J Plant Res (2005) 118:285–294
Digital Object Identifier (DOI) 10.1007/s10265-005-0221-7

ORIGINAL ARTICLE

Hery Purnobasuki · Mitsuo Suzuki

Aerenchyma tissue development and gas-pathway structure in root of *Avicennia marina* (Forsk.) Vierh.

Received: February 4, 2005 / Accepted: June 8, 2005 / Published online: July 30, 2005

Abstract The aerenchyma differentiation in cable roots, pneumatophores, anchor roots, and feeding roots of the mangrove plant, *Avicennia marina* (Verbenaceae) was analyzed using a light microscope and scanning electron microscope. In all types, cortex cells were arranged in longitudinal columns extending from the endodermis to the epidermis. No cells in the cortex had intercellular spaces at the root tip (0–150 μm), and aerenchyma started developing at 200 μm from the root apex. The aerenchyma formation was due to cell separation (schizogeny) rather than cell lysis. The cell separation occurred between the longitudinal cell columns, forming long intercellular spaces along the root axis. During aerenchyma formation, the cortex cells enlarged longitudinally by 1.8–3.9 times and widened horizontally by 2.2–2.9 times. As a result, the aerenchyma had a pronounced tubular structure that was radially long, elliptical or oval in cross section and that ran parallel to the root axis. The tube had tapering ends, as did vessel elements, although there were no perforated plates. The interconnection between neighboring tubes was made by abundant small pores or canals that were schizogenous intercellular spaces between the wall cells. All aerenchyma tubes in the root were interconnected by these small pores serving as a gas pathway.

Key words Aerenchyma · *Avicennia marina* · Cortex cells · Radial pores · Schizogeny

Introduction

The gray mangrove, *Avicennia marina* (Forsk.) Vierh. (Verbenaceae), grows in estuaries where it is subject to tidal flooding and the soil is anaerobic. It develops a fairly complex root system that is quite similar to another mangrove plant, *Sonneratia alba* (Lythraceae). The gray mangrove has four types of roots: cable roots, pneumatophores, feeding roots, and anchor roots. Cable roots run horizontally and radially more than 10 m from the tree. Pneumatophores grow vertically upward from the cable roots and expose their tips to the air. Anchor roots grow vertically down to a depth of about 1 m from the cable roots. Feeding roots originate from the pneumatophores just under the ground surface and grow horizontally. At low tide, lenticels on the surface of the pneumatophores exchange gas between the atmosphere and internal root structure.

Roots of mangrove plants typically have an aerenchymatous cortex (Justin and Armstrong 1987; Hogarth 1999; Allaway et al. 2001). Aerenchyma is thought to be an important adaptation to flooded soils because it provides a mechanism for root aeration in an environment characterized by low oxygen concentrations (Pezeshki 2001; Vartapetian et al. 2003) and acts as a pathway for oxygen in respiring roots (Curran 1985; Curran et al. 1986). Despite the importance of aerenchyma for the survival of mangrove species, little is known about the development and organization of aerenchyma in mangrove root systems.

The manner of aerenchyma formation varies between species, with one characteristic being that aerenchyma can be produced in either a constitutive or an induced manner (Webb and Jackson 1986; Justin and Armstrong 1987; Drew et al. 2000). For many wetland species, formation of extensive aerenchyma is an integral part of normal root development and is considered constitutive and pre-adaptive (Jackson and Armstrong 1999). In other species, root aerenchyma is induced by environmental stress, notably low oxygen concentrations (Armstrong et al. 1994; Vartapetian et al. 2003).

Classically, formation of intercellular space is thought to be the result of schizogeny and lysigeny during development (Fahn 1990; Drew et al. 2000; Vartapetian et al. 2003). Schizogeny is the outcome of highly regulated and species-specific patterns of cell separation and differential cell expansion creating spaces between cells. In contrast,

H. Purnobasuki (✉)
Biologi FMIPA, Universitas Airlangga, Jl. Mulyorejo (Kampus C Unair), Surabaya, 60115, Indonesia
Tel. +62-31-5926804; Fax +62-31-5926804
e-mail: hery_purnobasuki@lycos.com

M. Suzuki
Botanical Garden, Graduate School of Science, Tohoku University, Kawauchi, Aobaku, Sendai 980-0862, Japan

lysigenous space arises from spatially selective death of grown cells. Lawton et al. (1981) reported that gas spaces in *Avicennia* are lysigenous in origin, while Curran (1985) reported a schizogenous origin. There is also no information about the three-dimensional networks of gas spaces in the root system of mangrove plants, because most studies are based only on observations using root cross sections.

This study examines the tissue differentiation of the four types of roots from the apical meristems and the three-dimensional structure of a mature aerenchyma system in *Avicennia marina* to discuss the relationships between tissue structure and habitat adaptation.

Materials and methods

All root samples were collected from adult trees (10–15 cm in trunk diameter at base, 0.5–1 m in height) of *Avicennia marina* growing naturally in Urauchi (24°23′ N, 123°46′ E) and the Komi estuary (24°19′N, 123°54′E) of Iriomote Island, Okinawa Prefecture, Japan, in March and December 2002 and July 2003. Sampled trees were distributed sparsely in the outer fringes of a coastal mangrove forest where the trees were flooded by high tides and easily affected by strong winds and tidal forces. The root systems of the trees were excavated during low tide, and cable root tips, pneumatophore tips, feeding root tips and anchor root tips were collected (Fig. 1). Five adult trees were used as samples, and individual samples of each root type were collected from more than five cable roots.

Light microscopy

The samples were prepared by the ordinary paraffin sectioning method. They were fixed in FAA (70% ethanol, 10% formalin, 5% acetic acid at ratio of 90:5:5), dehydrated through a graded series of ethanol and embedded in Paraplast Plus (Oxford Labs, USA) at 59°C. Sections were cut at a thickness of 10–12 μm with a rotary microtome (HM 350 Microm, Heidelberg, Germany), stained with safranin-fast green (O'Brian and McCully 1981; Sanderson 1994), and then permanently mounted with Bioleit (Oken Shoji, Tokyo). Observation was made under a light microscope (BX 50 Olympus, Japan). Microscopic images were recorded on Neopan F film (Fuji Film ISO 32/16°) for black and white prints with a microscope camera (Olympus PM-C35, Japan).

The size measurements and counts were made on the cortex cells of the different root types and were performed for five roots of each root type using a computer-assisted image analysis system (IPLab Version 3.5 Scientific Imaging Software) on digitized images taken by a digital camera attached to a compound microscope (BX 50 Olympus, Japan).

