

Full Length Research Paper

Anatomical characteristics of Nigerian variants of *Caladium bicolor* (Aiton) Vent. (Araceae)

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Four *Caladium bicolor* variants collected from different parts of Nigeria were subjected to anatomical comparison to enhance the taxonomic status of the species. Fresh samples (leaf and petiole) of these variants were fixed in formalin, acetic acid and alcohol (FAA), dehydrated in alcohol series, peeled or sectioned. Peeled specimens were stained with safranin, while sectioned ones were stained with Alcian blue and counterstained with safranin. Good preparations were mounted on slides, viewed and photographed with Optika B-1000 FL LED microscope. Epidermal cells from the variants are mainly pentagonal-hexagonal but rarely heptagonal while the anticlinal cell walls are mainly straight and partly arched/curved. Variants A, B and D are amphistomatic while variant C is hypostomatic. Isotricytic, anisocytic, tetracytic, anomocytic and contiguous stomata were observed among the taxa. The stomata index (SI) varied from 4.35 to 11.76 on the adaxial surface, and from 6.25 to 47.62 on the abaxial surface. Calcium oxalate crystals (druses, 8.18 to 19.09 μm and raphides, 21.82 to 68.18 μm) occur in all variants. Raphides are predominantly found in the petiole while druses and raphides are found in the midrib and petiole. The shapes of the adaxial surface of the midrib are relatively different from each other, and include curved (convex) and flat surfaces. The leaf lamina comprised one layer of palisade and spongy mesophylls each. The number of vascular bundles varied from 11 to 23 in the midrib. These characters can be used to distinguish these taxa especially when combined with the existing data on the species. The similarities among these variants point towards the same evolutionary origin; however may suggest that intraspecific or interspecific hybridizations may have produced the variants.

Key words: Amphistomatic, *Caladium*, druses, hypostomatic, midrib, petiole, raphides.

INTRODUCTION

Caladium bicolor (Aiton) Vent. belongs to the family Araceae and is characterized by various leaf colourations (Hutchinson and Dalziel, 1954). There are over 2000 cultivar names for *Caladium*, and over a hundred cultivars are grown today (Wilfret, 1993). The genus *Caladium*

comprises 12 species (Mayo et al., 1997), of which *C. bicolor* is the major source of cultivars. *C. picturatum* and *C. marmoratum* are now considered synonyms of *C. bicolor* (Madison, 1981). It is a monotypic genus that is found in most West African countries (Hutchinson and

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Table 1. Description of voucher specimens studied.

S/N	Species name	Herbarium number	Locality	Date of collection	Coordinates
1	<i>C. bicolor</i> (variant A)	UPH/V/1176	Ngor Okpala, Imo State	30/04/2015	N05° 25' 27.60"; E007° 11' 48.10"
2	<i>C. bicolor</i> (variant B)	UPH/V/1128	University of Port Harcourt, Abuja Park, Rivers State	19/09/2014	N04° 55' 29.80"; E006° 55' 40.20"
3	<i>C. bicolor</i> (variant C)	UPH/V/1126	Obiga-Asa, Ukwu-West L.G.A. Abia State	09/07/2014	N04° 55' 45.40"; E007° 14' 28.70"
4	<i>C. bicolor</i> (variant D)	UPH/V/1242	Opposite Afam Power Plant, Rivers State	15/04/2015	N05° 21' 49.20"; E006° 57' 30.00"

Dalziel, 1954).

In South and Central America other species of *Caladium* like *C. marmoratum* Mathieu, *C. picturatum* C. Koch, and *C. schomburgkii* Schott including *Caladium bicolor* (Aiton) Vent. have been reported (Birdsey, 1951; Hayward, 1950; Wilfret, 1993). Breeding of this species of plant has led to many variants (Wilfret, 1993). Also, intraspecific or interspecific hybridizations among these American species may have produced the cultivated species *C. hortulanum* and other variants of economic values (Hayward, 1950; Birdsey, 1951; Wilfret, 1993; Deng and Harbaugh, 2006a). Members of this genus are used as ornamentals and other economic purposes (Evans et al., 1992; Deng et al., 2005).

The anatomy of vascular and support tissues in the leaf and petiole in Araceae showed and inferred an interesting relationship among them (Keating, 2000, 2002, 2004; Goncalves et al., 2004). These could suggest correlations between changes in vascular bundle and collenchyma characters and the appearance and further evolution of the unisexual-flowered, aperiogonate aroids (Natalie et al., 2011). Morpho-anatomical character patterns seem to imply a major adaptive shift in the evolution of aroids (Hesse, 2006a, b), and could be used to distinguish closely related plants (Kemka-Evans et al., 2014; Osuji and Nwala, 2015).

