

Regular Article

Some anatomical features of *Basella* Linn.: their adaptive significance to water stress

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Basella is a vegetable consumed in South Western Nigeria. Three species and a variety namely *B. alba*, *B. cordifolia*, *B. rubra* and *B. alba* var. round are cultivated. This study examined the anatomical features of this genus that possess adaptive significance. Free hand peeling was done to get the abaxial and adaxial epidermis. Sections of the stem were done using Reichert Sliding Microtome at 15µm, Epidermal peels and sections were preserved in 50% ethanol. The peels and sections were stained in Safranin O, mounted with glycerol and viewed under microscope. Result shows that *B. rubra* has long bundles which is an adaptation to draw water compared to the other *Basella* species. *Basella rubra* has few abnormal cells which is an advantage for a survival in extreme conditions. Other anatomical features that confer on *B. rubra* the ability to survive in conditions of water stress include; multiseriate epidermis which prevents water loss, longer vessel length and wider vessel diameters which enable the plant to draw water from the soil and for effective distribution of water within the plant system, vessels are in multiples, closed areoles and absence of veinlet endings and differentiation of the leaf mesophyll. These anatomical characters ensure water conservation and survival in water stress areas.

Key words: Abnormal stomata, *Basella*, vascular bundles, vessel diameter, vessel length.

Basella Linn. belongs to the family Basellaceae. The vegetable is commonly referred to as Indian spinach which is commonly eaten among Yorubas in the South western Nigeria. Farmers and consumers reported there are two main types of *Basella*; the green and purple stemmed. However, there are three species and a variety under cultivation distinguished by the pigment on the stem, petioles and leaves and the shapes of the leaves. *Basella rubra* Linn. has purple pigment on the stem, leaves as well as the petioles. The leaf is ovate with a cordate base. *Basella alba* Linn. has green stems, leaves and petioles. The leaf is ovate and cordate at the

base. *Basella cordifolia* Lamk. has a green stem, leaves and petiole with elongated heart shaped or cordate leaves. This type has pigmentation on the node as well as the base of the petioles and the first two internodes above the soil level. *Basella alba* variety round, has green stem, leaves and petioles with oval to almost round leaves. All of them are annual/perennial herbs, procumbent, climbing and creeping.

They are commonly use as pot herbs. The vegetable is rich in Calcium, Iron, Proteins, Vitamins A, C, B, and B₂ (Palada and Chang, 2003; Grubbens and Denton, 2004; Roy *et al.*, 2010). *B. rubra* contains

Proteins, Calcium, Iron, Vitamin A, and B₂. They contain phytochemicals (Olajire and Azeez, 2011; Shruthi *et al.*, 2012.). Ethnobotanical uses of the vegetable include; treatment of boils and blisters (Kayode *et al.*, 2008), fertility enhancer in females (Mensah *et al.*, 2008). It has been reported that it is used in the treatment of malaria. Adenegan-Alakinde (2012) reported that it is used in the treatment of dysentery, constipations, gonorrhoea, boils and blisters by the people of Ondo state. Adetula 2006 described this vegetable of importance as underutilized. Members of this genus are commonly found in the rainforest region of Nigeria and they become increasingly difficult to find as one moves towards dryer parts of the country (Adenegan-Alakinde and Adedeji, 2011). Generally the information on the anatomy of the Genus *Basella* is scarce and the ones available are not based on the adaptive significance of these features. Anatomical features of plants are genetic as well as products of plants interaction with their environment. Plants develop various protective measures ranging from morphological to anatomical in a bid to survive hostile periods. This paper outlines variations in anatomical features of members of the genus that possess adaptive significance especially in water stressed conditions.

Materials and Methods

Free hand sections were done for all the leaves to get the abaxial and adaxial epidermis. Leaf venation was done by cutting the leaves midway between the base and the apex of the leaf and midway between the margin and the midrib. These sizeable portions were boiled in 90% alcohol to remove the chlorophyll for 30 minutes. The partially cleared leaves were washed in 3-4 changes of water to remove the alcohol then cleared in 5% solution of domestic bleach (parazone) until they were totally cleared. The cleared leaves were washed in several

