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Description and characterization of leaf anatomy of Asian Palmyra Palm – *Borassus flabellifer* L.

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Abstract

Anatomical investigation of Asian Palmyra palm *Borassus flabellifer* L. was carried out, with the following objectives, description and characterization of vascular, fibro vascular bundles and fibrous sheaths from leaf. Comparative anatomical studies were done between lower, middle and upper regions of leaf pinnae with reference to diversity of fibro vascular bundles and other tissues. Free hand section of Palmyra leaf lamina was taken for anatomical and histochemical studies. Leaf lamina segments were macerated using Jeffery's fluid to study leaf fibres. Staining was done using histochemical stains such as Safranin 'O', Toluidine blue- 'O', Sudan III, Oil red, Nile blue, and fluorochromes such as Acridine orange & Rhodamine. Leaf peelings were taken for stomatal studies and micrometric measurements. Parameters such as length & width of all cells, and wall thickness were made and the results were statistically analyzed. Leaf epidermis is covered by a thick waxy cuticle layer. Hypodermis is made of very thick wall cells. Stomatal arrangement and vascular bundles have significant correlation in designing the leaf anatomical architecture. The findings on leaf anatomy would be useful in comparative evolutionary and adaptation studies of other species and genera of Palmae. The leaf anatomical characteristics justify that Palmyra palms are well evolved species to adapt themselves to dynamic environmental factors like heavy wind coupled with dry and high temperature. Thus, the findings of this investigation are unique and shed more light on the evolutionary and adaptational features of Palmyra Palm. Comparative anatomical studies on lower, middle and upper region of palmyra leaf lamina, this research was done for the first time in Palmyra palm. The anatomical investigation appearances of the leaf has shed significant information, they are Typification of fibro-vascular bundles (four types) based on the association of vascular bundles with fibrous sheath is being reported for the first time in *Borassus flabellifer.* Studies on leaf anatomy of palm leaves using fluorescence microscopy is first of its kind. Thick waxy cuticle layer is present in abaxial and adaxial surface of the leaf lamina, the main function ascribe to the cuticle are protection from water loss due to this palmyra tolerate drought conditions. Another unique characteristic feature is branchparahexacytic stomata, this arrangement and vascular veins have significant correlation in designing the leaf anatomical architecture.

INTRODUCTION

The Palmyra palm, scientifically known as *Borassus flabellifer*, holds a significant place among ancient trees that have spread across continents, originating from Africa and extending to the Indian subcontinent due to human migration, as highlighted by Arunachalam et al. (2020). Particularly in the Asian region, this palm species has garnered popularity and is notably distributed in countries like India and Sri Lanka. A comprehensive study conducted by Davis and Johnson (1987) delved into the economic importance and various commercial uses of the Palmyra palm, emphasizing its diverse utility. Palms, as a family of trees, stand out as the most extensively utilized for non-timber forest products in tropical regions, as detailed by Ballick and Beck (1990), who meticulously cataloged over 390 products derived from around 200 genera of palms. Referred to as the "palmyra tree of life," this remarkable species boasts a staggering array of almost 800 applications, ranging from serving as sources of food and beverages to providing essential fibers, medicinal extracts, and even timber, as documented by Arulraj and Augustine (2008).

The leaves of palms, whether they are of the pinnate, palmate, or entire shape variety as mentioned by Davis and Johnson (1987), hold remarkable versatility in their use. Across different geographical regions, there exists a longstanding tradition of harnessing these palm leaves for an array of practical purposes such as constructing thatch roofs, weaving mats, and crafting cloth. Notably, the fibers obtained from the leaf blades exhibit exceptional durability, as they are characterized by their hardness and stiffness. In particular, the fibers derived from the leaf bases are highly sought after for their quality, serving as a crucial raw material in the manufacturing of brushes. This specific type of fiber has even garnered significant attention as an important export commodity from various regions of India, as highlighted by Arunachalam et al. (2020).

Palm leaves, renowned for their historical significance, served as writing materials long before the advent of paper, creating an indelible mark on ancient Tamil literature by preserving poems and cultural documents. This ancient practice not only encompassed the literary realm but also extended to the field of medicine, where valuable insights into medicinal properties, measurement techniques, and treatment methods were meticulously recorded and safeguarded on these resilient palm leaves. Over the centuries, the art of palm leaf inscriptions not only thrived but also evolved, showcasing the adaptability and timelessness of this ancient writing medium. (Davis and Johnson, 1987).

Palm leaf provides a complex expression of diversity within a single organ, related to the primary function of phytosynthesis. The leaf axis in a palm is divided into three components like leaf sheath, petiole, and rachis into the segmented blade. These parts have been presented in Tomlinson 1990 in terms of their functions and mechanical properties. Anatomy of palm leaves was examined in detail by Tomlinson (1958; 1961), *Borasseae*, an unusually high incidence of isolateral leaf symmetry was described. Most palms have leaves with an asymmetrical (dorsiventral) anatomy a well defined adaxial palisade mesophyll, and stomata primarily on the abaxial surface. However, most *Borassus* species lack a clearly defined palisade, and have stomata on both surfaces, isolateral anatomy (Tomlinson, 1961). Leaf symmetry is thought to be correlated with light exposure (Tomlinson, 1961) and aridity (Barrow, 1998). In the *Borasseae*, genera from exposed or arid habitats (*Bismarckia* Hildebr & Wendl, *Hyphaene* Gaertn and *Medemia* Wurttenb. Ex Wendl.) exhibit isolateral leaf symmetry, while those from shady, humid habitats (*Borasso dendron*, *Lodoicea* Comm. ex D C. and Satranala Dransf. & Beentje) show dorsiventral leaf symmetry. *Latania* Comm. ex Juss., a genus of coastal cliffs and savannas, has a somewhat intermediate leaf anatomy (Tomlinson, 1961). *Borassus* reflects this overall pattern as most species of this primarily arid zone palm have isolateral leaf symmetry, but *B. heineanus*, from the rain forests of New Guinea, has dorsiventral leaf anatomy (Bayton 2007).

