

Bamboo *Pleioblastus chino* var. *hisauchii* Characteristics Before and After Flowering

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Pleioblastus chino var. *hisauchii* is an important ornamental bamboo species that rarely flowers. Studies on the change in its material properties before and after flowering were lacking. In this paper, the anatomical, chemical, and mechanical properties of bamboo culms before and after flowering were studied by using the method of bio-wood science. The results showed that after flowering, the morphology and proportion of the fiber, vessel and vascular bundle decreased, and the openings of pits in the vessel wall were enlarged significantly; the contents of the main components such as extractives, lignin, holocellulose, cellulose and pentosan rose, while the ash content dropped. There was a decrease in density and modulus of rupture, and a pronounced fall in modulus of elasticity, while the microfibril angle and crystallinity increased. In general, the strength of bamboo flowering culms decreased and the ability to transport nutrients increased, which were closely related to the changes in internal structure and properties. This meant that bamboo flowering may be monitored or predicted by significant changes in some properties (such as pits and modulus of elasticity) and provide a reference for further research on the mechanism of flowering senescence and delayed flowering in bamboo.

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INTRODUCTION

Bamboo plants are abundant resources characterized by rapid growth, a broad scope of use, and strong regenerative ability (Xu *et al.* 2016). Studies have shown that bamboo does not flower until a long period of vegetative phase ends, and flowering is usually followed by withering (Janzen 1976). Bamboo flowering can lead to bamboo forest degeneration and jeopardize its utilization, causing tremendous losses for forestry production (Kumawat *et al.* 2014; Jiao *et al.* 2019; Yao *et al.* 2020). Currently, there are different theories regarding the causes of bamboo flowering, including periodic, environmental, nutritional, and free radical explanations (Ramanayake 2006; Wang *et al.* 2016b). Due to the unique reproductive characteristics of bamboo, the flowering phenomenon of bamboo was recorded a long time ago (Xiao *et al.* 2020). The inflorescence, fruit, and other reproductive organs were used as the principal basis for classifying bamboo (Paichieh 1986; Hordern 1993). In the past, bamboo research focused

primarily on the biological characteristics of the flowering cycle, flowering habits, structure of flowering organs, *etc.* (He *et al.* 1994; Guerreiro 2014). With the development of molecular biology in recent years, increased research has turned to transcriptome analysis of the molecular mechanism of bamboo flowering (Hisamoto and Kobayashi 2013; Biswas *et al.* 2016; Li *et al.* 2019).

Flowering is an important event in the plant cycle and bamboo flowering is accompanied by various physiological and biochemical changes (Turnbull 2011). Some studies found that the contents of endogenous hormones, such as GA3, ABA, and CTK, increased, while the contents of nutrient elements, such as N, P, and K, decreased after bamboo flowering, and the contents of several important metabolites, such as oil and soluble sugar, also changed (He *et al.* 2005; Wang and Zhou 2008; Xu *et al.* 2017). Most of these studies focus on the changes in main substance contents and metabolic characteristics, while the anatomical, chemical, physical and other basic properties that affect the utilization of bamboo are rarely studied. These properties are considered important because they affect the mechanical strength, durability, pulping, and papermaking of bamboo and a range of uses (Sekimoto and Kitamura 1976; Koch and Lybeer 2005; Shukla and Sharma 2017). In this paper, the anatomical, chemical, and mechanical properties of *Pleioblastus chino* var. *hisauchii* culms before and after flowering were measured according to the principles of bio-wood science, in order to analyze the differences and reasons in the properties of bamboo materials before and after flowering, and provide a theoretical foundation for the mechanisms of bamboo flowering and its regulation.

EXPERIMENTAL

Sample Collection and Preparation

Pleioblastus chino var. *hisauchii* with luxuriant branches and smooth culms is a type of ornamental bamboo species with strong adaptability, which has great economic and ecological value. However, the emergence of flowering has had a significant impact on its use.

The authors found a partial flowering phenomenon in the bamboo forest of *P. chino* var. *hisauchii* in the Bamboo Germplasm Resources Conservation Bank of Anhui Taiping Experimental Center, International Center for Bamboo and Rattan (118° 02' E, 30° 20' N, 200 m above sea level (ASL)) Huangshan, China. Three non-flowering and three flowering plants of *P. chino* var. *hisauchii* were collected with the same site conditions, which were approximately 4 years old and 3 m in height. The average diameter at the height of the eyebrows (1.5 m) of non-flowering bamboos was 2 cm with a wall thickness of 2.6 mm; the average diameter at the height of the eyebrows of flowering bamboos was approximately 2.5 cm with a wall thickness of 3 mm.