The naming and identification of *C. bicolor* cultivars is difficult. This is due in part to the lack of up-to-date reference material which illustrates all cultivars in colour, as well as the sale of cultivars without reliable names (Jin et al., 1999). Among the Araceae family, calcium oxalate deposits can be diagnostic, including their presence, type, diversity, occurrence and distribution have been noted to enhance the delimitation of members of this family (Osuji and Nwala, 2015; Osuji, 2013; Nurul et al., 2013; Mais and Amal, 2012; Gary, 2009) and raphide idioblasts are known as storage facilities for Aroids (Okoli, 1988; Okoli and Green, 1987; Okoli and McEuen, 1986).

Except the morphological description by Metcalfe and

Chalk (1968), there is a dearth of information on the biology and taxonomic status of Nigerian species of *Caladium*. The ornamental and economic values of these species and their variants underscore the need for their taxonomic characterization using different systematic lines of study. This study bridges the gap by providing information on the biology and comparative anatomical features (including calcium oxalate crystal types) of four *C. bicolor* variants in Nigeria. The results of the study shall enhance the establishment of the taxonomic status of the species.

MATERIALS AND METHODS

Source of materials

The materials for this study were collected from different parts of the country (Table 1), properly identified using Flora of West Tropical Africa (Hutchinson and Dalziel, 1954), and deposited at the University of Port Harcourt Herbarium. Live specimens are being maintained at the Ecological Center, University of Port Harcourt.

Petiole and midrib studies

Subsamples from the original materials (Table 1) were fixed in formalin, acetic acid and alcohol (FAA) for 12 h. Thereafter, the specimens were dehydrated in ethanol series of different concentration (30 and 50%) and stored in 70% ethanol until when needed. The leaf portion of the petiole, and the central portion of the midrib were sectioned following the method of Agbagwa et al. (2007). The sections were stained in Alcian blue and counter-stained with 1% Safranin red for two minutes, mounted on a slide, viewed and photographed with an Optika B-1000 FL LED microscope.

Epidermal studies

Foliar materials for epidermal studies were collected fresh from plants in the Ecological Centre. 0.5 to 1 cm square leaf cuttings were obtained from identical regions of each fresh leaf, generally from mid-way between the leaf base and apex of lamina including