SEM observation

The resin casting method (Fujii 1993; Mauseth and Fujii 1994) was used for SEM observation. Cable roots and pneumatophores were cut into cubes of about 5–8 mm in length on each side using a razor blade, dehydrated through a graded series of ethanol and *t*-butyl alcohol, and then freeze-dried at −10°C (HITACHI ES-2030 Freeze Dryer). The dried samples were immersed in styrene monomer and polyester resin with 1% benzoyl peroxide within gelatin capsules, evacuated for several minutes, and then polymerized in an oven at 60°C overnight. After removal of the gelatin capsule and trimming using a chisel and sandpaper, the samples were immersed in a 1:1 mixture of hydrogen peroxide and acetic acid for 1 day or more in an oven at 60°C to remove lignin. They were washed in water and treated with 64% sulfuric acid for 1 day to remove cell-wall polysaccharides. The obtained resin casts were rinsed with water and cleaned by agitation in water using a mini-supersonic cleaner. The samples were dehydrated again through a graded series of ethanol and *t*-butyl alcohol and freeze-dried. They were glued on specimen stubs, coated with platinum–palladium in a vacuum evaporator (HITACHI E-1030 Ion Sputter), and then observed and photographed using a scanning electron microscope (HITACHI S-4100 SEM).

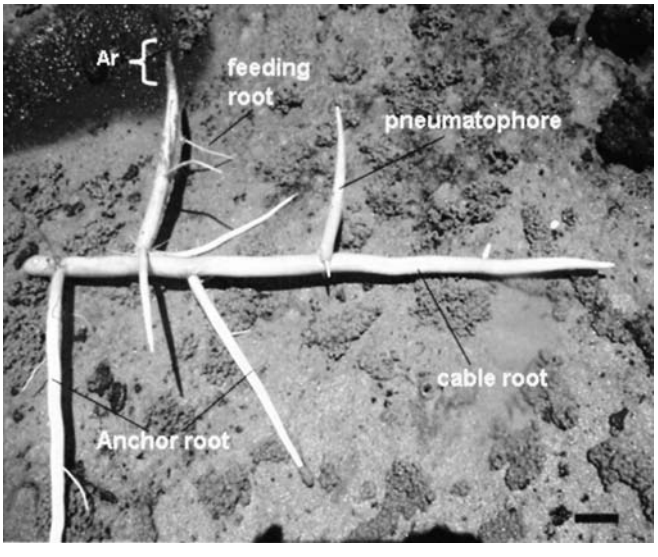

Fig. 1. Part of root system of *Avicennia marina* taken close to tip of cable root. *Bar* = 2 cm. *Ar* Aerial part of pneumatophore

Results

Root apical meristem

The root apical meristems of the four root types of *Avicennia marina* had fundamentally the same structure, except for root size. Cable roots were the thickest (3.63 mm in average diameter) at 2 mm from the root apex while feeding roots were finest (1.51 mm), and pneumatophores (2.06 mm) and anchor roots (1.89 mm) were intermediate

Table 1. Root diameters and cell numbers at different distances behind the root tip in four root types of *Avicennia marina*

Root types and distances behind the tip (mm)	Diameter (mm)	Thickness of cortex (mm)	Number of epidermal cells surrounding a root (in cross section)[a]	Number of radial files of cells in cortex (longitudinal section)[b]
Feeding roots				
2	1.51 ± 0.01	0.39 ± 0.01	272 ± 36	18.3 ± 0.5
6	2.01 ± 0.01	0.61 ± 0.01	326 ± 34	15.8 ± 0.4
10	2.32 ± 0.01	0.87 ± 0.02	392 ± 46	14.1 ± 0.8
14	2.56 ± 0.06	0.91 ± 0.01	395 ± 39	13.9 ± 0.3
18	2.85 ± 0.04	0.95 ± 0.01	396 ± 57	13.4 ± 0.5
20	3.02 ± 0.01	1.03 ± 0.06	402 ± 59	11.4 ± 0.5
Anchor roots				
2	1.89 ± 0.01	0.51 ± 0.01	344 ± 25	30.1 ± 1.2
6	2.52 ± 0.01	0.85 ± 0.05	385 ± 37	28.8 ± 0.8
10	3.13 ± 0.01	1.02 ± 0.05	404 ± 48	27.6 ± 0.8
14	3.79 ± 0.07	1.07 ± 0.02	428 ± 49	27.2 ± 0.4
18	4.23 ± 0.03	1.11 ± 0.02	430 ± 44	26.4 ± 0.5
20	4.86 ± 0.01	1.23 ± 0.04	433 ± 49	25.9 ± 0.5
Cable roots				
2	3.63 ± 0.01	0.95 ± 0.04	697 ± 45	45.1 ± 0.8
6	4.52 ± 0.01	1.22 ± 0.02	809 ± 55	43.2 ± 0.6
10	6.85 ± 0.02	1.34 ± 0.03	919 ± 63	39.8 ± 0.6
14	7.14 ± 0.03	1.43 ± 0.03	920 ± 52	39.8 ± 0.3
18	7.64 ± 0.03	1.53 ± 0.02	922 ± 53	39.4 ± 0.5
20	8.01 ± 0.01	1.79 ± 0.04	925 ± 66	38.2 ± 0.4
Pneumatophores				
2	2.06 ± 0.02	0.55 ± 0.01	381 ± 42	36.3 ± 0.5
6	3.13 ± 0.02	0.86 ± 0.02	409 ± 40	33.6 ± 0.8
10	3.88 ± 0.02	0.95 ± 0.03	519 ± 51	29.6 ± 0.5
14	4.38 ± 0.09	1.05 ± 0.06	524 ± 49	29.3 ± 0.4
18	5.59 ± 0.07	1.31 ± 0.02	527 ± 39	28.9 ± 0.3
20	6.35 ± 0.02	1.49 ± 0.04	530 ± 49	28.7 ± 0.5

[a]Measured by counting all the cells in epidermal layer as seen in Fig. 3a–c
[b]Measured by counting the columns of cell files as seen in Figs. 2 and 3d

(Table 1). The organization of the root apical meristem showed a closed system with an apparent boundary between protoderm and root cap (Fig. 2). The characteristic feature of the root was the distinct and extensive root cap, extending up 150 to 400 µm.

A broad lacunose cortex was another characteristic of the cross section of roots. Lacunae were distributed between hypoderm and endodermis and arranged in a honeycomb-like structure in all root types (Figs. 3, 4a–c). The lacunae were designated as aerenchyma because there was no liquid content when sample pieces were cut directly from excavated roots.

