International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 9 (1): 44-49, 2013
ISSN 1811-9700 / DOI: 10.3923/ijb.2013.44.49
© 2013 Asian Network for Scientific Information

Primary Structure and Intercellular Space Formation of Feeding Root in *Sonneratia alba* J. Smith

Hery Purnobasuki
Department of Biology, Faculty Sciences and Technology, Airlangga University,
Jl. Mulyorejo (Kampus C Unair) Surabaya-60115, Indonesia

Abstract: The aim of this research was to know the primary structure and intercellular space formation of feeding root in *Sonneratia alba* in order to relate their development and structure of their function as environmental adaptation in mangrove's root. The conventional histological techniques by Light Microscopy (LM) were used to get anatomical datas. This root has no cork covering. Cells of the meristem normally had dense cytoplasm and clear nucleus. Protoderm first appeared as a distinct layer at the edge of the tiers of cortical meristem. Root caps of feeding root of *S. alba* consist of two regions, i.e., weakly stained central columella or statenchyma and well-stained peripheral regions. The columella has 8-11 layers. The size of gas spaces is 100-320 µm. All the cells in the cortex appear round in cross section. Between 0-500 µm distances from the tip, few intercellular spaces and cortical cells are tightly packed. At distance more than 500 µm from the tip, cortical cells began to separate each other and resulted in the intercellular spaces between longitudinal files of the cortical cells. Changes of cortex cells with developing gas spaces suggested that cell separation (schizogenous) and enlargement of intercellular spaces has occurred to form aerenchyma in these plants. From its shape, structure and location, it seems clear that the primary structures of root assists the root in penetrating the soil and absorbs nutrient. The present study revealed that these plants have developed the structural adaptation in their roots as adaptation to their anaerobic habitat.

Key words: Cortical meristem, feeding root, intercellular space, schizogenous

INTRODUCTION

Mangrove communities, which typically grow in saline coastal areas of the tropics and subtropics, flourish in a stressful intertidal environment. *Sonneratia alba* J. Smith (The Mangrove apple) is one of mangrove species that a pioneering mangrove species and often grows in newly formed sand or mud flat at the seaward fringe of mangroves and sometimes even constitutes a pure sparse forest in the seaward zone. (Chapman, 1976; Tomlinson, 1986). This species is a fast-growing tree and can grow up to 8-10 m tall at its maturity in Indonesia. This species develops a fairly complex root system. In mature tree, the root system of *S. alba* is complicated and it has four root types (Fig. 1), i.e., cable roots, pneumatophores, feeding root and anchor roots (Purnobasuki and Suzuki, 2004).

Mangroves are highly adapted to the anaerobic environment, with various kinds of roots external structures (Gill and Tomlinson, 1975; Duke, 1992). The roots of mangrove plants are believed to absorb water efficiently from the soil under saline conditions. In order to maintain this water absorption power, the roots must respire at a high rate and have a unique anatomical structures. The underground tissues of mangrove, such as feeding roots require a large amount of oxygen for respiration varying degrees of waterlogging. McKee and Mendelssohn (1987) noted that, although the roots may cope with temporary periods of anaerobiosis, root function depends on the maintenance of aerobic conditions in their tissues. To withstand the situation, the structure of mangrove roots increases the availability of oxygen to roots growing in oxygen deficient sediment (Tomlinson, 1986).

The ecology and physiology of the root aeration of *Avicennia* spp. and other mangroves have been studied previously (Curran 1985; Curran *et al.*, 1986; McKee and Mendelssohn, 1987). However, anatomical studies of the mangrove roots are still very few (Chapman, 1944, 1976; Baylis, 1950; Fahn, 1982). Inspite of some works on the anatomy of roots of *Avicennia*, *Sonneratia* and *Rhizophora* (Chapman, 1940; Gill and Tomlinson, 1971, 1977) and on a few other genera (Ogura, 1940; Jenik, 1970), no critical investigations on the development and structure of the air conducting system in all root types of mangrove. In the most recent work, emphasis was given on oxygen tolerance, salt tolerance and aerial root architecture of the halophyte (Hogarth, 1999). Much remain to be learnt regarding root structure and function in wetland plants (Seago *et al.*, 2000).