Then, a 1-m-long section of bamboo culm from the height of the eyebrows upwards was collected from three flowering and three non-flowering bamboos and transported to the laboratory for later use. Samples of anatomy and density were taken from the internode at the height of the eyebrows, mechanical and microfibril angle samples were taken from the upper internode, and the remaining internodes were ground into powder for chemical properties and crystallinity samples (as shown in Fig. 1).

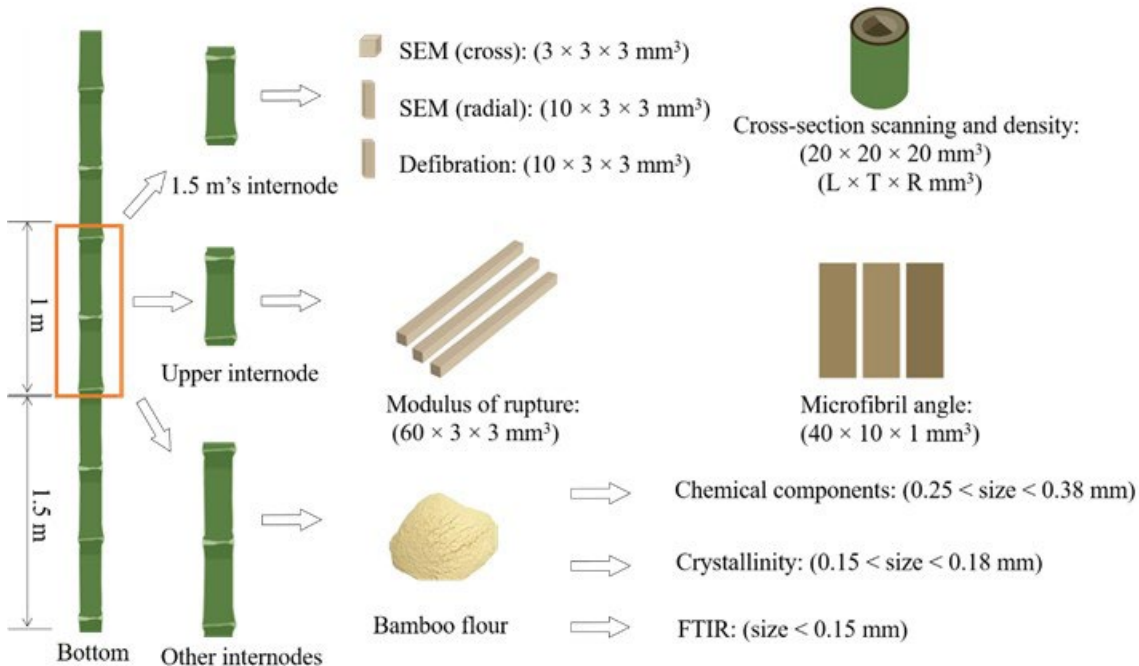


Fig. 1. The preparation of the samples

Anatomical Properties

2-cm-high samples ($20 \times 20 \times 20 \text{ mm}^3$, $L \times T \times R$) were taken from the internode at the height of the eyebrows, and the area ratios and morphologies of vascular bundles, basic tissues, and conducting tissues in the cross-section surface of the samples were determined *via* an environmental scanning electron microscope (ESEM, XL30ESEM-FEG; FEI, Hillsboro, OR, USA) and ImageJ software (National Institutes of Health, V1.8.0.112, Bethesda, MD, USA). A total of 30 small samples were measured in each group and the average value was reported as the final result. The stick-like samples ($10 \times 3 \times 3 \text{ mm}^3$) were put into test tubes containing a mixed solution of acetic acid and hydrogen peroxide (1:1), which were then placed in an oven at $80 \text{ }^\circ\text{C}$ to defiber for 6 h. The length, width, and double-wall thickness of the fiber were measured by automatic imaging equipment (CW4000; Leica, Wetzlar, Germany), and a total of 200 fiber cells were tested for each group. Finally, 10 cross-section ($3 \times 3 \times 3 \text{ mm}^3$) and radial-section ($10 \times 3 \times 3 \text{ mm}^3$) small blocks were made for each group, and the surface morphologies were observed *via* scanning electron microscopy (SEM, Quanta 200HV, FEI, Hillsboro, OR, USA).

Physical Properties

The density measurement took 2-cm-high samples ($20 \times 20 \times 20 \text{ mm}^3$) from the internode at the height of the eyebrows (the same as the samples used in the scanner), the air-dried, oven-dried, green, and basic densities of the bamboo were determined by the drainage method according to GB/T 15780 (1996). A total of 30 small samples were measured in each group, and the average values were reported.

The mechanical performance entailed long strip samples ($60 \times 3 \times 3 \text{ mm}^3$) that were made from the upper internode, and three-point bending tests were performed with a high-precision mechanical testing machine (SHIMADZU-AG-X, Shimadzu, Kyoto, Japan). The tangential bending strength and modulus were measured according to IS 8242-1976 (1977)

and ISO 22157-1 (2004). A total of 30 test samples were repeated for each group and the average value was reported.