Aerenchyma development

Root tissues became differentiated with distance from the root apex. Root diameter increased almost linearly from 2 to 20 mm from the root apex, and was 2.0–3.1 times thicker at 20 mm than at 2 mm (Table 1 and Fig. 5a). Due to the increase in root diameter, the number of epidermal cells also increased up to around 10 mm from the root apex (Fig. 5c) and was only 1.3–1.5 times greater than at 2 mm (Table 1 and Fig. 5c). The tangential diameter of the epidermal cells at 20 mm was 1.4–1.5 times that at 2 mm. Therefore the increase in the circumference of the root resulted from both the increase in cell number and cell size.

All cells in the cortex were apparently adjacent to each other in cross section near the root apex (0–100 µm from root apex). The cortex cells were round to isometric and lacked intercellular spaces (Fig. 4a) and were aligned in columns of longitudinal layers (cortical cell column). The layers were similarly extended between the endodermis and epidermal layers despite differences in root types (Figs. 2, 4d). The average number of cell layers varied among the four root types according to root diameter as follows: 18 (feeding roots), 30 (anchor roots), 36 (pneumatophores), and 45 (cable roots) at 2 mm from the root apex (Table 1).

The cortex also became thicker with increasing root diameter (Table 1 and Fig. 5b). The cortex width at 20 mm from the apex was 1.8–2.7 times larger than at 2 mm from the apex. Cortical cells at 0–200 µm from the apex were 9.3–20.5 µm and 18.7–21.3 µm in longitudinal and radial diameters, respectively (Table 2). These cells were enlarged about 1.8–3.9 times in the longitudinal direction and 2.2–2.9 times in the radial direction at 10–20 mm from the root apex. In contrast to the cell diameter increment, the number

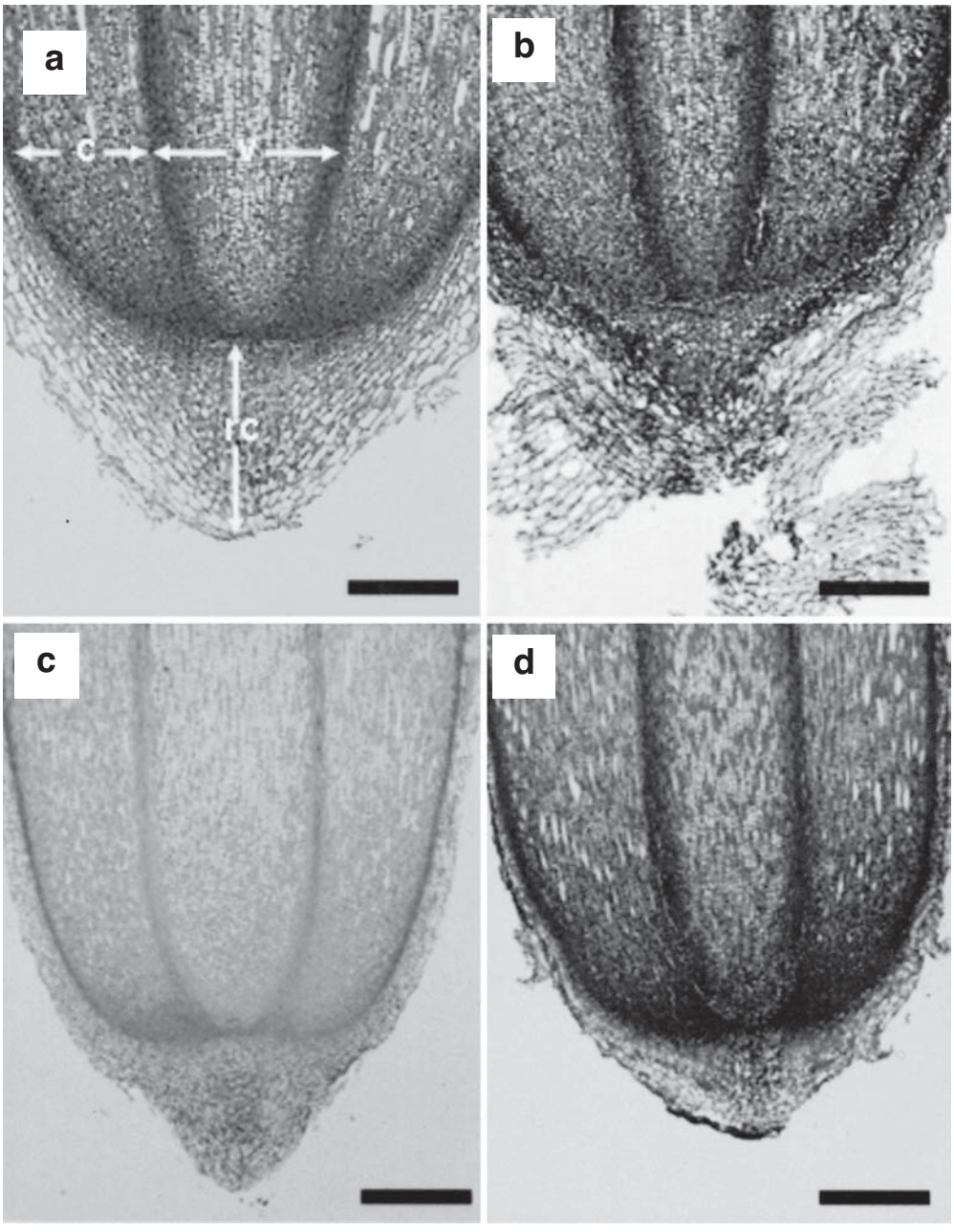

Fig. 2a–d. Median longitudinal sections of *Avicennia marina* root tips showing closed-type root structure with prominent root caps. **a** Feeding root (*bar* = 100 µm). **b** Anchor root (*bar* = 100 µm). **c** Cable root (*bar* = 400 µm). **d** Pneumatophore (*bar* = 400 µm). *c* Cortex, *rc* root 44cap, *v* vascular cylinder

of cortical cell layers decreased slightly during root development when counted in a cortex transect (Table 1 and Fig. 5). This decrease in cortical cell files was due to their looser, more irregular arrangement with increasing intercellular spaces (compare Fig. 4a–c).

