# Variations in the Anatomical Characteristics of *Bambusa pervariabilis* Culms with Age and Height

Elin Xiang  Yihan Guo  Shumin Yang  Xinge Liu
Genlin Tian  Jianfeng Ma  Lili Shang

**Abstract**

The anatomical characteristics of culms in *Bambusa pervariabilis* bamboo at different ages and heights were investigated by microscopy and image analysis. Among the two vascular bundle types found in culms, the broken-waist type was considered typical, with the following measurements: average proportion of fibrous tissue, 41.53 percent; length, 1.75 mm; slenderness ratio, 117; and Runkel ratio, 4.00. These values were close to those of the moso bamboo (*Phyllostachys edulis*), which is commercially relevant in China. Age and height significantly influenced the anatomical characteristics of *B. pervariabilis:* with an increase in age, both the length and double-wall thickness of the fiber gradually increased, whereas its lumen diameter decreased. The width of vascular bundles and the length, width, double-wall thickness, and lumen diameter of fiber markedly decreased from the bottom to the top. Therefore, *B. pervariabilis* is an ideal raw material for pulping and papermaking, and its performance is close to that of moso bamboo.

Sympodial bamboo, an important component of bamboo resources, is abundant in the southeast and southwest provinces of the subtropical and mild temperate regions in China. Owing to its fast growth, high cellulose content, and excellent mechanical properties, bamboo has been widely used as a raw material for pulp, paper and fiber-reinforced materials in China (Liu et al. 2012, Wang et al. 2014). The diversified use of sympodial bamboo has relieved the demand for moso bamboo (*Phyllostachys edulis* (Carrire) J. Houz), which is the prevalent species for commercialization in China (Peng et al. 2013). Numerous studies have been conducted, with focus on the species with a large diameter and high yield, such as *Bambusa rigida* (Huang et al. 2015), *Dendrocalamus brandisii* (Wang et al. 2016), *Neosinocalamus affinis* (Qi et al. 2014a), and *Dendrocalamus latiflorus* (Ren et al. 2014). The basic properties of sympodial bamboo with medium-sized and small diameters need to be fully understood for optimal use of sympodial bamboo.

Numerous studies have confirmed that the anatomical properties of bamboo are closely related to its physical and mechanical properties, ultimately influencing the use and performance of bamboo products (Li et al. 2007, Qi et al. 2014b, Huang et al. 2015, Zhan et al. 2015). The anatomical properties of bamboo significantly affect its durability and strength (Razak 1998, Wahab et al. 2010). Moreover, the fiber length of bamboo is strongly related to the strength of the culms and their pulping properties (Liese 1998, Londoño et al. 2002, Ren et al. 2014, Yang et al. 2016).

*Bambusa pervariabilis* McClure is a typical sympodial bamboo, mainly distributed in southern China. The fiber distribution of *B. pervariabilis* exhibits a microscopically graded architecture with favorable properties. This type of bamboo has been widely used in agriculture, handicrafts, pulp and paper manufacturing, and other traditional industries. The overall cultivation and use of sympodial bamboo have been studied, but the anatomical characteristics of bamboo have rarely been reported. Therefore, the anatomical characteristics and variation of bamboo with

The authors are, respectively, Graduate Student, Bamboo and Rattan Sci. and Technol. Lab., International Center for Bamboo and Rattan, and PhD Candidate, Research Inst. of Wood Industry, Chinese Academy of Forestry, State Forestry and Grassland Admin. of China, Beijing (xiangelin@163.com); and Graduate Student, Associate Researcher, Researcher, Associate Researcher, Associate Researcher, and Research Assistant, Bamboo and Rattan Sci. and Technol. Lab, International Center for Bamboo and Rattan, State Forestry Admin. of China, State Forestry Admin. of China, Beijing (512304624@qq.com, yangsm@icbr.ac.cn [corresponding author], liuxe@icbr.ac.cn, tiangenlin@icbr.ac.cn, Majf@icbr.ac.cn, Shangll@icbr.ac.cn). This paper was received for publication in September 2019. Article no. 19-00042.

Forest Prod. J. 70(1):72–78.
doi:10.13073/FPJ-D-19-00042

height and age were systematically analyzed in this study. The systematic characterization of these properties can contribute to the efficient utilization of *B. pervariabilis*.

