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Petiole Anatomy of the Genus *Basella* in South Western Nigeria

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ABSTRACT

The petiole anatomy of the genus *Basella* in south western Nigeria was investigated. Transverse sections from the median portion of the petiole were cut at 15 μm using Reicheirt sliding microtome and then stained in Safranin O for 5 min, rinsed in water thrice to remove excess stain and then counter stained in Alcian blue for 5 min. These were rinsed in water thrice to remove excess stain and then treated in series of grades of ethanol. Stained sections were mounted in 25% glycerol. Microscopic measurements were made with an ocular micrometer. Photomicrographs were taken using 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera. Boat shaped petiole outline, adaxial ribs and epidermal stomata were generic features. The rib length and vascular bundles were diagnostic. Rib length was $9,481.25 \pm 2.35 \mu\text{m}$ long in *B. rubra*; *B. alba*, $4112.50 \pm 2.50 \mu\text{m}$ long; *B. cordifolia*, $3,666.50 \pm 2.55 \mu\text{m}$ and *B. alba* round $6,126.50 \pm 1.56 \mu\text{m}$. Bundles in *B. rubra* were 3-4 collateral, 3 amphicribal bundles in *B. alba*, 3-4 amphicribal bundles plus an additional medullary bundle in *B. cordifolia* and *B. alba* round had 3-fused amphicribal bundles. The undulating rib epidermis was unique to *B. rubra*.

Key words: *Basella*, petiole, rib, vascular bundles

INTRODUCTION

Members of this genus are twinning sub succulent herbs with long, much branched stem. They are annual or biennial herbs. Fleshy leaves of *B. alba* and *B. rubra* are used as vegetables (Adeyemi, 2007). *Basella* is a good source of Vitamins A and C, the purple stemmed are especially delicious (Palada and Chang, 2003; Roy *et al.*, 2010). Two types of *Basella* are commonly known to farmers and consumers. The genus *Basella* Linn. was described in the Flora of West Tropical Africa as a monotypic that consists of *B. alba* (syn *B. rubra*) (Hutchinson and Dalziel, 1958). The species was described as having green or purple stems. Gill (1988) reported that only one species, *B. alba* is found in West Africa. But in reality different forms or taxa of *Basella* exist with descriptions different from that of Hutchinson and Dalziel (1958). Adeyemi (2007) reported that three main cultivars have been distinguished in Nigeria based on leaf shape and colour namely: *B. alba*, *B. rubra* and *B. cordifolia*.

Apart from the use of red-purple pigmentation in distinguishing the two species of *Basella* in Nigeria, information on the taxonomy of the genus is scanty and conflicting both within and outside Nigeria (Hutchinson and Dalziel, 1958; Sharma, 1961; Adeyemi, 2007; Gamble *et al.*, 1967; Henry *et al.*, 1987; Bittrich and Sperling, 1993; Warriar *et al.*, 1994; Palada and Chang, 2003; Greuter and Von Raab-Straube, 2006; Larkcom, 2007; Ozela *et al.*, 2007; Oladele and Aborisode,

2009; FAO, 1999; Roy *et al.*, 2010). Anatomy sometimes proves useful in individual identification especially materials that are not accompanied by floral parts or fruits and can be used to establish botanical identity of commercial samples of medicinal plants (Metcalf and Chalk, 1979). It has a lot of value in forensic Botany. Naik and Nirgude (1981) stressed the value of anatomical characters; they noted that anatomical characters provide additional features which along with other characters are of great taxonomic values in the classification and identification of plants. Essiett *et al.* (2010) reported that anatomical features are widely used in systematics for identification, for placing anomalous groups in satisfactory position in classification and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features.

Petioles provide many useful anatomical characters that are widely used in taxonomy and have been applied in the elucidation of phylogenetic relationship. According to Unamba *et al.* (2011) distinguishing features of the petiole that are of taxonomic value include variation in the shapes of the petiole, the variation in the number, arrangement and shapes of vascular bundles, types and mode of distribution of trichomes on the petioles, as well as the distribution of crystals in the petioles. Agbagwa and Ndukwu (2004) reported the variation in the number of bicollateral bundles in the petioles of the species of *Cucurbita* L.

