Anatomical Properties of Cultivated 3-Year-Old Bamboo Gigantochloa Levis and G. Scotechinii

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Abstract

The anatomical properties of 3-year-old tropical bamboos Gigantochloa levis and G. scotechinii. These bamboos were selected and harvested from the plantation plots located at the Forest Research Institute Malaysia in Kepong, Selangor. The studies focussed on the vascular bundles and fibres the located at the internodes and nodes seven, eight and nine at the outer, middle and inner cross-section of the bamboo culms. Data were taken on the sizes of the vascular bundle's length, vascular bundles width, fibre length, diameter, lumens diameter, walls thickness and fibre Runkle’s ratio between each of the bamboo species with the sample's location at the internodes, nodes, and locations in the cross-section of the bamboo culms. The two bamboo species exhibited similar in characteristics but having different sizes in anatomy and microstructure features. The results in the fibre's morphology studies showed that the fibres for each species possess different anatomy characteristics and properties in having different lengths, diameters, cell walls thickness and lumen sizes. The size of the vascular bundle is smaller at the outer position and become more prominent at the central locations.

Keywords: Cultivated Gigantochloa levis, G. scotechinii, anatomical studies, vascular bundles, fibres, microstructure features.

INTRODUCTION

Bamboo categorized as non-wood forest products are the best possible alternative to replace timber in the future. It took between three to four years for bamboo to mature before harvesting. Research in products utilization of bamboo in the modern era has recently intensified. Bamboo products are now used in the engineered bioresources products.

Bamboo, considered among the fastest-growing plant, has been the focus of research and development in recent years. However, the study of cultivated bamboo stands has so far mostly confined to selected species in silviculture and fertilisers application to enhance growth [1]. Properties of bamboo culms such as the anatomical structures and physical characteristics are known to influences on their durability and strength [2-6]. This is supported by Wahab et al., [2] in their studies on the anatomical and physical properties of cultivated Bambusa vulgaris. The information generated on the anatomical properties pf bamboo can be used to determine their possible proper utilisation. Currently, most of the bamboos are used for making traditional products such as handicraft, basketry, and high-value-added products. Gigantochloa species are considered the most popular among the bamboo-based industry in the tropical regions. These bamboos are easy to regenerate. They possess thick culms wall and having a uniform long length between the nodes and internodes. These make them suitable as materials for industrial usage.

This study aims to determining the anatomical and structural properties between bamboo G. levis and G. scotechinii. These properties investigated due to their strong relationship with strength, preservative absorption, distribution and likely pathways for colonization by micro-organisms [7-9].

RESEARCH METHODS

MATERIALS

Bamboo culms of cultivated species Gigantochloa levis, G.scotechinii and G.wrayi were harvested from the forest managed area in the FRIM.
Kepong, Selangor, Malaysia. Matured bamboo culms of 3-year-old were selected as they found to be most suitable as the for industrial uses. These culms with a diameter range from 10-18 cm in diameter were taken from randomly chosen clumps, depending on species. Each culm was marked and cut at nodes and internodes 7, 8 and 9. End-coating paint was applied to newly cut surfaces of the culms before transported to the laboratory. The applied paint reduces or preventing moisture evaporation and act as barriers in preventing fungal and insect attacks on the bamboo. The total number of the specimen taken was about ten (10) culms per species.

Sample Preparation

The bamboo culms were segregated according to the species, and further sub-segregated into nodes and internodes pieces. Specimen blocks intended for anatomical investigations were fixed in formalin-acetic acid (FAA) solution and kept in closed bottles. The FAA consists of 90% ethanol (70% concentration), 4% glacial acetic acid and 6% formaldehyde (37-48% concentration) [4]. Each culm was consistently cross-cut into samples consisting of position with and without nodes for subsequent respective studies.

Anatomy Assessment

The technique used by Wahab et al., [2, 3] and Latif and Tamizi [5] applied in measuring and counting in the distribution of the vascular bundles on the bamboo surface at the cross-section. The anatomical characteristic of the three (3) bamboo species with two locations (node and internode) and three (3) positions (outer, middle and inner layer) of the bamboo culm was studied.