## Materials and Methods

### Materials

*B. pervariabilis* bamboo culms age 1, 2, and 3 years were obtained from the bamboo plantation of Guangxi Academy of Forestry in Guangxi Province, China. Five culms with similar diameters were prepared for each age group for a total of 15 culms. Each culm was cut into 2-m segments from the roots. The key processes of sample preparation are illustrated in Figure 1. Variations in anatomical characteristics with the height of the culms were analyzed. Bamboo blocks measuring approximately 15 (axial) by 10 (tangential) by thickness mm (radial) were cut from the bottom (6th internode), the middle (12th internode), and the top (18th internode) for each of the sampled age groups (Fig. 1a), and immediately soaked in a formaldehyde alcohol acetic acid solution (5% formaldehyde, 5% acetic acid, and 90% ethanol; Fig. 1b). All samples were transported to the laboratory and stored at room temperature until processing was completed.

### Methods

*Determination of tissue proportion and vascular bundle morphology*.—To soften the samples, the fixed blocks were soaked in a thermostat water bath operated at 60°C for 2 weeks. Transverse-sections with a thickness of 20 μm were cut from the softened sample blocks by using a sliding microtome (Leica RM2010R, Wetzlar, Germany). The intact slices were randomly selected and dehydrated by freeze-drying. To analyze the morphological characteristics of the vascular bundles and the proportion of the cell tissue, the dehydrated transverse-sections were coated with gold and then observed using a field-emission scanning electron microscope (XL30-FEG-SEM, FEI, USA; Fig. 1c). The tissue proportion was calculated using the square grid method (Qi et al. 2013). After the transparent paper with a square grid was pasted onto the computer screen, the fibrous, ground, and conducting tissues were counted using the square grid method to calculate the tissue proportion, and 15 image fields were selected for each sample.

*Determination of fiber morphology*.—The remaining transverse-sections were stained with 0.1 percent safranin within 10 minutes, dehydrated with an alcohol series, and mounted in Canada balsam until permanent slices were finished (Fig. 1d). Subsequently, 30 measurements were determined for the fiber lumen diameter and fiber double-cell-wall thickness. The fixed blocks were rinsed several times with deionized water and artificially chipped into sticks (2 by 2 by 10 mm). The sticks were then macerated in a mixture of 30 percent hydrogen peroxide and glacial acetic acid (1:1 vol/vol) at 60°C under stirring until the separation of the fibers from their matrix (Hisham et al. 2006; Fig. 1e). The separated fibers were immediately transferred onto the glass slides to determine their length and width; 70 fibers from each sample were examined. Fiber lengths were measured in accordance with the standard applied for papermaking (Code of China 2011). The parameters of the fiber were observed using a digital microscope camera (DMLB2, Leica, Germany), and the quantitative data for the outer, middle, and inner parts across the culm wall were recorded separately.

*Statistical analysis*.—The slenderness ratio and Runkel ratio of the fiber were calculated based on Equation 1 and Equation 2, where $L$ is the length of the fiber, $D$ is the width of the fiber, $2w$ is the thickness of the double cell wall, and $d$ is the lumen width of the fiber:

$$\text{Slenderness ratio} = L/D \tag{1}$$

$$\text{Runkel ratio} = 2w/d \tag{2}$$

In addition, the experimental data were statistically analyzed and compared by analysis of variance (ANOVA) using the least significant difference method to determine the level of significance at $P \leq 0.05$.

