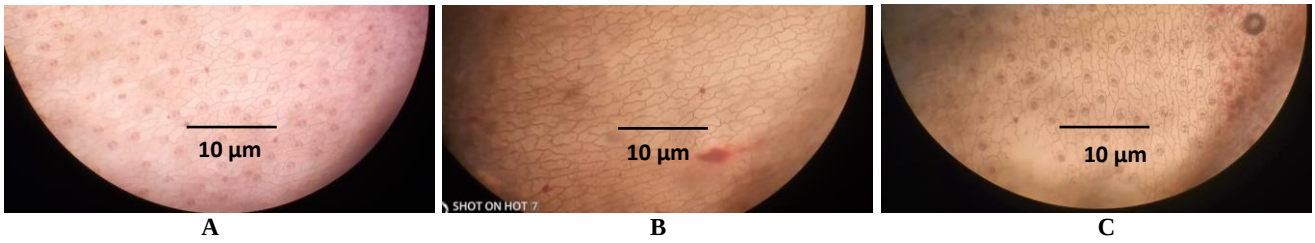

Figure 5. Foliar epidermal features of sterile frond of *P. bifurcatum.* A. Abaxial Surface, B. Adaxial Surface without stomata, C. Adaxial Surface with stomata (x100)

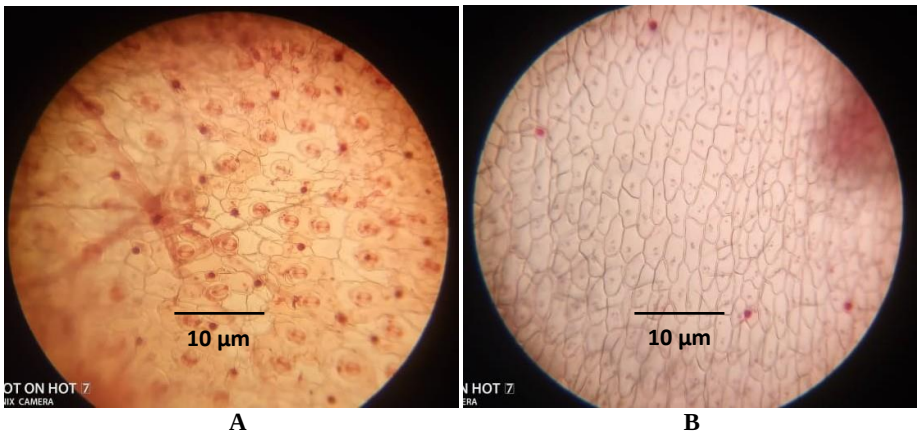

Figure 6. Foliar epidermal features of a fertile frond of *Platycerium superbum.* A. Abaxial Surface, B. Adaxial Surface (x100)

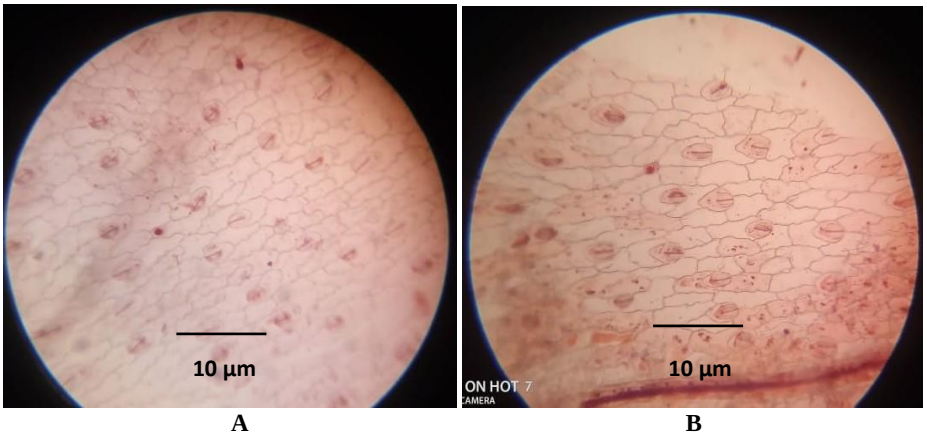

Figure 7. Foliar epidermal features of a sterile frond of *P. superbum.* A. Abaxial Surface, B. Adaxial Surface (x100)

Discussion

Morphological features are very diagnostic at the species level because they are usually employed in delimitation (Pryer et al. 2004); a mature *P. bifurcatum* can be as big as 3 feet across. Spores are produced in sporangia in the dark brownish masses (sori) on the underside of the tips of these fertile fronds, as Hoshizaki (1972) reported. Although *P. superbum* can look like a tangle of antlers at first, one can see the impressive 'nest' frond (sterile frond) on closer inspection. They are broad and multi-branching inhabit, and have short roots that are not prolific, as reported by Hoshizaki (1972) and Schneider et al. (2004). The *P. bifurcatum* and *P. superbum* have creeping rhizomes covered with scales; this is also similar with some species belonging to the family Polypodiaceae, mainly characterized by creeping rhizomes covered with varying scales (Smith et al. 2008). The leave or frond type of *P. bifurcatum* and *P. superbum* have the pinnate type of primary venation. On examination of the leaf venation traits, it has been observed that the 27 representative species of the family Polypodiaceae possess the pinnate type of primary venations (Tan and Buot 2020). The leaf nature of *P. bifurcatum* and *P. superbum* are evergreen, the leaf margins are lobed, and the sori are found on the lower

surfaces of the frond. This is similar to some species belonging to the family Polypodiaceae, which has evergreen leaves; the leaves are once-divided, with lobes or leaflets on opposite sides of the central axis, and the round sori are found on the underside of the frond (Tan and Buot 2020).

Epidermal anatomical characters have been regarded as important in the classification of vascular plants, and these characteristics are known to provide additional features, which, along with other characters, are usually of taxonomic value in the classification and identification of plants. Foliar epidermal features are essential in taxonomy and species delimitation (Uphof 1962; Scatena et al. 2005; Pryer et al. 2016). This study provides a comprehensive micro-morphology of the two ferns studied. The epidermal cell shape of the two species was irregularly shaped with thick, wavy anticlinal walls, as reported by Oloyede et al. (2011).