changes of water to remove the bleaching agent and then stored in 50% alcohol for anatomical studies. The transverse section of the stem was made using Reichert sliding microtome at 15µm. Sections were stained in Alcian blue for 3 minutes rinsed in water to remove excess stain and then counterstained in Safranin O for 3 minutes. These were rinsed in water to remove excess stain and treated with serial grades of alcohol and mounted in dilute glycerol for anatomical studies. Photomicrographs were taken with the aid of ACCUSCOPE 3013 Microscope with digital Camera mounted on the microscope. Stomata numbers per field were determined from an average of twenty counts on the adaxial and abaxial epidermis of the leaves. Stomata area was determined by measuring the length and width of the stomata using ocular micrometer and multiplying by Franco's constant (length X breadth X 0.7854). Stomata Index was determined using the formula:

$$\text{Stomata Index (S.I)} = \frac{S}{E + S} \times 100$$

Where S is number of stomata per unit area
E- Number of epidermal cells in the same unit area.

Results

Basella rubra:

Abaxial surface epidermis

Epidermal cells are polygonal, anticlinal walls are thin and wavy, paracytic and anisocytic stomata present and abnormal stomata (unopened stomata, stomata with one guard cell and stomata without guard cells) were observed. Stomata frequency is 2 per 100m². Stomata index, 27.30%. Guard cell area is 0.026±5.03mm.

Adaxial surface epidermis

Epidermal cells polygonal, anticlinal walls are thin, straight to curve, paracytic stomata present, cyclocytic stomata observed as well. No aborted stomata were observed. Guard

cell area is $0.035 \pm 2.15 \text{mm}$. Stomata frequency is 3 per 100m^2 Stomata index, 25.00%.

Venation: Venation is brochidodromous. Areoles are polygonal, closed, no veinlet ending observed. Area of areole is $305,370.00 \pm 1808.91 \mu\text{m}^2$.

Mesophyll: Mesophyll is clearly differentiated into palisade and spongy layers. There are two palisade layers (upper and lower) which consist of more or less short cylindrical shaped parenchyma cells while the spongy cells are more or less polygonal cells.

Transverse section of Stem: Thin cuticle, epidermis undulating, epidermal cells circular to oval, rectangular shaped cells, epidermis are uniseriate to multiseriate. The cortex is made up of parenchyma cells, 3-4 layers of non-continuous perivascular tissues.

Vasculature: 46-47 bundles, long collateral bundles arranged in rings, length of vascular bundles is between 0.15-0.63mm. Mean length $0.40 \pm 32.33 \text{mm}$. Phloem bundles are extended to form protrusions which are joined to form arc. Vessels are circular, oblique polygonal or cylindrical, vessels solitary, pore multiple 2-4, clustered 3-4, pore chain. Vessels are surrounded by fibres. Mean vessel diameter $0.15 \pm 3.5 \text{mm}$, mean vessel length $0.16 \pm 4.66 \text{mm}$.

Basella alba:

Abaxial surface epidermis

Epidermal cells are polygonal, anticlinal walls are thin, straight, paracytic and anisocytic stomata present, abnormal stomata (unopened stomata, stomata with one guard cell and stomata without guard cells) were observed. Stomata frequency is 4 per 100m^2 Stomata index, 30.77%. Guard cell area is $0.027 \pm 2.25 \text{mm}$.

Adaxial surface epidermis

Epidermal cells polygonal, anticlinal walls are straight to curve, paracytic and anisocytic stomata present. Stomata frequency is 4 per 100m^2 Stomata index, 28.57%. Guard cell area is $0.029 \pm 2.15 \mu\text{mm}$.

Venation: Venation is brochidodromous. Areoles are polygonal, veinlet endings 1-2 per areoles, may occur singly, linear-curve, Area of areole $0.71 \pm 3217.18 \text{mm}$.

Mesophyll: Mesophyll is not differentiated into palisade and spongy layers. Mesophyll is made up of polygonal parenchyma cells although some cells are circular or oval in shape. Crystal sands are numerous

Transverse section of Stem: Thin cuticle, epidermal cells circular to oval short rectangular cells, epidermis are uniseriate to multiseriate. The cortex is made up of polygonal circular to oval shaped parenchyma cells, 5-9 layers. 3-4 layered of non-continuous perivascular tissues.

Vasculature: Vascular bundles are short, collateral, 37-43, mean bundle length $0.067 \pm 13.86 \text{mm}$. Vessel pores are circular, oblique, polygonal and cylindrical, vessels solitary, pore multiple largely 2, pore clusters not observed. Mean vessel diameter $0.119 \pm 3.90 \text{mm}$, mean vessel length $0.117 \pm 3.80 \text{mm}$.

