Variation of Anatomical Characteristics within the Culm of the Three *Gigantochloa* Species from Indonesia

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The anatomical features of three valuable commercial *Gigantochloa* bamboo species growing in Indonesia, including *G. pseudoarundinacea*, *G. apus*, and *G. atroviolacea*, were investigated by optical microscopy. The relative crystallinity and crystalline width of the culm of the bamboo species were examined by an X-ray diffraction method. These species contained vascular bundle of type III. Vascular bundle density was higher in the outer part of bamboo culm than in the inner. Fiber portion decreased from the outer part to the inner part and *vice versa* for the parenchyma and vessel portions. Fiber length of all species was higher in the outer part than the inner part of the culm. There was a significant difference in the fiber percentage between the bamboo species. Significant differences were also found in vessel diameter and parenchyma cell dimensions among the bamboo species. There was a slight difference in the crystalline properties between the outer and inner parts of the culm and among the bamboo species. All parameters showed a variation in the radial direction of the three bamboo culm but did not show a consistent tendency along the vertical direction.

Keywords: Bamboo anatomy; Cell proportion; Gigantochloa; Relative crystallinity; Vascular bundle

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INTRODUCTION

Bamboos encompass a broad group of 1,250 species within 75 genera, most of which are relatively fast-growing in various types of soil, attaining stand maturity within five years. The stands of tall species may reach 15 m to 20 m, and the largest species, *Dendrocalamus giganteus*, grows to 40 m in height and 30 cm in culm diameter (Scurlock *et al.* 2000). The main distribution of bamboo comprises Africa and America, with 80% of the bamboo found in Asia Pacific including Indonesia (Mera and Xu 2014). Bamboo plays important roles in the lives of various peoples of the world. It is estimated that more than 2.5 billion people depend on bamboo for their economy and one billion people live in traditional bamboo houses (Lobovikov *et al.* 2007). Several studies show the potential of bamboo as an alternative to wood composite materials (Febrianto *et al.* 2012, 2015;

Hidayat *et al.* 2019; Maulana *et al.* 2019) and as a fuel source or biomass energy raw material (Park *et al.* 2018, 2019, 2020).

In Indonesia, there are 161 species of bamboo grown, 126 species of which are endemic (Widjaja *et al.* 2014). *Gigantochloa* is one of these endemic bamboo genera and is mostly found in Java and Sumatra. *Gigantochloa pseudoarundinacea*, *G. apus*, and *G. atroviolacea* are found only in cultivation and locally known as Andong, Tali, and Hitam bamboo, respectively. They are commonly used in the bamboo industry together with *Dendrocalamus asper, Bambusa blumeana,* and *Schizostachyum brachycladum*. In particular, *G. pseudoarundinacea* is a potential raw material for oriented strand board (Febrianto *et al.* 2015), while *G. apus* and *G. atroviolacea* are more suitable for composite beam (Cahyono *et al.* 2014).

Although these species have been used in various applications, information regarding the anatomical characteristics of the bamboo culms of *Gigantochloa* genus are very limited. Mustafa *et al.* (2011) reported that four species of *Gigantochloa* from Malaysia had different vascular bundle sizes and fiber morphology between species and along the horizontal position in the culms of each species. Nordahlia *et al.* (2012) reported that fiber length in *Gigantochloa levis* was not significantly affected by culm age; however, it was affected by culm height. Similar trends in the fiber length of *Gigantochloa atter* were also reported by Marsoem *et al.* (2015).

In this study, therefore, the anatomical characteristics in radial and axial directions of the culms of the three commercial bamboo species growing in Java, Indonesia, including *G. pseudoarundinacea*, *G. apus*, and *G. atroviolacea,* were investigated to give further valuable insight for effective utilization of such bamboo resources.