The stomatal type in the two species studied was a useful diagnostic characteristics for the delimitation of the species, as stated by Oloyede et al. (2011). Variations in types, arrangements, and distribution of stomata are characters that are taxonomically important at the generic level of classification, as reported by Oloyede et al. (2011). The stomata consist of two elongated guard cells surrounding a stomatal pore and are often surrounded by one or more subsidiary cells (Barclay et al. (2007). According to Adegbite (2008), most plants have numerous stomata on the abaxial surfaces than on the adaxial surfaces; this curtails excessive water loss through transpiration. Different stomatal types were observed and studied; these are anomocytic, diacytic, and amphidiacytic. The stomatal type on the abaxial surface of *P. bifurcatum* was diacytic and anomocytic, as reported by Oloyede et al. (2011), Khan et al. (2013), and Shabeena et al. (2014); the Stomatal type in *P. superbum* was diacytic amphidiacytic. On the adaxial surface, *P. bifurcatum* had anomocytic and diacytic stomata, which can be used to delimit the species for taxonomic purposes, whereas *P. superbum* had no stomata but Oloyede et al. (2011) reported diacytic and anomocytic stomata in this same plant in Osun State, Nigeria; the variation in this report maybe as a result of environmental condition.

The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. bifurcatum* was twenty-nine (29), followed by *P. superbum* twenty-two (22). Adedeji and Jewoola (2008) reported that the stomatal index is constant for any given, and the value is more uniform on the abaxial surface than the abaxial surface except in an isobilateral leaf. The adaxial stomatal index for *P. bifurcatum* was forty-three (43), and the highest for *P. superbum* was thirty-seven (37). This result conforms to the findings by Essiet and Iwok (2014) that the stomatal index is independent of the environment or size of the leaf surface and, thus, serves as a reliable identification tool.

Trichomes were absent on the abaxial and adaxial surfaces of *P. bifurcatum* while present on the abaxial and absent on the adaxial of *P. superbum*; this is due to the nature of the leaf surface of the fern species. Trichome types in plants are very useful for delimitating and identifying plants, even in the present study. Trichomes function in the reduction of the rate of transpiration in plants where they occur. They are also used for protection against insect infestation. The epidermal cell shape of the two species was irregularly shaped with thick, wavy, sinuous anticlinal walls, as Oloyede et al. (2011) reported.

Different stomatal types were observed and studied. These are paracytic, hemiparasitic, diacytic, and amphidiacytic. The stomatal type on the abaxial and adaxial surface of *P. bifurcatum* was paracytic and hemiparasitic, as reported by Oloyede et al. (2011), which can be used to delimit the species for taxonomic purposes, while *P. superbum* has diacytic and amphidiacytic stomata, as Oloyede et al. (2011) reported. The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. bifurcatum* was twenty-nine (29), followed by *P. superbum* twenty-two (22). Adedeji and Jewoola (2008) reported that the stomatal index is constant for any given, and the value is more uniform on the abaxial surface than the abaxial surface except in an isobilateral leaf.

Trichomes were absent both on the abaxial and adaxial surfaces of *P. bifurcatum* and *P. superbum.* This is due to the nature of the leaf surface of the fern species. Trichome types in plants are very useful for delimitating and identifying plants, even in the present study. Trichomes function in the reduction of the rate of transpiration in plants where they occur. Additionally, they are also used for protection against insect infestation.

In conclusion, studies on plants' morphological and foliar epidermal features have helped us understand the diversity of plant forms and how plants evolved over time. The two species of ferns *P. bifurcatum* and *P. superbum*, though they may look similar, the studies on their morphological and foliar epidermal features have proven that there is variation in the Root system, Climbing method, Habit/Form, Rhizomes, Frond appearance, the shape of the frond, stem bud scales, adaxial and abaxial surface, etc. Platycerium's morphological and foliar epidermal features should be investigated in other parts of Rivers State to provide more reviews for researchers and students worldwide.

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Effect of algal fertilization on the biochemical and phytochemical composition and antioxidant activity of tomato and pepper plants

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Abstract. *Baroud S, Tahrouch S, Hatimi A. 2024. Effect of algal fertilization on the biochemical and phytochemical composition and antioxidant activity of tomato and pepper plants. Cell Biol Dev 8: 36-44.* The aim of our study was to evaluate the effect of three brown algae, *Bifurcaria bifurcata, Cystoseira gibraltarica* and *Fucus spiralis*, on the biochemical and phytochemical composition of tomato and pepper plants. The algae were applied in two forms and at different concentrations: aqueous extract (0.5%, 1% and 2%) and amendment (C1, C2 and C3). The aqueous extract of *B. bifurcata* with its three concentrations showed the highest protein content in tomato leaves (217, 200 and 196.9 mg/g DM) and all aqueous extracts of *F. spiralis* showed high levels of total sugars (83.13, 83.08 and 75.38 mg/g DM). For pepper, the highest protein content was recorded for the 1% *C. gibraltarica* aqueous extract (196.57 mg/g DM). High levels of total sugars in pepper leaves were induced by the 2% *C. gibraltarica* aqueous extract (52.22 mg/g DM). Furthermore, the photosynthetic pigment content of the leaves of both vegetable crops (tomato and pepper) was generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae. In addition, tomato and pepper plants treated with aqueous extracts (spraying) or by amendment, showed a significant improvement in all phytochemical parameters and antioxidant activity. These three algae proved to be good candidates for the effective development of biostimulants to improve biochemical composition and phytochemical parameters. This study could provide important information on the identification and use of Moroccan algal resources in agriculture.

Keywords: Amendment, aqueous extracts, biochemical, biofertilization, phytochemical

INTRODUCTION

The main aim of an organic farming system is to optimize the health and productivity of soil, plants, animals and people, and to create an ecological balance and better functioning of the agro-ecological system (Dumont et al. 2013). This type of agriculture is based on the use of biostimulators as organic fertilizers made from dead leaves, grass clippings, vegetable garden waste, ashes, plant extracts (Cisse 2014). Seaweed-based fertilization is one of the fertilizers used by farmers to improve germination and growth of vegetable crops.

In Morocco, sea currents and hydroclimatic conditions rapidly favor for the development and expansion of marine algae such as brown seaweed and many species of algae grow rapidly and efficiently, (green algae) especially compared with land plants (Kindleysides et al. 2012). Brown algae are very abundant along the Atlantic coast, especially in the Cap Ghir Region. This biomass, while promoting research aimed at exploiting these brown algae, notably *Cystoseira gibraltarica*, *Bifurcaria bifurcata* and *Fucus spiralis*, in a number of fields, particularly agriculture.

