Anatomical Characteristics of *Gigantochloa scortechinii* Bamboo Rhizome in Relation with Hydraulic Conductance

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Structural development and modification of bamboo culm's anatomical characteristics occur during the maturation period. This process affects the conductivity efficiency in individual bamboo culms (above ground). The present study clarified this process in the sympodial type of bamboo rhizome (belowground). This study aimed to observe the anatomical characteristics of *Gigantochloa scortechinii* rhizome, examine their relationship with different study sites and rhizome ages, and investigate their relationship with hydraulic conductance. Destructive sampling on four consecutive rhizomes was conducted using a selective random sampling method. All rhizome anatomical characteristics were significantly different between study sites except parenchyma diameter, parenchyma lumen diameter, and fiber cell wall thickness. The results also indicated that the vascular bundle diameter, parenchyma diameter, parenchyma lumen diameter, parenchyma cell wall thickness, fiber diameter, fiber cell wall thickness, and fiber length increased with age, but radial to tangential ratio decreased with age. All measured characteristics including the conductance elements had no relationship with hydraulic conductance, except parenchyma diameter and parenchyma lumen diameter. The sizes parenchyma diameter and lumen diameter did not imply a determinant factor in hydraulic conductance. Further studies on rhizome chemical attributes should be carried out to isolate the cause of decreasing hydraulic conductance.

Keywords: Fiber; Parenchyma; Phloem; Vascular bundles; Xylem

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INTRODUCTION

The quality and health of a bamboo stand can be quantified based on the number of culms and age ratio in a clump, maximum growth of morphometric parameters, vigor, and physiological activity (Azmy *et al*. 1997; Ding *et al*. 1997; Banik and Islam 2005). The quality and health of stand in a clump have a close relationship with rhizome growth. Rhizomes are generally subterranean and form as the foundational organ for bamboo species by allowing rapid growth at the beginning of a new growing season. Rhizomes are important for nutrient uptake, storage, water absorption and conductance, and the vegetative reproduction system (Li *et al*. 1998; Liese 1998).

The vegetative reproduction strategy in sympodial bamboo species is to form a large number of rhizomes that are interconnected with each other and hence enable sustainable production. The growth performance, productivity, and life cycle of an individual rhizome in a bamboo clump are strongly related to the ontogenetically and physiologically age-related factors. For example, a new bamboo sprout in a 20-year-old bamboo clump is ontogenetically old but it is physiologically young (Londona 1992; Liese and Weiner 1996; Schweingruber 2007). The growth performance of new sprouts, however, solely depends on the stimulation of rhizome buds (encompass precipitation, relative humidity, soil moisture, and soil fertility) and the sources of energy and nutrients from its interconnected rhizomes. The new sprout has neither photosynthetic leaves nor a rooting system to ensure its rapid elongation (Zhang *et al.* 1996; Rodrigues *et al.* 2003).

Structural development and modification of the bamboo culm's anatomical characteristics occur during the maturation period (Fujii 1985; Alvin and Murphy 1988; Abd-Latif 1996; Liese and Weiner 1996; Londono *et al*. 2002; Gan and Ding 2005; Wahab *et al*. 2006). These changes include the thickening of the cell wall, deposition of additional lamella, and lignification that decreases the functional efficiency of conductance elements. The conductance elements must function for several years without secondary meristem. This leads to a breakdown in the conducting systems and dying of individual culms, both above and below ground.

This study examined the rhizome anatomical characteristics of *Gigantochloa scortechinii* Kurz ex Munro, including their relationship with different study sites, rhizome ages, and their relationship with hydraulic conductance.

EXPERIMENTAL

Sampling

Sampling was conducted at three different locations in Peninsular Malaysia (Table 1). Sampling was conducted using selective random sampling from healthy clumps of *Gigantochloa scortechinii* Kurz ex Munro. Infertile and congested clumps were ignored. Three clumps were selected for three replicates. Four consecutive rhizomes from each clump were selected, *i.e.*, new sprout (estimated to < one month), young (estimated to one year), premature (estimated to two years), and mature (estimated to three years) (Mohamed *et al*. 2019). The age estimation was based on the characters of culms (Banik 1993) and the number of rhizomes in a consecutive rhizome from the new sprout (Liese 1998). Only a complete set (four consecutive rhizomes) of a sectorial rhizome with complete plant parts (above and below ground) were chosen.

Determination of Anatomical Characteristics

Fresh samples were cut and sized (10 mm × 10 mm × 10 mm); (length × width × height) prior to examine using a LEO 1455 variable pressure scanning electron microscope (VPSEM; Oberkochen, Germany). The cross section (XS) cubes were prepared using a microtome. The cubes were oven-dried at 60 °C until a constant weight was reached to avoid the presence of steam when examined using the VPSEM. The sample was evaporatively coated using a sputter coater to obtain conductivity without affecting or reduce charging during observation. This method can provide an original figure from un-modified samples; it is cheaper and less time consuming than a conventional scanning electron microscope (PEO 1996).

The anatomical characteristics such as conducting elements, ground tissue, and supporting elements were observed. This study was conducted at the Microscopy Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang.

Table 1. Information of Three Different Study Sites

	Study Site		
Variation	Amanjaya FR	Kenaboi FR	Ayer Hitam FR
Position	5°37'12.46" N, 101°38'51.09" E	3°10'50.93" N, 101°58'37.60" E	3°0'16.17" N, 101°38'36.08" E
Elevation (m)	700	320	300
Annual precipitation (mm)	2000	2100	2100
Stand type	Natural stand	Natural stand	Planted
Soil type	Bukit Temiang series - red yellow (5YR 5/6, 5/8) to yellow red (5YR 6/6, 6/8, 7/6, 7/8), sandy clay loam, loose, well drained, from middle to highly slope	Beserah series - yellow chocolate (10YR 6/6, 6/8) to chocolate yellow (10YR 5/4, 5/6, 5/8), loamy with gravel, fine, loose, well drained and from middle to highly slope	Serdang series - chocolate yellow (10YR 5/4, 5/6, 5/8) to dark chocolate (7.5YR 4/6, 5/6, 5/8), fine sandy clay loam, loose, well drained and from small to highly slope
Disturbance	Nearly active forest production area, wildlife (elephant) habitat	Recreation and extreme activity (four-wheel drives)	Education and research area
Note: FR = Forest Reserve			

Determination of Fiber Length

Fresh sample blocks (10 mm length × 10 mm width × 20 mm height) were prepared before being chipped into matchsticks of 2 mm × 2 mm × 20 mm (length x width x height). The splints were boiled for 6 h. The splints were heated at 85 °C in a solution of 1.5 g of sodium chlorite dissolved in 25 mL distilled water and eight drops of acetic acid for several hours until the splints turned white in color. The white splints were washed carefully with distilled water to remove the solution.