EXPERIMENTAL

Materials

The three culms of 5-year-old Andong (*G. pseudoarundinacea*), Tali (*G. apus*), and Hitam bamboo (*G. atroviolacea*) were harvested from the bamboo garden of the Center for Research and Development (P3) Biomaterials, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia. The culms were cut at the second node above the ground and the branched top parts were removed, leaving three-quarters of the total culm height. These culms were divided into three equal-length parts consisting of top, middle, and bottom sections as per SNI 8020:2014 (2014). Information regarding the three bamboo species is shown in Table 1.

Table 1. General Morphological Information of the Three *Gigantochloa* Bamboo Species

	G. pseudoarundinacea			*G. apus*			*G. atroviolacea*		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
Diameter (mm)	68 ± 0.8	87 ± 0.8	94 ± 1.6	84 ± 0.7	88 ± 0.6	86 ± 1.2	48 ± 0.1	62 ± 0.8	65 ± 1.6
Culm Thickness (mm)	6.5 ± 0.2	9.2 ± 0.3	13.7 ± 1.2	11.5 ± 0.2	12.5 ± 0.4	14.2 ± 1.0	4.4 ± 0.2	6.2 ± 0.5	9.4 ± 1.0
Height (m)	16.1 ± 2.1			16.0 ± 0.8			15.6 ± 1.8		

Optical Microscopy

Anatomical characteristics of the bamboo species, such as vascular bundle type, vascular bundle density, fiber length, parenchymal dimensions, vessel diameter, and percentage of cell types were observed using an optical microscope (Nikon Eclipse E600, Tokyo, Japan) and an image analyzer (IMT i-solution lite, Version 9.1, British Columbia, Canada). Bamboo blocks were first softened in a boiling mixture of glycerin and water (50:50), and then sliced with a rotary microtome (Leica RM 2165, Wetzlar, Germany). The sections were stained with a 1% safranin solution and 1% Light Green SF yellowish solution and dehydrated with ethanol series (50%, 70%, 90%, 95%, and 99%) and xylene (Jeon *et al*. 2018a). The permanent slides were obtained using Canada balsam resin. For fiber length measurements, match-sized bamboo slivers were macerated with a mixed solution of glacial H_2O_2 and CH_3COOH with a ratio of 1:1 (v/v) and then heated at 60 °C until defibrillation (Franklin 1945).

In this study, the vascular bundle density was measured in the area of 4 mm^2. The percentage of cell types was determined as the proportion of the area of each cell type per the total area of the optical micrographs at 4x magnification. Data for vascular bundle density and percentage of cell types were collected 10 times and 5 times, respectively. The dimension of fiber, vessel, and parenchyma cells was measured with 40 replications.

X-ray Diffraction Analysis

Equatorial X-ray diffractograms in reflection mode were obtained using X-ray diffraction (Cu target, DMAX 2100V, Rigaku, Tokyo, Japan, 40 kV, 40 mA) installed at the Institute of Forest Science, Kangwon National University, Chuncheon, Korea. The relative crystallinity and crystallite width of the bamboo culms were analyzed using Segal's method (Segal *et al.* 1959) and Scherrer's equation (Burton *et al.* 2009), respectively.

Data Analysis

Analysis of variance and Duncan multiple range test were applied to test the significance of vascular bundle density, cells dimension, and cells proportion of the bamboo species using IBM SPSS Statistics, Version 21 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Characteristics of Vascular Bundles

Figure 1 shows the cross-section of the three *Gigantochloa* bamboo samples consisted of vascular bundles and ground parenchyma. The shape or pattern of vascular bundles in bamboo species is classified into 4 types (Liese 1980). Tropical bamboo generally has vascular bundles of types III and IV (Grosser and Liese 1971). Microscopic observations on the three bamboo species showed the vascular bundle of type III. This vascular bundle type consists of one vascular central strand and one fiber strand.

