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## RESEARCH ARTICLE

### STUDY OF FOLIAR EPIDERMAL CHARACTERS AND PETIOLE ANATOMY OF FOUR UNDERUTILIZED *ANNONA* (L.) SPECIES IN NIGERIA

\*Folorunso and Abayomi Ezekiel

Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

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#### ABSTRACT

Foliar epidermal characters and petiole anatomy of four underutilized *Annona* species in Nigeria were studied to facilitate identification of this novel source of fruits and also to bring the awareness of *Annona* fruits to the populace. *Annona* species being multipurpose trees have high nutritional value as a food and are sources of medicinal and industrial products. Petiole anatomy of the *Annona* species was undertaken using Light Microscope (LM), the foliar micromorphological study of the species of *Annona* was undertaken using Light Microscope (LM) and Scanning Electron Microscope (SEM). More anatomical characters which are of taxonomic value include smooth cuticular membrane, epidermal cells polygonal and occasionally isodiametric, stomatal cells paracytic, stomatal cells hypostomatic, guard cell shape elliptic and presence of peristomatal rim in all the *Annona* species. The nature of the anticlinal wall, cuticular wax granules, stomatal pore visibility, petiole outline, type of vascular bundle and crystal type are the notable endomorphological characters that provided useful specific distinctions.

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## INTRODUCTION

*Annona* L. which is a genus of tropical fruit tree are the fruits for the future, it belongs to the family Annonaceae (Nakasone and Paull, 1998). Despite their importance in family nutrition, their identification is still a major constraint to consumers. There are approximately 119 species in this genus of which seven and one hybrid are grown for domestic/commercial purposes. Five species of the genus *Annona* have been selected as important under-utilized species (International centre for underutilized crops, 2002.) The vegetative and floral morphological studies of four out of the five species categorised as important under-utilized had been reported (Folorunso and Olorode, 2006a). The taxonomic value of morphological characters has been stressed by several workers, including (Metcalf and Chalk, 1950, 1979; Adedeji and Illoh, 2005; Folorunso and Olorode, 2006b; Folorunso and Modupe, 2007; Abdulrahman and Oladele, 2010). *Annona* species being multipurpose trees have high nutritional value as food and are sources of medicinal and industrial products. The fruits are good sources of vitamin C and minerals such as calcium, phosphorus and potassium. They are also high in carbohydrates which are an excellent source of energy (International centre for underutilized crops, 2002). This work aims at studying the foliar anatomy and petiole morphological characters of *Annona* species to reveal some novel characters which could be used for their identification.

##### \*Corresponding author: Folorunso

Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

## MATERIALS AND METHODS

Foliar and floral materials of *Annona senegalensis* Pers. were collected from twenty accessions from the forest in the wild along Kwara State University Road, Malet (Latitude 8° 30' N and Longitude 5° 0' E). Foliar and floral materials of *Annona squamosa* L., *Annona reticulata* L. and *Annona muricata* L. were collected from twenty accessions within the campus environment of Obafemi Awolowo University, Ile-Ife (Latitude 7° 28' N and Longitude 4° 33' E). Voucher specimens were deposited in the herbarium of Obafemi Awolowo University, Ile-Ife (IFE). Foliar epidermal studies Sizeable portions of the mature leaves of the species were obtained from the standard median level of the plants. For Light Microscope study, the portions so obtained were macerated in Jeffrey's solution and incubated at 60 °C for 20 minutes. The abaxial and adaxial epidermis were separated by means of fine forceps and dissecting needle, stained with 10 % aqueous solution of safranin O for about 3 to 5 minutes, washed in water and mounted in glycerine. Stomatal index (I) and guard cell area were estimated for the abaxial and adaxial surfaces using the following formula:

$$\text{Guard cell area} = \text{Length} \times \text{Width} \times K$$

$$\text{Where } K = \text{Franco's constant}$$

$$= 0.78524$$

$$\text{Stomatal index (I)} = (S/E+S) \times 100$$

Where S = number of stomata per unit area. E = number of ordinary epidermal cells and subsidiary cells in the same unit

area. Length and width of guard cells were measured; all measurements in Light Microscope (LM) were made using a calibrated ocular micrometer with a 40 × objective. Determination of tannins and primastic crystals was carried out using standard procedures (METCALFE & CHALK, 1979).