MATERIALS AND METHODS

Sources of Plant Material

Mature leaves of Palmyra tree were collected from the Tirunelveli region at different places of Manonmaniam Sundaranar University campus (Lat N 08^{o} 76' 43.4''. Long E 077^{o} 65' 09.9"), Alangulam (Lat N 08^{o} 52' 13.8''. Long E 077^{o} 28' 57.7"), Thiruchendur (Lat N 08^{o} 31' 0.94''. Long E 078^{o} 05' 18.9"), Udankudi (Lat N 08^{o} 23' 14.5''. Long E 078^{o} 01' 09.5"), of Tirunelveli district south India. Leaf specimens were fixed in a fixative solution containing formalin acetic alcohol (FAA) (Berlyn & Mikshe, 1976) for prolonged use. After fixation for seven days leaf materials were transferred to 70 % ethyl alcohol. Fresh materials were given preference for anatomical, histochemical and maceration studies and observation using Fluorescence microscopy.

Free Hand Sectioning and Staining

Palm leaves were cut into small pieces of about 1-2 cm long; free hand sections were made using sharp knife or blade. Sections were floated in water held in a Petri dish and very thin sections were stained using light microscopic stains such as toluidine blue 'O' (Brien *et al*., 1964; Fisher, 1985) and safranin **(**Krishnamoorthy, 1988**)** and fluorochromes like acridine orange (Armstrong, 1956) and rhodamine. Histochemical reagents such as I_2KI (Haridass and Suresh Kumar, 1985), oil red (Lillie, 1965), and Sudan III (Chiffelle and Putt, 1951) were also tested. After proper staining, sections were placed on very clean slides and mounted using either sterile distilled water, dilute glycerin or 20% of calcium chloride solution (Herr, 1992). Very thin cover slips were used for mounting temporary or semi-permanent slides. Such good sections mounted on clean slides (thickness is 1.35mm) were observed under the light and fluorescence microscope (Nikon 80 i).

Maceration of leaf

Maceration of leaf segments was done using maceration mixture (10% Nitric acid, 10% Chromic acid) prepared by dissolving 10 g of Potassium chromate in 100 ml of 30% acetic acid (Jeffrey, 1917). 10% of Nitric acid and 10% Chromic acid were mixed in equal volumes before use. Slivers of the leaf samples were taken from three different regions vise basal, middle, and apical (1- 2 cm in length) were prepared and immersed in maceration mixture in 20-100 ml vials or bottles, left the set up undisturbed for 24-48 hrs to accomplish complete maceration of fibres. Maceration was checked using a fine brush, if the slivers have softened it was assumed that maceration is completed. They were boiled for few minutes under low flame to ease the process prior to macerated elements were repeatedly washed in distilled water to remove all traces of acid and further stored in 100 % alcohol. Macerated elements include stomatal layers, fibres, vessels, tracheids and crystals. Macerated fibres were stained in Safranin and Toluidene blue 'O' before observation. Excess stain was washed with distilled water to accomplish ideal color contrast for microscopic observation and photography. Calculation of stomatal index from leaf midrib was achieved using the formulae described by Chisom *et al*., (2015).

RESULTS

General Leaf Anatomical Characterization of Palmyra Palm – *Borassus flabellifer* L.

Palmyra tree is one of the World's most widely distributed palm species. Palmyra is an erect tree about 70 to 100 ft in height. A normal crown contains 30-40 palmate leaves, which are in duplicated and strongly costapalmate. Leaves are leathery, grey green, fan shaped, held along the midrib, and strong grooved petiole. Adaptive tissues are occurring in all parts of Palmyra tree so it is survives in all habitats, productive epi cuticular wax, cuticle, epidermis, hypodermis, palisade mesophyll parenchyma, fibro vascular bundles, fibrous sheath and stomata, all these cells can be seen in a Palmyra leaf in cross sectional view. All these cells are play an important role in these leaves, especially fibro vascular bundles and fibrous sheath are plays an important role, because of these characteristics palm leaf possess extraordinary strength. Palmyra leaves are getting stronger due to toys and hand craft are made from Palmyra leaves. The present anatomical investigation reports a detailed leaf anatomy and comparison is made between different of the leaf.

Thus, palms undoubtedly are a species-rich and ecologically ample clade is due to diversification in their leaf anatomy. Functional significance is clearly attributable to many leaf lamina anatomical characters that show obvious variation and diversification. Because fibers assume much of the load-bearing capacity of the lamina (Schwendener, 1874; Vincent, 1982, 1991), these structural differences may be expected to reflect corresponding differences in biomechanical attributes. Isobilateral lamina histology, in which the adaxial and abaxial leaf surfaces and mesophyll are alike, so that (disregarding the polarity of the vascular bundles) they are about mirror-image equivalents again, varying functional attributes likely accompany this major structural difference, because isobilateral leaves, with stomata and palisade mesophyll positioned at both leaf surfaces, can maximize the distribution of carbon dioxide (Mott *et al*., 1982 ; Slaton and Smith, 2002) and light (Vogelmann and Martin, 1993 ; Vogelmann *et al.,*1996) within the lamina. In high light environments, isobi-lateral leaves are therefore capable of greater rates of photosynthesis per unit biomass than their dorsiventral counterparts (Smith *et al.,* 1997). These and other characters, involving the epidermis, hypodermis, and transverse veins, collectively constitute much of the most readily perceptible anatomical variation within palm leaves.