The peculiarities of mangrove habitats in littoral conditions require on efficient air conducting system, aerenchyma in their roots. As in other plant species, the root initiation and development is very important for their mature structure. With regard to a well-developed tree, the root tips of feeding root define the structure that formed at different development stages through the plant growth. In the present study, primary structure in feeding roots of *S. alba* was investigated from the anatomical aspects in order to reveal adaptative meaning of the air conducting system in mangrove roots.

MATERIALS AND METHODS

The feeding root samples were taken from adult trees (10-15 cm in trunk diameter at base, 0,5-1 m tall) of *S. alba* which growth in Surabaya beach (7°12'28.82"S 112°46'33.35"T), East Java Province in Java Island, Indonesia, at March, 2010 and September, 2010. Around the sample trees, we excavated root system (Fig. 1) of the trees during low tide and collected feeding root tips. We collected more than twenty tips.

Histology: The samples of feeding root tips were fixed in the field. The samples were prepared for embedding in paraffin wax and sectioning. They were fixed in FAA (70% ethanol, 10% formalin and 5% acetic acid in the volume ratio 90:5:5). The air in the tissue was evacuated using an oil rotary vacuum pump. The samples were dehydrated in an ethanol series and embedded in parafin (Oxford Lab, USA) at 59°C. Longitudinal thin sections in 9-12 μm were cut using a rotary microtome (HM 350 Micron, Germany), stained in Safranin-Fast Green (O'Brian and Cully, 1981; Sanderson, 1994) and permanently mounted using entellan. Finally, all the observation was carried out using light microscope (BX 50, Olympus, Japan). Microscopic images were taken by microscopy camera (PM-C35, Olympus, Japan) and recorded on Fuji Film Neopan F ISO 32/16° films for black and white prints. The image data of root anatomy were taken using computer assisted image analysis (version 3.5 scientific imaging software, IP Lab USA) by digitizing the images with a digital camera attached to a compound microscope. The results were analyzed using one-way analysis of Variance (ANOVA, minitab version 13, $\alpha = 0.05$).

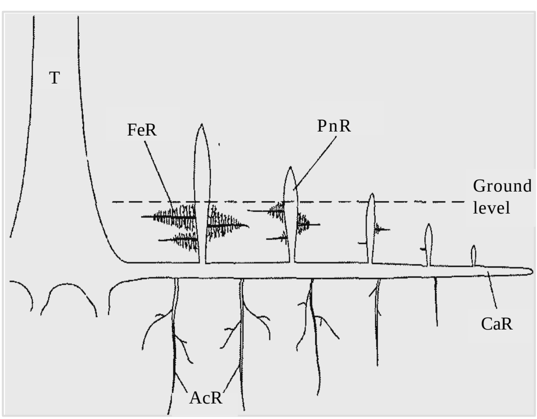

Fig. 1: Simplified diagram of mature *S. alba* root system showing different types of roots. CaR (cable roots), primary roots with lateral horizontal growth. PnR (pneumatophores), aerial roots with upward vertical growth from CaR. FeR (feeding roots), lateral horizontal roots grown from PnR. AcR (anchor roots), vertical roots grown downward from CaR. T, trunk. Bar = 2 cm

RESULTS

External morphology of feeding roots: This species develops a highly specialized root system mainly consisting of cable roots and pneumatophores (also see Troll *et al*., 1931; Tomlinson, 1986). The tap root in *S. alba* only exerts its function at the early stage for a short period. Afterwards, a large number of cable roots originate from the trunk base and extend horizontally around the tree, few meters deep in the substrate and up to the radius of 2-3 times of tree height. Detail morphology of feeding root of *Sonneratia alba* are as follow.

