Characterization of the Vascular Bundles in *Thyrsostachys oliveri*

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The characteristics of vascular bundles vary significantly across different heights in bamboo. Thus, a comprehensive understanding of vascular bundles is crucial for the identification and efficient utilization of bamboo materials. In this work, the structure and type of vascular bundles in *Thyrsostachys oliveri* at different heights were investigated. The results revealed substantial differences in the types of vascular bundles in *T. oliveri* at various heights within the bamboo. The radial diameter exhibited no significant differences in the radial variation of fiber sheath area at different heights, whereas the tangential diameter showed differences in the radial variation between the upper and lower middle parts of the bamboo. This study provides insights into the structural variations of vascular bundles in *T. oliveri* at different heights, which are beneficial for optimizing the use of bamboo materials in various applications.

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INTRODUCTION

Although most traditional building materials such as cement and stone possess excellent physical properties, they are not sustainable and often cause irreversible environmental damage. There is a global trend toward sustainability and the search for sustainable building materials has become imperative. Bamboo, a fast-growing and eco-friendly material with excellent mechanical properties, has gained attention (Shen *et al.* 2019). Bamboo has been used widely as a structural material in China. For example, the use of bamboo plywood in the construction industry has expanded rapidly (Feng *et al.* 2012). With the advent of green building standards, new bamboo processing techniques and bamboo composite materials are expected to be widely adopted in the building materials sector (Molla and Belay 2024; Oliveira *et al.* 2024). Bamboo is primarily composed of vascular bundles and basic tissues. The alternate structure of vascular bundles and basic tissues gives bamboo unique mechanical and functional properties, such as excellent tensile, compressive, and flexural strength, with balanced strength in all directions, thereby enhancing its stability and durability (Durbha 2024).

Vascular bundles, primarily present in the bamboo wall, consist of a bundle structure made up of xylem and phloem (Jiang *et al.* 2024a). Vascular bundles serve dual roles as both transport and mechanical systems in bamboo (Li *et al.* 2024b). Xylem conduits in the vascular bundle transport water and inorganic salts, while sieve tubes in the phloem transport nutrient solutions and organic matter. Fiber cells in the vascular bundles have small lumens, thick walls, and a high length-to-diameter ratio, which significantly influence the mechanical properties of bamboo (Liu *et al.* 2022). The taxonomy of bamboo is based on the morphology of its rhizomes and leaves (Clark *et al.* 2015), but dissecting bamboo vascular bundles can aid in the taxonomic identification of bamboo.

The physical properties and anatomical structure of *Phyllostachys* and other bamboo species have been investigated extensively (Xu *et al.* 2021; Jiang *et al.* 2024b), revealing distinct characteristics in different bamboo species (Stanley *et al.* 2024). *Thyrsostachys oliveri* Gamber, a tree-like species in the Poaceae family, is mainly distributed in Southeast Asia, including Myanmar, Thailand, and Malaysia. It has been introduced and cultivated in Yunnan, Fujian, and Guangdong provinces in China (Chaowana *et al.* 2021; Zhang *et al.* 2022). It is primarily used for ornamental purposes due to its elegant appearance, small leaves, and high branches. It has a relatively straight stem with long internodes, and it typically grows to a height of 12 to 20 m, with some individuals exceeding 20 m (Banik 2016). This bamboo species is fast-growing, maturing in approximately three to five years. The timber of *T. oliveri* is hard and tough, with a moderate xylem density, making it suitable for construction and bamboo tools (Chaowana *et al.* 2021).

Previous studies on *T. oliveri* have focused on its biological characteristics, genome, cultivation, and genetic breeding, but little attention has been paid to the morphological features of the vascular bundles (Chen 2006; Li *et al.* 2024a). In this study, the vascular bundle identification system was employed to analyze *T. oliveri* from a morphological perspective, providing a solid foundation for its efficient utilization in future applications. The vascular structure of bamboo gives it high strength and lightweight properties, making it suitable for building frames, roofs and walls. For example, bamboo is commonly used to build temporary structures and earthquake-resistant houses (Li *et al*. 2021).

EXPERIMENTAL

Sampling Location and Environment

Bamboo samples were collected from Bamboo Garden of Huaan (25°00′36″N, 117°32′27″E), which is in the Fujian province of China. The sampling site is located at an elevation of over 150 meters above sea level, with an annual average temperature of 17.5 °C and an average annual precipitation of 1450 mm. The site falls within the climate transition zone between the middle subtropical and south subtropical zones.