The initial formation stage of intercellular space was observed within 200 µm from the root tip in all root types. Beyond this, cells separated more prominently followed by radial and longitudinal expansion of cells with arm formation, resulting in enlargement of intercellular space to form aerenchyma (Fig. 4). There was no observed collapse or dissolution of cells in the cortex in any of the four root types, suggesting a schizogenous origin of intercellular space. Random schizogenous cell separation was observed in the cortex cross section at 3 mm from the root apex (Fig. 4a–c). The intercellular spaces were radial, long and slit-like in the early stage (Fig. 4a), becoming wider with increasing distance from the root apex (Fig. 4b), and finally becoming large and radially elongated, elliptical or oval lacunae at maturity (Fig. 4c). The size of mature aerenchyma in cross section varied among root types and was widest in cable roots (253 and 115 µm in radial and tangential diameter, respectively) and narrowest in anchor roots (135 and 49 µm); pneumatophores (189 and 81 µm) and feeding roots (143 and 56 µm) were intermediate (Table 3).

In longitudinal section, the appearance of cell separation was dissimilar to that in the cross section. The cortical cells were arranged in longitudinal rows called cortical cell col-

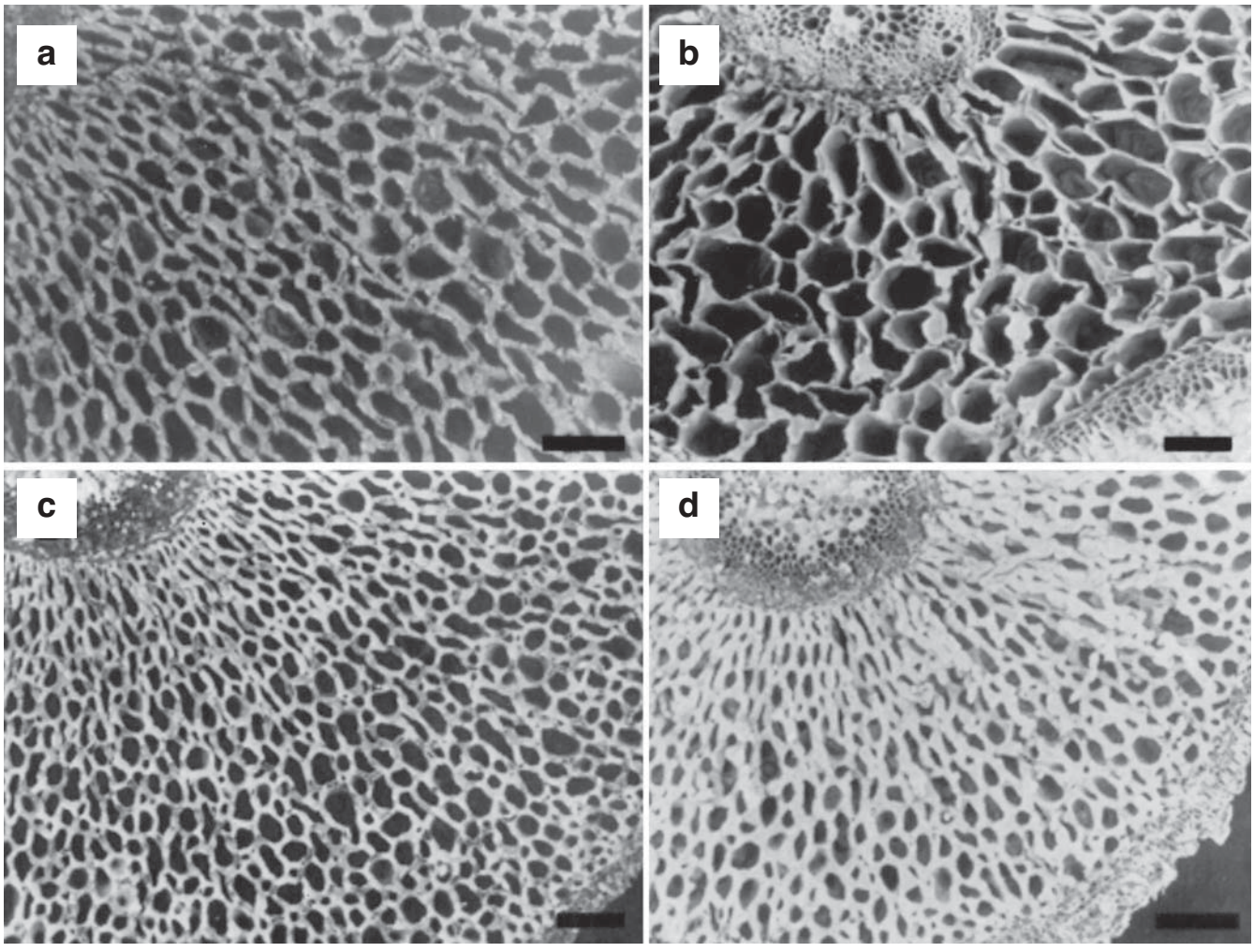

Fig. 3. SEM observation of cross sections of *Avicennia marina* roots showing honeycomb-like arrangement of aerenchyma in cortical area. **a** Cable root (*bar* = 333 μm). **b** Feeding root (*bar* = 150 μm). **c** Pneumatophore (*bar* = 300 μm). **d** Anchor root (*bar* = 250 μm)

Table 2. Cortex cell size from longitudinal sections taken at distances of 0–0.2 mm (no intercellular spaces) and 10–20 mm (well-developed intercellular spaces) from the root apex of *Avicennia marina*

Root types	Distance from the apex meristem					
	0–0.2 mm		10–20 mm		Ratio	
	Longitudinal	Radial	Longitudinal	Radial	Longitudinal	Radial
Feeding roots						
Mean (μm)	9.3	17.9	36.0	39.6	3.9	2.2
SD	0.4	2.1	6.7	5.7		
Anchor roots						
Mean (μm)	13.7	21.3	37.5	47.0	2.7	2.2
SD	1.9	2.1	3.1	6 .1		
Cable roots						
Mean (μm)	20.5	18.7	36.7	54.0	1.8	2.9
SD	3.3	2.2	3.6	3.6		
Pneumatophores						
Mean (μm)	17.4	18.7	44.5	44.8	2.6	2.4
SD	2.4	3.5	8.3	3.8		

