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The Importance of Foliar Anatomy in the Taxonomy of the Genus Alocasia (Schott) G. Don

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Abstract

The anatomy of the epidermis, transverse sections of the leaves and petioles of three species of *Alocasia* (Schott) G. Don in Nigeria were examined in this study in order to report any anatomical characters of taxonomic importance among them. The species are *Alocasia cucullata* (Lour.) Schott, *A. macrorrhiza* (L.) G. Don, and *A. plumbea* (Schott) G. Don. Standard procedures were followed in preparing the epidermal peels, transverse sections of the leaves, and the three regions of the petiole: proximal, median and distal regions. Generic characters among the species include polygonal to irregular epidermal cell shape, a straight anticlinal wall pattern, elliptic and circular-shaped stomata, brachyparacytic stomatal complex type, round abaxial petiole outline and the presence of druses, raphides, and starch grains in the petiole. The presence of peltate trichomes on the adaxial surface of *A. cucullata* as well as additional anomocytic stomata complex on both of its surfaces and mucilaginous cells in *A. plumbea* which are the diagnostic features were discussed. Other diagnostic features include the presence of druses on the abaxial surfaces of *A. macrorrhiza* and *A. plumbea*, highest stomata index in *A. macrorrhiza*, collenchyma type, tannins and adaxial petiole outline. The *Alocasia* species can be separated based on their leaf anatomical features.

Keywords: Alocasia, Leaf anatomy, Mesophyll, Mucilaginous cells, Petiole anatomy, Stomatal complex, Trichome.

1. Introduction

Alocasia (Schott) G. Don, tribe Colocasieae, is a genus of broad-leaved rhizomatous perennials in the Family *Araceae*. Their leaves are usually glossy, and they can be easily identified wherever they grow (Bown, 2000). The genus has been reported to be the largest in the family with about one-hundred species distributed in the tropical, subtropical and temperate regions of the world (Boyce, 2008).

Species of this genus are also found growing in different habitats and exhibiting diverse habits. An example is *Alocasia macrorrhiza* which grows as a weed or as a creeping plant with aerial roots to help support it. The same plant is wild in Malaysia and is naturalized in many areas of the tropics. Another *Alocasia* with the ability to climb and produce aerial roots is *A. amazonica*, which is available for cultivation under the name African Mask. Some other species of *Alocasia* including *A. cucullata*, *A. sanderiana* (also known as Kris Plant, a native of the Philippine Islands), and *A. plumbea* are being cultivated as houseplants and ornamentals, and are naturalized throughout the tropics (Bown, 2000; Ivancic *et al.*, 2009).

The morphological attributes of the members of this genus have been variously described (Hussey and Keighery, 1997; Bown, 2000). The large cordate to hastate, occasionally peltate (especially in juveniles) leaves grow to a length of 20 - 90 cm on long petioles. The thick, glossy leaves often have marked margins or colourful midribs and veins. *Alocasia* species may flower at any time during the growing season. Flowers are spathe about $4\frac{1}{2}$ inches long which grow at the end of a short stalk, but are not conspicuous; often hidden behind the leaf petioles (Hussey and Keighery, 1997). Suratman and Suranto, (2016) evaluated the morphological, anatomical and isozyme variation among twenty accessions of giant taro (*A. macrorrhiza* (L.) G. Don) from different collection sites in Central Java (Indonesia). Chromosome number 2n = 28, 42, 56, 70 and 84 has been reported for the genus *Alocasia* (Mayo *et al.*, 1997); this affirms that polyploidy occurs in the genus.

This work is intended to fill the knowledge gap in the area of foliar and petiole anatomy of the genus *Alocasia*. Three species found growing in the South Western part of Nigeria were used for this research. They are *Alocasia cucullata* (Lour.) Schott, *Alocasia macrorrhiza* (L.) G. Don and *Alocasia plumbea* (Schott) G. Don.

