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LEAF ANATOMICAL ADAPTATION OF EIGHTEEN MANGROVE PLANT SPECIES FROM THE SUNDARBANS IN BANGLADESH

PARVEEN RASHID, KISHWAR JAHAN SHETHI* AND ASFAQUE AHMED

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

Investigation on leaf anatomical adaptation of 18 mangrove plant species was carried out. Among the 18 species 13 were dorsiventral and five were isobilateral type. All the species had special stomatal structure and variable cuticle layer to minimize transpiration. Most of the species had succulent leaves with leaf thickness ranging from around 232 to 1363 µm. As an indication of salt secretion, both glandular and non-glandular trichomes were observed in several species. Although presence of single to multilayered hypodermis might effectively function as water storage tissue**,** several studied mangrove plant species e.g. *Cynometra ramiflora* L., *Phoenix paludosa* Roxb., *Pongamea pinnata* (L.) Pierre, *Sonneratia apetala* Buch. - Ham., *S. caseolaris* (L.) Engl. and *Xylocarpus moluccensis* (Lamk.) M. Roem. showed complete absence of hypodermis. This might be due to moderate saline condition. In addition, marked terminal tracheids in mesophyll tissue of a number of species might help with capillary water storage within the leaf. To enhance mechanical support several species were found to develop considerable amount of diverse sclereids within the mesophyll tissue and surrounding vascular bundle. Although maximum anatomical adaptations are common for plants growing in saline habitat it may be suggested that these features were differentially developed in plants specifically grown in mesohaline zone.

Introduction

A typical mangrove formation embraces an idiosyncratic assemblage of plant community, including shrubs and trees, dominating on low deltaic islands and sheltered estuaries where regular tidal influence of sea water prevails. These habitats are affected by humidity, precipitation, salinity, substrate and temperature. Plants are well adapted to the changing biological, chemical and physical traits of this environment through various xeromorphic properties including morphology, anatomy and physiology (Atkinson *et al.* 1967, Waisel 1972, Zimmermann 1983). Sundarban Mangrove Forests (SMF), the largest single tract mangrove forest of the world has the widest range of mangrove species in its tidal influenced saline soil. The SMF is located in the estuary of the River Ganges, in south-west coastal areas of Bangladesh and some part of West Bengal. The Sundarbans was originally extended about 40,000 sq, km but with continual agricultural and anthropogenic invasion the forest area reduced to a greater extent at around 16,000 sq km by 1930s (Blasco 1977). The Sundarbans is now restricted to around 10,000 sq km, stretching between the Baleshwar river, Bangladesh (about 60%) and the Hooghly river, India (about 40%) (Ahmed *et al.* 2018). Different saline zones, namely oligohaline, mesohaline and polyhaline occurs within SMF (Nazrul-Islam 2003) which is arbitrary depending on the seasons. Mesohaline zone is the comparatively moderate saline zone where both fresh water and saline water have influenced the plants whereas oligohaline zone is influenced mainly by fresh water and polyhaline zones have influence of the sea water. Such salinity gradients have been shown to influence species distribution and also the morphology of the plants (Maricle *et al.* 2009) and encompass a different kind of adaptive mechanism for those plants. The adaptive mechanisms of

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*Author for correspondence: <kishwar.botany@du.ac.bd>.