After maceration, splints were stained with safranin-O to contrast the fibers. Distilled water in the splints was dehydrated through an alcohol series of 50%, 70%, 95%, and absolute ethanol for 2 min in each solution. The splints were washed with xylene and mounted on a 25 mm × 75 mm slide. Two drops of Canada balsam were introduced, and samples were dried in an oven at 60 °C for three days. Fibre length was examined under an Olympus SZX-ILLK200 stereomicroscope (Tokyo, Japan). This study was conducted at Laboratory of Biocomposite Technology, Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, Serdang.

Determination of Hydraulic Conductance

Culms at selected rhizomes were felled at the culm base and connected to a high-pressure flow meter (HPFM) with a water-tight seal before hydraulic conductance was measured. Water was placed under pressure by compressed air that was controlled with a pressure regulator into the culm base opposite to the normal water flow during plant transpiration. This routine was conducted with rapidly changing the delivery pressure, P (MPa), simultaneously measuring water flow, F (kg s^{-1}), and hence hydraulic conductance was estimated from the slope of the F *versus* P plot (Tyree *et al.* 1994; 1995). The untested culms and belowground parts were avoided, not subjecting them to any cuts or major injuries, so as to reduce the degree of disturbance of the interconnected rhizome system.

The hydraulic conductance trend inside the belowground system where four rhizomes connected consecutively (as a rhizome system) can be used as a measure of their resistance inside. In the present study, the HPFM was used instead of another method (such as evaporative flux and thermal dissipation probe method) because the HPFM permits the determination of hydraulic conductance in a short time, allows determination while the water flows in state of reverse direction, and could reduce the error factors (such as osmotic changes and transpiration of the aboveground part) (Tyree *et al*. 1995; Tsuda and Tyree 1997; 2000; Do and Rocheteau 2002).

Statistical Analysis

The normality and homogeneity test of variances were performed before analysis using Univariate analysis of variance. Two factors (study site and rhizome age) were involved in the analysis with a total samples, n = 84. An equal sample, n=28 for each study site and n = 21 for each rhizome age, were measured and analyzed. The relationship between anatomical characteristics with each study site, rhizome age, and hydraulic conductance was analyzed using bivariate (Pearson) correlation. The statistical analysis was conducted using IBM SPSS statistics software version 21.0 (Armonk, NY, USA).

RESULTS AND DISCUSSION

Vascular Bundles Distribution

The vascular bundles size and distribution were anecdotally observed to change continuously from inside towards the periphery of the rhizome wall (Fig. 1a, 1b, and 2a). Twisted small vascular bundles between larger vascular bundles were observed (Fig. 2d, 2e, and 2f). Regardless of the study site and rhizome age, the vascular bundle distribution ranged from 62 to 68 bundles cm^{-2}. Table 2 shows that the vascular bundle distribution was significantly different ($p < 0.05$) between study sites, which could be related to the intrinsic conditions such as altitude, precipitation, and the disturbance level.

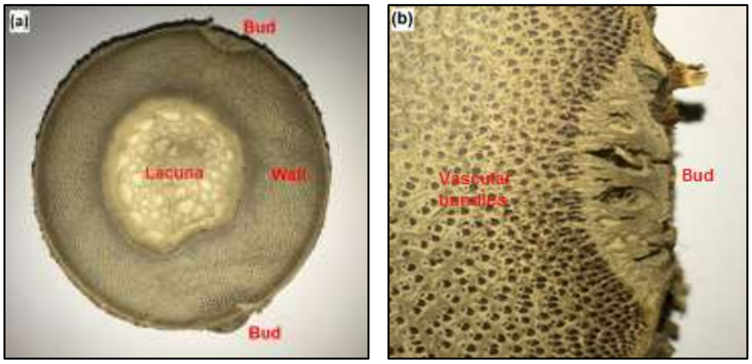

Fig. 1. Transverse section of rhizome wall with lateral buds (a), and lateral bud (b)

Furthermore, the vascular bundle distribution per centimeter square at Ayer Hitam FR was significantly different from those at Amanjaya and Kenaboi FR (Table 3). These results could be related to the smaller size and thinner wall (data not shown) of rhizomes at Ayer Hitam FR compare to rhizome at Amanjaya and Kenaboi FR. These results implied that the rhizome at Ayer Hitam FR formed in an immature planted clump. The morphology of bamboo such as height and diameter of new individual culms (rhizome in this study) increases over consecutive years after planted (Liese and Wiener 1996). Vascular bundle distribution, however, was not significantly different with rhizome age (Table 2). The results in Table 4 indicate that no significant correlation was found between vascular bundle distributions with rhizome age, which implied that the number of vascular bundles does not increase or decrease when rhizome age increases.

