

Original Research Article

LEAF EPIDERMAL ANATOMY IN FIVE VARIETIES OF *CAPSICUM ANNUUM* L. SOLANACEAE

ABSTRACT

Leaf epidermal features were studied in five varieties of *Capsicum annuum* L. to evaluate the reliability of the characters in the taxonomic consideration of these varieties. These varieties in most cases are not easily recognized especially using their dry samples. Varieties studied include var. *abbreviatum*, var. *annuum*, var. *accuminatum*, var. *grossum* and var. *glabriusculum*. Two types of stomata (anisocytic and tetracytic) were identified in the five varieties and the leaves were amphistomatic. There is high frequency of anisocytic stomata (66.67 – 95.24%) on both surfaces compared to tetracytic stomata (4.76 – 33.33%). Trichome is absent on both leaf surfaces of variety *grossum*; it is present on both surfaces of variety *accuminatum* while it is epitrichomatic and hypotrichomatic on varieties *glabriusculum* and *annuum* respectively. The shapes of the epidermal cells of the upper and lower surfaces are irregular and undulate in all the taxa studied. These features were found to be of good and reliable taxonomic use for delimiting the five varieties of *Capsicum annuum*.

Key words: *Capsicum annuum*, varieties, epidermal features, taxonomy, trichomes

INTRODUCTION

Capsicum annuum is a domesticated species of the plant genus *Capsicum* in the family Solanaceae (Night shade), native to southern North America and northern South America (Latham, 2009). Linnaeus originally proposed two species of *Capsicum* - *C. annuum* and *C. frutescens*. Since that time there has been no agreement as to whether the principal cultivated peppers constitute one or two species. Because of the similarity present in both, the two species are morphologically somewhat difficult to distinguish. However, *C. annuum* normally has white corollas and single pedicels, whereas *C. frutescens* has waxy, greenish-white corollas and frequently paired pedicels at the nodes (Nwachukwu *et al.*, 2007). Of the varieties of peppers now in commercial cultivation in Nigeria, all but

27 bell peppers are referred to as *C. annuum*. The species encompasses a wide variety of shapes and sizes
 28 of peppers, both mild and hot, ranging from bell peppers to chilli peppers (Zhang *et al.*, 2002). The ripe
 29 fruit can be red, yellow, green, orange, white, purple, blue, or brown depending on the specific cultivar
 30 (Dave and Paul, 2009). The single flowers are an off-white (sometimes purplish) colour while the stem
 31 is densely branched and up to 60 cm tall. The fruit is a berry which may be green, yellow or red when
 32 ripe (OECD, 2011). The species is a source of popular sweet peppers and hot chillis with numerous
 33 varieties cultivated all around the world. Sweet peppers are very often used as a bulking agent in ready-
 34 made meals and take-away food, because they are cheap, have a strong flavour, and are colourful. The
 35 colourful aspect of peppers increases the visual appeal of the food, making it more appetizing. Foods
 36 containing peppers, especially chilli peppers, often have a strong aftertaste which is due to the presence
 37 of capsinoids in peppers (Grubben, and Denton, 2004).

38 Capsicum terminology is very confusing with pepper, chilli, chile, chili, aji, paprika and capsicum all
 39 used interchangeably to describe the plant (Mcmullan and Livsey, 2013). The term “pepper” is the
 40 most common terminology used in Nigeria. This species is the most common and extensively
 41 cultivated of the five domesticated capsicums. There has been much argument and debate amongst
 42 botanists and taxonomists as to number and classification of Capsicum species. After much argument
 43 and amendment, it is now widely accepted that the genus capsicum consists of five domesticated
 44 species and twenty-six wild species. Due to ease in cross pollination and hybridization of new varieties,
 45 there is now a wide range of varieties available making classification an increasingly difficult task
 46 (Mcmullan and Livsey, 2013). *Capsicum annuum*, a Latin word – annuum meaning ‘annual’ is actually
 47 an incorrect designation given that peppers are perennials under suitable conditions (Katzer, 2008).
 48 Nwachukwu *et al* (2007) showed annual herb in *C. annum* and perennial herb in *C. frutescens*