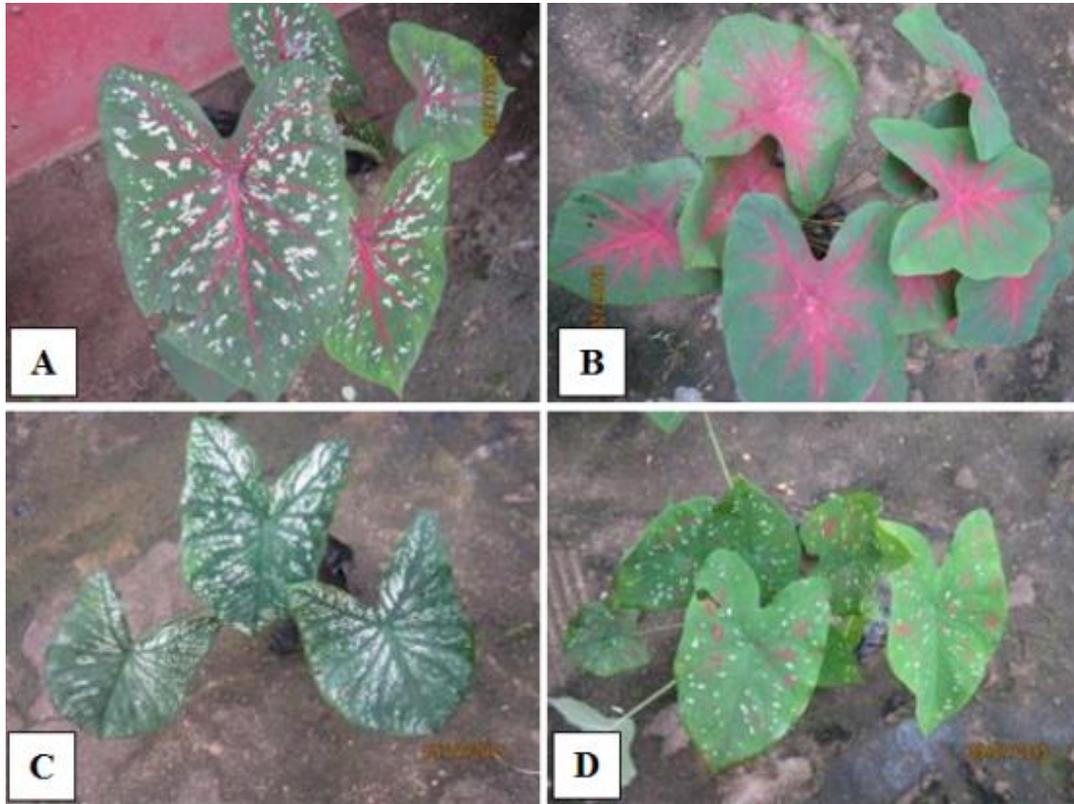


Figure 1. (A-D) Variants of *C. bicolor* collected from different locations in Nigeria.

the mid-rib. The adaxial and abaxial epidermal peels were obtained using sharp pointed forceps. Peels were stained with 1% safranin or alcian blue, rinsed with distilled water to remove excess stain and mounted in a drop of pure glycerol on clean glass slides. A cover glass was placed over the drop and sealed with nail varnish to prevent dehydration (Okoli and Ndukwu, 1992). The epidermal features that were observed include: organization of the epidermis, arrangement of the epidermal cells, nature of trichomes, shape of epidermal cells and nature of the anticlinal cell wall of the leaf epidermis, stomatal types, density and index. The stomatal index (SI) was determined based on Metcalfe and Chalk (1979), while the terminology for the stomatal type is taken after Malvey (2004).

Statistical analysis

The average (mean), standard deviation (STD) and range were determined using IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Four variants of *C. bicolor* were identified. These specimens display a variety of colors including red, white, pink, yellow and green, and are tagged *C. bicolor* var. A, B, C and D (Figure 1). The coloration of the collections conformed to previous reports by Metcalfe and Chalk (1968). These authors reported that the genus *Caladium* in West Africa is monotypic with various colorations.

Epidermal characteristics

No variation was observed on the shapes of the leaf epidermal cell among the different variants of *Caladium* studied. For instance, the shape of the upper (adaxial) and the lower (abaxial) epidermal surfaces in variant A, C and D are mainly pentagonal-hexagonal but rarely heptagonal, while the anticlinal cell wall are mainly straight and partly arced/curved. However, in variant B the shape of the adaxial and the abaxial epidermal surfaces are pentagonal-hexagonal (Figures 2 and 3, Table 2).

Variants A, B and D are amphistomatic (with stomata on both surface of the leaf) while variant C is hypostomatic (stomata on only one surface). This character makes this variant different from the other variants studied. The stomata types observed among the different *Caladium* variants are isotricytic, anisocytic, tetracytic, anomocytic and contiguous (Figures 2 and 3, Table 2). Among these stomata types, tetracytic and anomocytic stomata were dominant (Figures 2 and 3) and were observed on both leaf surfaces of variants A, B and D while in variant C they were recorded only on the lower surface (Table 2).

Isotricytic and anisocytic occurred only on the lower leaf surface of variant D. The occurrence of the same stomata type among these variants of *Caladium*