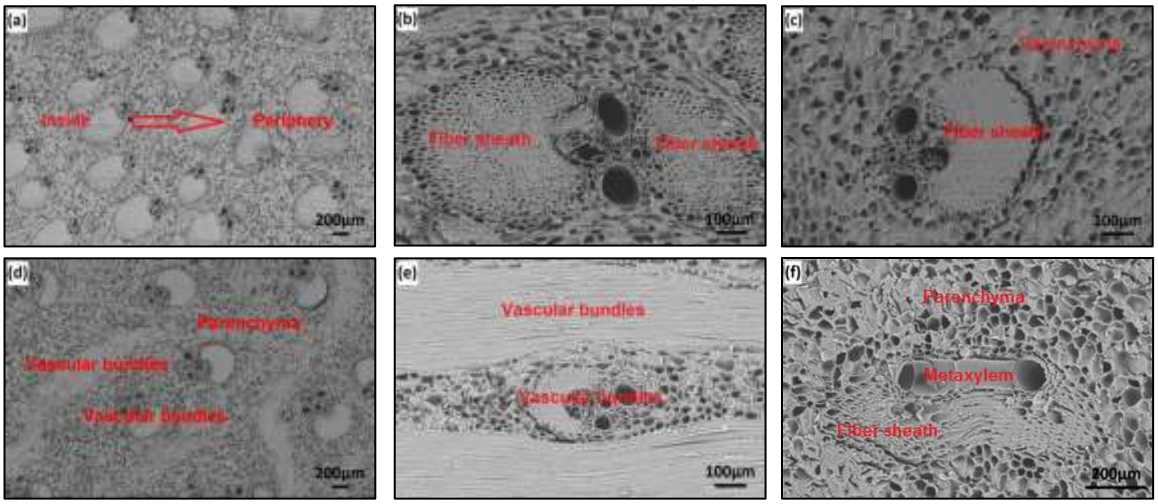

Fig. 2. Vascular bundles. (a) Sizes and distribution differ from inside towards the periphery, (b) with two fiber sheath, (c) with one fiber sheath, (d, e) twisted small vascular bundle between the main vascular bundle, and (f) transverse section of twist angle of a small vascular bundle

Vascular Bundles Diameter

In general, the vascular bundle diameter ranged from 450.25 to 994.20 µm. The large variation in vascular bundle diameter could be due to its position in the rhizome wall zonation (Fig. 1a and 1b) and also influenced by two types of vascular bundles developed (Fig. 2b and 2c). The vascular bundle diameter was highly significantly different ($p < 0.01$) with study site and rhizome age (Tables 2 and 3). There was no significant difference between vascular bundle diameter with the interaction of study site and rhizome age. The results in Table 4 indicate the vascular bundles diameter had a weak negative relationship ($r = -0.246$) with study site. A moderate positive relationship ($r = 0.343$) was found between vascular bundle diameter with rhizome age, which indicates that the diameter slightly increased with rhizome age.

Radial to Tangential Ratio and Protoxylem Diameter

Regardless of the study site and rhizome age, the radial to tangential ratio ranged from 1.60 to 2.19. Tables 2 and 3 show that radial to tangential ratio was significantly different ($p < 0.05$) with study site, and highly significantly different ($p < 0.01$) with rhizome age. However, the radial to tangential ratio was not significantly different with the interaction of study site and rhizome age. There was no significant correlation ($r = 0.061$) between the ratio with study site, but there was a strong negative correlation ($r = -0.611$, $p < 0.01$) between the ratio with rhizome age. This result contradicted the study by Wang *et al*. (2016), where the ratio was not significantly different with age.

Table 2. Summary Analysis of Variance on Anatomical Characteristics and Hydraulic Conductance of *G. scortechinii* Rhizome

S.O.V	DF	F values and Statistical Significance						
		Vascular Bundle Distribution cm^{-2}	Vascular Bundle Diameter (µm)	Radial to Tangential Ratio	Protoxylem Diameter (µm)	Metaxylem Diameter (µm)	Phloem Diameter (µm)	Parenchyma Diameter (µm)
S	2	3.75*	21.68**	3.46*	28.07**	9.21**	18.75**	0.60ns
A	3	0.21ns	5.39**	19.38**	2.12ns	1.64ns	0.21ns	16.47**
S x A	6	0.17ns	0.46ns	0.63ns	1.60ns	0.92ns	2.98*	6.45**
		Parenchyma Lumen Diameter (µm)	Parenchyma Cell Wall Thickness (µm)	Fibre Diameter (µm)	Fibre Lumen Diameter (µm)	Fiber Cell Wall Thickness (µm)	Fiber Length (µm)	Hydraulic Conductance
S	2	0.67ns	4.58*	13.45**	34.82**	2.24ns	31.72**	22431.88**
A	3	13.60**	35.05**	11.37**	6.70**	27.31**	26.42**	158174.13**
S x A	6	5.99**	4.27**	8.73**	7.89**	5.63**	3.83**	6162.85**

Note: S.O.V = source of variation; S = site; A = age; DF = degree of freedom; ns = not significant (F; $p > 0.05$, * = significant (F; $p < 0.05$), ** = highly significant (F; $p < 0.01$)

Table 3. Duncan's Multiple Range Test on the Effects of Site and Age on Anatomical Characteristics and Hydraulic Conductance

