

Leaf epidermal studies of three species of *Euphorbia* in Akwa Ibom State

UA Essiett, HC Illoh² and UE Udoh

¹Department of Botany and Ecological Studies, University of Uyo, Uyo

²Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State

ABSTRACT

Leaf epidermal studies of three species of *Euphorbia* in Akwa Ibom State are described. The mature stomata were paracytic, staurocytic, anisocytic, anomocytic, laterocytic and brachyparacytic stomata. The paracytic stomata was the commonest. Abnormalities notice here include vertical and parallel contiguous stomata, stomata with one guard cell, unopened stomatal pore, one subsidiary cell shared by two stomata variously orientated. Parallel and vertical contiguous stomata were distributed on both surfaces of *E. hirta* and *E. heterophylla* but absent in *E. milli*. Leaves are amphistomatic in *E. hirta* and *E. heterophylla*, but hypostomatic in *E. milli*. Uniseriate non-glandular trichomes were distributed on both surfaces of *E. hirta* and *E. heterophylla* but absent in *E. milli*. Other systematically useful characters are stomata index, guard cell area and the shape of the anticlinal cell walls can also be used for distinguishing the species.

Keywords: *Euphorbia* species, Epidermal, Stomata, Nigeria, Euphorbiaceae.

INTRODUCTION

Family *Euphorbiaceae*, the spurge family consisting of about 322 genera and 8910 species are predominantly cosmopolitan with strongest representation in the humid tropical and sub tropical regions of both hemisphere [1, 2]. It is the sixth largest family in the world [3]. *Euphorbiaceae* is characterized by the unisexual and mostly apetalous flowers, floral glands, the tricarpeal syncarpous pistil and schizocarpic capsular with tree cocci and persistent columella or rarely with drupeous fruits, the inter-relationships among the genera are not sufficiently understood.

Euphorbia hirta L. is a slender stemmed annual hairy plant with many branches from the base to the top, spreading up to 40cm tall reddish or purplish in colour. Leaves are opposite, elliptic- oblong to oblong-lanceolate, distichous, 1-2.5cm long. *Euphorbia heterophylla* L. is commonly called spurge weed. It is a hardy, ruderal species growing between 30 to 70cm in height. The leaves at the upper end of the stalk close to the cyathium, have a striking scarlet red colouration. Leaves are mainly 2-4 lobed and 4-7cm long by 1.5-3cm wide.

Euphorbia milli des Maul. is commonly called crown-of-thorns Christ weed, and is classified as a succulent plant with thick fleshy leaves and stems adapted for water storage. The stems are 5-7 sided, greyish. Brown, branched and up to 2-3cm in height with many prominent grey spines. The leaves tend to be obovate (wider near the tip) up to 3.5cm long and 1.5cm broad. The leaves have entire margin and are spirally arranged on the stem.

E. hirta is also believed to be used in the treatment of snake bite [4]. Not only do the leaves cure snake bites, applying crushed leaves to a wound can also stop bleeding and it contains anti-inflammatory agents that can speed the healing of pimples, wounds and boils while *E. heterophylla* acts as a purgative. The decoction of the leaves of *E. heterophylla* when eaten purges [4]. The decoction of the roots of *E. hirta* is used to heal various female disorders. It increases lactation in nursing mothers who are not producing enough milk, but it should never be administered to pregnant women, as this may induce miscarriage [5].

Folk remedial combination for piles contain aqueous extracts of the two plant species *E. hirta* (*Euphorbiaceae*) and *Gomphrena celosioides* (*Amaranthaceae*) [6]. *E. hirta* is traditionally used in the treatment of worm infestation, communicable disease like gonorrhoea and various skin infections like pimples [7].

Taxonomic significance of foliar epidermis in some members of the family *Euphorbiaceae* in Nigeria has been investigated by [8]. Foliar epidermis is one of the most noteworthy taxonomic characters from the bio-systematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis [9], [10], [11], [12]. Although taxonomists lately realized the importance of microscopic features of the epidermis, taxonomic monographs are now considered incomplete without them [13].

Epidermal cells are quite variable in their configuration. The characters, which have been proven to be of systematic value, are cuticular characters, epidermis, stomata, subsidiary cells and trichomes [14]. The taxonomic significance of epidermal morphology is well documented in botanical literature [15].

In this present study, the investigation of stomatal and trichome types in some *Euphorbia* species is an attempt to reveal additional characteristics for *Euphorbia* species, which might be useful for identification and assessment of the taxonomic relationships among species studied at the generic and species level.

