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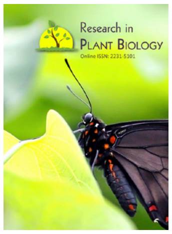

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Taxonomic significance of leaf architecture in the genus *Basella* Linn. in Nigeria

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ABSTRACT

This study seeks to use the leaf architecture as a taxonomic tool for members of the genus *Basella* Linn. in Nigeria, namely, *B. rubra* Linn., *B. alba* Linn., *B. cordifolia* Lamk. and a form, *B. alba* round because the taxonomy of the genus *Basella* Linn. is not clear and there is no information on the leaf architecture of the genus. For studies of the venation pattern of the leaves, sizeable portions were taken from the standard median portion of mature leaves. Cleared leaves were stored in 50% alcohol for anatomical studies. Leaves were stained in Safranin O for 3 minutes and counterstained in Alcian blue and then were rinsed in water to remove excess stain. Stained cleared leaves were treated in serial grades of alcohol for differentiation and dehydration. Stained materials were mounted in dilute glycerol for microscopic examination. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera. The areolar area was calculated from the length and breadth of the areoles measured using a micrometer inserted into the microscope eyepiece. *Basella* species have common generic features. Areoles are closed in *B. rubra* but others have veinlet endings. The area of areole is diagnostic because in *B. rubra* the area is 305,370±1808.91 μm^2; *B. alba* is 501,796.75±3217.38 μm^2, *B. cordifolia*'s area of areole is 396,394.75±2670.67 μm^2 while that of *B. alba* round is 540,058.75±3702.28 μm^2. The arrangement of druses in the areoles is also diagnostic.

KEYWORDS: Architecture, Areoles, Diagnostic, Druses, Veinlets

INTRODUCTION

Hickey (1979) described leaf architecture as the position and shape of elements that constitute the external expression of leaf structure including venation pattern, marginal configuration, leaf shape and glandular position. According to him, dicotyledonous leaves possess a consistent and recognizable pattern of leaf architectural organization at all levels from the subclass to the species. Since then a number of authors have successfully used leaf architecture as a diagnostic tool in delineating species. Leaf architecture is useful in the identification of tropical plants that rarely flower and plant fossils whose reproductive organs are absent. Praveen (2017) noted that veins constitute one of the visible leaf traits or characteristics of the leaf and that they represent the vascular structure of the organ extending from the petiole and transport water and nutrients between the leaf and stem which is important in maintaining the leaf water status and photosynthetic capacity.

There are a number of leaf architecture parameters that are useful as taxonomic tools. Colombo *et al.* (2007) noted that leaf architecture, especially the size and shape of the areoles and free ramifications have been useful in distinguishing between the species of *Viola tineorum* and *V. ucriana*. Lima *et al.* (2019) reported that the arrangement of the areola and the free venuses were different and unique for each taxa and differentiated the taxa in the Family Rubiaceae Juss. The number of secondary veins and angle of divergence vary from species to species and within the same species (Adeniran *et al.*, 2020). Areole size and shape are variable; the simple veinlets may be curved or hooked. An important aspect of foliar architecture is the minor venation pattern. According to many authors, the vein islet number is more or less constant for a species and could be used as a specific character (Mishra *et al.*, 2011). Masungsong *et al.* (2019) described some leaf architectural features that unify as well as differentiate the accessions of five *Cucumis* Linn species: these include lamina shape, primary vein size, and secondary vein spacing (Lima *et al.*, 2019). Maulia and Susandarini (2019) reported that leaf shape, lamina size, and midrib width with calcium oxalate crystals are diagnostic characters for the identification of agar wood-producing species (*Aquilaria malaccensis* Lam. and *Gyrinops versteegii* (Gilg) Domke. Ornamentation of the veins and course of traces in the lamina are useful additional characteristics (Sehgal & Paliwal, 2008; Praveen, 2017).

Buot (2020) described leaf architecture as basically important in species identification; useful in addressing nomenclatural issues as well as confirming and reinforcing the classification or grouping of certain taxa like sections and families. Leaf architecture is a key diagnostic feature used to identify fossil and extant plants. Fortunato *et al.* (2017) expressed the importance of leaf vein patterns in recognizing botanical origin and quality control of plant materials, especially medicinal herbs because they are mostly sold in fragments of dried materials. Adeniran *et al*. (2020) enumerated that the secondary angle of divergence areola shape and nerve endings as leaf architectural characters that were of taxonomic value in the Family Annonaceae in delimiting the genus even to the species level.

Leaf architecture is genetically fixed and geographical positions do not have an impact on its structure (Pulan & Buot, 2014; Masungsong *et al*., 2019). They are therefore useful morpho-anatomical tools for species delineation and of increasing importance in phylogenetic and ecological studies.

