

THE VALUE OF LEAF MICROMORPHOLOGICAL IN THE TAXONOMIC DELIMITATION OF *EMILIA* CASS, (ASTERACEAE) SPECIES

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ABSTRACT

Studies were carried out on the leaf epidermis of the three species of *Emilia*: *E. coccinea* (Sims) G. Don, *E. praetermissa* Milne-Redhead, and *E. sonchifolia* (L) DC using light microscope. Epidermal cells in the species are irregular with anticlinal wall pattern sinuous in *E. coccinea*, sinuous in *E. sonchifolia*, and straight to arcuate in *E. praetermissa*. Epidermal cell dimensions were highest in *E. praetermissa* with 52.50 μ m and 49.64 μ m for the length and breadth respectively. The epidermis is hypostomatic with anomocytic stomata in all species, semi-contiguous to contiguous stomata in *E. coccinea*, and *E. sonchifolia*. Stomata index (S.I), frequency and size differed in all species. While S.I of 34.80 in *E. sonchifolia* was the highest in the genus, *E. coccinea* had the lowest S.I of 21.90. Stomata ledges occur in *E. coccinea* and *E. praetermissa*. Simple unicellular trichomes were observed only on the abaxial surface of *E. coccinea*. The usefulness of these data to the taxonomic delimitation of the *Emilia* is discussed.

KEYWORDS: *Emilia*, Epidermis, Stomata, Taxonomy, Trichome

INTRODUCTION

Emilia Cass. consists of three species in Nigeria (*E. coccinea* (Sims) G. Don, *E. praetermissa* Milne-Redhead, and *E. sonchifolia* (L) DC), which occur as regrowths in cleared forests, abandoned farmlands, edges of footpaths and track roads. Morphological features of these species have been described by Hutchinson and Dalziel (1963), Olorode and Olorunfemi (1973), and Akobundu and Agyakwa (1987). The species are semi-erect with hollow, weak stems, which do not usually branch below the inflorescence. The leaves are simple and alternate, semi-fleshy and ovate; upper leaves are sessile and dilated at their bases. Burkill (1995) reported that leaves of these species are used as vegetables.

Olorode and Olorunfemi (1973) and Olorode (1974) established a basic chromosome number of $n = 5$ for the genus. They further reported a diploid chromosome number of 10 ($2n=10$) for both *E. coccinea* and *E. sonchifolia*, while *E. praetermissa* had $2n=20$ (tetraploid). Thus, *E. praetermissa* is a hybrid of *E. coccinea* and *E. sonchifolia*, the diploid progenitors. Taxonomic data on the genus especially in relation to the phylogenetic relationship among the species is very scanty. No known study had been carried out on aspects of micromorphology and microanatomy. The present study investigates foliar epidermal characteristics (epidermal cells, stomata, trichome etc) of these three species with the aim of improving the information on their taxonomy. Similar characters have been reported and utilized in taxa elucidation by Metcalfe and Chalk (1979), Patil and Patil (1987), Oladele (1990), Edeoga and Qsawe (1996), Ogundipe and Akinrinlade (1998), and Agbagwa and Ndukwu (2001). A taxonomic key based on the outstanding epidermal characteristics of the species has been developed to further demonstrate the value of these microanatomical features in taxa delimitation of *Emilia*.

MATERIALS AND METHODS

Fresh mature leaves of the three species (*E. coccinea*, *E. praetermissa*, and *E. sonchifolia*) were collected from living plants grown in the Botanic Garden of University of Port Harcourt, Nigeria. Samples for study were all taken from identical regions of the leaf, usually midway between the base

and apex of the lamina including the margins. The adaxial and abaxial leaf surfaces of the species were peeled following the methods of Cutler (1978) as modified by Okoli and Ndukwu (1992). The peels were bleached for 3 to 5 minutes with parazone (domestic bleach), and washed in several changes of clean water. With camel hairbrush the peels were transferred to 10% aqueous solution of safranin for 5 minutes, and subsequently washed in clean water before mounting in glycerin. The slides were examined under a light microscope at Objective Lens 10 and 40 and photomicrographs of the epidermis taken using LEITZ DIAPLAN microscope fitted with LEICA WILD MPS 52 camera at Objective Lens 40. All quantitative measurements were made with an ocular eyepiece graticule at Objective Lens 40. 10 different peels were examined per species.