MATERIALS AND METHODS

The fresh leaves of three species of *Euphorbia* (*E. hirta*, *E. heterophylla*, and *E. milli*) were collected from a bush in Uyo Local Government Area of Akwa Ibom State. Anatomical studies were carried out using the methods below.

MICROSCOPIC EXAMINATION

Small sizeable portions of the leaves of the specimens were obtained from standard median level of matured and well-expanded leaves. Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade taken from a standard median portion of the leaves on a clean glass slab, with the surfaces to be studied facing down. The specimens were irrigated with water holding it downwards from one end, and then the epidermis above the desired surface was scraped-off carefully with a sharp razor blade. The loose cells were washed away from the epidermal peels with the aid of soft camel hair brush and water until the desired epidermis below was reached. The epidermal peels were stained in 1% aqueous solution of Safranin O for 4-8 minutes, rinsed carefully in water to remove excess stain and mounted in 10% glycerol. Guard cell area was calculated by multiplying their length and width by Francós constant which is 0.7854. The stomatal index was determined according to [16] using the formula:

$$\frac{S}{E+S} \times \frac{100}{1} = \text{Stomatal Index (SI)}$$

Where S = number of stomata per unit area
E = number of epidermal cells in the same area.

The partially cleared leaves were further cleared in 5% domestic bleach for about 20-30 minutes under sunlight. The portions were again washed in several changes of water and stored in 50% ethyl-alcohol for anatomical studies. The cleared portions of the leaves were stained in 1% aqueous solution of safranin 0 for 3-5 minutes then washed in 3-4 changes of water to remove excess stain and mounted on 10% glycerol and specimens were observed at X40 objective magnification.

All microscopic measurements were made with the aid of an ocular micrometer; these measurements were converted by the ocular constant with respect to the power under which they were taken with a Motic microscope version 2.0ml.

RESULTS

Epidermal Cells

The epidermal cells on both surfaces are mainly polygonal and irregular on the epidermis in the three species. The smaller cell is recorded in *E. hirta* (9.8µm) Table 1. The epidermal cell on the abaxial surface are highly undulated in *E. milli* (25. µm) Table 1. The epidermal cell on the abaxial surface is highly undulated for all the species, while the adaxial surface are undulate.

The anticlinal cell walls are highly undulated for abaxial surfaces, while it is undulate for *E. hirta* and *E. heterophylla*, but straight in *E. milli*. Epidermal cell size is higher in adaxial surface *E. milli*. In the surface view, prominent papillae gives the impression being structure in centre of cell lome in *E. heterophylla* (Plate 1a-b) but absent in *E. hirta* and *E. milli*.

Stomata

Generally, stomata occur on both surfaces, the leaves are amphistomatic except in *E. milli* which is hypostomatic. *E. heterophylla* has the smallest stomata (12.3 μm) (Table 1) while the largest stomata were found on *E. milli* (24.3 μm) Table 1.

The mature stomata were staurocytic, anisocytic anomocytic, brachyparacytic and laterocytic stomata occurring in both surfaces of *E. hirta*, *E. heterophylla*, except in *E. milli*. There are irregularly and evenly distributed on both surfaces in some cases and their axes are oriented in different directions. Paracytic stomata were abundant in both surfaces. Stomata index varied between the two species on both adaxial and the abaxial surface (Plate 2a-f). The highest stomatal index was found in the adaxial surface of *E. hirta* (57.1) and the lowest stomatal index were found on the adaxial surface of *E. milli* (14.2). Guard cell area in abaxial surface of *E. hirta* (2144.4) are longer than those on the adaxial surface of *E. heterophylla* (243.8).

