Analyzing Fiber and Vascular Bundle Characteristics, and Micro-Mechanical Properties of *Oligostachyum sulcatum*

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The structure of vascular bundles and the mechanical properties of fibers are crucial factors determining the utilization of bamboo. This study investigated the structure of vascular bundles and evaluated the morphological and micromechanical properties of the fibers in *Oligostachyum sulcatum*. The results showed that the fiber length and width of *O. sulcatum* meet the requirements of raw materials for the papermaking process. However, the fiber content in *O. sulcatum* is relatively low, which may increase the cost of papermaking. The vascular bundle growth exhibited non-uniformity, especially at the top part, with no discernible pattern in bundle area changes. The nanoindentation testing demonstrated that the bamboo's indentation modulus of elasticity (IMOE) and hardness values were comparable to those of moso bamboo (*Phyllostachys edulis)*, suggesting its potential as a substitute in engineering applications.

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Keywords: Oligostachyum sulcatum; Nanoindentation; Fiber; Indentation modulus of elasticity; Hardness

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INTRODUCTION

As society advances, it faces environmental challenges such as plastic pollution, prompting researchers to investigate sustainable and eco-friendly materials (MacLeod *et al*. 2021). Bamboo, characterized by its unique gradient structure and rapid growth, is a renewable and resource-rich material. It has been employed in various applications, such as automobile interiors, pipes, and wind turbine blades (Holmes *et al*. 2009; Thilagavathi *et al*. 2010; Shi *et al*. 2019). From a mechanical perspective, bamboo comprises two main components: fibers and parenchyma cells, thus rendering it a natural composite material (Shao *et al*. 2018). Specifically, vascular bundles provide bamboo with stiffness and rigidity, while parenchyma cells contribute to its excellent ductility. The combination of these two elements grants bamboo immunity against damage from wind and snow. Consequently, bamboo has emerged as an excellent material for a wide range of architectural and structural applications (Yu *et al*. 2005; Xiao *et al*. 2010). In comparison to timber, the abundance and rapid growth rate of bamboo have also driven the emergence of numerous bamboo-based products in the market (Hung *et al*. 2013; Kathirvel and Ramachandran 2014; Asim *et al*. 2023).

Oligostachyum sulcatum, native to Fujian and Zhejiang of China, can attain a height of up to 12 m and a diameter reaching 6.2 cm (Shi *et al*. 2022). *O. sulcatum* demonstrates suitability for horticultural applications. Moreover, its leaves possess medicinal properties, while its bamboo shoots offer a distinct flavor, contributing to its high economic value and promising cultivation prospects (Li 2007). However, the focus of research efforts has predominantly concentrated on moso bamboo (*Phyllostachys edulis*), while research reports on *O. sulcatum* are relatively scarce. In addition, previous studies on *O. sulcatum* have mainly focused on the cells of leaves, cultivation, and genetic aspects, with little attention given to its culm walls, particularly fiber morphology and mechanical properties (Huiru and Zhunan 1995; Wang *et al*. 2012). In this study, the microstructure and micromechanics of *O. sulcatum* were investigated in terms of fiber properties and its potential as an industrial raw material using Franklin's method, nanoindentation, and the vascular bundle recognition model. The results provide a scientific basis for the industrial application of natural biodegradable materials.