Basella Linn. in Nigeria was described as a monotypic genus consisting of *B. alba* synonym *B. rubra* in the Flora of West Tropical Africa (FWTA) (Hutchinson & Dalziel, 1958) however three species namely, B. *rubra* (Sperling & Bittrich, 1993); *B. cordifolia* Lamk (Winters, 1963; Ozela *et al*., 2007; FAO, 2010); *B. alba* (Hutchinson & Dalziel, 1958). In addition to these a form yet to be accorded a status, *B. alba* var round was reported by Adenegan-Alakinde (2014). Studies are ongoing to resolve the riddle associated with the taxonomy of the genus. Information on the leaf architecture of the Genus *Basella* is not available. This is the first attempt to look into the vein architecture of members of the genus. Therefore this study seeks to find out if leaf architectural features could be useful in confirming and reinforcing the report of authors on the taxonomy of the genus *Basella*.

MATERIALS AND METHODS

Venation studies were carried out from ten sizeable portions taken from the standard median portion of mature leaves of each species. Leaf fragments for venation studies were first boiled in 90% alcohol for 30 minutes, rinsed in 4 changes of water to remove the alcohol, and this were further boiled in 10% sodium hydroxide solution for 10 minutes, rinsed in 4 changes of water to remove the hydroxide. The partly cleared leaflets were further cleared in 5% of the solution of domestic bleaching agent parazone. The cleared leaves were washed in 4 changes of water to remove the bleaching agent. These were preserved in 70% ethanol until when needed. Major venation pattern was studied with the help of a photographic enlarger. For the study of minor venation patterns, small bits were cut from the central part of the leaf skeletons (excluding midrib and marginal parts), stained with safranin and mounted in dilute glycerol.

The length and breadth of the areoles (50 per species) were measured using a micrometer inserted into the microscope eyepiece from which the area was calculated.

RESULTS

The summary of qualitative foliar attributes of the Genus Basella is shown in Table 1 while Table 2 shows the folial architectural attributes and areolar areas of the *Genus Basella*.

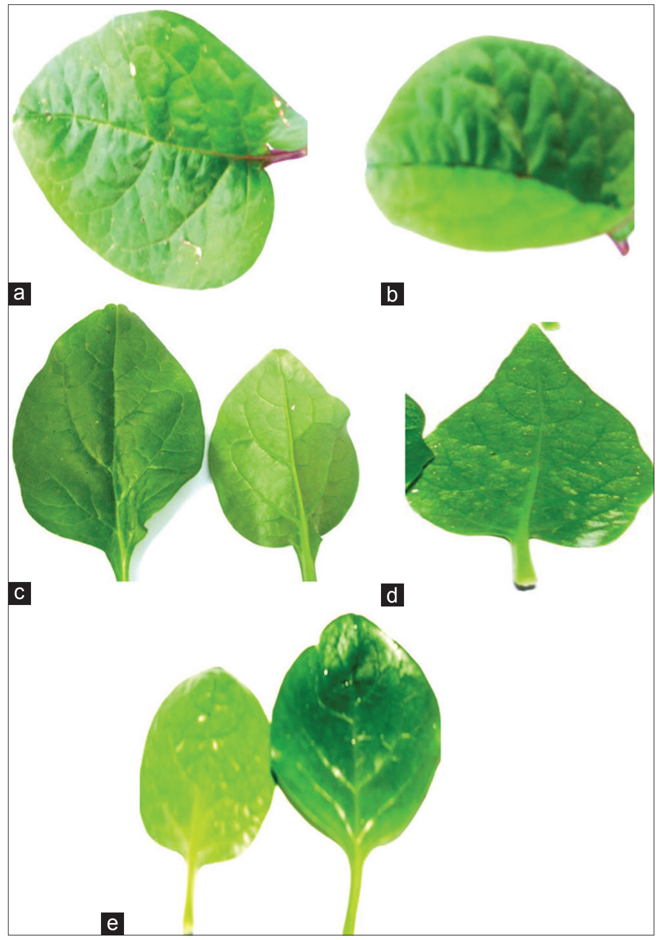

Figure 1: Leaf morphology of *Basella* species. a, b) Adaxial and abaxial leaf surfaces of *B. rubra*, c) Adaxial and abaxial leaf surfaces of *B. alba*, d) Adaxial and abaxial leaf surfaces of *B. cordifolia* and e) Abaxial and adaxial leaf surfaces of *B. alba* round