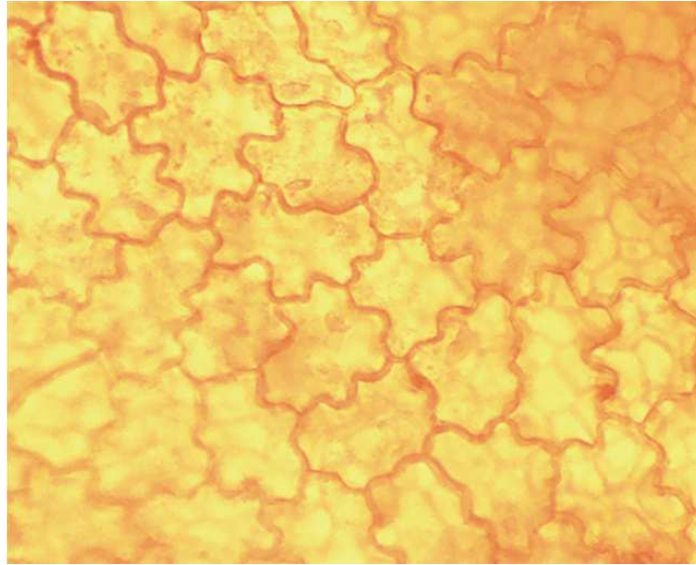


Plate 1A: Epidermal Cells of *Euphorbia milli* (upper surface) x 400

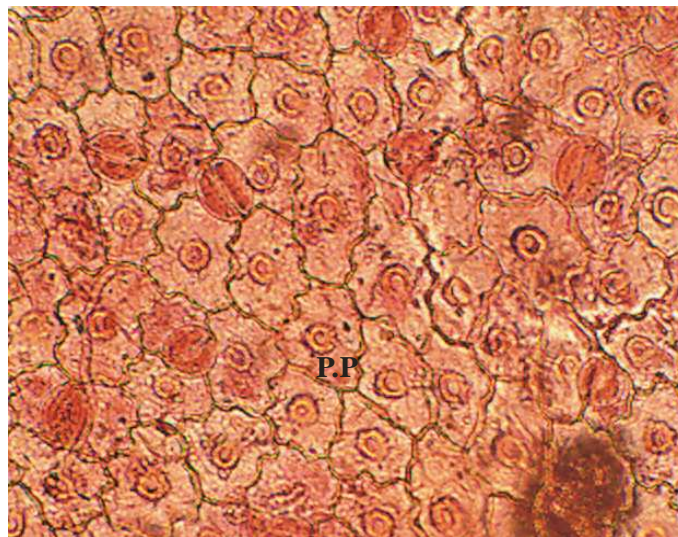


Plate 1B: P.P: Prominent Papillae in *Euphorbia heterophylla* (upper Surface) x400

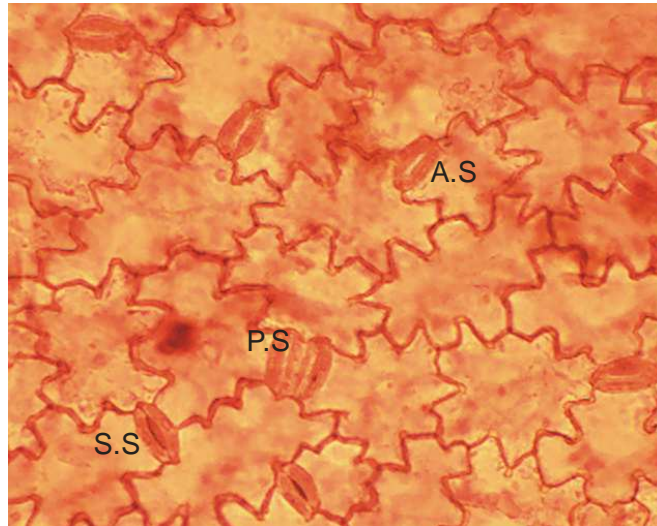


Plate 2A: P.S: Parallel Contiguous, A.S: Anisocytic and S.S: Staurocytic Stomata of *E. hirta* (lower surface) x400