Sample Preparation

The *T. oliveri* bamboo rings were taken from the internode parts at every two sections of internode from the base to the top of bamboo. A total of ten samples were collected, labeled sequentially from one to ten, with one being the base segment and ten being the topmost segment, as illustrated in Fig. 1. The cross-section of all bamboo rings were first flattened using a belt sander (LX-SM46-750MF, China) and then sanded with 320-mesh sanding pads.

Acquiring Vascular Bundle Distribution Characteristics

A high-resolution flatbed image scanner (EPSON PERFECTION V850 PRO) was used to scan the sanded cross-sections of the samples in 16 grayscale mode at a resolution of 9600 ppi to obtain high-definition grayscale maps. The vascular bundles were identified and their area and location information were extracted using the vascular bundle identification model developed by the authors' group based on the YOLO algorithm (Li *et al.* 2021c). The model detects vascular bundles on the bamboo culms and then performs the local binarization using the K-means clustering algorithm. The fiber volume fraction was defined as the area of the fiber sheath within the end face of the sample divided by the cross-sectional area of the sample. Similarly, the distribution density of vascular bundles was defined as the number of vascular bundles divided by the cross-sectional area of the sample.

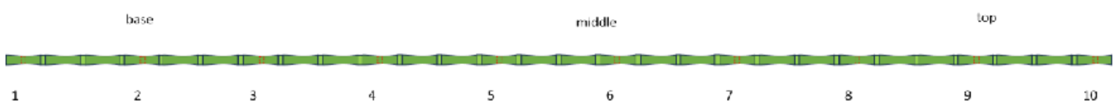

Fig. 1. Sample preparation

RESULTS AND DISCUSSION

The excellent mechanical properties of bamboo are based on its unique structure. The morphology, size, and distribution density of the vascular bundles directly influence the plant's mechanical properties (Hartono *et al.* 2022; Jiang *et al.* 2024b). The mechanical strength of *T. oliveri* is determined by characteristics of the vascular bundles and the fiber sheath.

Types of Vascular Bundles in *Thyrsostachys oliveri*

The tissue pattern of bamboo culms gradually changes with increasing height. The type, size, and pattern of vascular bundles vary markedly. For example, the structure in an internode from the culm base exhibits a significant difference from one taken from the middle, which also differs considerably from one taken from the top. Traditional methods of describing anatomical characteristics based on three orientated sections cannot be applied to bamboo. To characterize *T. oliveri*, it is necessary to describe the tissue pattern from several internodes at different positions along the culm. An in-depth study of more than 250 culms showed that 4 to 8 internodes in the axial direction are sufficient to explain the axial variation in bamboo (Grosser and Liese 1971).

According to Wen and Chou's criteria for categorizing vascular morphology, vascular types were classified as double-broken type, broken type, slender waist type, open type, and semi-open type. The vascular bundles in *T. oliveri* were classified according to the criteria for classification of vascular bundles proposed by Wen and Chou (1984). From the middle to the top in *T. oliveri*, the major type of vascular bundle in the middle zone was classified as the typical broken-waist type (Fig. 2). The broken-waist type vascular bundle consisted of two parts: a central vascular strand and a fiber strand. The fiber strand was located inside the central strand, with a sheath in the intercellular space (protoxylem), which was generally smaller than the other components. Near the epidermis, there were 1 to 2 layers of undifferentiated vascular bundles, which lacked sieve tubes and ducts and consisted only of fibrous strands. These bundles were irregularly shaped—some cap-shaped, some jar-shaped—and were densely arranged, forming the hard outer wall of the culm. There were 1 to 3 rows of semi-differentiated vascular bundles that began to develop conductive tissue, though their arrangement remains relatively dense (Wen and Chou 1984). Vascular bundles near the inner wall exhibited the open vascular bundle type. The open vascular bundles consisted of a single part: the central vascular bundle, with supporting tissue composed solely of sclerenchyma sheaths (Fig. 2a). From the middle to the base in *T. oliveri*, the vascular bundles in the middle and in the inner walls of the culm exhibited greater variation. The major type of vascular bundle in the middle zone was either the typical broken-waist type or the typical double-broken-waist type. The double-broken-waist type vascular bundle consisted of two parts: fiber groups and the central vascular bundle. The morphology of vascular bundle near the inner wall included both open vascular bundles and semi-differentiated vascular bundles, which were arranged in a staggered fashion. In combination with the double-broken-waist type, the broken-waist type was more prevalent in the middle and upper parts (Fig. 2b). The basic double-broken-waist type, however, was almost entirely confined to thick-walled basal internodes (Grosser and Liese 1971).