EXPERIMENTAL

Materials

Three three-year-old defect-free and straight *O. sulcatum* were collected from the International Center for Bamboo and Rattan (ICBR) Taiping Experimental Center in Huangshan, Anhui Province, China (longitude 117′13″ to 118′53″E, latitude 29′24″ to 30′31″N). The microwave softening method was used in this study. The bamboo blocks were taken from the base, 1.5 m (at 1.5 m above the ground), and top of the bamboo, respectively (Fig. 1). The dimensions of the bamboo block were 15 mm (L, longitudinal), 20 mm (T, tangential), and t mm (R, radial, thickness of the bamboo culm wall). The bamboo blocks were submerged entirely in distilled water and subsequently heated in a microwave oven for a duration of 10 min at a consistent temperature of 100 °C. Subsequently, bamboo blocks were promptly cooled in cold water and maintained at a temperature of 25 °C. This procedure was reiterated 40 times to attain the desired level of softening. Owing to the presence of air within the bamboo block, the entire block undergoes expansion when subjected to heat. Conversely, when immersed in cold water, the bamboo block contracts, thereby drawing a portion of the water into its interior. After this process is repeated, the voids inside the bamboo will be saturated with water. Water and heat are used as plasticizers to reduce the glass transition temperature of bamboo, thereby reducing the rigidity of bamboo. Simultaneously, the microwave heating process effectively reduces the starch content within the bamboo block, and the water in the bamboo makes the slice more transparent.

The softened bamboo blocks were individually sectioned with a slicer (Leica RM2265, German) to a thickness of 20 μm. Complete sections were stained with saffron stain, and permanent sections were made for subsequent observation. Fiber width measurements were conducted utilizing a microimaging system (Nikon, E200MV, Japan) under 400x total magnification (40x objective and 10x eyepiece). The remaining bamboo material, which had been sectioned, was divided into strips approximately 1 cm in length and placed in test tubes for fiber dissociation via the method described by Franklin (1954). This process was considered complete when the bamboo strips had been transformed into a white flocculent mass. Fiber length was quantified using a microimaging system with a total magnification of 20x (2x objective and 10x eyepiece).

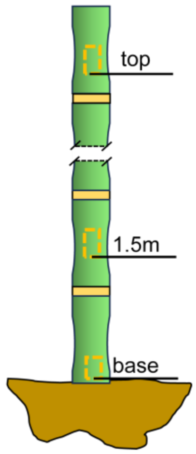

Fig. 1. Sample preparation

Measurement of Vascular Bundles

Three rings were taken from each of the bases, 1.5 m, and top, sanded with 320-mesh sanding pads, and then placed in the scanner. (PERFECTION V850 PRO, EPSON, Japan). The scanner was set to a resolution of 9600 ppi and scanned a cross-section of the sample in 16 grayscale mode. The information on the vascular bundles of the samples was obtained by using the recognition model of vascular bundles developed independently by the group based on the YOLO_V3 model (Redmon and Farhadi 2018). The model detects vascular bundles on the bamboo culms and then performs local binarization using the K-means clustering algorithm to obtain the length, width, and area of the vascular bundles.

Nanoindentation Test

The nanoindentation technique has emerged as a powerful tool for probing the mechanical properties of various materials at the micro and nanoscale.

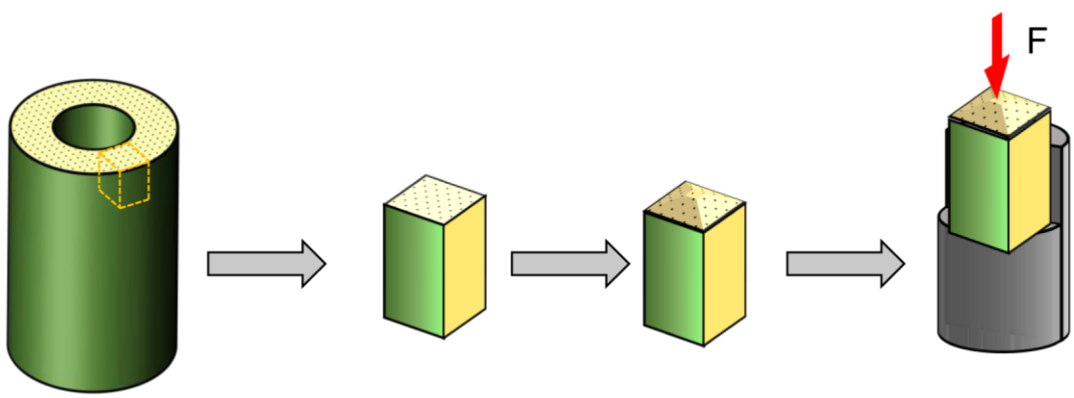

Fig. 2. Sample preparation for nanoindentation test

Despite its widespread application in characterizing synthetic materials, this method remains eminently suitable for investigating the mechanical behavior of biological systems, such as wood and plant fiber cell walls, at relatively small scales (Gindl and Schöberl 2004).