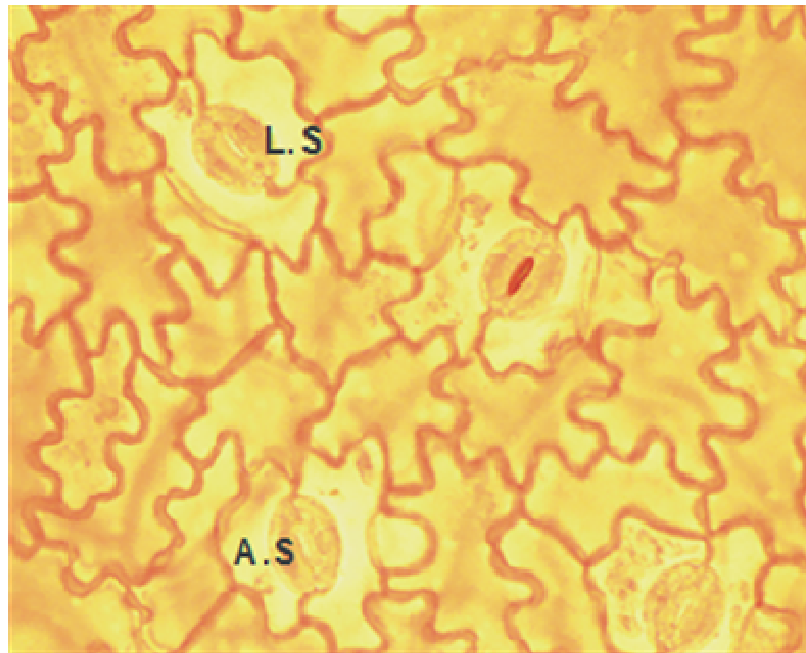


Plate 2B: L.S: Laterocytic and A.S: Anisocytic Stomata of *E. milli* (upper surface) x400

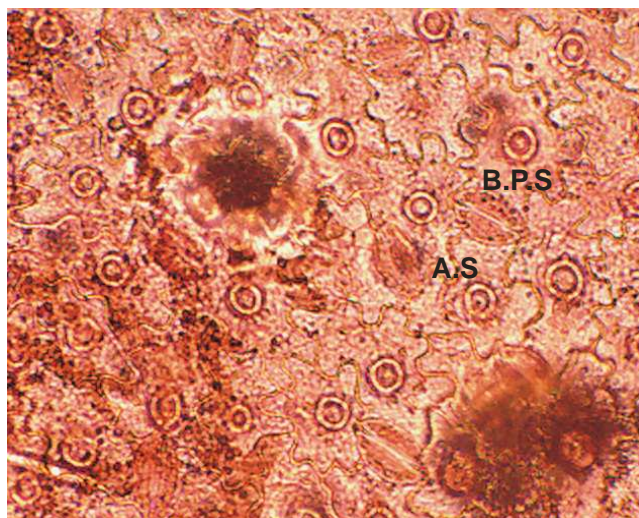


Plate 2C: A.S: Anisocytic and B.S: Brachyparacytic Stomata sharing one subsidiary cell of *E. heterophylla* (lower surface) x400

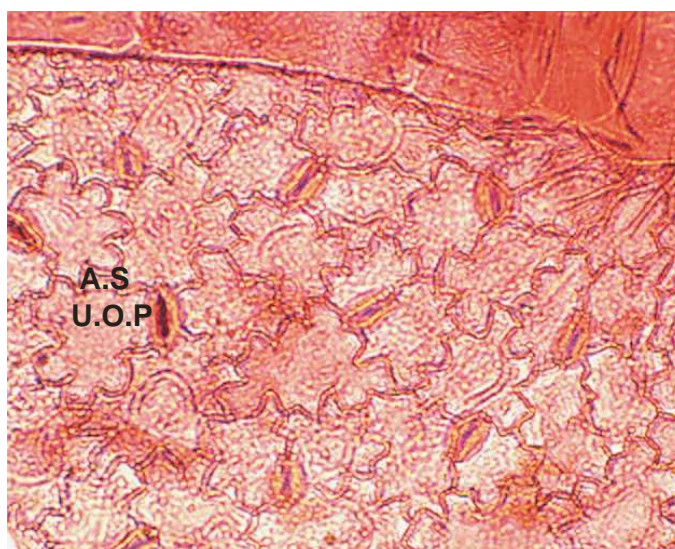


Plate 2D: A.S: Anisocytic with Unopen pore Stomata of *E. hirta* (upper surface) x400 subsidiary cell of *E. heterophylla* (lower surface) x400

Abnormal Stomata

Various abnormal stomata were observed in *E. hirta* and *E. heterophylla*, these include vertical contiguous stomata, parallel contiguous stomata with one guard cell, unopened stomatal pore, one subsidiary cell shared by two stomata (Plate 3a-b).

Hairs

The morphology of trichomes including their size, shape and frequency which are of characteristics interest. Trichomes are scanty present at the upper surface of *E. hirta*, non glandular uniseriate hairs are present with pointed apices or tapering apices were found in *E. hirta* and *E. heterophylla*, but absent in *E. milli*. The sizes of trichome found on *E. hirta* varied from 343.8 μm to 265.8 μm in *E. heterophylla* respectively (Plate 4a-b). *E. hirta* was 343.8 long and 16.5 wide while *E. heterophylla* was 265.8 long 12.2 wide.