Feeding roots commonly arise from pneumatophores slightly beneath the soil surface (0.7-1 cm) (Troll *et al*., 1931). Most of them are restricted to the swollen region of pneumatophores immediately below the ground surface (Fig. 2). Their density and proximity to each other is dependent on the rate of accretion of their growth (Chapman, 1944). Feeding roots usually experience weak secondary growth at their base. They normally branch very often to form densely clothing of numerous fine roots without root hairs nor a cork covering. The actual absorption takes place in these ultimate finest roots that growth around the pneumatophores. Feeding roots can reach 0.3 in length and has 1.74 mm in diameter in young and 3 mm in diameter in old roots (Table 1).

Anatomical structures of feeding roots: The root apex of feeding roots is quite similar to that of general terrestrial plants except for the absence of root hair formation. This root also has no cork covering. Cells of the meristem normally had dense cytoplasm and clear nucleus (Fig. 3). Protoderm first appeared as a distinct layer at the edge of

Table 1: Root system architecture unit of *Sonneratia alba*

Root type	Woody axis	Gravitropism	Mean diameter of young root (mm)	Mean diameter of old root (cm)	Growth	Maximum length (m)	Symmetry
Cable roots	W	↔	6.96±0.05 n = 10	3.8±0.26 n = 10	I	25.00	R
Pneumatophores	W	-	6.85±0.04 n = 10	3.6±0.32 n = 10	D	0.35	R
Feeding roots	NW	↔	1.74±0.06 n = 10	0.3±0.02 n = 10	D	0.30	R
Anchor roots	NW	+	2.25±0.41 n = 10	0.5±0.03 n = 10	D	0.60	R

W: Woody, NW: Non woody, +: Positive orthogravitropic, *orthogravitropic (growth in the direction of gravity), -: Negative orthogravitropic, ↔: diagravitropic *diagravitropic (growth perpendicular to gravity), I: Indefinite growth, D: Definite growth, R: Radial symmetry, #young roots taken close to the cable root tip, #old roots taken close to the basal of cable roots

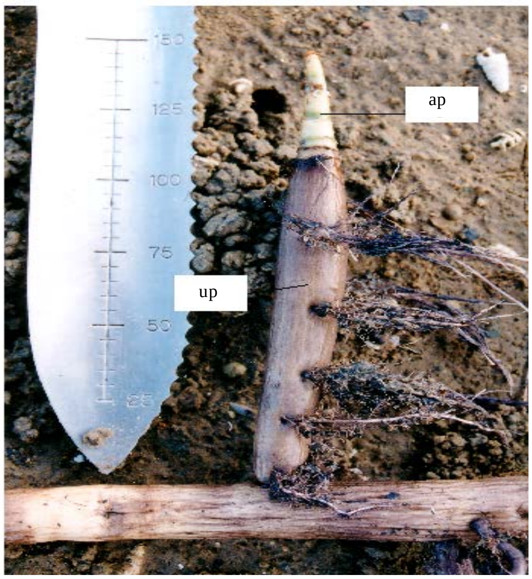

Fig. 2: Morphological structure of feeding roots that growth in pneumatophore of *S. alba*. The aerial part of pneumatophore (ap) is pencil like with a light green or gray green, flaky bark and hard. The underground part (up) is brown, swell, soft, coarse and many feeding roots growth laterally from this part