	Site			Age			
Properties	Amanjaya FR	Kenaboi FR	Ayer Hitam FR	New Sprout	Young Rhizome	Pre-mature Rhizome	Mature Rhizome
Vascular Bundle Distribution cm^{-2}	63.73 ± 0.20b	63.64 ± 0.24b	64.46 ± 0.22a	63.79 ± 0.27a	63.93 ± 0.24a	64.00 ± 0.30a	64.07 ± 0.26a
Vascular Bundle Diameter (µm)	771.82 ± 15.94b	848.91 ± 13.51a	712.55 ± 17.00c	730.63 ± 25.49c	769.37 ± 16.95bc	785.30 ± 18.58ab	825.74 ± 20.37a
Radial to Tangential Ratio	1.83 ± 0.02ab	1.78 ± 0.02b	1.85 ± 0.02a	1.94 ± 0.02a	1.82 ± 0.02b	1.76 ± 0.02c	1.74 ± 0.02c
Protoxylem Diameter (µm)	34.20 ± 0.31b	35.17 ± 0.36a	32.21 ± 0.20c	34.12 ± 0.54ab	33.39 ± 0.37b	34.40 ± 0.41a	33.53 ± 0.39ab
Metaxylem Diameter (µm)	112.44 ± 2.75a	116.52 ± 2.99a	99.52 ± 3.10b	107.53 ± 3.73a	114.19 ± 3.44a	104.48 ± 3.91a	111.77 ± 3.70a
Phloem Diameter (µm)	127.31 ± 3.20b	145.32 ± 4.97a	112.82 ± 3.53c	127.04 ± 4.47a	130.23 ± 5.09a	126.53 ± 7.06a	130.14 ± 4.94a
Parenchyma Diameter (µm)	43.27 ± 1.89a	43.63 ± 1.48a	41.83 ± 1.79a	34.68 ± 2.04b	44.55 ± 1.32a	48.16 ± 1.52a	44.24 ± 1.76a
Parenchyma Lumen Diameter (µm)	40.16 ± 1.74a	40.98 ± 1.49a	39.02 ± 1.67a	32.83 ± 1.99c	41.76 ± 1.36ab	44.93 ± 1.43a	40.68 ± 1.66b
Parenchyma Cell Wall Thickness (µm)	1.56 ± 0.11a	1.33 ± 0.05b	1.41 ± 0.09ab	0.92 ± 0.06c	1.40 ± 0.05b	1.62 ± 0.08a	1.78 ± 0.09a
Fiber Diameter (µm)	17.89 ± 0.49b	20.53 ± 0.68a	22.00 ± 1.09a	17.85 ± 0.79c	20.34 ± 0.94b	19.28 ± 0.67bc	23.09 ± 1.14a
Fiber Lumen Diameter (µm)	3.94 ± 0.36c	7.12 ± 0.64b	9.24 ± 0.72a	7.70 ± 0.68a	7.17 ± 0.85a	4.77 ± 0.47b	7.43 ± 1.08a
Fiber Cell Wall Thickness (µm)	6.97 ± 0.28a	6.70 ± 0.20ab	6.38 ± 0.39b	5.07 ± 0.35c	6.58 ± 0.23b	7.26 ± 0.21a	7.83 ± 0.26a
Fiber Length (µm)	1303.87 ± 37.28b	1376.90 ± 37.08b	1650.53 ± 37.22a	1202.57 ± 38.00c	1381.51 ± 43.55b	1555.79 ± 46.47a	1635.20 ± 40.02a
Hydraulic Conductance ($m^2\ s^{-1}\ MPa^{-1}$)	3.073 ± 0.378c	3.390 ± 0.196b	3.797 ± 0.304a	4.795 ± 0.142a	3.680 ± 0.071b	3.065 ± 0.104c	2.140 ± 0.210d

Note: Mean values with the same letter in the same row at each factors are not significant different (F; $p > 0.05$); ± = standard error, FR = Forest Reserve

The SEM images show that the vascular bundles of *G. scortechinii* rhizome consists of both one (Fig. 3a) and two (Fig. 3b) protoxylem, which is in agreement with a previous study by Abd-Latif (1996) on *G. scortechinii* culms. Regardless of the study site and age, the protoxylem diameter was between 30.45 and 39.19 µm. The protoxylem diameter was significantly different ($p < 0.01$) between study sites, but there was no significant difference with rhizome age (Tables 2 and 3).

Table 4 indicates a moderate negative relationship ($r = -0.413$) between protoxylem diameter and study site. A larger average protoxylem diameter was found at Kenaboi FR, followed by Amanjaya FR and Ayer Hitam FR (Table 3). Thus, the protoxylem diameter is more likely affected by intrinsic study site conditions rather than age-related factors. The modifications during protoxylem maturation occur in the cell wall of rings, where thickening and lignification occur throughout the year (Liese 1987; Yoshizawa *et al.* 1991).

Table 4. Correlation Coefficient Analysis of Anatomical Characteristics with Site, Age, and Hydraulic Conductance

Properties	Spatial	Age	Hydraulic Conductance
Vascular Bundle Distribution cm^{-2}	0.247*	0.086ns	-0.097
Vascular Bundle Diameter (µm)	-0.246*	0.343**	-0.087
Radial to Tangential Ratio (µm)	0.061ns	-0.611**	0.087
Protoxylem Diameter (µm)	-0.413**	-0.043ns	0.022
Metaxylem Diameter (µm)	-0.311**	0.020ns	0.064
Phloem Diameter (µm)	-0.241*	0.025ns	0.154
Parenchyma Diameter (µm)	-0.065ns	0.400**	-0.470**
Parenchyma Lumen Diameter (µm)	-0.055ns	0.350**	-0.466**
Parenchyma Cell Wall Thickness (µm)	-0.131ns	0.682**	-0.325
Fibre Diameter (µm)	0.376**	0.367**	-0.232
Fibre Lumen Diameter (µm)	0.573**	-0.095ns	-0.127
Fibre Cell Wall Thickness (µm)	-0.153ns	0.634**	-0.219
Fibre Length (µm)	0.355**	0.413**	-0.174

Note: * = significant (F; $p < 0.05$); ** = significant (F; $p < 0.01$); ns = not significant (F; $p > 0.05$)

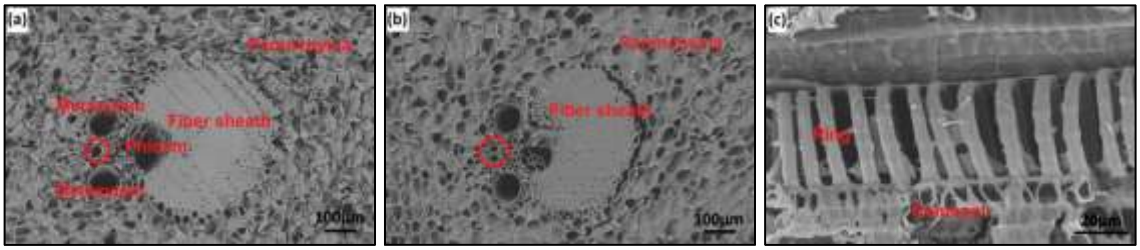

Fig. 3. Vascular bundle with one protoxylem (in red circle) (a), vascular bundle with two protoxylem (in red circle) (b), and individual isolated ring with remnant membranes (c).