49 The use of leaf micromorphological and epidermal features has been found to be of immense interest in
 50 taxonomy and have been used by many authors in plant identification (Sapir *et al.*, 2002; Agbagwa and
 51 Ndukwu, 2004; Noraini and Cutler, 2009; Soladoye *et al.*, 2010; Sharma *et al.*, 2010). An excellent
 52 review of the application of morphological features in systematic studies is shown in the works of
 53 Okwulehi and Okoli, 1999, Chakrabarty and Gupta, 1981 and Olowokudejo, 1990, Edeoga and Eboka
 54 2000, Edeoga and Ikem 2001 and Stern, 2000.

55 The present study assessed the relevance of leaf epidermal features in five varieties of *C. annuum* in
56 order to evaluate the reliability of these characters in the taxonomic consideration of the *C. annuum*
57 varieties studied.

58 MATERIALS AND METHODS

59 Living fresh materials were used in this study. The leaves of *Capsicum annum* varieties were obtained
60 from plant stands of the Research farm, Department of Biological Sciences, Gombe State University.
61 Photographs of all the specimens collected were taken with digital camera for picture database during
62 the field trips. The specimens were identified and authenticated at the Herbarium in the Department of
63 Plant Biology, University of Ilorin, Ilorin, Kwara State and National Center for Genetic Resources and
64 Biotechnology, Ibadan, Oyo State, Nigeria.

65 Epidermal peels of the leaf surfaces of the varieties were made using the method of Metcalfe (1960).
66 The abaxial and adaxial surfaces of the leaves surfaces were carefully sectioned with razor blade (free-
67 hand section) and placed on microscope slides. The preparations were stained with 1% safranin and
68 50% glycerol or Formalin Acetic Acid (FAA) and observed under a light microscope and the leaf
69 anatomical features were then examined.

70 Using 35 fields of view at X40 objective as quadrats, the numbers of subsidiary cells per stoma was
71 noted to determine the frequency of the different stomatal complex types and were expressed as
72 percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001).
73 Terminologies for naming stomatal complex types followed those of Dilcher (1974). The stomatal
74 densities were determined as the number of stomata per square millimeter (Stace, 1965). The stomata
75 observed were viewed with the light microscope and were calculated in unit area using the stomatal
76 index (S.I.) formular as shown below: $S.I. = \frac{S}{S + E} \times 100$, where *S* and *E* mean numbers of stomata
77 and epidermal cells respectively within the particular area under investigation

78 The mean stomatal sizes were determined by measuring length and breadth of guard cells using an eye
79 piece micrometer. Samples of 35 stomata were used. The method followed those of Franco (1939) and
80 Wilkinson (1979)

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84 **Statistical analysis**

85 All data were processed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test
 86 (DMRT). Computer software **was used was** SPSS version 17.0. A probability value of 0.05 was used as
 87 bench mark for significant difference between parameters