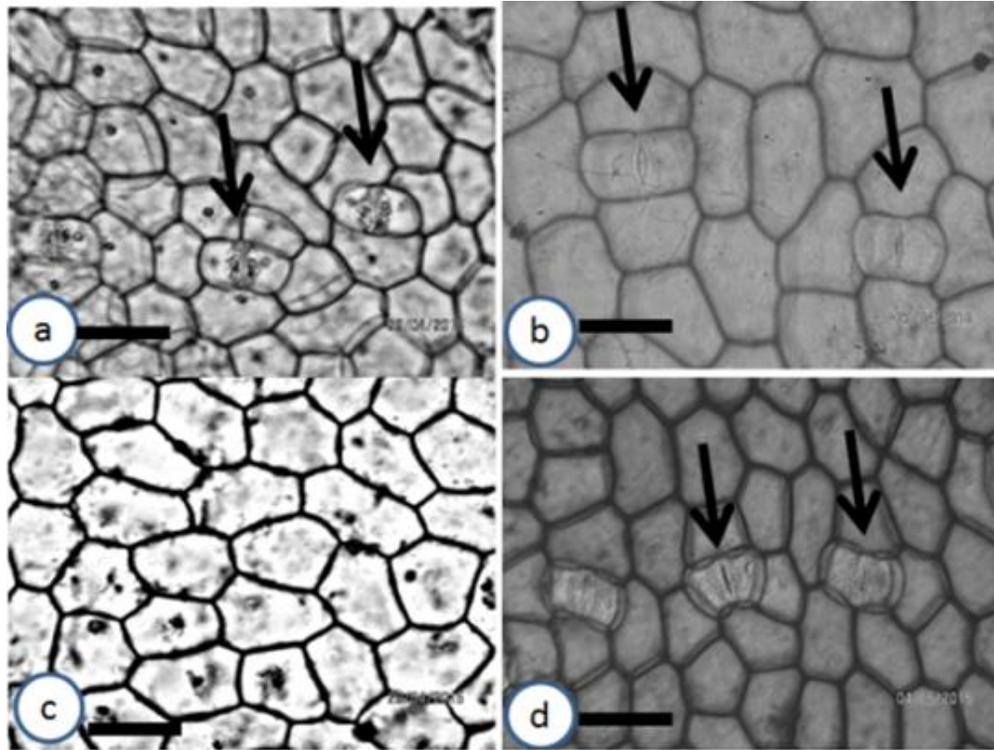


Figure 2. Upper epidermal surface of *C. bicolor* variants A to D. Bar = 36 μ m and arrows show stomata.

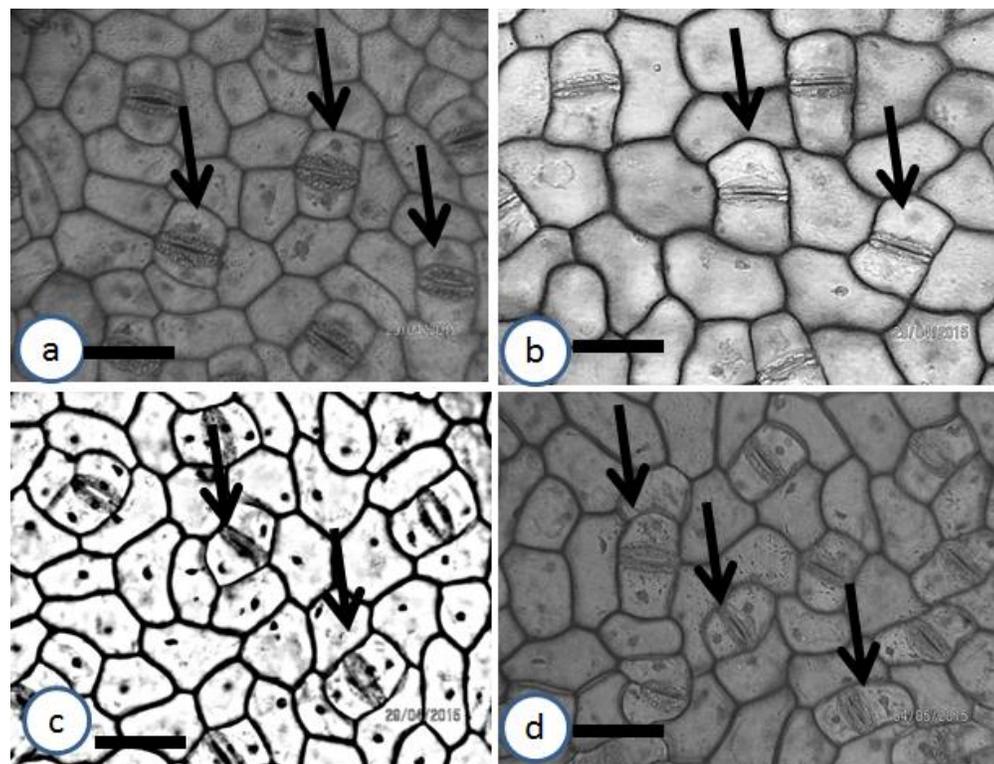


Figure 3. Lower epidermal surface of *C. bicolor* variants A to D. Bar = 36 μ m and arrows show stomata (a – d).

Table 2. Upper and lower epidermal characteristics of the *Caladium* variants studied.