The rings in protoxylem cell are associated with each other by a membrane, where the membrane is the original primary wall extensions before it gets ruptured during the growing phase (Liese 1998). The protoxylem lumen with individual isolated rings and remnant membranes (Fig. 3c) were observed in this study, but the tyloses were barely observed in the cells. Tyloses are often observed in protoxylem (developed from the surrounding parenchyma) of monopodial bamboo culms, which affect its conductance ability (Liese 1998; Ito *et al.* 2015).

Metaxylem Diameter and Phloem Diameter

The metaxylem comprises two large vessels, surrounded by one or two layers of sclerenchyma sheath (Fig. 4a, 4b). Metaxylem serves for water transport within culms and rhizomes, and its high conductivity is vital for foliage transpiration. The metaxylem diameter ranged from 78.3 to 150.6 µm. The structure of separating rim at the inner side of vessel, two distinct walled zonation (S1 and S2) (Fig. 4a, 4b), and the developed small branching lumens (Fig. 4c) inside the vessel lumen were observed. The observed vessels were axially oriented with branches for interconnection that formed vascular anastomoses (Fig. 4d). These observations corroborated a previous study on bamboo culm nodal zones (Ding and Liese 1997).

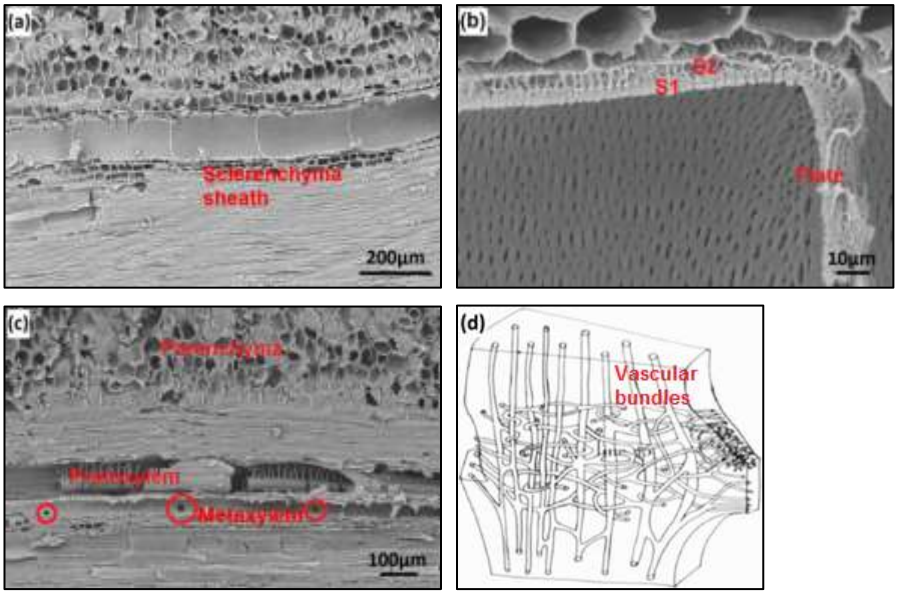

Fig. 4. Metaxylem surrounded by one or two layers of sclerenchyma sheath, separating rim at inner side of the metaxylem, and two distinct walled zonations (a), structure of inner side wall possess large simple perforation with numerous pit, two distinct walled zonations (S1 and S2) and perforation plate (b), small branching lumen developed to form the vascular anastomoses (c), and diagram of vascular anastomoses by Ding and Liese (1997) (d)

Figure 2f shows the transverse section of twist angle of small vascular bundles in the form of vascular anastomoses. This observation explains the unusual vascular bundle structure found in *Phyllostachys pubescens* rhizome by Ito *et al.* (2015), where the vascular bundles consist of two pairs of metaxylem. Table 2 shows that metaxylem diameter was significantly different ($p < 0.01$) with respect to study site, where the bigger metaxylem diameter was found at Kenaboi FR, followed by Amanjaya FR and Ayer Hitam FR (Table 3). The metaxylem diameter was not significantly different ($p > 0.05$) with rhizome age.

The phloem in the *G. scortechinii* rhizome consists of large, thin-walled sieve tubes and small companion cells, surrounded by fiber sheaths and sclerenchyma sheaths (Fig. 5a, 5b, and 5c). The companion cells were interconnected by sieve pores as explained by Liese (1998). Regardless of study site and rhizome age, the phloem diameter ranged from 86.4 to 193.3 µm. The phloem diameter was highly significantly different ($p < 0.01$) with study site, but not significantly different with rhizome age (Table 2). A significant difference ($p < 0.05$) but weak correlation ($r = -0.241$) was found between phloem diameter with study site. Correlation coefficient analysis also showed that there was no significant correlation between phloem diameter and rhizome age (Table 4).

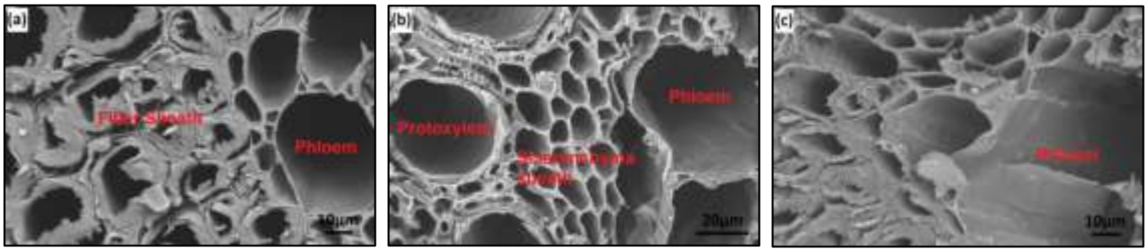

Fig. 5. Phloem consists of large, thin-walled sieve tube, and small companion cells, surrounded by fiber strand (a), phloem consist of large, thin-walled sieve tube separated with metaxylem and protoxylem and sclerenchyma sheath (b), and inner side structure of phloem with smaller companion cell separated by thin wall (c).