Vascular Bundles

Method of measuring the vascular bundles' distribution and fibre dimensions was also adopted from the technique used by Wahab et al., [2, 3] and Latif and Tamizi [5].

Vascular bundle size

The measuring tools of the scanning electron microscope were used in measuring the sizes of the vascular bundles [10].

Determination of Fiber Morphology

Bamboo Maceration

The bamboos were split to size 20 mm x 10 mm x thickness than was cut tangentially and divided into 3 equal sections (inner, middle and outer layer). Each section was split radially into match stick sizes using a sharp knife. Macerates were prepared from match-stick size's bamboo by placing them in a solution containing glacial acetic acid (M=60.05g/mol) and hydrogen peroxide (30% and M=34.01 g/mol) at ratio 1:1. The bamboos in the solution were heated over a water bath inside a fume chamber for 2-3 hours until it becomes soft and white. One or two drops of sodium hydrogen carbonate crystals were added to neutralize the acid before the mixture was decanted and washed with distilled water. A through shaking of the mixture was done to separate the individual fibers. Safranin was used to colour the extracted fiber to red. One hundred undamaged or unbroken fibres were measured for their length (L), fibre widths (d), lumen diameter (l) and cell wall thickness (w). Quantimeter Image Analyzer equipped with Lecia Microscope and Hipad Digitizer (Quantimet 520, Cambridge Instruments) was used to observe and measured at computer images at 10 x (length), 100 x (diameter) and 100 x (lumen) magnifications. The calculations of felting factor (L/d), Runkel’s ratio (2w/l), and coefficient of the suppleness or flexibility ratio (l/d) carried out in accordance to Mustafa et al., [10].

Cell Wall Structure in Electron Microscopy

The scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to analyse the fine structure of the bamboo cell walls. For SEM analysis, the samples were then selected and cut into a smaller size for the shorter duration of pre vacuum process. The surface section of samples was cut using high speed microtome blade to ensure the smooth surface. The samples went through pre vacuum process on a thin plate before the Auram coating process took place (about 20 nm) to ensure the efficient conductivity for the analysis process. The apparatus for the coating process is called ‘sputters coater’ Fison SC 515. Scanning analysis was performed using ‘Leica Cambridge S - 360’, with magnification up to 4000 times. The samples for TEM analysis were dehydrated in an ethanol series and embedded in Spurr resin. For cell wall structure of bamboo fiber, they were chose according to species and position in bamboo culm and cut into pieces of 2 x 3 blocks. Samples were then dehydrated in an ethanol series and embedded in Spurr resin (Epon), which polymerized for 24 hours at 60°C. Transverse sections (1µm) were cut from the embedded material, using the Sorvall ultra microtome (MT 5000) and stained with 1% Toluidine Blue for lignin distribution determination. This gives a high contrast to lignin rich structure such as middle lamellas and cell corners. The section was viewed under the polarized light microscope (Nikon YS2-H). Ultra-thin sections (0.1µm) were obtained from embedded samples, stained with 2% uranyl acetate and lead citrate and finally viewed under TEM (energy filter - Zeiss Libra®120).

RESULT AND DISCUSSION

Vascular Bundle Distribution

The result for the vascular bundle's distribution on the two (2) selected Gigantochloa species are shown in Table 1. The mean number of vascular bundle for G. scortechinii was 6.38 bundle/4 mm² and G. levis at 4.33 bundle/4 mm². These were in agreement with Wahab et al., [3] and Latif [11] finding in the number of vascular
bundles in *G. scortechinii*. The anatomical features within and between culm of different or even the same bamboo species may vary as the individual characteristic of the bamboo itself [7, 10-12]. Different number of vascular bundle in the node and the internode sections were observed in the bamboo culm. The distributions of vascular bundles in the internodes were higher than the nodes. The vascular bundles were also observed to be higher in the number and more compacted in the outer layers of the bamboo culm than those in the inner layers. This is acknowledged by other researchers [13, 5, 14], Li [15] in her studies on a monopodial bamboo *P. pubescens* found that the numbers of vascular bundles were higher compared to the sympodial bamboo species. Hisham et al., [14] studied on the anatomical, physical and chemical properties the characterization of bamboo *G. scortechinii* at different ages found that the number of vascular bundle’s increases from the inner zone towards the outer zone. Similar trend was reported in sympodial bamboo *Phyllostachys pubescens* [16]. This indicates that bamboo possesses long and small vascular bundle at the outer zone, but short and big inner the inner zone [6].

