Biota Vol. 16 (2): 309–315, Juni 2011 ISSN 0853-8670

Structure of Lenticels on the Pneumatophores of Avicennia marina: as Aerating Device Deliver Oxygen in Mangrove's root

Struktur Lentisel pada Pneumatofor Avicennia marina: sebagai Alat Pengantar Oksigen Pada Akar Mangrove

Hery Purnobasuki

Dept. of Biology, Faculty of Sciences and Technology, Airlangga University Jln. Mulyorejo (Kampus C Unair), Surabaya-60115 E-mail: herypurba@unair.ac.id or herypurba@yahoo.com

Abstract

Lenticels on the pneumatophores of *Avicennia marina* were studied by Scanning Electron Microscopy (SEM) in order to relate their development and structure of their function as aerating deliver oxygen in mangrove's root. The results reveals that lenticels varied in size and range in morphology from classical crater-like with a mass of fluffy tissue in the centre. The lenticels are composed of complementary (filling) tissue, which consists of thin walled spheroidal cells by intercellular spaces. The cells may be suberized by positive stained of safranin. As it grows, the complementary tissue ruptures the periderm and the lenticels become functional as part of air conduit systems.

Key words: Lenticels, pneumatophores, root conduit systems

Abstrak

Telah dilakukan penelitian terhadap lentisel pneumatofor Avicennia marina menggunakan mikroskop cahaya dan mikroskop pemindai electron untuk mengetahui keterkaitan perkembangan dan struktur dari fungsi lentisel sebagai alat pengantar oksigen pada akar mangrove. Hasil penelitian menunjukkan bahwa struktur lentisel bervariasi dalam hal ukuran dan morfologi, mulai dari bentuk seperti kawah dengan jaringan lunak di bagian tengahnya. Lentisel tersusun dari jaringan pelengkap yang tersusun atas sel-sel berdinding tipis dan membentuk ruang-ruang interseluler. Sel-sel tersebut mengandung lignin yang terdeteksi positif dengan pewarnaan sfranin. Dalam pertumbuhannya, sel-sel pelengkap merusak lapisan peridermis dan selanjutnya lentisel berfungsi sebagai bagian dari sistem saluran penghantaran udara.

Kata kunci: Lentisel, pneumatofor, sistem saluran akar

Diterima: 21 Februari 2011, disetujui: 31 Mei 2011

Introduction

In well oxygenated soil, there is little difficulty in obtaining the oxygen needed for respiration. This is not so in waterlogged soils, and special aerating devices are required. *Avicennia marina* is a common mangrove species on tropical and subtropical sea shores, swamps and stream banks (Chapman, 1976; Tomlinson, 1986). In mature tree, the root system of *A. marina* is complicated and it has fourroot types, i.e. cable roots, pneumatophores, feeding root, and anchor roots (Purnobasuki and Suzuki, 2004; 2005a; 2005b). Pneumatophores

grow vertically upward and expose their tip in air. The pneumatophores are covered by an impermeable periderm. At low tide, lenticels on the surface of the pneumatophores allow gas exchange between the atmosphere and the internal structure of root. Therefore, the pneumatophores has important function as a highly specialized ventilation mechanism, enabling the plant to survive in anaerobic soil and extreme conditions in the shore.

Despite the importance of lenticels for survival and gas transport of mangrove roots, very little is known about the development and organization of lenticels tissue in mangrove root system. The structure of pneumatophores of *Avicennia marina* has been studied previously by Gordon and Dubinsky (1992), however there is still no clear how this air conducting network develop, especially the structure of lenticels in this species.

The high flooding tolerance of certain species of forest trees such as mangrove has been attributed to one or more adaptive mechanism, one of this is ventilation structures of lenticels on their specialized root. Stems and roots that increase in thickness by secondary growth are generally bordered on the outside by a periderm, a protective tissue of secondary origin. Lenticels are circumscribed parts of the periderm in which the phellogen is more active and periodically produces a tissue with numerous intercellular spaces. Because of this relatively open arrangement of cells and the continuity of the intercellular spaces with those in the interior of the stems, the stem lenticels are supposed to provide pathways for transpiration and gas exchange (Rosner and Kartusch, 2003).

The present study examines the structures and organization of lenticels of the pneumatophores of *A. marina* and to assess the relationships between tissue structure and habitat adaptation.

Materials and Method

Pneumatophore samples were taken from adult trees (10–15 cm in trunk diameter at base, 0.5–1 m tall) of *Avicennia marina* (Forsk.) Vierh., which grow naturally in Urauchi (24°23 'N, 123°46'E) and Komi estuary (24°19'19N, 123°54'E), Iriomote Island, okinawa Prefecture, Japan. The sampled trees are sparsely distributed in seaward outer fringes of mangrove forest where plants are flooded by all high tides and easily influenced by strong winds and tidal forces. Around the sampled trees, we collected carefully root of the trees during low tide and collected pneumatophore.