Leaf Anatomy of *Borassus flabellifer*

Transverse sections of apical, middle and basal portion of *B. flabellifer* adaxial side of leaf were studied and observed under light, polarized and fluorescence microscopic modes. Notable variations occur in the size and thickness and function of all cells. In the palmyra leaves the epicuticular wax and cuticle in primary a barrier wall, these epicuticular wax provides covering and protection to the leaf lamina of *B. flabellifer.* A thick film of epicuticular wax is present as a translucent layer above the cuticle. Thin films of epicuticular waxes are up to 8 µm thick on the adaxial leaf surface and up to 10 µm on the abaxial surface. Presence of this thick film of epicuticular waxes covering the leaf lamina was observed in all selected individual trees and is related to the glossy green nature of the adaxial and abaxial surfaces, respectively, in the intercostal regions. The film of epicuticular waxes may also appear discontinuous in transverse section, probably due to preparation of the material. The detachment of the layers of epicuticular waxes was observed even in fresh and little-processed pinnae. Presences of epidermis this is first layer of cell, these epidermis are presence on adaxial and abaxial surface of the leaves. It is a single layer, very smallest cells in this leaf. In surface view under light microscope, the epidermis of *Borassus flabellifer* shows hexagonal in shape. Brachyparahexacytic stomata were identified both on the abaxial and adaxial leaf surfaces, where they occur in groups separated by epidermal cells associated with the intercostals veins in transverse sections of apical, middle and basal portions of pinnae, On both surfaces, epidermal cells possess straight to curved anticlinal walls arranged in longitudinal rows. Very thick wall cell of hypodermis is present in the Palmyra leaf of adaxial and abaxial region. Most of the hypodermis is rectangular in shape; some of the cells are oval or circular in shape. Mesophyll parenchyma cells is otherwise known as palisade parenchyama, it is thin wall cells, the cell size and numbers are various from apical to basal region of the leaf. Fibro vascular bundles and fibrous sheath play an important role in palmyra leaves, and it is this fiber that give leaves their flexibility. So many hand craft products are made from their palm leaves.

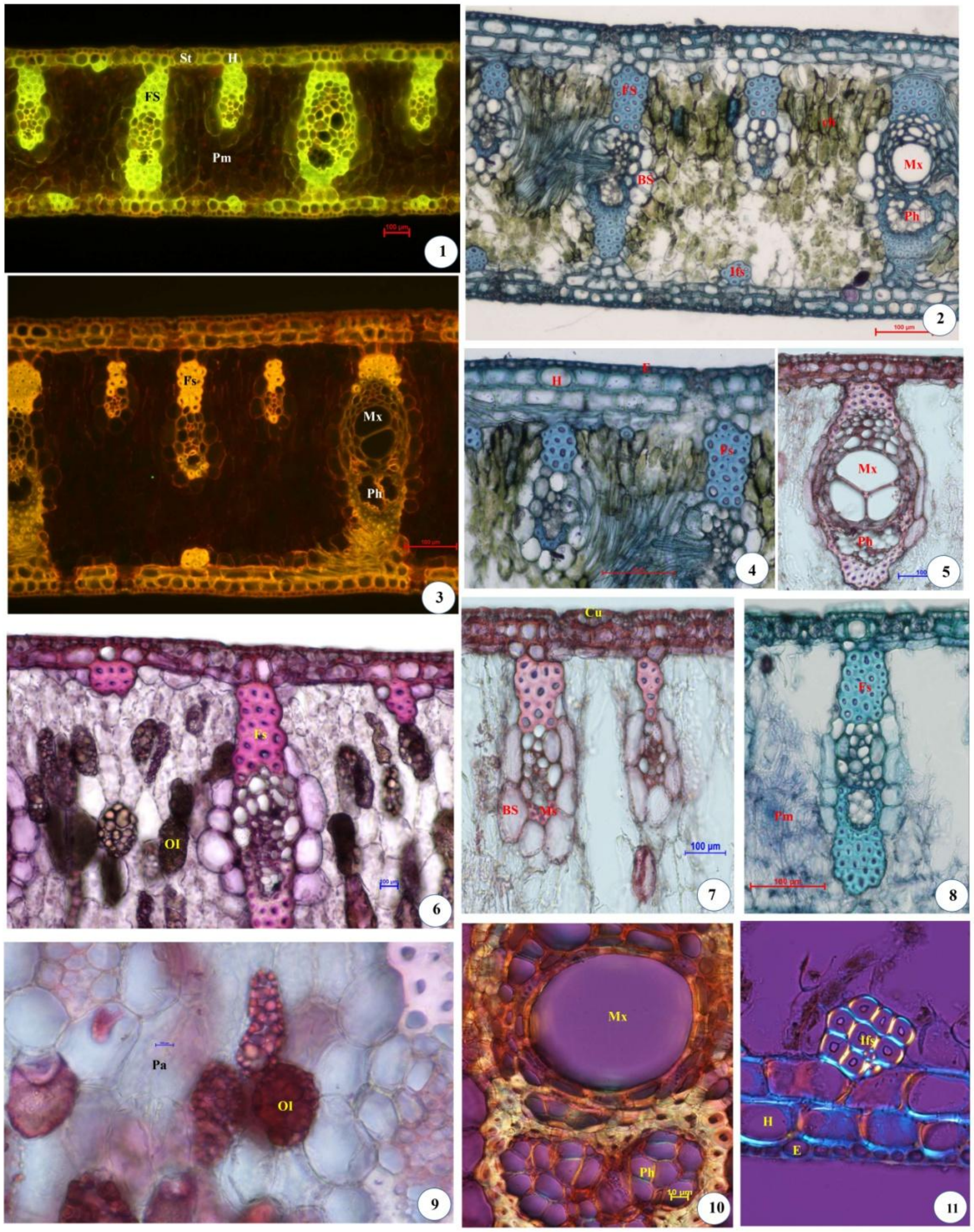


Figure 1

1. Low magnification showing Different types of fibro vascular bundles under blue light excitation. 2. Section stained with TBO showing mesophyll parenchyma and type II, type III, fibro vascular bundles. 3. Section stained with Rhodamine 'B' observed under blue light excitation dark yellow color indicated fibro vascular bundles. 4. Close up view of adaxial side, stained with TBO showing lignified epidermis, hypodermis, with fibro vascular bundles. 5. Enlarge view of Type IV fibro vascular bundles, large

