

Macroscopic and Microscopic Characteristics of 2- and 4-Year-Old *Schizostachyum brachycladum*

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Abstract: Anatomical of cell wall structure on *Schizostachyum brachycladum* examined. The harvested two-year-old and four-year-old bamboo culms segregated into the bottom, middle and top portions. The samples then undergo the Light Microscopy (LM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to determine their structure such as a vascular bundle, parenchyma, and sclerenchyma. Results show the surface of bamboo was visualized by LM to decide on their structural figure. In this part, 2-year age indicated that higher numbers of vascular bundle and average of mean compared to the 4-year *S. brachycladum*. Followed by a specific study of cell wall structure using SEM with highlighted 4-year *S. brachycladum* had more complex of morphology structure compared to the 2-year-old. Later on, TEM illustrated to shows most depth anatomically structure of bamboo such as middle lamella, primary and secondary walls.

Keywords: Bamboo morphology, *S. brachycladum*, light microscopy, scanning electron microscopy, transmission electron microscopy, young bamboo, mature bamboo, bamboo anatomy.

INTRODUCTION

In the world's bamboo estimated at 31.5 million hectares and have over 75 genera and 1250 species across 21 countries [1]. In Peninsular Malaysia bamboo were covered at about 7% from forest land, means estimated as 421,722 ha, of forest area with has about 59 bamboo species (25 cultivated species and 34 indigenous species) [2]. Bamboo is a vernacular term for members of the family Poaceae [3] and subfamily Bambusoideae with included both herbaceous bamboo or Olyreae tribe and woody bamboos or Bambuseae tribe [4]. Then automatically bamboo also included in grass family is Graminae, but the characteristics of bamboo are woody stems [2].

Traditionally, among the Malay folks, bamboo (*S. brachycladum*) was utilized as a container in cooking Lemang, and it is as one of the famous rice cakes. The shoots were edible and had a good taste [5]. Bamboo is the second most crucial nontimber resource and ranked third after timber and rattan. Others, bamboo also is a fast-growing species and can mature within 3 to 4 years after cultivating [6] and [7]. The distinctive part of bamboo is a stem or called as culm,

which has upper ground part that contains most of the woody material [23]. It was cheap material and occupy high mechanical properties among woody materials is currently being recognized as an option to wood [8]. Anatomically, the whole culms of bamboo comprise 40% fibers, which consisted of the vascular bundle and sclerenchyma. The remaining 60% of the total culm are considering parenchyma [9]. Studies in the bamboo culms anatomical structure undertaken by researchers so far have confined to species with thick culms wall. Rare previous studies have ever recorded on the bamboo with thin culms wall. Thin culms wall bamboo species such as *S. brachycladum* has been used for making flooring and panels by the rural communities as they simple to process and work with [5].

In this study, light microscope (LM) used for macrostructure viewing employed to the bamboo surface while scanning electron microscope (SEM) and transmission electron microscope (TEM) applied for in-depth visualization microstructure of bamboo sample. The LM used for counting the significant number of the vascular bundle in the standardized surface area. The SEM provided for producing a scanned image at the spatial resolution and in low acceleration voltage area

[10]. Also, available in wide range of magnifications, full three dimensions (transverse cross-section, tangential section, and radial section), excellent depth of field and straightforward specimen preparation [11]. On the other hand, TEM used to illustrate rare specimens (tissue section, molecules, etc.) through which electrons can pass generating a projection image [12].

MATERIALS AND METHODS

Two-year-old and four-year-old culms of *S. brachycladum* randomly selected and harvested from the forest areas in Jeli, Kelantan. Bamboo culms chose with diameters in the range from 5 to 6 cm. Specimen were segregated into portions bottom, middle and top. The culms were cut approximately 30 cm from ground level. Samples were split to 10 mm x 10 mm cubes for the structure of cell wall prepared separately on Light Microscopy (LM) and Scanning Electron Microscopic (SEM). TEM sample was reduced to the small pieces (diameter = 0.5 mm) and preserved into 3% concentration of glutaraldehyde.