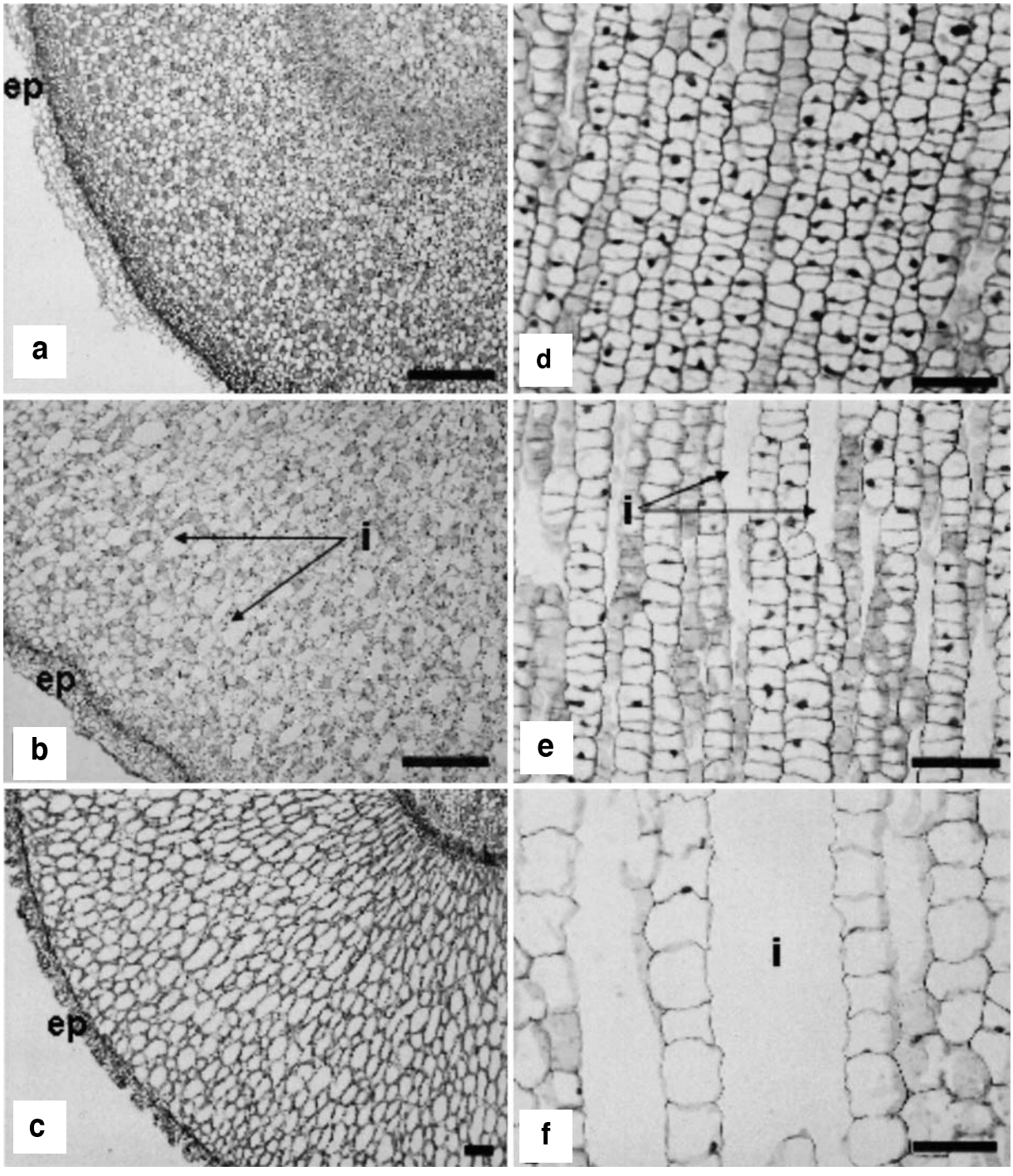

Fig. 4. Serial cross sections (**a**–**c**, *bars* = 200 μm) and longitudinal sections (**d**–**f**, *bars* = 50 μm) of pneumatophores of *Avicennia marina* at different distances from root apex to show development of intercellular space (aerenchyma) in cortex. **a** 3 mm. **b** 9 mm. **c** 20 mm. **d** 0.2 mm. **e** 6 mm. **f** 12 mm. *ep* Epidermis, *i* intercellular space

Table 3. Total average of aerenchyma diameter in mature roots of *Avicennia marina*

Root types	Distance from the root apex (mm)	Radial diameter (μm)	Tangential diameter (μm)
Feeding roots	20	143.5 ± 34.7	56.1 ± 16.2
Anchor roots	20	134.5 ± 25.4	49.3 ± 8.6
Cable roots	50	252.5 ± 45.2	115.2 ± 16.7
Pneumatophores	50	235.1 ± 42.2	103.5 ± 14.1
Average		188.9 ± 58.8	80.95 ± 23.3

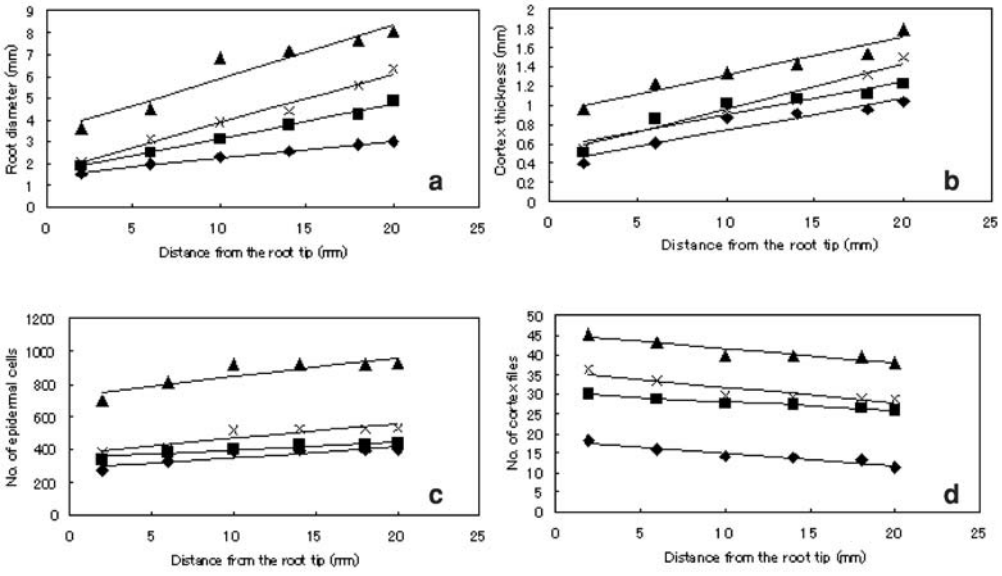

Fig. 5. Root diameter (**a**), cortex thickness (**b**), cell number of epidermal cells (**c**) and number of cortex files (**d**) at different distances from root tip in four root types of *Avicennia marina*. *Triangles* Cable roots, *crosses* pneumatophores, *squares* anchor roots, *diamonds* feeding roots

umns (Fig. 4d). From 0–200 µm from the root apex, there were few intercellular spaces and cortical cells were tightly packed (Fig. 4d). Beyond 6 mm from the root apex, cortical cells were clearly separated from each other and there were some intercellular spaces in the longitudinal direction (Fig. 4e, f). The cell separation developed regularly along the cortical cell columns, forming longitudinal long intercellular spaces (Fig. 4e, f).