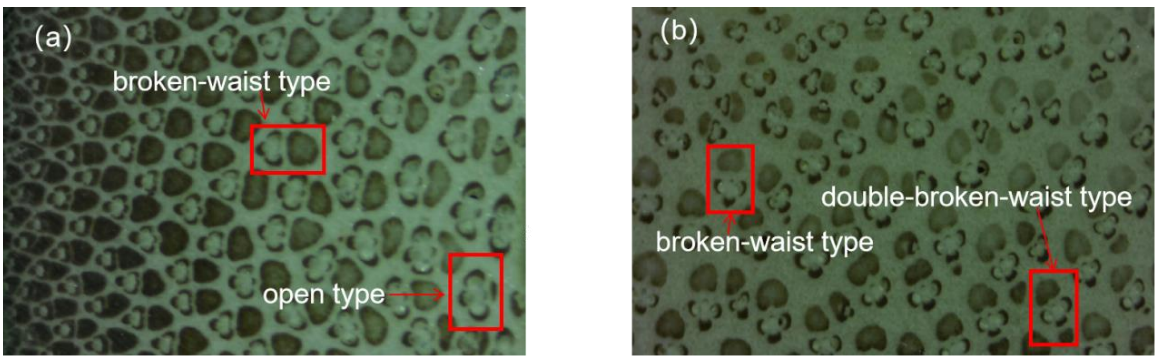

Fig. 2. (a) Upper and middle vascular bundle types; (b) middle and bottom vascular bundle types

High Variability in Fiber Volume Share

Bamboo can be considered a composite material because it consists of cellulose fibers embedded in a lignin matrix. Cellulose fibers are aligned along the length of the bamboo, providing maximum tensile and flexural strength, as well as rigidity in that direction (Lakkad and Patel 1981). The middle layer consists of fibers, veins, and sap conduits, which are randomly arranged in the transverse section and encased in a tissue known as parenchyma. On average, culms consist of 30% parenchyma, 60% fibers, and 10% veins and sap conduits (Tong *et al.* 2005). The percentages of fibers and parenchyma may vary depending on species and directly influence the physical and mechanical properties of bamboo that are closely associated with the fiber volumetric ratio. The mechanical properties vary at different height and ages of the bamboo culm. The fiber density in the cross-section of a bamboo culm varies with both thickness and height. Sixty to seventy percent of the total weight of bamboo is contributed by 40% fibers (by volume), while the rest weight is from parenchyma, vessels, and capillaries. The present study showed that the percentage of fiber volume from top to bottom were 43.9%, 45.4%, 47.7%, 47.9%, 46.8%, 42.6%, 38.1%, 39.6%, 35.1%, and 36.9% (Fig. 3). An increasing trend in the fiber volume fraction was observed from top to bottom, with the fiber volume fraction at the base being significantly smaller than at the middle and top. The goodness of fit (R^2) of the quadratic functions was 0.66. This phenomenon is likely due to the fact that the bottom part is primarily responsible for water absorption and support during the early stage of bamboo growth, while the upper and middle nodes continue to grow and lignify over time. As a result, more fibrous structures are developed. The average volume share of fiber in *T. oliveri* is 42.4%, which is higher than the 23.7% fiber volume fraction of common bamboo species such as *Phyllostachys edulis* and 25.7% fiber volume fraction of *Phyllostachys sulphurea* (Xu *et al.* 2022). It can be inferred that *T. oliveri* may possess better mechanical properties than *Phyllostachys*.