Meta xylem, phloem and bundle sheath cells are distinctly seen. 6. Sections are stained with Nile red, showing mesophyll cells with oil bodies. 7. Section stained with Saffranin showing close up view of Type I and Type II fibro vascular bundles. 8. Close up view of Type III fibro vascular bundles, early developed xylem and phloem with fibrous sheath is distinctly seen. 9. Section are stained with oil red, close up view of oil containing mesophyll cells, oil bodies are distinctly seen. 10. Enlarged polarized view of fibro vascular bundles, showing large Mata xylem, phloem. 11. Close up view of abaxial side, observed under polarized view, showing epidermis, hypodermis, and isolated fibrous sheath.

Cuticle

Cuticle is a layer made of hydrophobic cutin and form a covering on the outer epidermal wall in the apex, middle and base regions of the *B. flabeliffer* leaf (Fig. 7). In Palmyra palm leaves the thickness of cuticle layers varies from 2 µm to 6 µm from base to terminal region. The average length of leaf cuticle is 3.34 µm. Cuticle appears pink in color after staining with safranin, blue in TBO, and dark yellow color in Rhodamine. Due to presences of epicuticular wax and cuticle in the adaxial and abaxial surface of the leaf, so Palmyra leaves do not dry in all environmental condition. The thickness of cuticle may be influenced by environmental conditions. Species under intense light or water deficit usually have thicker cuticles (Esau, 1976; Arruda *et al*., 2009; Kosma *et al.,* 2009). Sandy coastal ecosystems are constantly subjected to water and nutritional deficits.

Epidermis

Epidermal cells are Presences in adaxial and abaxial surface of the leaf lamina. It is a protective layer of the leaf; the epidermal cells are covered by cuticles. Epidermis is single layered and composed of flattened cells on the upper surface and very small cells, approximately hexagonal or sometimes oval to rectangular. Epidermal is extended these layer ranges from 4 µm to 22 µm with an average of 11.95 µm in thickness (Fig.4, 11).

Hypodermis

Presences of hypodermis are below the epidermis, hypodermal layers are thick walled cells and these cells are rectangular in shape and larger than epidermis. It is a colorless without chloroplast. Single layer of hypodermis is present in apex region of the palm leaf but two layers of hypodermis is occur in basal and middle regions of the same leaf. The hypodermal layer consists of thick-walled cells; larger fibro-vascular bundles are connected to the hypodermal layer. The cells of hypodermal layer are rectangular in shape. Hypodermal layer varies from 9 µm – 49 µm (average 29.95 µm) and width maximum of 24 µm to minimum width of 11 µm (Average 15.69 µm) (Fig.1, 2, 3).

Hypodermal cells are unusual among all palms in being thick walled and lignified. The biomechanical hypothesis discussed is supported by correlations between Caryoteae (Coryphoideae) and those Arecoideae that both possess the cross-laminated surface layer type. Among all palms, these lineages have the correspondingly lowest proportion of mechanical tissue in their laminae. The low volume fraction of mechanical tissue in Arecoideae may be explained by both phylogenetic contingency with a subsequent correlated loss of fiber bundles in contact with the surface layers. However, the loss of mechanical tissue from Caryoteae is remarkable within their phylogenetic context because they are embedded within a clade that otherwise exemplifies the deployment of abundant fibers for structural support.

Mesophyll

Mesophyll cells are composed of approximately 6-9 layers of parenchymatous cells. The mesophyll cells contain photosynthetic pigments of chloroplasts, and some tissues filled with oil bodies. Mesophyll cells are very compactly arranged without any space and they are oriented parallel to each other. It is a thin wall cell, apical region its contains 5-7 layers of mesophyll cell (Fig. 1), but 8-10 layer of mesophyll cells are present in the middle and basal region (Fig. 2, 3), so the thickness of basal region of the leaf greater than the thickness of apical region of the leaf. The mesophyll cells cover a larger portion of leaf with a maximum length of 55 µm and minimum of 15 µm (average 30.09 µm) and in width maximum of 36 µm and minimum of 9 µm (average 20.3 µm).

Isolated Fibrous sheath & Non-vascular fibers bundles

In the cross section of palm leaf, the presence of fibrous sheath was observed in adaxial and abaxial side of leaf and these cells are connected to the hypodermis. In the apex region of the fibrous sheath cells are connected with the epidermal layer. In the apex region of leaf 3 to 5 fibrous sheaths are present on both lower and upper surfaces (Fig.1). At the time of leaf development fibrous sheaths are present in apex region which might later develop in to new small vascular bundles, but the basal fibrous sheath cells remain as such with 3 to 10 layers of fibrous sheath. These fibrous sheaths are also called as non-vascular fiber bundles (lacking of vascular tissues) distributed immediately below the adaxial hypodermis and larger non-vascular fiber bundles usually occur near the abaxial surface.