The greenhouse is a structure designed to house vegetable crops in more favorable or safer conditions than in the open air (Osentowski 2015). This structure protects plants by controlling the climate to obtain optimal growth conditions or minimize health risks. Greenhouse cultivation plays an important economic role in the marketing of off-season products. This technique makes it possible to grow plants in better conditions than those found in the natural environment, and therefore to obtain better-quality products.

Pot cultivation is the practice of growing plants, including vegetable plants, exclusively in pots instead of planting them in the ground (Mills 2012). Pot cultivation is a method used by farmers in areas where the soil or climate is unsuitable for the crop in question. As such, this method is useful for scientific trials before moving on to full-ground cultivation. In addition to the optimal conditions offered by the greenhouse, seaweed fertilization could significantly improve the growth and yield of greenhouse crops.

Some studies indicate that algal extracts can partially substitute fertilizers (Hong et al. 2007; Zodape et al. 2010) because they contain both minor and major mineral elements. Saccharides from algal extracts can act as elicitors of plant defensive mechanisms (Khan et al. 2009). Algae-based fertilizers contain a wide variety of plant growth-promoting substances such as auxins, cytokinins and betaines (Khan et al. 2009). These substances can influence the development of the aerial and root parts of plants (Durand et al. 2003). In addition, macronutrients (N, P, K, Ca and Na) and micronutrients (Fe, Zn, Mn and Cu) can promote fruit growth and yield (Möller and Smith 1998). These algal extracts can also increase phytochemical

parameters (Lola-Luz et al. 2014). Positive responses include improved plant growth and fruit quality, as well as overall plant vigour and pathogen resistance (Khan et al. 2009). For example, Ali et al. (2016) showed that the application of aqueous extracts of *Ascophyllum nodosum* algae increased the chlorophyll 'a' and 'b' content of tomato plants. In this context, our objective is to carry out a greenhouse experiment to test the effects of three brown algae: *B. bifurcata, C. gibraltarica* and *F. spiralis* on the biochemical and phytochemical parameters of pepper and tomato leaves and fruit.

MATERIALS AND METHODS

Plant material

Three brown seaweeds, *C. gibraltarica, B. bifurcata* and *F. spiralis* were collected at low tide, in the coastal area of Cap Ghir, (30°38'37 "N, 09°53'20 "W), located about 43 kilometers northwest of Agadir Morocco. All algal species identified by algal specialist Prof Chfiri INRH Agadir. The algae species harvested are carefully washed and dried, before being ground to a fine powder.

The experiments were carried out using certified tomato seeds (*Solanum lycopersicum*) of the Campbell variety marketed by Technisem, and pepper seeds (*Capsicum annuum*) of the Roldan variety.

Treatment preparation

Two types of treatment are used: amendment and spraying.

Amendment: Seaweed powder is applied to the crops in specific concentrations: C1 concentration (2.5 grams of powder per pot), C2 concentration (5 grams of powder per pot) and C3 concentration (10 grams of powder per pot).

Algal extract: Tomato and pepper plants are regularly sprayed with an aqueous algal extract in three different concentrations (0.5, 1 and 2%).

Preparation of soil improvers

Three amendments are prepared based on the concentrations used in organic farming (25 kg/100 m^2). Each pot contains 5 kg of substrate made up of a mixture of 75% soil and 25% peat. Three amendments are determined: C1 (2.5 grams of powder per pot), C2 (5 grams of powder per pot) and C3 (10 grams of powder per pot).

Preparation of algal extracts

Five grams of powder of each algal species are added to 100 mL of distilled water under magnetic stirring for 24 hrs. The recovered supernatant is filtered, and the aqueous extracts obtained are then stored in a cool place. These extracts are designated as stock solutions and coded according to genus and species: *C. gibraltarica* (C g), *B. bifurcata* (B b) and *F. spiralis* (F s). The stock solution of each alga was diluted with water to three concentrations (0.5, 1 and 2%).

Setting up greenhouse cultivation

The seeds of the two vegetable plants (tomato and pepper) were germinated in honeycomb plates containing peat. After 25 days of germination, 400 plants were selected at the four-leaf stage and transplanted into five-liter pots containing 5 kg of a mixture of 75% soil and 25% peat. Ten pots were used for each algal treatment, with one plant per pot. Each pot receives 50 mL/week of algal extract in three concentrations (0.5, 1 and 2%). For the amendment, the treatment is also represented by three increasing concentrations C1 (2.5 g/pot), C2 (5g/pot) and C3 (10 g/pot). At the same time, we used a water-only control and a witness chemical fertilizer (Maxi Greene: N:20, P:20, K:20).

Spraying with aqueous extracts was applied two weeks after sowing at a rate of 50 mL/week for three months. Fertilization was carried out when the plants were transplanted, using the three different concentrations determined above. All pots were irrigated with 50 mL of water every other day during the growing period. After 90 days of cultivation (Figure 1), the tomato and pepper fruits were harvested and the plants carefully removed and washed. We then measured leaf biochemical parameters (proteins, total sugars and chlorophyll pigments) and phytochemical parameters (flavonoids, total phenols and antioxidant activity).

Figure 1. Tomato and pepper plants after 90 days of cultivation

Determination of biochemical parameters

Determination of total sugars (Dubois et al. 1956)

20 mg of algal powder homogenize with 2 mL of ethanol 70% (v/v), the mixture is centrifuged at 2000 g. After recovery of the supernatant, the pellet is rinsed twice with ethanol 70% (v/v). To the supernatants thus combined, 16 mL of distilled water are added. 200 µL of the solution to be determined is added to 200 µL of a 5% aqueous phenol solution, then 1 mL of concentrated sulfuric acid is quickly introduced into the reaction medium. The vortexed mixture is allowed to stand for 10 min and then placed in a water bath for 10 to 20 min at a temperature of 30°C. The optical density is read at 490 nm using the visible IC 6400 spectrophotometer. The blank is the reaction mixture without sample. The values obtained are converted into sugar content in mg/g of Dry Matter (DM).

Protein assay

The method of Lowry (Lowry et al. 1951) consists in forming a complex between the peptide bonds and copper sulfate in alkaline medium. This complex then reduces the phosphomolybdic and phosphotungstic acids of the Folin-Ciocalteu reagent to give a second complex of blue color, measured by spectrophotometer (Frolund et al. 1995).

The assay reagent (solution R) is prepared extemporaneously from three solutions, respecting the order of addition of the solutions and stirring after each addition:

- Solution C: copper sulfate at 10 g/L,
- Solution B: sodium/potassium tartrate (Na/K) at 20 g/L,
- Solution A: Sodium carbonate (Na_2CO_3) at 20 g/L and soda (NaOH) 0.1 mol/L.

