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Morphological and Leaf Epidermal Features of Some *Capsicum* Species (Solanaceae) from Nigeria

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ABSTRACT

Investigation on the leaf epidermal morphological features was carried out on five species of the genus *Capsicum* in the family Solanaceae collected from different regions in the Southwestern part of Nigeria in search of taxonomic and diagnostic characters that could be employed for their classification and identification. The species are *C. annuum* L., *C frutescens* L., *C. chinense* jacq., *C. baccatum* L., and *C. chacoense* Hunz. All the species possessed either anomocytic or anisocytic stomata type on both adaxial and abaxial surfaces except for *C. chinense* which showed paracytic stomata type on both surfaces. The leaves of *C. baccatum, C. annuum, C. chinense* and *C. frutescens* are amphistomatic, while that of *C. chacoense* are epistomatic. The similarities and overlaps observed in the cell shape, stomata index and anticlinal wall pattern of the species provide evidence for their genetic and evolutionary relationship and justification for their taxonomic grouping.

Keywords: Anatomy, *Capsicum*, Leaf epidermal, Macro-morphology, Micro-morphology, Nigeria, Solanaceae, Taxonomy

INTRODUCTION

The genus *Capsicum* belongs to the family Solanaceae. Members of the family are mostly herbs and shrubs and are usually cultivated as annuals (Dutta 2005). The larger genera are *Solanum* (1,500spp), *Cestrum* (250spp, mostly American), *Physalis* (100spp), *Nicotiana* (100spp) and *Capsicum* (50spp) (Dutta, 2005). The family is widely distributed throughout tropic and temperate region of the world with centre of diversity occurring in central in Central and South America and Australia (Aunay, 2001).

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Capsicum is an economically important genus in the Solanaceae, encompassing around 25 species, native to tropical and temperate regions of the Americas (Aunay, 2001). Five of its members *C. annum, C. frutescens, C. chinense*, *C. baccatum*, and *C. pubescens* were domesticated by American Indians and were exploited in global scale after Columbus owing to their valuable fruits, with pungent fruits being used as spices and sweet ones as vegetables, i.e. they are mostly cultivated worldwide because of their spicy and nutritional value. In addition, the genus has medicinal and ornamental uses. The stem is erect, woody and hairy; leaves are alternate, simple and exstipulate. The fruit is a berry and the seeds are minute endospermic with a straight or curved embryo. The taxonomic value of epidermal morphology is well documented in botanical literature (Mbagwu & Edeoga, 2006; Adedeji, 2004). The result of epidermal characters studied by Nwachukwu *et al*. (2007) in *C. annuum* and *C. frutescens* showed little variation except for their number of stomata. Adedeji *et al*. (2007) also reported that *C. annuum* can be separated from *C. frutescens* and *C. chinense* on the basis of the organographic distribution of their trichomes.

Information on detailed anatomical structures of the genus is still fragmentary; hence, this present work provides a useful means of generating data to enhance further identification and delimitation of the taxa in the region.

MATERIALS AND METHODS

The seeds used were collected from four different states in the southwestern Nigeria. They were sundried, sown and then raised to maturity on a small portion of land from where matured leaf samples were collected for the study. Collections were also made from the field to facilitate comparison.

Matured leaves were measured for each species at comparative position. Macro characters measured include leaf length and width (taken at a widest part of the leaf) and petiole. Qualitative characters such as leaf shape, margin and apex were also noted. Micro characters such as number of epidermal cell per view (x 400), thickness of the cell wall, size of the epidermal cell (at the widest point), number of stomata per view (x 400), length and width of stomata and stomata index were also measured.

Epidermal preparation was by obtaining sizeable portion (5mm²-1cm²) of the mature leaves from the transverse section of each leaf soaked in concentration solution of trioxonitrate (v) acid (HNO_3) for a period of about 18-24 hours. The appearance of air bubbles on the surface of the leaf fragments indicated their suitability for separation. They were then transferred into water in a Petri dish from where the upper and lower epidermises were carefully peeled. Selected specimens were stained in Safranin and counterstained in Fast green. Stained specimens cleared in clove oil to remove excess stain were mounted on slides with DPX mountant.

Stomata frequency was estimated from an average of ten counts. Length and

width of stomata were measured at X400 magnification using ocular micro-meter. The measurements were later converted to microns using a pre-calibrated stage micrometer.

The stomata index (I) was obtained by expressing the number of stomata per unit area as a percentage of the total number of cells in the same unit area using Salisbury (1927) method as modified by Hussin *et al.* (2000).

$$I = \frac{S}{S + E} \times 100$$

Where, I represents the stomata index, S, number of stomata per unit area and E, number of ordinary epidermal cells in the same unit area.

