



STUDIES ON ECOLOGICAL ANATOMY OF THE MANGROVE FERN *ACROSTICHUM AUREUM* L.

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ABSTRACT: *Acrostichum aureum* L. is the only fern in the mangrove ecosystem of the Indian Coast. Anatomical adaptations of the fern in relation to its habitat has been studied. Epidermal cells on both surfaces of pinnae are sinuous walled with thick cuticle. Adaxial epidermal cells exhibited a greater density than abaxial surfaces. Stomata are anomocytic and hypostomatic, mesophyll with two layers of elongated closely set palisade cells followed by loosely-arranged spongy cells with plentiful lacunae. Leaf margin has lignified cells. Endodermis of the vascular bundles have casparian strips; sclerenchymatous bundle sheath is also present. Outer root cortex is aerenchymatous and inner cortex is sclerenchymatous. Rhizome sections revealed presence of starch granules. Sporophyll shows sporangia spread densely over the entire lower surface except over midrib, forming a reddish brown mat and are mixed with many peltate orange brown paraphyses. Anatomical results were analyzed considering the interaction of this fern with abiotic factors. The plant has hydrophytic and xerophytic anatomical adaptations.

Key words: *Acrostichum aureum*, Mangrove, Ecological anatomy, Anomocytic stomata, Peltate paraphyses.

INTRODUCTION

A plant's adaptation to its environment is one of the most important issues in evolutionary biology [1]. In order to survive in different environments, the morphological traits of widely distributed plant species often vary significantly. Mangroves are the good example for the study of salinity tolerance adaptations. They develop diverse mechanisms associated with anatomic or physiological characteristics to regulate salt absorption and exclusion, such as ultra filtration, salt-secretion [1]. Biochemical, physiological and morphological adaptive mechanisms are found in plants that experience water shortage [2]. Several structural characteristics may contribute to decrease in the rate of water loss, such as reduction of leaf surface, increased thickness of epidermal cell walls, increased thickness of cuticle, and stomata located on the abaxial surface [3, 4]. These characteristics in mangroves make them ideal ecological models of adaptation to salinity tolerance. Anatomical features of different plant organs are considered an adaptive response to habitat ecology of a species [5]. *Acrostichum* is the only pteridophyte genus found in the mangrove ecosystem. It grows luxuriantly in brackish water habitats also. Three species of *Acrostichum* which are reported from mangrove habitat viz. *Acrostichum speciosum*, Willd. an obligatory halophyte [6]. *Acrostichum danaeifolium* Langsd & Fisch. is the least salt tolerant species of genus the *Acrostichum* [7] and *Acrostichum aureum* L. is the only species from the Indian coast. This rhizomatous fern shows clumped distribution and produces golden yellow leathery fronds and hence aptly called golden leather fern. Powdered rhizome paste is used in wounds treatment and fronds are used as antimicrobial agent [8]. The present work is aimed to elucidate the anatomical and epidermal features particularly helpful to adapt to the mangrove ecosystem.

MATERIALS AND METHODS

Fully expanded pinnae, roots and rachis were collected. Epidermal preparations were made by peeling out the epidermis. Anatomy of petiole, root and pinnae was studied in serial microtome sections. For microtome, small portions of fern parts were fixed in Carnoy's fixative.

These were later dehydrated with ethanol and butanol series. Infiltration and embedding of the material was done in paraffin wax (Merck). Transverse sections were cut at 10 -15 μm thickness using microtome (Leica RM 2145). Staining was carried out using PAS and 2% saffranin stain and mounted in D.P.X. Morphometric studies were conducted using Leica Q Win software. Various histochemical tests were performed to ascertain the nature and depositions and cell substances. Lignin and cutin were tested by obtaining red colour with Phloroglucinol and Sudan IV. Presence of starch grains in rhizome was detected by staining fresh section with Lugols iodine solution [9]. For SEM, mature sporophylls were taken, fixed in Carnoys solution, dehydrated with alcohol series and kept in desiccators for further dehydration. The samples were then mounted on stubs, sputter-coated with gold and subjected to scanning electron microscope (JEOL). For fluorescence study, fresh hand sections were viewed with fluorescence microscope (Nikon Eclipse 50I).