88 **RESULTS AND DISCUSSION**

89 The results of the leaf epidermal features in the varieties of *C. annuum* investigated are summarized in
 90 Table 1 and Figs 1 - 8. The shapes of the epidermal cells of the upper and lower surfaces are irregular
 91 and undulate in all the taxa studied. The taxa were amphistomatic having stomata on both the adaxial
 92 (upper) and abaxial (lower) surfaces of the leaf. The distribution of stomata on the upper and lower
 93 surfaces of the varieties revealed anisocytic and tetracytic types of stomatal types (Figs 1 - 8).
 94 Generally, there is high frequency of anisocytic stomata (66.67 – 95.24%) on both surfaces compared
 95 to tetracytic (4.76 – 33.33%). This confirms the intraspecific relationship between *C. annuum* varieties.
 96 The stomatal index range from 1.61% - 13.69% in the upper surface of the taxa studied and from
 97 7.81% (lowest) in *C. annuum* var. *abbreviatum*, to 56.60% (highest) in *C. annuum* var. *annuum* in the
 98 lower surfaces. This implies that the stomatal index is therefore highest on the lower epidermis and
 99 lowest on the upper epidermis in the five varieties. Duncan Multiple Range Test (DMRT) – one-way
 100 ANOVA also revealed that there is no significant difference between the stomatal sizes of var.
 101 *abbreviatum* and var. *annuum*. The stomatal density is higher on the abaxial surface of var.
 102 *abbreviatum* and lower on adaxial surface of var. *accuminatum*. The stomatal density like index is also
 103 generally higher on the abaxial surface than in the adaxial surface in the five taxa. Moreover, the
 104 stomatal sizes of var. *accuminatum*, var. *grossum* and var. *glabriusculum* show no significant
 105 differences. A remarkable distinguishable feature of trichomes was observed in the varieties of *C.*
 106 *annuum* studied. There was absence of trichome on both surfaces of var. *grossum* and *abbreviatum* but
 107 present on both surfaces of var. *accuminatum* (Figs 9 and 10). However, trichomes are present on the
 108 adaxial surface of var. *annuum* and on the abaxial surface of var. *glabriusculum*. Hence, according to
 109 Greulach (1973) abundant dead hairs on leaf surface have been thought to form a boundary layer of
 110 moist air, and generally thought to help keep the leaf cool and also to prevent rapid wind current from
 111 passing close to the surface and thus removing water vapour from transpiring and so reduce the rate of
 112 transpiration and living hairs could be expected to increase the evaporating surface greatly. The

113 absence and presence of trichomes in the taxa are of taxonomic importance and can be used to delimit
114 species and varieties.

115 The leaf epidermal features observed in these investigations are of systematic value because they are
116 reasonably constant in the taxa studied (Table 2 and Figs 1 - 8). There are continuous epidermal cell
117 shape (irregular) and anticlinal wall pattern (undulate) in all the varieties. The epidermal cell density on
118 the abaxial surface is generally low ($10.00 - 39.00 \text{ mm}^2$) and high on the adaxial surfaces ($38.86 -$
119 103.20 mm^2). The results show a close relationship among the varieties. Olowokudejo (1990) made
120 similar observation in the genus *Anona*. Furthermore, the use of leaf epidermal features (epidermal cell,
121 stomata and trichomes) in Systematics has become popular and distinctive and have been used as a
122 great taxonomic tool at the levels of family, genus and species. Works of Paliwal (1967); Shah and
123 Gopal (1972); Gill (1982); Edeoga (2000); Edeoga and Osawe (1996) are typical examples.

Table 1: Stomatal parameters in some varieties of *Capsicum annuum*

Taxa	Surface	Stomatal complex type	Frequency (%)	Stomatal Density (mm ²)	Stomatal size (μm)	Stomatal index (%)
<i>C. annuum</i> var. <i>abbreviatum</i>	Abaxial	Tetracytic	25.42	1.80±0.20	53.25±4.35 ^a	7.81±0.93 ^a
		Anisocytic	74.58	25.50±0.51		54.39±1.82 ^d
	Adaxial	Tetracytic	29.00	1.63±0.32	91.36±8.02 ^c	2.23±0.48 ^c
		Anisocytic	71.05	4.00±0.80		5.46±0.58 ^e
<i>C. annuum</i> var. <i>annuum</i>	Abaxial	Tetracytic	24.71	4.20±1.24	56.88±2.45 ^a	31.23±4.43
		Anisocytic	75.29	12.80±1.01		56.60±3.75 ^d
	Adaxial	Tetracytic	33.33	1.00±0.24	51.19±1.04 ^a	2.08±0.43 ^c
		Anisocytic	66.67	2.00±0.55		1.94±0.54
<i>C. annuum</i> var. <i>accuminatum</i>	Abaxial	Tetracytic	25.00	3.40±0.93	90.38±4.01 ^b	15.75±2.06 ^b
		Anisocytic	75.00	9.00±1.12		21.13±1.74
	Adaxial	Tetracytic	4.76	0.50±0.02	77.89±6.62 ^c	2.73±0.19 ^c
		Anisocytic	95.24	2.20±0.32		6.21±0.14 ^e
<i>C. annuum</i> var. <i>grosso</i>	Abaxial	Tetracytic	11.40	1.73±0.37	75.37±4.10 ^b	10.83±1.77 ^{ab}
		Anisocytic	88.60	13.44±0.51		42.72±1.74
	Adaxial	Tetracytic	18.84	1.30±0.24	81.37±4.36 ^c	5.03±0.91
		Anisocytic	81.16	5.60±0.20		13.09±0.99
<i>C. annuum</i> var. <i>glabriusculum</i>	Abaxial	Tetracytic	14.89	2.71±0.20	84.06±9.94 ^b	16.22±0.84 ^b
		Anisocytic	85.16	15.50±1.44		51.54±4.94 ^d
	Adaxial	Tetracytic	23.81	1.00±0.00	55.30±5.23 ^d	1.61±0.02 ^c
		Anisocytic	76.19	3.20±0.37		13.69±0.99 ^e