RESULTS

Macromorphological Characters

The leaves of the species examined are mostly simple and alternate with all possessing an entire margin. The leaf shape ranges from ovate to lanceolate. *C. annuum, C. chinense* and *C. frutescens* are pubescent, while *C. baccatum* and *C chacoense* possess glabrous leaf surface (Table 1). A thorough study of the species revealed variation in the sizes of leaves of each as the largest was observed in *C. chinense* and the smallest in *C. frutescens*. From the analysis carried out on the length, the largest is *C. annuum* (7.6 cm) and smallest in *C. chacoense* and *C. chinense* with both having a record of 2.4 cm. The lowest length/width ratio is 1:1 in *C. chacoense, C. annuum* with 2:1, *C. frutescens,* with 3:1 and the highest in *C. chinense* with 4:1 (Table 2). *C. Chinense* has the longest petiole and fruit stalk with the mean number of 13 cm and 11.4 cm repectively, while *C. chacoense* has the shortest petiole with the mean number of 6.1 cm, and *C. annuum* has the shortest fruit stalk of 4.3 cm (see Table 2).

Epidermal Cell

The shapes of epidermal cells on both the adaxial and abaxial surfaces are polygonal and mostly irregular in all the taxa. The anticlinal walls are curved, slightly straight and straight. The irregular cells usually have curved to slightly straight anticlinal

TABLE 1
Comparison of some qualitative morphological features of *Capsicum*

Taxa	Character codes			
	1	2	3	4
C. annuum	+	–	–	+
C. chinense	+	–	–	+
C. frutescens	+	–	–	+
C. baccatum	–	+	+	–
C. chacoense	–	+	+	–

Legend to character codes: 1 = Ovate leaf shape, 2 = Lanceolate leaf shape, 3 = Pubescent leaf, 4 = Glabrous leaf, + = Presence, − = Absence

TABLE 2
Morphological characters of the studied specimens [min (mean ± S.E) max]

Taxa	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Fruit length (cm)	Fruit stalk (cm)
C. annuum	6.7(8.3 ± 0.3)10	3.1(3.3 ± 0.4)3.5	7.8(9.9 ± 0.4)11	2.4(2.9 ± 0.2)3.8	3.2(4.3 ± 0.3)5.7
C. chinense	6(8.9 ± 0.7)12	2.8(4.6 ± 0.5)6.5	9.2(13 ± 0.9)17	7(8.5 ± 0.2)9.4	9(10.5 ± 0.3)12.3
C. frutescens	5.4(5.9 ± 0.9)6.4	1.2(1.7 ± 0.8)2	6.9(7.6 ± 0.2)8.3	5.5(5 ± 0.1)6.4	7.8(8.3 ± 0.1)8.6
C. baccatum	5(5.7 ± 0.2)6.5	3(3.4 ± 0.1)3.9	6.6(7.2 ± 0.2)7.8	2.4(2.8 ± 10.1)3	4.4(5.1 ± 0.2)5.8
C. chacoense	4.5(5.3 ± 0.2)5.9	1.3(1.4 ± 0.1)1.6	5(6.1 ± 0.2)6.8	7.5(9 ± 0.3)11	10(11.4 ± 0.4)14

TABLE 3
Comparison of some epidermal features of *Capsicum* species (Adaxial)

Taxa	Character codes						
	1	2	3	4	5	6	7
C. annuum	+	-	+	-	+	-	-
C. chinense	+	-	-	-	-	+	-
C. frutescens	-	+	-	+	+	-	-
C. baccatum	+	-	-	+	+	-	-
C. chacoense	+	-	+	-	-	-	+

Legend to character codes: 1 = Anomocytic stomata, 2 = Anisocytic stomata, 3 = Irregular cell shape, 4 = Polygonal cell shape, 5 = Slightly straight anticlinal wall, 6 = Curved anticlinal wall, 7 = Curved/slightly straight anticlinal wall, + = Presence, − = Absence.

TABLE 4
Comparison of some epidermal features of *Capsicum* species (Abaxial)

Taxa	Character codes							
	1	2	3	4	5	6	7	8
C. annuum	+	-	-	+	-	-	-	+
C. chinense	-	-	+	+	-	+	-	-
C. frutescens	+	-	-	+	-	-	+	-
C. baccatum	-	+	-	+	-	-	-	+
C. chacoense	-	-	-	-	+	-	+	-

Legend to character codes: 1 = Anomocytic stomata, 2 = Anisocytic stomata, 3 = Paracytic stomata, 4 = Irregular cell shape, 5 = Polygonal cell shape, 6 = Slightly straight anticlinal wall, 7 = Curved anticlinal wall, 8 = Curved/slightly straight anticlinal wall, + = Presence, − = Absence.

