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***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

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

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

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

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

## MATERIALS AND METHODS

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

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

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

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

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**Statistical analysis**

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

**RESULTS AND DISCUSSION**

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

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

The leaf epidermal features observed in these investigations are of systematic value because they are reasonably constant in the taxa studied (Table 2 and Figs 1 - 8). There are continuous epidermal cell shape (irregular) and anticlinal wall pattern (undulate) in all the varieties. The epidermal cell density on the abaxial surface is generally low (10.00 – 39.00 $mm^2$) and high on the adaxial surfaces (38.86 – 103.20 $mm^2$).The results show a close relationship among the varieties. Olowokudejo (1990) made similar observation in the genus *Anona*. Furthermore, the use of leaf epidermal features (epidermal cell, stomata and trichomes) in Systematics has become popular and distinctive and have been used as a great taxonomic tool at the levels of family, genus and species. Works of Paliwa1 (1967); Shah and 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 (%) |
|---|---|---|---|---|---|---|
| *C. annuum* var. *abbreviatum* | 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] |
| *C. annuum* var. *annuum* | 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 |
| *C. annuum* var. *accuminatum* | 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] |
| *C. annuum* var. *grossum* | 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 |
| *C. annuum* var. *glabriusculum* | 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 |
|---|---|---|---|---|---|
| *C. annuum* var. | Abaxial | 18(26.40)25 | Irregular | Undulate | Absent |
| *abbreviatum* | Adaxial | 70(77.86)88 | Irregular | Undulate | Absent |
| *C. annuum* var. | Abaxial | 7(10.00)14 | Irregular | Undulate | Absent |
| *annuum* | Adaxial | 89(103.20)112 | Irregular | Undulate | Present |
| *C. annuum* var. | Abaxial | 23(39.00)58 | Irregular | Undulate | Present |
| *accuminatum* | Adaxial | 32(39.67)44 | Irregular | Undulate | Present |
| *C. annuum* var. | Abaxial | 16(17.75)20 | Irregular | Undulate | Absent |
| *grossum* | Adaxial | 28(38.86)48 | Irregular | Undulate | Absent |
| *C. annuum* var. | Abaxial | 11(16.50)19 | Irregular | Undulate | Present |
| *Glabriusculum* | Adaxial | 55(61.60)68 | Irregular | Undulate | Absent |

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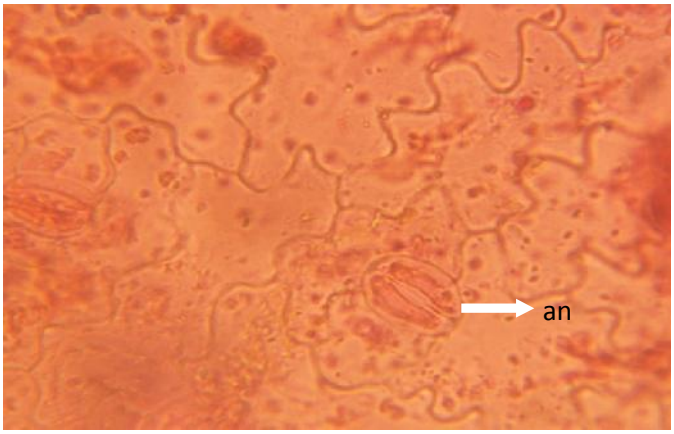


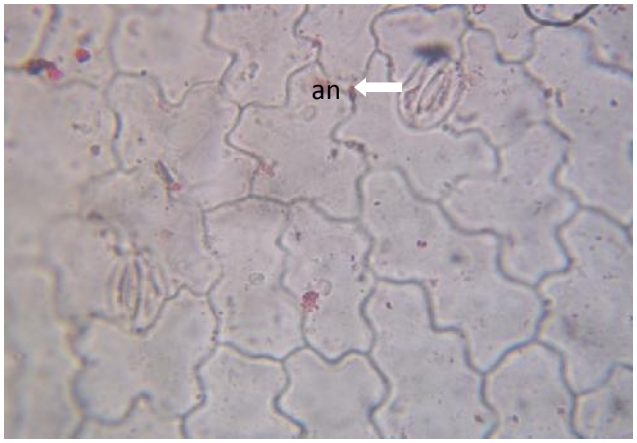




Figure 1: Abaxial surface of var. *abbreviatum* showing anisocytic (an) stomata x600