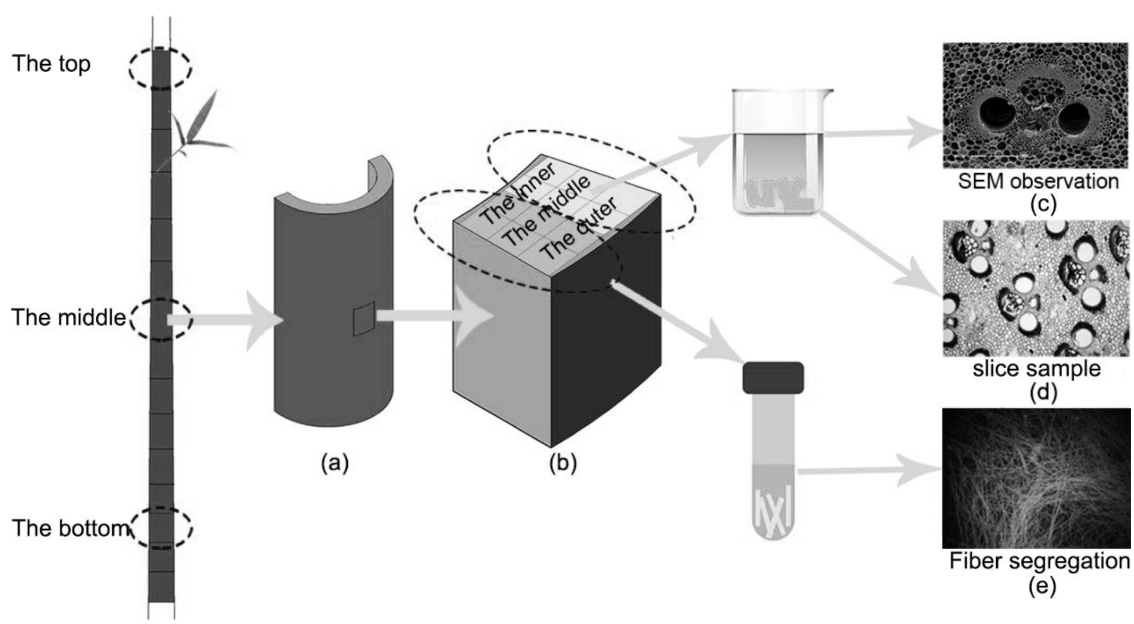


*Figure 1.—Schematic of the preparation of the original samples from* B. pervariabilis.

## Results and Discussion

### Tissue proportion

Bamboo is a natural two-phase reinforced composite, with the fiber as the enhanced phase and the parenchymatous ground tissue as the matrix. Thus, tissue proportion is an important factor that determines the structure, physico-mechanical characteristics, and pulp properties of bamboo culms. Many studies have reported that the fibrous tissue proportion exhibits a significant positive correlation with the basic density (Santhoshkumar and Bhat 2015), tensile strength (Qi et al. 2013), compressive strength (Xie et al. 2017), and elastic modulus of bamboo. The fibrous tissue proportion also affects the pulping properties of bamboo—the greater the fibrous tissue proportion, the higher the pulp yield (Lian 2012).

The statistical results for the tissue proportion of culms at different ages and heights are shown in Figure 2. The average proportions of the fibrous, ground, and conducting tissues were 41.53, 41.59, and 18.05 percent, respectively. The fibrous tissue proportion of the sample was significantly higher than that of the moso bamboo (26.46% to 32.28%; Yang et al. 2011) and close to that of *Neosinocalamus affinis* (39% to 43%; Qi et al. 2013). No significant differences in tissue proportion between the age groups was found (Fig. 2a), indicating that the 1-year-old bamboo had a slightly lower fibrous tissue proportion than the 2- or 3-year-old bamboo. Variations in tissue proportion with the culm height are presented in Figure 2b. The proportion of fibrous and conducting tissues slightly increased from the bottom to the top of the culm, whereas the proportion of ground tissue markedly decreased. This finding is consistent with the report by Santhoshkumar and Bhat (2015) that the percentage of fibrous tissues was comparatively higher at the top than the bottom and middle zones in *Dendrocalamus strictus*. Similar trends were observed in *N. affinis* in one study, which also suggested that the increase in fiber proportion contributed to increases in basic density, compressive strength, and shear strength (Xie et al. 2017, Huang et al. 2018).

Compared with those in the study by Wei et al. (1999), the proportion of fibrous tissues in *B. pervariabilis* in the current study was 5 percent lower, whereas that of conducting tissue was 3 percent higher. These differences could be attributed to the conditions at the site where the bamboo was grown. With respect to tissue proportion, *B. pervariabilis* is an ideal raw material for pulp and paper and performs better than the moso bamboo.

