Diagnostic Significance of Leaf Epidermal Features in the Family Cucurbitaceae

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Abstract: Background: Herbal medicine is a popular means of medical management in some parts of the world especially in Africa. Practitioners and students use fresh and dried specimens of herbs for their work and research. The source of dry samples is usually in the local markets where, adulteration of herbal materials takes place. When herbs are dried and folded, identification and authentication become rather difficult. An approach to overcome the problem of identification is the use of anatomical features of the plant materials, especially the plant epidermis. The family Cucurbitaceae has many herbaceous species with medicinal properties, hence its choice for this study. **Results:** The species in which stomata were found to be present on the adaxial epidermis include *Luffa cylindrica*, *Cucumis melo*, *Lagenaria breviflorus*, *Lagenaria siceraria*, *Citrullus colocynthis*, *Citrullus lanatus*, *Luffa acutangula* and *Momordica foetida*. The species in which stomata were absent on the adaxial epidermis include *Telfairia occidentalis*, *Momordica charantia*, *Trichosanthes cucumerina*, *Coccinia barteri*, *Coccinia grandis* and *Cucumis sativus*. *Cucumis melo* stands out as a species with high stomatal density of 800 mm^{-2} and above on both leaf surfaces possibly due to the small size of the stomata while *Cucumis sativus* has a low stomatal density of 45 mm^{-2} and stomata occurring on the abaxial leaf surface only. *Momordica foetida* has stomata on both leaf surfaces while *Momordica charantia* has stomata on abaxial surface only. *Lagenaria breviflorus* is characterized by paracytic and diacytic stomata and *Lagenaria siceraria* by paracytic and staurocytic stomata. *Telfairia occidentalis* is characterized by paracytic stomata and *Trichosanthes cucumerina* by diacytic stomata. **Conclusion:** An indented dichotomous key was constructed to highlight the diagnostic significance of the epidermal features in the family.

Key words: Cucurbitaceae, epidermal cells, stomata, trichomes, taxonomy

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 on 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 (Davis and Heywood, 1963).

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 dorsiventral anatomy: the upper (adaxial) and lower (abaxial) surfaces

have somewhat different construction and may serve different 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, 1964; Van Wyk *et al.*, 1982; 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.

Morphologically the Cucurbitaceae are mostly prostrate or climbing herbaceous annuals comprising about 90 genera and 700 species that are further characterized by commonly having 5 angled stems and coiled tendrils. The leaves are alternate and usually

palmately 5-lobed or divided; stipules are absent. The flowers are actinomorphic and nearly always unisexual. The perianth has a short to prolonged epigynous zone that bears a calyx of 3-6 segments or lobes and 3-6 petals or more frequently a 3-6-lobed sympetalous corolla. The androecium is highly variable, consisting of basically 5 distinct to completely connate stamens that frequently are twisted, folded or reduced in number. The gynoecium consists of a single compound pistil of 2-5 carpels, generally with one style and as many style branches or major stigma lobes as carpels and an inferior ovary with one locule and usually numerous ovules on 2-5 parietal placentae or 3 locules with numerous ovules on axile placentae. The fruit is a type of berry called a pepo (Jeffrey, 1962).

To complement the above morphological description, leaves of fourteen species in three plant families namely Cucurbitaceae were studied anatomically to elucidate their taxonomic potentials with aim of using features such as stomata, epidermal cells and trichomes to diagnose and classify various taxa in the family.

MATERIALS AND METHODS

Leaves of 14 species of Cucurbitaceae family were harvested for anatomical study. Leaf cuticles were macerated in concentrated nitric acid or trioxonitrate (v) acid, rinsed in distilled water, stained in 1% aqueous safranin solution and mounted in dilute glycerine. Parameters on stomata determined were stomatal density, stomatal index and stomatal size (Dilcher, 1974) and frequency of each complex type was expressed as percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). Epidermal cell size was determined as product of length and breadth of

a cell based on a sample size of 35. Index of similarity of leaf surface followed the formula of Philips (1959). Statistical analysis of data consisted of Analysis of Variance and Duncan Multiple Range Test (Duncan, 1955).