Parenchyma and Lumen Diameter, and Cell Wall Thickness

The parenchyma in *G. scortechinii* rhizomes was vertically elongated with short cube-like ones, more rectangular in shape like the parenchyma in monopodial bamboo species (*P. pubescens*) (Tekpetey *et al.* 2010). The parenchyma varied in size, and it was connected with pits on all side walls (Fig. 6a, 6b, 6c, and 6d) rather than on only tangential wall as such found in bamboo culm internodes (Liese and Tang 2015).

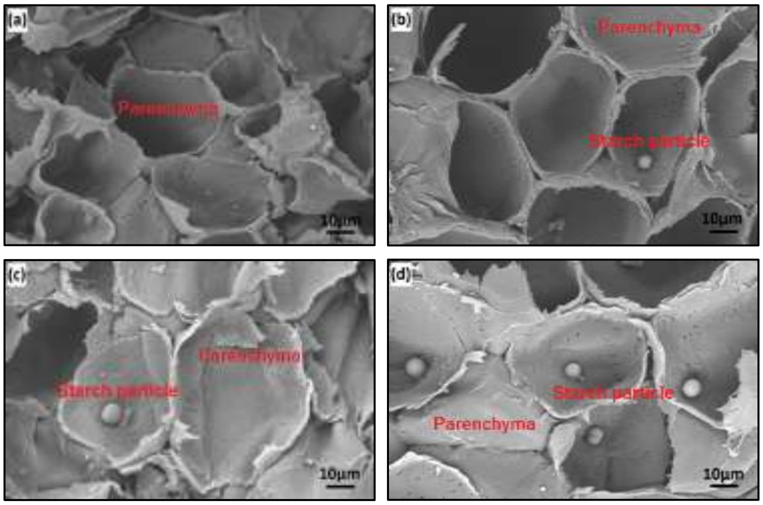

Fig. 6. Parenchyma in new sprout (a), young rhizome (b), premature rhizome (c), and mature rhizome (d). The starch particle was also observed filling in some parenchyma cells (b, c, and d).

The parenchyma diameter ranged from 24.8 to 60.7 µm. Starch particles of the round or ellipsoidal types were observed in some parenchyma cells; they were more frequent in mature rhizomes, followed by premature and young rhizomes. No starch particles were observed in parenchyma cells in a new sprout (Fig. 6a, 6b, 6c, and 6d). The low frequency of starch particles is related to the period of sampling, as the starch content is higher in the dry season than in the rainy season (Abd-Latif 1996; Wahab *et al.* 2006). Magel *et al.* (2005) explained that the mobilization of starch occurs before a new shoot is sprouted to accommodate the growing season.

The parenchyma diameter was not significantly different with respect to study site, but it was significantly different ($p < 0.01$) with rhizome age and the interaction of study site and rhizome age. The results in Table 4 indicate an insignificant correlation ($r = -0.065$) between parenchyma diameter with study site. A moderate positive correlation ($r = 0.400$, $p < 0.01$) was found between parenchyma diameter with rhizome age. The range of parenchyma lumen diameter in this study was 22.62 to 57.70 µm.

Analysis of variance showed that parenchyma lumen diameter was not significantly different with study site, but significantly different ($p < 0.01$) with rhizome age and the interaction of study site and rhizome age (Table 2). The variation of parenchyma lumen diameter had no relationship with study site ($r = -0.055$). A significant ($p < 0.01$) moderate positive correlation ($r = 0.350$) was found between parenchyma lumen diameter with rhizome age. This result indicates that parenchyma lumen diameter increased with rhizome age.

Regardless of study site and rhizome age, the parenchyma cell wall thickness ranged from 0.46 to 2.62 µm. The parenchyma cell wall thickness was significantly different ($p < 0.05$) with study site, and it was highly significantly different ($p < 0.01$) with rhizome age and the interaction of study site and rhizome age (Table 2). Table 3 indicates that the oldest rhizome was highest in parenchyma cell wall thickness, followed by premature, young, and new sprout rhizome, at 1.78, 1.62, 1.40, and 0.92 µm, respectively, which is in agreement with previous work (Wahab *et al.* 2006; Liese and Tang 2015). The variation of parenchyma diameter, lumen diameter, and cell wall thickness shows that maturing occurs, such as thickening by deposition of additional lamella with consequent lignification.

However, these results contradicted a study by Hisham *et al.* (2006) on the same bamboo species (*G. scortechinii*) culms, where the parenchyma cell wall thicknesses were not significantly different with culms age. According to Table 4, parenchyma cell wall thickness has no significant correlation with study site, but had a strong positive correlation ($r = 0.682$, $p < 0.01$) with rhizome age. This result indicates that parenchyma cell wall thickness increased when rhizome age increased.

Fiber and Lumen Diameter, Cell Wall Thickness, and Length

The fiber was grouped in a fiber sheath in vascular bundles. The individual fiber is characterized by their slender-form, long and tapered ends; the fork end type was not observed in this study. Numerous pits were observed inside fiber lumen walls, as shown in Fig. 7a.

Fiber diameter ranged from 12.0 to 33.6 µm. The variation in fiber diameter was caused by the a) cell position in rhizome wall where the fiber near the epidermis as well as near rhizome lumen are thinner and possess one or more lamella less than in the middle wall, b) position within length of culms (Londono *et al.* 2002) (as well as in rhizome in this study), and c) their position in a vascular bundles such as direct contact with the surrounding parenchyma, protoxylem, and phloem (Liese and Weiner 1996).

These effects can be observed in Fig. 1c and 7b.

Table 2 indicates that there were highly significant differences ($p < 0.01$) between fiber diameter with study site, rhizome age, and the interaction of study site and rhizome age. These results agreed with a study by Abd-Latif (1996) on bamboo culms, except on the interaction of both main effects. In contrast, Hisham *et al.* (2006) found that fiber diameter was found not significantly different with *G. scortechinii* culm age. The Pearson correlation analysis (Table 4) showed a moderate positive correlation ($r = 0.376$, $p < 0.01$) between fiber diameter with study site. The analysis also showed a moderate positive correlation ($r = 0.367$, $p < 0.01$) between fiber diameter with rhizome age. The positive correlation indicated that fiber diameter increased when rhizome age increased.