**Vascular Bundle Length**

The results on the the measurement of the vascular bundles length is shown in Table-2. The higher mean of vascular bundle length at internodes were *G. levis* (1171.14 µm) followed by *G. scortechinii* (787.19 µm). The vascular bundle length at the nodes were *G. levis* (1193.89 µm) followed by *G. scortechinii* (1078.20 µm). The vascular bundles lengths were longer at the node than the internodes. The mean average of vascular bundle length for outer layer position was 748.54 µm, middle layer 1013.25 µm and for inner layer was 1131.42 µm. The vascular bundles lengths were longer at the middle than at the outer and inner periphery.

**Vascular Bundle Width**

The results on the measurement of the vascular bundles width is showed in Table-3. The higher mean of vascular bundle width at internodes were *G. levis* (798.26 µm) and *G. scortechinii* (544.63 µm). Significant difference in the vascular bundle width between the internodes and nodes at different position are noted. The internodes vascular bundle width was 585.42 µm and for nodes was 630.70 µm. The vascular bundles width was observed to be higher at the node. The mean average of vascular bundle width for outer layer position was 467.23 µm, middle layer 599.76 µm and for inner layer was 757.19 µm. It shows the significant different between the vascular bundles in samples position. Vascular bundle width was widened at the inner and smaller toward the outer periphery position. This is due to the size of vascular bundle which were smaller and compact at the outer layer, compared to the inner layer of bamboo culms.

**Fiber Morphology**

**Fiber Length**

The results for the fiber lengths study of the various *Gigantochloa* species are showed in Table-5, Plate 1 and 2. The statistical analysis shows significant different in the fiber length between the bamboo species. The higher length was obtained from the *G. levis* (2039.98 µm) follow by *G. scortechinii* (1745.27 µm). The internode fiber length was 2074.24 µm and for node was 1672.62 µm. It shows there was significant different between position at internode and node. The fiber length was higher at the internode compare to the node. At the internode, the anatomy structure was constant but at the node it was quite twisted. The anatomical factor, maybe contribute the different of fiber length between two position. The mean of the fiberer length for outer layer position was 1698.52 µm, middle layer 2060.41 µm and for inner layer was 1861.35 µm. These show that there were significant different in the fiber length at the internodes, nodes and between the cross-sectional position of the bamboo. Similar observations were made at the internodes and nodes, which shows that the fiber possess longer fiber at the middle layer of bamboo. The same trends were also observed for all the three bamboo species in this study. The fiber length shows considerable differences between species as well as variations within one culm [17]. The mean average for fiber length in genera *Gigantochloa* from this study was 1600 - 2000 µm. The result obtained by Hisham et al., [14] studies on *G. scotechinii* was between 2350 - 2630 µm. While Ireana 2009 [20], study on *B. blumeana* found the length of fiber was around 2900 µm. *B. vulgaris* fiber length is 3600 - 4700 µm [2], 1940 - 2430 µm [11]. The fiber length of *G. levis* and *G. scortechinii* in this study ranged 1750 – 2040 µm while the fiber from *P. Pubescens* which growth in large areas of China, Japan, Taiwan and Indochina was about 1300 µm length [13]. Liese [13] studied the structure of bamboo in relation to its properties and utilization. He noted that the fibers contribute 60-70% by weight of the total culm tissue. Certain species generally have shorter fibers, such as *Phyllostachys edulis* (1.5 mm), *Ph. pubescens* (1300 µm), other longer ones like *Dendrocalamus giganteus* (3200 µm), *Oxytenanthera nigrociillata* (3600 µm), *D. membranaceus* (4300 µm).