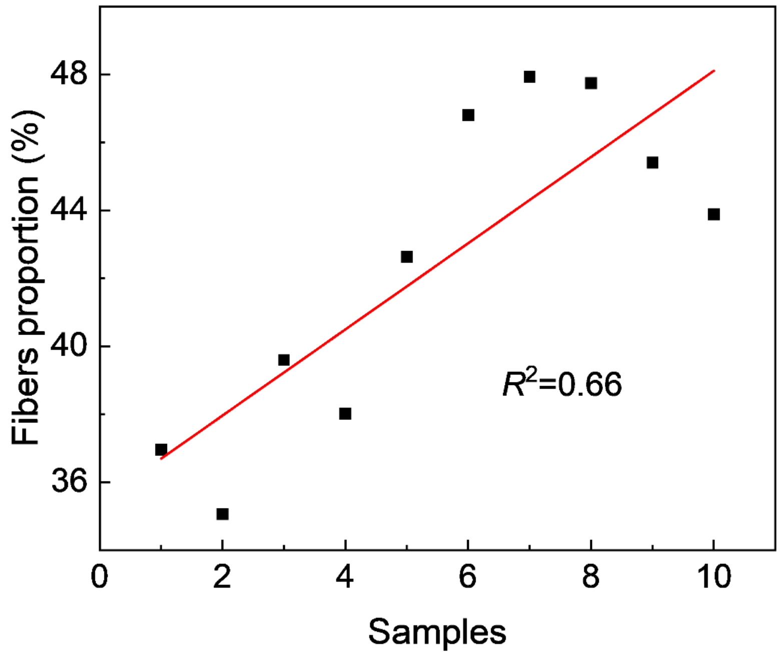

Fig. 3. Percentage of fiber sheath area

High Variability in the Density of Vascular Bundle Distribution

The density and number of vascular bundles are considered as the two most crucial characteristics for identifying bamboo species. The number of vascular bundles, counted from the bottom to the top of the bamboo, were 4285, 3745, 3475, 2764, 2259, 2237, 2006, 1778, 1587, and 1317, respectively. A strictly decreasing trend in the number of vascular bundles was observed from the bottom to top of bamboo. This finding aligns well with previous reports (Grosser and Liese 1971; Mohmod *et al.* 1993; Kelemwork 2009). Generally, the radial diameter of the vascular bundles decreased more with height than the tangential diameter. However, the density of the vascular bundles gradually increased from the bottom to the top. According to Liese (1985), smaller vascular bundles are more densely distributed than larger ones, resulting in higher density and mechanical strength in the outer zone compared to both the inner and middle zones.

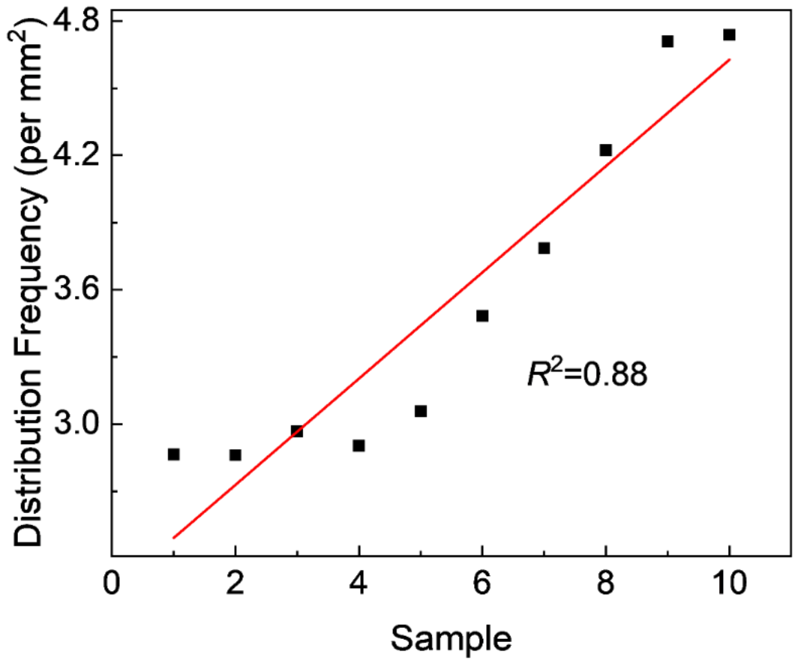

Fig. 4. Density of vascular bundle distribution

From the bottom to the top of the bamboo, the fiber bundle densities were 2.8, 2.8, 2.9, 2.9, 3.0, 3.4, 3.7, 4.2, 4.7, and 4.7/mm² (Fig. 4). The density of vascular bundles in *T. oliveri* showed a gradual increase from the base to the top. The R^2 value of the quadratic function was 0.88. The density of fiber distribution in the cross-section of bamboo is a crucial factor affecting its physical properties (Lo *et al.* 2004). Gradient variation in the longitudinal density of bamboo is a significant factor contributing to the changes in its mechanical properties (Li *et al.* 2024a).

Variation in the Morphologic Characteristics of Vascular Bundles

The dimensional characteristics—radial diameter, chordal diameter, and vascular bundle area—were investigated at 10 different heights of *T. oliveri.*

Variation in Radial Diameter of Vascular Bundle

From the inner to the outer skin of *T. oliveri*, the radial diameters of vascular bundles increased initially and then decreased, with the maximum radial diameters occurring in the middle of the bamboo wall. The rate of increase in the radial diameter of vascular bundles decreased gradually from the inner to the middle part of the bamboo, while the rate of decrease increased progressively from the middle to the outer cortex. This trend in the radial diameter of vascular bundles was consistent across the ten different heights in *T. oliveri* studied. A quadratic fit analysis, based on the radial diameter of vascular bundles and their distance from the inner bark, showed that the goodness of fit for the quadratic functions at the ten different heights in *T. oliveri* ranged from 0.596 to 0.884 (Fig. 5).

