

MORPHO-ANATOMICAL STUDIES OF GREY MANGROVE (*AVICENNIA MARINA*) LEAF LOCATED IN INTER-TIDAL ZONE SANDSPIT BACKWATER, PAKISTAN.

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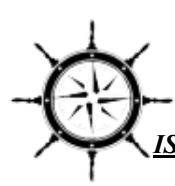
Abstract

Marine plants show a wide range of morphological and anatomical features under saline conditions. *Avicennia marina* (Forsk.) Vierh. is the dominant mangrove species and has been extensively studied, but the anatomical structures especially the electron microscopy received no considerable attention in Pakistan. The study describes the detailed leaf anatomy of the grey mangrove *Avicennia marina* in the Sandspit backwater mangrove forest of Pakistan, focusing on electron microscopy observations. The key anatomical features observed from the top to bottom surface include: i. Cuticle ii. Adaxial Epidermis iii. Hypodermis iv. Mesophyll and v. Abaxial Epidermis with derivatives. The results finds that the grey mangrove's leaf is hypostomatic holding cryptophore (sunken) stomata seem adapted for stressful condition while the presence of salt glands on both surfaces, allowing the plant to excrete excess salt. This arrangement is a significant xerophytic adaptation for survival in the high-salinity, potentially water-stressed environment. This specialized anatomy demonstrates significant adaptations (xeromorphic and halophytic) enable *A. marina* to thrive in the challenging intertidal mangrove ecosystem. It might be a possible explanation of the ecologically broad local distribution and the wide latitudinal range of this species.

Key Words: Mangrove, *Avicennia marina*, Morphology, Anatomy, Leaf, Adaptations.

Introduction

Avicenniaceae is a small but widespread and distinct mangrove family, characterized by several unique features, including: Anomalous secondary thickening (unusual growth in girth), distinctive leaf anatomy, characteristic pollen, incipient vivipary and seedling morphology. These characteristics are notably found in the genera like *Aegiceras*, *Aegialitis* and *Avicennia*, all of which exhibit an intermediate form of vivipary known as crypto-viviparous germination. The genus *Avicennia* is an exclusively pan tropic mangrove genera, generally considered pioneers of mangrove forests consists of eight species worldwide including: five in the Indo-West Pacific and three others in the Atlantic East Pacific. They can be found in a wide array of environments within tropical and subtropical sheltered tidal areas, thriving despite varying salinity levels. In the tropics, they frequently share their habitat with other specific types of mangroves, such as *Rhizophora*. In cooler subtropical and temperate zones, species of *Avicennia* are often the primary tree or shrub in the area. A particularly resilient, cold-tolerant variant is found as far south as 38° 45' S in southern Australia, distinguishing this as the most widespread among all mangrove genera. In Southwestern Asia, gray mangroves grow in discrete associations along the coasts of the Arabian Gulf as well as



the eastern and western shores of the Red Sea (Marin *et al.*, 2010). Globally, climatic, edaphic, physiographic, and biotic factors influence the distribution and biodiversity of mangroves and their associated communities (De-Santiago *et al.*, 2013). Mangrove growth varies by location, with tall, dense forests found in high-rainfall tropical areas and smaller, shrub-like forms in arid regions like the Arabian Peninsula due to factors such as low rainfall and high salinity (Naidoo, 2006; Sheppard *et al.*, 2010). Their sizes can vary from small shrubs and dwarf trees up to 30 meters high trees (Peter and Sivasothi, 1999). These variations are adaptations to different environmental conditions, as are the common morphological and anatomical specializations found globally in mangroves that allow them to thrive in harsh coastal habitats (Tomlinson, 1986 & Saenger, 2002). Understanding these adaptations is crucial for predicting how mangroves will perform in different environments (Ward *et al.*, 2016, Nazim *et al.*, 2018).