The materials for SEM observation were fixed in ethanol and formaldehyde, air-dried and mounted on stubs coated with gold in a sputter coater, and examined in a JOEL JSM 840A SEM at the Electron Microscope Centre of the University of Plymouth, United Kingdom. Photomicrographs were obtained. Transverse sections of the petioles were cut using a Reichert sliding microtome at a thickness between 10 – 15 µm. The specimens were stained in alcian blue for three minutes, rinsed in four to five changes of water, counterstained in Safranin O for three minutes, washed thoroughly and mounted in 25 % glycerol. Illustrations were made with camera lucida fitted to an M20 Wild microscope.

## RESULTS

### Foliar Anatomy

#### *Annona senegalensis* (Fig. 1 a and b)

Epidermal cells polygonal, occasionally isodiametric with straight anticlinal wall 43.2 - 50.4 µm long and 14.4 - 25.2 µm wide on both surfaces. The adaxial surface is smooth, with flakes of wax and short uniseriate non-glandular trichomes. Stomata are paracytic and elliptic, slightly sunken with a raised peristomatal rim and narrow aperture on the abaxial surface. Stomatal pore is visible. Anticlinal wall densely pitted, the trichomes are non-glandular, uniseriate and ranges between 57.6 – 64.8 µm long. Guard cell area is 41 µm<sup>2</sup>, stomatal index is 37.03 %. Fine flakes of wax cover the entire surface.

#### *Annona squamosa* (Fig. 1 c and d)

Epidermal cells polygonal, occasionally isodiametric with straight anticlinal wall 21.6 - 32.4 µm long and 18 - 28.8 µm wide on both surfaces. The adaxial surface is smooth with reticulum of short ridges and long uniseriate non-glandular trichomes. On the abaxial surface, stomata are paracytic and elliptic, sunken with reticulum of short ridges. Stomatal pore is visible. Peristomatal rim is absent and anticlinal walls are without pitting. The trichomes are non-glandular, uniseriate and ranges between 72 – 352.8 µm long. Guard cell area is 48.5 µm<sup>2</sup>, stomatal index is 21.07 %.

#### *Annona reticulata* (Fig. 1 e and f)

Epidermal cells polygonal, occasionally isodiametric with straight anticlinal wall 25.2 – 32.4 µm long and 21.6 – 28.8 µm wide on the adaxial surface. The adaxial surface is smooth and has long uniseriate non-glandular trichomes. Epidermal cells polygonal occasionally isodiametric 25.2 - 43.2 µm long and 18 - 36 µm wide on the abaxial surface with (sinuous) tight, frequent, U- shaped curves of shallow amplitude anticlinal wall. Stomata are paracytic and elliptic, sunken with a raised peristomatal rim and narrow aperture. Stomatal pore is visible. Anticlinal wall is without pitting. Guard cell area is 40

µm<sup>2</sup>; stomatal index is 32.2 %. There is a reticulum of rounded ridges on this surface.

#### *Annona muricata* (Fig. 1 g and h)

Epidermal cells polygonal, occasionally isodiametric with wavy anticlinal wall 43 – 50 µm long and 14 – 28 µm wide on the adaxial surface. There are scales of wax and short uniseriate non-glandular trichome on the adaxial surface. Epidermal cells polygonal, occasionally isodiametric with straight anticlinal walls 54 – 64.8 µm long and 28.8 – 43.2 µm wide on the abaxial surface. Stomata are paracytic and elliptic, with a raised peristomatal rim and narrow aperture. Stomatal pore is visible. Scales of wax are on the entire surface. Anticlinal wall is without pitting. Guard cell area is 35.3 µm<sup>2</sup>, stomatal index is 20.3 %. Tannins are present on both surfaces.

### Petiole Anatomy

#### *senegalensis* (Fig. 2a - c)

Outline: Semi-circular in proximal section, circular with little projections of two ribs in the median section, distal section is heart shaped. Epidermis uniseriate with some non-glandular uniseriate, bicellular trichomes. The cuticle is thick. Cortex comprised of three zones, the outer zone is composed of lacunar collenchyma, 5 – 6 layers and an inner zone of polygonal/isodiametric parenchyma cells of 8 – 10 layers. Groups of brachysclereids are scattered in the parenchyma cells. In the distal section, 4 – 8 layers of sclerenchyma cells surround the amphicribal vascular bundle, both brachysclereids and fibres are present.

Vasculature: Vascular bundles are bicollateral.