### Vascular bundle morphology

*Frequency of vascular bundle.*—The vascular bundle morphologies of *B. pervariabilis* culms at different heights are presented in Figure 3. In accordance with the vascular bundle classification by Liese (1998), two vascular bundle types—the broken-waist type and the semiundifferentiated type—were observed from the bottom zone to the top zone of *B. pervariabilis* culms. The typical vascular bundle morphology is classified as the broken-waist type, consisting of a central vascular strand and a fiber strand. The transverse-section of the bamboo culms consists of numerous vertical oval-shaped vascular bundles that are smaller near the periphery and then become sparser and larger toward the middle of the culm. Meanwhile, the inner layer consists of vascular bundles that are widely arranged and transformed into oval-shaped bundles arranged sideways (Fig. 3; Table 1). No significant differences in the frequency of the vascular bundles were observed in all age groups. However, the vascular bundle of bamboo culms in the Age 2 group was more concentrated than those in the Age 1 and Age 2 groups. Similar trends in *Bambusa vulgaris* were described by Wahab et al. (2010). The distribution of vascular bundles from the bottom to the top in all age groups exhibited an increasing trend. The vascular bundles with a more concentrated distribution near the top endow the top of the culm with excellent mechanical strength (Grosser and Liese 1971, Liese 1998, Londoño et al. 2002, Wang et al. 2016).

*Size of vascular bundle.*—No significant differences in the radial and tangential widths of the vascular bundles were found between the samples with different ages (Table 1). Both radial and tangential widths in the Age 2 group were less than those in the Age 1 and Age 3 groups. From the bottom to the top of the culm, the width sharply decreased, with the radial width decreasing significantly more acutely than the tangential width. Similar trends were reported by Hisham et al. (2006), Kelemwork (2009), and Wahab et al. (2010).

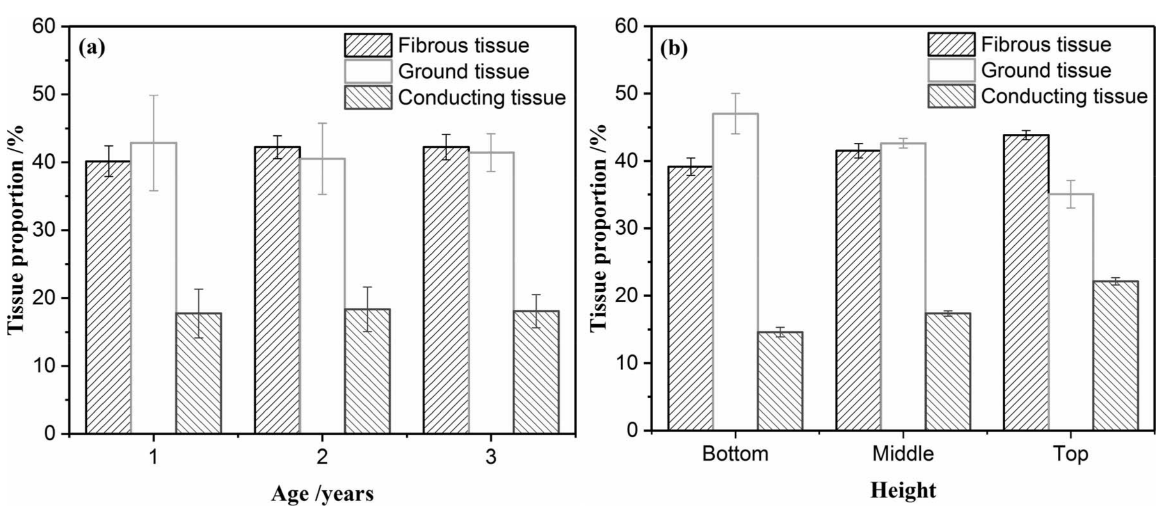


*Figure 2.—Proportions of the fibrous, ground, and conducting tissues in* B. pervariabilis *at different ages (a) and heights (b).*

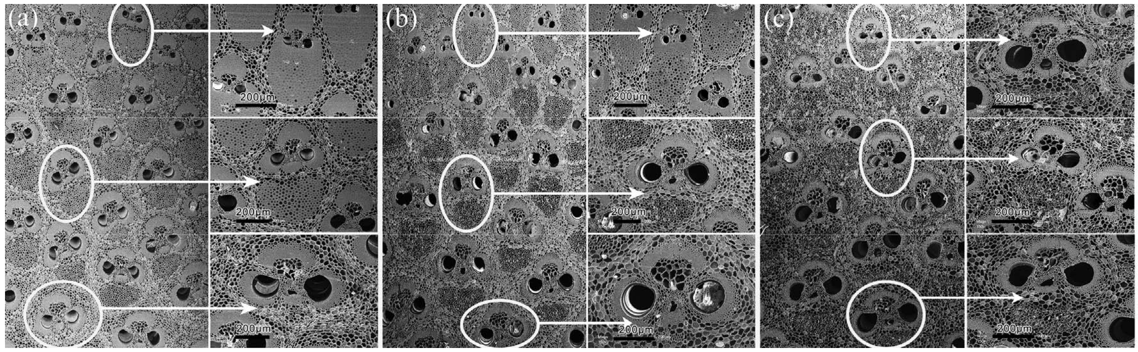


*Figure 3.—Vascular bundle types in the transverse-section: broken-waist type and semiundifferentiated type in the bottom (a), middle (b), and top (c). Arrow direction: from the inner to the outer zone of bamboo culms. Bar = 200 $\mu$m.*

