

Leaf Epidermal Studies in Four Species of *Euphorbia* (Euphorbiaceae)

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ABSTRACT

Leaf epidermal morphology of four plant species belonging to the Euphorbiaceae family was studied: *Euphorbia heterophylla* L., *E. hirta* L., *E. hyssopifolia* L. and *E. prostrata* Aiton. The study revealed several interesting characters such as an anticlinal cell wall pattern (ACWP), epidermal cell wall shape (ECWS), epidermal cell size (ECS), stomatal complex type (SCT), frequency of stomatal complex types (FSCT), stomatal density (SD), stomatal size (SS), stomatal index (SI) and trichomes are constant and variable within and between the species. There are some relationships between the four species. For instance, leaves are hypostomatic in all species; SCT is anisocytic in three species except one, *E. hyssopifolia* and 100% occurrence of one type of SCT; the ACWPs are of different types; they are straight, curved, round and undulate in the species studied. The ECWS were found to be polygonal and irregular but in *E. heterophylla* the shapes are isodiametric. The above leaf features are thus of great taxonomic significance in understanding the relationship between and within species of the family.

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INTRODUCTION

Plant classification has been a subject of discussion among plant taxonomists and systematists over the years. Plants are classified and reclassified as soon as new evidences arise, and this is going to be a continuous exercise over some years to come. Most plants are classified based on external morphological structures such as flowers and fruits. These structures are not always available to plants because they are seasonal in production. Due to this reason, other means of classification need to be involved; one of which is anatomical studies especially of leaves.

The epidermis is the outer layer of cells covering the leaf, it has several functions: protecting the plants against water loss by transpiration, regulation of gaseous exchange, secretion of metabolic compounds and absorption of water. Most leaves show dorsoventral anatomy: the upper (adaxial) and lower (abaxial) surfaces have somewhat different construction and may serve other functions (Hardie, 2009). It can also be used as taxonomic character delimiting plants. Some characters or features on the epidermis, which are useful taxonomically, include several differentiated cell types: epidermal cells, subsidiary cells, guard cells and epidermal hairs (i.e.

trichomes). These features have been used previously to resolve some taxonomic problems or to contribute to ever increasing taxonomic data base in some genera and even families of plants (Watson, 2006; Van Wyk *et al.*, 2008; Ren *et al.*, 2007; Saheed and Illoh, 2010). Continuity and/or discontinuity of the characters can be used to show relationships and differences between plant taxa within a genus or between families.

Leaves of four species in the plant family Euphorbiaceae were studied anatomically to elucidate their taxonomic potentials with the aim of using features such as stomata, epidermal cells and trichomes to delimit species within a genus and genera between families.

MATERIALS AND METHODS

Collection of Specimens

Leaf specimens of four species belonging to the family Euphorbiaceae (Table 1) were collected from various locations in Kwara State, Nigeria. These species were taken to the Herbarium Unit of the Department of Biology, University of Ilorin, Ilorin, Kwara State, Nigeria for identification and documentation.

Table 1: Lists of the Plant Species studied

Species	Place of Collection
<i>Euphorbia heterophylla</i> L.	Beside School Clinic, University of Ilorin, Kwara State
<i>Euphorbia hirta</i> L.	Along Chapel of Light, Oke-Odo, Kwara State
<i>Euphorbia hyssopifolia</i> L.	Opposite De-Dona hostel, Oke-odo, Kwara state
<i>Euphorbia prostrata</i> Aiton	Behind Abuja hostel, University of Ilorin, Kwara State

Isolation of Leaf Epidermal Layers

A leaf segment of an area of 1 cm square from each specimen is cut and immersed in a concentrated solution of nitric acid

for maceration. The upper (adaxial) and lower (abaxial) are separated with dissecting needle and forceps. These samples were picked from acid and rinse with clean water.

Determination of Frequency of Stomatal Complex Type (SCT)

Prepared slides of macerated cuticles from the leaves of the species were observed. Different types of stomatal complex present in each specimen were also observed using 35 fields of view at x40 objectives as quadrats. The number of subsidiary cells per stoma were noted, and recorded to determine the frequency of the different stomatal complex types present in each specimen. The frequency of each complex type was expressed as the percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). The terminologies for naming SCT followed those of Dilcher (1974).

Determination of Stomatal Density (SD) and Stomatal Index (SI)

The SD was determined as the number of stomata per square millimeter (Stace, 1965).

The SI was determined as follows: $SI = S/E + S \times 100$

Where S = numbers of stomatal per square millimeter

E = number of ordinary epidermal cells per square millimeter

Determination of Stomatal Size (SS)

The mean stomatal size of a species was determined as the product of the length and breadth of guard cells.

Determination of Epidermal Cell Size (ESC)

Epidermal cell size was determined as the product of the length and breadth of a cell based on a sample size of 35.