2. Materials and Methods

2.1. Epidermal Studies

The scrape technique of Metcalfe (1960) adopted by Arogundade and Adedeji (2016) was used to obtain the epidermal peels of both the adaxial and abaxial surfaces of the leaves. The median portion of well-expanded leaves

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were peeled by placing the desired epidermal surface face down on a glass slide and then all tissues above the required epidermis were scraped off with a sharp blade. The epidermal peels were then stained in 1 % aqueous Safranin O, and mounted in dilute glycerine for microscopic examination. Photomicrographs of the epidermis were taken for both the adaxial and the abaxial surfaces.

The epidermal cell shape, anticlinal cell wall pattern, and stomata type were studied. The epidermal cell area was calculated by multiplying the length and width of the epidermal cells. Also calculated was the Stomata Indices (S.I) for the two surfaces using the formula below according to Metcalfe and Chalk (1979):

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

Where S.I = Stomata Index

S = Number of stomata

E = Number of ordinary epidermal cells plus the subsidiary cells in the same unit area.

2.2. Transverse Sections of the Leaf and the Petiole

The transverse sections of the leaves and the three regions of the petiole - the proximal, median and distal

regions of the tree species were cut with the aid of a Reichert sliding microtome at a thickness of $8 - 15 \mu m$. The sections were stained with Safranin O, and counterstained with Alcian blue. After that, they were made to pass through a series of ethanol (50, 70, 80, 90 % and absolute) for differentiation and dehydration. The sections were then mounted in 25 % dilute glycerin solution for examination under the microscope.

2.3. Microscopy

Observation and examination of the peels and sections were made using Olympus XSZ-107BN light microscope. Photomicrographs of the sections were made using photo micrographic apparatus- microscope with built-in-camera optics.

3. Results

3.1. Epidermal Studies

Tables 1 and 2 give the summary of the important qualitative and quantitative foliar epidermal features of the adaxial and abaxial surfaces of the species respectively. Figure 1 shows the photomicrographs of the two surfaces for all the species.

Table 1. Summary	y of important	qualitative foliar	epiderma	l features of the	e adaxial and	d abaxial	surfaces of	the studied species

Species	Surface	Epidermal cell shape	Anticlinal wall pattern	Stomata shape	Stomata type	Other features on surface	
A. cucullata	Adaxial	Polygonal	Straight	Elliptic, Circular	Brachyparacytic Anomocytic	Scales	
	Abaxial	Polygonal to Irregular	Straight	Elliptic, Circular	Brachyparacytic Anomocytic	Nil	
A. macrorrhiza	Adaxial	Polygonal to Irregular	Straight	Elliptic, Circular	Brachyparacytic	Nil	
	Abaxial	Polygonal to Irregular	Straight to wavy	Elliptic, Circular	Brachyparacytic Anomocytic	Druses	
A. plumbea	Adaxial	Polygonal to Irregular	Straight	Elliptic, Circular	Brachyparacytic	Nil	
	Abaxial	Polygonal to Irregular	Straight	Elliptic, Circular	Brachyparacytic Anomocytic	Mucilaginous cells and Druses	

Table 2. Summary of important quantitative foliar epidermal features of the adaxial and abaxial surfaces of the studied species

Species	Surface	Epidermal area (µm ²)			Stomata area (µm ²)			Stomata index (%)		
		Minimum value	Maximum value	Mean value	Minimum value	Maximum value	Mean value	Minimum value	Maximum value	Mean value
A. cucullata	Adaxial	523.6	935.0	720.12	214.2	306.0	275.81	2.33	6.25	3.64
	Abaxial	510.0	1142.4	768.94	238.0	336.6	274.31	7.62	10.64	9.24
A. macrorrhiza	Adaxial	374.0	680.0	532.85	190.4	299.2	229.98	5.84	8.70	7.41
	Abaxial	224.4	816.0	489.74	183.6	272.0	244.53	8.98	12.36	10.34
A. plumbea	Adaxial	299.2	822.8	526.32	183.6	272.0	217.87	4.29	6.56	5.40
	Abaxial	336.6	856.8	565.22	238.0	336.6	282.61	8.21	12.59	10.82

3.1.1. A. cucullata

On the adaxial surface, epidermal cells are largely polygonal with a straight anticlinal wall. They vary in size, shape, and arrangement. The epidermal cell area ranges between 523.6 μ m²-935.0 μ m²; with a mean value of 720.12 μ m². The leaf is amphistomatous and stomata

are restricted to the non-venous regions; brachyparacytic occasionally anomocytic, elliptic in shape, occasionally circular. Stomata size ranges between 214.2 μ m² -306.0 μ m²; with a mean value of 275.81 μ m² and the stomata index ranges between 2.33 -

69

6.25 %; with a mean value of 3.64 %. Scales are present (Figure 1 A and B).