RESULTS

The 14 species studied can be categorized into 2 groups namely (1) those in which stomata are absent on the adaxial epidermis or occur on abaxial surface only. These include *Coccinia barteri*, *Coccinia grandis*, *Cucumis sativus*, *Momordica charantia*, *Telfaria occidentalis* and *Trichosanthes cucumerina* and (2) those in which stomata occur on both adaxial and abaxial leaf surfaces. These include *Citrullus lanatus*, *Citrullus colocynthis*, *Cucumis melo*, *Lagenaria breviflorus*, *Lagenaria siceraria*, *Luffa acutangula*, *Luffa cylindrica* and *Momordica foetida*. Six types of stomatal complex were observed in the family. These are namely paracytic, diacytic, anisocytic, actinocytic, cyclocytic, staurocytic (Fig. 1, Table 1). All of them occur with a frequency of 100. The range of stomatal density was from 17.57 mm⁻² in *Momordica foetida* to 870 mm⁻² in *Cucumis melo*. Stomatal size also varied from 10.00 µm² in *Cucumis melo* to 64.00 µm² in *Trichosanthes cucumerina* (Table 1). The pattern of anticlinal wall, epidermal cell shape, epidermal cell size and distribution of trichomes are as indicated in Table 2 and 3.

Cucumis melo stands out as a species with high stomatal density of 800 mm⁻² and above on both leaf surfaces possibly due to the small size of the stomata while *Cucumis sativus* has a low stomatal density of 45 mm⁻² and stomata occurring on the abaxial leaf surface only. *Momordica foetida* has stomata on both leaf

Table 1: List of the plant species studied

Species	Place of collection
<i>Coccinia barteri</i> (Hook. f.) Keay	Beside Rasmus Hostel, Obaleye Street, Oke-Odo, Ilorin, Kwara State, Nigeria.
<i>Coccinia grandis</i> (L.) J. Voigt	Behind Hope Hospital, along Oyan Road, Osun state, Nigeria.
<i>Citrullus lanatus</i> (Thunb.) Matsum.	Inside Biological Garden, University of Ilorin, , Kwara State, Nigeria
<i>Citrullus colocynthis</i> (L.) Schred.	Opposite LTCM, Obaleye Street, Oke Odo, Tanke, Ilorin, Kwara State, Nigeria
<i>Cucumis melo</i> Mill.	Beside Deeper life Church, behind Glorious House of God, Osere, Ilorin, Kwara State, Nigeria
<i>Cucumis sativus</i> L.	Church Street Area, Gaa-Akanbi, Ilorin, Kwara State, Nigeria
<i>Lagenaria breviflorus</i> (Benth.) Roberty	Oke Adini Area, off Basin Road, Ilorin, Kwara State, Nigeria
<i>Lagenaria siceraria</i> (Molina) Standl.	Church Street, Gaa-Akanbi Area, Ilorin, Kwara State, Nigeria
<i>Luffa acutangula</i> (L.) Roxb.	Behind Lagos Hostel, university of Ilorin, Kwara State, Nigeria
<i>Luffa cylindrica</i> (L.) Roem.	University of Ilorin Campus, Ilorin, Kwara State, Nigeria
<i>Momordica charantia</i> L.	Besides K-Dorms, opposite AP Petrol Station, Oke-Odo, Ilorin, Kwara State, Nigeria
<i>Momordica foetida</i> Schum.	Rasmus Hostel, Obaleye Street, Oke-Odo, Ilorin, Kwara State, Nigeria
<i>Telfaria occidentalis</i> Hook. f.	Beside K-Dorms, Opposite AP Petrol Station, Oke-Odo, Ilorin, Kwara State, Nigeria
<i>Trichosanthes cucumerina</i> L.	Oke-Adini Area, off Basin Road, Ilorin, Kwara State, Nigeria