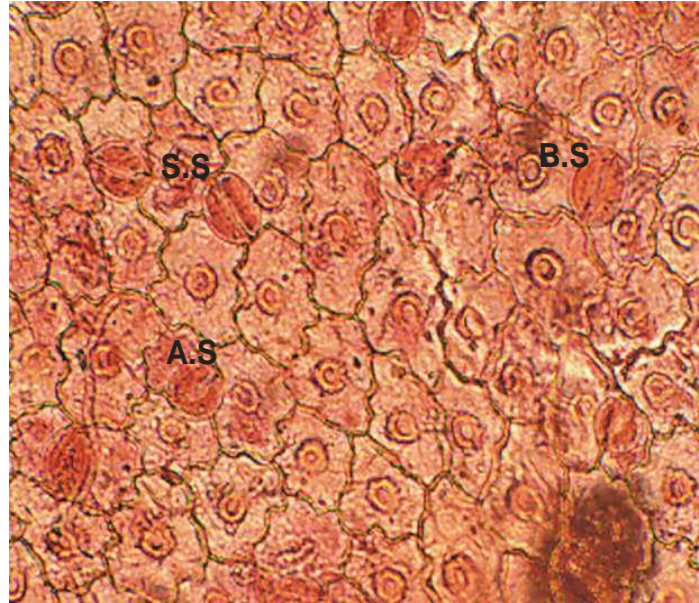


Plate 2E, B.S: Brachyparacytic, S.S: Staurocytic and A.S: Anisocytic Stomata of *E. heterophylla* (upper surface) x400

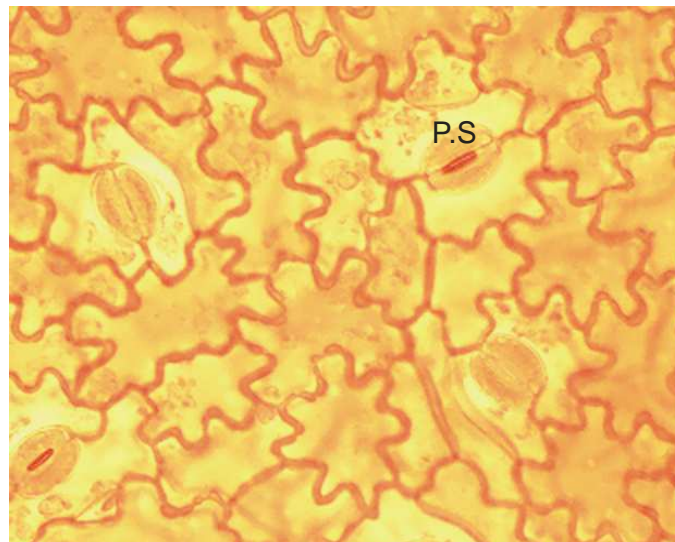


Plate 2F: Paracytic Stomata of *Euphorbia milli* (lower surface) x400

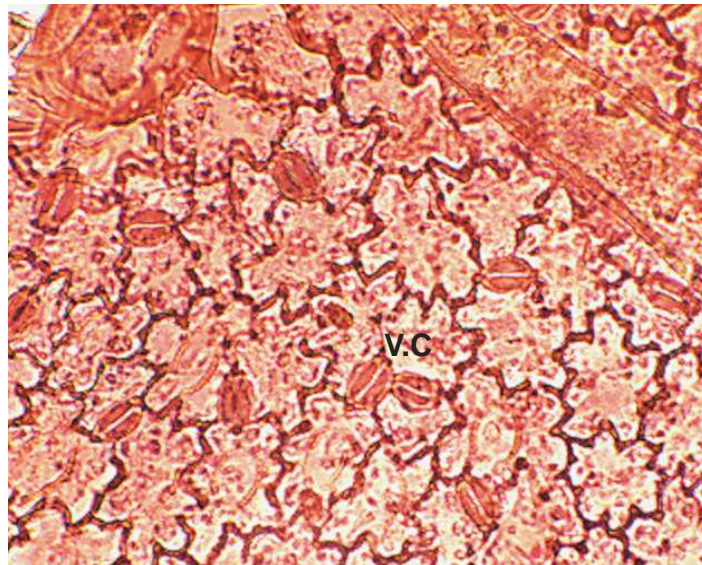


Plate 3A: V.C: Vertical contiguous stomata of *Euphorbia hirta*(upper surface) x400