*Table 1.—Characteristics of the vascular bundles in* B. pervariabilis *at different ages and heights.*[a]

| Variation | Parameters | Frequency (no./cm$^2$) | Tangential width (μm) | Radial width (μm) |
|---|---|---|---|---|
| Age | 1 yr | 193.67 ± 34.06 A | 571.85 ± 49.62 A | 803.06 ± 14.52 A |
| | 2 yr | 209.67 ± 53.35 A | 518.55 ± 10.23 A | 742.73 ± 65.71 A |
| | 3 yr | 208.00 ± 55.15 A | 556.48 ± 28.46 A | 765.25 ± 48.48 A |
| Height | Bottom | 171.00 ± 40.52 BC | 568.51 ± 38.53 A | 811.62 ± 10.06 A |
| | Middle | 182.11 ± 18.36 B | 561.22 ± 42.44 A | 780.53 ± 40.50 AB |
| | Top | 250.33 ± 19.22 A | 521.15 ± 13.24 A | 733.16 ± 52.48 B |

[a] Means with the same letter in each column are not significantly different at $P \leq 0.05$ probabilities.

## Fiber morphology

Apart from tissue proportion, anatomical characteristics of fibers such as length, double-wall thickness, lumen diameter, slenderness ratio, and Runkel ratio are also significant parameters for evaluating the pulp quality of fibers in raw materials (Anupam et al. 2016).

*Length, width, and slenderness ratio of fibers.*—Compared with a short fiber, a long fiber provides higher tensile strength and tearing strength (Zhan et al. 2015). Fiber length in the 1,448.91- to 2,410.94-μm range, with an average of 1,953.30 μm, is considered to be that of a long fiber. The morphology of the fiber and statistical results are presented in Figure 4 and Table 2. There was a slight decrease in length with age, and this variation was mainly attributable to the maturation of bamboo in the Age 1 group (Nordahlia et al. 2012). Fiber length also varied significantly with culm height, and the largest mean (1.99 mm) was observed in the middle of the culms (Table 2). This finding is consistent with previous studies (Wang et al. 2011, Zhan et al. 2015) in which fiber length was affected by internode length, and the middle internodes were longer than the bottom and top internodes.

Fiber width significantly affected the crossover area of the fiber. Generally, a wider fiber presents a larger crossover area per fiber, which is favorable for the production of paper with improved quality and strength (Anupam et al. 2016). Age did not significantly affect fiber width, and the largest mean was measured in the Age 2 group (Table 2). This observation was consistent with the report by Wang et al. (2011) that fibers complete their width growth in 1 or 2 years. From the bottom to the top of the culm, the mean width of fiber gradually and significantly narrowed, which may be caused by the tapering structure of bamboo culms (Table 2).

The length-to-width ratio of the fiber is also known as the slenderness ratio of the fiber. The pulp obtained from wood with thin-walled fibers provides dense and well-bonded sheets, whereas that from wood with thick-walled fibers produces bulky sheets with high tear resistance (Anupam et al. 2016). In this study, the slenderness ratio of the *B. pervariabilis* fiber ranged from 104.24:1 to 163.79:1, with an average of 140.01:1. This average is higher than that of wood fibers, indicating that fiber exhibits superior tensile strength and tear resistance. The age of the bamboo culms exerted no significant effect on the slenderness ratio of the fiber. However, there was significant difference between slenderness ratios by height, whereby the minimum mean was observed in the bottom of the culm (Table 2).