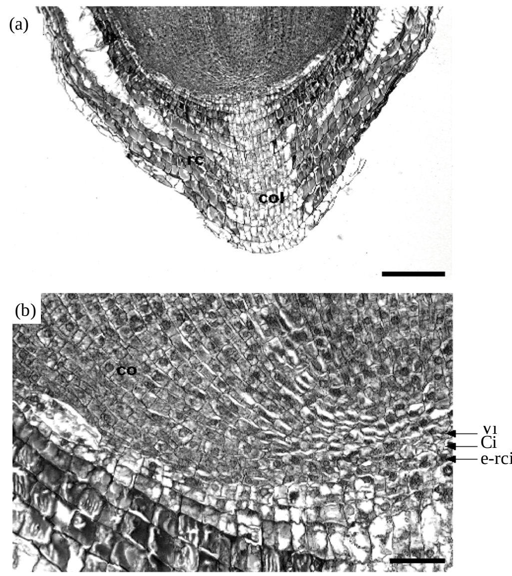

Fig. 3(a-b): Median longitudinal sections of feeding roots of *S. alba* on the apical meristem region. A. Root apical region that showing clearly root cap (rc) with columella region (col) in the middle part with lightly staining and densely staining in the peripheral part of the root cap (Bar = 200 μm). B. Magnification of cortical layer (co) in lateral part region of apical initials, showing clearly cortical layers (Bar = 50 μm). ci. cortical initial; e-rci. epidermis-root cap initial; pr. peripheral region; and vi. vascular initials

the tiers of cortical meristem (Fig. 3a, b). The meristem divides periclinally and anticlinally became to cortex area (240 μm in thickness) and pith (160 μm in thickness). Anticlinal divisions (with the reference to the root surface) are responsible for the maintenance of the apical groups. It is somewhat difficult to establish the precise boundaries for the apical group in longitudinal sections.

The most striking feature of the root is a distinct and extensive root cap with quite long files of cells. Root caps of feeding root of *S. alba* consist of two regions, i.e., weakly stained central columella or statenchyma (Fig. 3a, col) and well-stained peripheral regions (Fig. 3a, pc). Columellar initials are in direct contact with the cortex and epidermal initials. This root has such distinct columellar initials not joined by the vascular initials or overlying the cortical initials (Fig. 3a, b). The columellar initials also have more lightly stained cytoplasm than adjacent cells. The number of longitudinal layers of columella is quiet different between the root types. The columella consists of horizontal layers of regularly arranged cells. The columella layers are labeled from the meristem to the root tip according to the method of Sack and Kiss (1989). In feeding roots, the columella has 8-11 layers.

The each columella layer has statocytes cells horizontally arranged in longitudinal section (Fig. 3a).

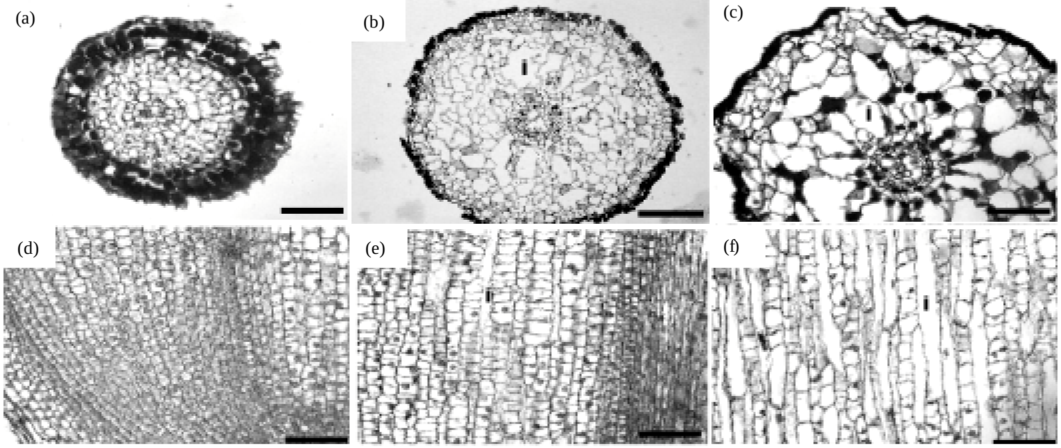

Fig. 4(a-f): Serial cross sections (a-c) and longitudinal sections (d-f) of feeding roots of *Sonneratia alba* at difference distances behind the root apex. A. 200 μm (Bar = 50 μm), B. 1 mm (Bar = 100 μm), C. 3 cm (Bar = 50 μm), D. 200 μm (Bar = 50 μm), E. 1 mm (Bar = 50 μm) and F. 3 cm (Bar = 50 μm). i. Intercellular space

These statocytes divide anticlinally and cells situated on both sides of the columella begin to enlarge in radial diameter with development of vacuoles, become stainable with Safranin and thus become peripheral cells. Cells at the tip of columella and outermost peripheral cells are slashed out by soil in feeding roots (Fig. 3a).

