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Kranz Features in Leaf Anatomy of Native Date Palm [*Phoenix sylvestris* (L.) Roxb.] Grown in Bangladesh

Md. Mahabubur Rahman[1]*, Most. Saila Samrin Nisat[1], Most. Sherin Sultana[1], Most. Dil Afrose[1], Md. Emam Hasan[1], Rubaiyat Sharmin Sultana[2]

[1]Faculty of Agriculture, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh
[2]Department of Botany, University of Rajshahi, Bangladesh

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ABSTRACT

Anatomical aspects in leaf of the native date palm [*Phoenix sylvestris* (L.) Roxb.] of Bangladesh were examined. Leaf peelings and transverse sections were stained with 1% safranin to thoroughly investigate surface and internal structures, respectively. Both adaxial and abaxial surfaces revealed various cellular structures, encompassing stomata, cork cells, prickles and distinct types of epidermal cells. Tetracytic amphistomata were noticed in *P. sylvestris* (L.) Roxb. with the number of 349/mm2. The mesophyll layer was composed of chloroplast-rich parenchyma cells aligned radially and resemble a ring-like appearance of mesophyll cells around the chlorophyllous bundle sheath. Around two mesophyll cells span the distance between vascular bundles in this species. By the above features, it was indicating that C4 plant characteristics were present in the experimented plants. The vascular tissues were surrounded by an exterior layer of bundle sheath cells and an inner mesotome sheath. In the vascular bundle, the xylem and phloem were distinctly identified. The sclerenchyma cells were observed in small and large clusters beneath the upper and lower epidermal layers. Examining the anatomical traits of the plant in this study will aid in clarifying taxonomic issues within the Arecaceae family.

Keywords: Anatomy, C4 plant, Date palm, Leaf, *Phoenix sylvestris*

***CORRESPONDENCE**
mahabuburkyotouniv@gmail.com
Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

1. INTRODUCTION

Date palms are the ancient trees on the Asian and African continents, farmed for their delicious, edible, and medicinal fruits. Evergreen date palms are members of the Arecaceae family (Angiosperms, monocotyledons), which comprises over 200 genera including well over 2,500 species. Phoenix is the sole genus in the Phoeniceae tribe of the Coryphyoideae subfamily with around 14 species native to the tropical or subtropical regions of southern Asia and Africa (Al-Alawi et al., 2017). *Phoenix sylvestris* (L.) Roxb. is one of the closely related wild species of date palm that grown for mainly jaggery production (Faruquei et al., 2020). Date palms are strongly defined by the clipped stubs of old leaf bases, pinnate leaves about 7 meters long with stiff leaflets that are gray-green in color (Hussain et al., 2021). Even though Phoenix plants are unique, their taxonomy was not well-established until recently. Several different taxonomic treatments have been developed, with some variations in species names and applications. Uhl and Dransfield (1987) noted that even with the genus's considerable ease of recognition and economic significance, the species is still poorly understood and dire need of a thorough revision. Palms are classified above the genus level based on leaf characteristics, including shape and splitting. Analyzing the leaf blade structure has been advocated as an