The sample containing the fibers of interest was fashioned into a rectangular geometry. The fibers under investigation were positioned at the center of one rectangular end face. Subsequently, this end face underwent a pyramidal shaping process utilizing a slicing instrument, positioning the target fiber at the apex of the newly formed pyramidal structure. Finally, the apical region was polished with a diamond blade, yielding a nanoindentation test specimen with the desired fiber exposed for analysis (Fig. 2).

A Triboindenter (Hysitron, Minneapolis, MN, USA) with a Berkovich diamond top (top diameter ≤100 nm) was used for this nanoindentation test. The test was divided into three phases: the loading phase, the holding phase, and the unloading phase. The loading phase was loaded at a rate of 50 μN/s for 5 s, at which point the maximum load of 250 μN was reached, followed by the holding phase (Fig. 3a, b). To avoid the creep of the material affecting the test, the duration of the holding phase was 6 s, followed by the unloading phase. The unloading phase lasted 3 s. The hardness and indentation modulus of elasticity (IMOE) of the sample were calculated using the following equations,

$$E_r = \frac{\sqrt{\pi}}{2}\frac{S}{\sqrt{A_c}} \tag{1}$$

$$\frac{1}{E_r} = \frac{1-v^2}{E} + \frac{1-v_i^2}{E_i} \tag{2}$$

$$H = \frac{P}{A_c} \tag{3}$$

where E and H are the indentation modulus of elasticity and hardness of the samples, respectively, P is peak load, A_c is the projected area at peak load, E_r is the reduced elastic modulus, and E_i and v_i are the modulus of elasticity and Poisson ratio of the tip, respectively. For the diamond tip, E_i is 1141 GPa and v_i is 0.07. v is the Poisson ratio of 0.22 for the samples (Yu *et al*. 2011). To maintain the accuracy of the measurement results, the surface of the sample was scanned both before and after the test. The Pauta criterion was applied to reject bad values and ensure that there were more than 30 valid data.