Basella cordifolia:

Abaxial surface epidermis

At the abaxial surface, epidermal cells are polygonal, anticlinal walls are thin, undulating, paracytic and anisocytic stomata present, abnormal stomata (unopened stomata, stomata with one guard cell and stomata without guard cells) were observed. Stomata frequency is 3 per 100m^2 Stomata index, 17.65%. Guard cell area is $0.029 \pm 1.95 \text{mm}$.

Adaxial surface epidermis

Epidermal cells are polygonal, anticlinal walls are thin and undulating, paracytic stomata present. Aborted stomata observed (abnormal stomata (unopened stomata, stomata with one guard cell and stomata without guard cells, sickle-shaped stomata). Stomata frequency is 3 per 100m^2 Stomata index, 16.67%. Guard cell area is $0.030 \pm 1.10 \text{mm}$.

Venation: Venation is brochidodromous. Areoles are polygonal, veinlet endings may be linear or bifurcated, 1-6 veinlet endings per areole. Area of areole is $0.63 \pm 2670.67 \text{mm}^2$.

Mesophyll: Mesophyll is not differentiated into palisade and spongy layers. Mesophyll is made up of polygonal parenchyma cells although some cells are circular or oval in shape. Crystal sands are numerous

Transverse section of Stem: Thin cuticle, epidermal cells are elongated to circular-oval

and they are uniseriate-multiseriate. The cortex is made up of polygonal 8-12 layers of circular - oval shaped parenchyma cells. Two-layered perivascular tissues present.

Vasculature: Vascular bundles 27-30, mean bundle length is $0.164 \pm 19.20 \text{mm}$. Vessel pores circular, oblique, polygonal and cylindrical, vessels solitary, pore multiple 2-4, vessels are surrounded by fibres. Mean vessel diameter $0.15 \pm 2.87 \text{mm}$, mean vessel length $0.147 \pm 3.20 \text{mm}$.

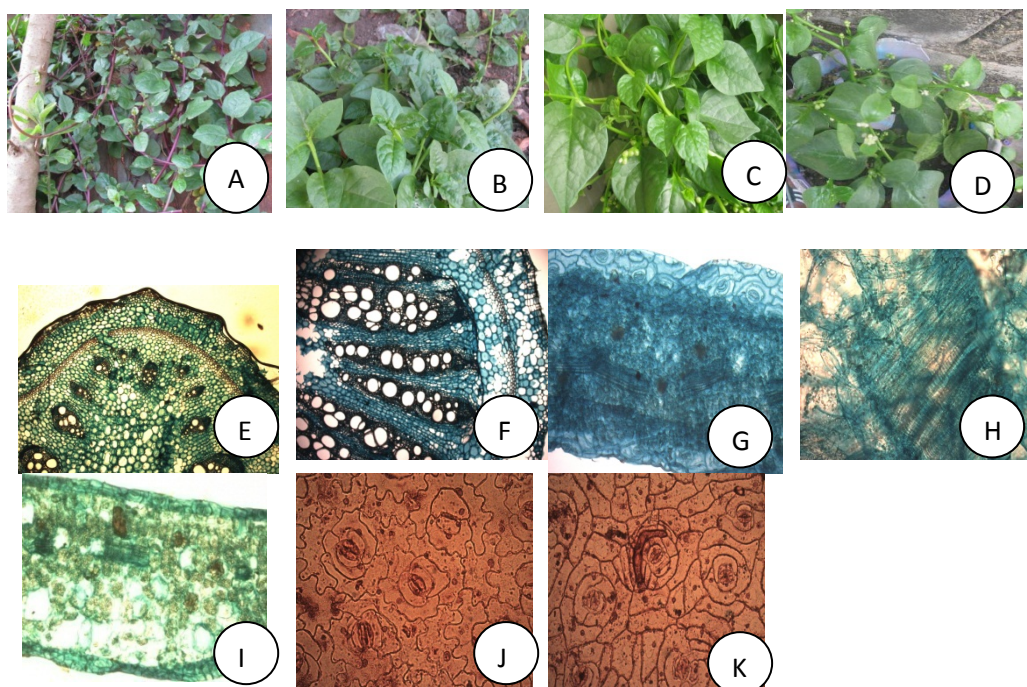


Plate 1. Legend: A- *B. rubra*; B- *B. alba*; C- *B. cordifolia*; D- *B. alba* var. round, E- Representative transverse section of the stem of *B. alba*, *B. cordifolia* and *B. alba* var. round, F- Transverse section of the stem of *B. rubra*; G-H-Transverse section of the lamina of *B. rubra* showing differentiated mesophyll and rib bundle; I- Undifferentiated mesophyll of *B. alba*, *B. cordifolia* and *B. alba* var. round; J-K-Representative adaxial epidermis of the four species.