Variation in Tangential Diameter of Vascular Bundles

There was a distinct trend in the tangential diameter along the radial variation at different heights. From the base to the middle of the bamboo (site 1 to site 5), the tangential diameter of vascular bundles increased initially and then decreased from the inner to the outer cortex, reaching a maximum in the middle of the bamboo wall. The rate of increase in the tangential diameter of vascular bundles decreased progressively from the inner to the middle part in bamboo, while the rate of decrease in the radial diameter increased progressively from the middle to the outer cortex. A quadratic fit analysis, based on the tangential diameter of vascular bundles and their distance from the inner bark of the bamboo, revealed that the goodness of fit for the quadratic function ranged from 0.7 to 0.836 at five different heights between the base and the middle. In contrast, from the middle to the top of the bamboo (site 5 to site 1), the tangential diameter of vascular bundles decreased from the inner to the outer epidermis. The rate of decrease increased progressively from the bamboo endodermis to the exodermis. For the five different heights between the middle and the top of the bamboo, the quadratic function had a goodness of fit between 0.759 and 0.903 (Fig. 6).

Variation in Fiber Sheath Area

Through studying the variation of the fiber sheath area in *T. oliveri*, it was observed that the fiber sheath area increased and then decreased from the inner epidermis to the outer epidermis, with the maximum value occurring at the middle of the bamboo wall. The rate of increase in the fiber sheath area gradually decreased from the inner to the middle part of the bamboo, while the rate of decrease in the radial diameter of vascular bundles increased from the middle to the outer cortex. A quadratic fit analysis of the fiber sheath area and the distance between the vascular bundles and the inner bark of the bamboo showed that the goodness of fit ranged from 0.544 to 0.791 across ten different heights between the base and the middle of the bamboo (Fig. 7).

Bamboo Gradient Variation

Similar to the gradient structural variation in *Phyllostachys*, structural gradient variation in *T. oliveri* was observed in this study (Gu *et al.* 2024; Jiang *et al.* 2024b). Based on the vascular bundle morphology, the vascular bundle types in *T. oliveri* were divided into five categories: double girdle-breaking, girdle-breaking, open, semi-differentiated, and undifferentiated. This morphological classification follows the available classification of bamboo wood vascular bundles (Wen and Chou 1984). The variation of vascular bundles in the axial versus radial direction has also been demonstrated (Grosser and Liese 1971). The bamboo tube, characterized by its hollowness, consists of a microstructure primarily made up of vascular bundles and longitudinally arranged parenchyma cells. In the radial direction of the bamboo wall, the density of vascular bundles decreased from the bamboo green layer (BGL) to the bamboo yellow layer (BYL), showing a distinct gradient pattern.