S/N	Species name	Lower epidermis				Upper epidermis			
		Shape of epidermal cell	Nature of anticlinal cell wall	Stomata type	Stomatal index (SI)	Shape of epidermal cell	Nature of anticlinal cell wall	Stomata type	Stomatal index (SI)
1	<i>C. bicolor</i> (variant A)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Mainly tetracytic and rarely anomocytic	25.93-37.5 (31.26±4.05)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Mainly tetracytic and rarely anomocytic	6.25 - 8.11 (7.08±0.94)
2	<i>C. bicolor</i> (variant B)	Pentagonal-hexagonal	Mainly straight and partly arced/curved	Mainly tetracytic and rarely anomocytic	22.22 - 37.50 (30.92±5.93)	Pentagonal-hexagonal	Mainly straight and partly arced/curved	Mainly tetracytic and rarely anomocytic	4.35- 11.76 (6.94±3.01)
3	<i>C. bicolor</i> (variant C)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Mainly tetracytic and rarely anomocytic	6.25-36.36 (19.93±10.15)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Absent	0.00
4	<i>C. bicolor</i> (variant D)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Isotricytic, anisocytic, tetracytic, anomocytic and contiguous stomata	26.67- 47.62 (36.64±7.68)	Mainly pentagonal-hexagonal but rarely heptagonal	Mainly straight and partly arced/curved	Tetracytic, anomocytic and contiguous stomata	4.55 - 8.82 (6.31±2.24)

Table 3. Number of vascular bundles in the midrib and petiole of the *Caladium* variants.

S/N	Species name	Petiole	Midrib
1	<i>C. bicolor</i> (variant A)	19	19
2	<i>C. bicolor</i> (variant B)	27	11
3	<i>C. bicolor</i> (variant C)	22	13
4	<i>C. bicolor</i> (variant D)	22	23

indicates very close phylogeny as shown in *Sphenostylis stenocarpa* (Hochst ex A. Rich) Harms by (Nyananyo and Osuji, 2007). The presence of isotricytic, anisocytic and contiguous stomata on variant D distinguishes it from the other variants. In the same vein, the presence of brachyparacytic stomata in the abaxial leaf epidermis of an accession of *Xanthosoma* 'Ede Uhie' and its absence in other cultivars indicate

divergent advancement in its evolution (Osuji and Nwala, 2015).

This suggests evolutionary divergence between *Caladium* variant D and the other variants. In a similar study among the Araceae, different stomata types namely anomocytic, actinocytic, paracytic, cyclocytic and transitional types between paracytic and cyclocytic have been noted (Wang and Zhao, 2002). They observed that the epidermal cells are nearly isodiametric in outline with straight, arched and undulate anticlinal walls. Striate ornamentation occurs on periclinal walls of epidermal cells in some species. They noted that though the stomatal apparatus types in Araceae are of little taxonomical significance at intra-family level of Araceae, the combined characters of stomatal apparatuses, the shape of anticlinal wall and ornamentation of cuticles in guard cells may be useful for species identification (Wang and Zhao, 2002; Osuji and Nwala, 2015).

The stomatal index (SI) varied from one variant to another. For instance, the stomatal index on the abaxial surface include- 25.93 to 37.5 (31.26±4.05), 22.22 to 37.50 (30.92±5.93), 6.25 to 36.36 (19.93±10.15) and 26.67 to 47.62 (36.64±7.68) for variants A, B, C and D respectively (Table 3). On the other hand, the stomatal index on the adaxial surface of the variants varied from 4.55 to 8.82 (6.31±2.24) in variant D to 6.25 to 8.11 (7.08±0.94) in variant A, and 4.35 to 11.76 (6.94±3.01) in B. The differences in stomata types, their distribution and indices on the leaf surfaces of these *Caladium* variants are diagnostic and could be used to distinguish among variants.

In this study, variants A and B have the same stomata types but the indices varied slightly from each other. Also, variant C has the same stomata types with A and B but it is hypostomatic while variant D has the same stomata types with these