Comparison with the fiber length of the Softwood (3600 µm), The fiber length of *Gigantochloa* (1600 - 2000 µm) genera was clearly shorter, but still longer than hardwood (1200 µm). In fact it is longer than the *Eucalytus spp* which was popular as a source of short fibers pulp for paper industry [18, 19]. The short-fiber pulp using by paper mill in Malaysia was the *Eucalytus spp* imported from Brazil. The fibers are combined long fibers pulp from the Softwood imported from Canada. There was a great potential that *Gigantochloa* fibers can be use as a pulp for future in Malaysia.
Fibre Diameter

Table 5 shows the fibre diameter obtained from the study. The larger fibre diameter were *G. levis* (22.67 µm) followed by *G. scortechinii* (17.26 µm). The fibre diameter at different position showed that the node has larger fibre diameter at 22.04 µm and internodes at 18.23 µm. Significant different existed between the fibre diameter in make at the internodes and nodes. The fibre diameter at different position showed that the outer layer was 18.49 µm, middle layer 22.36 µm and inner layer 19.56µm. The fibre diameter of the *Gigantochloa* genera in this study ranged between 17-22.8 µm. The previous studies on the fibre diameter for *G. scortechinii* were 26 µm [14], 23-37 µm [11], while studies on the species of bambusa genera found that; fibre diameter for *B. blumeana* were 12.0 µm [20], *B. vulgaris* was 16.9-18.0 µm [2], 20-42 µm [11]. This study found that the fibre diameter were smaller than the previous studies. The diameter of the fiber of this study was 17-22.8 µm and is smaller than the Softwood (35 µm) and hardwood (25 µm). The comparison between the fibre diameter on this study showed that the fibre diameter *Gigantochloa* genera (17-22.8µm) was bigger than *Eucalytus spp* (15.5 - 16.3µm).

Lumen Diameter

The results on the measurement of the lumen diameter of the two *Gigantochloa* species are shown in Table 5. The lumen diameter for *G. scortechinii* and *G. wrayi* were 8.60 µm and 4.75 µm, respectively. The results obtained in *G. scortechinii* were almost the same as obtained by Tamizi *et al.*, [10] and Hisham *et al.*, [14], but smaller than those obtained by Latif [11]. The lumen diameter for *Bambusa* were 1.6 µm for *B. blumeana* [20], 2.3-2.6 µm for *B. vulgaris* [2]. The lumen diameter for *Eucalytus spp* was 8.5-9.5 µm. The mean average for lumen diameter at different position showed that for the lumen diameter at node was 6.18 µm and for the internodes was 4.43µm. The results showed the lumen diameter were larger at the node compare to the internodes. The mean average for lumen diameter at difference position showed that at the outer layer was 5.44 µm, middle layer was 5.51 µm and at the inner layer was 5.96 µm. The result showed the lumen diameter was largest at the inner and smaller toward the outer layer and it was a significantly different.

Wall Thickness

The results on the measurement of the wall thickness between the two bamboo species are shown in Table 5. *G. levis* with 9.34 µm possess thicker wall compare to the *G. scortechinii* at 4.30 µm. The wall thickness at different position showed that for the wall thickness at node was 7.02 µm and for the internodes was 6.90 µm. The result showed the wall thickness are thicker at the node as compare to the internodes and it was a significantly difference between this two position. The wall thickness at difference position showed the outer layer was 7.03 µm, middle layer 8.43 µm and at the inner layer was 6.80 µm. The result showed the wall thickness are thicker at the outer and thinner toward the inner layer. From this study the fibre wall thickness for both species ranged 4.3-9.34 µm. The fibre wall thickness for *G. scortechinii* obtained by Hisham *et al.*, [14] was 8-10 µm and Latif [11] was 12.5-30.1 µm. The fibre wall thickness of *G.wrayi* (9.02 µm), *G.brang* (9.34 µm) was thicker than *B. blumeana* which was 5.01 µm (Ireana, 2009), *B. vulgaris* which was 7.1-7.6 µm [2], 2.5-13.3 µm [11]. As a comparison, the fibre wall thickness of *G.scortechinii* almost similar with fiber wall thickness of *Eucalytus spp* which was 4.3 µm and 3.29-3.86 µm [21], respectively.

Analysis of Varianace

The analysis of variance (ANOVA) for the above studies is shown in Tables 4 and 5. Table 4 shows the ANOVA on the vascular bundle distribution, vascular bundle length and vascular bundles width between the bamboo species at nodes and internodes. Table 5 shows the ANOVA on the length, fibre diameter, fiber lumen diameter, fiber wall thickness and Runkle’s ratio between the bamboo species, position and position.