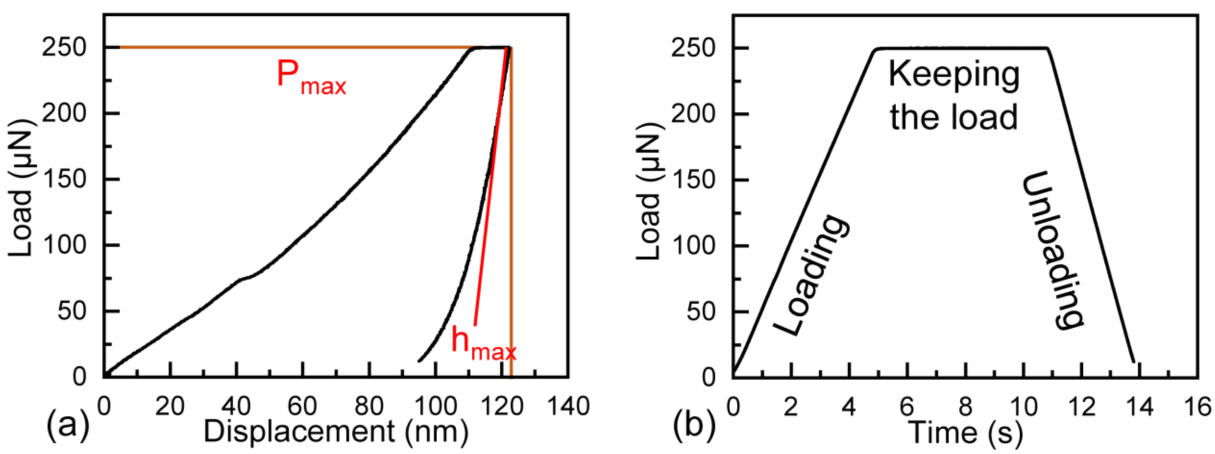

Fig. 3. (a) Typical force-displacement curves; (b) force-displacement curves

RESULTS AND DISCUSSION

Morphological Characteristics of Fibers

The morphological characteristics of bamboo fibers play important roles in determining their mechanical strength. Therefore, they serve as crucial indices for evaluating its suitability as a structural material. Typically, the morphological characteristics of bamboo fibers mainly encompass indicators such as length, and width. Fiber length and width significantly impact the viability of bamboo as a raw material for papermaking. In the study, it was found that the fiber lengths at the base, 1.5 m, and top of the bamboo were 1.85, 2.06, and 1.79 mm, respectively (Fig. 4a). The widths of the fibers were 16.8, 13.0, and 14.1 μm, respectively. Overall, the fibers at 1.5 m were both finer and longer than those at the base and top. According to the International Association of Wood Anatomists (IAWA) standard for grading fiber length, the fibers of *O. sulcatum* are long fiber (more than 1600 μm) (Guo *et al*. 2014). The variation patterns of fiber length and width in *O. sulcatum* are consistent with that of *Fargesia edulis* (Tang *et al*. 2015).

Bamboo can be used in papermaking (Tan *et al*. 2020). The quality of the paper depends mainly on the raw material. The fiber length and width of the raw material were crucial factors affecting the quality of the paper (Larsson *et al*. 2018). Specifically, fiber width is related to the structural properties of the paper, such as porosity, effective pore size, and water absorption, while fiber length is not only positively correlated with the structural properties of the paper but also with its tensile properties. Considering the fiber requirements for papermaking raw materials, *O. sulcatum* possesses favorable properties that render it a potential feedstock (Su *et al*. 2011). Similarly, fiber length affects the mechanical properties of the material, suggesting that composites incorporating *O. sulcatum* may exhibit enhanced mechanical properties (Zhang *et al*. 2018). However, considering pulp yield and economics, the fiber content of the raw material needs to exceed 50%, *i.e.* less than 50% non-fibrous cells (vessel, ray cells, and parenchyma cells), which often accompany the fibers in the raw material and negatively affect the paper properties. The present work showed that the percentage of fibers at the base, 1.5 m, and top were 21.3%, 31.0%, and 32.4%, respectively. The percentage of fibers at the base was significantly smaller than that at 1.5 m and the top, while the difference in the percentage of fibers at 1.5 m and the top were not significant (Fig. 4b). The fiber content of *O. sulcatum* is relatively low, which may lead to higher production cost when using it as a papermaking material.

Therefore, the challenge of cost-effective fiber screening from *O. sulcatum* for papermaking is crucial, necessitating the optimization of pulp properties and preparation processes as a feasible solution. This work showed nonsignificant variations in fiber length between the base, 1.5 m, and the top of *O. sulcatum*. However, significant variations in fiber width were exclusively detected between the base and top. This may be attributed to the phenomenon that the base of bamboo is subjected to greater external loads than the top. The widening of fibers at the base of the bamboo, driven by natural selection, is essential for withstanding greater loads and maintaining their original shape (Dai *et al*. 2024).

Different parts of bamboo fibers exhibit distinct morphological characteristics (fiber length and width). Therefore, in the process of bamboo utilization, it is necessary to reasonably select specific parts corresponding to various application domains, thereby enhancing their efficiency of utilization.

Moreover, this study exclusively focused on the morphological characteristics and mechanical properties of axial segmented fibers. The morphological characteristics and mechanical properties of fibers from different radial parts, as well as the effects of growth years and growth environment on bamboo, were not considered. Therefore, further research on *O. sulcatum* is still needed to assess whether it can be used for furniture, structures, and construction.