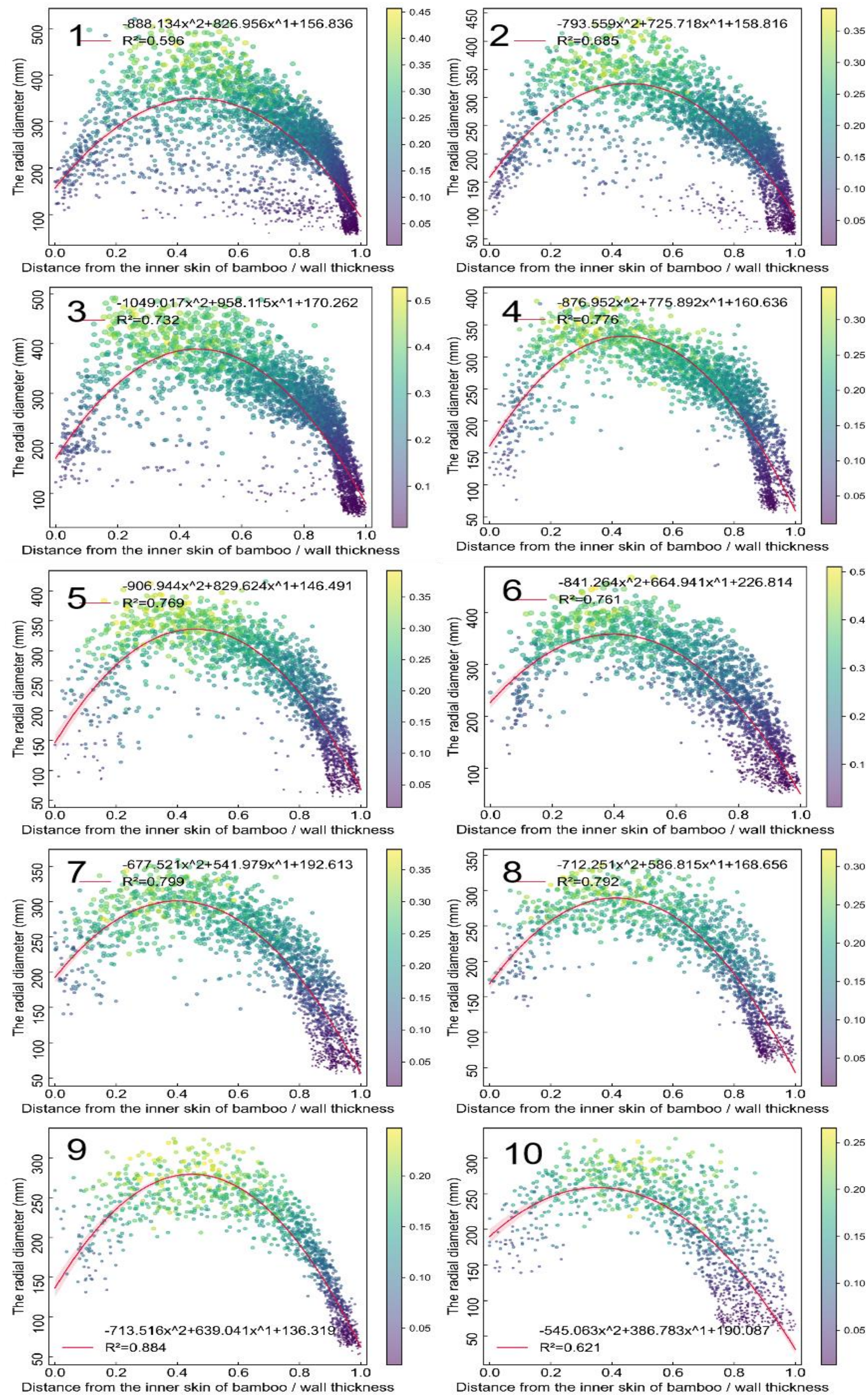

Fig. 5. Radial diameter of parts 1-10

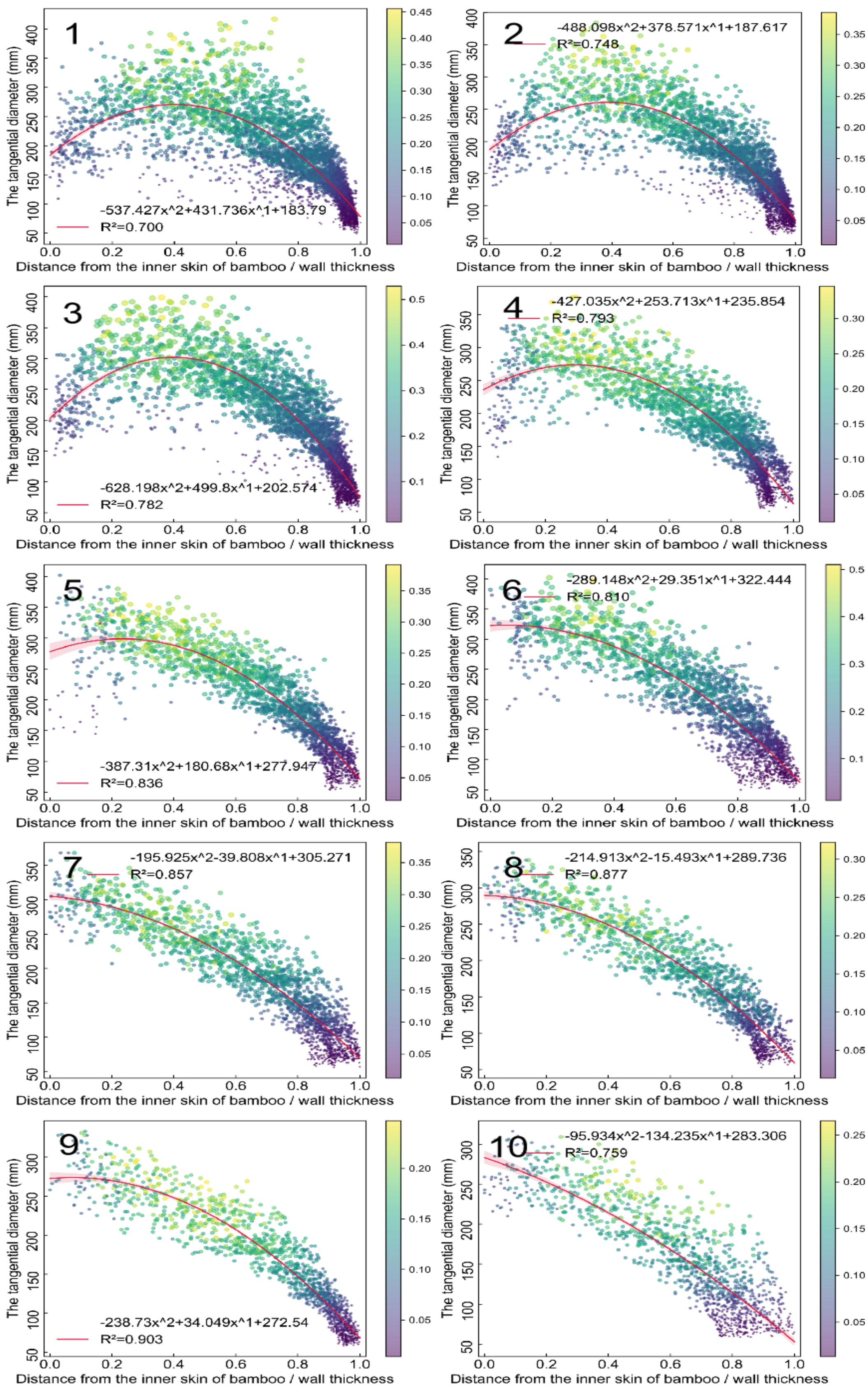

Fig. 6. Tangential diameter of parts 1-10

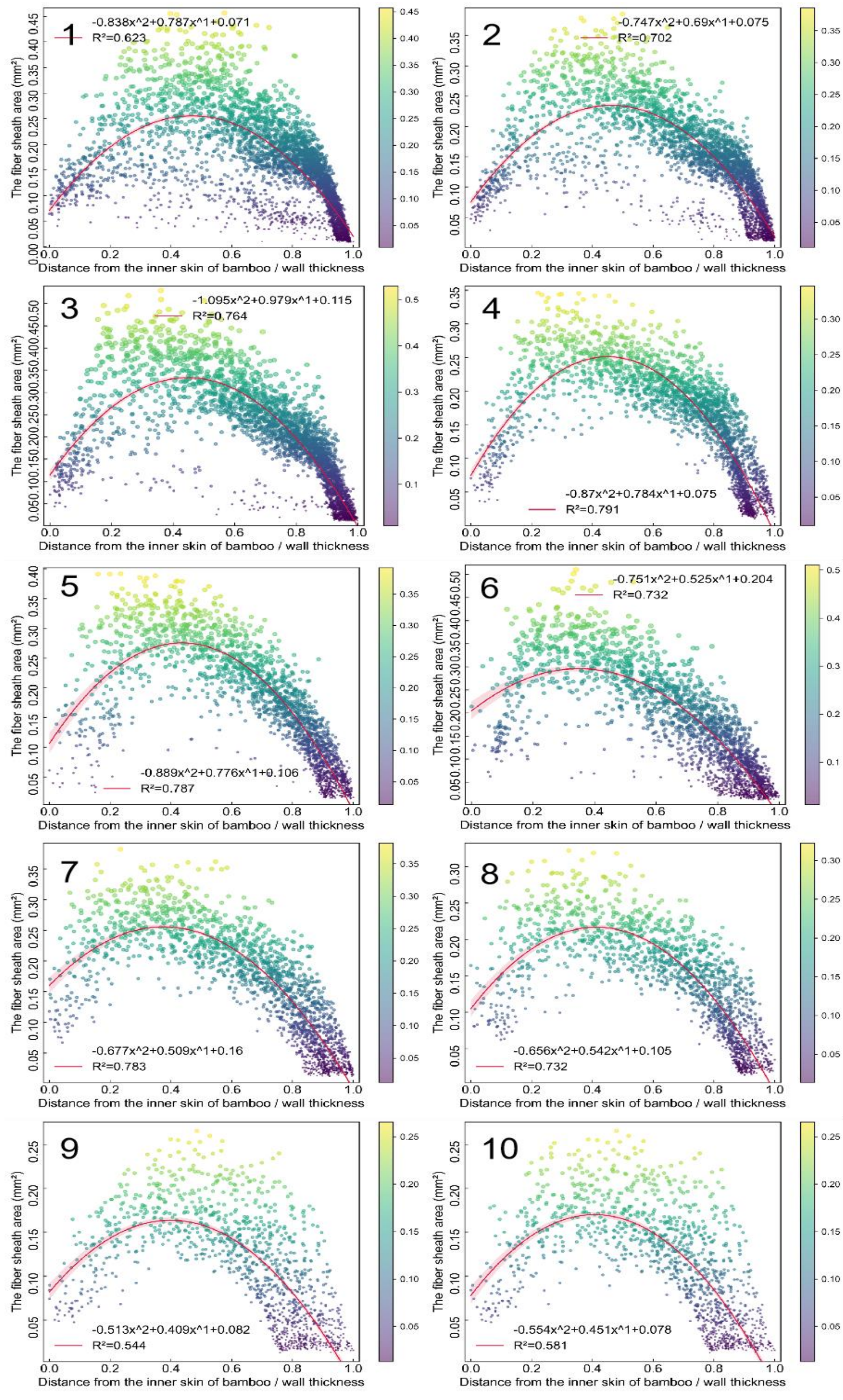

Fig. 7. Area of parts 1-10

The gradient structure of bamboo has evolved through continuous optimization. The vascular bundles on the inner side of the bamboo wall were large and sparsely distributed, while those on the outer side were small but densely distributed. The middle part of the bamboo wall acted as a transition layer, with the size and density of vascular bundles gradually changing between the inner and outer sides, confirming the gradient variation of bamboo material (Gu *et al.* 2024). Vascular bundles are the basic tissues for transporting water and nutrients in the plant body and also play an important role in the mechanical properties of the plant. The length and width pattern of the vascular bundle, *i.e.*, its geometric dimensions and proportions, determines the way in which it transmits and disperses stresses during support and resistance to bending (Zou *et al.* 2009). It has been found that thin-walled cells and vascular bundles act as energy absorbers to prevent and delay fracture extension (Zou *et al.* 2021). These findings provide additional design guidance for the development of bamboo composites with superior properties. Whereas the underlying physiological mechanisms for the variation in vascular bundle morphology at different heights may be related to physiological functions, in