Protein extraction *(Lowry et al. 1951)*

0.1 g of algal powder is ground in 1 mL of lysis buffer to extract the proteins. The extract is centrifuged at 13000 g for 10 min.

Lysis buffer is prepared by mixing 8 mL of 1M Tris-HCl pH=6.8, 2 mL of β-mercaptoethanol, 10 mL of SDS and 80 mL of water

Assay method *(Lowry et al. 1951)*

To 10 µL of the supernatant are added 990 µL of water and 5 mL of solution R (3 mL of solution C, 3 mL of solution B and 300 mL of solution A). The tubes are incubated for 10 min in the dark, then 0.5 mL of a 50% (v/v) Folin-Ciocalteu reagent solution is added and the mixture is vortexed. The stabilization of the color takes a few minutes. The intensity of the color obtained is evaluated by measuring the absorbance at 750 nm using the visible IC 6400 spectrophotometer. At the same time, a calibration line of Bovine Serum Albumin (BSA) (2mg/mL) is performed. Protein concentrations are expressed in milligram per gram of dry matter (mg/g DM) of sample.

Determination of chlorophylls and carotenoids

0.5 g fresh frozen leaves are ground and homogenized with 50 mL acetone (90:30; v/v), the extract is then centrifuged at 3500 g. The recovered supernatant is run through a visible IC 6400 spectrophotometer, either directly or after dilution. Optical Density (OD) is read at different wavelengths: 470 nm for carotenoids, 645 nm for chlorophyll "b" and 663 nm for chlorophyll "a" (Lichtenthaler 1987):

Concentrations are calculated from the following formulas (Lichtenthaler 1987):

$$Chlorophyll\ "a"\ (chl"a")(mg/gFM) = (11.75 \times DO663 - 2.35 \times DO645) \times \frac{50}{500}$$

$$Chlorophll\ "b"\ (chl"b")\ (mg/gFM) = (18.61 \times DO645 - 3.96 \times DO663) \times \frac{50}{500}$$

$$Carotenoids\ (mg/gFM) = \left((1000 \times DO470) - (2.27 \times Chl"a") - \frac{(81.4 \times Chl"b")}{227}\right) \times \frac{50}{500}$$

Determination of phytochemical parameters

Extraction

50 mg of algae powder, put in an Eppendorf tube, are homogenized in 1 mL of Methanol-water (8:2, v/v). The mixture is sonicated for 20 min and then centrifuged for 15 min at 10000 g. The extract obtained is used for the quantification of phenolic compounds (total phenols and total flavonoids) and for the determination of the antioxidant activity of the different algae.

Determination of total phenols

25 µL of algal extract, previously prepared, 110 µL of Folin-Ciocalteu reagent is added, shaking for 3 minutes and then 200 µL of sodium carbonate is added to the mixture. Then 1.9 µL of distilled water is added and vortexed. After a 30-minute incubation in the dark, the Optical Density (OD) of each sample is measured by spectrophotometer at 750 nm (Makkar 2003). The calibration range is performed by gallic acid. The OD values obtained are then transformed into the unit microgram of gallic acid equivalents per milligram of dry matter (µg GAE/mg DM).

Determination of total flavonoids

The dosage of flavonoids is carried out using two different methods:

Method of Andary (Andary 1990):

2 mL of algal extract are added with 100 µL of the reagent (2 amino-ethyl diphenyl borate) (Neu 1956). The OD reading is done at a wavelength of 404 nm. The flavonoid content is calculated according to the following formula:

$$T\ flavonoïdes = Aext \times 0.05 \times 100/Aq \times Cext$$

Where:

Aext: Absorption of the extract

Aq: Absorption of quercetin (0.05 mg/mL)

Cext: Concentration of the extract in mg/mL

The results are given in micrograms of quercetin equivalents per mg of dry matter (µg quercetin/mg DM).

Jay's method

The determination of flavonoids is performed according to the method of Jay (1975) as described by Harnafi et al, (2007) with a difference in the extraction solvent. To 1 mL of algal extract are added 0.5 mL of aluminum chloride ($AlCl_3$), left to stand for 30 minutes at room temperature, then the OD is measured at 430 nm by a visible IC 6400 spectrophotometer. The calibration range is performed by

quercetin. The OD values obtained are then transformed into the unit µg quercetin equivalents/mg Dry Matter (DM).

Determination of antioxidant activity

950 µL of a methanolic solution of DPPH (0.1 mM) are added to 50 µL of methanolic extract of the sample to be analyzed. After 30 min, the absorbance of the mixture is measured at 517 nm. The ability to trap the DPPH radical is calculated according to the following formula (Loo et al. 2008).

$P = (A1-A2)/A1X100$

Where:

P : Percentage of radical trapping

A1 : Absorbance of the control (DPPH solution without extract)

A2 : Absorbance in presence of extract

The DPPH- test is not quantitative, it allows to compare different extracts according to their capacity to trap DPPH- and thus, to appreciate the qualitative variations of phenolic compounds. The evaluation of the anti-free radical activity must be interpreted with precaution because the absorbance of DPPH- at 515-520 nm decreases under the action of light, oxygen, according to the pH and the type of solvent added to the antioxidant.

Statistical analysis

For each analysis three repetitions are carried out. The data are processed by the STATISTICA software, version 6.0. The Analysis of Variance (ANOVA) is used to determine the degree of significance. Means are compared using Duncan's tests at the probability threshold ($P<0.05$).

RESULTS AND DISCUSSION

Effect of algal fertilization on protein and total sugar content of tomato and pepper leaves

The protein contents of the two vegetable crops (tomato and pepper) were generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae (Table 1). However, the extracts had no effect on the pepper. In fact, we note that in tomatoes, the addition of extracts or amendments significantly increased protein content compared with the control. Tomato plants sprayed with aqueous extracts of the three brown algae had significantly higher protein contents than the control, up to 217 mg/g DM. The aqueous extract of *B. bifurcata* with its three concentrations showed the highest protein values in tomato leaves (217, 200 and 196.9 mg/g DM), followed by the three concentrations of *C. gibraltarica* (201, 197.65 and 192.07 mg/g, DM).

Fertilization with algal amendment also increased protein content in tomato plants. In fact, the results obtained show values significantly different from the untreated control. The highest values for protein content in tomato leaves were obtained with *F. spiralis* C1 amendment (208.91, 198.32 and 196.9 mg/g DM), followed by *B. bifurcata* (199.41, 198.66 and 191.9 mg/g DM).