Means with similar indices in each column are not significantly different ($p \leq 0.05$)

128 **Table 2: Epidermal cells, anticlinal cell-wall patterns and multiseriate unicellular trichomes in varieties of *Capsicum annuum***

Taxa	Leaf Surface	Epidermal Cell Density (Range and Mean)	Epidermal Cell Shape	Anticlinal Cell wall pattern	Trichome
<i>C. annuum</i> var. <i>abbreviatum</i>	Abaxial	18(26.40)25	Irregular	Undulate	Absent
<i>C. annuum</i> var. <i>annuum</i>	Adaxial	70(77.86)88	Irregular	Undulate	Absent
<i>C. annuum</i> var. <i>annuum</i>	Abaxial	7(10.00)14	Irregular	Undulate	Absent
<i>C. annuum</i> var. <i>annuum</i>	Adaxial	89(103.20)112	Irregular	Undulate	Present
<i>C. annuum</i> var. <i>accuminatum</i>	Abaxial	23(39.00)58	Irregular	Undulate	Present
<i>C. annuum</i> var. <i>accuminatum</i>	Adaxial	32(39.67)44	Irregular	Undulate	Present
<i>C. annuum</i> var. <i>grossum</i>	Abaxial	16(17.75)20	Irregular	Undulate	Absent
<i>C. annuum</i> var. <i>grossum</i>	Adaxial	28(38.86)48	Irregular	Undulate	Absent
<i>C. annuum</i> var. <i>Glabriusculum</i>	Abaxial	11(16.50)19	Irregular	Undulate	Present
<i>C. annuum</i> var. <i>Glabriusculum</i>	Adaxial	55(61.60)68	Irregular	Undulate	Absent

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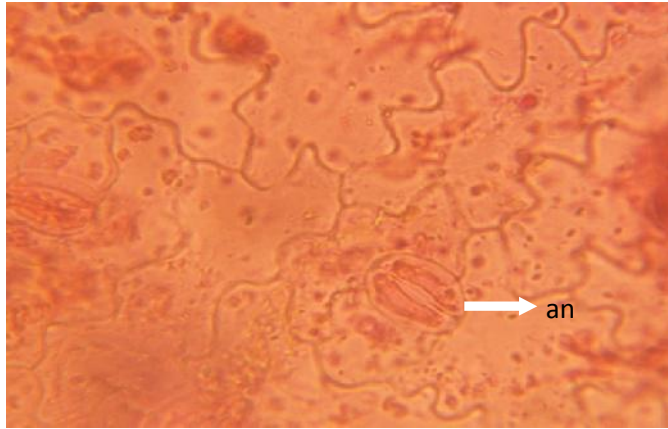


Figure 1: Abaxial surface of var. *abbreviatum* showing anisocytic (an) stomata x600

