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Anatomical Study on *Commelina diffusa* Burn f. and *Commelina erecta* L. (Commelinaceae)

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ABSTRACT: *Commelina diffusa* Burn. f. and *Commelina erecta* L. (Commelinaceae) are known from tropical and subtropical regions of the world. In the present work, the leaf epidermal characters, midrib and stem anatomy were studied in relation to their taxonomic values. Voucher specimens collected from different parts of Abia, Rivers, Bayelsa and Delta States were analysed. The samples were fixed in FAA, dehydrated in series of ethanol (50%, 70% and 90%), peeled/sectioned, stained in 2% aqueous solution of Safranin O, counter stained in Alcian blue for about 3-5 minutes, mounted in glycerine, viewed and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. Comparative foliar epidermal features, midrib and stem anatomical characters of the two *Commelina* species indicated that the leaf epidermal characters showed close similarity among the species though with few distinguishing features while the anatomical features of the midrib and lamina could be used to distinguish these species.

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The genus *Commelina* belongs to the family Commelinaceae commonly known as Spiderwort family with 42 genera and 650 species (Cabezas *et al*., 2009). The cosmopolitan genus *Commelina* is the largest in the family Commelinaceae containing some 200 species (Mohsin *et al*., 1987). They are widely distributed over the tropical, sub-tropical and warm temperate regions with main centres in Africa and Asia (Hutchinson and Dalziel, 1954; Mohsin *et al*., 1987; Faden, 1998). Among this genus, the chromosome number and morphology varied (Jones and Jopling 1972; Mauro *et al.,* 2005) and majority of the species have basic chromosome number of x = 15, while the basic numbers of x=10, 11, 12, 13 and 14 are restricted to a few species only (Federov 1969). *Commelina* species are used in the treatment of many diseases (Hillocks, 1998) some of which include leprosy and leucoderma (Mukerjee, 2006), chilling the blood and performed blood clotting functions (Kaur and Das, 2011), diabetes, skin diseases and atherosclerosis (Ujowundu *et al*., 2008) and other economic, medicinal and ethnobotanical values.

In Commelinaceae, numerous taxonomic treatments have been produced but no consensus as to which character or characters should be used to define relationships among the genera has been reached (Evans *et al.,* 2000). Despite this, in some members of this family such as in *Aneilema* and *Murdannia,* anatomical characters have been employed in delimitating and resolving some taxonomic problems among them (Tomlinson, 1966 and 1969; Faden and Hunt, 1991). There are also other works on the anatomy of *Commelina* (Paula *et al.,* 2010; Oladipo and Ayo-Ayinde, 2014) and their morphological attributes (Mahbubur Rahman *et al*., 2015; Jyoti and Krishna, 2009). Stebbins and Jain (1960) gave a detailed description of the structure and stomatal ontogeny in leaves of *Commelina diffusa* while Kaushik (1971) provided information on the structure of the stomatal apparatus in five other species of *Commelina*. Jyoti and Krishna (2009) have morphologically described some members of this genus however; the close morphological similarities among these species make it difficult to differentiate easily. Among the Nigerian species of *Commelina,* little is known about the anatomical features of the species. Therefore this study focused on the comparative leaf epidermal characteristics, anatomy of stem and midrib of these two species in other to complement the existing taxonomic information.

MATERIALS AND METHODS

The species used for this study are *Commelina erecta* and *C. diffusa* Burn.f. collected from different parts of Abia, Rivers, Bayelsa and Delta States. For the purpose of comparative anatomical work, small sizeable portions of the leaves (about 4mm x 4mm) were cut from the median parts of matured and well expanded leaves, that is, midway between the base and the apex.

Epidermal peels of the adaxial and abaxial surfaces of the leaves of the five species each were also made. The required epidermis was obtained using the scrape method of Metcalfe (1989). The leaf samples of each

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species were placed on a clean glass slide with the required surface turned facedown, the epidermis above being irrigated intermittently with distilled water was placed in a Petri dish was carefully scraped with a sharp razor blade (or a dissecting knife) until the epidermis underneath was reached and the adhering long tissues removed with a Carmel hair brush (Cutler, 1978). The epidermal peels were stained in 2% aqueous solution of Safranin O for about 3-5 minutes after which they were serially dehydrated using, 50%, 70% and 90% ethyl ethanol for 3-4 minutes interval each. The epidermal peels of the leaf specimens of the species were mounted in dilute glycerine solution after they were washed in 3-4 changes of water. For each of the species, about 20-25 measurements of the length and width of guard cells were taken from the adaxial and abaxial epidermal surfaces using a calibrated ocular micrometer at x40 objective lens. Stomata number/ frequency (which is the average number of stomata per square millimetre of leaf) was calculated. The epidermal peels were observed under a light microscope at x40 magnification. Features like types and distribution of trichomes, stomata, and shape of the epidermal cells were observed, measured and documented.