On the abaxial surface, epidermal cells are largely polygonal, occasionally irregular with a straight anticlinal wall. They vary in size, shape and arrangement. Epidermal cell area ranges between $510.0 \,\mu m^2$ - $1142.4 \,\mu m^2$ with a mean value of 768.94 μm^2 . Stomata are restricted to the non-venous regions; brachyparacytic occasionally anomocytic, and they are elliptic in shape, occasionally circular (Figure 1C). Stomata size ranges between 238.0 μm^2 -336.6 μm^2 with a mean value of 274.31 μm^2 and the stomata index ranges between 7.62 - 10.64 % with a mean value of 9.24 %.

3.1.2. A. macrorrhiza

On the adaxial surface, epidermal cells are polygonal to occasionally irregular with a straight anticlinal wall. They vary in size, shape and arrangement. The epidermal cell area ranges between 374.0 μ m²-680.0 μ m² with a mean value of 532.85 μ m². Leaf is amphistomatous and stomata are restricted to the non-venous regions; brachyparacytic, elliptic in shape, occasionally circular (Figure 1D). Stomata size ranges between 190.4 μ m² - 299.2 μ m² with a mean value of 229.98 μ m², and the stomata index ranges between 5.84 - 8.70% with mean value of 7.41%.

On the abaxial surface, epidermal cells are polygonal to occasionally irregular with a straight to wavy anticlinal wall. They vary in size, shape and arrangement. Epidermal cell area ranges between 224.4 μ m²-816.0 μ m² with a mean value of 489.74 μ m². Stomata are restricted to the non-venous regions; they are brachyparacytic, occasionally anomocytic, and are elliptic in shape, occasionally circular. Stomata size ranges between 183.6 μ m² -272.0 μ m² with a mean value of 244.53 μ m² and stomata index ranges between 8.98 - 12.36 % with a mean value of 10.34 %. Druses are present (Figure 1E).

3.1.3. A. plumbea

On the adaxial surface, epidermal cells are polygonal to irregular with a straight anticlinal wall. They vary in size, shape and arrangement. Epidermal cell area ranges between 299.2 μ m² - 822.8 μ m² with a mean value of 526.32 μ m². The leaf is amphistomatous, and stomata are restricted to the non-venous regions; they are brachyparacytic and elliptic in shape, occasionally circular (Figure 1F). Stomata size ranges between 183.6 μ m² - 272.0 μ m² with a mean value of 217.87 μ m², and stomata index ranges between 4.29 – 6.56 % with mean value of 5.40 %.

On the abaxial surface, epidermal cells are polygonal to irregular with a straight anticlinal wall. They vary in size, shape and arrangement. Epidermal cell area ranges between $336.8 \ \mu\text{m}^2$ - $856.8 \ \mu\text{m}^2$ with mean value of $565.22 \ \mu\text{m}^2$. Stomata are restricted to the non-venous regions; they are brachyparacytic, occasionally anomocytic. Shape is elliptic, occasionally circular. Stomata size ranges between $238.0 \ \mu\text{m}^2$ - $336.6 \ \mu\text{m}^2$ with a mean value of $282.61 \ \mu\text{m}^2$ and stomata index ranges between $8.21 - 12.59 \ W$ with a mean value of $10.82 \ W$. Mucilaginous cells and druses are present (Figure 1G).