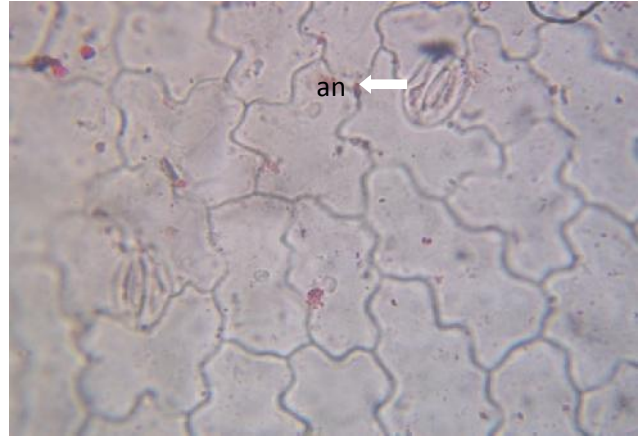


Figure 2: Adaxial surface of var. *abbreviatum* showing anisocytic stomata x600



Figure 3: Abaxial surface of var. *annuum* showing anisocytic stomata x600

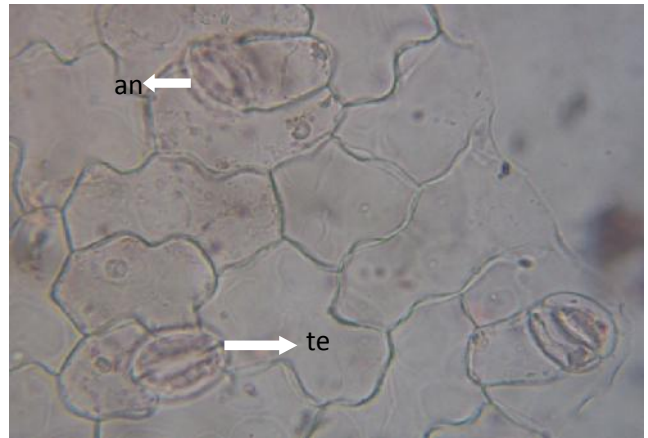
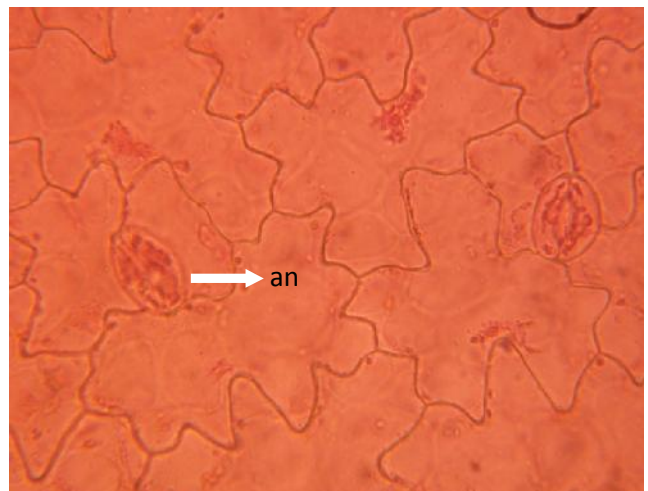
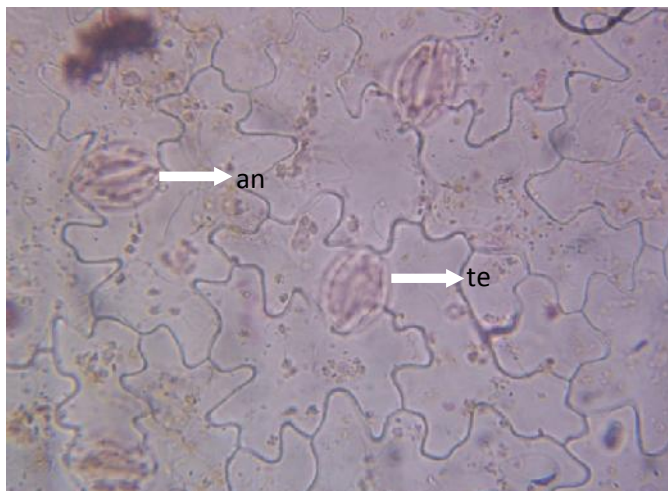
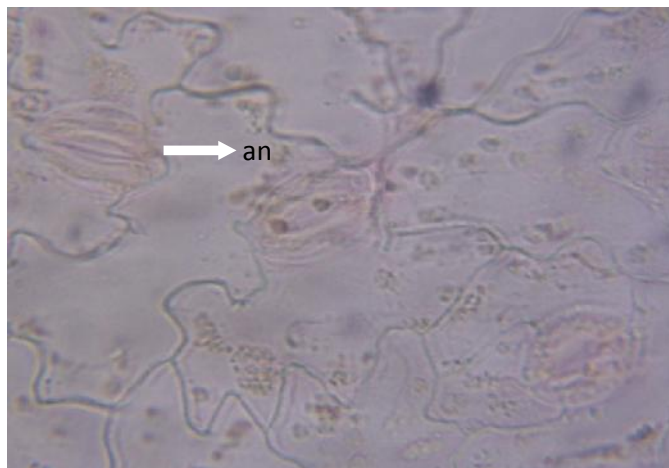