RESULTS

EPIDERMIS: The epidermis is composed of single layer of cells covered by smooth cuticle. It is thick on the upper surface (Fig. 1A) while thin on the lower surface. Basically, the epidermal cells are irregular in the species, anticlinal wall pattern sinuous in *E. coccinea*, sinuous in *E. sonchifolia* and straight to arcuate in *E. praetermissa* (Figs 1A-D). The costal cells are mostly tetragonal. The epidermal cell wall of *E. praetermissa* was thicker than the other two species. Epidermal cell lengths of 52.50 μ m and 49.64 μ m on the adaxial and abaxial surfaces of *E. praetermissa* respectively were the highest in the genus. The minimum length and breadth of 17.25 μ m and 2.80 μ m respectively were recorded in *E. sonchifolia*. The frequency of epidermal cell per field view at Objective Lens 40 on the upper leaf surface of the species range from 256 in *E. praetermissa* to 428 in *E. coccinea*. The trend is similar on the lower surface with 340 in *E. praetermissa* and 508 in *E. coccinea*.

STOMATA: The leaves were hypostomatic in all species; the stomata were randomly distributed and irregularly oriented in the intercostal areas. The stomata in all the species are anomocytic with each stoma surrounded by 3 to 4 epidermal cells. Semi-contiguous to contiguous stomata were observed in *E. praetermissa* and *E. sonchifolia* (Figs 1C & D). These were, however, more frequent in *E. praetermissa*. Stomata in *E.*

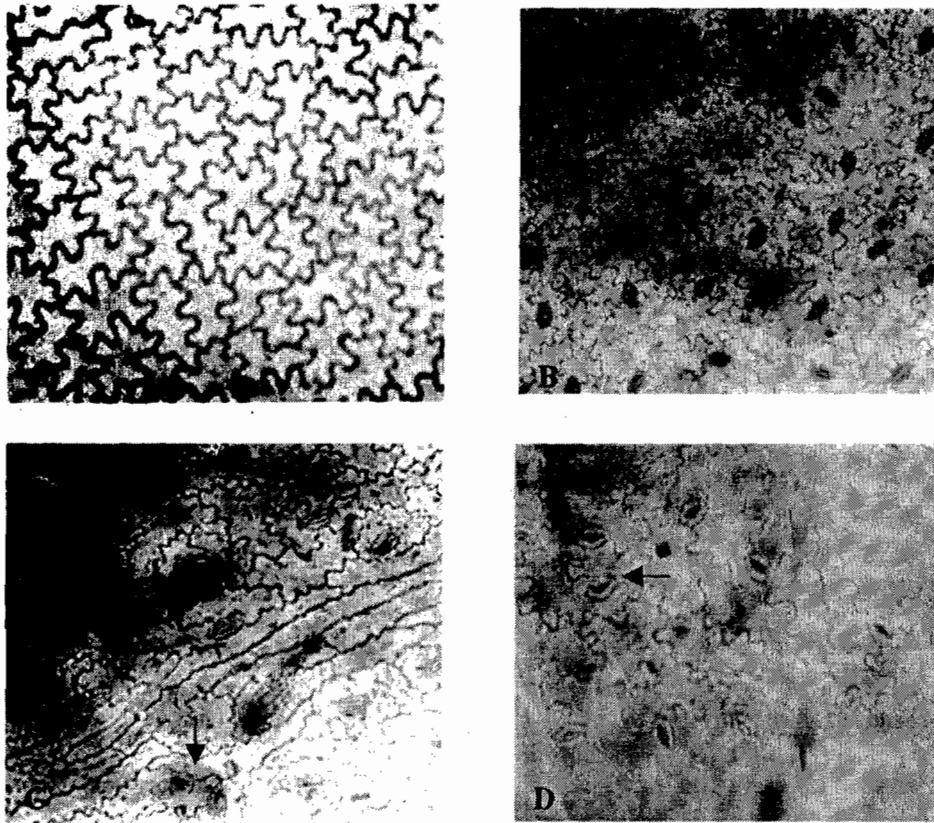


Figure 1A-D: A- showing adaxial epidermis of *E. coccinea* with sinuous wall pattern. B- abaxial epidermis of *E. coccinea*. Arrow points to a simple unbranched trichome. C- *E. praetermissa*. Arrow indicates a semi-contiguous stomata. D- *E. sonchifolia*. Mag. X 400