mangrove plants to the changing environment could be investigated through morphology, anatomy and physiology. Salinity has a direct influence on the leaf architecture of the plants (Chapman 1976). Leaves of mangrove habitat have evolved several distinctive characteristic features on cuticle, mesophyll zone, stomatal structure, hypodermis and vascular bundle of main vein which help them to photosynthesize optimally and serve a good vegetative growth under tidal influenced varied saline condition. Several studies have been conducted on the architecture, specially the micromorphology**,** of mangrove leaves from the Indian mangrove species (Seshavatharan and Srivalli 1989, Fitzgerald *et al.* 1992, Das and Ghose 1993, Ramassamy and Kannabiran 1996, Das 1999, Nabilah *et al.* 2011). At the same time, very little work (Malik and Bhosal 1983, Datta *et al.* 2007) have been done to demonstrate the influence of these highly variable habitats on the physiology of mangrove species. Recently in Bangladesh, some work have been done on species diversity and changes in forest and land cover (Nazrul-Islam 2003, Ahmed *et al.* 2018), effects of salinity on species diversity (Ahmed *et al.* 2011), soil properties (Ataullah *et al.* 2017) and heavy metals contamination (Ataullah *et al.* 2018). However, anatomical adaptation of members of gramineae e.g. *Porteresia coarctata* T. (Rashid and Sarker 2004), *Myriostachya wightiana* Hook. f. (Rashid and Ahmed 2011) growing in the coastal area of Bangladesh which are also available in the Sundarbans have been reported. Though the Sundarbans of Bangladesh have a wide variation of mangrove species no substantial information are available on the anatomy in relation to their adaptation**.** Therefore during the present investigation leaf anatomy of different species of mangrove plants in relation to the adaptive significance from mesohaline zone of Bangladesh SMF was carried out to have an idea of the combined effects of fresh water and saline water on the adaptation of these mangrove species. Such study will also enlighten the present existing knowledge on how to raise active vegetation on the vast newly accreted char in saline prone coastal areas of Bangladesh.

Materials and Methods

For anatomical study fresh leaves of 18 different mangrove species (Table 1) were collected in saturated polythene bags from different areas of mesohaline zone of Bangladesh Sundarbans (Fig. 1) and recorded by Ecology and Environment Laboratory, Department of Botany, University of Dhaka. Free hand sectioning of leaves were done at a position approximately half-way between the base and apex of a sector from one side of the lamina with the help of a razor blade. The sections were stained in safranin, mounted in 20% glycerol and studied with the help of a light microscope (Carl Zeiss Lab. A1 microscope). Microphotographs of the sections were taken using Axiocam ERc 5s digital camera attached with computer through Axio Vision Release 4.8.2 software. Measurements of important anatomical features, namely leaf thickness, cuticle thickness, epidermis diameter and hypodermis diameter were also estimated using stage micrometer and oculometer. An average of five random observations was taken from each plant species for each parameter and values were calculated for mean ± standard error using the Microsoft Excel 2011. All anatomical studies were carried out in the Plant Physiology, Biochemistry and Plant Nutrition Laboratory, Department of Botany, University of Dhaka.

Results and Discussion

Eighteen species belonging to 11 different families of mangroves from the Sudarbans were studied. Out of 18 species the lamina of 13 were dorsiventral while **in** the rest five species, namely *Pongamea pinnata* L. Pierre. (Fig. 4D), *Brownlowia tersa* (L.) Kosterm., *Phoenix paludosa* Roxb. *Sonneratia caseolaris* (L.) Engl. and *S. apetala* Buch. **-** Ham. (Fig. 3F) the lamina were isobilateral. It is interesting to note that though Chapman (1976) on the basis of transverse section of leaves classified *Excoecaria agallocha* L. as isobilateral, it was found to be dorsiventral since mesophyll was demarcated into adaxial palisade parenchyma and abaxial spongy parenchyma. This observation is in conformity with the findings of Das and Ghose (1996).