Busuioc and Ifrim (2004) reported on histo-anatomical aspects of the leaf structure of *B. alba* and *B. rubra*. They noted that the petiole outline of *B. alba* is incompletely circular, with plane adaxial surface and with two small lateral adaxial ribs while the outline of the transverse section of the petiole of *B. rubra* is semicircular with two obvious lateral wings and an adaxial depression. The vascular area is represented by seven bundles which contained collenchyma layers in phloemic position. Anatomical parameters have contributed greatly to taxonomy. For instance in delimiting species of a genus and also establishing affinities between members of the same genus.

The study reports on the additional evidence based on petiole anatomy of the genus occurring in south western, Nigeria.

MATERIALS AND METHODS

Transverse sections of the petiole were cut from the median portion of the materials available using Reichert sliding microtome at 15 μm . The sections were stained in Safranin 0 for 5 min, rinsed in water thrice to remove excess stain and then counter stained in Alcian blue for 5 min. These were rinsed in water thrice to remove excess stain and then treated in series of grades of ethanol: 50, 70, 80, 90 and 100% with two changes in 100% ethyl alcohol to remove water molecules, (dehydration process) and excess stain (differentiation process). The dehydrated and differentiated sections were mounted in 25% glycerol treated with thymol crystals to prevent fungi growth. Microscopic measurements were made with an ocular micrometer. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera.

RESULTS

***Basella rubra* (Roxyb)**

Outline: Cuticle of *Basella rubra* is thin. The petiole outline is boat or saucer shaped with extended ribs. There is a deep depression on the adaxial epidermis (Fig. 1). Epidermis is undulating and biseriate (Fig. 1b and c). The mean rib length is $9,481.25 \pm 2.35 \mu\text{m}$. Ribs have ridge bundles (Fig. 1e). Epidermal cells are cylindrical and perpendicular to the surface of the petiole. Adaxial and abaxial epidermises have stomata. Epidermal cells are polygonal cylindrical cells (Fig. 1d).