Also calculated was the stomata index. The stomata index is expressed by the formula, Stomata index (S.I) = 100S/(S+E), Where S = number of stomata per unit area, E = number of ordinary epidermal cells.

RESULTS AND DISCUSSION

The results of this research showed lots of similarities and differences in the epidermal and anatomical characters in the *Commelina* species studied. The summary of both the qualitative and quantitative stem and foliar epidermal characters of the two *Commelina* species studied are presented in Tables 1, 2 and 3 while the photomicrographs of the epidermal cells, midrib, leaf lamina and stem are presented in Figures 1, 2 and 3.

Epidermal characteristics

***Commelina erecta* L. (Figures 1A, B, C and Table 1):** The epidermal cells are generally pentagonal to heptagonal, partly polygonal with straight or curved anticlinal wall on both the adaxial and abaxial surfaces. The adaxial epidermal cell size varied from 25.4 to 51.3 µm long and 14.6 to 18.8 µm wide while the abaxial epidermal cell size varied from 20.5 to 27.4 µm long and 13.7 to 24.5 µm wide. Stomata on both leaf surfaces are anomocytic, tetracytic and paracytic, parallel to partly parallel in orientation (Table 1). The stomatal index ranged from 2.30 – 5.66 (3.81±1.30) on the adaxial surface to 20.00 – 24.55 (22.22±2.13) on the abaxial surface. The adaxial and abaxial epidermal surfaces are hairy with uniseriate eglandular trichomes (Figures 1C and D).

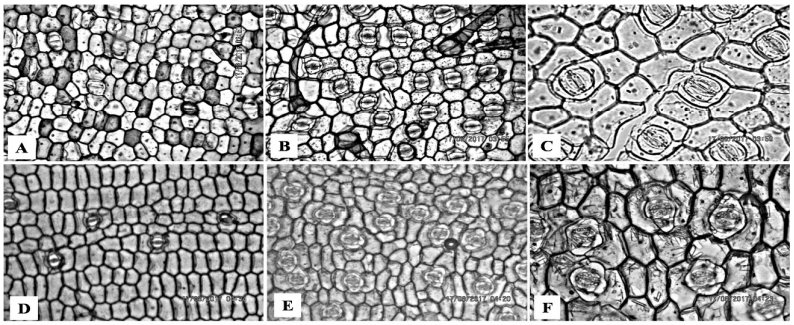

Fig 1: Epidermal features of *C. erecta* (A) upper epidermis and (B and C) lower epidermis and *C. diffusa* (D) adaxial epidermis and (E and F) abaxial epidermis

***Commelina diffusa Burn. f. (Figures 1D, E, F and Table 1*):** In *C. diffusa,* epidermal cells are pentagonal to heptagonal, partly polygonal on both adaxial and abaxial surfaces with straight or curved anticlinal wall (Figures 1D, E and F). Cell size varied from 37.6 to 40.3 µm long and 17.1 to 20.5 µm wide on the adaxial surface, 33.5 to 39.6 µm long and 16.3 to 20.6 µm wide on the abaxial surface. Stomata on both leaf surfaces are tetracytic, parallel to partly parallel in orientation (Table 1). The stomatal index ranged from 2.88 – 4.00 (3.45±0.56) on the adaxial surface to 20.69 – 40.91 (27.39±9.45) on the abaxial surface. The adaxial epidermal surface is glabrose while the abaxial surface is hairy with uniseriate eglandular trichomes. The epidermal cells of the two species studied are pentagonal to heptagonal and partly polygonal. In contrast, Oladipo and Ayo-Ayinde (2014) noted that the epidermal cells in *Aneilena* and *Commelina* (Commelinaceae) are polygonal. Also, the anticlinal walls are straight or curved. These characters inferred that there is interspecific relationship between the species as opined by Oladipo and Ayo-Ayinde (2014). Among Commelinaceae family, Stebbins and Jain (1960) documented stomata consisting two guard cells whose aperture is oriented parallel to the long axis of the leaf. Similarly, we observed that the stomata aperture of the species studied are parallel to the leaf axis. Furthermore, the range of sizes of epidermal cells recorded in this work collaborates with the work

of Oladipo and Ayo-Ayinde (2014) who reported that the sizes of the epidermal cells on the adaxial and abaxial leaf surfaces of *C. diffusa* as 6.85 – 20.56 µm^2 and 15.17 – 35.34µm^2 respectively.