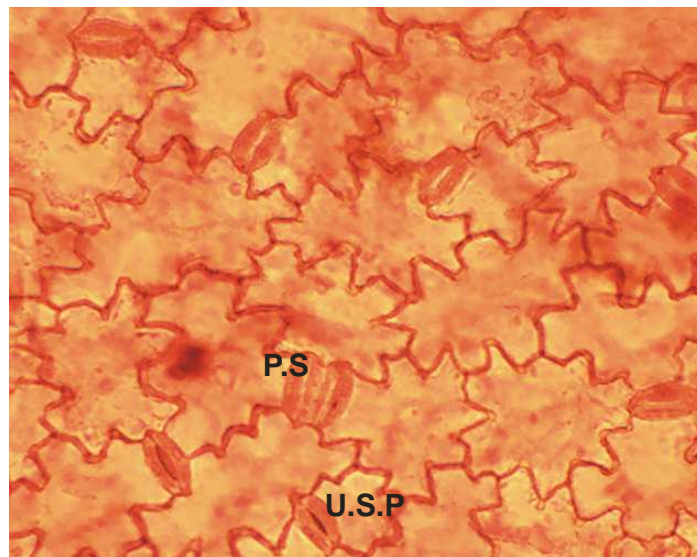


Plate 3B: P.S: Parallel contiguous Stomata and U.S.P: Unopened Stomata pore of *E. hirta* (lower surface) x400