RESULTS

Adaxial surface of the leaf is more dark green in color than abaxial. Epidermal cells on both surfaces of pinnae are sinuous walled (Fig. 1- A) and cutinized. Epidermal cells are elongated near veins and midrib portion. Stomata are hypostomatic. Guard cells of the stomata are entirely surrounded by a single epidermal cell. Guard cells are bean shaped. Stomatal complex is anomocytic. The adaxial epidermal cells were smaller than abaxial epidermal cells. Abaxial cell breadth was greater than that of epidermal cells of adaxial surfaces. Consequently, adaxial epidermal cells exhibited a greater density than abaxial surfaces (Table 1). Epidermal cells of petiole are hexagonal in shape (Fig.1-B). In the petiolar portion, stomata are few and are irregularly distributed. Anatomically, the leaf is distinctly bifacial, the upper half being characterized by a hypoderm of collenchymatous tissue, and beneath this, two layers of elongated, closely set, palisade cells followed by loosely-arranged spongy mesophyll cells with plentiful lacunae. Mucilaginous cells are also found in mesophyll. (Fig. 1-F). The lower side of the mesophyll is frequently interrupted by the stomatal air chambers. Midrib portion has 2-3 layers of sclerenchymatous hypodermis. Vascular bundles of midrib region are hippocampus. Xylem lies in the centre of the vascular strand. It is plate like with several protoxylem points in exarch condition. Xylem is surrounded by phloem. Single layers of endodermis and pericycle are also present. Endodermis shows casparian thickenings. Leaf show 2-3 layer of lignified cells in the marginal region (Fig.2-G). Thick cuticle is present on both epidermis. Histochemical test revealed the presence of lignin and cutin.