Anatomical structure under Light Microscopy (LM)

The studies on parenchyma fiber, cell wall thickness, and vascular bundle were conducted according to methods described by [21] and [13]. Each part from *S. brachycladum* was cut into a cross section with 20mm x 20mm x thickness (depending on species of bamboo thickness) as a sample dimension. Then, the samples were immediately scrutinized and photographed using a light microscopy Model Olympus SZX9, Olympus Optical, Japan.

Anatomical structure analysis using Scanning Electron Microscopy (SEM)

S. brachycladum visualized under Scanning electron microscopy (SEM) to characterize the morphology structure such as a vascular bundle, parenchyma fiber, vessel and structure of sclerenchyma [13]. The SEM micrographs were taken from the cross-section of samples into 5mm x 5mm size of samples block. The samples with the dry condition were coated with gold by an ion sputter coater (Polaron SC515, Fisons Instruments, and United Kingdom). Then, the samples visualized by Scanning Electron Microscope LEO SUPRA 55 VP, Field Emission SEM, Carl-Zeiss, Oberkochen, Germany.

Anatomical Structure on Transmission Electron Microscopy (TEM)

The fresh of *S. brachycladum* with different ages taken from a cross-section of sample into 2.0 x 0.5 mm size of the sample block. According to Spurr's resin method (1969), samples were fixed in Karnovsky's fixative with 3% glutaraldehyde and washed with 0.1 M phosphate buffer. The specimen was postfixed in 1% Osmium tetroxide and was left in for 2 hours. Next, the samples dehydrated with ethanol 50%, 75%, 95%, 100% of concentration and 100% of acetone then a mixture of acetone and Spurr's resin (1:1) used for the embed sample. Transverse section of thickness one μm was cut using Sorvall Ultra Microtome (MT500) with a diamond knife. The part stained with 2% uranyl acetate and lead citrate. Transverse section examined with a LEO in column EFTEM with OMEGA spectrometer, Zeiss, Libra 120.

RESULTS AND DISCUSSION

Structural of *S. brachycladum* under light microscopy (LM) analysis

The anatomical structure cell of parenchyma and vascular bundle on *S. brachycladum* was characterized in different ages (2-year and 4-year) and portions (top, middle, and bottom) as showed as in Figure 1. *S. brachycladum* culm consists of parenchyma, vascular bundle, sclerenchyma, and vessel. Moreover, the area of each image (a; b; c; d; e and f) standardized to uniformed scale which is under 50 magnification. From the observation, the bottom portion from 2-year and 4-year had less compact on some vascular bundles compared than another image (b, c, e and f) while on the bottom portion of 2-year and 4-year also were visualized more clearly and utterly vascular bundle structure. Based on the previous study, believed that the vascular bundle is smaller and more numerous at the peripheral zone of bamboo culm while in the inner parts the vascular bundle becomes larger and fewer as shown in Figure 1 [14]. Proven by [22] study, the vascular bundle is oval and varies in sizes depending on the location or position as well as species of bamboo.

According to Table 1 and Table 2, the statistical analysis of homogeneous and ANOVA was indicated that the 4-year *S. brachycladum* had a significant difference with a mean of the vascular bundle based on the surface area counted which is 8.00, 8.00 and 14.33 at the bottom, middle and top, respectively. 2-year *S. brachycladum* had not significantly different with the average mean value shows 9.00, 10.00 and 16.33 for the bottom, middle, and top, respectively. According to [15] the most content in culm tissue is parenchyma with about 50%,

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followed by fiber for 40% and the rest vascular bundle (vessel and sieve tubes with companion cells) at about 10% as shown in Figure 1, but with some variation in purpose, the different of sclerenchyma and parenchyma were also can classify based on their contrasted by the darker coloured is sclerenchymatous tissue and lightly stained is the parenchymatous ground tissue. In another hand, the anatomical structure of the bamboo culm supported for understanding the basic physical characterization of bamboo and is responsible for fiber dimension to vary cross and along the bamboo culm [17].