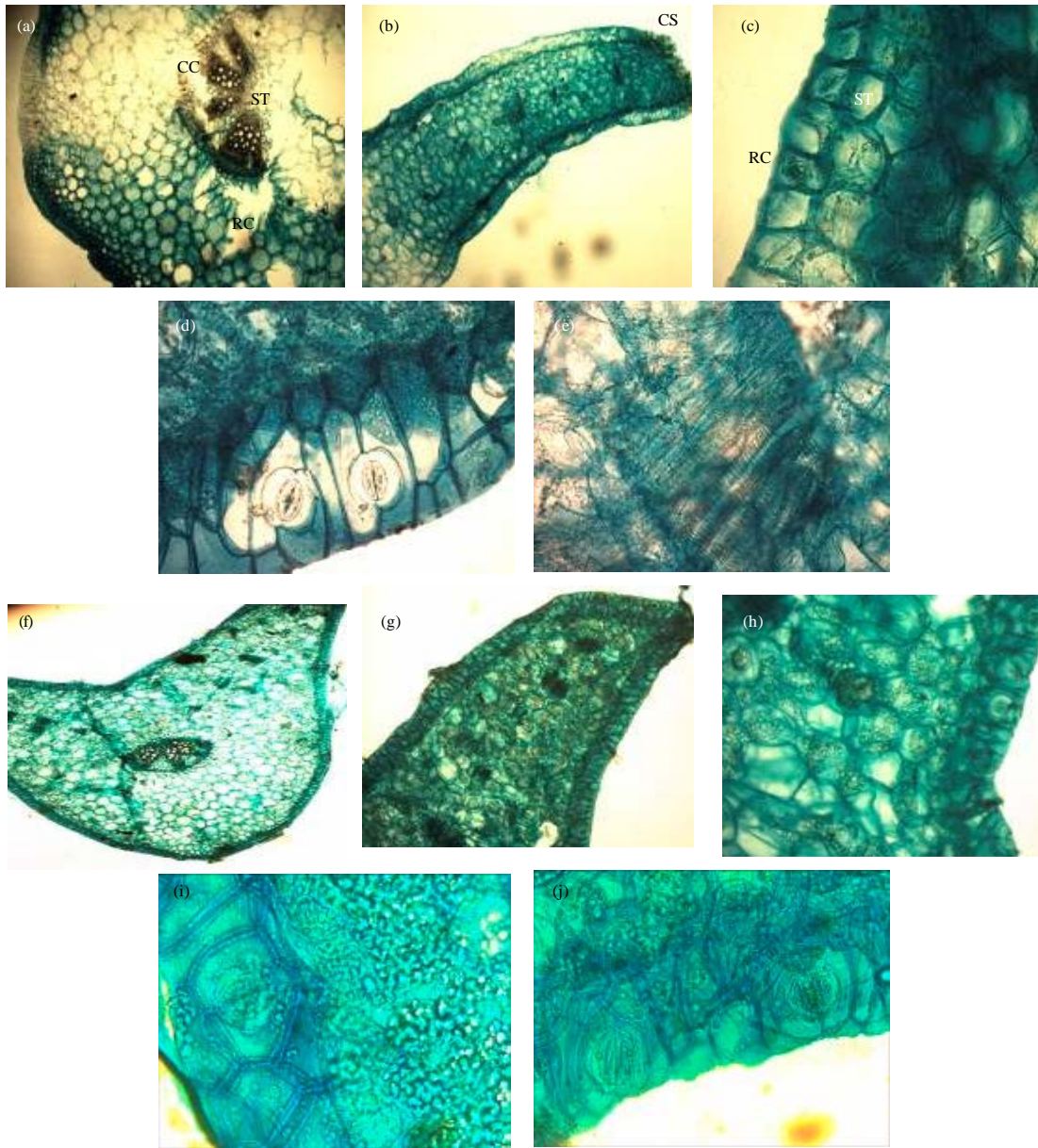


Fig. 1(a-j): Petiole anatomy of *Basella rubra* and *Basella alba*, (a) Petiole outline of *B. rubra* showing collateral vascular bundles, (b) Extended adaxial rib of *B. rubra* showing undulating epidermis, (c) Biseriate epidermis of the petiole of *B. rubra* (d) Petiole epidermis of *B. rubra* showing stomata and cylindrical cell, (e) Rib bundle in *B. rubra*, (f) Petiole outline of *B. alba* showing amphicribal vascular bundles, (g) Extended adaxial rib of *B. alba* showing rib bundles, (h) Petiole of *B. alba* showing crystals and biseriate epidermis and (i and j) Petiole epidermis of *B. alba* showing crystal sands, cylindrical cells, rectangular cells, stomata, AD: Adaxial depression, BEP: Biseriate epidermis, CC: Cylindrical cells, CO: Collenchyma, CR: Crystals, RB: Rib bundle, ST: Stomata, VB: Vascular bundle

Cortex: Cortical cells are polygonal collenchyma and parenchyma cells. Octahedric druses and rosette crystals were observed.

Vasculature: Vascular bundles collateral closed; 3-4 bundles. Rib bundles lined the length of each rib (Fig. 1a).

Crystals: Crystal druses are present and housed in the parenchyma cells.

***Basella alba* (Linn.)**

Outline: The petiole outline is boat or saucer shaped with short ribs. There is a slight depression on the adaxial epidermis (Fig. 1f). Mean rib length is $4,112.50 \pm 2.50$ μm . Cuticle is thin and not striated. Epidermal cells are uniseriate in some portion and biseriate in others. Epidermal cells could be short rectangular, polygonal or short cylindrical cells. The cylindrical cells are perpendicular to the surface of the petiole. Stomata are found on the epidermis and the ribs (Fig. 1i, l).

Cortex: Cortical cells are made up of thin walled polygonal, circular or oval collenchyma and parenchyma cells. Crystal druses are found in the cortex and they are housed in the parenchyma cells. Cortical bundle sheath present in the cortex.

Vasculature: Vascular bundles amphicribal, 3 in number and they are free. Rib bundles lined the length of each rib (Fig. 1f).

Crystals: Crystal druses are present and housed in the parenchyma cells. Crystal sands are circular and abundant.