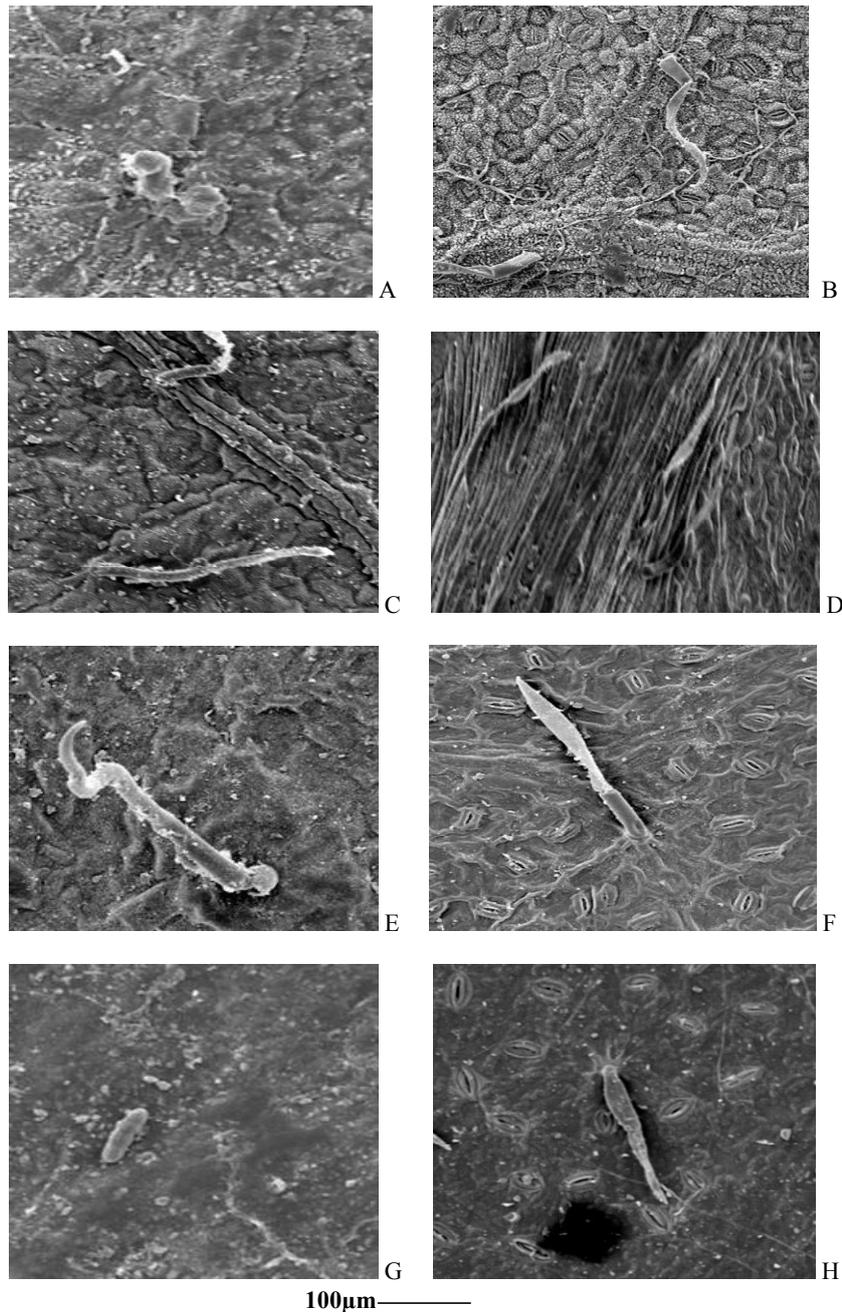
Crystals: Druses of Calcium oxalate and crystal sand are present in both cortical and pith parenchyma of both proximal and median sections. Tannins are present in all the sections.

#### *squamosa* (Fig. 2d - f)

Outline: Generally round in all the three regions, there are two prominent ribs in the distal region. Epidermis uniseriate with non-glandular uniseriate, bicellular trichome. Cuticle is thin. Cortex comprised of three zones, the outermost collenchyma 4 – 5 layers in proximal region, 5 – 6 layers in median layer and 3 – 4 layers in the distal region. The innermost zone of parenchyma is 8 – 10 layers in both proximal and distal regions, 6 – 8 layers in median region. Sclerenchyma cells surround the bicollateral vascular bundle in all three regions. Groups of brachysclereids are scattered in the parenchyma cells. Tannins are present in both cortical and pith parenchyma of all the sections.