Nonvascular fiber bundles (fiber sheath) and fibro vascular bundles (longitudinal veins) of both types of distribution (surface vs. mesophyll) are common throughout palms and co-occur in many taxa. These two features have an evolutionary outline in which loss of fiber bundles free in the mesophyll are concentrated among lineages that possess fiber bundles in contact with surface layers indeed this is the only highly significant correlation between mesophyll fibers and any of the other characters studied. Whereas fiber bundles associated with the surface layers are of strong mechanical significance (Schwendener, 1874; Vincent, 1982, 1991) unlignified mesophyll fiber bundles may possibly also effect hydration of the mesophyll because they are moreover not or only weakly lignified (Tomlinson and Fisher, 2005).

Fibro-vascular bundles

Vascular bundles are together with sclerenchyma fibrous sheath is called as fibro vascular bundle; these characteristic features are unique in monocot family. A single fibro vascular bundles are contains sclerenchyma fibre, bundles sheath cells, mestome, xylem, and phloem. In the present study four types of bundles have been identified and characterized based on the nature of association to the fibrous sheath with vascular strand and varying sizes of the bundles. There are four types of fibro-vascular bundles present in *B. flabellifer* leaf, two are small fibro-vascular bundles, and two are large fibro-vascular bundles. The small fibro-vascular bundles are connected with cells of adaxial epidermal layer. More number of thick wall and very thick wall fibre are found in these leaves. Due to this the leaves are very flexible and strength. The number and size of the fibro vascular bundles and isolated fibrous sheath was extended, so thickness of leaf also increases. The length of fibro-vascular bundles ranges from 124.82 µm to 394.97 µm with an average of 244.47 µm. (Fig.1, 2, 3).

Such compact arrangement of fibro-vascular bundles was observed in the rind of Sugarcane (Saravanan, 1996) and Sorghum (Manimekalai *et al.,* 2002). The fibrous cells are referred as girders when they extend from the epidermis to serve as a mechanical support for the vascular bundles (Metcalfe, 1971). However, the fibrous cells that are not in direct contact with the vascular bundles are known as fiber strands. Pattern and distribution of vascular bundles in the clum varies significantly. The peripheral region (rind) has more number of densely arranged vascular bundles, a feature commonly observed in many members of Cyperaceae and Gramineae.

Vascular bundles types

Anatomical features with special reference to the vascular bundles and their association with fibrous strand shows considerable variations. In view of the association of fibrous sheath with vascular bundles the term fibro-vascular bundles have been used. The fibro-vascular bundles are compactly arranged. Vascular bundles are typically collateral. Though, different types of vascular bundles have been recognized based on shape and size, typification based on association with fibrous cells has not been known earlier from palmae, thus forming first report of this kind.

Type I

Very small vascular bundles enclosed by three layers of cells, the outer most being radially arranged parenchymatous cells. Inner to this is the bundle sheath cell and followed by poorly developed mestome layer, xylem and phloem are not well developed. The vascular bundle is directly attached (without the intervention of parenchymatous cells) to a sclerenchyma thick walled fibrous sheath which in turn is attached to the two layered hypodermis at the adaxial side of the leaf (fig.7).

Type II

Small vascular bundles similar to type I, but bundle sheath cells and mestome cells are larger than type I, the vascular bundle is attached to a sclerenchyma fiber, the length of fibrous sheath is longer than type I; the sclerenchyma fibrous sheath is connected to the hypodermis (Fig. 4).

Type III

Large vascular bundles showing well differentiated xylem and phloem elements. The vascular bundle is covered by fibrous sheath at both phloem and xylem poles. The phloem cap sclerenchyma fibrous sheath is connected to the adaxial hypodermal layer; sclerenchyma fibrous sheath below the xylem is connected to abaxial hypodermal cells. Along the side of vascular bundle there exists a mestome layer which on either side is covered by large bundle sheath cells. Presence of chloroplast is explicit. The number of bundle sheath cells on one side (which side) ranges from 4- 6 (fig. 8).

Type IV

Very large vascular bundle similar to type III but has well developed metaxylem and phloem, the metaxylem and phloem are larger than that present in type III bundles. Similarly the number of mestome layer cells is also more than type III. The bundle sheath cells are quite larger than any other type. The vascular bundles are connected to both phloem and proto xylem poles. The protoxylem lacuna is also very large.

Sclerenchyma fiber exist as a single layer around the protoxylem cavity however, at the connecting point near the abaxial hypodermis two – four layers of fibrous sheaths are present (Fig. 5).

Details of cellular measurement in *Borassus flabellifer* leaf lamina

Parameters	Mean ± SD (µm)
Cuticle length	3.34 ± 0.85
Epidermis length	11.95 ± 3.59
Epidermis width	7.62 ± 1.78
Hypodermis length	29.95 ± 11.04
Hypodermis width	15.69 ± 3.26
Palisade parenchyma length	30.09 ± 7.61
Palisade parenchyma width	20.3 ± 5.28
Oil cell length	159.1 ± 59.93
Oil cell width	328.34 ± 56.65
Meta xylem length	46.92 ± 26.38
Bundle sheath cell length	26.32 ± 4.93
Sclerenchyma single cell length	15.26 ± 2.78
Vascular bundle length	244.47 ± 6.68
Stomata length	131.87 ± 16.99
Guard cell length	39.03 ± 13.49
Subsidary cells longitudinal length	148.61 ± 52.41
Subsidary cells oval length	62.84 ± 24.95

Presence of oil bodies in *B. flabellifer* leaf

Some Palm leaves and midrib possess aromatic compounds due to the presences of plenty of oil cells and oil glands. *B. flabellifer* leaf and midrib are also aromatic. The presence of oil cells and glands was confirmed by observing the sections stained with Sudan III under light microscope. The palmyra leaf oil cells average length is 27 µm - 52 µm and width is 25 µm – 15 µm. (Fig. 9).