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

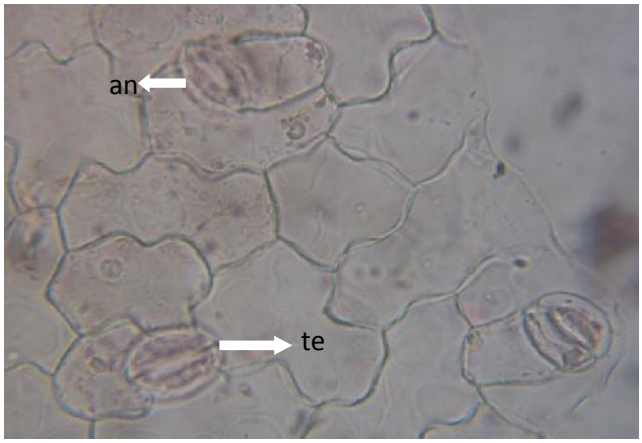
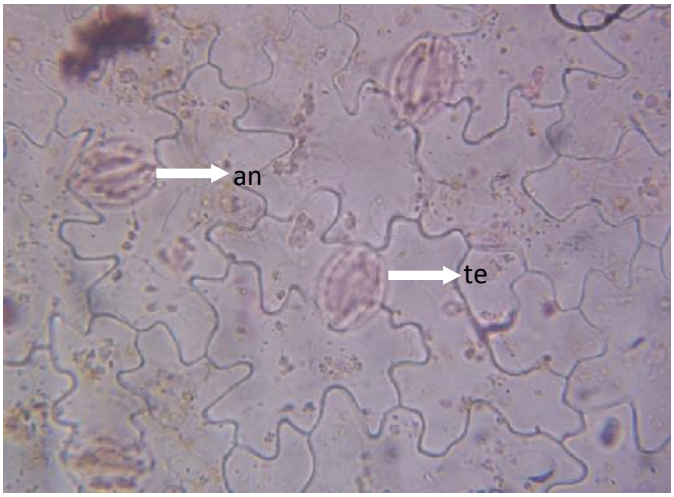



Figure 3: Abaxial surface of var. *annuum* showing anisocytic stomata x600

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



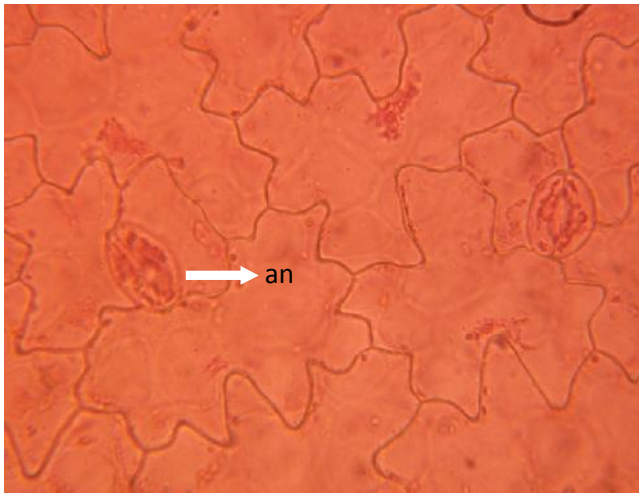



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Figure 5: Abaxial surface of var. *accuminatum* showing anisocytic and tetracytic stomata x600