Figure 1. Genus *Alocasia* – Leaf epidermal surfaces

A and B. Adaxial epidermis of lamina of *A. cucullata;* C. Abaxial epidermis of lamina of *A. cucullata;* D. Adaxial epidermis of lamina of *A. macrorrhiza;* E. Abaxial epidermis of lamina of *A. macrorrhiza;* F. Adaxial epidermis of lamina of *A. plumbea;* G. Abaxial epidermis of lamina of *A. plumbea;* G. Abaxial epidermis of lamina of *A. plumbea;* G.

Legend: BP-Brachyparacytic stomata, AN-Anomocytic stomata

SC-Scales, MU-Mucilaginous cell Scale bar: $A - G = 125 \mu m$

3.2. Transverse Sections of the Leaf

A summary of the important features of the transverse sections of the leaf is shown in Table 3 while the photomicrographs are shown through Figures 2, 3 and 4.

3.2.1. A. cucullata

The Upper and lower epidermis are one-layered with uniseriate cells. Palisade and spongy layers are differentiated. The mesophyll thickness ranges from 134 μ m to 268 μ m. Palisade mesophyll cells are present only on the upper surface of the leaf and the mid-rib. They are cylindrical in shape, and packed together while the spongy mesophyll cells are oval to oblong or irregular in shape with air spaces in between them. Angular collenchyma cells are present in the mid-rib, and the vascular bundle type is Collateral. Few raphides and druses are present (Figure 2 A- H).

3.2.2. A. macrorrhiza

The upper and lower epidermis are both onelayered with uniseriate cells. Palisade and spongy layers are differentiated. The mesophyll thickness ranges from 174.2 μ m to 294.8 μ m. Palisade mesophyll cells are present only on the upper surface of the leaf, and the midrib. They are cylindrical in shape, and packed together. On the other hand, the spongy mesophyll cells are oval to oblong or irregular in shape with air spaces between them. Lacunar collenchyma cells are present in the mid-rib, and the vascular bundle type is Collateral. Raphides, druses, tannins, and starch grains are present. (Figure 3 A - H).

3.2.3. A. plumbea

The upper and lower epidermis are both one-layered with uniseriate cells. The mesophyll thickness ranges from 187.6 μ m to 348.4 μ m. Palisade and spongy layers are differentiated. Palisade mesophyll cells are present only on

the upper surface of the leaf and the mid-rib. They are cylindrical in shape and packed together, while the spongy mesophyll cells are oval to oblong or irregular in shape with air spaces between them. Lacunar collenchyma cells are present in the mid-rib, and the vascular bundle type is Collateral. Druses, an abundance of raphides and starch grains are present. (Figure 4 A - G).

Table 3. Summary of the Features on the Transverse Section of the Leaves of the Alocasia studied Species.

Characters	No. of	Collenchyma	No. of	Mesophyll	Thickness	Raphides	Druses	Tannins	Starch	
Species	Cells of Upper & Lower Epidermis	Cell Type	Layers of Palisade Mesophyll	Minimum (µm)	Maximum (µm)	Average (µm)	(+/-)	(+/-)	(+/-)	grains (+/-)
A. cucullata	1	Angular	1-2	134.0	268.0	180.63	+	+	-	-
A. macrorrhiza	1	Lacunar	1-2	174.2	294.8	209.93	+	+	+	+
A. plumbea	1	Lacunar	1-2	187.6	348.4	248.70	+	+	-	+



Figure 2.Transverse Section of the Leaf of A. cucullata A - Outline of transverse section. B - Mid-rib region. C - Leaf lamina. D - Leaf lamina. E & F - Raphides. G – Druses. H -Palisade layer and angular collenchyma cells. Scale bars: A= 15 μ m, B= 40 μ m, C-F= 120 μ m.

Figure 3. Transverse Section of the Leaf of A. macrorrhiza A - Outline of transverse section. B - Mid-rib region. C - Leaf lamina. D - Leaf lamina. E - Raphides and druses. F - Lacunar collenchyma cells. G – Druses. H - Vascular bundle and tannins Scale bars: A= 15 μ m, B= 40 μ m, C-H= 120 μ m.