Regardless of study site and rhizome age, fiber lumen diameter ranged from 1.00 to 18.86 µm. The results in Table 2 indicate that fiber lumen diameter was not significantly different ($p > 0.05$) with study site, but was highly significantly different ($p < 0.01$) with rhizome age, and the interaction study site and rhizome age. Table 4 shows that there was no correlation between fiber lumen diameter with rhizome age ($r = -0.095$). These results suggested that the increasing rhizome age did not influence the variation of fiber lumen diameter, as indicated by a small F value (6.70) in Table 2. Similar findings were reported by Abd-Latif (1996) and Hisham *et al.* (2006).

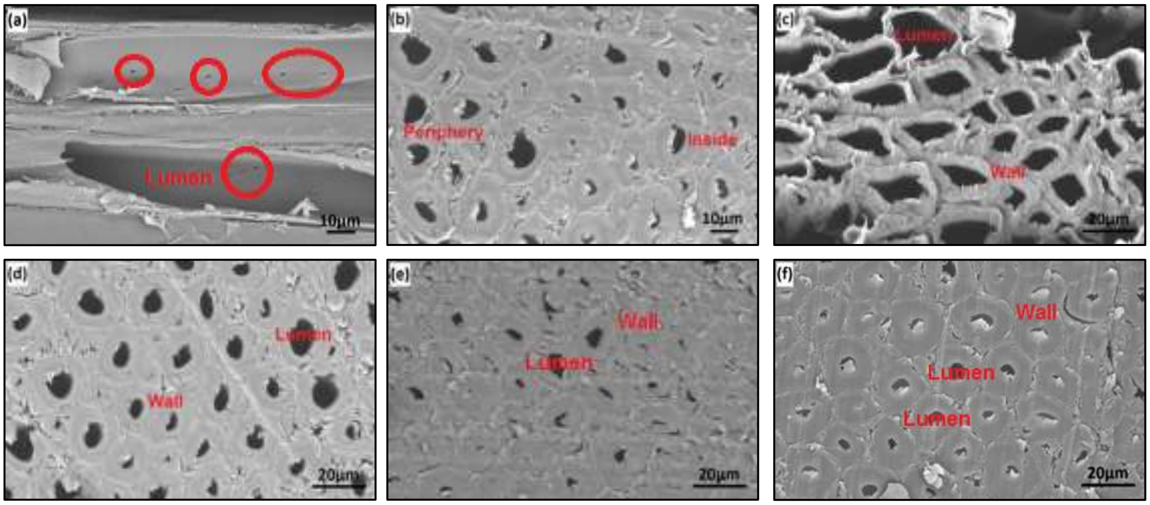

Fig. 7. Pits cavity (in red circle) inside the fiber lumen wall (a), sizes of fiber lumen varies from inside to periphery in a vascular bundle (b). Fiber structure in new sprout (c), young rhizome (d), premature rhizome (e), and mature rhizome (f)

Furthermore, fiber cell wall thickness ranged from 2.07 to 9.96 µm. Regardless of rhizome age, fiber cell wall thickness was not significantly different with study site, but significantly different ($p < 0.01$) with rhizome age, which agreed with Abd-Latif (1996). The fiber cell wall thickness was significantly different ($p < 0.05$) with the interaction of study site and rhizome age (Table 2). Fiber cell wall thickness had no significant correlation with study site, as the Pearson correlation value was -0.153, but fiber cell wall thickness had a strong positive correlation with rhizome age ($r = 0.634$, $p < 0.01$). This result illustrates that fiber cell wall thickness increased with rhizome age.

Liese (1998) explained that the thickening of cell walls (regarding parenchyma and fiber) during development of their structure occurs due to the deposition of additional lamella layers but not due to the thickening of the existing cell walls. The anatomical changes during the development of fiber structure have been reported in other bamboo species (such as Fujii 1985; Alvin and Murphy 1988; Liese and Weiner 1996; Londono *et al.* 2002; Gan and Ding 2005).

A wide range of fiber length (339.0 to 2660.7 µm) was observed in *G. scortechinii* rhizomes (Fig. 8a, 8b). This could be due to the closed position or the unclear zonation between the internodes and nodes in rhizome walls. Liese and Tang (2015) reported that the fibers in culm nodes are shorter than in the internodes. Table 2 indicates that there were highly significant differences ($p < 0.01$) between fiber length with study site, rhizome age, and the interaction of study site and rhizome age. The correlation coefficient indicates a significant ($p < 0.01$) moderate positive correlation ($r = 0.355$) between fiber length with study site and with rhizome age, with $r = 0.355$ and 0.413, respectively. The positive correlation between fiber lengths with rhizome age illustrates that the fiber length increased with increasing rhizome age.