Fiber Runkle’s ratio

Table 6 shows the fiber Runkle’s ratio value for every species, position and position. Runkle’s ratio of fibre in this study was higher than 1.0 for *G. wrayi* and *G. levis*. *G. scortechinii* was the only species has the value of Runkle’s ratio less than 1.0. The Runkle’s ratio value more than one, this main the fiber properties was hard and difficult to felting during the paper production. The quality of the paper will be gross and poor bonding if Runkle’s ratio value more than one. If the Runkle’s ratio less than one, it indicates the fiber has a thin fiber wall and easily to felting. The quality of the paper will be better and bonding will be good. This indicates that *G.scortechinii* could be a source to replace short-fiber pulp that was imported from abroad. *Eucalytus spp*, the Runkle's ratio is less than 1.0, namely 0.7 and 0.8 [21].
was even shorter fiber than *G.scortechinii*. The Runkle's for hardwood and Softwood was 0.4-0.7 and 0.35 respectively. Kenaf has Runkle's ratio of 0.5-0.7 to prove they are good fiber felting power. Runkle's ratio for *G.scortechinii* was lowest than value one, which was 0.97 to prove it can still be used.

**Microstructure Study**

The microstructure studies on the *Gigantochloa levis* and *G. scortechinii* are shown in Plates 1 to 6. Plates 1 & 2 shows the ultrastructures of the internodes and nodes focusing at the vascular bundles of the *G. levis*, and *G. scortechinii* taken using the SEM. Plates 3 and 4 shows the images of the bamboo species taken using TEM focusing on the fibres cells. The cells wall of the fibres clearly indicates that they possess more than two layers, which were S1, S2, S3 and Sn... All the two bamboo species show clear that they belong to the bamboo is bamboo in class type III [4]. They possess a vascular sheath fibre and one fibre strand. Even though all the cells are similar in shape but they are however different in sizes in position at internodes and nodes, and position in the bamboo at either the outer, middle and the inner layers. The distribution of the vascular bundles per mm², vascular length, vascular bundle width are shown in Tables 1, 2, 3 and 4. The fibres length, diameter, lumen diameters, wall thickness and the Runkle’s ratio are given in Tables 5 and 6.

**Table-1: Number of vascular bundle (per 4 mm²) between *G. levis* and *G. scortechinii***

| Position | Location | *G. levis* | *G. scortechinii* |
|----------|----------|------------|-------------------|
|          | Outer    | 7.46 (±1.72) | 13.24 (±1.75)    |
| Internode| Middle   | 3.00 (±0.54) | 6.44 (±1.12)     |
|          | Inner    | 2.54 (±0.40) | 3.50 (±0.64)     |
|          | Mean     | 4.33 (±0.87) | 7.73 (±1.17)     |
|          | Outer    | 5.94 (±3.40) | 10.55 (±1.77)    |
| Node     | Middle   | 3.56 (±1.03) | 5.80 (±1.40)     |
|          | Inner    | 2.87 (±0.88) | 2.75 (±1.13)     |
|          | Mean     | 4.12 (±1.77) | 6.37 (±1.43)     |

Values in bracket represent the standard deviation.

**Table-2: Vascular bundle length (µm) between *G. levis* and *G. scortechinii***

| Position | Location | *G. levis* | *G. scortechinii* |
|----------|----------|------------|-------------------|
|          | Outer    | 928.73 (±303.07) | 625.77 (±232.89) |
| Internode| Middle   | 1176.49 (±144.23) | 882.32 (±74.07)  |
|          | Inner    | 1408.20 (±210.11) | 853.60 (±110.02) |
|          | Mean     | 1171.14 (±219.13) | 787.19 (±138.99) |
|          | Outer    | 769.09 (±129.79)  | 785.40 (±193.88) |
| Node     | Middle   | 1387.66 (±65.41)  | 999.55 (±157.05) |
|          | Inner    | 1424.92 (±99.85)  | 1449.64 (±172.69) |
|          | Mean     | 1193.89 (±98.35)  | 1078.20 (±174.54) |

Values in bracket represent the standard deviation.