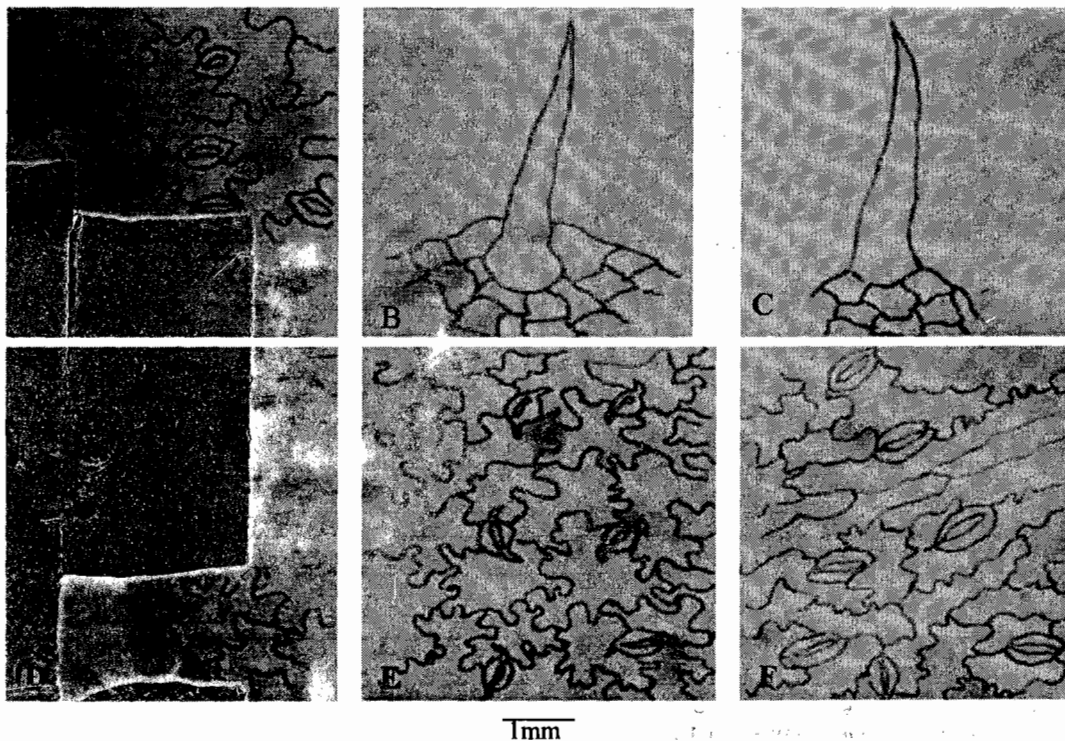


Fig. 2A - F: *Emilia* species. A - adaxial epidermis. B - section of the abaxial epidermis of *E. coccinea* showing clearly the stomata with ledges. C, D - abaxial epidermises of *E. praetermissa* and *E. sonchifolia* respectively. E, F - simple unbranched trichomes in *E. coccinea*.

coccinea was characterized by the occurrence of two stomatal ledges (upper and lower ledges) as shown in Fig. 2B. In *E. praetermissa* only one ledge was observed. Ledges were scarcely present in *E. sonchifolia*. The frequency of stomata per unit area varies from 115 in *E. praetermissa* to 210 in *E. coccinea*, while the stomatal index was found to be highest in *E. sonchifolia* (34.80) and lowest in *E. coccinea* (21.90). Stomatal dimensions of 42.70 μ m and 29.28 μ m for the length and breadth respectively in *E. coccinea* were the highest in the genus. The least were observed in *E. sonchifolia* (20.50 μ m and 12.20 μ m for length and breadth).

TRICHOMES: Among the three species, trichomes were observed only on the abaxial surface of *E. coccinea*. The trichomes are unicellular and simple. They are distributed with a reduced frequency of 25 per field view and a low trichome density of 0.7.