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approach to identifying different palm species. Plant anatomy plays a role in understanding plant evolution, taxonomy, and physiology. Such studies highlight the importance of plant anatomy in bridging the gap between extant and fossil plants, supporting the study of evolutionary developmental biology, and shedding light on the physiology and ecology of plants (Saini et al., 2023). The productivity of plants is impacted by various environmental and biological factors. The most significant aspect is photosynthesis. Understanding the photosynthetic pathway allows us to interpret a variety of essential ecological aspects. Leaves are specialized plant structures that perform photosynthesis with either one of three types of pathways, viz. C3, C4 and CAM. The anatomical leaf structures among these types are known to be quite different from one another (Esau, 1979; Fahn, 1990). Leaf anatomy provides an easily distinguished difference between C3 and C4 plants. Analyzing leaf anatomical features can enable researchers to reliably distinguish photosynthetic subtypes, providing valuable insights into their evolutionary adaptations and ecological roles. The Arecaceae family has received some attention since different photosynthetic pathways are to be found within this family. Leaf anatomy plays a crucial role in identifying plants within the Arecaceae family. In certain genera of this family, *Oenocarpus* and *Butia* species have shown that leaf anatomy, including features like isobilateral mirrored mesophyll, amphistomatic leaves, and secondary vascular bundles with sclerenchymatous sheath reinforcement, can provide valuable characteristics for species identification (Silva & Potiguara, 2008; Sant'Anna-Santos et al., 2018). Typically, this family follows the C3 photosynthetic pathway, but some species exhibit C4 characteristics or CAM adaptations, particularly in arid environments (Röser, 1994). As a result, this family provides an ideal subject for comparative analysis of leaf vascular systems in plants that differ in photosynthetic types and ecological characteristics. C3 photosynthesis only involves mesophyll cells, but C4 photosynthesis requires the cooperation of both mesophyll and bundle sheath cells inside the Kranz anatomy. The C3 plant bundle sheath cells are tiny and typically lack chloroplasts, evenly distributed throughout the mesophyll cells. After the finding of C4 photosynthesis from grass leaves, C3 photosynthesis was quickly associated with non-Kranz anatomy, and C4 photosynthesis with Kranz anatomy (Laetsch, 1968; Ueno et al., 2006). In C4 photosynthesis, ambient CO_2 is first fixed in the mesophyll cells, followed by decarboxylation and refixation in the bundle sheath cells (Hatch, 1987). In the early stage of C3 plant studies, it was reported that C3 leaves have a less dense vascular system than C4 leaves (Takeda & Fukuyama, 1971). Based on various literature, the majority of research on identifying photosynthetic types or subtypes has focused predominantly on common species like wheat, rice, maize, oats, etc. Other species with less economic importance have not drawn much attention, although some may have peculiar structural features. Proper identifications are crucial for date palm commercialization and medical applications. Such identification ambiguities can be solved using anatomical attributes. Thus, the study aimed to characterize the anatomy of *Phoenix sylvestris* (L.) Roxb. focusing on anatomical features of leaf.

2. MATERIALS AND METHODS

Naturally grown fresh, mature, healthy and diseased-free leaves of *Phoenix sylvestries* (L.) Roxb. were taken for the experiment. Leaf blades of the plant were used for the anatomical study; they

were carefully selected to ensure a representative sample. By free hand, several peeling-off of leaf blade were performed surface study. The cross-sections of the leaf blades prepared using a common stainless razor blade (commercially Feather blade) for inside inspection. Each peeling-off and section were cleaned with sodium hypochlorite, rinsed in water, dyed with 1% aqueous safranin and then mounted in 10% glycerol (Vieira et al., 2002) on a glass slide. Thin cut sections were observed under a bright field microscope fitted with a digital camera. Microphotographs were taken to study the gross leaf surface and cross-sectional anatomy of leaf blades at different magnifications. The cell anatomical characteristics of leaf blades were measured quantitatively using ImageJ software (Mathers et al., 2018).

3. RESULTS

3.1. Dermal Tissue System

The dermal tissue system of the leaf blade was primarily comprised of epidermal cells. There were two layers of epidermis, viz., an upper and another lower epidermis. Both epidermal layers were composed of compactly arranged, almost oval or rectangular, thin-walled cells and possess distinct thin layer cuticles for *Phoenix sylvestries* (L.) Roxb. (Fig. 1D). The epidermal cells, which were both long and short, were organized in parallel rows on both sides and had sinuous anticlinal walls. Both short and long longitudinal cells were observed at the intercostals and costal zones, in which cork cells persisted (Fig. 1A). The long cells were elongated having no stomata where the short longitudinal cells having stomata were lower in number. Compared to the coastal zone, the intercostal zone had fewer cell rows. Occasionally two long cells were united by the cork cells (Fig. 1A). Prickles were present in coastal zone for this species. Bulliform cells on the upper epidermis were not distinguished from epidermal cells, despite being a common characteristic of the Arecaceae family. Stomata appeared longitudinally and parallel in rows on both the abaxial and adaxial surfaces, therefore, the leaves were amphistomatic and present isobilateral character. Two dumbbell-shaped guard cells were formed at a stomata. Each guard cell was surrounded by triangular subsidiary cells that were not the prominent because of overlying prickles. In this species exhibit a tetracytic stomata structure with the guard cells were encircled by four subsidiary cells, consisting of two lateral and two polar cells. The stomata exhibited a diameter of 28.22 µm for *P. sylvestris* as noted in Table 1. The