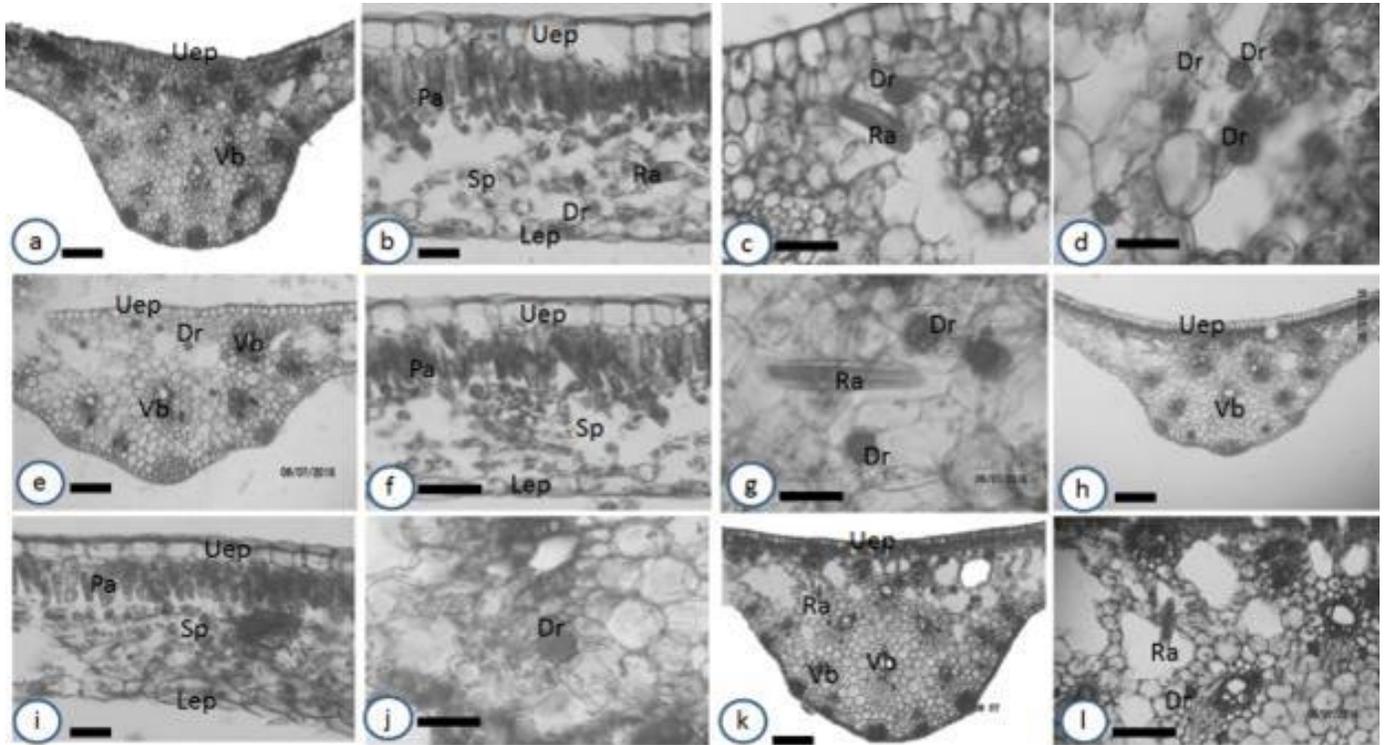


Figure 4. Midrib and leaf lamina of *C. bicolor* variants- (a-d) – variant A; (e-g) – variant B; (h-i) – variant C and (k-l) – variant D; Vb = vascular bundle; Uep = upper epidermis; Pa = palisade mesophyll; Sp = spongy mesophyll; Lep = lower epidermis; Dr = druse crystal; Ra = raphide bundle; bar = 40µm.

variants in addition to isotricytic, anisocytic and contiguous stomata. This finding however conforms with Osuji and Nwala (2015) who noted the presence of stomata on both upper and lower epidermis of both *Colocasia* and *Xanthosoma* spp. They further noted that stomata were more on the lower epidermis than the upper epidermis. Also, they observed epidermal variations and differences in stomatal indexes within the cultivars of *Xanthosoma* and *Colocasia* and suggested that this could account for their ecological adaptation to variation in the degree of wetness of the environment. Also, stomata types and nature of the epidermal cells have been of diagnostic importance in other members of angiosperm namely *Emilia* (Ndukwu and Agbagwa, 2006), *Abrus* (Agbagwa and Okoli, 2006), *Vernonia* (Kemka-Evans et al., 2014) and *Ixora* (Essienn and Umoh, 2014).

Shape of midrib and petiole

The shapes of the adaxial surface of the midrib are relatively different from each other. In variants A (Figure 4a) and C (Figure 4h), the shapes are curved (convex); in variant B (Figure 4e), it is flat while in variant D it is relatively flat (Figure 4k). This character is fairly diagnostic among the different variants. The leaf lamina comprised one layer of palisade and spongy mesophylls each

(Figures 4b, f and i). The upper epidermal cells in the lamina of all the variants are isodiametric or oval. They elongate periclinally (Figures 4b, f and i). Also, the shapes of the petiole in all the variants studied are oval (Figures 5a, c, e and g). These similarities confirm that the variants have the same evolutionary origin and are of the same monotypic taxon (Metcalf and Chalk, 1968); however intraspecific hybridizations may have produced the variants (Hayward, 1950; Birdsey, 1951; Wilfret, 1993; Deng and Harbaugh, 2006b).