Table 2: Leaf epidermal features in some selected members of cucurbitaceae

Species	Leaf surface	Anticlinal cell wall pattern	Epidermal cell wall shape	Epidermal cell size (μm)
<i>Coccinia barteri</i>	Adaxial	Curved	Irregular	24.01d
	Abaxial	Straight	Polygonal	40.00b
<i>Coccinia grandis</i>	Adaxial	Curved	Polygonal	16.50e
	Abaxial	Curved	Polygonal	18.07e
<i>Citrullus lanatus</i>	Adaxial	Round	Polygonal	35.21c
	Round	Abaxial	Polygonal	25.68d
<i>Citrullus colocynthis</i>	Adaxial	Undulate	Polygonal	24.31d
	Abaxial	Curved	Polygonal	28.69cd
<i>Cucumis melo</i>	Adaxial	Undulate	Irregular	8.21g
	Abaxial	Undulate	Irregular	8.70g
<i>Cucumis sativus</i>	Adaxial	Curved	Polygonal	10.11g
	Abaxial	Curved	Irregular	12.00f
<i>Lagenaria breviflorus</i>	Adaxial	Undulate	Irregular	22.00d
	Abaxial	Curved	Polygonal	18.20e
<i>Lagenaria siceraria</i>	Adaxial	Curved	Irregular	13.80f
	Abaxial	Undulate	Irregular	63.30a
<i>Luffa acutangula</i>	Adaxial	Curved	Polygonal	30.20c
	Abaxial	Curved	Polygonal	35.57c
<i>Luffa cylindrica</i>	Adaxial	Curved	Polygonal	25.68d
	Abaxial	Curved	Polygonal	24.00d
<i>Momordica charantia</i>	Adaxial	Straight	Polygonal	12.71f
	Abaxial	Undulate	Irregular	42.26b
<i>Momordica foetida</i>	Adaxial	Curved	Polygonal	30.00c
	Abaxial	Curved	Polygonal	36.60bc
<i>Telfaria occidentalis</i>	Adaxial	Straight	Polygonal	14.11f
	Abaxial	Curved	Polygonal	40.23b
<i>Trichosanthes cucumerina</i>	Adaxial	Straight	Irregular	16.00ef
	Abaxial	Curved	Irregular	21.54d

Means with same letters are not significantly different at $p < 0.05$

Table 3: Stomatal and trichome features in some selected members of Cucurbitaceae

Species	Leaf surface	Type*	Stomata				Trichome
			Density (mm^{-2})	Size (μm^2)	Index (%)		
<i>Coccinia</i>	Adaxial	-	-	-	-	-	Absent
<i>Barteri</i>	Abaxial	Actinocytic	19.73h	62.00a	2.04g	-	Present
<i>Coccinia</i>	Adaxial	-	-	-	-	-	Absent
<i>Grandis</i>	Abaxial	Actinocytic	80.92d	38.00c	7.38e	-	Present
<i>Citrullus</i>	Adaxial	Anisocytic	95.37d	35.00c	12.89d	-	Absent
<i>Lanatus</i>	Abaxial	Anisocytic	82.24d	15.00f	6.37e	-	Present
<i>Citrullus</i>	Adaxial	Diacytic	26.32h	35.00c	5.66ef	-	Absent
<i>Colocynthis</i>	Abaxial	Cyclocytic	51.97ef	30.00cd	8.99e	-	Present
<i>Cucumis</i>	Abaxial	Anisocytic	821.00a	10.00g	4.29f	-	Absent
<i>Melo</i>	Adaxial	Anisocytic	870.00a	12.00g	34.25a	-	Present
<i>Cucumis</i>	Adaxial	-	-	-	-	-	Absent
<i>Sativus</i>	Abaxial	Paracytic	45.39f	21.00d	11.26d	-	Present
<i>Lagenaria</i>	Adaxial	Diacytic	38.82g	28.00d	3.90f	-	Absent
<i>Breviflorus</i>	Abaxial	Paracytic	120.39c	18.00e	12.80d	-	Present
<i>Lagenaria</i>	Adaxial	Staurocyclic	75.00d	63.00a	8.00e	-	Absent
<i>Siceraria</i>	Abaxial	Paracytic	196.05b	56.00b	21.06b	-	Present
<i>Luffa</i>	Adaxial	Actinocytic	46.05f	27.00d	5.97ef	-	Absent
<i>Acutangula</i>	Abaxial	Actinocytic	49.34f	20.00de	5.89ef	-	Present
<i>Luffa</i>	Adaxial	Actinocytic	39.47g	19.00e	4.28f	-	Absent
<i>Cylindrica</i>	Abaxial	Staurocyclic	56.59e	23.00d	5.30ef	-	Absent
<i>Momordica</i>	Adaxial	-	-	-	-	-	Present
<i>Charantia</i>	Abaxial	Diacytic	148.03c	15.00f	20.07b	-	Present
<i>Momordica</i>	Adaxial	Actinocytic	17.57h	30.00c	1.35	-	Absent
<i>Foetida</i>	Abaxial	Actinocytic	52.63ef	27.00c	6.05e	-	Present
<i>Telfaria</i>	Adaxial	-	-	-	-	-	Absent
<i>Occidentalis</i>	Abaxial	Paracytic	212.17b	35.00c	20.99bc	-	Present
<i>Trichosanthes</i>	Adaxial	-	-	-	-	-	Absent
<i>Cucumerina</i>	Abaxial	Diacytic	151.32c	64.00a	18.36c	-	Present