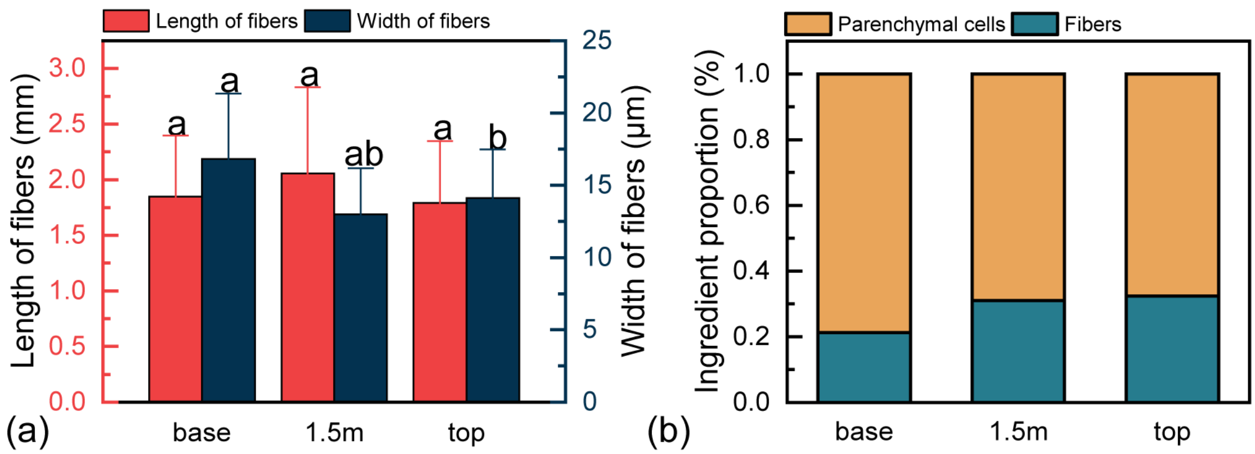

Fig. 4. The fiber length and width (a), percentage of fibers and parenchymal cells (b).

Morphological Characteristics of Vascular Bundles

The radial diameter of vascular bundles at the base, 1.5 m, and top of *O. sulcatum* gradually increased and then decreased along the radial direction (from the outer to inner part). The goodness of fit (R^2) of the quadratic functions were 0.509, 0.353, and 0.184, respectively (Fig. 5a1, 6a1, 7a1). The tangential diameter of vascular bundles at the base and 1.5 m gradually increased along the radial direction, while those at the top gradually decreased along the radial direction.

The R^2 of the quadratic function was 0.745, 0.744, and 0.336, respectively (Fig. 5a2, 6a2, 7a2). The change in the fiber sheath area along the radial direction of the base, at 1.5 m, and the top was similar to that of length. Nevertheless, the R^2 of the quadratic function was lower, with values of 0.361, 0.319, and 0.066, respectively (Fig. 5a3, 6a3, 7a3). The ratio of radial diameter to tangential diameter of the vascular bundles at the base and 1.5 m gradually increased along the radial direction, while that at the top increased along the radial direction before decreasing.

The R^2 of the quadratic function was 0.334, 0.61, and 0.217, respectively (Fig. 5a4, 6a4, 7a4). Among the three parts, the vascular bundles at the top had the lowest fit. The vascular bundles at the base and 1.5 m of the bamboo were mainly concentrated in the area near the outer epidermis of the bamboo. The vascular bundles in this area were small in volume and not fully differentiated, while the vascular bundles near the inner epidermis of the bamboo were large in volume and relatively highly differentiated.