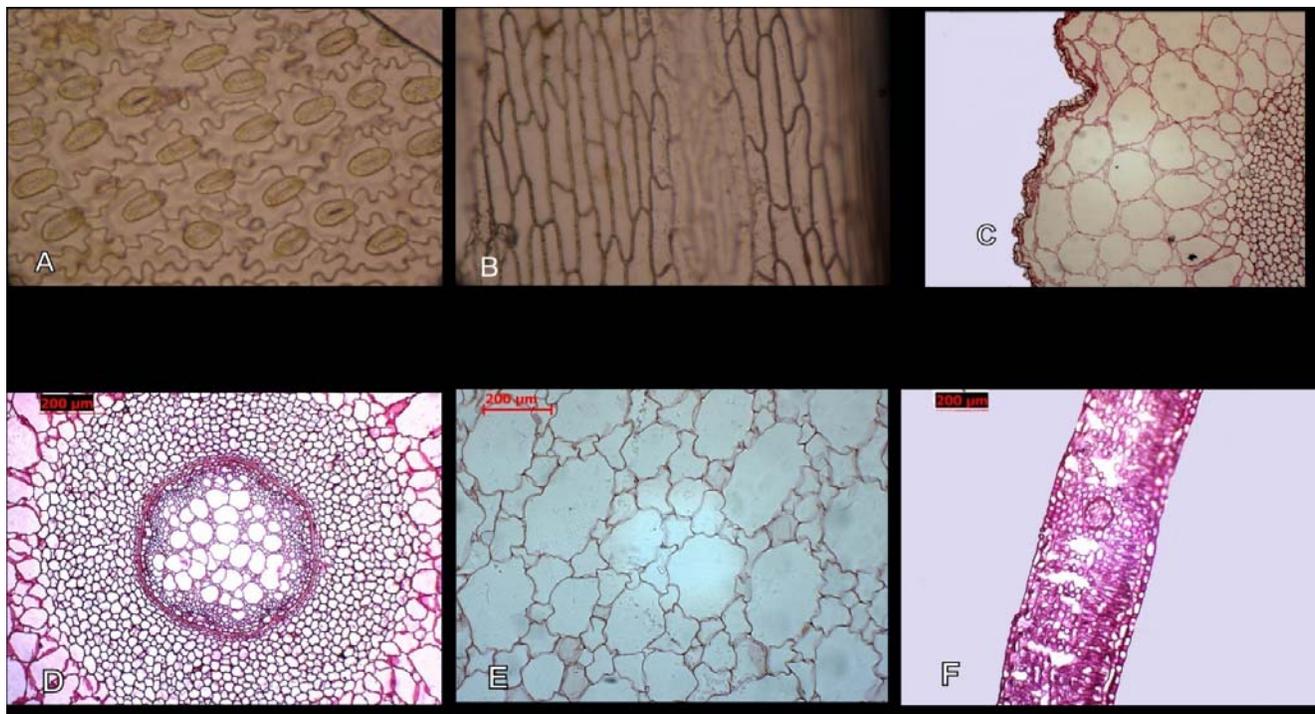


Fig.1 *Acrostichum aureum* L. (A). Leaf epidermis of with sinuous wall and stomata. (B) Epidermis of petiole. (C) T.S of root with aerenchymatous cortex. (D) Root cross section showing sclerenchymatous inner cortex surrounding the stele. (E) Petiole cortex with air spaces. (F) Leaf transverse section with palisade and spongy mesophyll.

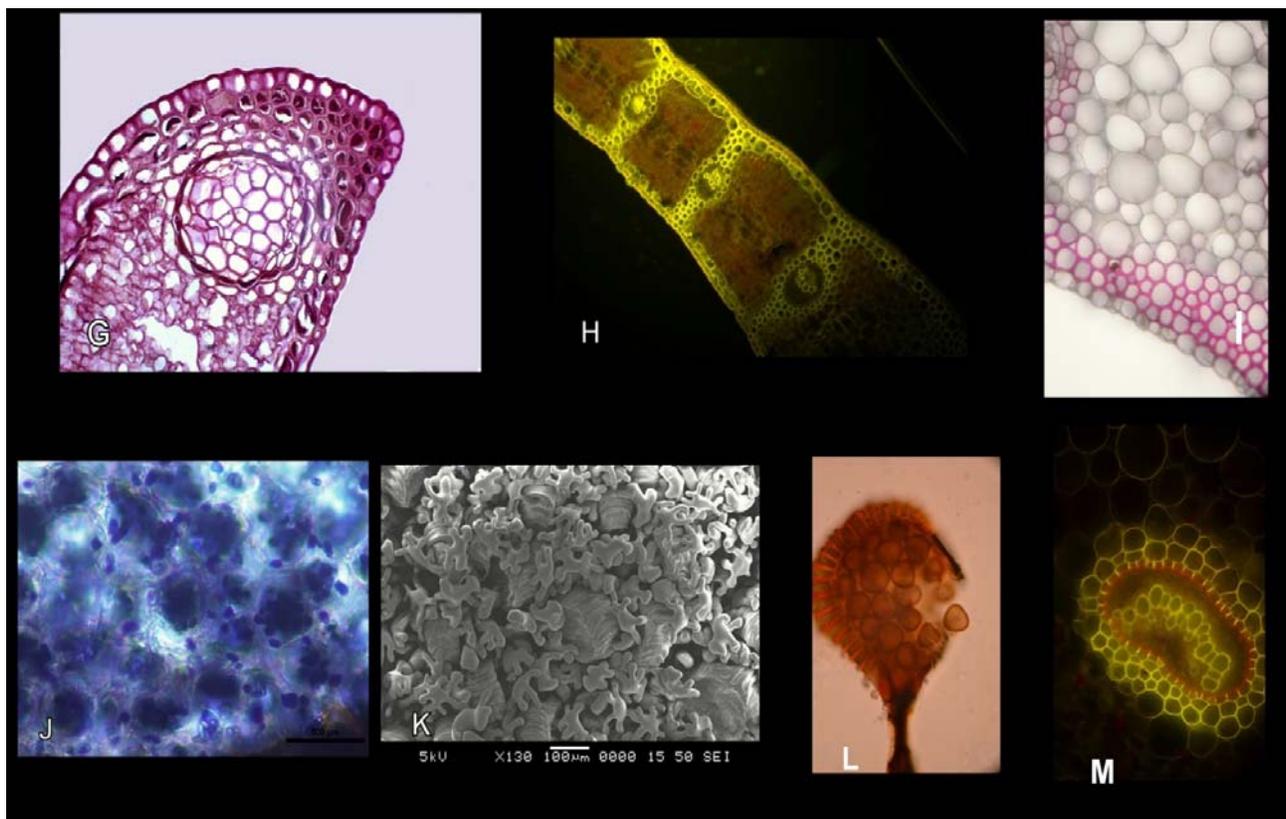


Fig.2 *Acrostichum aureum* L. (G) Leaf margin showing cells with lignified walls. (H) Bundle sheath extension in the laminar region. (I) Lignified hypodermis in the midrib. (J) Rhizome cross section showing starch granules. (K) Scanning electron micrographs of sporophyll showing peltate paraphyses and sporangia. (L) Sporangia with spores. (M) Fluorescence micrograph of vascular bundle showing endodermis with casparian thickenings.