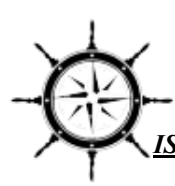
Avicennia marina is the dominant species and has been extensively studied in Pakistan, but the structure especially the electron microscopy received no considerable attention. Though, these features are necessary to understand the functional processes of a mangrove tree (Nazim *et al.*, 2015 & Khan *et al.*, 2021) these plants inhabiting marine environments have adapted features which help them to thrive under adverse conditions (Tomlinson, 1986). Anatomy of leaf show xeric adaptation for conservation of water (Naskar and Rabindranath, 1999) the structures typically show physiological and morphological adaptations to survive in an anaerobic, waterlogged and saline conditions. *Avicennia spp.* has salt glands that exclude the salt (Borkar *et al.*, 2009), water storage tissues which plays an important role in conservation of water (Nair and Govindakutty, 1972). These features are often displayed in the morphology and anatomy of plant, as the most obvious adaptations initiate throughout the plant body (Dickson, 2000). The microscopic anatomy of the leaf of *A. marina* seems very consistent and has been described by numerous authors (Areschoug, 1902; Song, 1960; Rao and Sharma, 1968; Rao, 1971; Fahn and Shimony, 1977; Saenger, 1982; Clough, 1984 a,b; Chen, 1984; Chiang, 1984; Hsiao and Chen, 1988; Roth, 1992; Das and Ghose, 1993, 1996; Das, 1999; Naskar and Mandal, 1999; Sheue *et al.*, 2000; Sasomsapatawee *et al.*, 2017).

The present study aims to describe the leaf morphometry, salient features of internal structure and surface micro-morphological adaptive characteristics of *A. marina* under Scanning Electron Microscope (SEM) in order to understand the adaptations and the global distribution of this species.

Materials and Methods

Study site: The inter-tidal zone of the Sandspit backwater mangrove forest was selected for the present study; the site is dominated by monospecific stands of *A. marina* with some other halophytes and some non-halophytes on higher ground. As this site is near to Karachi so suffers from anthropogenic activities (pollution, grazing, cutting or logging) which stresses this mangrove environment (Nazim *et al.*, 2015).

Sampling: Six month's healthy leaves were randomly collected from the tip of the 35 different trees for morpho-anatomical findings (Fig. 1). The morphological study based on; leaf form, leaf size, leaf arrangement, leaf margin and leaf surface. The samples were preserved in 4% formalin solution and fixed in glutaraldehyde (2%), prepared in cacodylate buffer pH 7.0. The plant material



was dehydrated in acetone 50, 70, 90 and 100% (2x). Then the samples were covered with gold (Au) in a Sputter Coater Balzers SCD 050. The observations were made in a Scanning Electron Microscope JEOL – 840 in Centralized Laboratory, University of Karachi.



Figure 1. The opposite arrangements of *A. marina* leaves on plant.

Results and Discussion

Morphological Characteristics: The leaf is thick, small and arranged in opposite pairs with obscure smooth leaf veins (Ghafoor, 1984) on a short petiole. The petiole's length ranges from 12 mm to 15 mm with an average of 14.64 ± 1.39 while the leaf length is between 35 mm and 100.7 mm averaging of 72.45 ± 5.67 mm and the leaf width is between 15 mm and 32 mm, thru an average of 24.56 ± 4.73 mm with an average leaf area 226.50 ± 13.1 mm² (Table 1). Leaf thickening occurs when plants are exposed to high light intensity in a habitat (Tobing *et al.*, 2022). The adaxial (upper) surface of the leaf is typically shiny dark green and glabrous (hairless) or has an obscure powdery coating while the abaxial (lower) surface has a distinct grey-to-white powdery appearance. The leaf morphology shows halophytic characteristics with xeromorphic adaptations, designed to conserve water and manage high salt concentrations. These include: reduced leaf size, leathery appearance, thickness, succulent form, (Fig. 2 A&B).