Table 1: Adaxial and abaxial Epidermal features of *C. diffusa* and *C. erecta*

Character	Species name	
	***C. erecta* L.**	***C. diffusa* Burn. f.**
Adaxial epidermis		
Epidermal cell shape	Pentagonal to heptagonal, partly polygonal	Pentagonal to heptagonal, partly polygonal
Anticlinal wall pattern	Straight or curved	Straight or curved
Stomata type	Anomocytic, tetracytic and paracytic	Tetracytic
Trichome type	Uniseriate egalandular trichome	None
Epidermal cell size (µm)	25.4 – 51.3 x 14. 6 -18.8	37.6 – 40.3 x 17.1 – 20.5
Stomatal index	2.30 – 5.66 (3.81±1.30)	2.88 – 4.00 (3.45±0.56)
Stomata orientation	Partly parallel to each other	Parallel to each other
Abaxial epidermis		
Epidermal cell shape	Pentagonal to heptagonal, partly polygonal	Pentagonal to heptagonal, partly polygonal
Anticlinal wall pattern	Straight or curved	Straight or curved
Stomata type	Anomocytic, tetracytic and paracytic	Tetracytic
Trichome type	Uniseriate egalandular trichome	Uniseriate egalandular trichome
Epidermal cell size (µm)	20.5 – 27.4 x 13.7 – 24.5	33.5 – 39.6 x 16.3 – 20.6
Stomatal index	20.00 – 24.55 (22.22±2.13)	20.69 – 40.91 (27.39±9.45)
Stomata orientation	Partly parallel to each other	Partly parallel to each other

Among *Jatropha* species, stomata type has been employed in their delimitation (Dehgan, 1980). Similarly, *C. diffusa* had only tetracytic stomata while *C. erecta* had anomocytic, tetracytic and paracytic stomata. The stomata types are the same with the work of Kaushik (1971) on *Commelina.* The presence of paracytic stomata in *C. erecta* is an indication that in the evolutionary tree it is more primitive than *C. diffusa*. Also, stomatal index varied slightly among the species studied. For instace, the stomatal index in *C. erecta* varied from 2.30 – 5.66 on the adaxial surface and 20.00 – 24.55 on the abaxial surface while in *C. diffusa* it varied from 2.88 – 4.00 on the adaxial surface to 20.69 – 40.91 on the abaxial surface. Though this character has been reported to be highly constant in certain plant species and could be used to delimit them (Olatunji, 1983), this overlap in stomatal index among the species suggests that this character is not dependable and could not be used to make clear taxonomic distinction between the studied species.

Anatomy of midrib and leaf lamina

***Commelina diffusa* Burn. f. (Figures 3A, B, C and Table 2):** In this species, the midrib has 1 - 3-concentric vascular bundles (Figure 3A and Table 2). The adaxial cuticle is V-shaped and hairy (Figure 3D). The lamina is 34 – 56 µm thick, palisade mesophyll 1 – 3-layers, spongy mesophyll 2 – 3-layers, adaxial/abaxial epidermis 1-layer and oval in shape with intercellular air spaces.

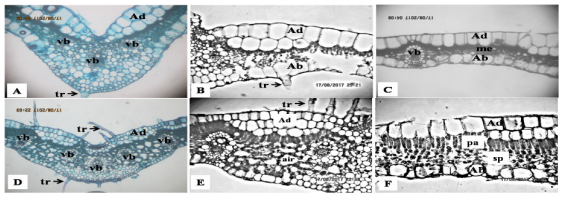

Fig 3: Midrib and lamina of (A – C) *C. diffusa* and (D – F) *C. erecta* (Note: A and D = midrib, B and E = lamina towards the midrib, C and F = lamina, tr = trichome, vb = vascular bundle, Ad = adaxial epidermis, air = air space, pa = palisade mesophyll, sp = spongy mesophyll, Ab = abaxial epidermis, me = mesophyll)

Table 2: Anatomical features of midrib and leaf lamina of *C. diffusa* and *C. erecta*

Character	Species name	
	C. erecta	***C. diffusa***
Midrib		
Hairy	Adaxial and abaxial	Abaxial surface only
Trichome type	Eglandular and serriated	None
Shape of adaxial cuticle	V-shaped	V-shaped
Number of vascular bundle	5	1 – 3
Vascular bundle type	Concentric	Concentric
Leaf lamina		
Thickness of mesophyll (µm)	25 – 43	34 – 56
Spongy mesophyll	3 – 5 layers	2 – 3 layers
Palisade mesophyll	1 – 2 layers	1 – 3 layers
Adaxial/abaxial epidermis	1-layer, oval in shape	1-layer, oval in shape
Intercellular air space	Absent	Present