A section near the root apex (0-300 μm) showed that an outer zone of large cells without contents was formed first, which may partly form the root cap. Some layers of this root cap readily break off (Fig. 3) and was replaced by a layer of cells somewhat compressed, though these cells expanded and formed a layer outside the epidermis. Beneath this zone, a few layers of cells formed an outer cortical zone with small, round and closely aligning cells, followed by an inner cortical zone of cells that began to separate from each other with developing intercellular spaces (Fig. 4b, c and f).

At the growing apex the cells of the cortex were regularly rounded and are arranged in rows that radiated out from the stele. The endodermis was well defined (Fig. 4c) and outside the epidermal layer, there are several layers of root cap cells. Even at an early stage the cells of the inner and the outer cortexes differed in shape and cells of the outer zone were more extent size and rectangular and composed of polygonal inner ones (Fig. 4c). Shortly behind the apex, the cells of the inner cortex rounded off and air spaces develop and enlarge. In the feeding root, the size of gas spaces is 100-320 μm.

All the cells in the cortex appear round in cross section. In longitudinal view, cortical cells were tightly packed and appear in files parallel to the root axis near the apex (Fig. 4d, e). Outside the cortex generally three layers of small and rectangular cells composed of a multilayered epidermis (Fig. 4c, d).

Observations of longitudinal sections confirmed findings from cross sections. Between 0-500 μm distances from the tip, few intercellular spaces and cortical cells are tightly packed (Fig. 4a). At distance more than 500 μm from the tip, cortical cells began to separate each other and resulted in the intercellular spaces between longitudinal files of the cortical cells (Fig. 4c, f).

Root hairs were never observed in the present study, but also other marsh plants often lack root hair. Therefore, It could not be concluded that persistent cap layers are fundamentally responsible for the lack of hairs in mangrove roots (Baylis, 1950).

DISCUSSION

It is noteworthy that the root system of *Sonneratia alba* is effectively the same as that of many other mangroves in which part of the root system projects upward from a subterranean system as aerial root. This situation is illustrated diagrammatically in Fig. 1 with functionally equivalent parts closely similar in the same way. In mangroves with pneumatophores of various types, pneumatophores are connected by underground horizontal system. Functionally, the aerial part of pneumatophores have important role of oxygen pathway for underground roots (cable root, anchor root and feeding root).

Changes of cortex cells in root sections (transversal and longitudinal) with developing gas spaces suggested that cell separation (schizogenous) and enlargement of intercellular spaces (Fig. 4) has occurred to form aerenchyma in these plants. Although there are fairly many studies on air conduction and static morphology of already differentiated aerenchyma tissues within the root system of mangrove plants, developmental anatomy of aerenchyma formation is rather few. For example, Lawton *et al.* (1981) studied physiological anatomy of *Avicennia marina* in relation to ion transportation in its root system. They reported that air spaces in root cortex of *A. marina* were randomly distributed in transverse section and formed lysigenously in origin. This is the only paper that declares the lysigenous origin of root cortex aerenchyma of this species. Several papers suggested schizogenous origin of aerenchyma in root cortex of this species basing on anatomical description of aerenchyma for example, Baylis (1950) and Curran (1985) but not conclusive. Any cell destructions or lyses were not observed among cortical cells during the tissue differentiation of root cortex in the present study.

Furthermore, the cell alignment in longitudinal columns is established just behind of root apex (Fig. 4f). The number of cell column in more matured parts where the intercellular spaces are well developed is the same or a little fewer than that of younger part near the apex without intercellular spaces. According to development in longitudinal sections, the cell columns increase distance to adjacent columns forming longitudinal intercellular spaces between them. A similar aerenchyma formation was also observed in some wetland plants, *Sagittaria lancifolia, Thalia geniculata* and *Pontederia cordata* (Longstreth and Borksenious, 2000), *Filipendula ulmaria* and *Caltha palustris* (Smirnoff and Crawford, 1983). Lawton *et al.* (1981) did not present evidence to support intercellular space formation of *S. alba* in the present study.