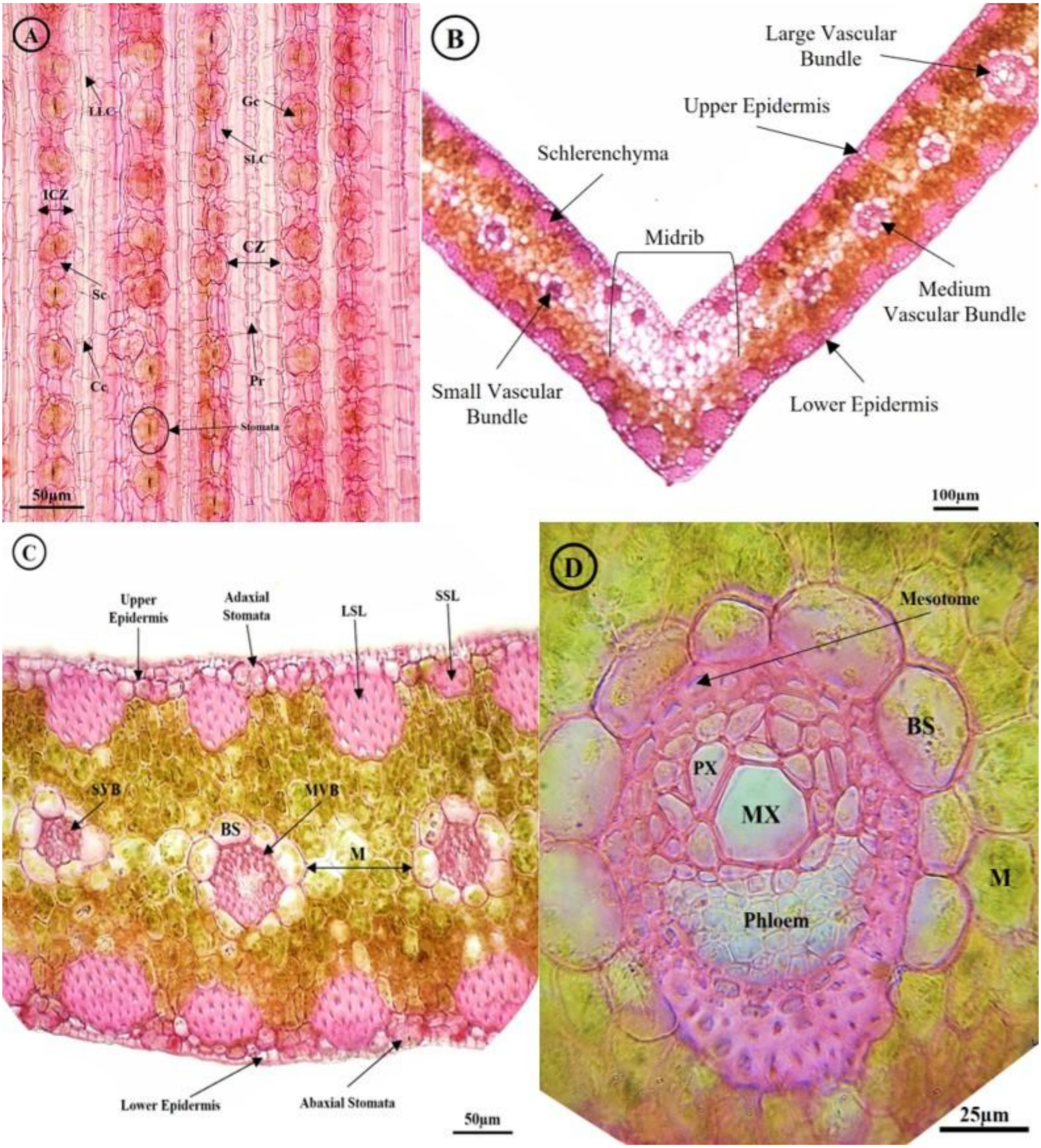

Fig. 1 Anatomy of experimented plant leaf of *Phoenix sylvestris* (L.) Roxb. (A) Surface layer of leaf blade taken from peeling, (B) Transverse section of leaf blade showing midrib, (C) A close up view of transverse section showing distance between vascular bundle in leaf blade, (D) A close up view of a large sized vascular bundle. LLC-Long Large Cell, Gc-Guard cell, ICZ-Intercostal Zone, CZ-Costal Zone, SLC-Short Large Cell, Sc-Subsidiary cell, Cc- Cork cells, Pr-Prickle, LSL- Larger Sclerenchyma, SSL- Short Sclerenchyma, SVB-Secondary Vascular Bundle, BS-Bundle Sheath, MVB-Multiseriate Vascular Bundle, M- Mesophyll, PX-Proto Xylem, MX-Meta Xylem.