***Basella alba* var. round:**

Abaxial surface epidermis

Epidermal cells are polygonal, anticlinal walls are thin, straight to curve. Paracytic and anisocytic stomata present, abnormal stomata also observed. Stomata frequency is 4 per 100m^2 Stomata index, 30.77%. Guard cell area is $0.033 \pm 3.05 \text{mm}^2$.

Adaxial surface epidermis

Epidermal cells polygonal, anticlinal walls more or less straight, occasionally curved, paracytic and anisocytic stomata present. Abnormal stomata (unopened stomata, one guard cell and stomata without guard cell) were observed. Stomata frequency is 2 per 100m^2 . Stomata index, 20.00%. Guard cell area is $0.028 \pm 1.95 \text{mm}^2$.

Venation: Venation is brochidodromous. Areoles are polygonal, veinlet endings linear, singly divided or bifurcated, 2-6 vein let endings per areole. Area of areole is $0.73 \pm 3702.28 \text{mm}^2$.

Mesophyll: Mesophyll is not differentiated into palisade and spongy layers. Mesophyll is made up of polygonal parenchyma cells although some cells are circular or oval in shape.

Transverse section of Stem: Thin cuticle, epidermal cells are elongated or circular to oval short shaped cells, epidermis grooved,

uniseriate to triseriate. Cortex 5-7 layered, polygonal circular to oval shaped collenchyma cells. 3-4 layered continuous perivascular tissues.

Vasculature: 29-31 collateral bundles arranged in a ring, mean bundle length $0.041 \pm 6.30 \text{mm}$. Vessel pores are circular, oblique, polygonal and cylindrical, vessels solitary, pore multiple, 2, vessels surrounded by fibres. Mean vessel diameter $0.119 \pm 3.70 \text{mm}$, mean vessel length $0.096 \pm 1.80 \text{mm}$.

Table 1: Summary of Foliar Anatomical Features

Characters	<i>Basella</i> species			
	<i>B.rubra</i>	<i>B. alba</i>	<i>B.cordifolia</i>	<i>B.alba</i> var. round
Abaxial Stomata Frequency/ 100m ²	2	4	3	2
Adaxial Stomata Frequency/ 100m ²	3	4	3	4
Abaxial stomata index (%)	27.30	30.77	17.65	30.77
Stomata index (Adaxial) (%)	25.00	28.57	16.67	20.00
Guard cell area (Abaxial) (mm)	0.026±5.03	0.027±2.25	0.029±1.95	0.033±3.05
Guard cell area (Adaxial) (mm)	0.035±2.30	0.029±2.15	0.030±1.10	0.028±1.95
Venation pattern	Brochido dromous	Brochido dromous	Brochido dromous	Brochido dromous
Veinlet endings (µm)	None	1-2	2-6	2-6
Mean area of Areole (mm)	0.55 ±1808.91	0.71	0.63 ±2670.67	0.73.75 ±3702.28
		±3217.38		

Table 2: Summary of Lamina and Stem Anatomical Features

Characters	<i>Basella</i> species			
	<i>B.rubra</i>	<i>B. alba</i>	<i>B.cordifolia</i>	<i>B.alba</i> var. round
Messophyll	Differentiated into 2 layers of palisade and spongy layer	Homogenous	Homogenous	Homogenous
Epidermis	Uniseriate - triseriate	Uniseriate - triseriate	Uniseriate - triseriate	Uniseriate - triseriate
Vasculature	Collateral, 46 long bundles Vessels solitary, pore multiples and in chains	Collateral, 37-43, short bundles vessels solitary	Collateral, 27-30 short bundles vessels solitary	Collateral, 29-31 oval short bundles vessels solitary
Mean length of vascular bundle (mm)	0.39±32.33	0.067±13.86	0.164±19.20	0.041±6.30
Mean vessel length (mm)	0.16±4.66	0.117±3.80	0.148±3.20	0.096±4.40
Mean vessel diameter	0.15±3.50	0.119±3.90	0.15±2.87	0.119±3.70