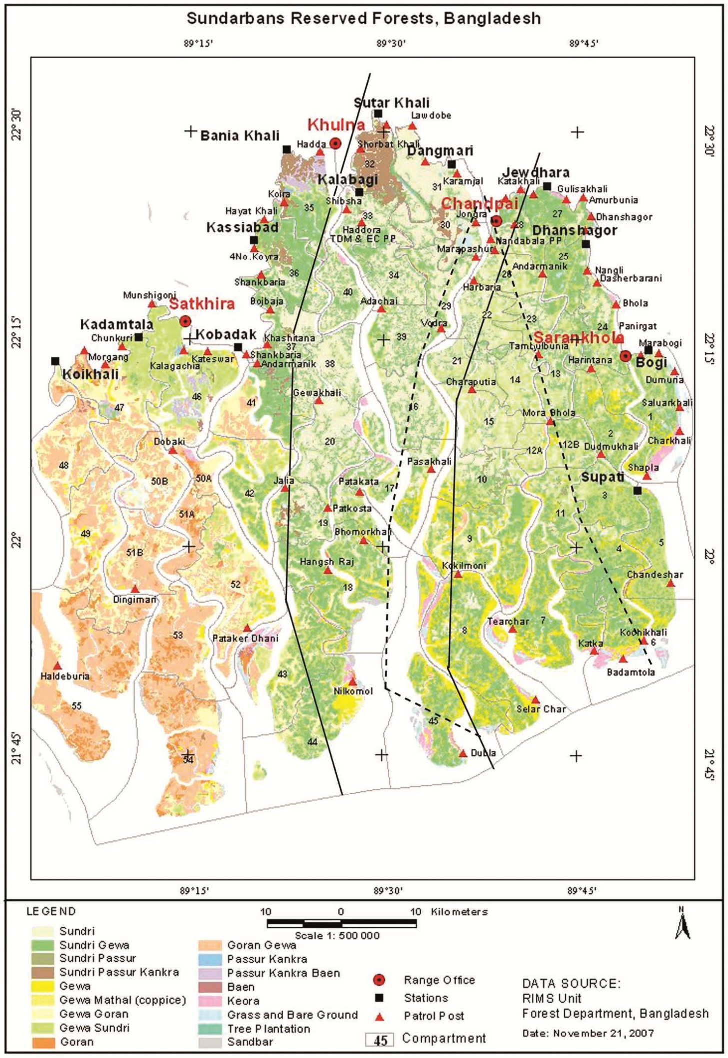

Fig. 1. Map showing the total area of Sundarban Mangrove Forests (SMF) where mesohaline zone demarcated by solid lines and oligo-mesohaline zone demarcated by dashed lines which becomes oligohaline during monsoon season and mesohaline during winter. Demarcation of the ecological zones was adopted from Nazrul-Islam (2003). Source of image: RIMS unit, Forest Department, Bangladesh

Two types of leaf hairs or trichomes, namely glandular and non-glandular were observed in the studied species. Glandular hairs were present on both adaxial and abaxial surfaces of *Acanthus ilicifolius* L. and only on adaxial surface of *Aegiceros corniculatum* Blanco. However, Das (1999) reported glandular hairs on both surfaces of *A. corniculatum*. Glandular type capitate hairs were visible on abaxial surface of *Avicennia officinalis* L. (Datta *et al.* 2007). Non-glandular hairs were followed on abaxial surfaces of *A. ilicifolius* while present on abaxial surface of *Rhizophora apiculata* Blume. Stellate hairs (non-glandular) were abundantly present on abaxial surface of *Heritiera fomes* Buch. - Ham. In *Vitex trifolia* L. hook shaped, multicellular, non-glandular hairs were found on abaxial and single-celled, non-glandular hairs on adaxial surface. The single-celled trichome could be a notable characteristic for *V. trifolia*, since it is general feature for root hair. Fig. 2A-H demonstrated different types of hairs studied. Hairs might be concerned with salt secretion, protection and therefore important in relation to adaptive nature. However, other studied materials displayed no such structure on their surfaces. Sunken stomata are another criterion of mangrove to minimize transpiration and they were observed in several samples e.g. *A. ilicifolius* (Fig. 2 I), *A. corniculatum*, *Xylocarpus moluccensis* (Lamk.) M. Roem., *P. pinnata*, *P. paludosa*. Guard cells have cuticular beak-like outgrowths on either the outer side or both outer and inner side of the stomatal pore in species like *Bruguiera gymnorrhiza* (L.) Lamk. Stomata are usually restricted to the abaxial surface of dorsiventral leaves and in isobilateral leaves are equally distributed on both surfaces except in *P. paludosa* where restricted to the abaxial surface only.