Table 1: Statistical analysis of homogenous subsets due to the age for light microscopy (LM) on *S. brachycladum*

Parameter	4-year-old	2-year-old
Bottom	8.00 ^a ± 1.00	9.00 ^a ± 2.00

happens according to species. So, vascular bundle appearance is important to observe the differences between and within species [16]. For identification

Middle	8.00 ^a ± 1.00	10.00 ^{ab} ± 4.36
Top	14.33 ^b ± 2.31	16.33 ^b ± 3.79

Table 2: ANOVA for light microscopy (LM) on *S. brachycladum* due to the age

Parameter	SS ^a	SS ^b	d _{f^a}	d _{f^b}	MS _a	MS _b	F	Sig.
4-year-old	66.556	14.667	2	6	34.778	2.444	14.227	0.005
2-year-old	94.889	74.667	2	6	47.444	12.444	3.812	0.085

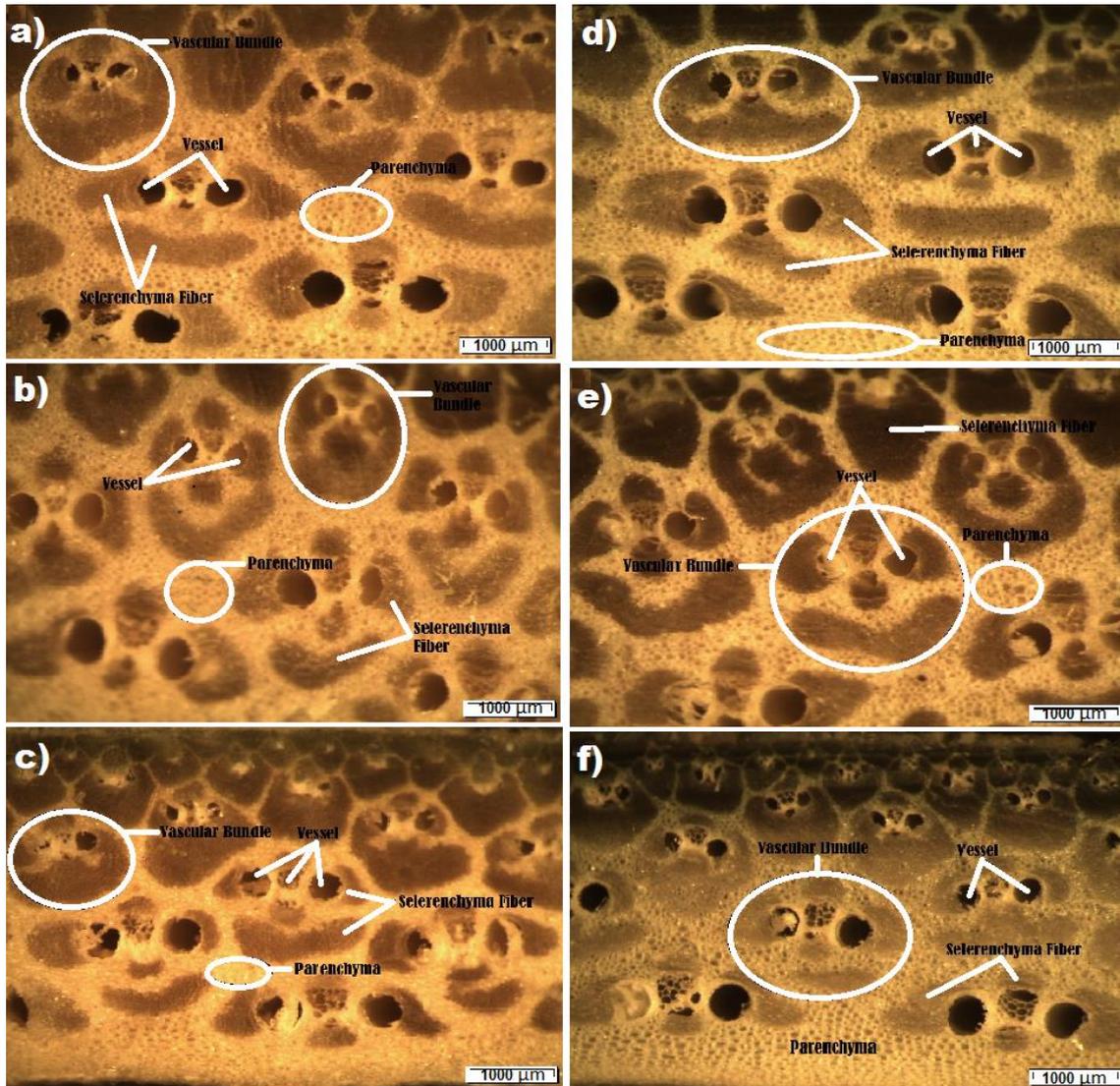


Figure 1: Light microscopy *S. brachycladum* which is (a), (b) and (c) are the bottom, middle and top for 4-year age, respectively while (d), (e), and (f) are the bottom, middle and top for a 2-year age, respectively.

Structural of *S. brachycladum* under scanning electron microscopy (SEM) analysis

The Scanning Electron Microscopy (SEM) viewed more clearly cell wall structure of the parenchyma, sclerenchyma, vascular bundle and vessel compare to the light microscopy (LM). But, SEM was still limited to observe in depth cell wall fiber inside individually. Figure 2, (a) and (b) represented as 4-year *S. brachycladum* while (c) and (d) represented as 2-year *S. brachycladum*. There were illustrated in both image and consisted not too much of difference between others. In-depth observation on Figure 2 (a)

and (b) show parenchyma fiber had a thicker wall and stronger texture compared to the Figure 2 (c) and (d) with believing that had high capabilities to support the massive load. Based on the previous study, the number of layers is mostly restricted from two to four in parenchyma wall whereby with the herringbone pattern of fibrillar arrangement [18] and [15]. Moreover, as reported by [20] on bamboo based the parenchymatous ground cell was outlined by a thin cell wall and large cell lumen with diameter average to 15 mm while thick cell wall and small cell lumen characterized on sclerenchyma fiber with diameter around 5mm and.