#### *Reticulata* (Fig. 2g - i)

Outline: Semi-circular in proximal section, round in shape with two ribs in one end in both median and distal section. Epidermis uniseriate with non-glandular uniseriate and bicellular trichomes. Cuticle is thick. Cortex comprised of three zones, the outermost zone of collenchyma cells are 5 – 8 layers in the proximal region, 4 – 6 layers in the median region and 3 – 4 layers in the distal region. The innermost zone of



**Fig. 1.** Adaxial and abaxial epidermal surfaces of *Annona* species. (A) *A. senegalensis* adaxial surface; (B) *A. senegalensis* abaxial surface; (C) *A. squamosa* adaxial surface; (D) *A. squamosa* abaxial surface; (E) *A. reticulata* adaxial surface; (F) *A. reticulata* abaxial surface; (G) *A. muricata* adaxial surface; (H) *A. muricata* abaxial surface.

isodiametric/polygonal parenchyma cells is 10 – 15 layers in the proximal region, 10 – 12 layers in the median region and 8 – 10 layers in the distal region. Groups of brachysclereids are scattered in the parenchyma cells. Sclerenchyma cells surround the amphicribal vascular bundle in the distal region. Crystals: Druses of Calcium oxalate and crystal sand are present in both cortical and pith parenchyma of the proximal section. Tannins are present in both cortical and pith parenchyma of all the three sections.

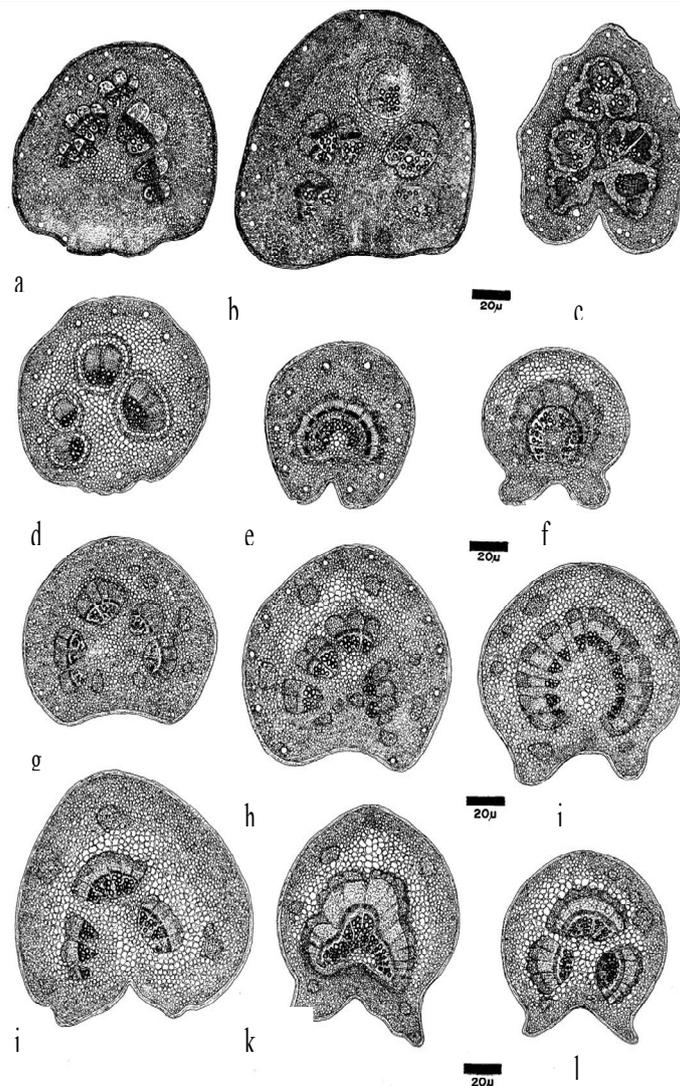
#### **Muricata (Fig. 2j - l)**

Outline: Generally round in all the three regions with two prominent ribs. Epidermis uniseriate. Cuticle is thick.

Cortex comprised of three zones, the outermost collenchyma cells 4 – 5 layers in the proximal region, 3 – 4 layers in both the median and distal sections. Parenchyma cells 15 – 20 layers in the proximal section, 8 – 10 layers in both the median and distal sections. Groups of brachysclereids are scattered in the parenchyma cells. Sclerenchyma cells surround the amphicribal vascular bundle. Tannins are present in both cortical and pith parenchyma of all the three sections.