There were no gas spaces in the region near the root tip and cortex cells were round in cross section and were generally arranged in radial files with contact between and adjacent files. The earliest formation of gas spaces is revealed by intercellular spaces typically begins schizogenously randomly within 300 μm distance from the ground meristem tip. The gas spaces were enlarged by increased distance between cortex cells because the number of files of cells changed little as the distance from the root tip (Table 2). The mechanism of gas space enlargement is unknown, it should be involved the complex mechanism (Longstreth and Borksenious, 2000).

The anatomy of cortical aerenchyma in *Avicennia marina* and *Sonneratia alba* studied here were closely similar to that of *Avicennia marina* var. *australasica* reported by Allaway *et al.* (2001); var. *resinica* by Baylis (1950); *Avicennia nitida* by Chapman (1944) and *Sonneratia alba* by Troll *et al.* (1931).

Table 2: Root diameter and cortical cell numbers at difference distances from the root tip in feeding roots of *Sonneratia alba*

Distance from tip in feeding root (mm)	Diameter (mm)	Cells in outer cortex (cross sectional) (n)	Radial files of cells in the inner cortex (longitudinal view) (n)
2	0.95±0.02	368±8.57	22±6.01
6	1.25±0.02	366±7.03	17±5.23
10	1.68±0.03	365±8.24	16±3.63
20	1.72±0.02	370±7.98	14±4.03

Intercellular space development in response to anaerobic conditions may involve important role between maintaining physiological function and the need to reduce tissue respiration. Intercelular space system can provide benefits to the plant in terms of facilitating oxygen transport and increasing metabolic efficiency. Eliminating cortex tissue can impede other root functions such as water and mineral uptake and transport (Moog, 1998). The present study revealed that these plants have developed the structural adaptation in their roots as adaptation to their anaerobic habitat.

CONCLUSION

This root has no cork covering. Cells of the meristem normally had dense cytoplasm and clear nucleus. Protoderm first appeared as a distinct layer at the edge of the tiers of cortical meristem. Root caps of feeding root of *S. alba* consist of two regions, i.e., weakly stained central columella or statenchyma and well-stained peripheral regions. The columella has 8-11 layers. The size of gas spaces is 100-320 μm. All the cells in the cortex appear round in cross section. Between 0-500 μm distances from the tip, few intercellular spaces and cortical cells are tightly packed. At distance more than 500 μm from the tip, cortical cells began to separate each other and resulted in the intercellular spaces between longitudinal files of the cortical cells. Changes of cortex cells with developing gas spaces suggested that cell separation (schizogenous) and enlargement of intercellular spaces has occurred to form aerenchyma in these plants. From its shape, structure and location, it seems clear that the primary structures of root assists the root in penetrating the soil and absorbs nutrient.

REFERENCES

Allaway, W.G., M. Curran, L.M. Hollington, M.C. Ricketts and N.J. Skelton, 2001. Gas space and oxygen exchange in roots of *Avicennia marina* (Forssk.) Vierh. Var. Australasica (Walp.) Moldenke ex N.C. Duke, the Grey Mangrove. Wetl. Ecol. Manage., 9: 211-218.

Baylis, G.T.S., 1950. Root system of the New Zealand mangrove. Trans. R. Soc. New Zea., 78: 509-514.

Chapman, V.J., 1940. The functions of the pneumatophores of *Avicennia nitida* Jacq. Proc. Linn. Soc., 153: 228-233.

Chapman, V.J., 1944. 1939 Cambridge University Expedition to Jamaica-Part 3. The morphology of *Avicennia nitida* Jacq. and the function of its pneumatophores. J. Linn. Soc., 52: 487-533.

Chapman, V.J., 1976. Mangrove Vegetation. HJ Kramer Publishing, Vaduz.

Curran, M., 1985. Gas movement in the roots of *Avicennia marina* (Forsk.) Vierh. Aust. J. Plant Physiol., 12: 97-108.