**Table-3: Average vascular bundle width (µm) between *G. levis* and *G. scortechinii***

| Position | Location | *G. levis* | *G. scortechinii* |
|----------|----------|------------|-------------------|
|          | Outer    | 610.61 (±129.41) | 824.41 (±141.22) |
| Internode| Middle   | 723.01 (±93.36)  | 494.11 (±73.87)  |
|          | Inner    | 1061.18 (±103.56) | 527.62 (±101.20) |
|          | Mean     | 798.26 (±108.78) | 501.38 (±105.43) |
|          | Outer    | 570.89 (±89.88)  | 478.04 (±46.60)  |
| Node     | Middle   | 752.93 (±76.08)  | 593.74 (±72.24)  |
|          | Inner    | 637.48 (±73.18)  | 591.88 (±92.30)  |
|          | Mean     | 720.43 (±79.71)  | 587.89 (±70.38)  |

Values in bracket represent the standard deviation.
Table 4: Analysis of variance for anatomical properties between bamboo species, position & position

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|-------------------------------------------------------|
| Anatomical Properties                                  |
| No. Vascular bundle | Vascular bundle length | Vascular bundle Width |
|---------------------|------------------------|-----------------------|
| Species             | G. levis               | G. scortechinii       |
| Position            | Internode              | Node                  |
| Location            | Outer layer            | Middle layer          |
| Inner layer         |                        |                       |

Table 5: Analysis of variance for fibre morphology between species, position & position

| Table 5: Analysis of variance for fibre morphology between species, position & position |
|-------------------------------------------------------|
| Fibre Morphology                                     |
| Fibre Length | Fiber Diameter | Lumen Diameter | Wall Thickness | Runkle’s Ration |
|---------------|----------------|----------------|----------------|-----------------|
| Species       | G. levis       | G. scortechinii|
| Position      | Internode      | Node            |
| Location      | Outer layer    | Middle layer    |
| Inner layer   |                |                 |

Values followed by the same letter in a column is not significant different at 95% probability level.

Table 6: Runkle’s ratio of various Gigantochloa species

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|--------------------------------------------------------|
| Sample Position | Location | G. levis | G. scortechinii |
|-----------------|----------|----------|-----------------|
| Outer           | 4.71 (±2.80) | 2.16 (±1.50) |
| Internode       | 7.19 (±3.09) | 1.42 (±0.95) |
| Node            | 5.44 (±2.09) | 0.97 (±0.78) |
| Outer           | 4.62 (±2.09) | 1.71 (±0.96) |
| Node            | 5.29 (±2.64) | 1.06 (±0.64) |
| Inner           | 4.64 (±2.55) | 0.79 (±0.57) |

Values in bracket represent the standard deviation.
Plate -1: SEM images of the vascular bundles at internodes and nodes of the *G. levis*

Note: SEM image at the internodes and nodes of *G. levis* and *G. scortechinii*. Distribution of vascular bundle (left), Isometric view (central) and vascular bundle image (right).

Plate 2: SEM images of the vascular bundles at internodes and nodes of the *G. scortechinii*
CONCLUSION

The anatomical structure of the two bamboos varies significantly. The distribution and the size of the vascular bundle are differences between species. The vascular bundle of the two species shows a similar pattern and are classified under Type III consisting of a single vascular sheath fibre and one fibre strand. But, the vascular bundle size was significantly different between position (nodes and internodes) and location (outer, middle and inner layers).

The fibre morphology of each species possesses different measurement in size especially in the length, diameter, lumen diameter and wall thickness. The study identified that there were differences in fiber dimensions on the position between the nodes and internodes, and as well as the location at the outer, middle and inner layer within the same species. The length of the fiber was longer at the internode than the node. The middle layer has the longest fiber length compare to the outer and inner layer.

Ultra structures properties of both *G. levis* and *G. scortechinii* shows different characteristics than wood. The fiber of both bamboos has a small lumen and thick wall thickness. Wall thickness of wood only has two layers which were S1 and S2, while in bamboo the fiber has more than two layers, which were S1, S2, S3 and Sn.

The *G. scortechinii* fiber has great potential to be a resource of fiber production. It will be a source to replace short fiber from of hardwood, to combine with soft wood pulp for paper production in the future.

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