Figure 4: Adaxial surface of var. *annuum* showing anisocytic and tetracytic (te) stomata x600



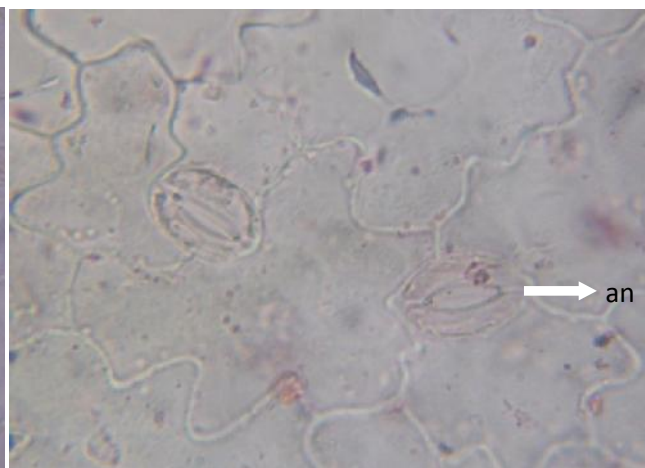
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Figure 5: Abaxial surface of var. *accuminatum* showing anisocytic and tetracytic stomata x600



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Figure 6: Adaxial surface of var. *accuminatum* showing anisocytic and tetracytic stomata x600



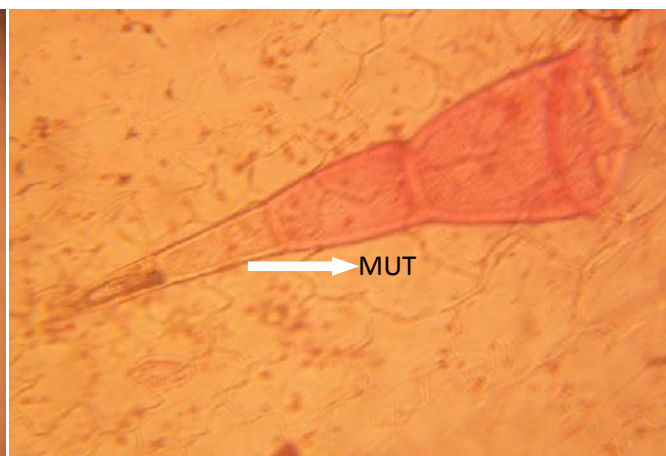
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Figure 7: Abaxial surface of var. *grossum* showing anisocytic and tetracytic stomata x600

Figure 8: Adaxial surface of var. *grossum* showing anisocytic stomata x600



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Figure 9: Multiseriate unicellular trichomes (MUT) var. *annuum* x400

Figure 10: Multiseriate unicellular trichomes (MUT) var. *accuminatum* x400

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145 REFERENCES

- 146 Agbagwa, I.O. and Ndukwu, B. (2004). The value of morpho-anatomical features in the
 147 systematics of *Cucurbita* L. (Cucurbitaceae) species in Nigeria. African Journal of.
 148 Biotechnology, 3(10): 541-546.
- 149 Chakrabarty, T. and Gupta, (1981). Morphological studies on three herbaceous species of
 150 railway tract. Proc. *International Academic Science Plants*, 90:305-315.