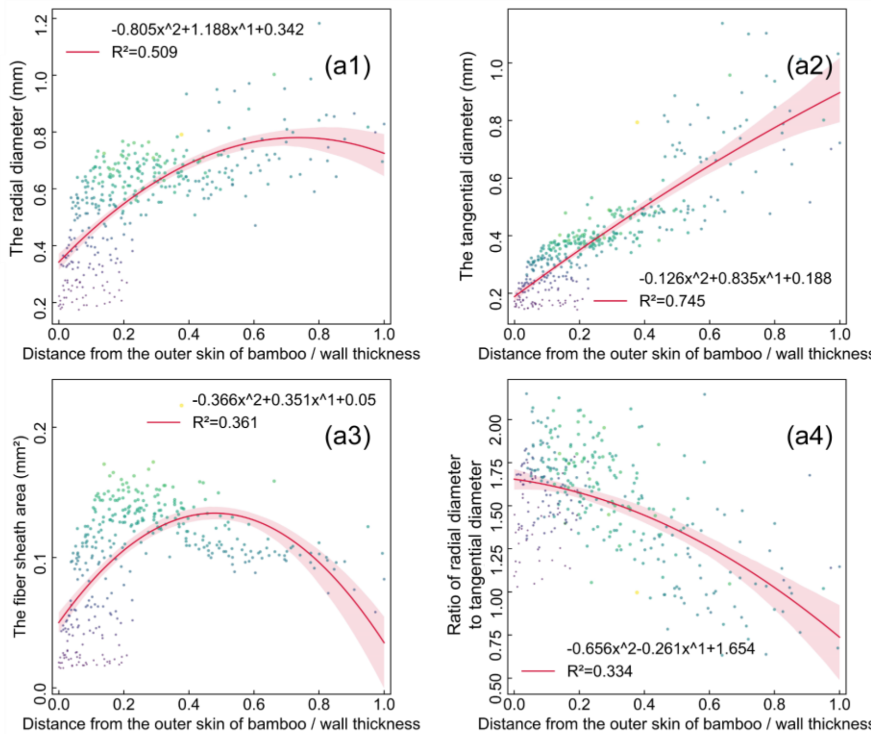

Fig. 5. Radial diameter (a1), tangential diameter (a2), area (a3), and Ratio of radial diameter to tangential diameter ratio (a4) of vascular bundles at the base

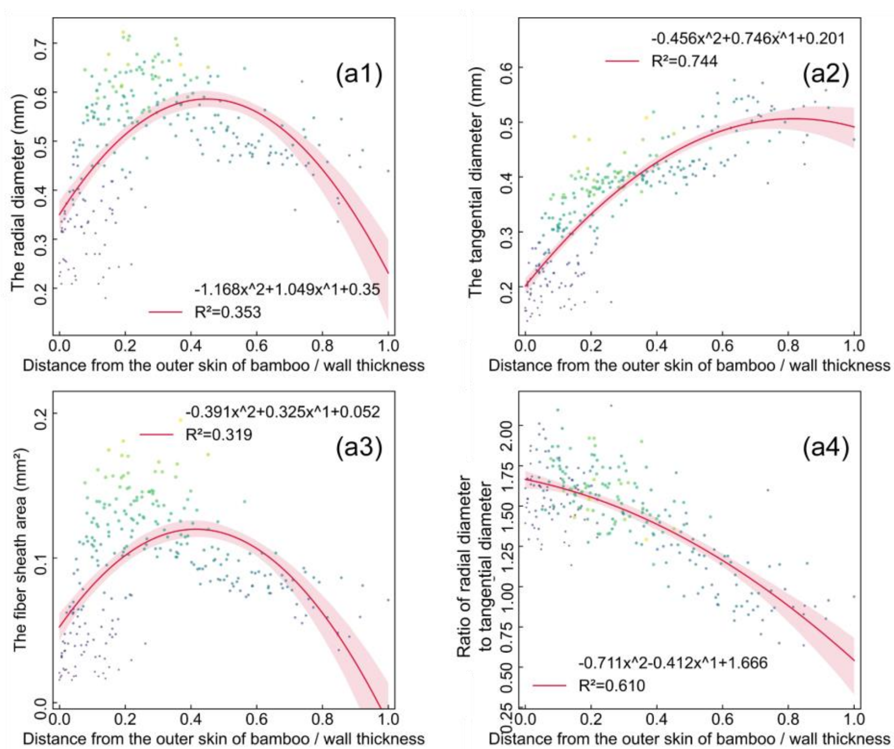

Fig. 6. Radial diameter (a1), tangential diameter (a2), area (a3), and Ratio of radial diameter to tangential diameter ratio (a4) of vascular bundles at 1.5 m