number of stomata was variable when the epidermises of the two surfaces were compared. The adaxial surface had a lower stomatal density, while the abaxial surface had a higher concentration. The stomatal density per leaf area were 349/mm^2.

Table 1 Diameter of different cells *Phoenix sylvestris* (L.) Roxb

Different plant cells	Diameter in µm ($\bar{x}$±S.E.)
Stomata	28.22±0.72
Mesophyll cell	33.82±1.55
Schlerenchyma cell	14.17±0.55
Bundle sheath	29.12±1.77
Mesotome	10.44±0.58
Metaxylem	20.95±1.81
Protoxylem	11.53±1.07
Phloem	7.76±0.41

Here, $\bar{x}$ is Mean value and S.E. indicates Standard error

3.2. Ground Tissue System

The ground tissue system of the leaves displayed a well-organized mesophyll tissue. Between two epidermis layers, a mesophyll layer was present, composed of parenchyma fortified with chloroplasts although it was not differentiated into palisade and spongy parenchyma as a characteristic of monocot plant. The mesophyll cells were tightly organized, with few intercellular spaces (Fig. 1D) and sub-stomatal chambers. The average diameter of mesophyll cells was 33.82 µm (Table 1). A ring-like structure around the vascular bundle was adjoined to mesotome sheath that was notifying kranz structure of C4 plants. The number of two mesophyll cells was present between the vascular bundles. The sclerenchyma cells with small and large clusters observed beneath the both lower and upper epidermis layer. The upper side had more cells, while the lower side had larger sclerenchyma cluster (Fig. 1C). The average diameter of the sclerenchyma cells recorded 14.17 µm (Table 1). Bundle sheath cells were round or oval shape with thin cell wall and possess chloroplasts (Fig. 1B). Table 1 displayed the average diameter of bundle sheath cells that was measured 29.12 µm.

3.3. Vascular Tissue System

The vascular bundle was collateral and closed; the phloem lies towards the lower epidermis and the xylem towards the upper epidermis in leaf blades (Fig. 1D). Three different sizes of vascular bundles were observed where small bundles being least in number. The medium bundles were almost the same in number as those found between the large vascular bundles. The distribution of three vascular bundles was uneven in the leaf blade. There was no vascular bundle in the midrib (Fig. 1C). Each vascular bundle was surrounded by a double sheath consisting of an inner mesotome sheath and another outer layer of bundle sheath (Fig. 1B). Mesotome sheath cells measured 10.44 µm in diameter with thick cell walls. Xylem and phloem were found within the same radius. The xylem is composed of vessel (meta and proto xylem), fibers and xylem parenchyma elements. The large vascular bundle clearly located the meta and protoxylem. The average diameter of metaxylem and protoxylem cells were 20.95 and 11.53 µm, respectively (Table 1). The phloem portion was clearly detectable, but phloem components were not separately identified. The mean diameter of phloem cells was 7.76 µm as noted in Table 1. The midrib shape was predicted to be more or less conical towards the abaxial. The center area of the midrib featured thin-walled cells and it was meet up by the large oval-shaped cells (Fig. 1C).