the basal vascular bundles, the part close to the ground requires stronger mechanical support, and thus the vascular bundles are denser and have thicker cell walls. The middle vascular bundle, in the middle of the culm, tends to be more evenly distributed to meet the transportation needs as well as the mechanical strength. Apical vascular bundles, where the top vascular bundles have a lower density and thinner cell walls to accommodate rapid growth and transportation of photosynthetic products (Zhao *et al.* 2024).

Variations in the number of circles within bamboo's vascular bundles affect the deposition and distribution of nanomaterials (Tao *et al.* 2011a; Ye *et al.* 2014). The integrated biorefinery process can convert bamboo feedstock into multifunctional nanocomposites, and the cellulose in bamboo is more readily converted into cellulose nanocrystals than wood-based kraft pulp (Du *et al.* 2013; Tao *et al*. 2011b).

The Significance of Bamboo Classification

With the continuous advancement of scientific techniques, the identification and classification of bamboo have become more accurate. Although genetic classification methods are improving, they cannot displace the method of the micromorphological identification of bamboo. The arrangement, size, and distribution of vascular bundles play a crucial role in bamboo classification. Through studying the vascular bundle characteristics of ten species of scattered bamboo in Huangshan City, it was found that the radial diameter of *Pleioblastus simonii* (Carr.) Nakai was significantly larger than that of *Chimonobambusa tumidissinoda*, and the tangential diameter of *Oligostachyum lubricum* was much larger than that of *Chimonobambusa tumidissinoda*. These differences in vascular bundle characteristics help distinguish various bamboo genera. To identify bamboo more accurately, it is essential to integrate various scientific methods, including genetics, anatomy, and other relevant tools.

CONCLUSIONS

1. There are differences in the shape, density, type, and arrangement of vascular bundles at different heights of *T. oliveri*. Because vascular bundles are a key factor affecting bamboo's mechanical strength, optimizing the economic efficiency across different heights of *T. oliveri* is still necessary.

2. The primary vascular bundle types in *T. oliveri* are girdle-breaking in the upper-middle section, while the lower-middle section predominantly features double girdle-breaking and co-existing girdle-breaking distributions.
3. The volume fraction of fiber sheath and vascular bundle density both showed a positive correlation with the distance from the bamboo's outer epidermis.
4. The radial diameter and area of vascular bundles followed the same trend in the radial direction at different height sites, while the tangential diameter exhibited a different trend between the upper and lower sections.
5. The vascular bundle characteristics in *T. oliveri* exhibited gradient variation across different axial and radial locations.

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