***Basella cordifolia* (Larmk)**

Outline: The petiole outline is boat or saucer shaped with short ribs. Mean rib length is $3,666.50 \pm 2.55$ μm . Cuticle is thin and not striated (Fig. 2). Adaxial epidermis has median depression (Fig. 2b). Epidermal cells are uniseriate, biseriate and triseriate (Fig. 2c). Epidermal cells could be short cylindrical to circular cells arranged parallel to the surface. In some portion of the epidermis, the cells are made up of single cylindrical cells which are perpendicular to the surface of the petiole. Stomata are found on the epidermis and the ribs. Epidermal cells have protrusions rib bundles present on the ribs (Fig. 2b).

Cortex: Cortical cells are made up of thin walled polygonal, circular or oval parenchyma and collenchyma cells. Crystal druses are found in the cortex and they are housed in the parenchyma cells.

Vasculature: Vascular bundles amphicribal, 3 in number and they are free forming an arch and an additional medullary collateral bundle (Fig. 2b).

Crystals: Crystal druses are present and housed in the parenchyma cells. Crystal sands are abundant. Rosette crystals observed (Fig. 2c).

***Basella alba* round**

Outline: The petiole outline is boat or saucer shaped with short ribs. Mean rib length is $6,216.50 \pm 1.56$ μm . Cuticle is thin and not striated. Epidermis is biseriate (Fig. 2f) but in some