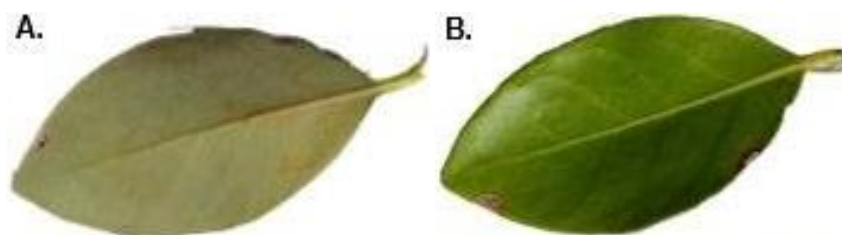


Figure 2. showing A. abaxial surface, B. adaxial surface of *A. marina* leaf.

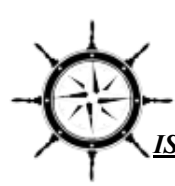


Table 1. Morphological characteristics of leaf.

Structures	Measurements
Leaf Form	Elliptic, Oblong, Ovate
Leaf Arrangement	In Opposite Pairs
Leaf Margin	Smooth
Leaf Surface	Hairs, Glands
Petiole Length	14.64 ± 1.39 mm (12 mm-15 mm)
Leaf Length	72.45 ± 5.67 mm (35 mm - 100.7 mm)
Leaf Width	24.56 ± 4.73 mm (15 mm -32 mm)
Leaf Area	(226.5±13.1mm ²)

Anatomical Characteristics: The cross-section of a leaf under scanning electron microscope reveals several distinct layers (from the top surface down) ; 1. Cuticle, 2. Adaxial epidermis (featuring epidermal derivatives glandular trichomes/salt glands), 3. Hypodermis tissue, 4. Mesophyll tissue (which is differentiated into upper palisade parenchyma and lower spongy parenchyma tissue & several vascular bundles each containing both xylem and phloem and 5. Abaxial epidermis (containing epidermal derivatives glandular & non-glandular trichomes and stomata) (Fig. 3). Surya and Hari (2017a) also found that in *A. marina* leaf the fundamental tissue arrangement is consistent, further study is necessary to fully grasp the variations in adaptation.

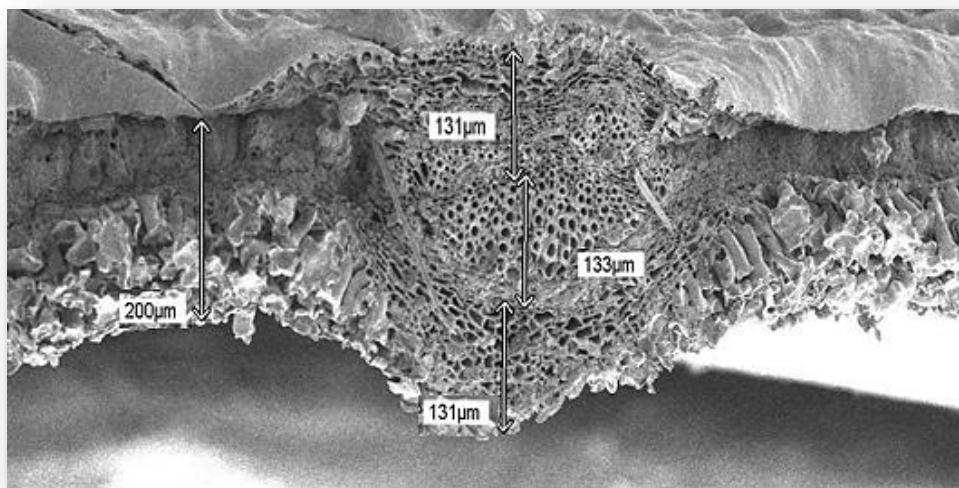
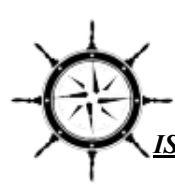


Figure 3. The section of leaf showing cuticle (a), upper epidermis (b), salt glands (c), Hypodermis tissue (D), xylem (E), Phloem (F), parenchymal palisade tissue (G), spongy tissue parenchyma (H), lower epidermis (I), and no glandular trichomes (J), stomata (K).

1. **Cuticle:** A thick waxy cuticle is clearly present on both the upper and lower epidermis including the surface of the trichomes, a common feature in xerophytic and halophytic plants to minimize water loss (Li *et al.*, 2018). Relative to plants with thin cuticle layers, those possessing anatomically thicker cuticles are more resistant leaves (Yeats and Rose, 2013).