Maximum species have succulent leaves with higher leaf thickness and thin cuticle (Table 1). Maximum leaf thickness was observed in *S. apetala* (1363.50 ± 0.55 µm) followed by *S. caseolaris* (939.30 ± 0.34 µm). Considerable thin cuticle was found in *R. apiculata* (23.04 ± 0.56 µm), followed by *R. mucronata* Poir. (10.24 ± 0.45 µm) and *A. corniculatum* (7.86 ± 0.55 µm). Mangrove leaves are generally thick and succulent, which can be associated with the extra water storage capacity and leaf succulence of mangroves increases with the increase of substrate salinity (Wehe 1964). Thickness of cuticle may affect the exposure of thylakoid membranes to sunlight and thus controls the rate of carbon assimilation. Succulent and thick leaves with cutinized epidermis might help to prevent non-stomatal water loss (Das 1999) and regarded as an adaptive feature (Waisel 1972).

Colorless and larger cells beneath epidermis generally known as hypodermis were observed in most mangrove species which often function as water storage tissue (Das 1999). Single layer hypodermis was found in *E. agallocha*, two layer in *R. mucronata*, three layers in *X. granatum* and even five layers in *A. officinalis* (Fig. 3B, 3C, 3D and 3E). However, hypodermis was reported to be absent from several species studied here as in *X. moluccensis* (Fig. 3A), *Cynometra ramiflora* L., *P. paludosa*, *P. pinnata*, *S. apetala* and *S. caseolaris*. According to Zimmermann (1983) terminal tracheids helped with capillary water storage and provided mechanical support to the leaves. Naskar and Palit (2015) reported except in some species of *Bruguiera* all common mangrove genera show frequent development of groups of enlarged terminal tracheids at vein endings. During the present study, prominent terminal tracheids in the mesophyll of species, namely *S. apetala, B. gymnorrhiza* (Fig. 3F and 3G, respectively), *E. agallocha*, *A. ilicifolius*, *X. moluccensis*, *A. officinalis*, *P. pinnata* were noticed. Nonetheless, several anatomical characteristics to conserve water are mostly species specific and unique to mangrove plants as such characteristics are not found in their genetically close relatives (Naskar and Palit 2015).

Large number of xylem vessels in conspicuous vascular bundles serves for better production. *A. ilicifolius* carried three large vascular bundles in midrib and *H. fomes* had vascular bundle sheath extending upper and lower epidermis in lamina along with main vascular bundle. Structure of vascular bundles showed diversity across the studied species e.g. kidney shaped and closed in *H. fomes*, oval shaped in *A. ilicifolius*, lip shaped and open as in *P. pinnata*, *S. caseolaris*, fairy ring shaped in *R. apiculata* and cresent shaped in *X. granatum* (Fig. 4 F-K). *Acrostrichum aureum* L. collected species from Pteridaceae showed 5 - 6 circular, scatter vascular bundles in mid vein of frond (Fig. 4L). Vascular bundles of midrib region included one or sometimes two large metaxylem and a few protoxylem. Phloem elements were present on the abaxial side of