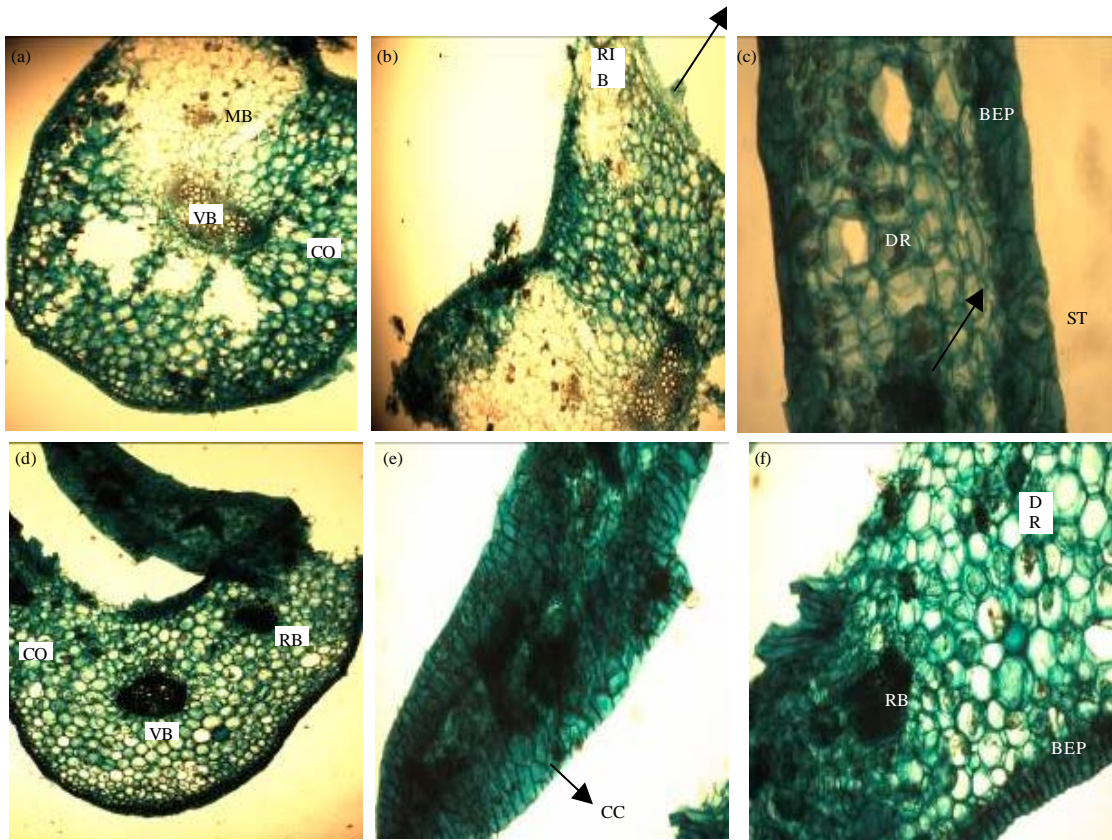


Fig. 2(a-f): Petiole anatomy of *Basella cordate* and *Basella alba* round, (a) Petiole outline of *B. cordate* showing amphicribal vascular bundles, collenchyma and medullary bundle, (b) *B. cordifolia* showing adaxial depression, showing rib and protrusion on the epidermis, (c) Petiole rib of *B. cordifolia* showing biseriata epidermis, stomata, (d) Boat shaped outline of *B. alba* round showing fused amphicribal, collenchyma and rib bundle, (e) Petiole rib of *B. alba* round showing cylindrical cell and (f) Portion of petiole rib of *B. alba* round showing biseriata, druses and rib bundles, AD: Adaxial depression, BEP: Biseriate epidermis, CC: Cylindrical cells, CO: Collenchyma, CR: Crystals, DR: Druses, MB: Medullary bundle, PR: Protrusion, RB: Rib bundle, ST: Stomata, VB: Vascular bundle

portions uniseriate. Epidermal cells could be short rectangular, circular or oval cells which are arranged parallel to the surface. In some portion of the epidermis, the cells are polygonal and cylindrical cells (Fig. 2e) which are perpendicular to the surface of the petiole. Stomata are found on the epidermis and the ribs. Epidermis has grooves.

Cortex: Cortical cells are made up of thin walled polygonal, circular or oval collenchyma and parenchyma cells. Crystal druses are found in the cortex and they are housed in the parenchyma cells.

Vasculature: Vascular bundles amphicribal, 3 and fused. Five rib bundles lined the length of each rib (Fig. 2d).

Crystals: Crystal druses are present and housed in the parenchyma cells. Crystal sands are abundant.

DISCUSSION

Petioles provide many useful anatomical characters and are widely used in other aspects of taxonomy and have been largely applied to the elucidation of phylogenetic relationship (Unamba *et al.*, 2011). The distinguishing characters of taxonomic value include the variation in shapes of the petioles, the variation in the number, arrangement and shape of vascular bundles, type and mode of distribution of trichomes on the petioles as well as types and distribution of crystals in the petioles (Essienn *et al.*, 2010).

The petiole anatomy of *B. rubra* and *B. alba* was reported by Busuioac and Ifrim (2004). The petiole was described as not completely circular, having two small lateral adaxial ribs in *B. alba* while it was semi-circular with two obvious lateral wings in *B. rubra*. In this study the outline of the petiole of all the forms is boat/saucer shaped with adaxial ribs lined with rib bundles. The boat shape and the presence of adaxial ribs are common to all the species but the length of the ribs differs from one form to another. *Basella rubra* has an extended rib, $9,481.25 \pm 2.35 \mu\text{m}$ long while the other forms have ribs that can be described as short. The rib length of *B. alba* is $4112.50 \pm 2.50 \mu\text{m}$, *B. cordifolia*'s rib length is $3,666.50 \pm 2.55 \mu\text{m}$ and *B. alba* round rib length is $6,126.50 \pm 1.56 \mu\text{m}$. The rib length is useful in delineating the forms. The pattern and distribution of tissues in the petiole are similar in all the forms with few exceptions. The epidermis consists largely cylindrical cells but *B. alba* in addition have short rectangular cells, *B. cordifolia* has circular cells in addition to the cylindrical cells while *B. alba* round has rectangular cells, circular and oval shaped cells in addition to the cylindrical cells. This shows point of divergence among the forms. Stomata are found in the epidermis of all the forms.

Vascular bundles in the petiole of the four forms differ from one another. Bundles in *B. rubra* are collateral and 3-4 in number. It is amphicribal 3 and free in *B. alba*, 3-4 free amphicribal plus an additional medullary bundle in *B. cordifolia* and *B. alba* round has 3-fused amphicribal bundle. The *Basella* forms can be divided into two groups based on their vasculature: those with collateral bundles and the forms with amphicribal bundles. Undulating rib epidermis is unique to *B. rubra*.

This study showed that the genus *Basella* is not a monotypic genus as reported in the Flora. Similarities in the petiole anatomical features show taxonomic affinity. The vasculature and length of the adaxial ribs of the *Basella* types show that they are different and as such should be treated as distinct taxa. *Basella alba* round is not assigned any taxonomic status in literatures. The use of round in describing this type is for convenience in this study. This collection needs to be recognized as different taxa because of its distinct petiole anatomical feature.

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