2. **Adaxial Layer:** Fig. 3 showing the outermost uniseriate layer of the leaf containing parenchyma cells. The thickness of the layer is approximately 200 μm while length ranges between 100.6 μm and 133.0 μm (Table 2). The scattered sunken glandular trichomes (specialized multicellular salt glands) are observed within crypts.

Glandular Trichomes (Salt Glands): Fig. 4a screening the presence of glandular trichomes, the derivatives of upper surface of the leaf at 500 X magnification. The cells of upper epidermal layer are typically compact, and the trichomes extend outward from the surface. Fig. 4b showing a single glandular trichome (salt gland) with measured area of the gland is 1500.6 μm , lies within cavities in the upper epidermal layer of the leaf Khan *et al.*, (2021) which are specialized hairs found on plants. These glands help the leaf to actively secrete salt and allowing the plant to maintain a stable internal salt concentration (Naidoo, 2006). These multicellular structures found on both upper and lower epidermal layers as described by Suriya and Hari, (2017a, 2017b, & 2017c) consisting several types of cells: Basal collecting cells, Stalk cells and Secretory cells (Naidoo, 2025). The thick cuticle and compact cells of the upper epidermis along with the trichomes help *A. marina* to conserve water and limit the excessive transpiration (Suriya and Hari, 2017a).

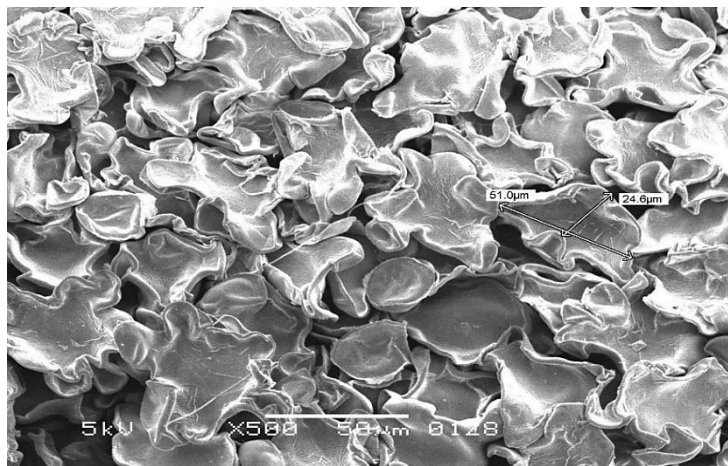
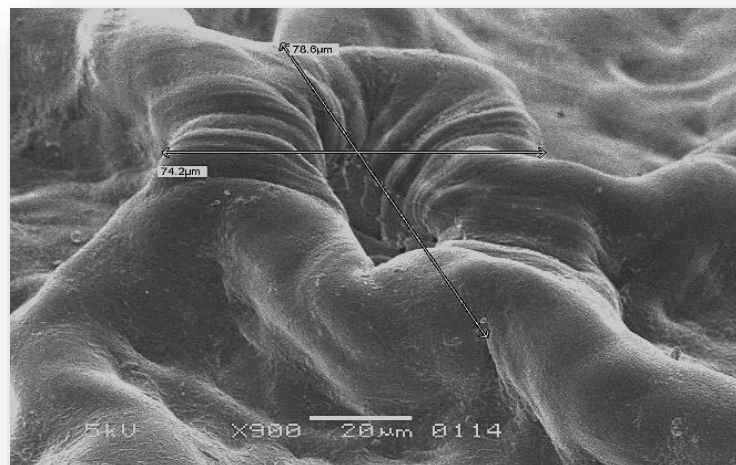
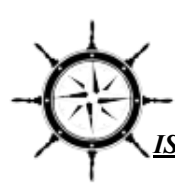


Figure 4a. Salt glands on the upper surface of leaf with measurements

Figure 4b. Showing salt glands with measurements.