Table 2 shows the vascular bundle density of the three *Gigantochloa* bamboo species. Vascular bundle density in the outer part of the bamboo culms was higher than that in the inner part. However, there were almost no changes and no fixed tendency of vascular bundle density in the vertical direction. In addition, there were significant differences in vascular bundle density in the horizontal direction of the bamboos. Vascular bundle density of Tali (*G. apus*) bamboo was significantly different from the Andong (*G. pseudoarundinacea*), and Hitam (*G. atroviolacea*) bamboo species.

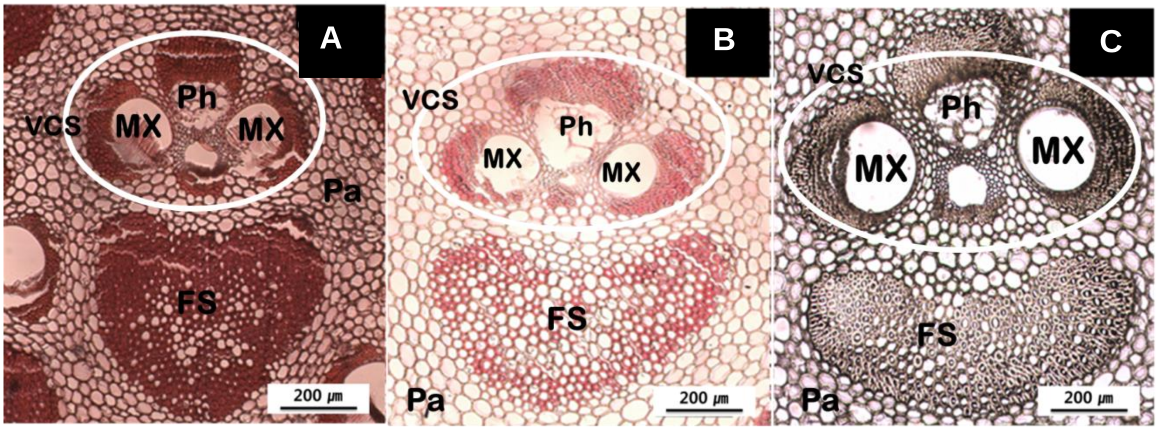

Fig. 1. Vascular bundles in the cross-sections of *G. pseudoarundinacea* (A), *G. apus* (B), and *G. atroviolacea* (C); VCS: vascular central strand, Pa: parenchyma cells, FS: fiber strand, MX: metaxylem vessels, Ph: phloem vessels

Table 2. Vascular Bundle Density (Number/4 mm^2) in the Three *Gigantochloa* Bamboo Species

Species	Outer Part				Inner Part			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
G. pseudoarundinacea	15 ± 1.1	15 ± 0.9	15 ± 0.8	15 ± 0.9^{Ab}	6 ± 0.8	5 ± 1.1	4 ± 0.5	5 ± 1.1^{Ba}
G. apus	17 ± 2.0	17 ± 2.4	16 ± 1.5	17 ± 2.0^{Bb}	4 ± 0.5	4 ± 0.5	4 ± 0.7	4 ± 0.5^{Aa}
G. atroviolacea	13 ± 1.5	15 ± 2.9	14 ± 1.2	14 ± 2.1^{Ab}	6 ± 0.8	6 ± 0.7	4 ± 0.7	5 ± 1.0^{Ba}

Notes: Numbers in the same column followed by different capital letters are significantly different at 5% level. Numbers in the same row followed by different lower case letters are different at 5% significant level.

The significant difference in vascular bundle density between the inner and outer parts may be due to the increasing size of the vascular bundles in the inner part of the culm; thus, the number per unit area was decreased. Several studies have shown a similar trend in vascular bundle density along the horizontal position. Mustafa *et al.* (2011) found that number of vascular bundles of various Malaysian *Gigantochloa* species decreased from the outer part to the inner part of the culm, both in the node and internode. Vascular bundle number near epidermis of the culm of *Phyllostachys pubescens* was higher than that in the near pith part (Jeon *et al.* 2018b). Quite recently, Darwis *et al.* (2020) reported using a non-linear regression equation that the number of vascular bundles in the culm of *G. pruriens* decreased from the outer layer to the inner.