SEM Observation

Pneumatophores were cut into cubic of 5–8 mm length pieces using razor blade for SEM preparation using Resin Casting Method

(Mauseth and Fujii, 1994). The samples were observed and photographed on SEM (Hitachi S-4100). The tissue was fixed in FAA and dehydrated in a graded ethanol-t butyl alcohol and freeze dried at -10°C (Hitachi ES-2030 Freeze Dryer). The dried samples were embedded in styrene monomer-polyester resin with 1% benzoyl peroxide in the gelatin capsule and vacuum for several minutes. The samples were put at the oven 60°C overnight for polymerization. After removing gelatin, samples were trimmed using a chisel or sand paper. The trimmed sample was immeresed in a mixture of hydrogen peroxide-acetic acid (1:1) to remove lignin for one day or more at the oven 60°C, after that is was washed in water and put on the sulfuric acid 64% to remove cell wall polysaccharides for one day. The resin cast was rinsed with water and cleaned by agitation in water using mini supersonic cleaner. The sample was dehydrated again in a graded ethanol-t butyl alcohol and freeze dried. The dried sample was glued to the specimen stub that coated with conductive carbon tape. The sample was coated with platinum palladium in vacuum evaporator (Hitachi E-1030 ion sputter), observed and photographed on Scanning Electron Microscope (Hutachi S-4100).

Results and Discussion

The surface of pneumatophores of *A. marina* had many small spots or mere pimples of lenticels. Lenticels spread in the surfaces of pneumatophores and only clearly showed at the region of aerial part of these roots. These varied in size and range in morphology from classical crater-like (Fig. 1) with a mass of fluffy tissue in the centre to unopened pustules.

The clorenchyma of the pneumatophores cortex beneath seemed to be responsible for overall green color in the surface of aerial part of pneumatophores. In *A. marina*, there were two kinds of pneumatophores, the smooth and the rough types. The rough type, which was found at the region of pneumatophores above the surface of water, had numerous lenticels, which project markedly from surface, whereas the smooth type (young pneumatophores) had fewer or no lenticels, submerged underground (Fig. 1B).

The lenticels of this species is composed of complementary (filling) tissue, which consists of thin walled spheroidal cells by intercellular spaces (Fig 2 and 3). They concave disc, stacked with no intercellular matrix (Fig 3C and D). The cells may be suberized by positive stained of safranin.

Lenticels appear up to 0.5 cm below the pneumatophore tip and the uppermost lenticels are mere pimples on the surface of the pneumatophores in A. marina. As it grows, the complementary tissue ruptures the periderm and the lenticel become functional (Fig. 2D). After that, it supposed that a suberized layer is the meristem beneath formed by the complementary tissue of the lenticel. Eventually the meristem begins to form complementary tissue inside this suberized layer eventually ruptures and is cast off along with all of the old complementary tissue. A scar remains at the edges of the lenticels (Fig. 2D) shows a short section of the suberized layer and some complementary tissues. From the presence of several sets of scar tissue in some lenticels it is obvious that this may be happen many times in the life of one lenticel.

The lenticels development of these roots briefly describe as follows. The parenchymatous

cells adjoining the aerenchyma tubular structure in the cortex increase in size and divide. The give daughter-cells resulting rise to complementary tissue which fills up the 'airchamber' in regular form of files formation. A curved cell-layer, convex toward the inside, undergoes tangential divisions and becomes the 'lenticellar meristem' (Fig. 2A), which continually cuts off additional complementary cells on its outer side. The pressure of the expanding regular steadily files of complementary tissue causes the epidermis layer to bulge outwards and finally to burst and make the epidermis layer become rupture; the complementary tissue than protrudes in places, hence to rough surface of the lenticels. On older pneumatophores, which are already covered with periderm, the lenticels arise from the phellogen, which at certain points, produces complementary tissue with abundant intercellular spaces in place of the normal uninterrupted layers of cork (Fig. 2 and 3B) and thus becomes locally converted into lenticelullar meristem. The layer of cork above a developing lenticel suffers the same fate as the epidermis in the previous explanation, being distended and finally ruptures by the growing complementary tissue.



Figure 1. Morphological structure of pneumatophores as gas exchange in *A. marina*. The lenticels mere pimples or spot on the surface of pneumatophores (A–B), Bar= 2 cm. The chlorenchyma of the pneumatophore cortex beneath may be responsible for overall green color and the dot line reveals the ground level. C. Root tip of pneumatophores with many lenticels, appearance as crater-like. Bar= 8 mm. D. More distance for root tip with young lateral root (feeding root) growth. Bar= 4 mm. fe. feeding roots; lc. Lenticels.

