Pak. J. Bot., 50(5): 1865-1869, 2018.

FOLIAR EPIDERMAL MORPHOLOGY OF SOME MEMBERS OF SUBFAMILY DODONAEOIDEAE - SAPINDACEAE

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Abstract

Dodonaeoideae Burnett is a subfamily of flowering plants in the soapberry family Sapindaceae Juss. Leaf epidermal characteristics of some species in the subfamily Dodonaeoideae were studied with the aid of a compound light and scanning electron microscopes in order to evaluate their reliability as taxonomic markers. Both qualitative and quantitative assessments were carried out using standard methods and the species studied included *Zanha golungensis* Hiern, *Dodonaea viscosa* (L) Jacq. and *Majidea fosterii* (Sprague) Radlk. Both *Z. golungensis* and *M. fosterii* are hypostomatic with stomata restricted to the abaxial surface while *D. viscosa* is amphistomatic. Their epidermises are composed of cells of various shapes from polygonal in *Z. golungensis* and adaxial surface of *D. viscosa* species, to irregular *M. fosterii* and abaxial surface of *D. viscosa*. The anticlinal wall patterns vary on abaxial and adaxial surfaces of each species, from straight in *D. viscosa* to undulate in *M. fosterii*. Anomocytic stomata are present in *M. fosterii* and *D. viscosa* species while paracytic stomata type is found in *Z. golungensis.* There is variation in the stomata size, number, length and width of the three species. Stellate trichomes were observed on the adaxial surface of *Z. golungensis* while epicuticular wax is granular in all taxa and mainly especially on the adaxial surface. Also, striations were observed on both surfaces of *Z. golungensis.* The range of variation in the epidermal characters between the species under investigation renders them of value for taxonomic purposes. An artificial dichotomous key for identifying the species is presented.

Key words: Epidermal cells, Sapindaceae, Stomata, Taxonomic Markers, Trichomes.

Introduction

The role of anatomical data in traditional taxonomy has been long recognized owing to the fact that variation within species, genera or a family is usually reflected in anatomical features. Leaf epidermal features such as stomata, trichomes and other characters are useful anatomical tools and this has been established by several authors including Dilcher (1974), Metcalfe & Chalk (1950, 1979), Kadiri & Ayodele (2003), amongst others. However, asides from the studies by Radlkofer (1886, 1890, 1933) and Muller & Leenhouts (1976), and Solereder (1899, 1908) not much has been done on the description of the subfamily Dodonaeoideae of the family Sapindaceae using anatomical data. The Dodonaeoideae as defined by Radlkofer (1890, 1933) is a subfamily of the soapberry family Sapindaceae Juss. Which is comprised of two subgroups: Doratoxylon group (Doratoxyleae, *Ganophyllum and Zanha*, without *Averrhoidium*): indehiscent berry-like fruits and Dodonaea group (Cossinieae, Dodonaeeae, *Arfeuillea, Averrhoidium, Eurycorymbus, Euphorianthus, Harpullia* and *Majidea*): dehiscent fruits. In West Africa, the subfamily is represented by three genera namely: *Dodoneae, Majidea* and *Zanha*, all of which are known to be of economic and medicinal importance (Hutchinson and Dalziel, 1958; Keay *et al*., 1964; Burkhill, 2000). There are few reports on the foliar epidermal characters of *Dodonaea* (Venkatesh *et al.*, 2008; Al-Aani *et al.*, 2016) however; little has been done on the other two genera. Consequently, this research aims at carrying out foliar anatomical characterization of members of the subfamily Dodonaeoideae represented in west Africa with a view to delimit and show the relationships among the plant species.

Materials and Methods

Source of sample used: The representative specimens deposited in the herbaria of University of Lagos Herbarium (LUH) were collected with permission and investigated in the Department of Botany, University of Lagos, Nigeria. The provenances of the specimens are presented in Table 1.

Epidermal preparations: The adopted method follows Kadiri (2003) and Ogundipe *et al.,* (2008). For the study, 2-3 cm² portions were cut from the standard median portion of the lamina near the mid-rib. The leaf pieces were dissolved in Mccartney bottle containing Nitric acid for 5 h. Tissue disintegration was indicated by the bubbles and the epidermises were transferred into Petridishes containing cold water for cleansing before they were separated with forceps and mounting needle.

Table 1. List of specimens studied.