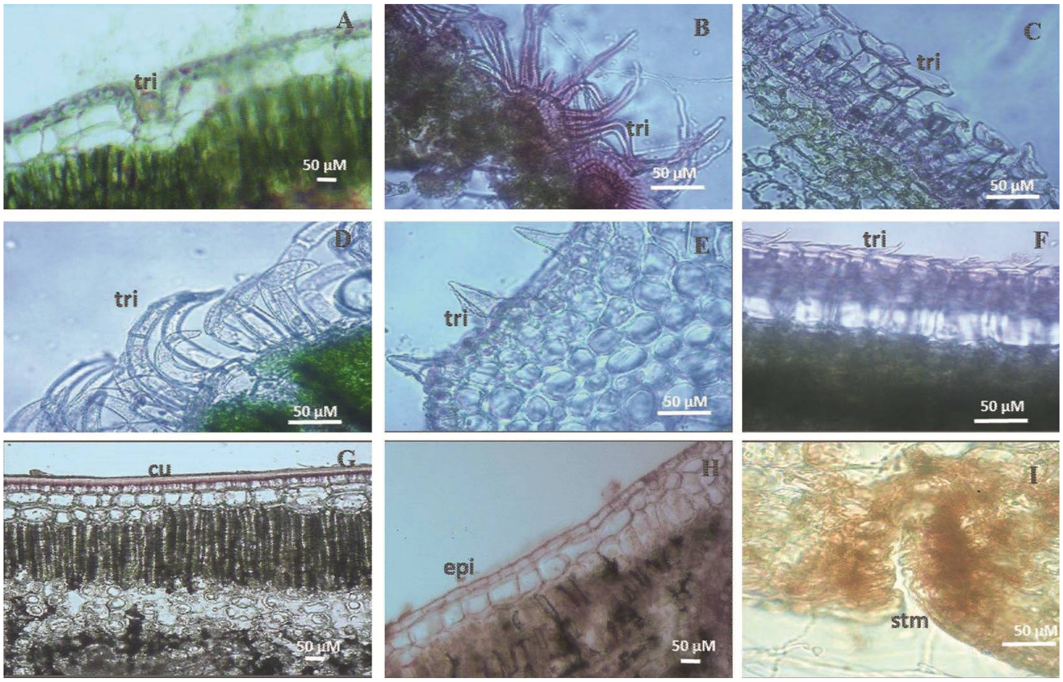

Fig. 2. Transverse sections of leaves showing various hairs (A) glandular in *A. ilicifolius* under 100X, (B) *H. fomes* with stellate, (C) *A. officinalis* with capitate, (D) and (E) multicellular and unicellular hairs respectively on *V. trifolia*, (F) papillary hair on *X. moluccensis* under 400X magnification; (G) and (H) presenting thick cuticle on *R. mucronata* and thin cuticle on *H. fomes*, respectively under 100X magnification; (I) sunken stomata on upper epidermis of *A. ilicifolius* under 400X magnification. Tri = Trichome, cu = Cuticle, epi = Epidermis and stm = Stomata.

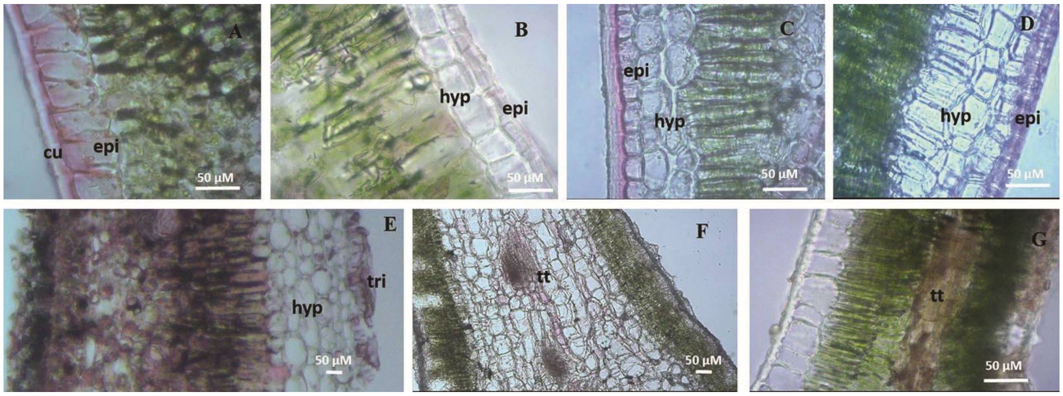

Fig. 3. Different layers of hypodermis beneath epidermal layer. (A) no hypodermis in *X. moluccensis*, (B) one layer in *E. agallocha*, (C) two layers in *R. mucronata*, (D) three layers in *X. granatum* under 400X and (E) five layers in *A. officinalis* under 100X magnification. Terminal tracheids in (F) *S. apetala* under 100X and (G) *B. gymnorrhiza* under 400X magnification. Cu = Cuticle, epi = Epidermis, hy = Hypodermis, tri = Trichome and tt = Terminal tracheids.

Table 1. Measurements of cuticle, epidermis, hypodermis and leaf thickness in case of studied materials in micrometer (µm) based on average of five transverse sections from each material.