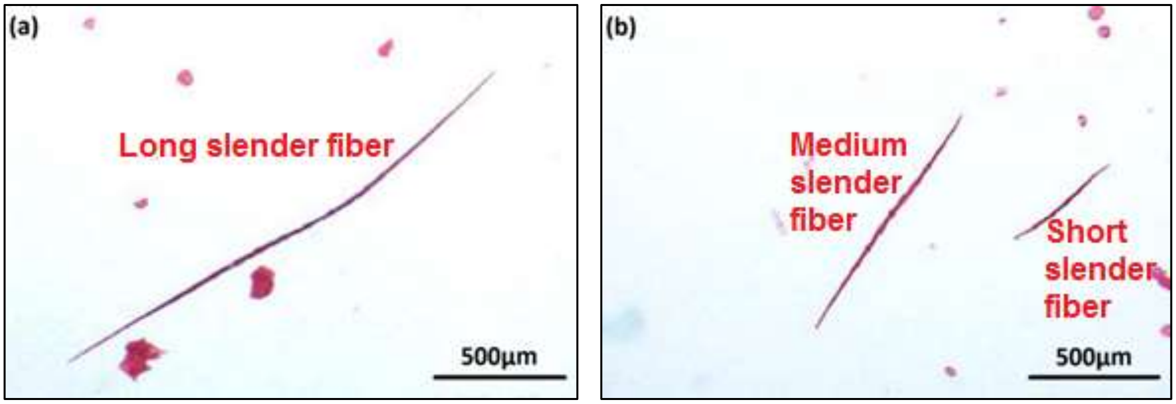

Fig. 8. Long (a), medium, and short slender fiber (b) with both tapered end

Hydraulic Conductance

The hydraulic conductance of rhizomes ranged from 1.289 to 5.333 $m^2 s^{-1} MPa^{-1}$. Table 2 shows that the hydraulic conductance was highly significantly different ($p < 0.01$) with study site, rhizome age, and their interaction. Higher hydraulic conductance was found in Ayer Hitam FR, followed by in Kenaboi FR and Amanjaya FR, with values of 3.797, 3.390 and 3.073 $m^2 \cdot s^{-1}$ MPa^{-1}, respectively. The new sprout rhizome demonstrated the highest hydraulic conductance (4.795 $m^2 s^{-1} MPa^{-1}$), followed by the young (3.680 $m^2 s^{-1} MPa^{-1}$), pre-mature (3.065 $m^2 s^{-1} MPa^{-1}$), and mature rhizome (2.140 $m^2 s^{-1} MPa^{-1}$).

There was no significant correlation ($r = 0.283$) between hydraulic conductance with study site, but there was a significant correlation ($r = -0.918$) with rhizome age (Table 4). The negative correlation indicates that hydraulic conductance decreased with age. Higher hydraulic conductance in younger *versus* older rhizomes illustrates that the hydraulic resistance increased up to an average of 23% at each increment of age. During the late phase of culms elongation, fiber cell walls in the phloem cap are actively thickening and almost reach their full secondary cell wall deposition during the first growing season. This thickening results in multilayered cell walls that begin around the edge of conducting elements (phloem and metaxylem), and continuously thicken towards the outer part of a vascular bundles (Bhat 2003; Gritsch *et al.* 2004).

Like other bamboo species, *G. scortechinii* is unable to grow new conductance elements and, hence, may show vulnerability to metaxylem dysfunction due to cavitation (Johnson *et al.* 2009; Marine 2009) and metaxylem implosion (Cochard *et al.* 2004), which lead to loss of hydraulic conductivity (Tyree and Zimmerman 2002). The growing organs develop to mature phase with continuous changes in density, strength properties, and chemical constituents (Liese 1998; Hisham *et al.* 2012; Xu *et al.* 2014; Wang *et al.* 2016).

Relationship of Anatomical Characteristics with Hydraulic Conductance

The results in Table 4 indicate that only parenchyma diameter and parenchyma lumen diameter had a significant ($p < 0.01$) relationship ($r = -0.470$ and -0.466, respectively) with hydraulic conductance. The negative relationship of parenchyma diameter and parenchyma lumen diameter with hydraulic conductance illustrates that hydraulic conductance decreases with increasing parenchyma diameter and parenchyma lumen diameter. However, the sizes of parenchyma diameter and lumen diameter do not imply a direct determinant factor of hydraulic conductance unless the parenchyma cell wall thickness also showed a significant relationship with hydraulic conductance. This is because the size of parenchyma diameter and lumen diameter varied with the location such as near vascular bundles or the distance from periphery of rhizome.

The results show that parenchyma cell wall thickness did not have a significant relationship ($r = -0.325$) with hydraulic conductance. The parenchyma cell wall thickness showed a weak negative relationship with hydraulic conductance, where the p-value of the correlation coefficient was nearly significant (0.053).

The conductance elements such as protoxylem, xylem, and phloem diameter did not have a significant relationship ($r = 0.022$, 0.064, and 0.154, respectively) with hydraulic conductance. This study did not measure the conductance elements cell wall thicknesses, which are more likely to determine the relationship with hydraulic conductance due to the irregular or uneven thickness of cell wall membranes (Figs. 3, 4, and 5). Furthermore, chemical substances such as ash content, cellulose, lignin, and extractives show a significant relationship with anatomical changes during the maturation of bamboo culms (Abd-Latif 1996; Hisham *et al.* 2006; Li *et al.* 2007; Wang *et al.* 2016; Mohamed *et al*. 2019). The senescent effect has consequences during the natural dying of culms. This effect includes blocking off water conductance in metaxylem by tylosses and slime substances, and the phloem callose occlusions that decrease the functional efficiency (Liese and Weiner 1996; Liese and Tang 2015).

In the present study, the accumulated substances could be lost during the boiling process of sample preparation. Furthermore, the differences between living and dead cells or substances that accumulate were hard to determine using the sputter coater method. It can be measured or viewed by staining with 1% potassium permanganate prepared in citrate buffer (Cho *et al.* 2008) or with 1% safranin-O and counter-stained with 1% aqueous Alcian Blue (Wang *et al.* 2016). Cho *et al*. (2008) stained samples with 0.0001% acrifl avine to obtain confocal images for lignin fluorescence.

The hardness of rhizome samples directed the choice of sample preparation method in this study. Rao (1985) reported the differences between soft tissues in bamboo shoot and hard tissues in mature bamboo culms and also between the nodes and internodes. Liese and Weiner (1996) used different approaches to obtain smooth surfaces between soft and extremely hard samples of 12 different ages of *Phyllostachys viridiglaucescens* culms. Therefore, further studies on chemical substances in relation to hydraulic conductance could improve the understanding regarding relationship with age and hydraulic conductance.

CONCLUSIONS

1. All measured anatomical characteristics were found significantly different with study site except for parenchyma diameter, parenchyma lumen diameter, and fiber cell wall thickness.
2. Vascular bundle diameter, parenchyma diameter, parenchyma lumen diameter, parenchyma cell wall thickness, fiber diameter, fiber wall thickness, and fiber length increased with increasing rhizome age, while radial to tangential ratio, protoxylem diameter, and fiber lumen diameter decreased with increasing rhizome age.
3. The hydraulic conductance was significantly different with study site and rhizome age. The negative correlation indicated that the hydraulic conductance decreased with increasing rhizome age.
4. All measured characteristics including the conductance element had an insignificant relationship with hydraulic conductance, except the parenchyma diameter and lumen diameter.

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