RESULTS AND DISCUSSION***Euphorbia Heterophylla***

Stomata are absent on the adaxial but present on the abaxial surface. The stomatal complex type is anisocytic. The shapes of the epidermal cells were isodiametric on adaxial surface and irregular on the abaxial surface. Trichomes are absent on adaxial surface only (Tables 2 and 3). Abaxial cells (11.00 μm) are smaller than adaxial cells (13.50 μm), and 11.00 μm is the smallest epidermal cell size recorded among the four species.

Table 2: Leaf Epidermal Features in Some Selected Members of Euphorbiaceae Family

Species	Leaf Surface	Anticlinal Cell Wall Pattern (ACWP)	Epidermal Cell Wall Shape (ECWS)	Epidermal Cell Size (μm) (ECS)
<i>Euphorbia heterophylla</i>	Adaxial	Curved	Isodiametric	13.50
	Abaxial	Undulate	Irregular	11.00
<i>Euphorbia hirta</i>	Adaxial	Curved	Irregular	14.63
	Abaxial	Curved	Irregular	15.80
<i>Euphorbia hyssopifolia</i>	Adaxial	Straight	Polygonal	15.70
	Abaxial	Undulate	Irregular	14.00
<i>Euphorbia prostrata</i>	Adaxial	Undulate	Irregular	15.25
	Abaxial	undulate	Irregular	17.33

Euphorbia Hirta

Stomata are present on the abaxial surface. The stomatal complex type is anisocytic. The epidermal cells are irregular on both surfaces. Trichomes are present on abaxial surface but absent on adaxial surface. Stomatal density (263.16 per mm^2) is the highest of the four species, though almost identical to that of *E. hyssopifolia*. Stomatal size (60.65 μm) is intermediate to large, and the stomatal index is 21.98% (Tables 2 and 3).

Euphorbia Hyssopifolia

Stomatal complex types on abaxial surface were anomocytic. Stomatal are absent on adaxial surface. Epidermal cells are polygonal on adaxial surface, but irregular on abaxial surface. Trichomes are absent on both surfaces. Adaxial cells (15.70 μm) are larger than abaxial cells (14.00 μm) (Tables 2 and 3).

Table 3: Stomatal Features and Trichome in Some Selected Members of Euphorbiaceae Family

Species	Leaf surface	STOMATA					
		Type	Frequency (%)	Density (mm^2)	Size (μm)	Index (%)	Trichome
<i>Euphorbia heterophylla</i>	Adaxial	-	-	-	-	-	Absent
	Abaxial	Anisocytic	100.00	246.45	40.00	20.90	Present
<i>Euphorbia hirta</i>	Adaxial	-	-	-	-	-	Absent
	Abaxial	Anisocytic	100.00	263.16	60.65	21.98	Present
<i>Euphorbia hyssopifolia</i>	Adaxial	-	-	-	-	-	Absent
	Abaxial	Anomocytic	100.00	263.09	73.80	25.68	Absent
<i>Euphorbia prostrata</i>	Adaxial	-	-	-	-	-	Absent
	Abaxial	Anisocytic	100.00	249.87	42.00	27.33	Absent

Euphorbia Prostrata

Stomata are absent on adaxial surface only. Stomatal complex type present on adaxial surface is anisocytic. The epidermal cell shapes are irregular on both surfaces. Trichomes are

absent on both surfaces. Abaxial cells (17.33 μm) are larger than adaxial cells (15.25 μm), and 17.33 μm is the largest epidermal cell size recorded among the four species (Tables 2 and 3).

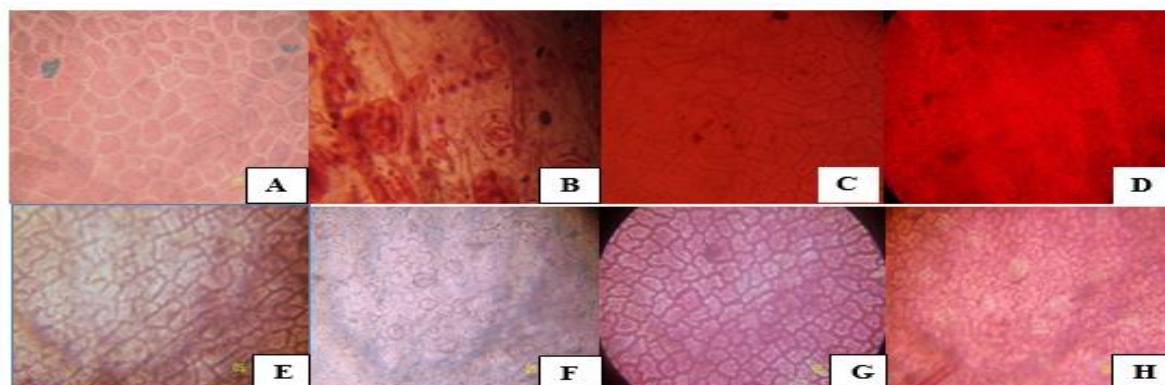


Figure 3: Leaf surfaces in some members of Euphorbiaceae family: *Euphorbia heterophylla* (a and b), a - adaxial surface showing no stomata, b - abaxial surface showing anisocytic stomata; *Euphorbia hirta* (c and d), c - adaxial surface no stomata, d - abaxial surface showing anisocytic stomata; *Euphorbia hyssopifolia* (e and f), e - adaxial surface showing no stomata, f - abaxial surface showing anomocytic stomata; and *Euphorbia prostrata* (g and h), g - adaxial surface showing no stomata, h - abaxial surface showing anisocytic stomata