Cell Proportions

Table 3 shows the cell proportions of the three *Gigantochloa* bamboo species. Bamboo species and horizontal position have a significant effect on the proportion of each cell. The percentage of fiber cells in the outer part was higher than that in the inner part, but parenchyma and vessels in the outer part were of lower portion than the inner part for all three of the bamboo species. Andong (*G. pseudoarundinacea*) bamboo showed the lowest fiber percentage and highest parenchyma proportion among the three species studied. Vessel portion in the inner part of Hitam (*G. atroviolacea*) bamboo showed a somewhat higher value compared to the other bamboo species. Meanwhile, differences in vertical position on fiber and parenchyma cell portion do not show a consistent tendency for all bamboo species. Vessel cell portion tended to increase from the bottom culm to the top culm position. Darwis *et al.* (2020) reported a similar trend in *G. pruriens*, with an increase in the parenchyma percentage from the outer to the inner part and *vice versa* for the vascular bundles. They also reported that parenchyma percentage in the bottom position was lower than that in the middle and top positions. The different portions in the outer and inner part of the culm could be due to different vascular bundle densities. Because the vascular bundle frequency decreases in the inner part, parenchyma ground tissue would occupy more space (Huang *et al.* 2015).

Table 3. Cell Proportion (%) of the Three *Gigantochloa* Bamboo Species

Species	Position	Fiber		Parenchyma		Vessel	
		Outer	Inner	Outer	Inner	Outer	Inner
G. pseudoarundinacea	Top	49 ± 1.4 A	29 ± 3.5 A	42 ± 1.6 A	61 ± 1.9 AB	8 ± 0.9 A	11 ± 2.3 A
	Middle	52 ± 1.6 A	31 ± 1.3 A	41 ± 1.8 A	60 ± 1.8 A	7 ± 0.3 A	9 ± 0.9 A
	Bottom	52 ± 2.3 A	29 ± 1.2 A	41 ± 2.1 A	63 ± 1.2 B	7 ± 0.2 A	8 ± 0.6 A
	Mean	51 ± 2.2 Ab	30 ± 2.3 Aa	41 ± 1.7 Ba	61 ± 2.2 Ab	7 ± 0.9 Aa	9 ± 1.8 ABa
G. apus	Top	54 ± 1.4 A	30 ± 1.4 B	39 ± 1.2 B	62 ± 2.2 A	7 ± 0.7 A	8 ± 1.0 A
	Middle	57 ± 0.5 B	32 ± 0.8 C	37 ± 1.1 A	59 ± 2.3 A	6 ± 0.6 A	8 ± 1.7 A
	Bottom	58 ± 0.9 B	27 ± 0.9 A	36 ± 1.1 A	66 ± 1.3 B	6 ± 0.3 A	7 ± 0.9 A
	Mean	57 ± 2.5 Bb	30 ± 2.4 Aa	37 ± 1.9 Aa	62 ± 3.3 Ab	6 ± 0.7 Aa	8 ± 1.3 Ab
G. atroviolacea	Top	62 ± 0.7 A	29 ± 0.9 A	28 ± 1.1 A	58 ± 1.5 A	11 ± 1.5 C	13 ± 1.6 B
	Middle	54 ± 8.4 A	29 ± 1.2 A	41 ± 8.0 B	59 ± 0.6 A	5 ± 0.9 B	12 ± 1.1 B
	Bottom	59 ± 1.4 A	26 ± 2.5 A	38 ± 1.7 **B**	67 ± 2.8 B	3 ± 0.5 A	7 ± 0.8 A
	Mean	58 ± 5.5 Bb	28 ± 2.0 Aa	35 ± 7.3 Aa	61 ± 4.4 Ab	6 ± 3.6 Aa	11 ±2.9 Bb

Notes: Numbers in the same column followed by different capital letters are different at 5% significant level among vertical position. Numbers in the same row followed by different lower case letters are different at 5% significant level between outer and inner part for each type of cell.