**Table 1: Morphometric analysis of surface features of *Acrostichum aureum* L.
Epidermis characteristics**

Length of adaxial epidermal cells (μm)	108.6 ± 4.71
Breadth of adaxial epidermal cells (μm)	76.8 ± 4.52
Number of adaxial epidermal cells (mm^{-2})	103 ± 15.7
Length of abaxial epidermal cells (μm)	118 ± 8.16
Breadth of abaxial epidermal cells (μm)	115.8 ± 5.75
Number of abaxial epidermal cells (mm^{-2})	58 ± 12.5
Length of petiolar epidermis (μm)	250 ± 7.54
Breadth of petiolar epidermis (μm)	37.2 ± 3.85
Stomata	
Length of the guard cells (μm)	73.6 ± 2.61
Width of the guard cells (μm)	37.33 ± 3.99
Length of stomata (μm)	62.93 ± 1.50
Width of stomata (μm)	6.36 ± 0.047
Number of stomates (mm^{-2})	30 ± 5.6
Stomatal index	49.2 ± 3.4
Stomatal complex	Anomocytic

\pm Standard deviation

The rachis has a groove towards the inner side. Single layered cutinized epidermis is followed by 4 - 6 layers of sclerenchymatous hypodermis, middle cortex is made up of aerenchyma tissue (Fig.1- E). Stele is dictyostelic with several meristemes. Number of vascular bundles varies from younger to matured petiole. Endodermis shows casparian thickening, pericycle is followed by 2-3 layers of sclerenchymatous bundle sheath (Fig.2-M). Metaxylem is arranged towards the centre and protoxylem arms is exarch, xylem is surrounded by phloem tissue. Root section is circular in outline; exodermis is made up of 2 layers of lignified cells. Ground tissue is aerenchymatous, with large air spaces (Fig.1- C). The cells lining the lacunar chambers are very irregular in shape, and connect up with one another by numerous short arms with large air spaces followed by inner 5-7 layers of sclerenchyma tissue surrounding the stelar region (Fig.1-D). Xylem and phloem are arranged in alternate manner followed by a layer of pericycle and endodermis. Pith is parenchymatous with large intercellular spaces. Rhizome sections revealed presence of starch granules in the cortex. Leaf traces are present which are surrounded by sclerenchymatous tissue. Scanning electron microscopy of mature sporophyll shows sporangia spread densely over the entire lower surface except over midrib, forming a reddish brown mat over the surface and mixed with many peltate, orange brown paraphyses (Fig.2-L), having a slender thin walled, 2 – 3 celled long stalk and a lobed terminal cell. Sporangia are globose with a stalk and an annulus of 20-22 cells. Paraphyses are arranged in an interlocking manner.