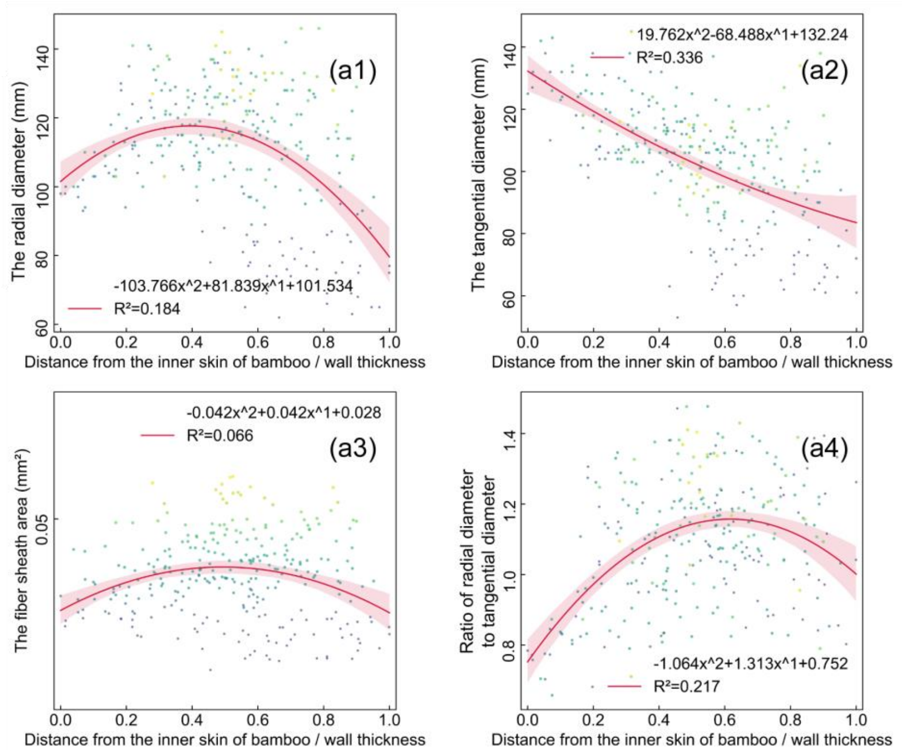

Fig. 7. Radial diameter (a1), tangential diameter (a2), area (a3), and Ratio of radial diameter to tangential diameter ratio (a4) of vascular bundles at the top

The Mechanical Properties of Fibers

O. sulcatum showed excellent micro-mechanical properties. The IMOE at the base, 1.5 m, and top of the bamboo were 20.59, 22.54, and 21.78 GPa, respectively. The hardness of cell walls was 604, 603, and 605 MPa, respectively (Fig. 8). Overall, the IMOE and hardness of the fibers at 1.5 m exhibited a significant discrepancy when compared to those of the fibers at the base and top. Conversely, the IMOE and hardness of the fibers at the base and the top demonstrated no significant difference.

The average IMOE and hardness of *O. sulcatum* were 21.7 GPa and 604 MPa, respectively, which were similar to those of moso bamboo (22.0 GPa and 530 MPa), and the IMOE was slightly higher than that of *Bambusa arundinacea* (20.8 GPa and 497 MPa), while the hardness was much higher than that of *B. arundinacea* (Dai *et al*. 2023b; Huang *et al*. 2016). Compared to the world's largest bamboo, *Dendrocalamus sinicus* (24.1 GPa and 626 MPa), the IMOE and hardness of *O. sulcatum* were lower (Dai *et al*. 2023a).