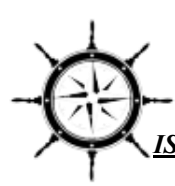
3. **Hypodermis:** A multilayered tissues (4 or 5 layers) thin-walled parenchymatous cells can easily be seen beneath the adaxial epidermis (Fig. 3). Though this can vary depending on the specific environmental conditions, particularly salinity and light intensity (Fitzner, *et al.*, 2023). The cells are isodiametric (circular/oval) in shape and highly vacuolated, their primary function is water storage and conservation (Sasomsaptawee *et al.*, 2017). This is a key xerophytic adaptation, helping the plant cope with the physiologically dry conditions of a mangrove habitat where fresh water is scarce and high salinity makes water uptake difficult. Hypodermis also provides a thicker barrier against mechanical injury and excessive water loss through transpiration, increasing boundary-layer resistance to water vapor diffusion (Tobing *et al.*, 2022).

Table 2. The measurement of the parts of leaf under Scanning Electron Microscope

Parts	Length (µm)	Width (µm)
Upper Epidermis	100.6 – 133.0	-
Palisade Parenchyma	94.7- 126.0	-
Spongy Parenchyma	73.5 – 80.0	-
Vascular Bundle	196 – 221.0	298.6 – 391
Xylem Tissues	9.30 – 12.0	0.6 - 1.2
Phloem Tissues	127.4 – 134.0	-
Salt Glands	49.8 – 79.8	19.1 - 74.2
Stomata	30.9 – 41.0	41 – 42.2
Lower Epidermis	128.3- 155.0	-

4. **Mesophyll Network:** Beneath the hypodermis there is a distinct layer of mesophyll network, measuring around 126 µm, shows a more cellular structure with air spaces, where photosynthesis occurs. Fig. 2 clearly showing that the mesophyll contains two types of layers:

- i. **Palisade Mesophyll:** The layer is beneath the hypodermis showing elongated thick cells tightly packed together. Table 2 showing the length measurement of this layer is between 94.7 µm and 126.0 µm with 155µm thickness. The 2-3 layers of palisade tissue are the primary photosynthetic tissues, the elongated and columnar cells arranged perpendicular to the leaf surface maximizing light absorption (Fig. 3). The palisade parenchyma cells are efficient photosynthetic cells (Corrêa *et al.*, 2017), this is a form of anatomical adaptation of *A. marina* to ensure the plant can produce enough energy to survive in this challenging habitat.
- ii. **Spongy Mesophyll:** In Fig. 3 spongy mesophyll layer is seen below the palisade layer, the measurements "110µm" and "117µm" indicate the depth of this layer while the length ranges between 73.5 µm and 80.0 µm (Table 2). The well- developed spongy mesophyll consisting irregularly shaped parenchyma cells with fewer chloroplasts than



the palisade cells and many intercellular air spaces. According to Starzecki, (2015) a well-developed spongy parenchyma is a sign of efficient photosynthesis, due to its superior ability to manage carbon dioxide, move nutrients, and access water and light.

iii. **Vascular Bundle:** A vascular bundle, contains the xylem and phloem, is visible within the mesophyll surrounded by a sclerenchymatous sheath. The length ranges from 196 μm to 221.0 μm and width from 298.6 μm to 391 μm (Table 2).

a. **Xylem:** Fig. 5a highlights the xylem network, composed of dead, hollow, elongated cells include: vessels, tracheids, xylem parenchyma and xylem fibers provide the support and transport water and minerals to the plant. The xylem cells are greater in number, located towards the center of the vascular bundles near the upper (adaxial) side. The anatomical adaptation mechanism of *A. marina* under high water potential conditions leads to an increase in the number of xylem cells and a decrease in their individual size (Tobing *et al.*, 2022). The xylem of *A. marina* possesses hydraulic properties, highly resistant to embolism (the blockage of vessels by air bubbles), which is a key adaptation for managing water stress in its saline environment. Jiang *et al.*, (2021) stated that mangrove species with a wide latitudinal distribution provide a good opportunity to test their adaptive xylem variation over latitudes. The overall length and width of the xylem vessels are recorded as 221 μm and 391 μm respectively while the length of xylem cells (from protoxylem to metaxylem) is measured as 108 μm and 125 μm (Fig. 5a & 5b). The length of the vessel cells (narrow and pitted) of xylem ranges between 9.30 μm and 12.0 μm while width is between 0.6 μm and 1.2 μm in dimensions at 50 μm with 500 X magnification (Table 2 & Fig. 5b).