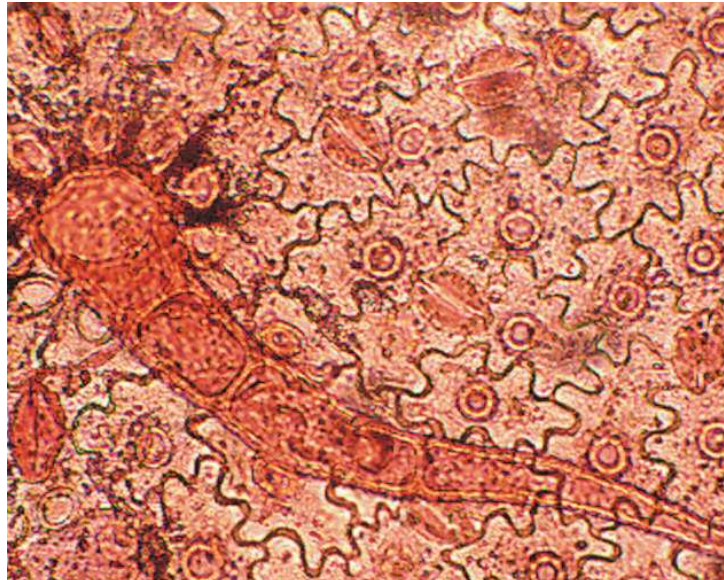


Plate 4A: Uniseriate Trichome of *Euphorbia heterophila* (lower surface) x400

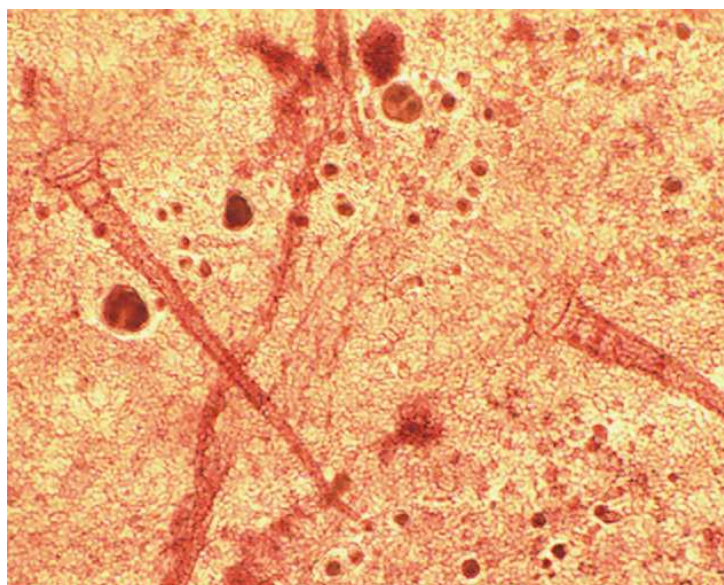


Plate 4B: Uniseriate trichome of *Euphorbia hirta* (lower surface) x400

Table 1 Epidermal Features of *E. hirta*, *E. heterophylla* and *E. milli*

Species	Stomatal size (µm)	Epidermal cell size (µm)	Non-glandular (µm)	Guard cell area (µm) ²	Stomatal Index (%)	Epidermal cell wall	Stomatal Distribution
<i>E. hirta</i>	Ab Ad 24.5x18.8 24x19	Ab Ad 15.5x 9.8 13.8x13.5	Ab Ad 103.8x103.3 343.8x16.5	Ab Ad 2144.4x605 2.2x35	Ab Ad 52.6x55.3 57.1x50.5	Slightly sinous to wavy	Amplistomatic
<i>E. heterophylla</i>	Ab Ad 24.3x15.3 12.3x17.15	Ab Ad 15.5 x12.8 14.3x 6.8	Ab Ad 265.8x12/2 195.8x16.2	Ab Ad 243.8x 622 309x 69	Ab Ad 50x55 53.3x47.3	Slightly sinous to wary	Amplistomatic
<i>E. milli</i>	Ab 24.3 x 15.3	Ab Ad 23.3x58 25.5x 48.8	- - - - -	Ad 963 x 71	Ab Ad 31.4x52.4 14.2x24.8	Slightly sinous to wary	Hypostomatic

Ad - Adaxial Surface (upper)
 Ab - Abaxial Surface (lower)
 L - Length
 B - Breadth

DISCUSSION

Taxonomic relevance of vegetative anatomy in taxa delimitation recognition has been reported by [17], [18], [19], [20].

The present investigation describe the epidermal structure of three species of *E. hirta*, *E. heterophylla*, and *E. milli*. The cell shape may be polygonal and irregular on the three species. The anticlinal cells are highly undulated on abaxial for *E. hirta* and *E. heterophylla* but straight in *E. milli*. The largest cell was in *E. milli*. The leaves were amphistomatic in *E. hirta* and *E. heterophylla* but hypostomatic in *E. hirta* and *E. heterophylla* but hypostomatic in *E. milli*. A combination of different types of stomata has also been observed here on the same surface of an organ in species investigated.

The presence and combination of different types of stomata on the surfaces of leaves can be as useful in classification and delimitation. Their findings in the three species of *Euphorbia* studied exhibited this; it is possible for most species to have more than three types of stomata. This has been shown by [21] on Solanaceae, [22] on the variation in the structure and development of foliar stomata in the *Euphorbiaceae* [23] on leaves anatomy of *Euphorbia*. The presence of various types of stomata in the taxa is of taxonomic interest in these studies, because it can be use to distinguish *E. hirta*, *E. heterophylla* in having paracytic, anisocytic, anomocytic, laterocytic on the adaxial but absent in *E. milli* this is in agreement with [21].

Abnormal stomata were parallel contiguous, vertical contiguous stomata with one guard cells, unopened pore one subsidiary cell shared by two stomata occurred on *E. hirta* *E. heterophylla*, such abnormality has been described by [23], in the studies of anatomy of *Euphorbia*. The importance of abnormalities in leaves have been the result of environmental factor as confirmed by [24].

The occurrence of curved walls in some of the species agreed with the suggestion of [10] that curved wall is a mesomorphic character and that environmental condition such as humidity play a significant role in determining the pattern of anticlinal cell wall.

The structural pattern of the epidermal wall on both adaxial surface can be taxonomically employed in taxa. Stomatal Index is independent of the environment size or portion of the leaf surface size of the intervening epidermal cell [16] and also highly constant for any given species.

The variation in stomatal index observed in these studies can be reasonable employed in delimiting in *Euphorbia* species. Stomatal index is highly constant for any given species and the value is more uniform upon the abaxial than the adaxial surface. The role of stomatal index in systematic work to separate species has also been reported by [25], [26] and [8].

The guard cell area in abaxial surface of *E. hirta* (2144.4) are longer than those on the adaxial surface of *E. heterophylla* (243.8) and Stomatal index can also be useful for Identification of the studied taxa. Many plant groups show great diversity in their indument, some of which are of taxonomic importance while ecological variation may affect the degree of hairness. The type of hair is usually constant in many species or species group. [27] and many researchers have found the presence or absence and types of trichomes on the epidermal surface as classification tools. [28], [19], [20].

[16] has long suggested that the types of epidermal trichomes can frequently delimit species, genera or families in plant. Difference in trichomes types were employed by [26] to delimit species in the *Vernonia* genus. In these studies, trichome occurred on both surfaces of *E. hirta* and *E. heterophylla* but absent in *E. milli*.