Cells Dimensions

Fiber lengths of the three *Gigantochloa* bamboo species are shown in Table 4. Fibers in the outer part of the bamboo culm were longer than those in the inner part. The analysis of variance showed that the bamboo species and horizontal position significantly influenced the fiber length. There was a significant difference in the length of the fibers between the outer and inner parts of the culm. However, this was not a consistent tendency in the fiber length along the vertical direction. In Tali bamboo, the middle part of bamboo culm had the lowest fiber length, while the highest fiber length was found in the middle part in Hitam bamboo. An increase of fiber length from the bottom to the top of the culm was found in Andong bamboo. Siam *et al.* (2019) reported fiber lengths of 13 Malaysian bamboo species, which decreased from the bottom to the top position. The variety in fiber length seen in the trend along the vertical position might depend on the species. Other research from India found that the longest fiber in the culm of *Bambusa mizorameana* was in the middle position and the shortest was in the bottom position (Kumar *et al.* 2017). In *G. pruriens*, the fiber length was reported to be the shortest in the inner and bottom of the culm (Darwis *et al.* 2020).

Table 4. Fiber Lengths (µm) of the Three *Gigantochloa* Bamboo Species

Species	Outer				Inner			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
G. pseudoarundinacea	2330 ± 232	2413 ± 395	2637 ± 506	2460 ± 412^{Bb}	2304 ± 285	2353 ± 373	2600 ± 428	2419 ± 383^{Ba}
G. apus	2404 ± 251	2392 ± 342	2457 ± 286	2417 ± 294^{Bb}	2321 ± 210	2184 ± 269	2408 ± 294	2304 ± 272^{Ba}
G. atroviolacea	2085 ± 230	2460 ± 581	2373 ± 206	2306 ± 409^{Ab}	2071 ± 150	2249 ± 349	2150 ± 247	2157 ± 267^{Aa}

Notes: Numbers in the same column followed by different capital letters are different at 5% significant level. Numbers in the same row followed by different lower case letters are different at 5% significant level.

The vessel diameter of the outer part of *Gigantochloa* bamboo ranged from 173.1 µm to 308.1 µm (Table 5). The largest vessel diameter was found in Andong bamboo, and there were significant differences in vessel diameters among the bamboo species. Jeon *et al.* (2018a) reported that vessel diameter of three Korean bamboos (*Phyllostachys genera*) ranged from 90.9 µm to 117.9 µm, which were smaller in diameter compared to that found in this study. Although, comparable results for vessel diameter of *G. pruriens*, which ranged from 130 µm to 197 µm have been reported (Darwis *et al*. 2020).

Table 5. Dimensions of Vessel and Parenchyma (µm) of the Three *Gigantochloa* Bamboos

Characteristics	Vessel Diameter on the Outer Part	Parenchyma Diameter on the Outer Part	Parenchyma Length	
			Radial	Tangential
G. pseudoarundinacea	308.1 ± 40.8^{c}	35.5 ± 3.6^{a}	101.4 ± 9.3^{Ab}	96.2 ± 11.9^{Ab}
G. apus	204.5 ± 34.8^{b}	36.7 ± 9.4^{a}	78.1 ± 21.7^{Aa}	87.2 ± 18.4^{Aa}
G. atroviolacea	173.1 ± 30.4^{a}	39.5 ± 3.8^{b}	120.4 ± 10.9^{Ac}	125.0 ± 9.8^{Ac}

Notes: Numbers in the same column followed by different lower case letters are different at 5% significant level. Numbers in the same row followed by different capital letter are different at 5% significant level.