DISCUSSION

The species shows both xeromorphic and hydrophytic characteristics. Pteridophytes are outstanding in their capacity to occupy marginal environments under extreme conditions [10]. *Acrostichum aureum* shows anatomical peculiarities of leaf, rachis, root, rhizome and sporophyll. Leaves with hypostomatic condition and sinuous anticlinal walls of epidermis deserves attention. Smith *et al.*, [11] suggested that bicolor laminar leaves containing stomata only in the abaxial epidermis may have evolved as a result of selective pressure to enhance light capture, while avoiding the detrimental effect of exposure to sunlight and minimizing water- loss due to transpiration. According to Watson [12] development of stress during the differentiation of the leaf is linked to sinuous anticlinal wall pattern in epidermal cells. Sinuous walls enable the epidermal cells to harvest maximum light striking upon the surface [13]. According to Haberlandt [14] presence of sinuous epidermal walls increases contact between adjacent cells, and may help to maintain leaf structure under mechanical stress and to substrate limitations causing leaf blades to roll up when water is scarce. Guard cells of the stomata are entirely surrounded by a single epidermal cell. This condition, described as “floating stomata” or adetostomy [15], is common in genus *Anemia* and *Pyrrosia* [16]. Leaves are exposed to varying environmental conditions more than any other plant organs, and the variation in their characters have been interpreted as adaptations to specific environments [17, 18]. The variations in the morphological and anatomical features of leaves in relations to the amount of sun exposure, the degree of salinity, or water availability have been reported for many species [19, 20 & 21]. Plants growing in habitats with contrasting environmental conditions of water availability may show distinct mesophyll architecture. Leaves of xeromorphic species tend to be more compact [22]. Salinity of the water and soil has a direct influence on the leaf architecture of the plants [23]. Mesophyll with plentiful lacunae is known to provide a large total surface area between the cells and the internal air [24]. Endodermal cells girdled by Casparian strips around the vascular bundles of the petiole and leaf blade is a common feature of ferns [25]. Higher sclerenchyma density and mucilage storage tissue is found in the mesophyll of this fern. According to Dickinson [4] this type of tissue distribution results in improved mechanical conditions and reduced tissue damage. According to Nikolopoulos *et al.*, [26]; Terashima,[27]; Morison *et al.*,[28] presence of bundle sheath extension in the laminar region helps to distribute light through thicker leaves, enhancing photosynthesis, and they may limit lateral CO₂ diffusion within the leaf. Bundle sheath extensions are more common in xeric, high-light species and upper canopy trees [29].

Thicker cuticle layers is an adaptation characteristic of plants growing in extremely dry habitat [30, 4]. Cuticular layer forms an ultimate barrier between the plant and atmosphere. According to Das [23], in mangroves, the thick cuticle plays an active role in restricting nonstomatal water loss. Presence of lignin and cutin was confirmed in histochemical tests. According to Brett & Waldron [31], the lignin molecule tended to fill all the spaces in the wall not occupied by macromolecules, which constituted an effective barrier to the penetration of several kinds of substances. The cutin, which was formed by different types of saturated hydroxy-fatty acids forming a three-dimensional polyester network, is associated with the control of water loss by transpiration because of its presence in cuticles [32]. Petiolar region also deserves attention with presence of lignified hypodermis followed by aerenchymatous cortex. Vascular bundles are distributed in the cortex and are with well developed endodermis and pericycle. Ogura [25] reported that Casparian strips around the vascular bundles of the petiole and leaf blade is a common feature of ferns.

In Angiosperms, this feature is always found in roots, frequently in underground or submerged stems, and rarely in the leaves [33]. Its function has been related to solute retention in the stele and sap flow canalization, thus forming closed, defined apoplastic routes [33, 34]. It is also essential for the protection of aerenchyma chambers in submerged plants [35]. Roots of this fern also show the presence aerenchyma. Aerenchyma tissues occur in other mangrove plants also [36]. It allows effective O₂ transport within the roots to avoid hypoxia [37]. Aerenchyma formation increases the porosity of roots, i.e. the volume of gas-filled spaces in relation to the total tissue volume, above the usual levels contributed by intercellular spaces [38]. According to Justin and Armstrong [39], higher porosity is characteristic of plants adapted to grow in anaerobic conditions, because it enhances the internal movement of gases. Rhizome parenchyma cells are filled with starch. According to Arpagaus [40], presence of starch filled organs help the wetland plants to survive and grow under anoxia condition by supplying free sugars. In the Rhizomatous ferns, leaf growth depends on the stored resources in the rhizome and frond biomass production approximately is linearly related to rhizome biomass [41]. This indicates the relative importance of the rhizome in plant growth and adaptation. Scanning microscope study of sporophyll revealed the presence of peltate paraphyses, which are spread uniformly on the abaxial surface of the sporophyll. Paraphyses are hairs interspersed among the sporangia; approximately one-third of fern species have paraphyses of one type or another and they are like indusia, believed to be protective perhaps stop the reproductive organs from drying out [42]. This is in contrast to other leptosporangiate fern families, such as the Polypodiaceae [43]. By this anatomical observation it's possible to conclude that, like higher Angiospermic mangrove plants, this fern also possess anatomical characters like presence of thick cuticle, hypostomatic condition, higher amount of sclerenchyma and arenchyma tissue and presence of sinuous epidermal walls, which are necessary for adapting to the mangrove ecosystem.

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