Table 1: Summary of Qualitative Foliar attributes of the Genus Basella

Basella species/ venation features	*B. rubra*	*B. alba*	*B. cordifolia*	*B. alba* round
Margin	entire	entire	entire	entire
Apex	Acute/ emarginate/ obtuse	Acute/ emarginate/ obtuse	Acuminate	round
Base	cordate	cordate	cordate	acute
Pigmentation	On the veins and leaf margin	None	None	None
Venation	Pinnate	Pinnate	Pinnate	Pinnate
Primary vein	Alternate	Alternate	Opposite	Alternate/ opposite
Texture	Succulent	Succulent	Succulent	Succulent

Table 2: Foliar architectural attributes and areolar areas of the Genus Basella

Features	Basella species			
	B. rubra	*B. alba*	*B. cordifolia*	*B. alba* round
Shape	Rectangular to polygonal and of various sizes.	Rectangular to polygonal and of various sizes.	Polygonal to rectangular.	Rectangular to polygonal and of various sizes.
Crystals	Crystal druses are abundant.	Crystal druses are abundant. There are few crystal sands.	Crystal druses, styloids and prismatic crystals are present.	Crystal druses are fewer
Arrangement of druses	Druses are on the veins (Figure 2a).	Druses scattered in the areoles (Figure 2b)	Druses are scattered (Figure 2c).	Druses are along the veins (Figure 2d).
Vein-let endings	Areoles are closed; without veinlet endings.	Veinlet ends singly, linear to curved or bifurcated.	Veinlet endings are linear or bifurcated.	linear, singly divided or bifurcated,
Vein-let endings per areole.	None	Veinlet endings per areole 1	1 – 6 veinlet endings per areole.	1-2 veinlet endings per areole.
Mean area of areoles (µm²)	305,370±1808.91[a]	501,796.75±3217.38[b]	396,394.75±2670.67[a]	540,058.75±3702.28[c]

*Means with the same superscript along a row are not significantly different at p≤0.0

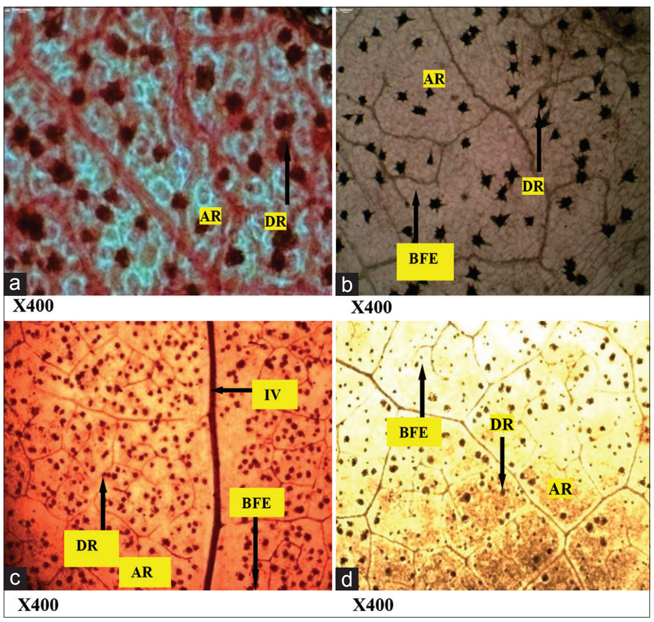

Figure 2: Venation pattern showing areoles and druses. a) Venation pattern showing areoles and druses in *B. rubra*, b) Venation pattern showing areoles and druses in *B. alba*, c) Venation pattern showing areoles and druses in *B. cordifolia* and d) Venation pattern showing areoles and druses in *B. alba* round. Legend: AR - Areole, BFE - Bifurcated endings, DR - Druses, IV - Intercoster vein

B. rubra

Leaves: Leaves are simple, margins entire, apex acute or emarginate, or obtuse, the leaf margin is lined with purple colouration (Figure 1a & b); leaves are glabrous, succulent, the shape is ovate and the base is cordate, leaves are arranged alternately on the stems. The leaf texture is succulent.

Venation: Veins are pigmented with a purple colouration. The main vein arises from the petiole to the apex of the leaf. The primary veins are arranged alternately on the main vein. Secondary veins join together to form a series of arches which do not terminate at the margin (Brochidodromous). Lateral veins are 4-5 pairs. Veins are more prominent at the abaxial surface than the adaxial surface of the leaf (Figure 1a).

Areoles: Rectangular to polygonal and of various sizes. Crystal druses are abundant and are found in the veins. Mean of areolar area is 305,370±1808.91 µm².

B. alba

Leaves: Leaves are simple, margins entire, apex acute or emarginate, or obtuse; leaves are green, glabrous, and succulent, the shape is ovate and the base is cordate, leaves are arranged alternately on the stems. The leaf texture is succulent.