Figure 2 shows the optical micrographs of radial and tangential sections of the three *Gigantochloa* bamboos. The diameters of the parenchyma cells on the outer part in the cross-sections of three *Gigantochloa* bamboos was 35.5 to 39.5 µm (Table 5). The lengths of parenchyma cells in the radial and tangential sections of the bamboos was 78.1 to 120.4

µm and 87.2 to 125.0 µm, respectively. There was a significant difference in the parenchyma diameter of *G. atroviolacea* compared to the other bamboo species. Parenchyma lengths between bamboo species were also significantly different. However, parenchyma length between the radial and tangential sections in each bamboo species were not significantly different. Wahab *et al.* (2010) found that parenchyma cell diameter of *Bambusa vulgaris* grown in Malaysia was 23.3 µm to 26.6 µm, which is lower than what was found in this study. Jeon *et al.* (2018a) reported that the diameter and length of parenchyma cells showed significant differences among three *Phyllostachys* bamboos.

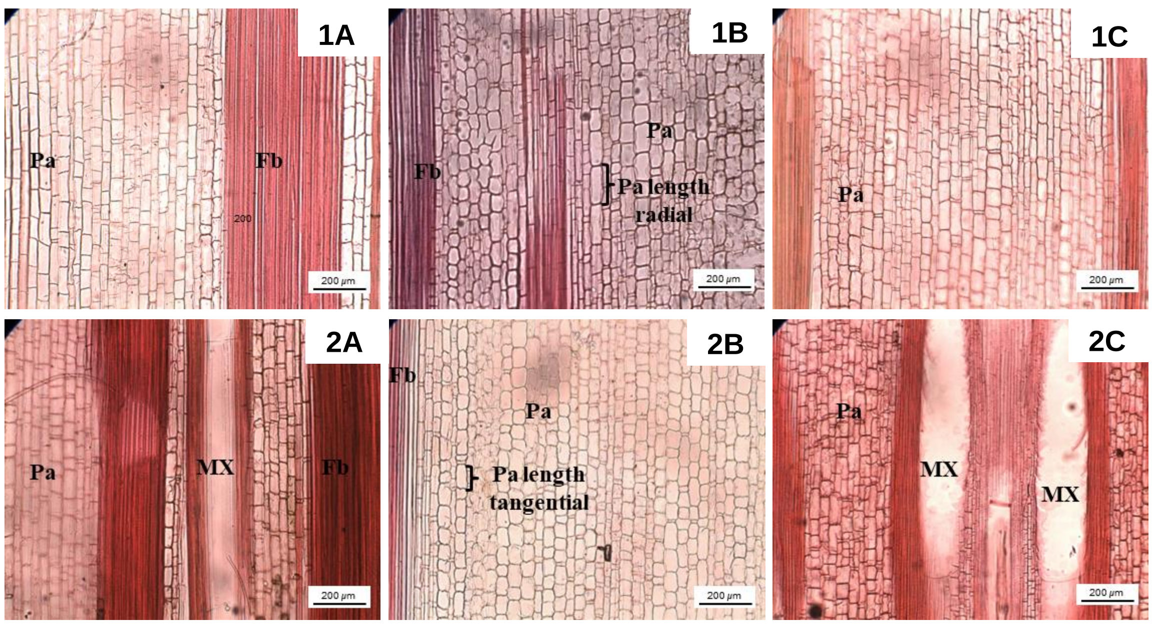

Fig. 2. Optical micrographs of radial (1A, 1B, and 1C) and tangential (2A, 2B, and 2C) sections of *G. pseudoarundinacea* (A), *G. apus* (B), and *G. atroviolacea* (C); Pa: parenchyma cells, MX: metaxylem vessels, Fb: Fiber bundles

Relative Crystallinity

Table 6 shows the crystalline properties in the culms of the three *Gigantochloa* bamboo species.