Stomata

Stomata are usually found on the aerial portion of the plants and especially on leaves. Each guard cell is accompanied by one or more subsidiary cells that are aligned parallel with it. These occur in the Convolvulaceae, Leguminosae, Magnoliaceae, and Rubiaceae, among others. This was formerly known as the rubiaceous types (Mauseth, 1988). The stomata is of brachyparahexacytic type in palmyra palm, it contains two guard cells and four subsidiary cells, two subsidiary cells on the sides arranged parallel to the guard cells are longitudinal and those occur on terminal junctions are oval connecting two other subsidiary cells (Fig. 13). Guard cells include prominent nucleus, starch and probably chloroplast.

Stomata index was calculated for 100 mm^2 in the upper and lower leaf surface and the surfaces of midrib. Stomatal index is more on the upper epidermis compared to the lower. It is 55.65 on the upper surface of the leaf and 46.86 on the lower surface. Photosynthesis and transpiration rates were always higher on the abaxial surface than the adaxial surface. CO_2 uptake rates increased as atmospheric CO_2 was increased up to the growth concentrations on both leaf surfaces. Above these values, CO_2 uptake on the abaxial surface was either stable or increased as CO_2 concentration increased. In marked contrast, CO_2 uptake rates on the adaxial surface were progressively inhibited at concentration above the growth CO_2 value, whether light was supplied directly to this or the abaxial surface (Driscoll *et al*,. 2006).

The length of stomatal apparatus (guard cells and two subsidiary cells) in leaf region varies from 93.65 µm and 288.67 µm. earlier study by Metcalfe and Chalk (1988) and Beerling and Woodward (1997) showed that large stomata resulted in low stomatal density while small stomata gave high stomatal density. The work of Abdul Rahaman and Oladele (2003) also showed this pattern where large stomata actually yielded low stomatal density and small stomata with high density in some vegetable species.

Some correlations do occur between the stomatal features (like density, index and size) and rate of transpiration in each species studied. Stomatal density has been identified to play a major role in water use efficiency of plants thus, its numerical strength on the leaf surface is essential (Wang *et al.,* 2007). The work of Spence *et al.,* (1986), Spence (1987), Royer (2001) and Wijad *et al*., (2005) also showed that the geometry and resulting mechanical properties of small stomata, enhanced the capacity of opening or maintaining open pores with lower guard cell turgor pressures, relative to the turgor of the surrounding epidermal cells. This is the important reason because of which Palmyra is able to survive very harsh and hot temperature during summer sending out less of water by transpiration. Thus, Palmyra is a hardy drought tolerant species.