Number of vascular bundle in the midrib and petiole

Variations in number and arrangement of vascular bundles in petiole and midrib have been used to differentiate species of the same genus or family (Ekeke and Mensah, 2015; Agbagwa and Ndukwu, 2004; Metcalfe and Chalk, 1979). For instance, in their studies, Ekeke and Mensah (2015) noted that the number of vascular traces in the midrib of members of Asteraceae varied from one species to another and is diagnostic. Also, Agbagwa and Ndukwu (2004) reported that the number of vascular bundles in the petioles of members of *Cucurbita* could be used to distinguish them (*C. moschata*, 10; *C. maxima*, 16 and *C. pepo*, 14).

In this study, the number of vascular bundles in the midribs and petioles varied from one variant to another

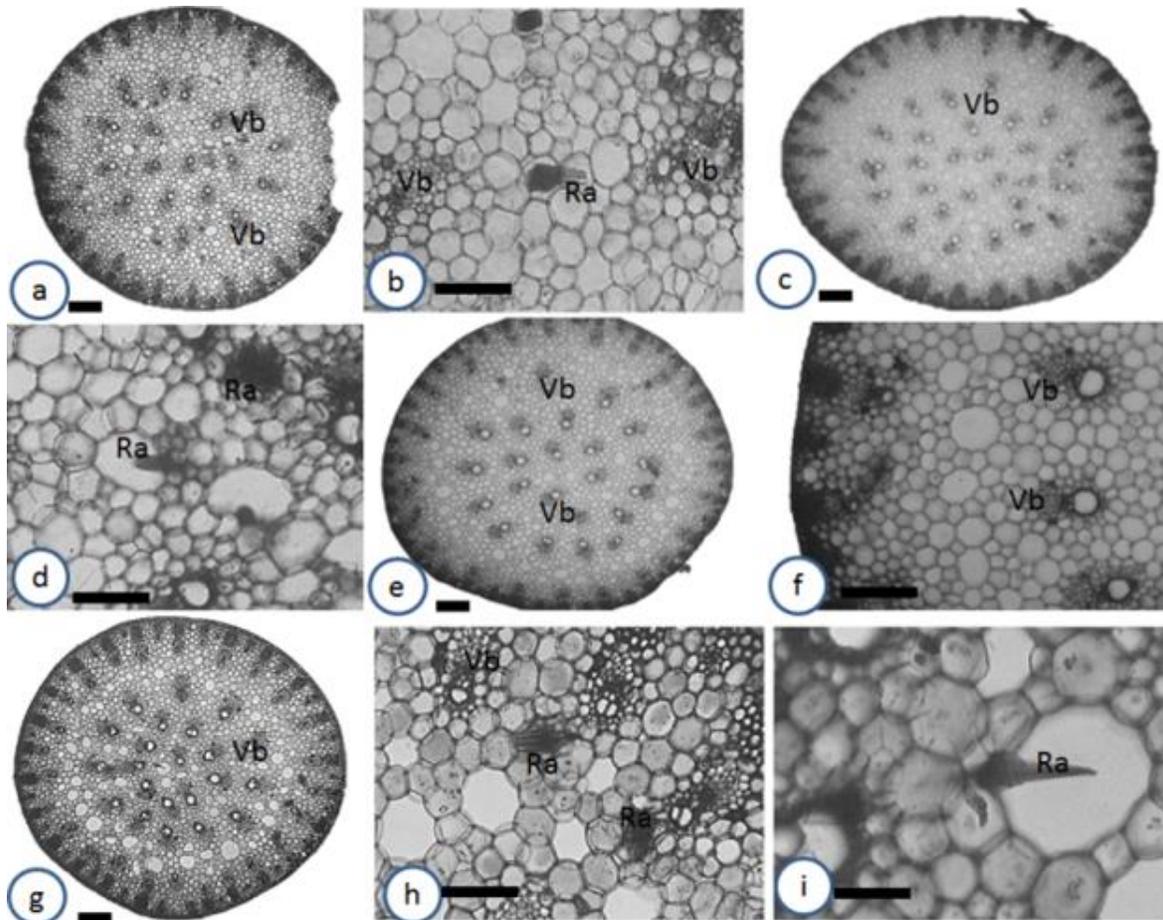


Figure 5. Petiole anatomy of *C. bicolor* (a-d) – variant A; (e-g) – variant B; (h-j) – variant C and (k-l) – variant D; Vb = vascular bundle; Ra = raphide bundle; bar = 42µm.