4. DISCUSSION

The analysis of the leaf structure of the studied species has exhibited features consistent with the Arecaceae family. In the Arecaceae epidermis, the surface view is divided into zones or prominent bands. Typically, two primary zones can be identified: intercostal and costal zones. The epidermis of the studied species was arranged similarly, consistent with the findings of Barfod (1988), Passos and Mendonça (2006). Moreover, the costal and intercostal regions of the studied species consist of both long and short cells. As reported by Metcalfe (1956), the shape and outline of these long cells can vary, ranging from shorter elements with straight, thin walls to longer elements with sinuous, thickened walls. The characteristics of the leaf epidermal layer have a major role in the systematics and characterization of wider groupings within the palm, especially within the subfamilies and tribes. Leaves epidermal features can elucidate taxonomic issues (Davila & Clark, 1990; Cai & Wang, 1994). This study identified cork cells, prickles, and tetracytic stomata order in rows as distinctive features of the leaf epidermal layer. Cork cells were observed between two long cells in the studied plant. As stated by Scarpella and Meijar (2004), cork cells limit water loss and enhance the plant's resistance to fungal and bacterial attacks. Prickles were also perceived in the studied plant between two large cells. Pandey et al. (2018) suggested that prickles play a crucial role in both physical protection and biochemical defense mechanisms in plants. Tetracytic stomata were also identified by Müller et al. (2017) and Din et al. (2020) in date palm species. The leaves of the studied plants exhibited a tetracytic amphistomatic characteristic, with a greater number of stomata on the abaxial side. Typically, tetracytic amphistomatic leaves have a higher quantity of stomata on the epidermis of the abaxial (lower) surface to prevent photoinhibition (Haworth et. al., 2018), since the adaxial surface is more exposed to sunlight due to the majority of the leaves being in the horizontal orientation (Hussain et al., 2021).

Mesophyll cells in the studied plant were aligned radially and resemble a ring-like appearance around the bundle sheath. In this study, it was observed that around two mesophyll cells were found between two vascular bundles, indicating a relatively tight arrangement of vascular bundles within the leaf blades of the studied plants. In studied species, bundle sheath cells encircled the vascular bundle entirely with uneven cells; these cells were contained of chloroplasts. The vascular bundles were closely obtained. The bundle sheath cells were small and large in size which uniformly distributed throughout the mesophyll cells. These features proved to be C4 plant (Maai et al., 2011). C3 plant bundle sheath cells were small and uniformly distributed throughout the mesophyll cells; they typically lack chloroplasts (Gowik & Westhoff, 2011). There were three different sizes of vascular bundles in the current study: large, medium, and small. Similar findings, three types of vascular bundle had been previously reported by Artschwager (1925), Al-Thahab et al., (2019), and Shareef et al., (2021). In this species, the vascular bundle encased the xylem and phloem, which was of the conjoint collateral closed type. The phloem was positioned near the lower epidermis, while the xylem was oriented towards the upper epidermis. This was a distinctive feature of the vascular bundle in the Arecaceae family (Alvarado & Jáuregui, 2011; Chen et al., 2022). Metaxylem and protoxylem regions were only observed in the large vascular bundle, which was one of the study's noteworthy aspects. The existence of xylem elements in large vascular bundles was identified in *Phoenix dactylifera* (L.) Roxb. (Shareef et al., 2021). The vascular bundles in the studied plant were surrounded by an exterior layer of bundle sheath cells and an inner sheath of mesotome. In this study, experimented species exhibited large quantity of sclerenchyma tissue on both sides of the leaf epidermis. Sclerenchyma tissue in the leaf is a crucial anatomical characteristic and its abundance is significant for species breeding efforts. The proportion of sclerenchyma within the mesophyll tends to be higher in the drier environment (Dani & Kovács, 2014; Khelil et al., 2016). Large spherical cells with thin walls were seen in the midrib's central area and the midrib itself had a conical form that tapered toward the adaxial side. A comparable outcome was reported in Allagoptera species (Pinedo et al., 2016), date palm (Bokor et al., 2019), and in Butia species (Noblick & Sant'anna-Santos, 2021) that the midrib area features rounded cells with a conical shape directed towards the abaxial side.

5. CONCLUSION

The present study has revealed valuable anatomical insights required for the accurate identification of *Phoenix sylvestris* (L.) Roxb. Key features observed in the mesophyll cells, displayed a radial arrangement, present the ring-like pattern along with shorter distance between the vascular bundle, which suggested typical features of the C4 plant. Although physiologically the plants of genus *Phoenix* are included C3. The vascular bundle was characterized by an inner mesotome sheath, an outer layer of bundle sheath cells. These findings underscore the significance of leaf blade anatomical studies in palm systematics, particularly given the numerous unresolved questions in the field. Further research will be necessary to clarify these findings and explore additional aspects of date palm anatomy.

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