- 151 Dave, D. and Paul W. B. (2009). The Complete Chile Pepper Book: A Gardener's Guide to
152 Choosing, Growing, Preserving, and Cooking. Timber Press. ISBN 978-0881929201.
- 153 Dilcher, D. L. (1974). Approaches to the identification of angiosperm remains. *Botanical*
154 *Review*, 40:1 – 157
- 155 Edeoga, H. O. (2000). Taxonomic studies on certain of *Dioscorea* L. (Dioscoreaceae) in Eastern
156 Nigeria. Ph.D. Thesis, University of Port-Harcourt, Nigeria.
- 157 Edeoga, H. O. and Eboka A. U. (2000). Morphology of the leaf epidermis and systematics in
158 some *Dissotis* species Benth (Melastomataceae). *Global Journal of Pure and Applied Science*,
159 6:371-374.
- 160 Edeoga, H. O. and Ikem, C. L. (2001). Comparative morphology of the leaf epidermis in three
161 species of *Boerhaavia* L. Nyctaginaceae. *Journal of Plant Anatomy and Morphology*, 1:14-
162 21.
- 163 Edeoga, H. O. and Osawe, P. I. (1996). Cuticular studies on some Nigerian species of *Senna*
164 Tourn. Ex Mill (Syn cassia ex.L) Leguminosae-Caesalpinoideae. *Acta Phyto tax Geobot.*,
165 47:41-46.
- 166 Esua, K. (1977). Anatomy of Seed Plants (2nd Edition). John Wiley and Sons, London, Pg 83-
167 97.
- 168 Franco, C. (1939). Relation between chromosome number and stomata in *Coffea*. *Botanical*
169 *Gazette*, 100: 817 - 818
- 170 Gill, L. S. and Karatela, Y. Y. (1982). Epidermal structure and stomatal ontogeny in some
171 Nigerian Curcubitaceae. *Willdenowia*, 12: 303-310.
- 172 Greulach, V.A. (1973). Plant Function and Structure. Macmillan Publishing Company; Lincoln,
173 United Kingdom. Pg 432.
- 174 GRIN, (2010). Germplasm Resources Information Network. United States Department of
175 Agriculture. 1997-01-22. <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?8904>. Retrieved
176 2010-07-29.
- 177 Grubben, G. J. H. and Denton, O. A. (2004). Plant Resources of Tropical Africa 2. Vegetables.
178 PROTA Foundation, Wageningen; Backhuys, Leiden; CTA, Wageningen
- 179 Katzer, G. (2008). Paprika (*Capsicum annum* L. [http://www.uni-](http://www.uni-graz.at/~katzer/engl/Caps_ann.html)
180 [graz.at/~katzer/engl/Caps_ann.html](http://www.uni-graz.at/~katzer/engl/Caps_ann.html). Retrieved February 21, 2011.