Species	Habit	Place of collection	Collector	Date of collection	Accession No.
Z. golungensis	Shrub	F.R.I.N (Ibadan)	Daramola, B.O	10-02-2011	LUH 3317
Z. golungensis	Shrub	F.R.I.N (Ibadan)	Adeyemi, T.O	08-Jul-2008	LUH 3462
D. viscosa	Shrub	LEKKI (swamp)	Kadiri, A. B.	24-07-2011	LUH 3119
D. viscosa	Shrub	ABU, Zaria	Adeyemi, T.O	02-Jun-2009	LUH 037
M. fosterii	Shrub	Limbe Botanic Gardens	Adeyemi, T.O	16-09-2009	LUH 1718

Tissue debris was removed from the epidermis with fine hair brush and rinsed for some time in water. Few drops of ethanol were added in turn to harden the cells. It was then stained with 1-3 drops of Safranin, and mounted in glycerine on the glass slide. The slides were labelled carefully and appropriately and viewed under the light microscope. Photographs of the micro-morphological features were taken at magnification x400 using photomicroscopic camera attached to a Pentium IV computer. Quantitative and qualitative characters of the leaf epidermis were assessed and stomatal index was calculated using the formula as reported by Stace (1965).

Data analysis: Twenty epidermal cells and stomata were randomly selected for measurement using micrometer eye piece. Statistical analysis was carried out using the data analysis tool (Microsoft 2007 Excel) and calculations include mean, standard deviation, standard error and stomata index.

Scanning electron microscopy (SEM): For scanning electron microscopy, Hitachi S-4700 SEM protocol was adopted. Approximately 8 mm^2 of the preserved dried leaves was cut with knifes under an OPTECH microscope and the surfaces were cleansed with a soft brush. With the aid of forceps, this was placed on labelled aluminium stubs covered with sticky tapes so that both adaxial and abaxial surfaces faced upward; they were placed in a sputter coater stub holder and coated with argon for 2-5 mins. The samples were exposed to infrared radiation and observed using Hitachi S-4700 Scanning Electron Microscope. Photographs were taken with the computer using the Hitachi program. The Study was carried out at Jodrell laboratory, Royal Botanic Gardens, Kew, UK.

Results

All the species investigated showed variation in their foliar epidermal characters which are useful in species delimitation. Anatomical characterization of the Dodonacoideae - Sapindaceae using light and scanning electron microscopy revealed that the surface features consisted of hairs, papillae and stomata.

Anomocytic stomata type was common in *Dodonaea viscosa* (Fig. 1A and 1B) and *Majidea fosterii* (Fig. 1C and 1D) but paracytic type was recorded only in *Zanha golugensis* (Fig. 1E and 1F). This observation was supported by Venkatesh *et al.,* (2008), who reported the presence of anomocytic stoma in *D. vicosa*. Members bear stomata on the abaxial surface only i.e., they are hypostomatic exceptions is however recorded in *Dodonaea viscosa* which is amphistomatic in nature with stomata ridges on the abaxial surface (Fig. 2). Anticlinal wall pattern ranges from straight to undulate form with polygonal or irregular shape. On both adaxial and abaxial epidermal surfaces of *D. viscosa,* the epidermal cell shape varies from polygonal to irregular respectively however it is entirely polygonal in *Z. golungensis* and irregular on both surfaces of *M. fosterii*. The anticlinal wall pattern is straight in *Z. golungensis* and *D. viscosa* however, it is undulate in *M. fosterii* (Table 2). Stellate multicellular trichomes are present on the adaxial surface of *Z. golungensis* (Fig. 1E) while none was observed on both surfaces of *D. viscosa* and *M. fosterii*. Also crystal deposits are seen on the adaxial surface of *D. viscosa* and *M. fosterii* while striations were observed on the surfaces of *Z. golungensis* (Fig. 2).

Table 3. Quantitative epidermal features of specimen studied.