In summary, the micromechanical properties of *O. sulcatum* are comparable to those of moso bamboo. However, it is worth noting that the micromechanical properties of *O. sulcatum* and moso bamboo, measured by nanoindentation, may not fully represent their macro mechanical properties. This is because the macro mechanical properties should take into account both the fibers and parenchyma cells of bamboo, which affect its mechanical properties differently. In contrast, nanoindentation solely focuses on individual fibers within bamboo. In addition, it is also important to note that the laboratory equipment used for micromechanics and macromechanics testing is different. Hence, confirming the similarity of the macroscopic mechanical properties between *O. sulcatum* and moso bamboo necessitates conducting further mechanical experiments.

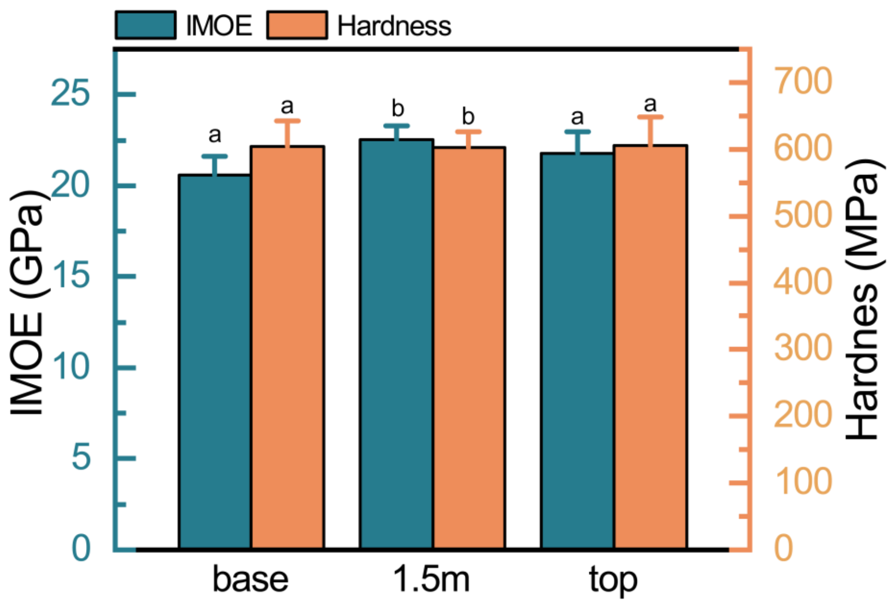

Fig. 8. IMOE and hardness of fibers at the base, 1.5m, and top.

CONCLUSIONS

1. The morphological characteristics of the fibers in *Oligostachyum sulcatum* meet the requirements for papermaking. However, the papermaking process using *O. sulcatum* as a raw material requires optimization to enhance economic efficiency.
2. Compared with the vascular bundles at the base and 1.5 m, the growth pattern of the vascular bundles at the top exhibited non-uniformity. Moreover, the radial and tangential diameters, as well as the fiber sheath area and the ratio of the radial to the tangential diameter of the vascular bundles at the top, did not exhibit any discernible pattern of change.
3. The indentation modulus of elasticity (IMOE) and hardness of fibers at 1.5 m significantly varied in comparison to those at the base and top of the bamboo. Overall, the average IMOE and hardness of *O. sulcatum* were similar to those of moso bamboo, suggesting the potential for *O. sulcatum* to replace moso bamboo in industrial applications.

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Author Contributions Statement

Kangjian Zhang: wrote the main manuscript. Peng Zhao: Data curation. Linpeng Yu: Experiments. Fukuan Dai: Review. Yuxuan Chen: Review. Youhong Wang: Funding acquisition, Resources. Genlin Tian: Methodology, Project administration. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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