In the case of peppers, treatment with aqueous extracts showed no significant effect on protein content, with the exception of the two concentrations of 1 and 2% *C. gibraltarica*. The highest protein content was recorded for the 1% aqueous extract of *C. gibraltarica* (196.57 mg/g DM). On the other hand, fertilization with algal amendments increased protein content (Table 1). In fact, the results obtained show values significantly different from the untreated control. The highest values for protein content in pepper leaves were obtained with *C. gibraltarica* C1 amendment (204.4 mg/g DM).

The three aqueous extracts of *F. spiralis* showed high levels of total sugars (83.13, 83.08 and 75.38 mg/g DM), followed by *C. gibraltarica* with its three concentrations (83.16, 82.75 and 43.77 mg/g DM). Fertilization by amendment also increased total sugar content, except for C3 amendment of *B. bifurcata* and *F. spiralis*. The highest statistical values for total sugar content in tomatoes were obtained with *F. spiralis* amendment C1 (83.08 mg/g DM).

Pepper plants sprayed with aqueous extracts of the three brown algae showed significantly lower levels than the control. The high levels of total sugars in pepper leaves were induced by the 2% aqueous extract of *C. gibraltarica* (52.22 mg/g DM). Fertilization by amendment also improved total sugar content. In fact, all *F. spiralis* amendments showed significantly lower values than the control. The highest values for total sugar content in pepper leaves were obtained with *F. spiralis* C3 (69.44 mg/g DM) (Table 1).

Effect of algae on the photosynthetic pigment content of tomato and pepper leaves

The photosynthetic pigment content of the leaves of the two vegetable crops (tomato and pepper) was generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae (Table 2). The use of algal fertilizer in the form of an amendment produced significantly better results than the use of aqueous extracts for both tomato and pepper crops. The aqueous extracts of the three brown algae showed a clear improvement in the quantity of chlorophyll 'a', chlorophyll 'b' and carotenoids in pepper plants compared with tomato plants.

Algal extracts of *B. bifurcata* at 1% and 2% and *C. gibraltarica* at 1% significantly improve leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control in tomato crops. Indeed, the 2% *B. bifurcata* extract is significantly effective (1.34 mg/g FM for chlorophyll 'a' and 0.8 mg/g FM for chlorophyll 'b'), followed by the *C. gibraltarica* extract at 1% (0.92 mg/g FM for chlorophyll 'a' and 0.48 mg/g FM for chlorophyll 'b') and *B. bifurcata* extract at 1% (0.9 mg/g FM for chlorophyll 'a' and 0.49 mg/g FM for chlorophyll 'b'). In pepper, algal extracts of *C. gibraltarica* at 1% and *F. spiralis* at 1% and *B. bifurcata* at 0.5% and 2% significantly improved leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer. *C. gibraltarica* at 1% gives highly significant chlorophyll 'a' and 'b' contents (1.47 mg/g FM, for chlorophyll 'a' and 0.65 mg/g FM, for chlorophyll 'b'), followed by *F. spiralis* at 1% (1.13 mg/g

FM, for chlorophyll 'a' and 0.45 mg/g FM, for chlorophyll 'b') and finally *B. bifurcata* (1.07 mg/g FM, for chlorophyll 'a' and 0.44 mg/g FM, for chlorophyll 'b' at 2% 1.06 mg/g FM, for chlorophyll 'a' and 0.37 0.44 mg/g FM, for chlorophyll 'b' at 0.5%) (Table 2).

Table 1. Effect of the algae *C. gibraltarica* (Cg), *B. bifurcata* (Bb) and *F. spiralis* (Fs) in the two treatments (spraying and amendment) at different concentrations on the protein and total sugar content of tomato and pepper leaves

Spraying	Tomato		Pepper	
	Proteins	Total sugars	Proteins	Total sugars
	mg/g DM	mg/g DM	mg/g DM	mg/g DM
Control	191.15±0.75 g	56±0.16 e	186.06±0.62 d	23.8±0.12 f
Witness	191.4±0.25 g	31.6±0.12 h	198.66±0.5 a	10.44±0.25 k
BB 0,5 %	196.9±0.25 e	77.83±0.16 c	181.89±0.25 f	28.47±0.12 e
BB 1 %	217±0.25 a	46±0.2 f	183.81±0.38 e	40.58±0.08 c
BB 2 %	200±0.25 b	56.38±0.26 e	185.73±0.62 d	19.52±0.31 g
CG 0,5 %	201±0.38 b	83.16±0.08 a	171.88±0.25 l	50.77±0.12 b
CG 1 %	197.65±0.25 d	43.77±0.26 g	196.57±0.14 b	19.88±0.2 g
CG 2 %	192.07±0.38 f	82.75±0.25 b	191.48±0.76 c	52.22±0.41 a
FS 0,5 %	191.9±0.5 g	75.38±0.25 d	173.8±0.38 k	38±0.16 d
FS 1 %	193.32±0.38 f	83.13±0.12 a	180.81±0.62 g	17.16±0.16 h
FS 2 %	199.24±0.38 c	83.08±0.08 a	177.64±0.25 h	10.25±0.22 k
Amendment				
Control	191.15±0.75 g	56±0.16 f	186.06±0.62 f	23.8±0.12 e
Witness	191.4±0.25 g	31.36±0.12 k	198.66±0.5 b	10.44±0.25 l
BB C1	199.41±0.25 b	55.58±0.08 f	171.38±0.25 l	18.66±0.16 g
BB C2	198.66±0.5 c	80.36±0.17 b	189.23±0.14 e	31.97±0.2 b
BB C3	191.9±0.25 g	51.83±0.16 g	196.9±0.5 c	17.05±0.12 h
CG C1	194.49±0.38 e	62.75±0.08 e	204.4±0.25 a	10.36±0.12 l
CG C2	196.24±0.52 d	70.27±0.09 d	176.22±0.38 k	21.94±0.25 f
CG C3	193.4±0.5 f	62.66±0.16 e	177.14±0.25 h	11.83±0.16 k
FS C1	208.91±0.25 a	83.08±0.08 a	184.31±0.38 g	31.33±0.16 c
FS C2	198.32±0.38 c	77±0.16 c	192.82±0.38 d	25.69±0.25 d
FS C3	196.9±0.25 d	43.8±0.2 h	188.81±0.52 e	69.44±0.2 a