Species	E. Cell width	E. Cell length	Stomata width	Stomata length	C.W thickness	No of E. cell	No of stomata	S.I.
	min(mean±S.E)max	min(mean±S.E)max	min(mean±S.E)max	min(mean±S.E)max	min(mean±S.E)max	min(mean±S.E)max	min(mean±S.E)max	(%)
Z. golungensis								
Abaxial	4(5.50 ± 0.35)8	6(7.60 ± 0.65)10	5(6.1±1.24)9	6(7.20±2.16)10	0.5(0.62±0.08)1	213(340±4)421	195(240±3)274	41.38
Adaxial	4(6.70 ± 0.65)10	7(9.92 ± 1.02)12	-	-	0.5(0.78±0.05)1	412(450±6)495	-	-
M. fosterii								
Abaxial	4(4.80±0.39)6	7(9.95±1.20)13	3(3.50±0.35)4	6(7.00±0.59)8	0.5(0.75±0.05)1	119(190±3)252	26(34±3)53	15.18
Adaxial	7(8.00±0.55)9	7(8.00±0.25)9	-	-	0.5(0.70±0.05)1	134(190±3)242	-	-
D. viscose								
Abaxial	4(6.10±0.29)9	5(9.40±2.12)13	3(4.00±0.54)5	6(6.90±0.46)8	0.5(0.80±0.06)1	115(210±3)271	29(46±2)56	17.97
Adaxial	5(7.15±0.31)9	10(12.10±2.12)15	4(5.50±0.35)6	6(7.50 ± 0.35)10	0.5(0.78±0.05)1	149(200±2.)257	27(36±3)45	15.25

KEY: E. Cell width – Epidermal cell width, E. Cell length – Epidermal cell length, C.W Thickness – Cell wall thickness, No of E. Cell – Number of epidermal cell, No of stomata – Number of stomata, Min - Minimum value, Mean – Average of observed values, S.E. – Standard error of the mean, Max - Maximum value, S.I (%) – Stomata index in percentage

Table 2. Qualitative epidermal features of the specimens studied.

Species	Epidermal cell shape	Anticlinal wall pattern	Stomata type	Trichomes
Zanha golungensis				
Abaxial	Polygonal	Straight	Paracytic	----
Adaxial	Polygonal	Straight	Absent	Stellate
Majidea fosterii				
Abaxial	Irregular	Undulate	Anomocytic	----
Adaxial	Polygonal/Irregular	Straight/Undulate	Absent	----
Dodonaea viscosa				
Abaxial	Irregular	Straight	Anomocytic	----
Adaxial	Polygonal	Straight	Anomocytic	----

Epidermal cell size varies from 4(5.50 ± 0.35)8 µm x 6(7.60 ± 0.65)10 µm to 4(6.70 ± 0.65)10 µm x 7(9.92 ± 1.02)12 µm in *Z. golugensis* on the abaxial and adaxial surface respectively while the stomatal size is 5(6.1±1.24)9 µm x 6(7.20±2.16)10 µm. In *M. fosterii*, epidermal cell size varies from 4(4.80±0.39)6µm x 7(9.95±1.20)13 µm to 7(8.00±0.55)9 µm x 7(8.00±0.25)9 µm on the abaxial and adaxial surfaces respectively while the stomatal size is 3(3.50±0.35)4 µm x 6(7.00±0.59)8 µm. Again in *D. viscosa*, epidermal cell size varies from 4(6.10±0.29)9 µm x 5(9.40±2.12)13 µm to 5(7.15±0.31)9 µm x 10(12.10±2.12)15 µm in *Z. golugensis* on the abaxial and adaxial surface respectively while the stomatal size ranges from 3(4.00±0.54)5 µm x 6(6.90±0.46)8 µm to 4(5.50±0.35)6 µm x 6(7.50 ± 0.35)10 µm. The highest number of stomata as well as epidermal cells was recorded in *Z. golugensis* while the least was observed in *M. fosterii* (Table 3).

Discussion

In spite of the fact that vegetative and floral characters are markedly modified in relation to the habitat and pollination mechanisms, anatomical characters have been found to be very useful in taxonomic studies. The leaf epidermal characteristics of the three species studied proved useful for identification and discrimination.

Stace (1965) highlighted stomata distribution as a reliable feature of angiosperm leaf which can be employed for delimiting taxa. In this study, anomocytic stomata were observed in all the taxa studied. This observation is consistent with that of Venkatesh *et al.*, (2008) and Pole (2010), who reported the presence of anomocytic stomata in *D. vicosa*. Also hypostomatic stomata was recorded in the species of *Zanha* and *Majidea* studied and this is similar to the report of Metcalfe and Chalk (1979) and Pole (2010). Our findings also corroborate the works of Buijsen, (1995) who reported polygonal epidermal cells with undulate anticlinal walls and crystals on the epidermal surface of *M. fosterii*.