Curran, M., M. Cole and W.G. Allaway, 1986. Root aeration and respiration in young mangrove plants [*Avicennia marina* (Forsk.) Vierh.]. J. Exp. Bot., 37: 1225-1233.

Duke, N.C., 1992. Mangrove Floristics and Biogeography. In: Tropical Mangrove Ecosystems, Robertson, A.I. and D.M. Alongi (Eds.). American Geophysical Union, Washington D.C., USA., pp: 63-100.

Fahn, A., 1982. Plant Anatomy. 3rd Edn., Pergamon Press, Oxford, New York, USA.

Gill, A.M. and P.B. Tomlinson, 1971. Studies on the growth of red mangrove (*Rhizophora mangle* L.) 2. Growth and differentiation of aerial roots. Biotropica, 3: 63-77.

Gill, A.M. and P.B. Tomlinson, 1975. Aerial Roots: An Array of Forms and Functions. In: The Development and Function of Roots, Torrey, J.G. and D.T. Clarkson (Eds.). Academic Press, London, UK., pp: 237-260.

Gill, A.M. and P.B. Tomlinson, 1977. Studies on the growth of red mangrove (*Rhizophora mangle* L.) 4. The adult root system. Biotropica, 9: 145-155.

Hogarth, P.J., 1999. The Biology of Mangrove. Oxford University Press, Oxford, New York.

Jenik, J., 1970. Root system of tropical trees 5. The peg-roots and the pneumathodes of *Laguncularia racemosa* Gaertn. Preslia (Praha), 42: 105-113.

Lawton, J.R., A. Todd and D.K. Naidoo, 1981. Preliminary investigations into the structure of the roots of the mangroves *Avicennia marina* and *Bruguiera gymnorhiza* in relation to ion uptake. New Phytol., 88: 713-722.

Longstreth, D.J. and O.N. Borksenious, 2000. Root cell ultrastructure in developing aerenchyma tissue of three wetland species. Ann. Bot., 86: 641-646.

McKee, K.L. and I.A. Mendelssohn. 1987. Root metabolism in the black mangrove *Avicennia marina* (L.) L: Response to hypoxia. Environ. Exper. Bot., 27: 147-156.

Moog, P.R., 1998. Flooding tolerance of *Carex* species. I. Root structure. Planta, 207: 189-198.

O'Brian, T.P. and M.E. Cully, 1981. The Study of Plant Structures Principle and Selected Methods. Termacarphi Pty. Ltd., Melbourne.

Ogura, Y., 1940. On the types of abnormal roots in mangrove and swamp plants. Bot. Mag. Tokyo, 54: 389-404.

Purnobasuki, H. and M. Suzuki, 2004. Root system architecture and gravity perception of a mangrove plant *Sonneratia alba* J. Smith. J. Plant Biol., 4: 236-243.

Sack, F.D. and J.Z. Kiss, 1989. Plastids and Gravity Perception. In: Physiology, Biochemistry and Genetics of Nongreen Plastids, Boyer, C.T., J.C. Shannon and R.C. Hardison (Eds.). American Society 01: Plant Physiologists, Rockville, MD., pp: 171-181.

Sanderson, J.B., 1994. Biological Microtechnique. BIOS Scientific Pub., Oxford.

Seago Jr., J.R., C.A. Peterson, L.J. Kinsley and J. Broderick, 2000. Development and structure of the root cortex in *Caltha palustris* L. and *Nymphaea odorata* Ait. Ann. Bot., 86: 631-640.

Smirnoff, N. and R.M.M. Crawford, 1983. Variation in the structure and response to flooding of root aerenchyma in some wetland plants. Ann. Bot., 51: 237-249.

Tomlinson, P.B., 1986. The Botany of Mangroves. Cambridge University Press, Cambridge, UK., Pages: 419.

Troll, W., D. Dragendorff and H. Fromherz, 1931. Uber die luftwurzeln von *Sonneratia* Linn. F. und ihre biologische bedeutung. Planta, 13: 311-473.