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different P≤0.05

Table 2. Effect of the algae *C. gibraltarica* (Cg), *B. bifurcata* (Bb) and *F. spiralis* (Fs) by the two treatments (soil watering and soil amendment) at different concentrations on the photosynthetic pigment content of tomato and pepper leaves

Spraying	Tomato			Pepper		
	Pigments (mg/g FM)			Pigments (mg/g FM)		
	Chl a	Chl b	Carotenoids	Chl a	Chl b	Carotenoids
Control	0.84±0.02d	0.27±0.04f	0.14±0.02 c	0.66±0.01g	0.25±0.01e	0.22±0.01 h
Witness	1.12±0.02b	0.89±0.02a	0.21±0.02 b	0.85±0.01d	0.47±0.01b	0.12±0.00 k
BB 0.5 %	0.8±0.02 d	0.41±0.01d	0.16±0.01 c	1.06±0.02c	0.37±0.13c	0.37±0.05 d
BB 1 %	0.9±0.03 c	0.49±0.01c	0.16±0.01 c	0.75±0.00e	0.28±0.01e	0.30±0.01 e
BB 2 %	1.34±0.07a	0.8±0.06 b	0.22±0.04 a	1.07±0.01c	0.44±0.01b	0.43±0.01 c
CG 0.5 %	0.79±0.02d	0.39±0.04d	0.17±0.02 c	0.6±0.01d	0.33±0.01d	0.54±0.01 a
CG 1 %	0.92±0.02c	0.48±0.01c	0.14±0.01 c	1.47±0.00a	0.65±0.01a	0.47±0.01 b
CG 2 %	0.71±0.07e	0.39±0.07d	0.13±0.03 c	0.73±0.00f	0.26±0.01e	0.27±0.01 f
FS 0.5 %	0.56±0.01f	0.28±0.01f	0.08±0.01 d	0.76±0.00e	0.30±0.01e	0.28±0.01 f
FS 1 %	0.6±0.01 f	0.33±0.01e	0.09±0.01 d	1.13±0.01b	0.45±0.01b	0.37±0.01 d
FS 2 %	0.71±0.04e	0.38±0.04d	0.10±0.01 d	0.71±0.01g	0.29±0.01e	0.24±0.01 g
Amendment						
Control	0.84±0.02h	0.27±0.04g	0.14±0.02 f	0.66±0.01k	0.25±0.01d	0.22±0.01 d
Witness	1.12±0.02f	0.89±0.02 b	0.21±0.02 c	0.85±0.01h	0.47±0.01c	0.12±0.00 e
BB C1	1.03±0.05g	0.59±0.01 e	0.16±0.01 e	0.73±0.03k	0.3±0.01 d	0.23±0.00 c
BB C2	1.5±0.15 d	0.8±0.03 b	0.22±0.05 c	1.21±0.01d	0.72±0.01a	0.37±0.01 b
BB C3	0.97±0.01g	0.55±0.01 f	0.11±0.02 g	0.93±0.01g	0.45±0.01c	0.26±0.02 c
CG C1	1.37±0.01e	0.72±0.02 c	0.16±0.01 e	1.51±0.02b	0.74±0.1 a	0.34±0.04 b
CG C2	1.85±0.07b	1.13±0.07 a	0.26±0.03 c	1.41±0.02c	0.75±0.11a	0.35±0.04 b
CG C3	1.96±0.08a	1.12±0.09 a	0.35±0.04 a	0.98±0.06f	0.59±0.07b	0.15±0.03 e
FS C1	1.7±0.01 c	0.55±0.08 f	0.18±0.01 d	1.72±0.01a	0.75±0.06a	0.41±0.03 a
FS C2	2.06±0.07a	1.3±0.07 a	0.29±0.02 b	1.15±0.01e	0.39±0.01c	0.36±0.01 b
FS C3	2.12±0.02a	1.22±0.02b	0.39±0.04 a	0.8±0.01 h	0.47±0.02c	0.24±0.01 c

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different P≤0.05

Table 3. Effect of *B. bifurcata* (BB), *C. gibraltarica* (CG) and *F. spiralis* (FS) algae by both treatments (spraying and amendment) at different concentrations on antioxidant activity (%), flavonoid content (µg/100 mg EqQ) and total phenols (µg/mg EAG) of tomato fruit and pepper