#### **DISCUSSION**

The result revealed many anatomical variations between the species of *Annona* studied. The characters present in all of



**Fig. 2: Petiole**

- (a) *A. senegalensis* proximal (b) *A. senegalensis* median (c) *A. senegalensis* distal  
 (d) *A. squamosa* proximal (e) *A. squamosa* median (f) *A. squamosa* distal  
 (g) *A. reticulata* proximal (h) *A. reticulata* median (i) *A. reticulata* distal  
 (j) *A. muricata* proximal (k) *A. muricata* median (l) *A. muricata* distal

them may be typical of the genus or family (Folorunso and Olaniyan, 2009). In floras and taxon identification literature, the target morphological and anatomical characters are those that separate taxa (Folorunso and Olorode, 2006b). Some anatomical characters found in the species of Annonaceae from West Tropical Africa were reported (Hutchinson and Dalziel, 1958). In the present work, more anatomical characters that are of taxonomic significance have been reported. These include smooth cuticular membrane, epidermal cell polygonal and occasionally isodiametric, stomatal paracytic, stomatal hypostomatic, guard cell shape elliptic and presence of peristomatal rim in all the *Annona* species. The trichome types in all the species are non-glandular and uniseriate. Traits of leaf epidermal tissue such as stomata size and shape as well as form of trichomes are valuable in taxonomy and identification of plant genera and species (Dickison, 2000; Scatena *et al.*, 2005; Munir *et al.*, 2011).

Some anatomical characters revealed possible interspecific relationships among the *Annona* species. The anticlinal wall on the adaxial surface is generally straight in all the species except in *A. muricata* where it is wavy. On the abaxial surface, the anticlinal wall is generally straight except in *A. reticulata* which has a U-shaped (sinuous) anticlinal wall with shallow amplitude. This anticlinal wall is densely pitted in *A. senegalensis* but not pitted in *A. squamosa*, *A. reticulata* and *A. muricata*. The presence of a densely pitted anticlinal wall, moderately sized epidermal cells, hydropoten cells and the highest stomatal index in *A. senegalensis* may be responsible for its survival in savannah regions. Hydropoten cells facilitate absorption of water and mineral salts, and may assist plants to survive in arid regions (Metcalf and Chalk, 1979). Among the four species of *Annona* studied, *A. senegalensis* and *A. muricata* shared more interspecific characters, both of them have wax granules either in the form of scales or flakes,

the stomatal rim is raised in them, they both have visible stomatal pore and short non-glandular uniseriate trichomes. The role of cuticular waxes as barriers to the transcuticular movement of many substances has been well documented. Wax limits the diffusion of water and solutes, while permitting a controlled release of volatiles that may deter pests or attract pollinating insects (Chowdhury *et al.*, 2005).

Petiole anatomy has been used considerably by several workers for plant classification and identification (Illoh and Inyang, 1998; Kaplan and Inceoglu, 2003). There is some consistency in shape in the median and distal regions of the petiole of all the taxa studied; in the median region, the outlines are all round, most especially at their dorsal view. Similarly, in the distal region, the occurrence of two ribs towards the ventral view is prevalent. Variations occur in the proximal region; in this proximal region, petiole outline is semi-circular in *A. senegalensis* and *A. reticulata* while it is round in both *A. squamosa* and *A. muricata*. In the distal region, the petiole is round in all the species except in *A. senegalensis* where it is heart shaped. The presence of bicollateral vascular bundles and thin cuticle delimit *A. squamosa* from the other species. Ribs are well developed and more conspicuous in *A. muricata* than in the other species studied. Apart from the taxonomic characters shared by the four species of *Annona*, *A. senegalensis* and *A. reticulata* shared more interspecific characters together. Most importantly, they both have druses of Calcium oxalate and crystal sand in both cortical and pith parenchyma, and the outline of their proximal region is semi-circular.

In conclusion, the results of this study revealed more interspecific characters which may be of taxonomic value in the classification of the genus *Annona*. However, each species has peculiar diagnostic characters that may assist its identification and delimitation from other species. Leaf epidermal features like cuticular membrane, wax ornamentation, peristomatal rim, stomata types, stomatal index, guard cell area, type of anticlinal wall, trichome types and presence of ergastic substances, all of which are described in this study are of taxonomic value in delimiting species of the same genus or genera within a family (Jayeola and Thorpe, 2000; Jayeola *et al.*, 2001; Ogundipe, 2004; Abdulrahman and Oladele, 2010). The petiole anatomy similarly revealed important characters for the identification of the *Annona* species studied.

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