Means with same letters are not significantly different at $p < 0.05$

surfaces while *Momordica charantia* has stomata on abaxial surface only. *Lagenaria breviflorus* is characterized by paracytic and diacytic stomata and *Lagenaria siceraria* by paracytic and staurocyclic

stomata. *Telfairia occidentalis* is characterized by paracytic stomata and *Trichosanthes cucumerina* by diacytic stomata.

Indented dichotomous taxonomic key is hereby constructed to assist in the process of plant identification using anatomical features:

1a	Stomata on adaxial epidermis present	2
2a	Number of subsidiary cells per stoma few (2-5)	3
3a	Trichome (abaxial) absent	<i>Luffa cylindrica</i>
3b	Trichome (abaxial) present	4
4a	Stomatal density 800 mm ⁻² and above; stomata anisocytic	<i>Cucumis melo</i>
4b	Stomatal density less than 800 mm ⁻²	5
5a	Stomatal density 100 mm ⁻² and above	6
6a	Stomatal complex (adaxial) diacytic	<i>Lagenaria breviflorus</i>
6b	Stomatal complex (adaxial) staurocytic	<i>Lagenaria siceraria</i>
5b	Stomatal density less than 100 mm ⁻²	7
7a	Stomatal complex (adaxial) diacytic	<i>Citrullus colocynthis</i>
7b	Stomatal complex (adaxial) anisocytic	<i>Citrullus lanatus</i>
2b	Number of subsidiary cells per stoma many (6 and above)	8
8a	Stomatal density (adaxial) more than 20 mm ⁻²	<i>Luffa acutangula</i>
8b	Stomatal density (adaxial) less than 20 mm ⁻²	<i>Momordica foetida</i>
1b	Stomata on adaxial epidermis absent	9
9a	Stomatal density (abaxial) 80 mm ⁻² and above	10
10a	Stomata (abaxial) paracytic	<i>Telfairia occidentalis</i>
10b	Stomata (abaxial) diacytic	11
11a	Trichome (adaxial) present	<i>Momordica charantia</i>
11b	Trichome (abaxial) absent	<i>Trichosanthes cucumerina</i>
9b	Stomatal density (abaxial) less than 80 mm ⁻²	12
12a	Stomatal density less than 20 mm ⁻²	<i>Coccinia barteri</i>
12b	Stomatal density more than 20 mm ⁻²	13
13a	Stomata (abaxial) actinocytic	<i>Coccinia grandis</i>
13b	Stomata (abaxial) paracyti	<i>Cucumis sativus</i>