Spraying	**Tomato**				**Pepper**			
	Antioxidant activity	**Flavonoids by $AlCl_3$**	**Flavonoids by NEU**	**Total phenols**	**antioxidant activity**	**Flavonoids by $AlCl_3$**	**Flavonoids par NEU**	**Total phenols**
Control	8.67 k	13.23±0.43	4.61±0.29 e	1.76±0.08 f	47.76 g	40.95±0.59 e	5.72±0.03 h	1.76±0.08 ef
Witness	10.71 h	32.38±1.64 a	3.36±0.04 f	8.6±0.12 a	86.73 a	67.04±0.43 a	8.06±0.04 c	1.68±0.08 f
BB 0.5 %	20 g	15.42±0.28 e	4.56±0.03 e	3.06±0.04 c	73.77 c	56.28±0.28 c	7.05±0.04 d	1.76±0.16 f
BB 1 %	24.19 c	13.23±0.16 f	5.39±0.02 d	2.72±0.08 d	38.84 k	35.14±0.28 g	4.37±0.04 l	1.2±0.08 g
BB 2 %	38.87 a	19.23±0.16 d	10.45±0.02 a	3.46±0.04 b	54.2 f	40.85±0.28 e	15.39±0.08 a	1.68±0.08 f
CG 0.5 %	23.88 d	23.42±0.28 c	5.58±0.03 d	3.54±1.08 b	75.59 b	56.57±0.28 c	6.19±0.07 f	2.02±0.04 d
CG 1 %	21.36 f	23.9±0.16 c	7.61±0.06 b	2±0.08 e	75.06 b	43.42±0.57 d	6.81±0.05 e	1.84±0.08 d
CG 2 %	24.34 c	23.33±0.43 c	6.17±0.04 c	2.4±0.08 de	48.59 g	38.47±0.32 f	7.04±0.08 d	2.64±0.08 c
FS 0.5 %	37.85 b	32.38±1.64 a	7.73±0.08 b	2.4±0.08 de	70.08 d	40.76±0.71 e	10.4±0.05 b	8.38±0.12 a
FS 1 %	21.45 f	24.57±0.28 c	6.10±0.05 c	3.49±0.12 b	54.1 f	43.52±0.32 d	5.93±0.06 g	8.26±1.22 a
FS 2 %	22.71 e	30.47±0.32 b	5.97±0.08 c	1.94±0.12 e	68.6 e	64±0.28 b	4.59±0.08 k	7.12±0.08 b
Amendment								
Control	8.67 f	13.23±0.43 f	4.61±0.29 c	1.76±0.08 k	47.76 m	40.95±0.59 l	5.72±0.03 k	1.76±0.08 g
witness	10.71 d	32.38±1.64 a	3.36±0.04 f	8.6±0.12 d	86.73 d	67.04±0.43 e	8.06±0.04 e	1.68±0.08 g
BB C1	27.85 a	32.19±0.16 a	4.99±0.08 b	2.69±0.04 h	65.22 g	75.8±0.43 b	7.69±0.05 g	2.66±0.46 e
BB C2	27.51 a	27.61±0.43 b	3.83±0.08 e	8.98±0.12 c	71.56 e	73.61±0.16 c	7.89±0.09 f	3.73±1.22 b
BB C3	8.37 f	17.42±0.28 d	9.17±0.05 a	11.70±0.04 b	90.3 c	58.85±0.28 g	9.37±0.06 c	2.88±0.08 e
CG C1	17.6 b	25.71±0.28 c	4.22±0.02 d	13.44±0.08 a	60.66 h	59.33±0.43 g	9.21±0.08 d	2.85±0.04 e
CG C2	9.84 e	12.85±0.28 f	3.4±0.03 f	8.72±0.08 d	68.97 f	55.71±0.28 h	10.7±0.08 a	4.8±0.08 a
CG C3	9.51 e	15.71±0.28 e	3.88±0.04 e	8.66±0.04 d	57.8 k	49.14±0.28 k	7.92±0.08 f	1.01±0.12 h
FS C1	9.87 e	16±0.28 e	2.53±0.04 h	8.4±0.08 e	56.69 l	70.19±0.43 d	8.15±0.05 e	3.32±0.08 d
FS C2	11.01 d	17.33±0.43 d	2.9±0.05 g	7.84±0.08 f	91.99 b	85.23±0.16 a	6.43±0.04 h	3.52±0.08 c
FS C3	12.68 c	29.33±0.16 a	2.64±0.04 h	7.28±0.08 e	92.52 a	64.57±0.28 f	9.79±0.04 b	2.33±0.30 f

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different $P \leq 0.05$. EqQ: Quercetin equivalent; EAG: Gallic Acid Equivalent

The *C. gibraltarica* and *F. spiralis* were also statistically effective in improving leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer in tomato cultivation. Indeed, *F. spiralis* at C3 and C2 and *C. gibraltarica* at C3 gave statistically the best results (2.12, 2.06 and 1.96 mg/g FM, respectively) for chlorophyll 'a'. For chlorophyll 'b' *F. spiralis* at C2 and *C. gibraltarica* at C2 and C3 (1.3, 1.13 and 1.12, mg/g FM, respectively) gave statistically the best results.

For the pepper crop, amendment with *F. spiralis* at C1 was statistically effective in improving leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer (1.72 and 0.75 mg/g FM, respectively). Followed by *C. gibraltarica* at C1 (1.51 mg/g FM, for chlorophyll 'a' and 0.74 mg/g FM, for chlorophyll 'b'), then the same alga at C2 (1.41 mg/g FM, for chlorophyll 'a' and 0.75 mg/g FM for chlorophyll 'b') and finally the amendment by *B. bifurcata* at C2 (1.21 mg/g MF, for chlorophyll 'a' and 0.72 mg/g MF, for chlorophyll 'b') (Table 2).

As far as carotenoids are concerned, the algal extracts of the three algae and the amendments significantly improved leaf content compared with the control and even with the chemical fertilizer for the pepper crop (Table 2). *C. gibraltarica* extract at 0.5% showed statistically maximum content (0.54 mg/g FM), followed by extract of the same alga at 1% (0.47 mg/g FM) and finally *B. bifurcata* extract at 2% (0.43 mg/g FM). As with chlorophyll 'a' and 'b', the 2% *B. bifurcata* extract was significantly effective on tomatoes (0.22 mg/g FM). *C. gibraltarica* and *F. spiralis* were statistically effective in improving leaf carotenoid content compared with the control and even with chemical fertilizers for tomato crops. Indeed, *F. spiralis* and *C. gibraltarica* at C3 gave statistically the best results (0.39 and 0.35 mg/g FM, respectively), followed by *F. spiralis* at C2 (0.29 mg/g FM). In peppers, *F. spiralis* at C1 was significantly effective (0.41 mg/g FM), followed by *B. bifurcata* at C2, *F. spiralis* at C2 and *C. gibraltarica* at C2 and C1 (0.37, 0.36, 0.35 and 0.34 mg/g FM, respectively).

Effect of algal fertilization on phytochemical parameters of tomato and pepper fruits

Both tomato and pepper plants grown in greenhouse pots, treated with aqueous extracts (spraying) or by amendment, showed a significant improvement in all phytochemical parameters (Table 3). Nevertheless, we note that the application of algal fertilizer in the form of aqueous extracts gives significantly better results than the amendment for tomato cultivation. On the other hand, the application of algal fertilizer in the form of an amendment gave significantly better results than the aqueous extract for peppers.

For tomato fruits, the aqueous extract of *B. bifurcata* at 2% showed a maximum value in antioxidant activity (38.87%), a maximum value in total flavonoids (10.45 µg EqQ/100mg DM, by NEU reagent) and a maximum content in total phenols (3.46 µg EAG/mg DM). On the other hand, the same alga (*B. bifurcata*) added as an amendment gave highly significant values for antioxidant activity (27.45%), total flavonoids (32.19 µg EqQ/100mg DM by $AlCl_3$; 9.17 µg EqQ/100mg MS by NEU reagent) and total phenols (11.7 µg EAG/mg DM). In addition, the 0.5% *F. spiralis* extract significantly improved antioxidant activity (37.85%) and total flavonoid content (32.38 µg EqQ/100mg DM by $AlCl_3$ and 7.73 µg EqQ/100mg DM by NEU reagent). Amendment with *F. spiralis* C3 also improved total flavonoid content (29.33 µg EqQ/100mg DM).