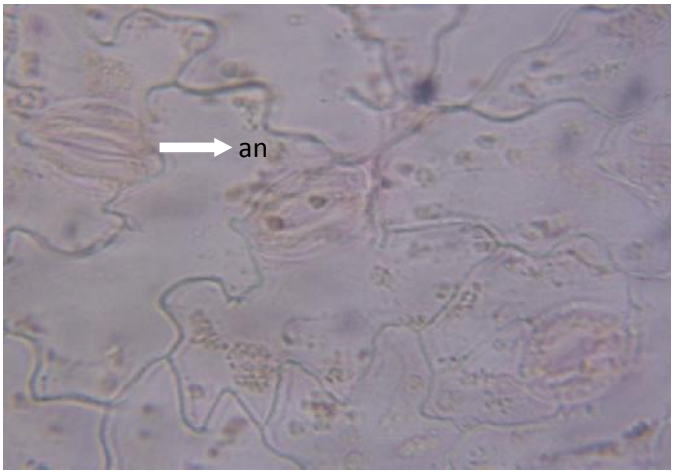



Figure 6: Adaxial surface of var. *accuminatum* showing anisocytic and tetracytic stomata x600

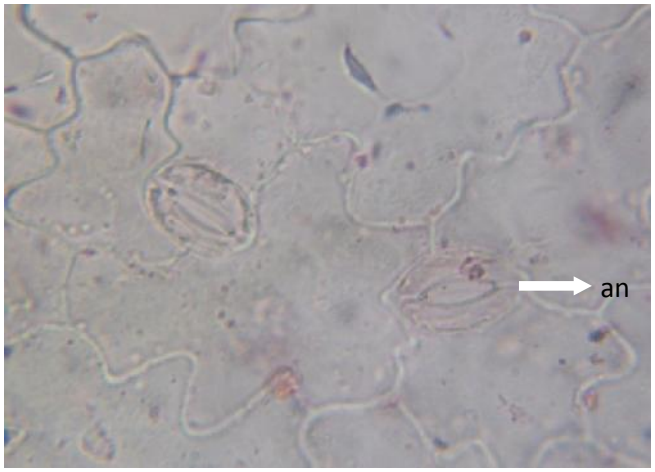
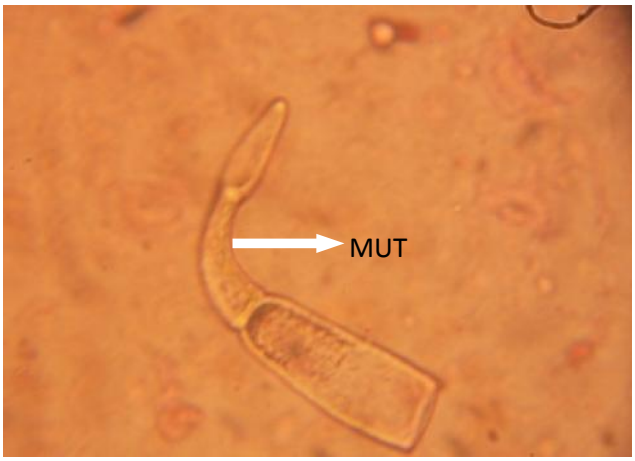



Figure 7: Abaxial surface of var. *grossum* showing anisocytic and tetracytic stomata x600

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



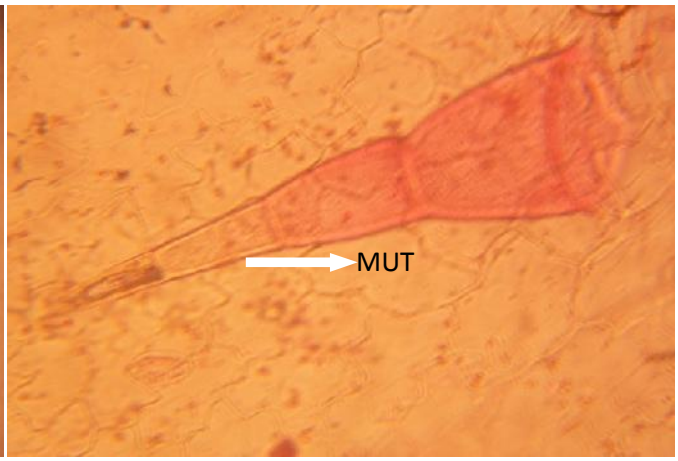




Figure 9: Multiseriate unicellular trichomes (MUT) var. *annuum* x400

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

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