DISCUSSION

The data obtained from the studies indicate a certain level of uniformity in the qualitative epidermal characters of the *Emilia* species investigated. For instance, cell shape is the same in all three species (Table 1). The predominant anticlinal wall pattern as demonstrated in Figs 1A-D is sinuous (wavy). This uniformity confirms their phylogenetic relatedness. However, it was relatively more sinuous in *E. coccinea* and occasionally straight to arcuate in *E. praetermissa*. Another taxonomic identity of the species is the occurrence of anomocytic stomata in all species and contiguous stomata in *E. praetermissa* and *E. sonchifolia*. Both anomocytic and contiguous stomata have been reported in the Asteraceae (Metcalfe and Chalk, 1950; Oladele, 1990; Sasikala and Narayanan, 1998). Amidst similarities in stomatal features that further suggest phylogenetic relatedness include the stomatal distribution pattern per unit area, the different stomatal indices

Table 1: Qualitative Foliar Epidermal Characters of *Emilia* species

Taxa	Leaf surface	Epidermal cell shape	Anticlinal cell wall pattern	Stomata type	Trichome type
<i>E. coccinea</i>	Adaxial	Irregular	Sinuous	Absent	Absent
	Abaxial	Irregular	Sinuous	Anomocytic, contiguous	Simple, unicellular
<i>E. praetermissa</i>	Adaxial	Irregular	Straight to arcuate	Absent	Absent
	Abaxial	Irregular	Straight to arcuate, sinuous	Anomocytic, contiguous	Absent
<i>E. sonchifolia</i>	Adaxial	Irregular	Sinuous	Absent	Absent
	Abaxial	Irregular	Sinuous	Anomocytic, contiguous	Absent

and dimensions as shown in Table 2. Patil and Patil (1987) investigated stomatal distribution, frequency, index and size in the leaves of 11 species and varieties of *Chlorophytum* and pointed out that these characters were significant at the level of the subgenera. Ogundipe and Akinrinlade (1998) and Agbagwa and Ndukwu (2001) reported similar observations in *Albizia* and *Cucurbita* species respectively. Esau (1965) and Metcalfe and Chalk (1979) observed that frequently elevated extensions of the cuticular membrane known as outer stomatal ledges or rims rise from the guard cell surface like an incompletely roofed dome, in protective manner over the stomatal pore. The presence or absence of this feature, as is in this genus, can confer higher taxonomic significance on the stomata. In *E. coccinea* for instance, the ledges occur on both polar and distal ends of the stomata as against *E. praetermissa* where it occurs on one side. However, this stomatal feature in *E. sonchifolia* is at best described as vestigial in this taxon (Fig 1D). It is to be noted that the hybrid *E. praetermissa* seems to have acquired this feature midway from its putative parents, as expected of a typical hybrid.

The seemingly large epidermal cell size and stomatal dimensions observed in *E. praetermissa* is particularly noteworthy. Olorode and Olorunfemi (1973) established a polyploid status for *E. praetermissa*. Swanson (1967) reported increase in size of certain structures as the first fact in detection of polyploids from their diploid progenitors. The results from the current studies therefore support reports of Olorode and Olorunfemi (1973) and Olorode (1974) on the polyploid status of *E. praetermissa*. Phylogenetically therefore, *E. praetermissa* is shown to have arisen from a hybridisation between *E. coccinea* and *E. sonchifolia*, a point which was actually proved by Olorode and Olorunfemi (1973) and Olorode (1974). The diploid hybrid may have undergone self-induced chromosomal doubling to produce the tetraploids. This may have occurred in order to circumvent the complications associated with unbalanced chromosome complements. The simple unicellular trichome occurring in this genus was confined to the abaxial surface of *E. coccinea*. Though the functions of these epidermal structures are not known, their taxonomic importance is well documented. They occur

sparingly as shown in Table 2, but the ability to isolate this character in an unknown species of *Emilia* clearly delimit the species in question as *E. coccinea*.

Table 2 Quantitative Foliar Epidermal Data of *Emilia* Species

Taxa	Leaf surface	Num of Epidermal cells/mm ²	Epidermal cell size (µm)		Num of stomata /mm ²	Mean stomata size (µm)		S.I	Num of epidermal cells surrounding stomata	Trichome characters	
			Length	Width		Length	Width			#/mm ²	Index
<i>E. coccinea</i>	Adaxial	428	20.50-27.50	5.00-6.00	-	-	-	-	-	-	-
	Abaxial	508	19.00-25.50	4.0-4.90	210	30.30 ± 2.40	12.90 ± 1.20	33.30	3 to 4	25	0.7
<i>E. praetermissa</i>	Adaxial	256	29.40-52.50	8.00-13.00	-	-	-	-	-	-	-
	Abaxial	340	27.60-49.64	8.00-13.00	115	42.70 ± 2.20	29.28 ± 1.30	21.90	3 to 4	-	-
<i>E. sonchifolia</i>	Adaxial	372	21.75-31.75	3.00-5.00	-	-	-	-	-	-	-
	Abaxial	456	17.25-27.25	2.80-3.20	150	20.50 ± 2.20	12.20 ± 1.30	34.80	3 to 4	-	-