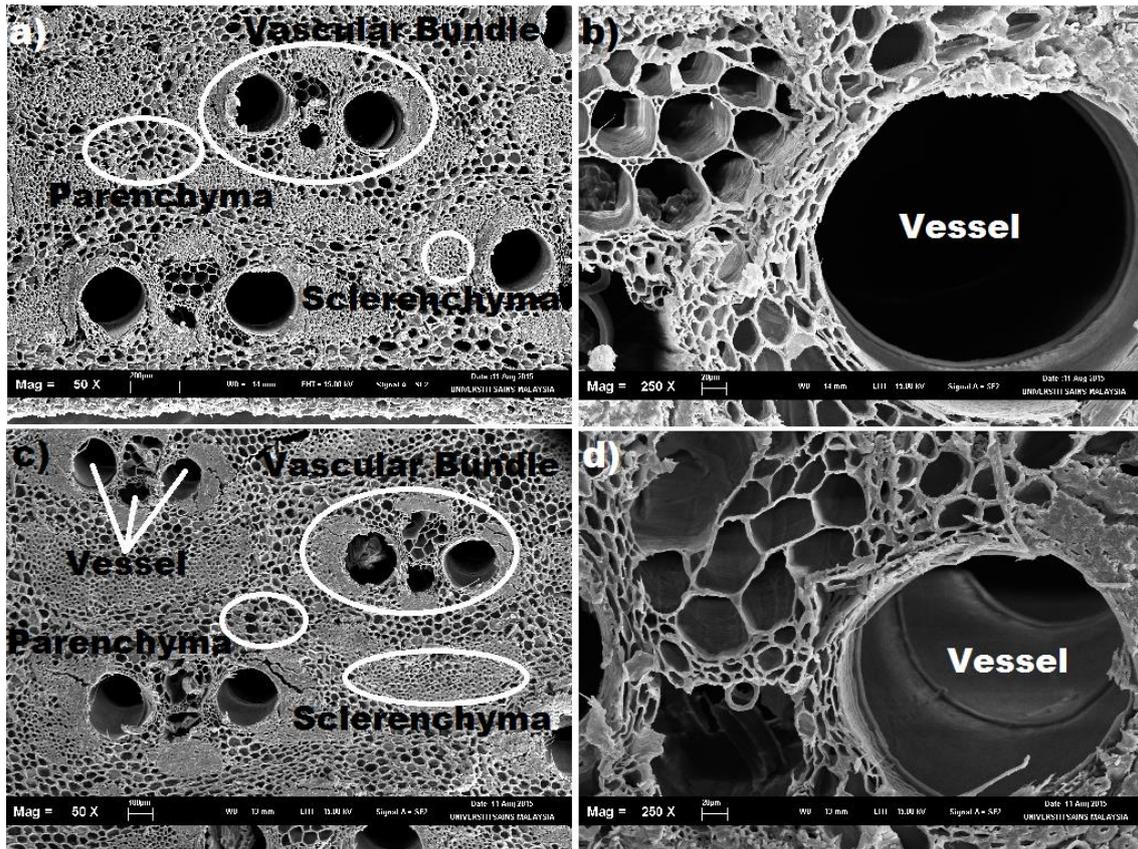


Figure 2: Scanning Electron Microscopy (SEM) of *S. brachycladum* which is (a) and (b) consist on 50x and 250x of magnification for 4-year-old, respectively while (c) and (d) consist on 50x and 250x of magnification for a 2-year-old, respectively.

Structural of *S. brachycladum* under transmission electron microscopy (TEM) analysis

Figure 3 (a), (b) represented as 4-year *S. brachycladum* and Figure 3 (c), (d) indicated of 2-year *S. brachycladum*. Figures illustrated that the cell wall structure of parenchyma cell with clearly using the Transmission Electron Microscopy (TEM) compared than to SEM and LM. The figure also shows that the primary wall (P), secondary wall (S) and middle lamella (ML) appeared as a thin layer. According to the study of the anatomical structure by [18], there are two types of microfibril orientation in bamboo which are the broader ones with fibrillar angle almost parallel to an axis and the narrow lamellae showing the fibrillar angle of 80 – 90° to the axis. Although the fibers in bamboo demonstrated that polylamellate nature with eight lamellae (S1-l, S2-t, S3-l, S4-t, S5-l, S6-t, S7-l, and S8-t) compare to the wood with only three lamellae (S1, S2, and S3). Next, the thick fibril layer which is parallel to the axis

is transcendent compared to the narrow lamellae. The thickness and structure of cell wall were affected the strength properties and density.

Figure 3 highlighted S1, and S2 layers consist of the lignin distributed homogeneously. However, the lignin level increased in the middle lamella (ML) and cell corner. Reported by [19] seen from the SEM and TEM images that possibly be the lignin-rich at corner middle lamella and it was the most durable in physically. Also, frequently show hemicellulose consisted more content on the S1 layer and cellulose more consists in the S2 layer. Based on [15] study, the walls of vessels on bamboo are characterized by a middle lamella and a primary wall together with a well-developed gap of the secondary wall into S1 and S2. Figure 3 highlighted 2-year *S. brachycladum* assume content more lignin and cellulose because the part had more numerous of cell corner and thicker middle lamella and S2 layer compared than 4-year *S. brachycladum*.