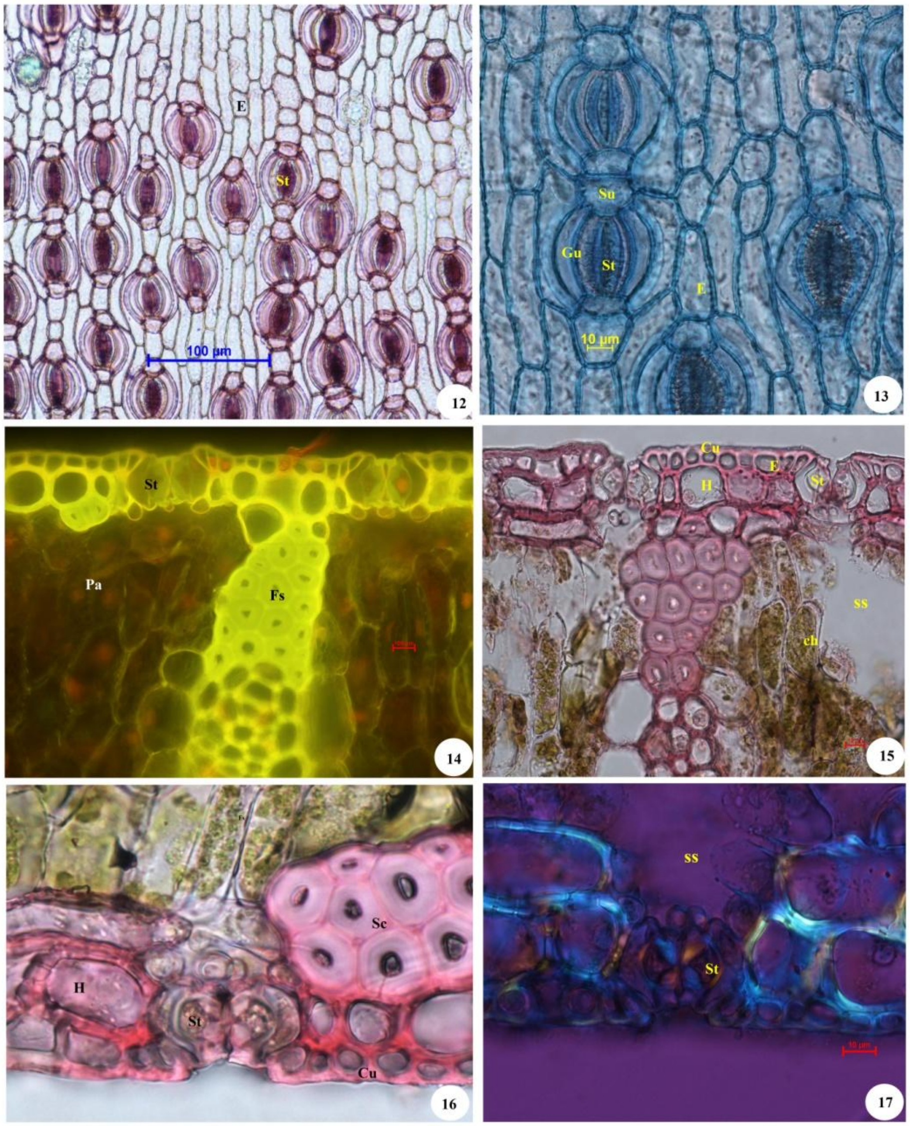


Figure 2. 12. Adaxial side of epidermal peel, showing epidermis with stomata. 13. Epidermal peel stained with TBO, observed under light microscope, guard cell, subsitary cells are distinctly seen. 14. Cross section of leaf stained with AO observed under blue light excitation showing Adaxial epidermis with closed stomata. 15. Section are stained with saffranin, adaxial epidermis, stomata, sub stoamtal cavity, are distinctly seen. 16. Close up view of Abaxial Stomata complex showing subsidiary cell with guard cells. 17. Polorized view of single stomata with stomata cavity

Calculation of Stomatal index in leaf:

Upper surface: The 1 cm^2 area of upper leaf contains 7,580 stomata.

$$SI=\frac{7580}{7580+6040}\times 100$$

= **55.65**

Lower surface: 1 cm^2 area of lower leaf contain 5,680 stomata

$$SI=\frac{5,680}{5,680+6440}\times 100$$

= 46.86

Fiber macerates

Microscopic and dimensional characteristics of fibers from *B. flabellifer* leaf

The *B. flabellifer* leaf is flexible and strength is due to the presence of isolated fibrous sheaths and fibro-vascular bundles. The isolated fibrous sheath and fibro-vascular bundles provide strength, thus fibers were isolated to study their cell wall properties. Four different types of fibers were observed based on wall thickness viz., very thick walled, and thick walled, thin walled and very thin walled (fig.19-24). Fibers have tapering, blunt and cleft ends. The surface layers of the palm leaf lamina epidermis and hypodermis evolve in close tandem and appear to function as a unit or meachanical skin of the leaf.

Critical to the mechanical stability of all but the smallest leaves of terrestrial plants is a network of strong, usually lignified cells that provide the scaffolding or internal skeleton around which other leaf tissues are organized (Lucas *et al*., 1991; Choong *et al*., 1992; Niklas, 1999; Roth-Nebelsick *et al.,* 2001) In palms and many other monocots, this network not only consists of veins but includes nonvascular fibers that, at least in palms, usually occur grouped in bundles that are either positioned in contact with the surface layers, or else may be independent of the surfaces and "free" in the mesophyll. The longitudinal veins of some palms have fibers or sclereids that effectively attach the vein along the length of its course to one or both surface layers. Thus, functioning much like an I - or T-beam, these cells reinforce the leaf via both material and, collectively, structural properties (Schwendener, 1874; Read and Stokes, 2006). The presence of fibers that ramify freely in the leaf lamina in genera belonging to three different sub-families of palmae (Tomlinson, 1961). Tomlinson and Fisher (2005) suggest that similar fibers in *Gnetum* leaves have a role in effecting hydration of the lamina in addition to mechanical support. The parallel loss of these fiber bundles in the major palm lineages associated with arid climate suggests this.