Discussion

Taxonomic values of anatomical features in plants are well discussed in literatures. Generally the information on the anatomy of the Genus *Basella* is scarce and the ones available are not based on the adaptive significance of these features. Anatomical features of plants are genetic as well as products of plants interaction with their environment. Plants develop various protective measures ranging from morphological to anatomical in a bid to survive hostile periods. This paper outlines variations in anatomical features of members of the genus that possess adaptive significance. Carlquist (1998) noted that wood anatomy has proven to be sensitive indicators of adaptation to diverse ecological regimes and may be seen with special clarity in families and genera that have diversified in recent geological time with little attendant extinction. According to him adaptation of wood of dicotyledons to varied ecological regimes is primarily related to vessel features; vessel diameter, vessel elements length, vessel density, vessel grouping and nature of growth rings and the occurrence of vasicentric tracheids. Kulkarni and Deshpande (2006) reported that anatomical markers are simple and cost effective characterization methodology for screening germplasm against drought resistance.

The abaxial and the adaxial epidermal cells are polygonal with thin straight to curved anticlinal walls on the adaxial surface while the anticlinal wall is wavy in *B. rubra*; undulating in *B. cordifolia* and straight to curved in *B. alba* and *B. alba* var round. Stace (1965) reported that the environmental conditions of a place such as humidity plays significant role in determining the pattern of the anticlinal walls. Straight to curved anticlinal walls is peculiar to species growing in drier environment. Members of the genus that have thin straight to curved anticlinal

walls (*B. alba* and *B. alba* var round) will likely acclimatize well to drier areas.

Stomata type is majorly paracytic in all the species in the genus but anisocytic is also observed. In addition to these cyclocytic stomata is observed in *B. rubra*. The occurrence of more than one type of stomata on the surface of a leaf has been reported (Essienn *et al.*, 2012). They noted that it is possible for one species to have more than three types of stomata. Abnormal stomata occurrence was reported in some Acalypha species (Essienn and Etukudo, 2012); Euphorbia species (Essienn *et al.*, 2012) and in the Loranthaceae (Ibrahim and Ayodele 2013). The presence of abnormal stomata was highlighted by Metcalfe and Chalk (1979). Abscisic acid (ABA) in-balance has been attributed to be responsible for abnormal stomata because it is the regulatory hormone found in the stomata. Abnormal stomata as also been attributed to responses to environmental factors. The main function of the stomata is regulation of water loss through transpiration. Presence of abnormal stomata especially closed stomata pore is likely one of the means of water conservation by these species. When stomata pores are closed water loss is prevented. Stomata frequency also has direct relationship with drought tolerance. Mishra *et al.* (2011) reported that lower stomata frequency could be considered as an adaptive feature towards preventing excessive water loss while higher stomata frequency reflects poor tolerance to drought under field conditions. *B. rubra* and *B. alba* round has stomata frequency of 2 per 100m² respectively while *B. alba* and *B. cordifolia* has 4 and 3 per 100m² respectively on the adaxial surface of their leaves. On the abaxial surface *B. rubra* and *B. cordifolia* have stomata frequency of 3 per 100m² respectively while *B. alba* and *B. alba* round have 4 per 100m². *B. rubra* has the least stomata frequency on the abaxial and adaxial surfaces among the *Basella* species, thus the species is

likely to be more tolerable of drought. Mishra *et al.* (2011) observed that the pattern and frequency of stomata on any leaf surface are under tight genetic control, however could be modified by environmental parameters such as the availability of carbon (IV) oxide.

Venation pattern is brochidodromous i.e veins form series of arches that do not terminate at the margins in all the species. Roth-Nebelsick *et al.* (2001) reported that there are two main functions of the leaf venation; transport of substances and mechanical stabilization which is based on the lignified xylem and sclerified elements that are associated with the conducting bundle system of the leaf. Roth-Nebelsick *et al.* (2001) also reported that the hierarchies of the leaf venation pattern show different degrees of reticulation. Based on the lower order veins (1^o and 2^o veins) two types of venation can be described; brochidodromous (closed) and craspedodromous (open). These exhibit differences in their transport behavior and are associated with different climates. Brochidodromous occurs in tropical flora while craspedodromous is common to floras in the temperate regions. A closed network provides for homogenous pressure distribution by rerouting the water flow to sites with higher rates of water loss.