TABLE 5
Variations in the epidermal cell sizes and cell wall thickness of the studied species

Taxa	Cell wall thickness per µm [min (max ± S.E) max]		Number of cell [min (max ± S.E) max]	
C. annuum	AD	AB	AD	AB
	2(3.3 ± 0.3)4	1(2.5 ± 0.3)4	48(56 ± 1.6)52	151(169 ± 4.4)189
C. chinense	1(2.6 ± 0.4)5	5(5.7 ± 0.3)7	8(14.8 ± 1.4)21	146(154 ± 1.5)160
C. frutescens	5(6.2 ± 0.4)8	1(3.6 ± 0.5)6	127(146 ± 3.6)160	88(97.3 ± 3.6)126
C. baccatum	3(4 ± 0.2)5	5(6.1 ± 0.3)7	46(62.7 ± 2.5)73	108(208 ± 5.5)235
C. chacoense	4(4.9 ± 0.3)6	1(2 ± 0.3)3	90(117.5 ± 5.3)136	15(19.4 ± 1.1)26

walls, while the polygonal cells usually have straight anticlinal walls. The thickness of the epidermal cell walls ranged from 2.6 µm in *C. baccatum* to 6.2 µm in *C. chacoense* on the adaxial surface and from 2.0 µm in *C. chinense* to 6.1 µm on the abaxial surface of *C. frutescens*. The number of cells on the abaxial surface is more than those on the abaxial surface of the studied species. The mean number ranged from 14.8 in *C. baccatum* to 146.6 in *C. chacoense* on the abaxial, while it ranged from 19.4 in *C. chinense* to 208.5 in *C. frutescens* on the adaxial (see Table 4).

Stomata

Fig.1 (a to j) show variation in the distribution and type of stomata among the taxa. Of the five species studied, *C. baccatum, C. annuum, C. frutescens* and *C. chinense* showed amphistomatic leaves, while only *C. chacoense* is epistomatic (Table 6). Most of the species possessed anomocytic stomata and anisocytic stomata type with only the abaxial surface of *C. chinense* possessing paracytic stomata.

DISCUSSION

The results of the morphological and epidermal features of the genus *Capsicum* studied revealed some stable diagnostic characteristics that could be used for taxonomic decision.

The variations in the type of trichomes possessed by the species is in accordance with the suggestion made by Roe (1971) that the genus exhibits great hair diversity which have been of considerable importance in

TABLE 6
Stomata characters for the studied taxa

Taxa	Stomata Length		Stomata Width		Stomata density		Stomata index %	
	AD	AB	AD	AB	AD	AB	AD	AB
C. annuum	42(48.7 ± 1.27)55	65(73.5 ± 1.68)82	20(25.9 ± 0.84)30	21(24.4 ± 0.67)27	10(13.8 ± 0.85)18	37(42.3 ± 1.05)48	19.8	20.0
C. baccatum	58(61.2 ± 0.83)65	52(61.1 ± 1.57)68	20(22.2 ± 0.55)25	20(24.2 ± 0.73)28	3(4 ± 0.26)5	21(33.6 ± 2.41)46	21.3	17.9
C. chacoense	65(74.9 ± 2.03)85	–	30(36.6 ± 1.20)41	–	8(11.8 ± 1.06)17	–	7.5	–
C. frutescens	50(60.5 ± 1.46)66	58(68 ± 2.41)80	18(22.2 ± 0.76)25	25(29.4 ± 1.21)35	11(15.3 ± 0.87)19	48(52.9 ± 1.08)58	19.6	20.2
C. chinense	50(58.3 ± 1.45)62	70(76.7 ± 1.63)85	20(24.3 ± 0.91)28	10(10.8 ± 0.29)12	25(30.8 ± 1.02)35	3(4.3 ± 0.34)6	20.8	18.1