Aerenchyma structure

The intercellular aerenchyma formed elongated tubes or canal structures with an elliptical-to-oval cross section and indeterminate length along the root axis (Fig. 3). A resin cast of this canal structure revealed ends (overlapping tips) that looked just like vessels (Fig. 6a–d). The canal structures were connected longitudinally to each other at the overlapping tips by very fine canals, but no perforated plates or transverse septa were observed at the tips (Fig. 6c, d). An 8-mm resin-cast sample showed some canals terminated at various heights while others were broken at the connection sites. Aerenchyma elements at the terminal were about 1.3–2.1 mm in length.

The aerenchyma canal was delineated by a layer of cortical cells (aerenchyma wall cells). The aerenchyma wall cells were thin-walled, transversely long rectangular or polygonal in lateral view (Fig. 7a, b) and I-, X-, Y-, or T-shapes in transverse view (Fig. 7d). In *Avicennia marina*, the aerenchyma canal was usually composed of four to six wall cells. The lateral inner surface of aerenchyma was sculptured with these wall cells in transversely elongated rectangular or polygonal shapes (Figs. 6a, 7b). The connection between aerenchyma canals was formed by very fine (7–8 µm in diameter) horizontal intercellular spaces (radial pores) formed by two or three adjoining wall cells (Fig. 7b, c).

Discussion

Aerenchyma development

Aerenchyma gas space is important for wetland plants because it allows for gas exchange in roots under anaerobic conditions. Aerenchyma is formed either via schizogeny or lysigeny. Schizogenous intercellular space is the outcome of highly regulated and species-specific patterns of cell separation and differential cell expansion, while lysigenous space is caused by spatially selected cell death (Armstrong 1979; Jackson and Armstrong 1999). Although there are few studies on air conduction and the static morphology of differentiated aerenchyma tissues in the root system of mangrove plants, few data exist on the developmental anatomy of aerenchyma formation. Lawton et al. (1981) studied the physiological anatomy of *Avicennia marina* in relation to ion transportation in its root system and reported that the air spaces in the root cortex were random in transverse section and lysigenous in origin. Their paper is the only work claiming a lysigenous origin of root-cortex aerenchyma in this species. A schizogenous origin of root-cortex aerenchyma in this species has been suggested in several papers (e.g., Baylis 1950; Curran 1985) based on anatomical observation.

This study found no cell destruction or lysis of cortical cells during tissue differentiation of the root cortex. Furthermore, alignment of cells in longitudinal columns was found just behind the root apex (Fig. 4d). The number of cell columns per unit area in more mature parts of roots was similar to that of younger parts (Table 1, Fig. 5d). The regular alignment of longitudinal cell columns in every part of the root cortex (Fig. 4d–f) indicates that periclinal division does not occur in cells after the establishment of regular cell alignment just behind the root apex. Along with development, the cell columns separated from the adjacent