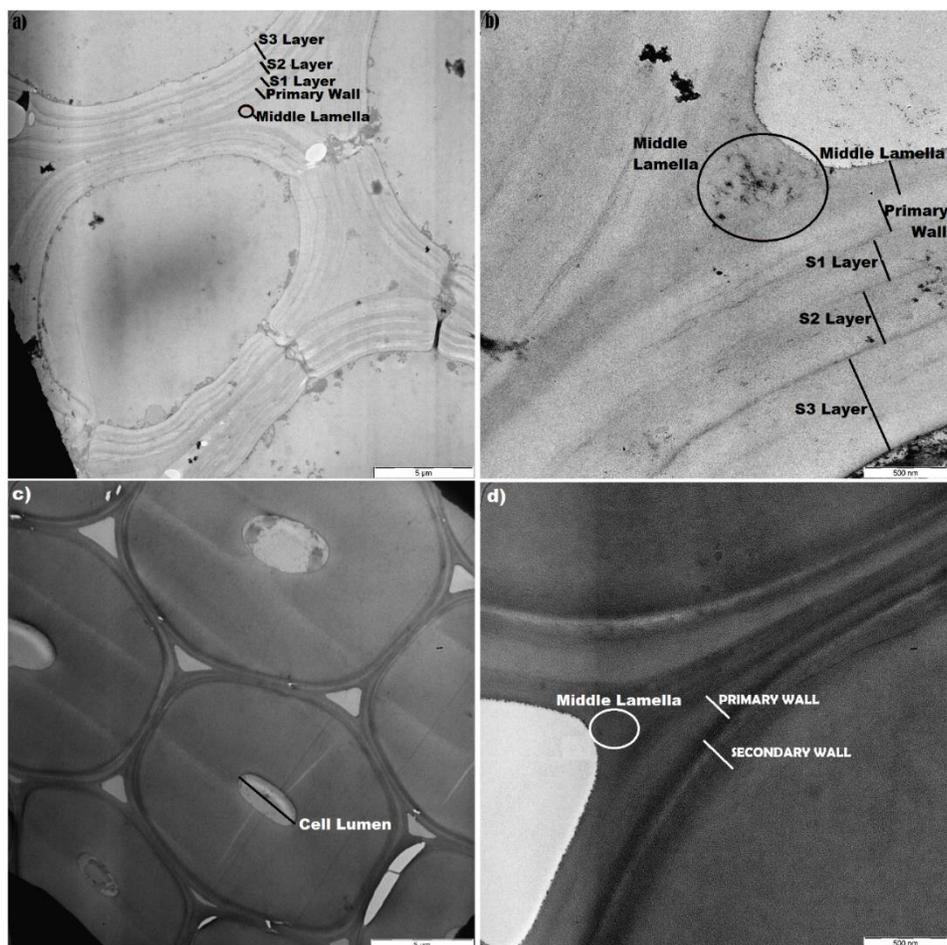


Figure 3: Transmission Electron Microscopy of *S. brachycladum* which is (a) and (b) consist on 1.3k and 10k of magnification for 4-year-old, respectively while (c) and (d) consist on 1.3k and 10k of magnification for a 2-year-old, respectively.

CONCLUSION

Study on the micrograph of the vascular bundle, parenchyma, sclerenchyma, and vessel of two-year-old and four-year-old *S. brachycladum* highlighted LM surface area of vascular bundle and parenchyma cell structure compared than with SEM and TEM. At the same magnification on surface area, LM analyses show that the 2-year *S. brachycladum* had a slightly higher percentage of vascular bundle compared to the 4-year *S. brachycladum*. SEM analyses highlighted not drastically different between 2-year and 4-year or on their magnification showed. TEM indicated 4-year-old consisted of more clear structure of layer (ML, Primary layer and Secondary layer) compared to the 2-year-old species. The overall 4-year *S. brachycladum* designated that most clear cell wall structure which is a vascular bundle, parenchyma, sclerenchyma fiber, and vessel compared to the 2-year *S. brachycladum*. An in-depth study on TEM has indicated that 4-year age had most clear of morphology structure of *S. brachycladum*. 4-

year also shows TEM analyses had some layer (primary layer, secondary layer, and middle lamella) that believed contained lignocellulosic compounds such as cellulose, hemicellulose, lignin, and other substances.

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