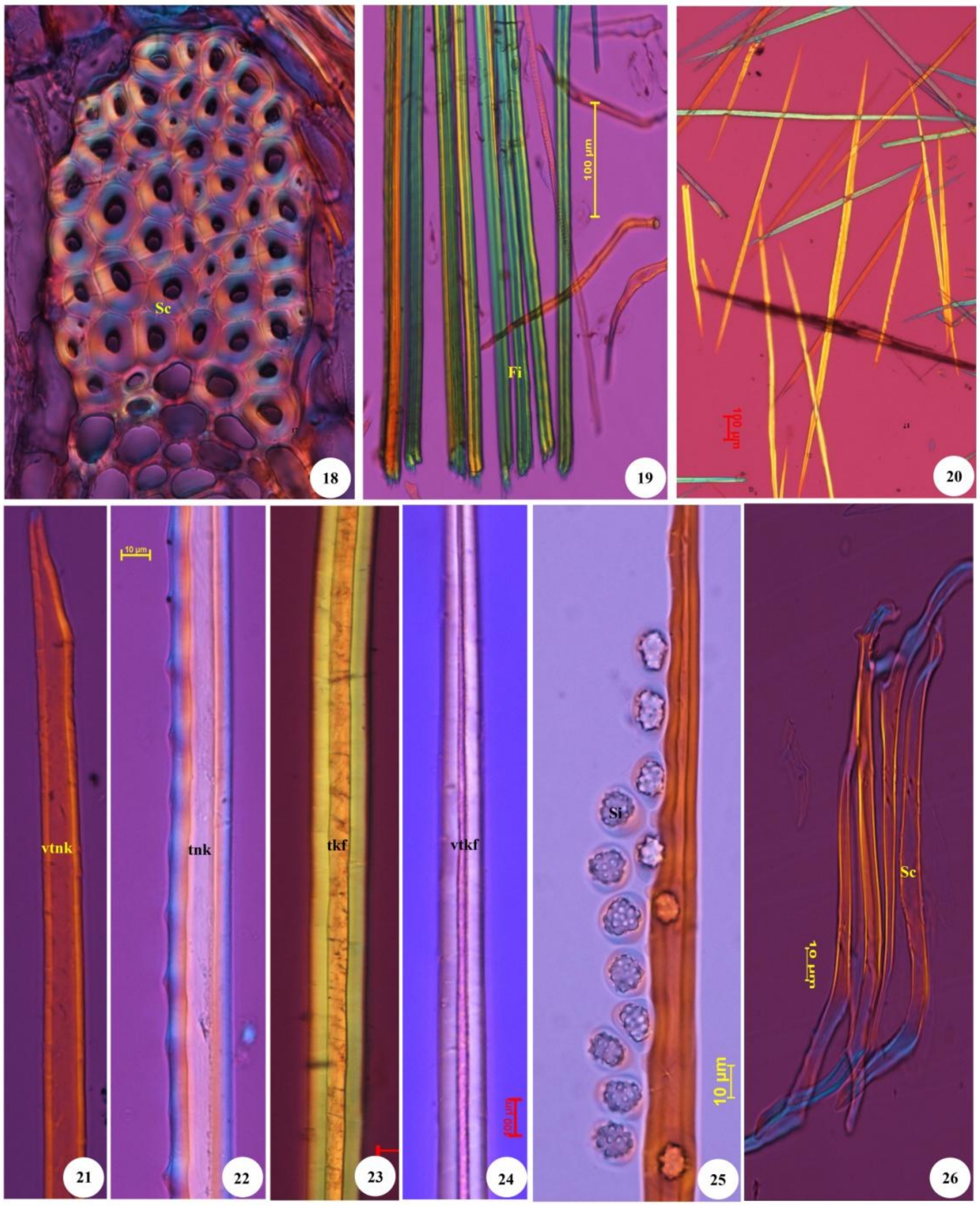


Figure 3

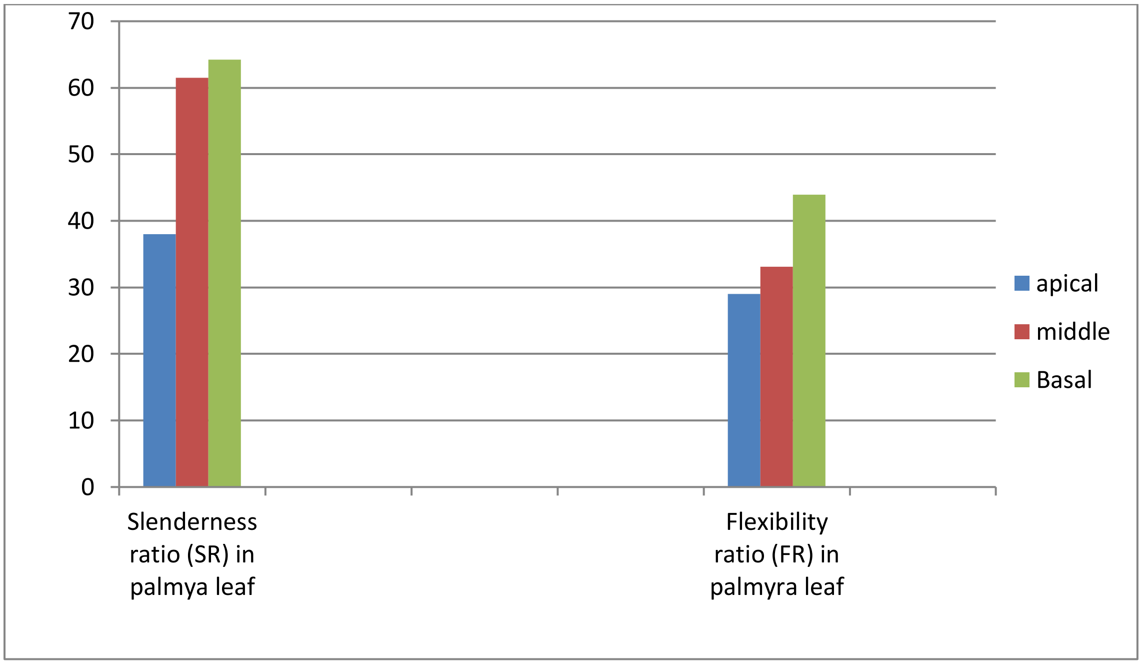
18. Cross section polarized view of sclrenchyma fibre. 19. A punch of fibre is Polarazied view. 20. Fires are separated. 21. Very thin walled fibre. 22. Thin walled fibre. 23. Thick fibre. 24. Very thin walled fibre. 25. Silica cell with fibre. 26. Sclereide fire.

Among the key strategies for structural support present within the possible range of variation in palm leaf lamina anatomy is the occurrence of sclerenchyma cells juxtaposed between the longitudinal veins and one or both surface layers. These cells, by bridging internal and superficial zones of the lamina, stiffen the lamina to an extent, possibly minimizing reliance on hydro static support by mesophyll cells. Groups of cells that bridge veins to the surface layers, frequently referred to as bundle sheath extensions, occur in many angiosperm families and characterize a functional class termed **heterobaric leaves**. These observations support the fact that the evolution of vein-bridging cells particularly in the form of fibers may have been a key innovation that allowed palms to occupy arid or cold climates. Thus, the leaf anatomical characteristics justify that Palmyra palms are well evolved species to adapt themselves to dynamic environmental factors like heavy wind coupled with dry and high temperatures. This may be a significant factor due to which the populations are dense in coastal belts rather than in lands.

Morphometric measurements of Palmyra leaf fire

Morphometric measurements	**Palmyra leaf fire**		
	apical	**middle**	**Basal**
Slenderness ratio (SR)	38.03	61.48	64.2
Flexibility ratio (FR)	29.01	33.12	43.97

Figure 4. Morph metric measurements of Palmyra leaf fire



Conclusion

The present study on leaf anatomy would be useful in comparative studies of other genera of Palmae. Thus, the findings of this investigation are unigue and shed more light on the evolutionary and adaptation features of Palmyra Palm. Thus, the leaf anatomical characteristics justify that Palmyra palms are well evolved species to adapt themselves to dynamic environmental factors like heavy wind coupled with dry and high temperatures. This may be a significant factor due to which the populations are dense in coastal belts rather than in lands.

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