Table 6. Crystalline Properties of Three *Gigantochloa* Bamboo Species

Species	Position	Relative Crystallinity (%)				Crystallite Width (nm)			
		Outer	Mean	Inner	Mean	Outer	Mean	Inner	Mean
G. pseudoarundinacea	Top	78	81 ± 2.3^{Ba}	74	77± 3.0^{Aa}	3.9	4.1 ± 0.16^{Bb}	3.7	3.8 ± 0.14^{Aa}
	Middle	82		80		4.1		3.9	
	Bottom	82		77		4.2		3.9	
G. apus	Top	77	78 ± 1.2^{ABb}	74	73 ± 0.6^{Aa}	3.4	3.4 ± 0.02^{Aa}	3.3	3.3 ± 0.05^{Aa}
	Middle	79		74		3.5		3.3	
	Bottom	77		73		3.4		3.3	
G. atroviolacea	Top	74	75 ± 2.3^{Aa}	72	74 ± 2.1^{Aa}	3.8	4.0 ± 0.16^{Ba}	3.2	3.8 ± 0.52^{Aa}
	Middle	78		76		4.0		4.1	
	Bottom	74		73		4.1		4.0	

Notes: Numbers in the same column followed by different capital letters are different at 5% significant level. Numbers in the same row followed by different lower case letters are different at 5% significant level.

The relative crystallinity and crystallite width in the outer part were higher than those in the inner part of the bamboos. There were some differences in the crystalline properties between the bamboo species. Andong bamboo had the highest relative crystallinity, while Hitam bamboo had the lowest. The smallest crystallite width was found in Tali bamboo.

Jeon *et al.* (2018a) reported comparable values in relative crystallinity from three *Phyllostachys* bamboo species. They also observed a similar trend along radial direction as was noted in the present study. This tendency could be caused by the higher vascular bundle density and fiber percentage in the outer parts compared to the inner parts of the bamboo culm. Other studies have shown higher crystallite width in tropical bamboo. Brito *et al.* (2012) showed that the crystallite width of *Bambusa vulgaris* was 5.7 nm. A similar crystallite width of 5.59 nm was also found in *Dendrocalamus aspe*r (Fatriasari *et al.* 2014). However, a study on temperate bamboo showed a similar trend in crystallite width along the horizontal position as found in this study. Wang *et al.* (2012) found that crystallite width of *Phyllostachys edulis* planted in China increased from about 2.9 nm in the inner part of the culm to 3.1 nm in the outer part. Crystallite widths of three *Phyllostachys* bamboos also increased from the inner part to the outer part of the culm; however, a consistent trend was not found among the three bamboos in the vertical position (Jeon *et al.* 2018a, 2018b).

CONCLUSIONS

1. The *Gigantochloa pseudoarundinacea, G. apus,* and *G. atroviolacea* bamboo had the vascular bundle of type III.
2. Vascular bundle density in bamboo culms was higher in the outer part than that of the inner part. There was a significant difference in the vascular bundle density in the radial direction among the bamboo species.
3. Fiber portion decreased from the outer part to the inner part and *vice versa* in the case of parenchyma and vessel portions. There was a significant difference in fiber percentage between the bamboo species. Vessel portion in Hitam bamboo showed a somewhat higher value compare to the other two bamboo species.
4. Fiber length in the outer part of culms was longer than that in the inner part. Andong bamboo had the largest fiber length and vessel diameter. There was a significant difference in fiber length between the outer and inner parts in the bamboo culms. Significant differences were also found in vessel diameter and parenchyma cell dimension among the bamboo species.
5. There was a slight difference in the relative crystallinity and crystallite width between the outer and inner parts of the culm and among the bamboo species.
6. All parameters showed a variation in the radial direction of the three bamboo culms but did not show a consistent tendency in the vertical direction.

In conclusion, it is revealed that vascular bundle density, vessel diameter, and parenchyma cell length can be used to identify the species among the three *Gigantochloa* bamboo species.

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