Furthermore, it can be seen that the shape, size and cell wall thickness of the species studied varied greatly, this according to Sheteolu and Ayodele (1997) is often genotypic in nature, and in many cases have definite taxonomic application. Ugbabe & Ayodele, (2008), also opined that the presence of large epidermal cells with thin walls is an adaptation for water storage. Examination of the leaf epidermal layer of *Z. golungensis*, *D. viscosa* and *M. fosterii* shows that there are wide variations in the morphology and distribution of stomata. Naturally these variations in the morphology and distribution of the adjacent epidermal cells raise the question of how far they can be reliably employed by systematic taxonomy.

Epidermal characters such as trichomes, crystal deposits and striations were useful in delimitation of the three species studied. Functionally, trichomes prevent herbivory, excessive heat and sunlight; and they are offensive to animals when in contact (Stace, 1965). In view of the foregoing observations, a key to the identification of each species based on observed characters is presented below:

Key to West African genera in the subfamily dodonaeoideae

1a. Stomata type Anomocytic, striations absent, epidermal cell width not more than 9 µm ……...…….. 2

2a. Anticlinal wall pattern undulate, stomata restricted to abaxial surface epidermal cell shape irregular, stomata width not more than 4 µm ……..... *M. fosterii*

2b. Anticlinal wall pattern straight, stomata present on both surfaces, epidermal cell shape polygonal, stomata width is up to 6 µm ...……..... *D. viscosa*

1b. Stomata type paracytic, striations present, epidermal cell width up to 10 µm ……. *Z. golungensis*

Conclusions

In conclusion, taxonomically both the quantitative and qualitative characters are useful in the practical identification of these three species studied. The features that emphasized the distinctiveness and differences in the three species when compared are the epidermal shapes, epidermal numbers, stomata types, stomata numbers and the anticlinal pattern.