- 181 Latham, E. (2009). The colourful world of chillies. *Stuff.co.nz*. [http://www.stuff.co.nz/life-](http://www.stuff.co.nz/life-style/food-wine/1756288)
- 182 [style/food-wine/1756288](http://www.stuff.co.nz/life-style/food-wine/1756288). Retrieved 2009-03-08.
- 183 McMullan, M. and Livsey, J. (2013). The Chileman. BOLVIAN GOLD (PI 260579): Baccatum.
- 184 Bolivia.
- 185 Noraini, T. And Cutler, D.F. (2009). Leaf Anatomical micromorphological characters of some
- 186 Malaysian *Parashorea* (Dipterocarpaceae). *Journal of Tropical Forest Science*, 21(2): 156-
- 187 167
- 188 Nwachukwu, C. U., Mbagwu, F. N., Onyeji, A. N. (2007). Morphological and Leaf epidermal
- 189 features of *Capsicum annuum* and *Capsicum frutescens* Solanaceae. *Nature and Science*, 5(3);
- 190 54-60
- 191 OECD, (2011). Organisation for Economic Co-operation and Development. *Safety assessment of*
- 192 *transgenic organisms: OECD consensus documents*. OECD Publishing. pp. 299–304.
- 193 Obiremi, E. O. and Oladele, F. A. (2001). Water conserving stomatal system in selected *Citrus*
- 194 species. *South African Journal of Botany*, 67:258 – 260
- 195 Okwulehi, I. C. and Okoli, B. E. (1999). Morphological and palynological studies in some
- 196 species of *Corchorus* L. Tiliaceae, *New Botanist*, 25: 87 – 101.
- 197 Olorode, O. (1984). Taxonomy of West African Flowering Plants. Longman Group Limited. The
- 198 Book Project, Obafemi Awolowo University, Ile-Ife, Nigeria.
- 199 Olowokudejo, J. D. (1990). Comparative Morphology of leaf epidermis in the genus *Annona*
- 200 (*Annonaceae*) in West Africa. *Phytomorphology*, 40: 407 – 422.
- 201 Paliwal, G. S. (1967). Structure and ontogeny of stomata in some Coryphylliaceae.
- 202 *Phytomorphology*, 16: 533 - 532.
- 203 Pandey, S.N. and Misra, S.P. (2009). Taxonomy of Angiosperms. Ane Books Pvt. Ltd. Parwana
- 204 Bhawa, Darya Ganj, New Delhi, India.
- 205 Sapir, Y., Shmid, A., Fragman, O., Comes, H.P. (2002). Morphological variation of the
- 206 *Oncocylus irises* (Iris: Iridaceae) in the southern Levant. *Botanical Journal of Linnean*
- 207 *Society*, 139: 369-382.
- 208 Shah, G. L. and Gopal, B. V. (1972). Some observation on the diversity of stomata and
- 209 trichomes in some species of *Dioscorea*. *Annals of Botany* 36: 997 – 1004.

- 210 Sharma A, Sehrawai S.K., Singhrot R.S. and Tele A. (2010). Morphological chemical
211 characterization of *Psidium* species. *Notulae Boanicae Horti Agrobotanici Cluj-Napoca* 38(1):
212 28-32.
- 213 Smith, P. G. and Heiser, Jr., C. B. (2000). Taxonomic and genetic studies on the cultivated
214 peppers, *Capsicum annuum* L. and *C. frutescens* L. *American Journal of Botany*, 38: 362-368.
- 215 Soladoye, M. Sonibare, M.A. and Chukwuma, E.C. (2010). Morphometric study of the genus
216 *Indigofera* Linn. (Leguminosae- Papilionoideae) in South-Western Nigeria. *International*
217 *Journal of Botany*. 6(3): 227-234.
- 218 Stace, C.A. (1965). Cuticular Studies as an Aid to Plant Taxonomy. *Bulletin of British Museum*
219 (Natural).
- 220 Website1 en.m.wikipedia.org/wiki/capsicum-annuum
221 http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200020513.
- 222 Wilkinson, H. P. (1979). The Plant Surface (Mainly Leaf), Part 1: Stomata. In Metcalfe, C.F. and
223 Chalk, L. (eds.). *Anatomy of Dicotyledons*, Vol. 1 (2nd ed.), Clarendon Press, Oxford. Chapter
224 10. P. 113
- 225 Zhang, Z., Lu, A and D'Arcy, W. G. (2002). *Capsicum annuum* Linnaeus, Sp. Pl. 1: 188. 1753.
226 *Flora of China*. 17. pp. 313–313.
- 227