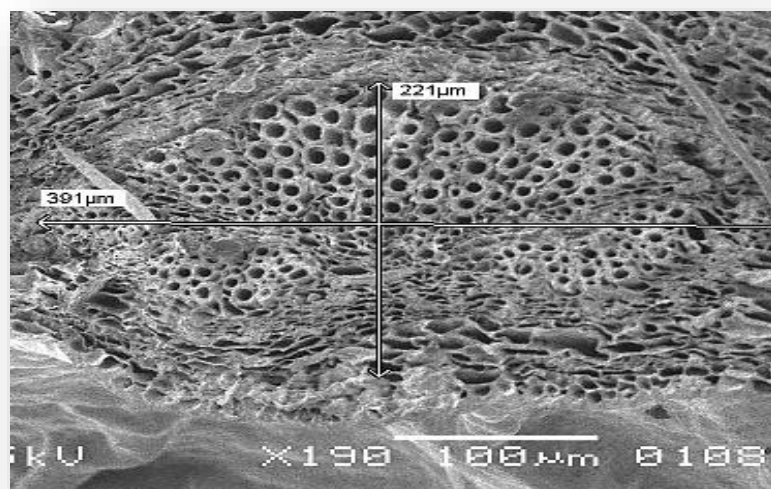


Figure 5a. Showing the measurements of the xylem tissues.

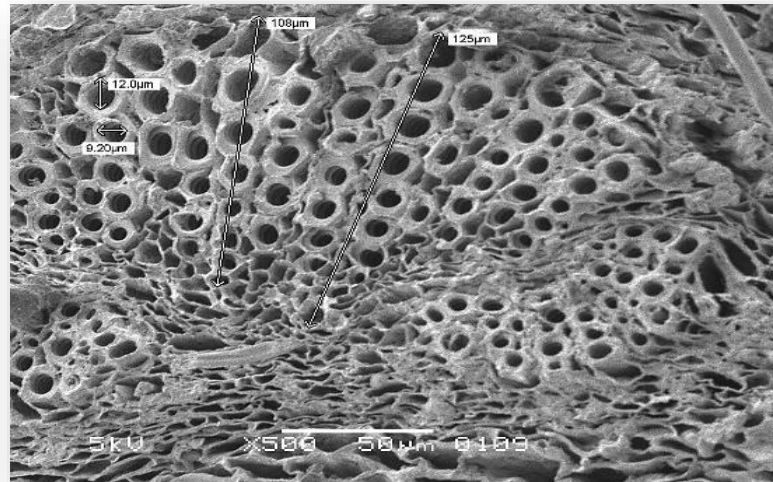
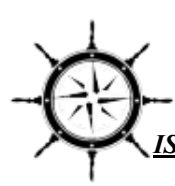


Figure 5b. Showing the measurements of xylem vessels (from protoxylem to meta xylem).

- b. Phloem:** Figures 6a & 6b show a SEM micrograph of phloem tissue at 500 X (50 μm), the living tissues responsible for transporting sugars and other nutrients from the leaves to other parts of the plant. The length of the phloem ranges between 127.4 μm and 134.0 μm while individual cells appear as 12.04 μm, 11.66 μm and so on at 700 X (10 μm). The large, interconnected sieve elements provide efficient pathways for nutrient movement, while the companion cells and parenchyma cells contribute to the tissue's metabolic activity and structural integrity. The phloem cells, along with the xylem, form the vascular bundles. They are typically located on the outer side of the vascular bundle. The phloem is composed of living cells, which appear as smaller, more irregularly shaped cells including sieve tubes, companion cells, phloem parenchyma, and phloem fibers (bast fibers) (Surya and Hari, 2017c). These are typically located towards the lower (abaxial) side of the leaf's vascular bundles.