Fig. 1: Leaf surface view of (a) *Coccinia barteri* showing actinocytic stoma, (b) *Coccinia grandis* showing actinocytic stoma, (c) *Citrullus lanatus* showing anisocytic stoma, (d) *Citrullus colocynthis* showing diacytic and cyclocytic stomata, (e) adaxial, (f) abaxial, *Cucumis melo* anisocytic stoma, (g) *Cucumis sativus* showing paracytic stoma, (h) *Lagenaria breviflorus* showing diacytic and paracytic stomata, (i) adaxial, (j) abaxial, *Lagenaria siceraria* showing staurocytic and paracytic stomata, (k) adaxial, (l) abaxial, *Luffa acutangula* actinocytic stoma, (m) *Luffa cylindrica* showing actinocytic and staurocytic stomata, (n) adaxial, (o) abaxial, *Momordica charantia* showing diacytic stoma, (p) *Momordica foetida* showing actinocytic stoma, (q) *Telfairia occidentalis* paracytic stoma and (r) *Trichosanthes cucumerina* showing diacytic stoma

DISCUSSION

Anatomical evidences observed in the 14 species of the family Cucurbitaceae (Table 1) seems to be enough diagnostic values for delimiting species and genera studied. Plant anatomy has been found to be 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 ordered manner (Olorode, 1984). In spite of the fact that vegetative and floral characters are markedly modified in relation to the habitat and pollination mechanisms, the preceding observation and the summaries of character variation in Table 2 indicated that the taxonomic application of the diversity of epidermal morphology in Cucurbitaceae cannot be over emphasized. The decision to choice 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; Abdulrahman and Oladele, 2010a; Abdulrahman and Oladele, 2010b).

It is worth note the presence of curved ACWPs on both leaf surfaces of *L. acutangula* and *L. cylindrica*. The ECWSs are polygonal on both leaf surfaces in all 14 species of Cucurbitaceae except in *C. barteri*, *C. sativus*, *L. breviflorus* and *M. charantia* and in *C. melo*, *L. siceraria* and *T. cucumerina* where, they are irregular on one surface and on both surfaces respectively (Table 3). The SD in Cucurbitaceae was very large in *C. melo* (821 and 870) on both leaf surfaces and smaller in *M. foetida* (17.57 μm) in adaxial surface (Table 3).

Trichome is another taxonomic significance feature in the species of the three families. Occurrences of trichomes on the abaxial surface of all the 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. Similar observation was made in two species of *Jatropha* by Abdulrahman (2009) where, *J. gossypifolia* with high trichome density and index transpired faster than *J. curcas* which possessed lower trichome density and index.

Stomatal frequency varies from one leaf to the other and these were observed among the species in the family studied. Homogeneous nature of stomatal complex type on leaves of the species of Cucurbitaceae is responsible for 100% occurrence of Stomatal Complex Types (SCTs) where, a single SCT present on a leaf

surface. Stomatal Density (SD) according to Esau (1977) was found to vary from one plant to another. All species of Cucurbitaceae studied possessed more SD in the abaxial surface than in the adaxial surface. This pattern is in corroboration with Oyeleke *et al.* (2004), Abdulrahman (2009), Abdulrahman and Oladele (2009) and Saadu *et al.* (2009). SI and SS are higher (ranging from 22.73-26.91%) and larger in the abaxial surface than in the adaxial surface in most species of Cucurbitaceae where, stomata occurred on both surfaces of leaves (Table 3). According to Esau (1965), stomata index varied on different part of the leaves or on different leaves of same plants. While Davis and Heywood (1963) considered stomatal size to be too variable as diagnostic features other researchers like Wilkinson (1971) had contrary view. He indicated that stomatal size shows a much wider range in some taxa than in others, as it may be sometimes be useful as diagnostic character when dealing with taxa in which size ranges are restricted. This present study conforms to Wilkinson's observation.

The above leaf epidermal features could be said to be taxonomically significant because of discontinuities that occurred within and between genus, genera within the family as shown on Table 2 and 3 and Fig. 1. The leaf epidermal features observed in all the fourteen species of Cucurbitaceae are enough taxonomic characters which could be implored to support hitherto external morphological characters used to classify plants in this family.

CONCLUSION

Based on epidermal features, some members of the family Cucurbitaceae can readily be distinguished from one another. Such epidermal features as on stomata and trichomes are thus useful tools for diagnostic and taxonomic works. The approach can be put to use in herbal medicine and forensic science where, identification and authentication of plant specimens are essential.

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