Structure of Lenticels on the Pneumatophores of Avicennia marina



Figure 2. Cross section of pneumatophore of A. marina at a lenticels which has undergone several growth cycles. A. closed lenticels (Bar=100 μm). B. some epiderm rupture and lenticels start to open (Bar = 150 μm). C–D. open lenticels (Bar=150 μm). ar. aerenchyma; cc. complementary tissue; m. meristem; pe. periderm; s. suberized tissue. Arrows indicate rupture suberized layers with old complementary tissues.



Figure 3. Longitudinal section of pneumatophore at a lenticel (A, B). and complementary cells (C, D). Mass of complementary cells (cc) make regularly alignment and there is a intercellular space (arrows) between them and there is no intercellular matrix on this space.

As it growth, the complementary tissue ruptures the periderm and the lenticels become functional. After a while, a suberized layer was formed by the meristem beneath the complementary tissue (Fig. 2A) of the lenticel. Eventually the meristem begins to form complementary tissue inside this suberized layer which ruptures and its cast off along with all of the old complementary tissue (Fig. 2D).

Observation of longitudinal sections of pneumatophores of A. marina gave similar section. However results in cross the observation showed that the mass of complementary cells make regularly alignment and that there were intercellular spaces or gaps between the adjoining files of complementary cells. These spaces or gaps may provide a connection between the aerenchyma tubular structures in the cortex area (Fig. 3A and B). It observed also there was no intercellular matrix on these spaces.

Lenticels have an important role on the gas pathway of root of A. marina as first gate that related to atmosphere directly. The SEM observations on the present study revealed different stages of the lenticels (Fig. 2). I suppose that these are different stages in the development of the lenticels; the partiallyopened one (Fig2A), a partially-mature lenticels (Fig 2B) and the fully-opened one (Fig. 2C and D). From the structural analyses I suggest that a developmental process taking places in the lenticels. The young immature lenticels are closed, with a relatively low number of complementary cells. Later. more complementary cells are formed, creating an increasing pressure inside the lenticels. When the pressure is high enough, the cork breaks open, forming the partially-opened state of the partially-mature lenticels. As the complementary cells continue to be formed, the additional pressures enlarge the opening, creating the fully-opened mature lenticels. Although gas exchange can occur in the partially-mature lenticels, the fully-opened mature lenticels is probably more effective in the aeration process, as its larger opening and the larger amount of complementary cells inside the lenticels, allow more air to diffuse into the lenticels and to more rapidly. Chapman (1939, 1940), using light microscopy, describes

lenticels of *Avicennia nitida* with and without an opening, but associated them with different kinds of pneumatophores. Thus, the suggested development stages for lenticels of *A. Marina* may be similar in some or all the species in the genus.

The presence of the complementary cells with no intercellular matrix will encourages free diffusion of air between the cells, whose concave shape also helps to fulfill this function. Since the reaction to Safranin was positive, it is possible that, because of their suberin and or cutin cover, the accumulation of complementary cells may act as a hydrophobic layer through which air may diffuse while water penetration is prevented.

In the pneumatophore describe in the present study, lenticels arise further down the pneumatophore than most subrisules and are a more permanent site of conductance. Lenticels seem to be rejuvenated several times throughout their functional life. Some lenticels are probably non functional, especially in an older pneumatophores on which the growth of algae is profuse. Pneumatophores which are covered in algae have lenticels which grow out in a fashion which is termed hypertrophy. Hypertrophic lenticels are functional and evident on regions of pneumatophores which are inundated most of the time. Hypertrophic lenticels were not studied here since the interest was mostly with aerenchyma development.

The periderm of pneumatophores is both water and air tight resulting in gases being kept in the root system and water out. The periderm covers all of the penumatophore except at the sites conductance, lenticels. Since lenticels are large and complex organs of gas exchange, they take time to produce. Therefore lenticels are not found in the tip most section of pneumatophores. If the pneumatophore is actively growing, then the dividing cells at the tip have a high oxygen demand. This demand could be a significant sink of the oxygen which the pneumatophore is supplying to the rest of the isolated root system. It is likely that this demand is met by the order structure like subrisules or horizontal structures in the pneumatophores(Allaway et al., 2001; Hovenden and Allaway, 1994). The observation that functional pneumatophores which are not

actively growing do not posses subrisules supports this hypothesis.