Areoles are the smallest areas of the leaf tissue surrounded by major veins and form a contiguous field over most of the leaf area. Areoles in the *Basella* species are well developed. Areoles are closed without vein endings in *B. rubra* but opened in the three other species; vein endings are linear or curved or bifurcated. Closed areoles without vein endings are adaptation for survival in extreme conditions because there are no veinlets to distribute water to the margins. Areoles are polygonal but vary in their sizes; area of areole in *B. rubra* is $0.55 \pm 1808.91 \text{mm}$, *B. alba* has areole size of $0.71 \pm 3217 \text{mm}$, *B. cordifolia*, $0.62 \pm 2670.67 \text{mm}$ while *B. alba* var round has areole size $0.73 \pm 3702.28 \text{mm}$.

B. rubra has the least areole size while the largest is found in *B. alba* var round. Small areole implies that there is small surface area for water loss, thus conserving water while large surface areas of areoles provide for more vein distribution and increased water that could be lost through transpiration. *B. alba* var round has the largest areole and the highest number of veinlet endings which may account for increased water loss.

Lamina is differentiated in *B. rubra* into spongy mesophyll sandwiched in between two palisade layers but undifferentiated in the three other species. Spongy mesophyll is associated with intercellular spaces that have double functions of aeration and passage for water. The presence of two layers of palisade and the compact arrangement of the cylindrical cells in *B. rubra* reduces water loss.

The stem anatomy of the *Basella* species shows that the epidermis is largely multiseriate but some portions are uniseriate. Multiseriate epidermis reduces water loss. De Micco and Aronne (2012) noted that drought resistant in plants is also achieved through multi-layered epidermis or parenchyma tissues responsible for water storage. Multilayered epidermis reduces water loss through transpiration. Epidermis have thin cuticle which is characteristic of mesophytic plants however the presence of grooves on the epidermis shows the possibility of adapting to drier environments. Cortical cells are collenchyma and these function as means of support. Bundles are collateral in all the species, but variation occur in the number, size and length of bundles and in the length and diameter of vessels. *B. rubra* has more vascular bundles (46), longer ($0.39 \pm 32.33 \text{mm}$), the mean vessel length is the longest ($0.16 \pm 4.66 \text{mm}$) while the vessel diameter is $0.15 \pm 3.50 \text{mm}$. Makbul *et al.* (2011) noted that vessel diameter are usually larger in drought stressed plants. Masrahi (2014) noted that increased vessel diameter greatly increases water conduction efficiency although there

may decrease in safety and this may render vessels more vulnerable to cavitation (the formation of water bubbles within the conduits resulting in breakage of water columns). However Carlquist (2001) noted that the ray parenchyma near the vessel group may show a dense accumulation of starch grains which apart from supporting growth, enhance water flow and reduce the risk of embolism by hydrolysis into soluble sugars which enter the vessels and reduce the solute potential of the sap.

Vessels in *B. rubra* are in multiples and chains in addition to the solitary vessels observed in others. Alves and Angyalossy-Alfons (2000) reported that vessels show a tendency towards grouping in dry environments, whereas they are more often solitary and only rarely grouped in humid environments. The multiplicity of vessels (in chains or groups of vessels) increases water conductivity. This vascular bundle attributes confers on this species of *Basella* the ability to draw water from the soil to the plants body thus increasing its chance for survival in extreme conditions. Also the presence of phloemic protrusions observed in *B. rubra* would allow for better distribution of photosynthates or products of photosynthesis.

Conclusion

This study has revealed that the *Basella* species have anatomical features that possess adaptive features useful for water management. Among these species, *B. rubra* has anatomical features that allow for its survival in extreme conditions. These features include;

- Multiseriate epidermis which prevents water loss
- Vascular bundles and vessel length are longer and wide vessel diameters which enable the plant to draw water from the soil and the effective distribution of water within the plant system. Vessels are in multiples.

- Closed areoles and absence of veinlet ending which ensures water conservation.
- Differentiation of the leaf mesophyll.

Adaptation in plants is based on many morpho-anatomical traits expressed in different organs at different levels. Since they are not mutually exclusive, different combinations of traits lead to different adaptive strategies. Moreover, various degrees of adaptation can coexist, allowing quite different biological forms to share the same environment. Therefore these anatomical characters can be incorporated into breeding programs. This information is useful for future breeding program in the genus *Basella*.

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