Scientific name	Family name	LS	Cuticle thickness (µm)	Epidermis diameter (µm)	Hypodermis diameter (µm)	Leaf thickness (µm)
Acanthus ilicifolius	Acanthaceae	D	Thick 5.12 ± 0.14	25.60 ± 0.78	102.4 ± 0.45	575.70±0.57
Acrostichum aureum	Pteridaceae	D	Thin 2.56 ± 0.11	10.24 ± 0.35	30.72 ± 0.11	606.00±0.74
Aegiceras corniculatum	Primulacase	D	Very Thick 7.68 ± 0.55	17.92 ± 0.35	115.20 ± 0.57	404.00±0.57
Avicennia officinalis	Acanthaceae	D	Thick 5.12 ± 0.55	15.36 ± 0.32	120.32 ± 0.82	484.80 ± 0.87
Brownlowia tersa	Malvaceae	I	Thin 2.56 ± 0.11	12.80 ± 0.17	20.48 ± 0.57	424.10 ± 0.88
Bruguiera gymnnorrhiza	Rhizophoraceae	D	Thick 5.12 ± 0.57	17.92 ± 0.20	28.16 ± 0.45	474.10 ± 1.02
Cynometra ramiflora	Fabaceae	D	Thin 2.56 ± 0.23	10.24 ± 0.51	Absent	303.00 ± 0.87
Excoecaria agallocha	Euphorbiaceae	D	Thin and Smooth 2.56 ± 0.23	20.48 ± 0.75	33.28 ± 0.11	474.70 ± 0.57
Heritiera fomes	Malvaceae	D	Thin 2.56 ± 0.11	12.80 ± 0.89	51.20 ± 0.57	454.50 ± 0.87
Phoenix paludosa	Arecaceae	I	Thin 5.12 ± 0.46	33.28 ± 0.87	Absent	232.30 ± 0.57
Pongamea pinnata	Fabaceae	I	Thin 2.56 ± 0.12	20.48 ± 0.51	Absent	303.00 ± 1.12
Rhizophora apiculata	Rhizophoraceae	D	Highly thick 23.04 ± 0.56	92.16 ± 0.72	76.80 ± 0.11	656.50 ± 75
Rhizophora mucronata	,,	D	Very Thick 10.24 ± 0.45	17.92 ± 0.23	38.40 ± 0.23	787.80 ± 0.57
Sonneratia apetala	Lythraceae	I	Thin 2.56 ± 0.11	25.60 ± 0.26	Absent	1363.50 ± 0.55
Sonneratia caseolaris	,,	I	Thin 2.56 ± 0.23	10.24 ± 0.11	Absent	939.30 ± 0.34
Xylocarpus moluccensis	Meliaceae	D	Thick 7.68 ± 0.43	71.68 ± 0.12	Absent	505.00 ± 0.27
Xylocarpus granatum	,,	D	Thin 2.56 ± 0.23	10.24 ± 0.82	94.72 ± 0.55	545.40 ± 0.12
Vitex trifolia	Lamiaceae	D	Thin 2.56 ± 0.21	10.24 ± 0.81	51.20 ± 0.12	323.20 ± 0.87

Mean value for five observations ± standard deviation. LS = Leaf symmetry, D = Dorsiventral, I = Isobilateral.

vascular bundle and separated from the xylem by parenchymatous cells. Ample of sclereids, fibres surrounding vascular bundles and mesophyll in species like *P. pinnata*, *S. caseolaris*, *R. apiculata*, *X. granatum*, *A. aureum* (Fig. 4H-L)**;** presence of lacunae in *A. corniculatum* (Fig. 4C) reflected adaptive nature to stressful mangrove habitat. Sclereids are involved in capillary water storage as well as mechanical support for leaves with reduced turgor pressure (Tomlinson 1986). The coriaceous nature of studied mangrove leaves might be result of occurrence of such sclereids. It was interesting to note that though *Heritiera fomes* Buch. **-** Ham. is a well-known mangrove species it had several peculiar leaf characters e.g. loosely arranged palisade parenchyma, extended vascular bundles towards hypodermis and thin cuticle which indicate that this species is not so much suitable to withstand high saline habitat and commonly adapted to mesohaline zones.