Although quantitative, the variation in trichome length observed in this study can be reasonable employed in delimiting the species.

CONCLUSION

Leaf epidermal characters are of taxonomy significance in the members of the family *Euphorbiaceae*. With this they can be separated and distinguished based on their stomata, epidermal cells as these features which are being influenced by environmental factors are present on almost every leaf surface. Therefore the stomata; epidermal cells are micro morphological features on leaves epidermal surface and can be used to identify separate or differentiate plant species.

REFERENCES

- [1] Webster, GL, *Bot. J. Linn. Soc.*, **1987**, 94: 3 – 46.
- [2] Webster, GL, *Ann Missouri Bot. Gard.*, **1994a**, 8(1): 3 – 32.
- [3] Radcliff-Smith, A, *Bot. J. Linn. Soc.*, **1987**, 94: 47 – 66.
- [4] Etukudo, I, *Ethnobotany: Conventional and Traditional Uses of Plants*. First Edition. Uyo: verdict Investment Ltd., **2003**, 191pp.
- [5] Patricia, BJ, Ezzeldir, MA, Emmanuel, AT, Cowan, B, Ibrahim, AA and Isah, MH, *African J. Biotech.*, **1998**, 9(16). 241 – 249.
- [6] Arys, KR and Agarwal, SC, *Ethnobotany*, **2001**, 13(1): 142 – 145.
- [7] Scandeeep, BP and Chandrakant, SM, *Biosystematics Contemporary Biology*. 1st Edition. London: Edward Arnold Ltd., **2011**, pp. 74 – 83.
- [8] Aworinde, DO, Nwoye, DU, Jayeda, AA, Olagoke, AO and Ogundele, AA, *Research Journal of Botany*, **2009**, 4: 17 – 28.
- [9] Bhatia, RC, *Phylotaxon.*, **1984**, 19: 381 – 385.
- [10] Stace, CA, *The taxonomic importance of leaf surface*. In: Heywood, VH and Moore, DM (eds.). Current concepts in Plant Anatomy. London: Academic Press, **1984**, pp. 67 – 94.
- [11] Jones, JH, *Ann. Missouri Bot. Garden*, **1986**, 73: 228 – 275.
- [12] Baranova, M, *Taxon*, **1972**, 21: 447 – 469.
- [13] Rejdali, FLS, *Bot. J. Linnean. Soc.*, **1991**, 104: 67-77.
- [14] Ellis, RP, *Bothalia*, **1976**, 12: 65 – 109.
- [15] Dehgan, B, *Bot. J. Linnaean. Soc.*, **1980**, 80: 257 – 278.
- [16] Metcalfe, CR and Chalk, L, *Anatomy of Dicotyledons*. 2nd Edition. Oxford: Clarendon Press, **1979**.
- [17] Tomblinson PB, *Bot. J. Linn. Soc.*, **1961**, 55, 779 – 809.
- [18] Korreasha and Seetharam, VN, *Phytomorphological*, **2000**, 50(3-4): 229-237.
- [19] Essiett, UA, *Biosystematic studies of some Nigerian Dioscorea species*. Ph.D. Thesis, Department of Botany and Microbiology, University of Uyo, Uyo, Nigeria, **2004**.
- [20] Essiett, UA and Akpabio, KE, *Int. J. Biotechnol and Allied Sci.*, **2009**, 4(1): 424 – 432.
- [21] Patel, RC and Inamdar, JA, *Ann. Bot.*, **1969** 35: 389-409.
- [22] Raju, SV and Rao, PN, *Bot. J. Linn. Soc.*, **2008**, 75: 69-97.
- [23] Kakkar, L and Paliwal, GS, *Beitr. Biol. Pfl*, **1972**, 48(2): 425 – 432.
- [24] Carr, SG and Carr, DJ, *Bot. J. Linn. Soc.*, **1990**, 102: 123 – 156.
- [25] Abdulrahaman, AA and Oladele, FA, *Nigerian Journal of Botany*, **2003**, 16: 144 – 150.
- [26] Isawumi, MA, *Nigerian Journal of Botany*, **1989**, 23: 94 – 100.
- [27] Okpon, ENU, *Morphological notes on the genus Cassia. l*. Notes from the Royal Botanic Garden. Edinburg, **1969**, 185-195.
- [28] Rollins, RC and Shaw, IA, *The genus Lesquerella (Cruciferae) in North America*. Harvard University Press, Cambridge, **1973**.