(Table 3). The number of vascular bundles in the petiole of the different variants are variant A (19), variant B (27), variant C (22) and variant D (22). In the midrib (Table 3), variant A has 19 vascular bundles, variant B (11), variant C (13) and variant D (23). This variation in the number of vascular bundles is diagnostic and could be used to distinguish the different *Caladium* variants. Though variants C and D had the same number of petiolar vascular bundle, the number of vascular bundle in their midrib differed and could be used to differentiate them. This finding therefore supports that the variation in number of vascular bundle in the petiole and midrib are diagnostic (Ekeke and Mensah, 2015; Agbagwa and Ndukwu, 2004; Metcalfe and Chalk, 1979) and however may suggest that intraspecific or interspecific hybridizations may have produced the variants.

Calcium oxalate types and sizes

Two main calcium oxalate crystal types were observed among the taxa studied. These are druses and raphides

(Table 4, Figures 4 and 5). The druses are ubiquitous, raphides are predominantly found in the petiole while druses and raphides are found in the midrib. The occurrence of some of these crystals is specific or restricted to some tissues. For instance, among the taxa studied, the occurrence of the raphides is specific. They are found in the midrib and petiole but not in the lamina. This observation has been made in *Dieffenbachia seguine* (Araceae) (Gray, 2009). He noted that druses appear to be nearly ubiquitous throughout this plant occurring in most of plant parts except in the ovaries.

Furthermore, he observed that different portions of the same organ may have different crystal types such as the leaf margins which have a greater density of druses than the lamina, as well as overlapping raphide bundles not present in the lamina (Gray, 2009).

In the lamina, the calcium oxalates (druses) are embedded in the palisade and spongy mesophylls. They are identified by their dark and spiny nature. The accumulation of the calcium oxalate crystals in the leaf suggests that the taxa are poisonous. This explains why the leaves are not normally consumed by animals and

Table 4. Anatomical characteristics of the petiole and midrib.

S/N	Species name	Petiole		Midrib		Calcium oxalate sizes Range (Mean±standard deviation) µm	
		Shape of petiole	Calcium oxalate type bundle	Shape of upper epidermal surface	Calcium oxalate type bundle	Druses	Raphides
1	<i>C. bicolor</i> (variant A)	Oval	Raphide	Curved (Convex)	Raphide and druse	10.91-13.64 (12.43±1.27)	21.82- 40.91 (31.52±7.85)
2	<i>C. bicolor</i> (variant B)	Oval	Raphide	Flat	Raphide and druse	10.91-15.00 (13.03±1.54)	31.36-68.18 46.82±13.91)
3	<i>C. bicolor</i> (variant C)	Oval	Raphide	Curved (Convex)	Raphide and druse	9.55-19.09 (13.03±2.74)	27.27-43.64 (39.85±5.05)
4	<i>C. bicolor</i> (variant D)	Oval	Raphide	Relatively flat	Raphide and druse	8.18-15.00 (11.52±2.27)	24.55-49.09 (36.21±8.35)

humans except after being properly processed. These crystals could therefore be a source of defense to the plant against herbivores. This observation is in line with previous studies on *Xanthosoma* and *Colocasia* (Osuji, 2013; Uno et al., 2001).

The size of druses varied from 8.18 µm in variant D to 19.09 µm in variant C which corresponds to smallest and largest sizes of druse found in *Caladium* variants studied (Table 3). However, the sizes of the raphides ranged from 21.28 µm in variant A to 68.18µm in variant B (Table 4). In similar study in Araceae, Gray (2009) recorded different sizes of crystals in *Dieffenbachia seguine*. In this present study, the sizes of the calcium oxalate observed among the different variants include; druses (8.18 µm to 19.09 µm) and raphides (21.28 µm to 68.18 µm). These ranges fall within 1.0 µm to 250 µm recorded in *Amorphophallus muelleri* (Nurul et al., 2013).

Though there is difference between this value and that recorded in *Caladium* variants, this result supports the placement of both genera in the same family as having the same evolutionary origin. They are genetically controlled (Mais and Amal, 2012) and are useful in germplasm characterization and classification. Furthermore,

the result conforms to the previous work in Araceae in the sense that druses and raphides were observed but in contrast because prismatic and styloid were not observed (Osuji, 2013; Nurul et al., 2013; Mais and Amal, 2012; Gray, 2009).

Conclusion

This study has revealed the different calcium oxalate crystals, their sizes and occurrence including the epidermal and anatomical characteristics of leaf, petiole and midrib among the different variants of *Caladium bicolor* in Nigeria. These are useful taxonomic characters for delimiting the variants especially when combined with the existing data on the species.

Conflict of interests

The authors have not declared any conflict of interests.

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