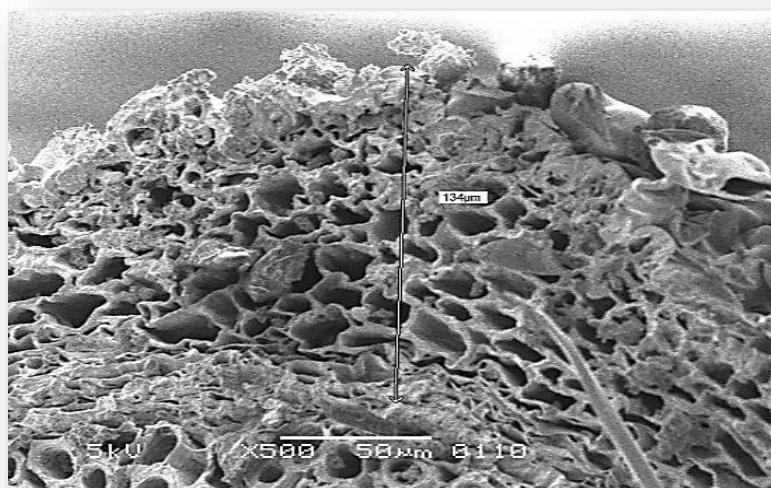


Figure 6a. Showing the measurement of phloem tissues

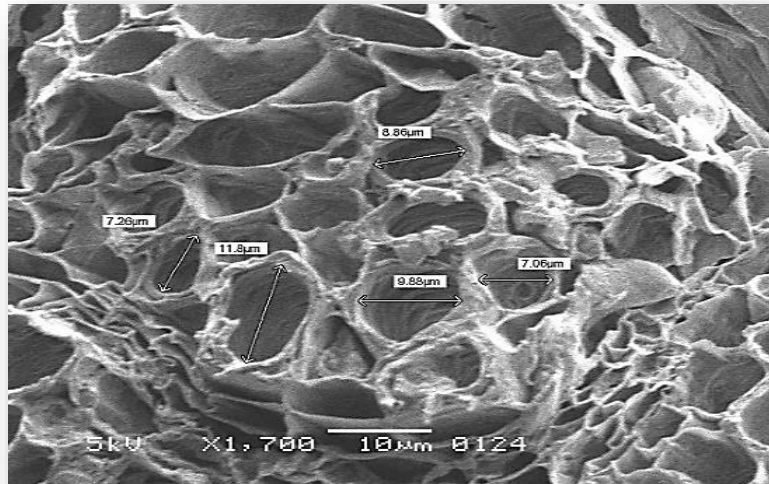
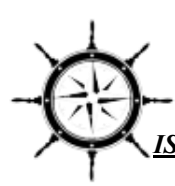


Figure 6 b. Showing the measurement of phloem sieves.

5. **Abaxial Layer:** The lower epidermis or the innermost layer of *A. marina* can be seen on magnification 200 μm approximately 80 μm thick, appears denser and more compact at the lower surface of the leaf having a length between 128.3 μm and 155.0 μm (Fig. 2 & Table 2). The image showing that the lower epidermis consists of stomata and, on its outer side there are both types of trichomes; the glandular (salt glands) and non-glandular. The trichomes seems to be adapted for high salinity to accumulates and excludes salt to outside. Both types of trichomes have found in the lower epidermis similar to those reported by Triperm, (1977; Fahn and Shimony, 1977). The abaxial surface is generally characterized by a dense covering of non-glandular hairs and the presence of stomata, which are typically absent on the adaxial surface (Nakata & Odata., 2013). The micrograph shows the intricate folded structure of the lower epidermis of leaf with numerous visible stomata pores. The image is magnified 180 times, with a scale bar indicating that the length of the bar is 100 micrometers (Fig. 7a).