Figure 4. Transverse Section of the Leaf of A. plumbea A - Transect of the outline of transverse section. B - Transect of the mid-rib region. C - Leaf lamina. D - Leaf lamina. E - Raphides. F - Druses. G - Lacunar collenchyma cells. Scale bars: A= 15 μ m, B= 40 μ m, C-G= 120 μ m

3.3. Transverse Sections of the Three Regions of the Petioles

3.3.1. A. cucullata

Proximal region: The petiole outline is circular. The epidermis is one-layered with uniseriate cells. The cortex is made up of one to five layers of parenchyma cells; the angular collenchyma cells occur as discontinuous bundles, and are separated by parenchyma cells. Collenchyma cells are present on both the adaxial and abaxial surfaces. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include spindle-shaped raphides. Druses are absent (Figure 5 A - G).

Median region: The petiole outline is circular. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to five layers of parenchyma cells; angular collenchyma cells occur as discontinuous bundles, and are separated by parenchyma cells. Collenchyma cells are present on both the adaxial and abaxial surfaces. Air spaces are present and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include spindle-shaped raphides, druses, and starch grains (Figure 6 A - G).

Distal region: The adaxial outline of the petiole is concave, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to five layers of parenchyma cells; angular collenchyma cells occur as discontinuous bundles on both the adaxial and abaxial surfaces. The collenchyma cells are separated by parenchyma cells. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include Unmodified and spindle-shaped raphides, druses, and starch grains (Figure 7 A - H).



Figure 5. Proximal region of A. cucullata

A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transects. E &F - Spindle-shaped raphides (Arrowed). G - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–G=100 μ m



Figure 6. Median region of *A. cucullata* A - Outline of petiole. B & C - Petiole transects. D - Druses (Arrowed). E & F - Spindle-shaped raphides (Arrowed). G - Vascular bundle. Scale bars: $A = 10 \ \mu m$, $B = 30 \ \mu m$, $C - G = 100 \ \mu m$.



Figure 7. Distal region of A. cucullata

A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transects. E - Druses (Arrowed). F & G - Spindle-shaped and unmodified raphides (Arrowed). H - Vascular bundle. Scale bars: A & B=10 μ m, C = 30 μ m, D–H=100 μ m

3.3.2. A. macrorrhiza

Proximal region: The adaxial outline of the petiole is convex, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to six layers of parenchyma cells; lacunar collenchyma cells occur as discontinuous bundles and are separated by parenchyma cells. Collenchyma cells are present on both the adaxial and abaxial surfaces. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include spindle-shaped raphides, druses, tannins, and starch grains (Figure 8 A -H).

Median region: The adaxial outline of the petiole is convex, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to six layers of parenchyma cells; lacunar collenchyma cells occur as discontinuous bundles and separated by parenchyma cells. Collenchyma cells are present on the abaxial surface only. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include spindle-shaped raphides, druses, tannins, and starch grains (Figure 9 A - G).

Distal region: The adaxial outline of the petiole is concave, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to five layers of parenchyma cell; lacunar collenchyma cells occur as discontinuous bundles, and separated by parenchyma cells. Collenchyma cells are present on the abaxial surface only. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include spindle-shaped raphides, druses, and starch grains (Figure 10 A - H).





Figure 8. Proximal region of A. macrorrhiza

A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D- Petiole transects. E - Spindle-shaped raphides (Arrowed). F - Spindle-shaped raphide and druse (Arrowed). G - Druse (Arrowed). H - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–H=100 μ m



Figure 9. Median region of *A. macrorrhiza* A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transect. E - Druses (Arrowed). F - Spindle-shaped raphide and druse. G - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–G=100 μ m



Figure 10.**Distal region of** *A*. *macrorrhiza* A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transect. E & F - Spindle-shaped raphides (Arrowed). G - Druses in the region (Arrowed). H - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–H=100 μ m.

3.3.3. A. plumbea

Proximal region: The adaxial outline of the petiole is flat, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to six layers of parenchyma cells; lacunar collenchyma cells occur as discontinuous bundles, and are separated by parenchyma cells. Collenchyma cells are present on both the adaxial and abaxial surfaces. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include numerous spindle-shaped raphides, very few druses, and starch grains (Figure 11 A - H).