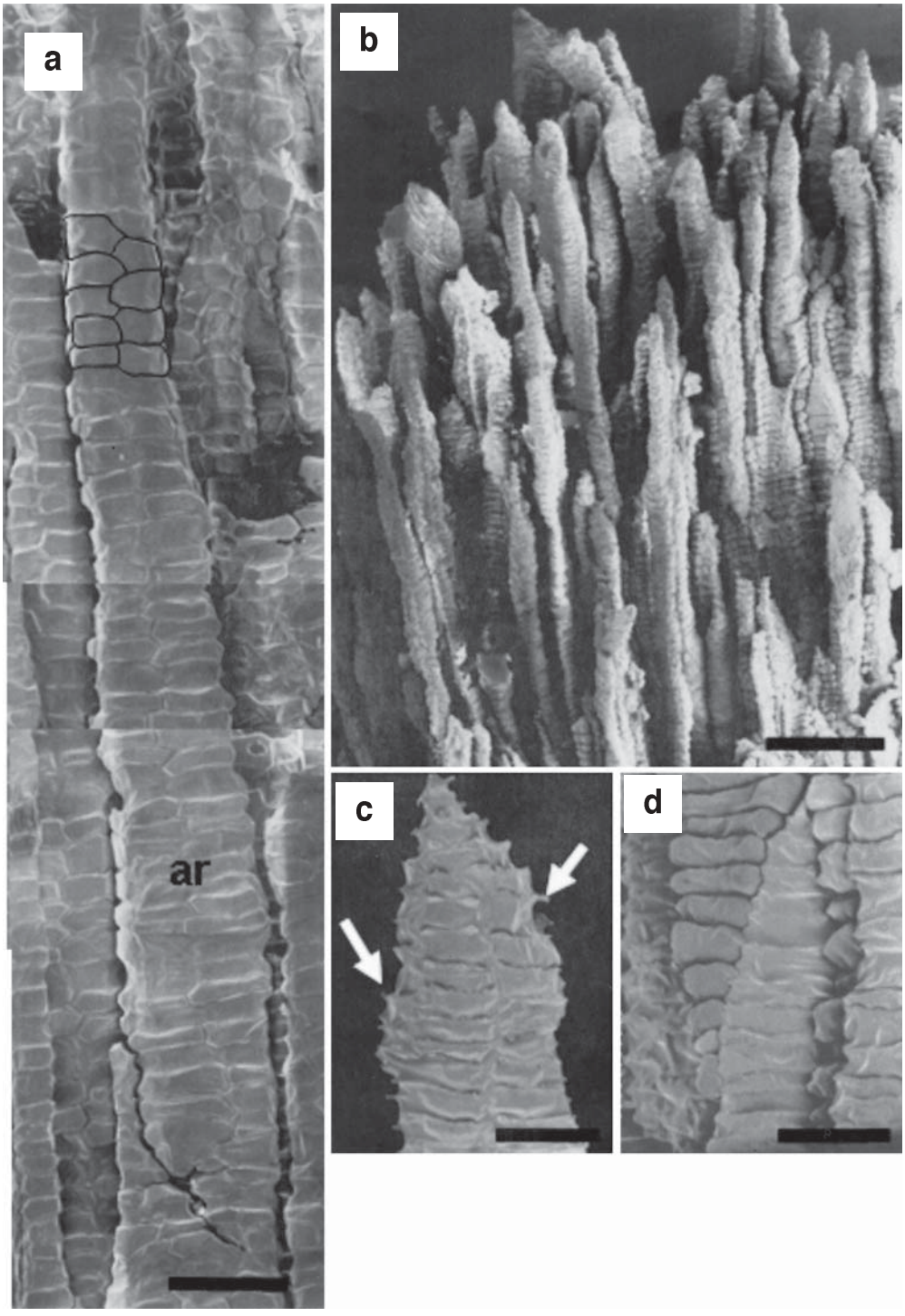

Fig. 6a–d. Longitudinal view of aerenchyma resin cast of cable root of *Avicennia marina* by SEM observation. **a** Inner surface view of tubular structure of aerenchyma (*ar*). There are no transverse septa interrupting longitudinal tubes. *Lines* drawn to emphasize impressed cell shapes of aerenchyma wall cells (*bar* = 200 µm). **b** Tips of aerenchyma tubes run parallel to root axis (*bar* = 420 µm). **c** Tip of aerenchyma tube without perforation. Fine prickle-like protrusions on surface of tips (*arrow*) are residues of fine pores (*bar* = 75 µm). **d** Overlapping tips between two tubes (*bar* = 75 µm)

columns, forming longitudinal intercellular spaces between them. This type of schizogenous aerenchyma formation was observed in all four *Avicennia marina* root types. The same condition has been observed in some wetland plants, such as *Sagittaria lancifolia*, *Thalia geniculata*, and *Pontederia cordata* (Longstreth and Borkhsenious 2000), *Filipendula ulmaria* and *Caltha palustris* (Smirnoff and Crawford 1983), as well as in another mangrove plant, *S. alba* (Purnobasuki and Suzuki 2004), with a similar root system to *A. marina*. Because Lawton et al. (1981) did not explain why they regarded aerenchyma in *A. marina* as being of lysigenous origin, it is difficult to reconcile this disagreement.

The differentiation of aerenchyma in the cortex followed the same general pattern in all root types we examined. Well-developed intercellular space was due to the great extension of cortical cells (aerenchyma wall cells) in the transverse direction with arm formation creating the T-, X-, I-, and Y-shaped wall cells (Table 2, Fig. 7d) as reported

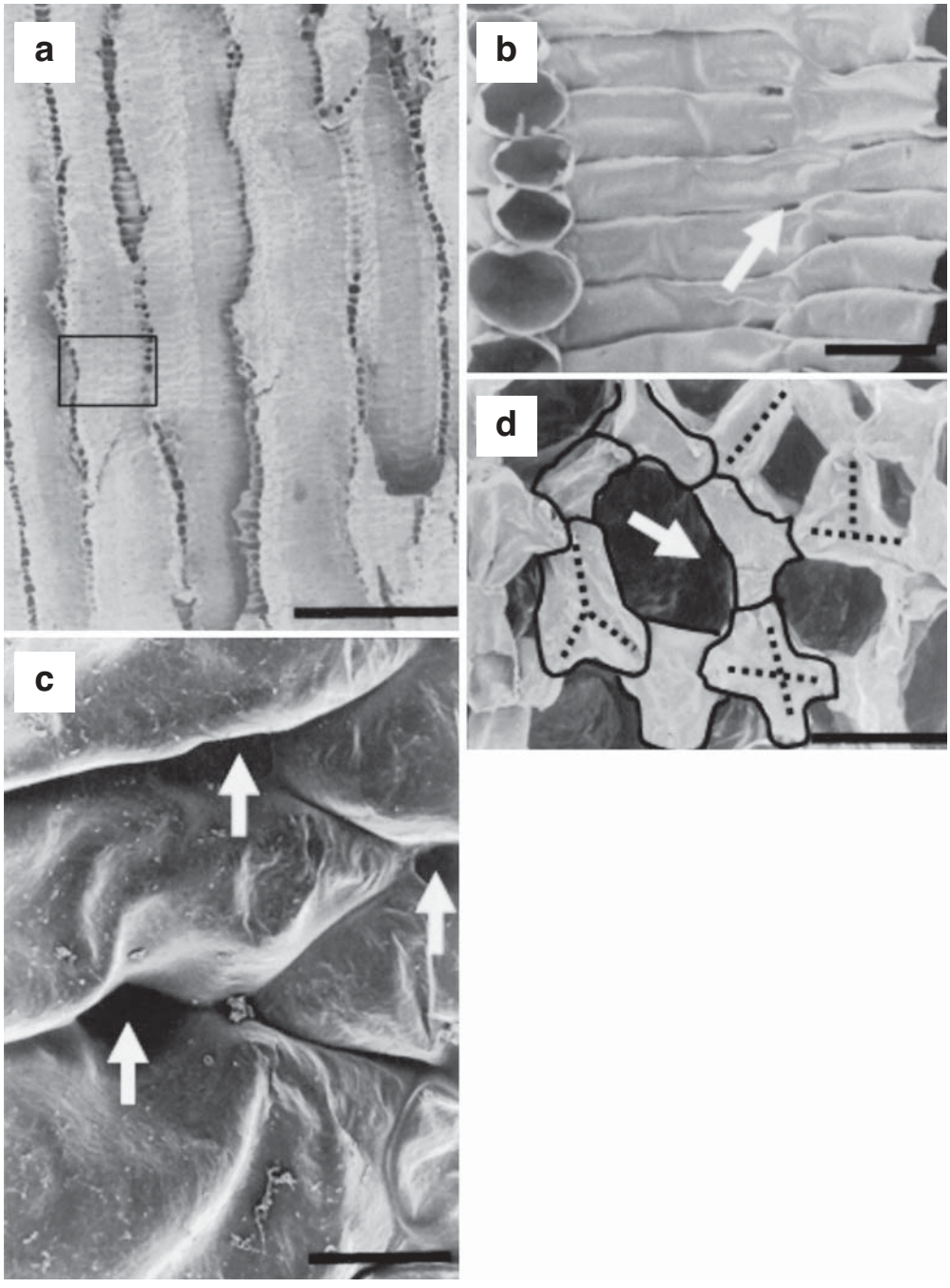

Fig. 7a–d. SEM observation of columnar cells with aerenchyma of cable root in *Avicennia marina* (not resin cast) showing pores in the walls of aerenchyma tubes. **a** Longitudinal section of cable roots at 4 cm from the tip (*bar* = 375 μm). **b** Magnification of the *marked area* of **a** (*bar* = 33 μm). **c** Higher magnification of aerenchyma wall cells showing the radial pores (*arrow*) (*bar* = 15 μm). **d** Cross section of cable root at 4 cm from the tip showing the position of radial pores (*arrow*) (*bar* = 60 μm). *Drawn lines* demarcate the cell shapes that composed the aerenchyma lacunae and the *dashed lines* show the I-, X-, Y-, and T-shaped wall cells united by the ends of their arms

by Baylis (1950). The "arm" cells were tightly packed at the arm tips forming a cell network with gas spaces in cross section looking just like the spongy tissue of leaves.