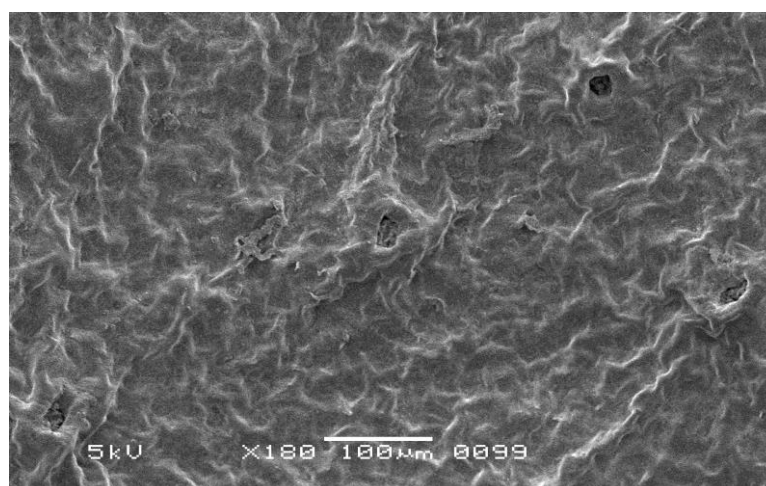
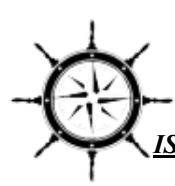


Figure 7a showing the stomatal pores at the lower epidermis



Stomata: The Fig. 7b shows cryptophore (sunken) stomata primarily or exclusively found on the lower (abaxial) surface. This characteristic confined that the leaves are hypostomatic. This adaptation helps to reduce water loss through transpiration, as the lower surface is less exposed to direct sunlight and air currents by creating a humid microenvironment around the stomatal pore (Khan *et al.*, 2020). Their function is critical for water conservation and gas exchange.

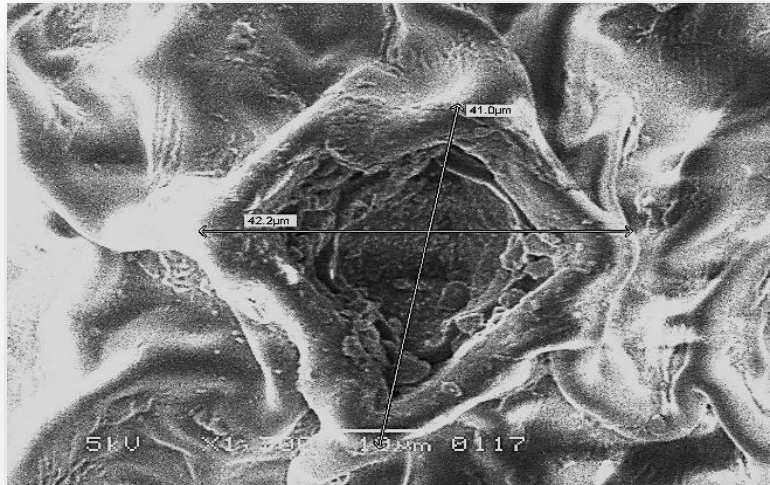


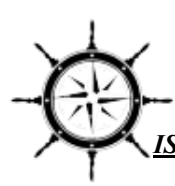
Figure 7b. Showing the stomata in leaf.

Conclusion

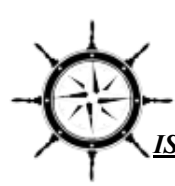
The leaf anatomy, characterized by a thick epidermis, a well-developed thick palisade layer, multi-layered hypodermis for water storage, the sunken stomata along with other features like thick cuticles and salt glands, are key adaptations that allow *A. marina* to thrive in high-salinity coastal habitats and potentially arid conditions. It has been observed that glandular trichomes are specialized mini-organs/salt glands for active salt excretion, a key adaptation to the saline mangrove habitat, while non-glandular trichomes provide a physical, protective layer and help with water conservation. These features collectively could be a possible explanation for the species' broad local distribution and wide latitudinal range. However, more detailed studies are required for a complete understanding of its global distribution and adaptive behaviors.

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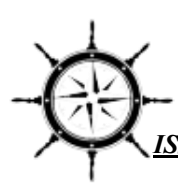
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