Discussion

Anatomical relationships and variations observed in the four species of Euphorbiaceae studied are indeed good taxonomic characters. Plant anatomy is very essential in plant taxonomy; the purpose is to develop a system of classifying plants in a way that all the differences and similarities are set out in an orderly manner (Olorode, 1984). Even though vegetative and floral characters are markedly modified about the habitat and pollination mechanisms, the preceding observation and the summaries of character variation in the species studied indicated that the taxonomic application of the diversity of epidermal morphology in Euphorbiaceae cannot be overemphasized. The decision to choose epidermal characters for this study was informed by earlier declaration that these characters represented genetic variations and have been used to solve taxonomic problems in certain other plant groups by taxonomists (Srivastava, 1978; Oladele, 1990; Adegbite, 1995; Nwokeocha, 1996; Ogunkunle and Oladele, 1997; Ogunkunle and Oladele, 2008; AbdulRahaman and Oladele, 2010a; AbdulRahaman and Oladele, 2010b).

The four species of *Euphorbia* showed different ACWPs including undulate and curved walls on both leaf surfaces of *E. prostrata* and *E. hirta* respectively. The ECWSs are irregular in at least one surface or both surfaces in the four species of *E. milii* (Table 2). The ECSs are closely related ranging from 11µm on abaxial surface of *E. heterophylla* to 17.33µm on abaxial surface of *E. prostrata* (Table 2).

Trichome is another taxonomic significance feature in the species of the three families. Trichomes are found in some species and absent in others. They are present on the abaxial surface in the two *Euphorbia* namely *E. heterophylla* and *E. hirta*. Occurrence of trichomes on the abaxial surface of the majority of species may support the claim that trichomes themselves transpire. According to Shields (1950), living trichomes, which themselves lose water do not protect the plants from excessive transpiration as do dead trichomes which form protective layers. A similar observation was made in two species of *Jatropha* by AbdulRahaman (2009) where *J. gossypifolia* with high trichome density and index transpired faster than *J. curcas* which possessed lower trichome density and index.

The results showed that the leaves of members of Euphorbiaceae family were discovered to be hypostomatic. *E. milii* was earlier observed by AbdulRahaman (2009) to possessed hypostomatic leaves as other species of *Euphorbia* studied in this present work. Thus possession of stomata on the abaxial surface only seems to be a common feature in the

genus *Euphorbia*. SCTs are mostly anisocytic in three species except in *E. hyssopifolia* with anomocytic type. Stomatal frequency varies from one leaf to the other and these were observed among the species studied. The homogeneous nature of SCT on leaves of all species studied is responsible for 100% occurrence of SCTs where a single SCT present on a leaf surface. SD according to Esau (1977) was found to vary from one plant to another. There is rarely any significant difference in the SD of three *Euphorbia* species while SD was generally found to be higher in the abaxial surface of the leaves than the adaxial surface. This pattern is in corroboration with some earlier works of (Oyeleke *et al.*, 2004; AbdulRahaman, 2009; AbdulRahaman and Oladele, 2009; Saadu *et al.*, 2009). According to Esau (1965), stomata index varied on different part of the leaves or on different leaves of the same plants. Though there are clear differences in the SS of *Euphorbia* species, the SI did not show such difference. While Davies and Heywood (1963), considered stomatal size to be too variable as diagnostic features other researchers like Wilkinson (1971) held contrary views. He indicated that stomatal size shows a much wider range in some taxa than in others, as it may be sometimes be useful as a diagnostic character when dealing with taxa in which size ranges are restricted. This present study conforms to Wilkinson's observation.

Apart from taxonomic implications, a way by which plant conserves water is by having higher number of stomatal on the abaxial surface of the leaves as seen in *Euphorbia hirta*, *Euphorbia prostrata*, *Euphorbia heterophylla* and *Euphorbia hyssopifolia*.

Conclusion

In conclusion, the four *Euphorbia* species studied share a common leaf surface: all are hypostomatic, with stomata only on the abaxial surface and a stomatal frequency of 100%. Stomatal density is also similar across species about 246 to 263 per mm². Therefore, the leaf epidermal features of the four species could be said to be taxonomically significant because of discontinuities that occurred within and between genuses, genera within the family.

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