***Commelina erecta* L. (Figures 3C, D, F and Table 2):** The midrib has 5-concentric vascular bundles (Figure 3D and Table 2). The adaxial cuticle is V-shaped and hairy on both adaxial and abaxial epidermal surfaces (Figure 3D). The lamina is 25 – 43 µm thick, palisade mesophyll 1 – 2-layers, spongy

mesophyll 3 – 5-layers and adaxial/abaxial epidermis 1-layer and oval in shape. Worthy to note is the difference in midrib and lamina anatomical features (Table 2). The variation in the size/thickness of the leaf lamina, layers/thickness of spongy and palisade mesophylls, number of vascular bundle in the midrib and presence of trichomes on the leaf surfaces and midrib could be used to distinguish these species. The adaxial surface of the midribs are V-shaped with concentric vascular bundles, parenchymatous cortex 9-10 layers in *C. erecta* and 7-10 layers in *C. diffusa.* The diagnostic values of these characters have been documented in *Emilia* species (Ekeke et al., 2016) and Asteraceae (Ekeke and Mensah, 2015).

Stem anatomy

***Commelina erecta* L. (Figures 4A, B, C, D and Table 3):** Stem of *C. erecta* is glabrose, oval with 16 pericycular vascular bundles (Figure 4B), 14 vascular bundles in the ground tissue (Figure 4C), continuous layer of sclerechymatous cells (2-layers thick), parenchymatous cortex 9 – 10-layers, intercellular air space (Figure 4C) and starch grains (Figure 4D and Table 3).

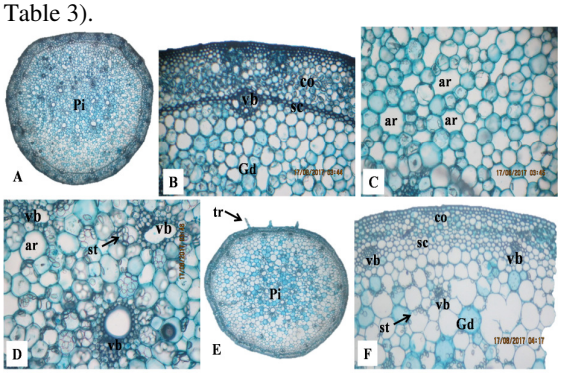

Fig 4: Stem anatomy of (A – D) *C. erecta* and (E and F) *C. diffusa* (**Note:** Pi – pith, Gd – ground tissue, tr – trichome, vb – vascular bundle, co - perenchymatous cortex, sc - sclerenchymatous cortex, ar – air space, st – starch grain)

Table 3: Anatomical features of *C. diffusa* and *C. erecta* stem

Character	Species name	
	C. erecta	***C. diffusa***
Hairy	No	Yes
Shape	Oval	Oval
Number of pericycular vascular bundle	16	20
Number vascular bundle in the ground tissue	14	16
Presence air space	Present	Absent
Presence of starch grains	+++	+
Nature of sclerenchymatous cortex	Continuous	Continuous
Layers of sclerenchymatous cortex	2	1
Layers of perenchymatous cortex	9 – 10	7 – 10
Layer of epidermis	1	1

***Commelina diffusa* Burn. f. (Figures 4E, F and Table 3):** In this species the stem is hairy (Figure 4E), oval in shape, with 20 pericycular vascular bundles, 16 vascular bundles in the ground tissue, continuous layer of sclerechymatous cells (1-layer thick), parenchymatous cortex 7 – 10-layers and starch grains (Figure 4F and Table 3).

Tomlison (1966, 1969) classified the trichomes in Commelinaceae into non-glandular micro-hair (clavate) trichomes, macro-hair and glandular hairs and further stated that *Commelina* species contain uniseriate trichomes with varying number of cells. In this study, we recorded only non-glandular uniseriate trichomes which occurred in different parts of the plants. Though there is slight variation in the occurrence of the trichomes in different parts of the plants, this type of trichome did not constitute dependable diagnostic character in the species studied

From the comparative foliar epidermal and stem anatomical characters of the two species of the *Commelina*, the leaf epidermal characters showed close relationship in the species though with few distinguishing features while the anatomical features of the midrib and lamina could be used to distinguish these species.

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