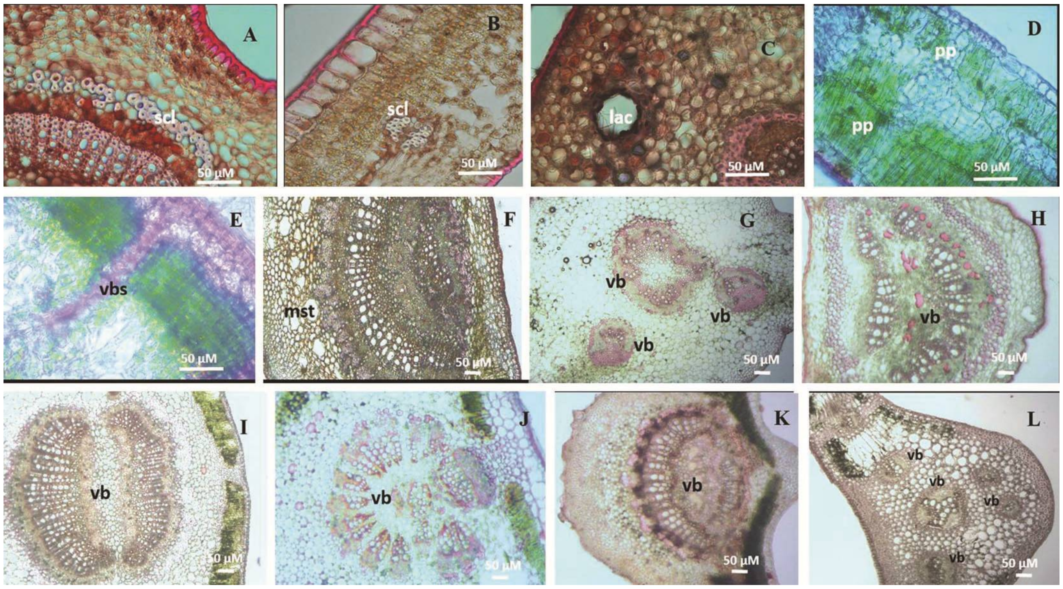

Fig. 4A and B. Sclereids in and around vascular bundle and in mesophyll of *X. granatum* respectively; C - lacuna in *A. corniculatum*; D - isobilateral leaf with two sided palisade parenchyma in *P. pinnata*; E - vascular bundle sheath extended up to both epidermal layer under 400X and (F) loosely arranged mesophyll tissue in *H. fomes* under 100X magnification. G - Three vascular bundles in *A. ilicifolius*; H - K. prominent vascular bundles with many xylem vessels and sclereids in *P. pinnata*, *S. caseolaris*, *R. apiculata* and *X. granatum* respectively and L - *A. aureum* collected only fern species under 100X magnification. Scl = Sclerenchyma, lac = Lacuna, pp = Palisade parenchyma, vbs = Vascular bundle sheath, vb = Vascular bundle.

Considering the studied species from the present investigation it may be inferred that **-** 1. Hair, cuticle, enlarged epidermal layer with succulent leaves helped to withstand variable and stressed mangrove habitat; 2. Hypodermis, terminal tracheid provided extra water storage and 3. For mechanical support prominent sclereids were found along with vascular bundles and within mesophyll tissue. Therefore, such typical characters of mangrove species of mesohaline zone might help to understand adaptation to the moderate saline environment and also have taxonomic value in genus differentiation. Besides, the study involving the plants grown under natural saline ecosystem is beneficial in understanding and managing salt stress tolerance (Lokhande and Suprasanna 2012). However, extensive study including anatomical, physiological and biochemical adaptation necessary for growth outlining mesohaline as well as other zones of saline environment will further facilitate the present understanding about **plant-**salt tolerance and adaptive ability.

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