In conclusion, similarities of epidermal features observed amongst the three species support their phylogenetic relationship. Certain features like large and contiguous stomata and epidermal cells reported in *E. praetermissa* is a confirmation of earlier reports of the polyploid status of the species. However, the species-specific occurrence pattern of

stomata ledges, and the presence of trichomes on the abaxial surface of *E. coccinea* establish the individuality of these species. Such features stand out for easy identification of the species. Based on the epidermal features as observed in the genus, the following artificial key has been constructed. Artificial identification Key to the species

- 1 Stomata ledge present 2
- 1' Stomatal ledge absent *E. sonchifolia*
- 2 Two stomata ledges, trichome present on foliar surface...
... *E. coccinea*
- 2' One stomatal ledge, trichome absent on foliar surface...
... *E. praetermissa*

REFERENCES

- Agbagwa, I.O. and Ndukwu, B. C. 2001. Epidermal Micromorphology of *Cucurbita* L. species in Nigeria. *JASEM*, 5(2):59 – 64.
- Akobundu, I. O. and Agyakwa, C. W. 1987. A hand book of West African Weeds. International Institute of Tropical Agriculture, Ibadan, Nigeria. 521pp.
- Burkill, H. M., 1995. The Useful Plants of West Tropical Africa. (Ed. 2), 3: Families J-L. Royal Botanic Gardens Kew.
- Cutler, D. F., 1978. Applied Plant Anatomy. Longman Inc. New York. 103 pp.
- Edeoga, H. O. and Osawe, P. I., 1996. Cuticular Studies of Some Nigerian Species of *Senna* Tourn. ex Mill. (Syn. *Cassia* Tourn. ex L.): Leguminosae – Caesalpinioideae. *Acta Phytotax. Geobot.* 47(1):41 – 46
- Esau, K., 1965. Plant Anatomy. 2nd ed. John Wiley & Sons, New York. 767pp.
- Hutchinson, J. and Dalziel, J. M., 1963. Flora of West Tropical Africa, Vol II. Crown Agents, Millbank London.
- Hutchinson, J. and J. M., Dalziel, 1963. Flora of West Tropical Africa: Vol. II, Crown Agents for Oversea Governments and Administrations.
- Metcalfe, C. R. and Chalk L., 1979. Anatomy of the Dicotyledons. 2nd ed. Vol. 1: Systematic anatomy of the Leaf and Stem, with a brief history of the subject. Clarendon Press, Oxford.
- Ogundipe, O. T. and Akinrinlade, O. O., 1998. Epidermal micromorphology of some species of *Albizia* Durazz. (Mimosaceae). *Phytomorphology* 48(4): 325 – 333.
- Ndukwu, B. C. and Okoli, B. E. 1992. Procedures for the Observation of Living Tissue (Vital Observations): In *Fic'd, Herbarium and Laboratory Techniques* (ed B.E. Okoli) pp: 30 - 38. Mbeyi & Associates (Nig.) Ltd, Port Harcourt.

- Oladele, F. A., 1990. Leaf epidermal features in *Vernonia amygdalina* and *Vernonia cinerea*. Nigerian Journal of botany, 3: 71-77.
- Olorode, O. 1974. Identification of the genomic complements of *Emilia praetermissa* (Senecioneae - Compositae). *Ann. J. Bot* 60(1): 55-60
- Olorode, O., and Olorunfemi, A., E. 1973. The hybrid origin of *Emilia praetermissa* (Senecioneae - Compositae). *Ann. Bot.* 37: 185 - 191.
- Patil, G. and Patil, V. P., 1987. Stomatal Studies in the genus *Chlorophytum* and their taxonomic significance. *Phytomorphology*, 37(2,3): 155-158.
- Sasikala, K. and R. Narayanan, 1998. Numerical evaluation of trichome characters in certain members of Asteraceae. *Phytomorphology*, 480: 67-81.
- Swanson, C.P., 1968. Cytology and Cytogenetics, 2nd ed. Prentice-Hall Inc. New Jersey. 596pp.