*Wall thickness, lumen diameter, and Runkel ratio.*—The measurements of the double-wall thickness, lumen diameter, and Runkel ratio of the fiber are summarized in Table 3. The wall thickness ranged from 7.04 to 8.75 μm, and the lumen diameter varied from 2.29 to 3.05 μm. Age exerted a significant effect on the wall thickness and lumen diameter of the bamboo fiber. As the age of the bamboo culm increased, the cell wall thickness generally increased, whereas the lumen diameter decreased owing to the formation of a multilayered structure and lignin deposition in the cell wall. The height of the bamboo culm significantly influenced the wall thickness but not the lumen diameter of the fiber, as shown by the sharp decrease in cell wall thickness from the bottom to the top of the bamboo culm, as well as slight narrowing of the lumen diameter of the fiber.

The wall-to-lumen ratio, also known as the Runkel ratio, was determined based on the double-wall thickness to lumen diameter, which is one of the important indexes for assessing the softness and interweaving of the fiber (Anupam et al. 2016). Significant differences in Runkel ratio were found between the bamboo samples of different age groups, with the average ranging from 2.56 to 3.02 and

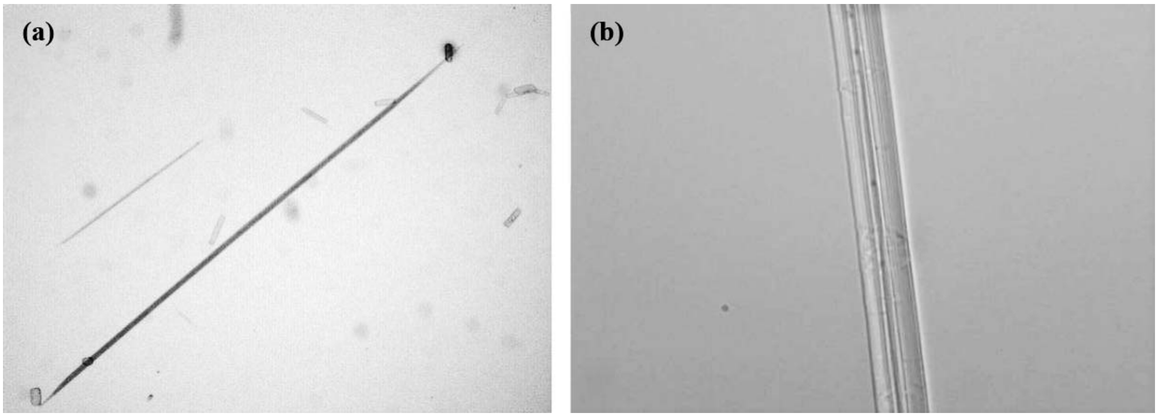


*Figure 4.—Measurement of the fiber length (a) and width (b) of* B. pervariabilis.

*Table 2.—Fiber characteristics (length, width, and slenderness ratio) of* B. pervariabilis *fibers with at different ages and heights.*[a]

| Variation | Parameters | Length (mm) | Width (μm) | Slenderness ratio (L/W) |
|---|---|---|---|---|
| Age | 1 yr | 2.25 ± 0.43 A | 14.72 ± 3.00 A | 158.89 ± 43.83 A |
| | 2 yr | 2.16 ± 0.36 B | 15.06 ± 3.38 A | 151.11 ± 38.45 A |
| | 3 yr | 2.14 ± 0.53 BC | 14.83 ± 5.08 A | 159.54 ± 63.09 A |
| Height | Bottom | 1.75 ± 0.64 C | 14.96 ± 4.32 A | 125.94 ± 50.03 B |
| | Middle | 1.99 ± 0.43 A | 14.28 ± 4.17 AC | 151.23 ± 50.95 AC |
| | Top | 1.96 ± 0.38 B | 13.32 ± 3.31 B | 159.27 ± 50.41 A |

[a] Means with the same letter in each column are not significantly different at $P \leq 0.05$ probabilities.

*Table 3.—Fiber characteristics (double-wall thickness, lumen diameter, and Runkel ratio) of* B. pervariabilis *fibers with different ages and heights.*[a]

| Variation | Parameters | Double-wall thickness (mm) | Lumen diameter (μm) | Runkel ratio (2w/d) |
|---|---|---|---|---|
| Age | 1 yr | 7.04 ± 1.70 C | 3.05 ± 0.97 A | 2.56 ± 1.07 C |
| | 2 yr | 7.57 ± 1.57 B | 2.83 ± 1.03 B | 3.02 ± 1.22 B |
| | 3 yr | 8.75 ± 2.21 A | 2.29 ± 1.02 C | 3.02 ± 1.22 B |
| Height | Bottom | 8.38 ± 2.28 A | 2.75 ± 1.07 A | 3.61 ± 2.12 A |
| | Middle | 7.59 ± 1.88 B | 2.73 ± 0.97 A | 3.22 ± 1.72 B |
| | Top | 7.39 ± 1.60 C | 2.70 ± 1.14 A | 3.40 ± 2.07 AB |

[a] Means with the same letter in each column are not significantly different at $P \leq 0.05$ probabilities.