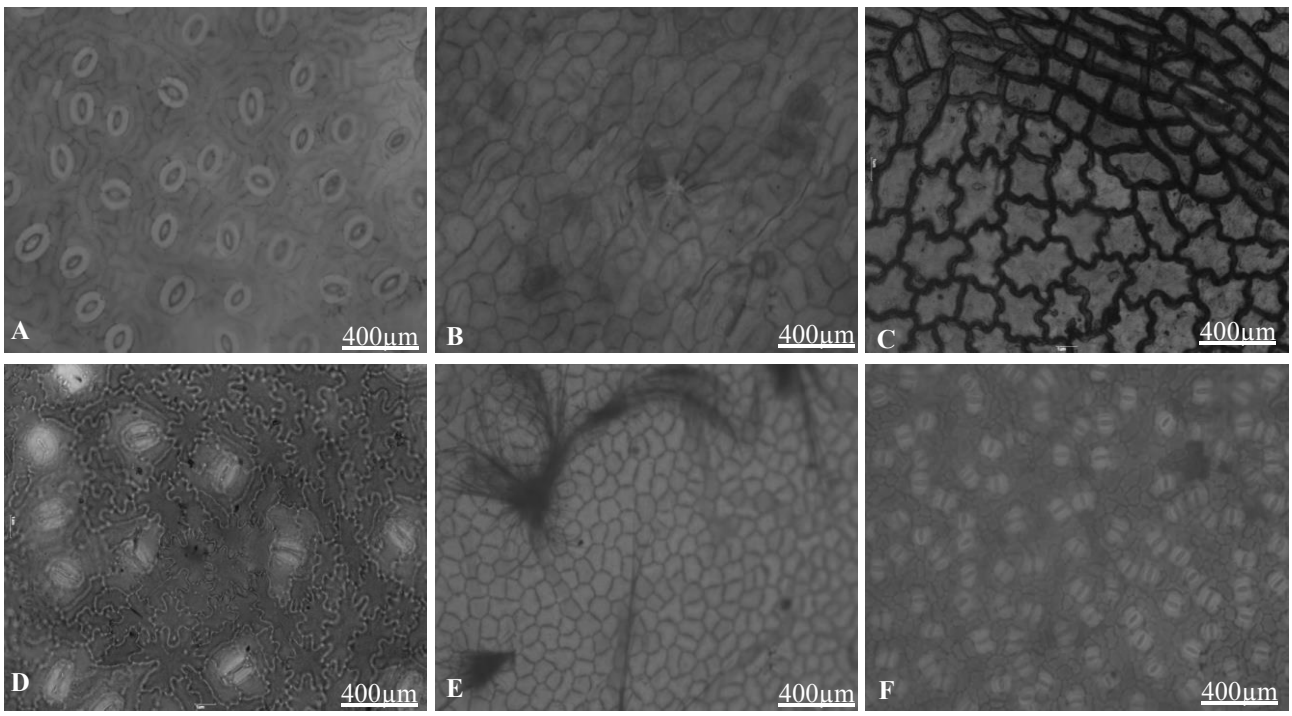

Fig. 1. Photomicrographs showing epidermal features of Dodonaeaoideae studied. **A:** Adaxial surface of *Dodonaea viscosa.* **B:** Abaxial surface of *Dodonaea viscosa.* **C**. Adaxial surface of *Majidea fosterii.* **D:** Abaxial surface of *Majidea fosterii.* **E:** Adaxial surface of *Zanha golugensis.* **F:** Abaxial surface of *Zanha golugensis.*

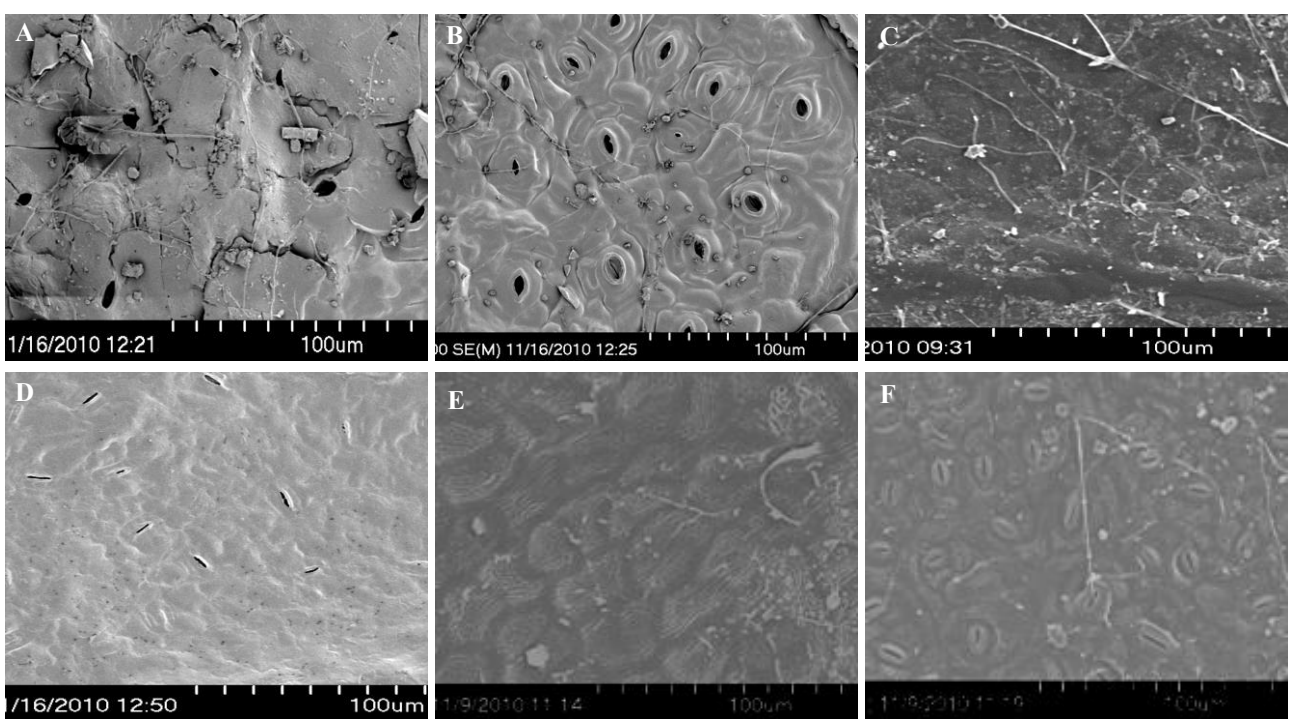

Fig. 2. Scanning Electron Micrograph showing epidermal features of members of Dodonaeaoideae studied. **A:** Adaxial surface of *Dodonaea viscosa.* **B:** Abaxial surface of *Dodonaea viscosa.* **C:** Adaxial surface of *Majidea fosterii.* **D:** Abaxial surface of *Majidea fosterii.* **E:** Adaxial surface of *Zanha golugensis.* **F:** Abaxial surface of *Zanha golugensis.*

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(Received for publication 28 September 2017)