For pepper fruits, antioxidant activity was significantly enhanced by the 0.5% and 1% aqueous extracts of *C. gibraltarica* (75.59% and 75.06%, respectively) and by the amendment with *F. spiralis* at C2 and C3, which recorded maximum values (92.52% for C3 and 91.99% for C2). For total flavonoids, the aqueous extract of F. spiralis at 2% recorded a significantly high value for $AlCl_3$ (64 µg EqQ/100mg DM), while it was *B. bifurcata* at 2% that gave a maximum value with the NEU reagent (15.39 µg EqQ/100mg DM). On the other hand, amendment fertilization showed a significant improvement in total flavonoid content with the $AlCl_3$ reagent compared with the control, with a maximum value obtained by the *F. spiralis* C2 amendment (85.23 µg EqQ/100mg DM), followed by the *B. bifurcata* C1 amendment (75.8 µg EqQ/100mg DM).

Amendment with *C. gibraltarica* to C2 showed a very highly significant value for total flavonoid content with NEU reagent (10.7 µg EqQ/100mg DM), followed by C3 from *F. spiralis* (9.79 µg EqQ/100mg DM). Total phenol content was significantly improved with the 0.5% and 1% aqueous extracts of *F. spiralis* (8.38 and 8.26 µgEAG/mg DM, respectively), followed by the 2% extract of the same alga (7.12 µg µg EqQ/100mg DM/mg MS) (Table 3). On the other hand, fertilization by amendment also showed a significant improvement over the untreated control, with a maximum value obtained by the C2 amendment of *C. gibraltarica* (4.8 µg/mg DM), followed by *B. bifurcata* at C2 (3.73 µg/mg DM) and finally *F. spiralis* at C2 (3.52 µg/mg DM).

Discussion

In order to assess the effect of algal fertilization on the biochemical and phytochemical composition of plants, we tested increasing concentrations of extracts and amendments of three brown algae (*B. bifurcata, C. gibraltarica* and *F. spiralis*) on two vegetable crops, tomato and pepper. In general, the results obtained after three months of cultivation in pots (greenhouse) are very satisfactory. The results show a significant improvement in all biochemical parameters. The quantity of photosynthetic pigments (Chlorophyll 'a'; 'b' and carotenoids) in both tomato and pepper crops was significantly improved by the addition of aqueous extracts and amendments of the three brown algae. Pigments play a vital role in plant photosynthesis. It is thanks to this phenomenon that plants absorb CO_2, which could be responsible for increasing the sugar, protein and organic matter content of both crops. Several studies have shown that algal fertilization improves the chlorophyll and protein content of *Zea mays* and *Phaseolus mungo* leaves (Lingakumar et al. 2004).

According to Whapham et al. (1993), the increase in the quantity of these pigments is a consequence of the uptake of magnesium, a major constituent of chlorophyll. Furthermore, aqueous extracts of the three brown algae showed a notable effect on the protein content of tomato and pepper leaves. Such an increase in protein content may be contributed to the increased availability and uptake of mineral elements (N, K, Ca, Na, Mg, Cu and Zn) present in algal fertilizers. Our results concur with those of Ashok et al. (2004) who show that the protein content of *Sorghum vulgare* increases when this plant is treated with the aqueous extract of *Hydroclathrus clathratus*. Our study shows that the total sugar content of tomato and pepper leaves was enhanced by aqueous extracts of the three brown algae at low concentrations. This could be explained by the fact that algal extracts stimulate various biological processes that increase carbohydrate levels in plants (Kumari et al. 2011). Similar observations were recorded in *Vigna catajung* treated with aqueous extracts of *Caulerpa racemosa* (Anantharaj and Venkatesalu 2001).

Algae contain macronutrients and microelements, amino acids, vitamins, cytokinin, auxins and abscisic acid that affect the cellular metabolism of treated plants, resulting in enhanced crop growth (Crouch and Van Staden 1993; Stirk et al. 2004). In addition, the presence of polysaccharides in algal extracts can enhance plant growth in a similar way to hormones (Rolland et al. 2002). Brown seaweed extracts also contain various betaine-type compounds (Ghoul et al. 1995). This molecule acts as a compatible solute that mitigates salinity-induced osmotic stress, and functions as a nitrogen source when provided in low concentration and as an osmolyte at higher concentrations (Naidu et al. 1987). This could often improve the biomass and fruit quality of vegetable crops, notably tomatoes and peppers.

Our results also showed that the application of aqueous extracts or amendment increased the phenolic compound content and antioxidant activity of tomato and pepper fruits. In general, the aqueous extracts were more effective than the amendment treatment in providing maximum levels of total phenols and flavonoids, as well as antioxidant activity, particularly for the two algae *B. bifurcata* and *C. gibraltarica*. Similar results have shown that aqueous extracts of *A. nodosum* algae increase the total phenol and flavonoid content of fruit (Fan et al. 2011; Lola-Luz 2014). The same *A. nodosum* algae can act as a stressor due to its bioactive components. This stress enhances the defense system, leading to an increase in phenolic compound content, which explains the increase in phenolic compounds after addition of algal extracts (Alghamdi 2017). As a result, increased production of various phenolic compounds improves plant resistance to pathogen infection (Levine et al. 1994). We have also noted that algal extracts can be a promising source of new biologically active substances and compounds essential for human nutrition (Jimenez-Escrig et al. 2012).

Phenolic compounds undergo a redox reaction with the complex of phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent. This reaction varies according to the number of hydroxyl groups (OH) of the phenolic compounds (Singleton et al. 1999). However, this method is non-specific because the reagent can react with some amino acids (tyrosine and tryptophan), reducing sugars and sulfur compounds (Boizot and Charpentier 2006). Bruneton (1999) reported that phenolic compounds are generally soluble in polar organic solvents and aqueous solutions and are poorly soluble in apolar organic solvents, hence the choice to extract optimally with methanol-water (80-20; v/v). Similar results have been reported by several studies using the same extraction system and conditions from different plant parts (Ahmed et al. 2016).

In conclusion, this work presents results on the study of the effect of three brown algae *C. gibraltarica, B. bifurcata* and *F. spiralis* on the biochemical and phytochemical parameters of two vegetable plants (tomato and pepper) pots grown in greenhouse. In general, fertilization with the three brown algae improved the biochemical and phytochemical parameters of tomato and pepper. In particular, the two algae *B. bifurcata* and *F. spiralis* showed high efficacy on all parameters studied. Our results showed that treatment with aqueous extracts had a higher positive effect than treatment with amendments. It can also be noted that the algal species affects the various parameters studied. These three algae proved to be effective and good candidates for the development of biostimulants to improve the parameters studied.

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