*Table 4.—Statistical comparison of the anatomical characteristics of several paper and pulp fibers.*

| Index | Fiber length | Slenderness ratio | Runkel ratio | Fibrous tissue proportion | Isolated methods | References |
|---|---|---|---|---|---|---|
| *B. pervariabilis* | 1.75 | 117.00 | 4.00 | 40.20 | 30% hydrogen peroxide and glacial acetic acid (1:1 v/v) | Determined |
| Moso bamboo | 2.24 | 164.00 | 6.20 | 32.00 | | Tian 2015 |
| *Neosinocalamus affinis* | 1.91 | 98.82 | 1.28 | — | | |
| Poplar | 1.08 | 41.00 | 0.25 | — | | Chen et al. 2009 |
| Eucalyptus | 0.67–0.81 | 39.4–48.4 | 0.54–1.75 | 22.00–50.00 | | Prinsen et al. 2012 |
| Hemp | 0.43 | 14.73 | 0.28 | — | | Li et al. 2013 |
| Cotton stalk | 0.74 | 37.66 | 0.44 | 77.28 | | Lin et al. 2012 |

abruptly increasing from the Age 1 group to the Age 3 group (Table 3). Moreover, the Runkel ratio was not significantly affected by the height of the bamboo culm; the bottom had the largest ratio. According to previous reports, the fiber Runkel ratio is preferably $< 1$ to obtain good pulp raw materials (Yang et al. 2008). The mean ratio indicated that the suitability of bamboo for pulp raw materials was inferior to those of hardwood and softwood.

## Anatomy-based papermaking potential

The performance of *B. pervariabilis* fibers as raw materials for pulping and papermaking is compared with that of other plant fibers—including wood, hemp, and cotton—in Table 4. The fiber length of *B. pervariabilis* is classified as long, and is evidently longer than those of poplar, hemp, eucalyptus, and cotton, but shorter than that of the moso bamboo. In addition, the slenderness ratio and Runkel ratio of *B. pervariabilis* fibers are comparable to those of the moso bamboo, and the proportion of the fibrous tissues is considerably higher. Therefore, the *B. pervariabilis* fiber is an ideal choice as a raw material for pulping and papermaking, and its performance is even close to that of the moso bamboo.

## Conclusions

The anatomical characteristics of *B. pervariabilis* culms with different ages and heights were investigated by microscopy and image analysis. The conclusions drawn from the findings are presented as follows:

1. The broken-waist and semiundifferentiated types are observed from the bottom to the top zone in *B. pervariabilis* culms; the broken-waist type is characterized by a typical vascular bundle morphology.
2. The measurements of the fibrous tissues of *B. pervariabilis* are as follows: average proportion, 41.53 percent; length, 1.75 mm; slenderness ratio, 117; and Runkel ratio, 4.00. These anatomical indexes are similar to those of the moso bamboo. Therefore, *B. pervariabilis* is a suitable raw material for pulping and papermaking and thus should be cultivated and promoted.
3. Age and height significantly affect the anatomical characteristics of *B. pervariabilis*. As age increases, the length, double-wall thickness, and Runkel ratio of the fiber significantly increases, whereas the lumen diameter of the fibers decreases. Meanwhile, the smallest slenderness ratio was that of the fiber in the Age 2 group. From the bottom to the top, the length, width, double-wall thickness, and lumen diameter of the fiber decreased gradually; in the middle, the length and Runkel ratio of the fiber were the maximum and the minimum, respectively. Thus, if *B. pervariabilis* is to be used as a raw material for pulping and papermaking, the age and position of the bamboo culms need to be considered.

## Acknowledgments

This study was conducted with the financial support of the National Science and Technology Support Plan (Grant No. 2015BAD04B03) and the National Science Foundation of China (Grant Nos. 31670565 and Grant Nos. 31370563).

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