Median region: The adaxial outline of the petiole is concave, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to six layers of parenchyma cells; lacunar collenchyma cells occur as discontinuous bundles, and are separated by parenchyma cells. Collenchyma cells are present on the abaxial surface only. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include numerous spindle-shaped raphides, very few druses, and starch grains (Figure 12 A - G).

Distal region: The adaxial outline of the petiole is concave, while the abaxial outline is round. The epidermis is one-layered with uniseriate cells. The cortex is composed of one to seven layers of parenchyma cells; lacunar collenchyma cells occur as discontinuous bundles, and are separated by parenchyma cells. Collenchyma cells are present on the abaxial surface only. Air spaces are present, and are surrounded by parenchyma cells. Vascular bundles are collateral, and are scattered throughout the ground tissue. The xylem is surrounded by xylem parenchyma. Cell inclusions include numerous spindleshaped raphides, druses, and starch grains (Figure 13 A -H).



Figure 11. Proximal region of *A. plumbea* A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transects. E & F - Spindle-shaped raphides (Arrowed). G - Druse (Arrowed).H - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–H=100 μ m.



Figure 12. Median region of *A. plumbea* A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transects. E - Spindle-shaped raphides (Arrowed). F - Druses (Arrowed). G - Vascular bundle Scale bars: A & B= 10 μ m, C = 30 μ m, D-G=100 μ m.



Figure 13. Distal region of *A. plumbea* A - Abaxial outline of petiole. B - Adaxial outline of petiole. C & D - Petiole transects. E - Spindle-shaped raphides and druse (Arrowed). F - Spindle-shaped raphides (Arrowed).G - Druse (Arrowed). H - Vascular bundle. Scale bars: A & B= 10 μ m, C = 30 μ m, D–H=100 μ m.

4. Discussion

The epidermal characters of the three species of Alocasia show more similarities than differences. This is actually expected since the three species are of the same genus. They all have polygonal to irregular epidermal cell shape, a straight anticlinal wall pattern, elliptic and circular-shaped stomata as well as brachyparacytic stomatal complex types. The few exceptions to these include the polygonal epidermal cell shape found only on the adaxial surface of A. cucullata, additional wavy anticlinal wall patterns found on the abaxial surface of A. macrorrhiza, and additional anomocytic stomatal complex found on both surfaces of A. cucullata and the abaxial surfaces of the other two species, that is, A. macrorrhiza and A. plumbea. In as much as the frequency of stomata can be affected by the environment, the specific stomata types are only determined by genetic factors (Hetherington and Woodward, 2003). Therefore, the species of Alocasia in this study can be separated based on the stomatal complex type. A. cucullata is the only species with anomocytic stomatal type on both the adaxial and abaxial leaf epidermal surfaces.

Suratman and Suranto (2016) conducted an anatomical epidermal research on twenty accessions of *A. macrorrhiza* (Giant Taro) in Indonesia, and reported the absence of stomata on the adaxial surfaces. This is contrary to the findings of this research. Brachyparacytic stomata are present on the adaxial surface of *A. macrorrhiza* and, interestingly, the stomata index is the highest on this same surface when compared with the other two species in this study. One factor that could be responsible for this observation is the fact that all the *A. macrorrhiza* accessions used for this study were found growing in

water-logged areas, so the higher stomata index is an indicator for higher transpiration rate. On the whole, there were more stomata on the abaxial surfaces than on the adaxial surfaces of all the species. Higher stomata frequency on abaxial surfaces in most plant species have been confirmed by several researchers (Muradoglu and Gundogdu, 2011; Suratman and Suranto, 2016). Mean epidermal cell area was higher on the abaxial surfaces of *A. cucullata* and *A. plumbea* which is a deviation from the usual adaxial epidermal cell area higher than the abaxial epidermal cell area.