The suberized layers whish are produced by the phellogen along with the complementary tissue in lenticels have been termed closing layers (Fahn, 1974). The production of a closing layer in the lenticels has been described previously (Fahn, 1974). Most authors postulate that the function of the closing layer is to hold together the rather loose aggregations of complementary cells. This may be the case in plant species which produce closing layers alternating regularly with lavers of complementary cells. The production of closing layer in the lenticels of A. marina is infrequent and may be a seasonal phenomenon. A closing layer is absent in many lenticels and I didn't found more than one intact closing layer in a lenticel such as was described for some species by Haberlandt (1914) although some lenticels have several rings of scar tissue which probably represent ruptured closing layer. The closing layer of A. marina is heavily suberized and it is likely that a lenticel with an intact closing layer is non-functional. In this species, the production of closing layer may function as an anti-fouling mechanism whereby the lenticels clears all setting algae and exposes a fresh conducting surface. Chapman (1940) referred to a closing layer at the point where the penumatophore emerges from cable root. This is a very different structure and the name should not be used for it. Three stages in the development of lenticels have been identified by Gordon and Dubinsky (1992), viz, immature, partially-mature and mature. I suggest an extension of this developmental series to include the production of a closing layer and its eventual rupturing. These two steps may occur many times during the life of lenticels.

The importance of lenticels for gas exchange has been demonstrated by measuring O_2 and CO_2 concentrations in the aerenchyma of *Rhizophora* roots. When the lenticels are occluded by smearing grease over the aerial potion of the root, O_2 declines continuously and CO_2 rises. Control roots showed fluctuations related to tidal level (Scholander *et al.*, 1955 and Hogarth 1999).

Conclusion

The development stages for lenticels of *A. Marina* may be similar in some or all the species in the genus. The complementary cells structures of mature lenticels help more effective in the aeration process, as its larger opening and the larger amount of complementary cells inside the lenticels, allow more air to diffuse into the lenticels and to more rapidly. This structure of lenticels fully supports the air conducting on the root system of *A. marina* to adapt on their habitat.

Acknowledgments

We thank to Prof. Tokushiro Takaso, University of Ryukyus and Prof. Mitsuo Suzuki, University of Tohoku for their great support in our field and laboratory research. We also thank Kazutaka Kobayashi, Takahisa Tanaka, Tomohiro Hasebe, Youichi Hasegawa, Masanori Seki and Hiroaki Terasawa for their field sampling assistance. Great appreciate for our colleague in Department of Biology, Faculty of Sciences and Technology, Universitas Airlangga for their supporting and pray.

References

- Allaway, W.G., Curran, M., Hollington, L.M., Ricketts, M.C. and Skelton, N.J. 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. Manag., 9: 211–218.
- Chapman, V.J. 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. 1940. The functions of the pneumatophores of *Avicennia nitida* Jacq. *Proc. Linn. Soc.*, 153: 228–233.
- Chapman, V.J. 1976. Mangrove vegetation. Cramer, Vaduz.
- Fahn, A. 1974. Plant Anatomy 4th Ed. Pergamon Press. New York.

Hery Purnobasuki

- Gordon, N.I.S. and Dubinsky, Z. 1992. Ultrastructure of the pneumatophores of the mangrove A. marina. South Afr. J. Bot., 58: 358-362.
 Gordon, N.I.S. and Z. Dubinsky, 1992. Ultrastructure of the pneumatophores of the mangrove A. marina. South Afr. J. Bot., 58: 358-362.
- Haberlandt, G. 1914. Physiology Plant Anatomy. London.
- Hogarth, P.J. 1999. The biology of the mangrove. Oxford University Press. Oxford-New York.
- Hovenden, M.J. and Allaway, W.G. 1994. Horizontal structures on the pneumatophores of *Avicennia marina* (Forsk.) Vierh. – A new site of oxygen conductance. *Ann. Bot.*, 73: 377–383.
- Mauseth, J.D. and Fujii, T. 1994. Resin-casting: A method for investigating apoplastic spaces. *Amer. J. Bot.*, 81: 104–110.
- Purnobasuki, H. and Suzuki, M. 2004. Aerenchyma formation and porosity in root of a mangrove plan, *Sonneratia alba J. Smith. J. Plant Res.*, 117: 465–472.

- Purnobasuki, H. and Suzuki, M. 2005a. Functional anatomy of air conducting network on the pneumatophores of a mangrove plant, *Avicennia marina* (Forsk.) Vierh. Asian J. Plant Sci., 4: 334–347.
- Purnobasuki, H. and Suzuki, M. 2005b. Aerenchyma tissue development and gas pathway structure in root of Avicennia marina (Forsk.) Vierh. J. of Plant Research, 118: 285–294.
- Rosner, S. and Kartusch, B. 2003. Structural changes in primary lenticels of Norway Spruce over the seasons. *IAWA J.* 24 (2): 105–116.
- Scholander, P.F., Van Dam, L. and Scholander, S.I. 1955. Gas exchange in the roots of mangroves. *Amer. J. Bot.*, 42: 92–98.
- Tomlinson, P.B. 1986. The botany of mangroves. Cambridge University Press. New York.