- denotes no stomata present

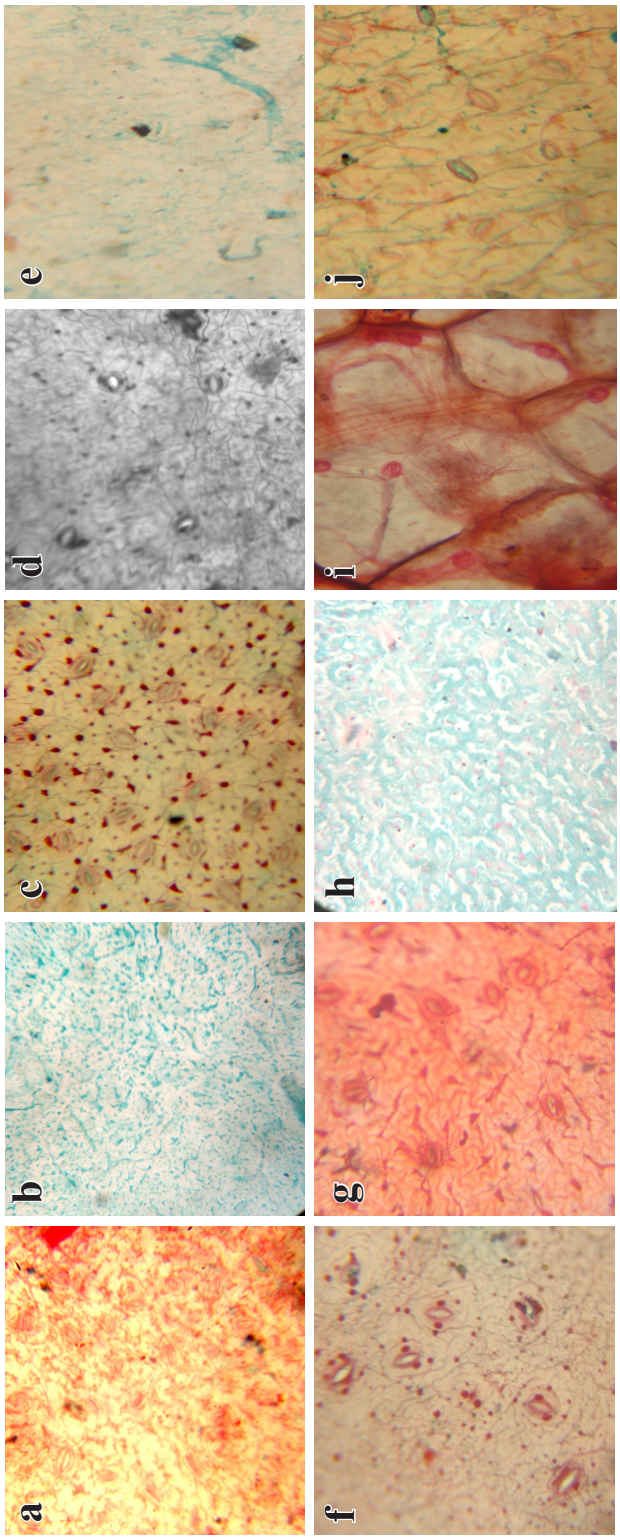

Fig.1 a) Abaxial surface of *C. annuum* showing Anomocytic stomata and irregular cell.
b) Adaxial surface of *C. annuum* showing Anomocytic stomata and irregular cell
c) Abaxial surface of *C. frutescens* showing Anomocytic stomata and irregular cell
d) Adaxial surface of *C. frutescens* showing Anisocytic stomata and polygonal cell
e) Abaxial surface of *C. chinense* showing paracytic stomata and irregular cell
f) Adaxial surface of *C. chinense* showing Anomocytic stomata and irregular cell
g) Adaxial surface of *C. chacoense* showing Anomocytic stomata and irregular cell
h) Abaxial surface of *C. chacoense* showing no stomata and polygonal cell
i) Adaxial surface of *C. baccatum* showing Anomocytic stomata and polygonal cell
j) Abaxial surface of *C. baccatum* showing Anisocytic stomata and irregular cell

comparative investigations in angiosperms. They are frequently present, easily observable and have been found to have variation patterns which correlate with other features of the taxa under investigation.

Morphologically, the vegetative features that separate the species from one another is in line with earlier works of Okwulehi and Okoli (1999), and Edeoga and Emeka (2000), who used comparative morphology of different species in establishing relationships relation among various taxa. The cell wall and shape varied among the species; *C. chinense* and *C. annuum* can be grouped together as they possess irregular shape both on the adaxial and abaxial surfaces, while *C. baccatum* and *C. frutescens* can also be grouped by their polygoanal and irregular shapes on the adaxial and abaxial surfaces, respectively. *C. chacoense* is the only species that possess irregular cell shape on the adaxial surface and polygonal cells on the abaxial surface. Stace (1965) suggested that environmental conditions, such as humidity, play a significant role in determining the pattern of anticlinal walls. The anomocytic type of stomata present in the species of *Capsicum* indicates that the species are phylogenetically related (Nwachukwu *et al*., 2007). The preponderance of stomata on the adaxial surfaces is probably an adaptation to water loss (Mbagwu *et al*., 2008). This is in agreement with Metcalfe and Chalk (1979), as well as Mbagwu and Edeoga (2006), who observed that stomata are usually more on the lower epidermis in the species of *Amaranthus* and *Vigna,* respectively.

Variation was also noted in the stomata density and index among species which is often a reflection of physiological responses, together with the combination of environmental factors and this can be of help in delimitation at the species level (Adegbite, 2008). Metcalfe and chalk (1979) suggested that while stomata density varies considerably with the age of leaf, stomata index is highly constant for a given species.

Variation shown by the data and figures of the characters such as stomata density, stomata index, stomata type, stomata length and width are therefore of taxonomic importance. Also, the similarities observed in stomata type and shape provide evidence for their genetic and evolutionary relationships and justification for their taxonomic grouping.

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