Mucilaginous cells were found occurring on the abaxial surface of *Alocasia plumbea* as distinct cells from the other epidermal cells. This is most likely responsible for the slimy nature of the leaves on squeezing, and clearly separates *Alocasia plumbea* from the other two species of the studied *Alocasia*. According to Bredenkamp and VanWyk (1999), mucilage in plants plays a role in the storage of water and food, seed germination and membrane thickening. Druses of calcium oxalate crystals were found on the abaxial surfaces of *A. macrorrhiza* and *A. plumbea*, but not on any of the surfaces of *A. cucullata*. This is another important foliar epidermal delimiting feature among the species. Adedeji and Illoh (2004) also separated some species of *Hibiscus* based on the presence of druses in them.

Scales, a type of trichome, also known as peltate hair, were encountered on the adaxial surface of *A. cucullata* bringing a clear-cut demarcation between *Alocasia cucullata* and the other two species of the *Alocasia* in this study. Trichomes function in the reduction of the heating effect of sunlight, thereby controlling the rate of transpiration in plants where they occur (Pandey, 2007). Trichomes types have been used severally in the classification of genera and even species (Gravano *et al.*, 1998; Adedeji and Illoh, 2004; Fróes *et al.*, 2015). Ina and Eka (2013) also separated some species of *Alocasia* in Indonesia based on the presence of multicellular trichome in just one of the ten species studied.

An interesting aspect of this research is the observation of definitive ways by which each of the species of *Alocasia* maintain its water retaining capacity. Each of these species has a unique feature on its epidermis which enables it to adapt to its environment in terms of water conservation. *A. cucullata* possess scales on its adaxial surface in order to reduce transpiration rate; *A. macrorrhiza* found growing in water-logged areas have the highest stomata index so as to increase its transpiration rate and *A. plumbea* possess mucilaginous cells in order to conserve more water in its system. These distinctive features are very important in the taxonomy of the genus.

The transverse sections of the leaves of the *Alocasia* species studied are similar in the number of upper and lower epidermal layers, the presence of palisade mesophyll on the adaxial surface of the mid-rib, differentiated palisade and spongy mesophyll layers, the number of palisade mesophyll layers in the leaf lamina and the presence of calcium oxalate crystals - raphides and druses. Notwithstanding, there are qualitative and quantitative features that can be employed in the delimitation of the species. Angular collenchyma cells, found in the transverse sections of the leaf of *A. cucullata* only, delimit it from the other two species with lacunar collenchyma

type. Tannins were also observed on the lamina surface of *A. macrorrhiza* only. Tannins are non-nitrogenous complex compounds, and are the derivatives of phenols (Pandey, 2007). Their primary function is to serve as protection for plants against injury, decay termites, and pests (Simpson, 2010). The highest mesophyll thickness was encountered in *A. plumbea*, and the least was seen in *A. cucullata*.

The adaxial outline of the petioles presents a delimiting feature among the species studied as it ranges from concave to flat to round and then to a convex outline. Two different combinations of each of these outlines were found in each of the species. It is round in the proximal and median regions of A. cucullata, and concave in the distal region. It is convex in the proximal and median regions of A. macrorrhiza, and concave in the distal region. It was found to be flat in the proximal region of A. plumbea and concave in the median and distal regions. The abaxial petiole outline, on the other hand, is a unifying feature as it is round in all the species. The distribution of cells in the transverse section of the three regions of the petioles of the Alocasia species is similar to that of the leaf. A one-layered epidermis with uniseriate cells was found in all the species. Also the exact collenchyma cell types present in the leaves were found in the petioles, and tannins were encountered only in A. macrorrhiza. These can also be employed in separating the species. Collateral vascular bundles scattered in the ground tissues were found in the three regions of all the species studied, and all the xylem cells are surrounded by xylem parenchyma. This affirms the monocotyledonous status of the genus Alocasia (Fahn, 1974; Simpson, 2010).

5. Conclusion

This study concludes that the species of *Alocasia* can be classified using the foliar anatomical characters such as the presence of peltate trichomes, mucilaginous cells, anomocytic stomata type, angular collenchyma cells, tannins, and adaxial petiole outline. Each of the three studied species was observed to have developed some unique features in order to improve or maintain their water retaining capacity. All these features are quiet diagnostic in the taxonomy of the species.

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