Aerenchyma structure

A main result of this study is the three-dimensional view of aerenchyma in the root cortex of *Avicennia marina* achieved by resin casting. The aerenchyma structure agrees fundamentally with descriptions based on observations made by Baylis (1950) using a light microscope, which noted "meshes of this network prove to be lacunae of limited extent." Based on SEM observations, Curran (1985) noted that the longitudinal tubular structure found in the aerenchyma is unlike the network of lacunae of limited extent described by Baylis (1950). Curran (1985) speculated that the reason for this disagreement was either the difference in age and growth conditions of the examined plants, or the difficulty of reconstructing three-dimensional images using a light microscope. Actually, inclined thin sections of tissues with long structures, such as vessels or aerenchyma, portray

only a limited length as shown in Fig. 10 of Baylis (1950). As shown in the present study, the aerenchyma system in the root cortex of *A. marina* is composed of abundant long canals or tubes running parallel to the long axis of the root, but apparently not in radial layers as reported for *A. nitida* (Chapman 1939, 1940). Although many researchers (e.g., Baker 1915; Curran 1985) consider aerenchyma tubes or canals as fairly long, no one has referred to the actual length. Our observation using resin casts (Fig. 6) gives a useful hint about length; since many tubes had one end within an 8-mm length (Fig. 6b), the full length is expected to be no more than 16 mm. This shortness is quite different with some herbaceous wetland plants such as *Nelumbo*, in which the major aerenchyma is very long (more than 30 mm) and fully extends throughout the petiole length.

The "sheet of cells" illustrated by Baylis (1950) can be reinterpreted as the wall cells of the aerenchyma tubes as Curran (1985) pointed out. The lateral SEM view of the cortical tissue clearly shows that the aerenchyma wall is constituted compactly of longitudinally short and transversely elongated wall cells looking like logs in the wall of a log cabin (Fig. 7b) with small "windows or ventilation holes" between the "logs" (Fig. 7c). Baylis (1950) noticed that "the sheet of cells separating them is freely perforated by small intercellular pores." Although Baylis (1950) described the pores in aerenchyma of pneumatophores, this study showed the presence of aerenchyma in the cortex cells of all root types. As Curran (1985) stated, these pores function as interconnections between tubes and support the radial movement of gas in aerenchyma tubes.

The tubular structure of aerenchyma helps explain the close agreement in cable roots between measured diffusion rates and rates predicted from simple theoretical models proposed by Curran (1985) that do not allow for tortuousity. Cortical cells are usually elongated along the root axis and joined together in files several or many cells long. Consequently, the longitudinal intercellular spaces are essentially tubular and nontortuous in character.

The highly elongated tubular structure is extensively connected, so the gas space forms a continuum creating internal long apoplastic gas-transport pathways with low impedance. This condition minimizes the risk of asphyxiation (lack of oxygen) in inundated organs. Moreover, the structure of aerenchyma is ideal for fulfilling the dual functions of a transport system and oxygen storage for respiration at high tides.

Acknowledgements We thank Prof. Tokushiro Takaso, Research Institute for Humanity and Nature, Kyoto, and the staff of Iriomote Station, Tropical Biosphere Research Center, University of Ryukyus, for their support in our field research. We also thank Kazutaka Kobayashi, Takahisa Tanaka, Youichi Hasegawa, Masanori Seki and Hiroaki Terasawa for their assistance in field sampling.

References

Allaway WG, Curran M, Hollington LM, Ricketts MC, Skelton NJ (2001) Gas space and oxygen exchange in roots of *Avicennia marina* (Forssk.) Vierh. var. *australasica* (Walp.) Moldenke ex N.C. Duke, the grey mangrove. Wetland Ecol Manage 9:211–218

Armstrong W (1979) Aeration in higher plants. Adv Bot Res 7:275–332

Armstrong W, Brändle R, Jackson MB (1994) Mechanism of flood tolerance in plants. Act Bot Neerl 43:307–358

Baker RT (1915) The Australian "grey mangrove". Proc R Soc NSW 78:509–514

Baylis GTS (1950) Root system of the New Zealand mangrove. Trans R Soc NZ 78:509–514

Chapman VJ (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 VJ (1940) The functions of the pneumatophores of *Avicennia nitida* Jacq. Proc Linn Soc 153:228–233

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

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

Drew MC, He CJ, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. Trend Plant Sci 5:123–127

Fahn A (1990) Plant anatomy, 4th edn. Pergamon Press, New York, 588 pp

Fujii T (1993) Application of a resin casting method to wood anatomy of some Japanese Fagaceae species. IAWA J 14:273–288

Hogarth PJ (1999) The biology of mangroves. Oxford University Press, Oxford, 228 pp

Jackson MB, Armstrong W (1999) Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. Plant Biol 1:274–287

Justin HFW, Armstrong W (1987) The anatomical characteristics of the roots and plant response to soil flooding. New Phytol 106:465–495

Lawton JR, Todd A, Naidoo DK (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 DJ, Borkhsenious ON (2000) Root cell ultrastructure in developing aerenchyma tissue of three wetland species. Ann Bot 86:641–646

Mauseth JD, Fujii T (1994) Resin-casting: a method for investigating apoplastic spaces. Am J Bot 81:104–110

O'Brian T, McCully ME (1981) The study of plant structure: principles and selected methods. Termacarphi, Melbourne

Pezeshki SR (2001) Wetland plant responses to soil flooding. Environ Exp Bot 46:299–312

Purnobasuki H, Suzuki M (2004) Aerenchyma formation and porosity in root of a mangrove plant, *Sonneratia alba* J. Smith. J Plant Res 117:465–472

Sanderson JB (1994) Biological microtechnique. Bios Scientific, Oxford, 224 pp

Smirnoff N, Crawford RMM (1983) Variation in the structure and response to flooding of root aerenchyma in some wetland plants. Ann Bot 51:237–249

Vartapetian BB, Andreeva IN, Generozova IN, Polyakova LI, Maslova IP, Dolgikh YI, Stepanova AY (2003) Functional electron microscopy in studies of plant response and adaptation to anaerobic stress. Ann Bot 91:155–172

Webb J, Jackson MB (1986) A transmission and cryo-scanning electron microscopy study of the formation of aerenchyma (cortical gas-filled space) in adventitious roots of rice (*Oryza sativa*). J Exp Bot 37:832–841