Venation: Veins are green. The main vein arises from the petiole to the apex of the leaf. The primary veins are arranged alternately on the main vein. Secondary veins join together to form a series of arches which do not terminate at the margin (Brochidodromous). Lateral veins are 3-4 pairs. Veins are more prominent at the abaxial surface than the adaxial surface of the leaf (Figure 1c).

Areoles: Rectangular to polygonal and of various sizes. Crystal druses are abundant and are scattered in the areoles. There are few crystal sands. Mean of areolar area is 501,796.75±3217.38µm².

B. cordifolia

Leaves: Leaves are simple, margins entire, and apex acuminate; leaves are glabrous, and succulent, the shape is ovate and the base is cordate, leaves are arranged alternately on the stems. The leaf texture is succulent.

Venation: Veins are green. The main vein arises from the petiole to the apex of the leaf. The primary veins are arranged opposite to one another on the main vein. Secondary veins join together to form a series of arches which do not terminate at the margin (Brochidodromous). Lateral veins are 3-4 pairs. Veins are more prominent at the abaxial surface than the adaxial surface of the leaf (Figure 1e).

Areoles: Rectangular to polygonal and of various sizes. Crystal druses are abundant and are scattered in the areoles; styloids and prismatic crystals are present. Mean of areolar area is 396,394.75±2670.67µm².

B. alba Round

Leaves: Leaves are green, simple, margins entire, apex round; leaves are glabrous, the shape is round and the base is acute, leaves are arranged alternately on the stems. The leaf texture is succulent.

Veins: Veins are green. The main vein arises from the petiole to the apex of the leaf. The primary veins are arranged alternately on the main vein. Secondary veins join together to form a series of arches which do not terminate at the margin (Brochidodromous). Lateral veins are 3-5 pairs. Veins are more prominent at the abaxial surface than the adaxial surface of the leaf (Figure 1d).

Areoles: Rectangular to polygonal and of various sizes. Crystal druses are fewer and are along the veins in the areoles. Mean of areolar area is 540,058.75±3702.28 μm^2.

DISCUSSION

Members of the Genus have features that are common to them, indicating phylogenetic affinity. These features include simple leaves, entire, globorous, and succulence of leaves. Others are prominent abaxial veins. Leaf venation pattern is bronchidodromous in the *Basella* species and the form. Tanninferous substances and crystal druses are found in the entire genus. Areoles were either well developed or imperfect and may be quadrangular, pentagonal or irregular in shape Areoles are also polygonal or polygonal with varied sizes. However, the areoles are closed with no vein let endings and the druses occurred on the veins in *B. rubra* which separates it from the others. The veinlet endings in *B. alba* are single, linear to occasionally curved and occasionally singly divided; veinlet endings is 1-2 per areole. *B. cordifolia* has veinlet endings that are linear or bifurcated, 1-6 veinlet endings per areole. *B. alba* round has linear veinlet endings, singly divided, 2-3 veinlet endings per areole. The veinlet endings are specific for all the species and the form studied. This result is in agreement with that of Lima *et al*. (2019) who pointed out that the free venuses are useful in delimitation of taxa in the Rubiaceae.

There are differences in the area of the areoles. The area of areole in *B. rubra* is 305,370±1808.91 μm^2, in *B. alba* is 501,796.75±3217.38 μm^2, *B. cordifolia* area of areole is 396,394.75±2670.67 μm^2 while in *B. alba* round the area of areole is 540,058.75±3702.28 μm^2. The areolar areas are significantly different from each other at $p \leq 0.05$. Despite the areole area being a quantitative attribute, it is useful as a diagnostic tool. Another feature useful in delineating members of the taxa is the arrangement of druses. Druses are found on the veins *B. rubra* but towards the middle of the areole in *B. alba* and scattered in *B. cordifolia* but arranged along the veins in *B. alba* round. This is in congruence with the report of Adenegan-Alakinde and Jayeola (2015) and Maulia and Susandarini (2019) that the presence and pattern of druses arrangement of the leaves are useful for separating the genus.

CONCLUSION

The study has increased our understanding of the architecture of the genus *Basella* and also showed that leaf architecture of plants can successfully be used to show affinity as well as separate taxa. Members of the Genus *Basella* (Baselllaceae), are distinguished based on the area of the areoles (though a quantitative parameter), the arrangement of druses and vein-let endings per areole. The study also reinforced the opinion that there are three species and a form of *Basella* occurring in the Southwestern part of Nigeria. *B. rubra* is distinct from the green stem species of *Basella*. Further studies need to be carried out on the green species to determine the genetic variability among them.

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