Microfibril Angle and Crystallinity

For the microfibril angle, small samples of internode were made into $40 \times 10 \times 1$ mm³ tangential chips from the upper internode. The microfibril angle was determined by X-ray diffraction (X'pert Pro, Royal Dutch Philips Electronics Ltd., Amsterdam, Netherlands). The main parameters were as follows: tube voltage 36 kV, tube current 20 mA, scanning speed 16°/min, rotation range of the sample stage 0 to 360°. The scanning intensity curve was imported into Origin data processing software (OriginLab, v9.5, Northhampton, MA, USA), the Gaussian function was used to fit the single peak, and then the 0.6T method was used to get the microfibril angle (Stuart and Evans 1995). Thirty samples were performed for each group and the average value was reported.

For the crystallinity, the remaining internodes were dried and ground into powder from 80 to 100 meshes (0.150 to 0.180 mm) and the crystallinity was determined *via* X-ray diffraction. The main parameters were as follows: tube voltage 36 kV, tube current 20 mA, scanning speed 4°/min, and the sample scanning range 5 to 40°. The relative crystallinity was calculated with the Segal method (Yang *et al.* 2010). Ten replicates were measured for each group and the average value was reported.

Chemical Compositions

The remaining internodes were dried and ground into powder from 40 to 60 meshes (0.25 to 0.38 mm), and the contents of extractives, holocellulose, lignin, cellulose, pentosan, and ash in the samples were determined according to GB/T 2677 (1995). Among them, the contents of pentosan and cellulose were assayed via methods of dibromide and nitric acid-ethanol, respectively. The other internodes were ground into powder less than 100 meshes (size < 0.15 mm), and the main functional groups in the samples were characterized by the tableting process and Fourier transform infrared (FTIR) (TENSOR II, Bruker Optics Inc., Billerica, MA, USA). Ten replicates were conducted for each group and the average value was reported.

Data Analysis

Data were processed and analyzed using SPSS22 (SPSS Inc., Chicago, IL, USA) and Origin v9.5 (OriginLab, Northhampton, MA, USA).

RESULTS AND DISCUSSION

Anatomical Properties

The vascular bundles of *P. chino* var. *hisauchii* were open (Fig. 2a), and the internode cells were arranged axially (Fei *et al.* 2016). The parenchymal cell wall was multilayered and there were a few starch granules and single pits in the cavity and wall (Figs. 2b, 2d, 3b, and 3d). Generally, there were tough fiber sheaths at the outer edge to counter the external load and deformation (Liu *et al.* 2014b), and the fiber sheaths were composed of fiber cells that were the primary source of bamboo's strength. The figures show that the cross-section of the fiber cell is almost circular with a thick cell wall (Figs. 2c and 2e), while there are still some single small and round pits in the cell wall (Figs. 3c and 3e).

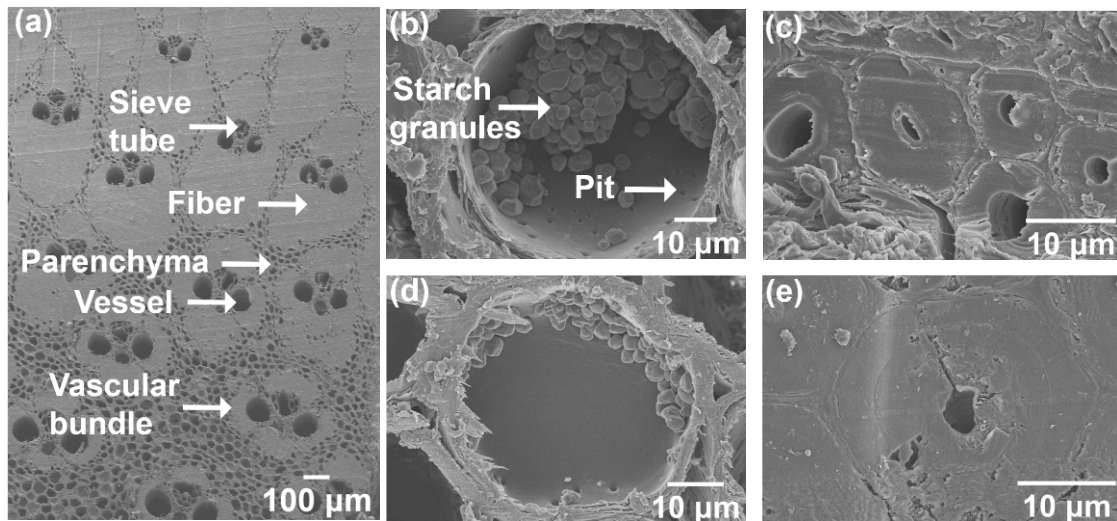


Fig. 2. The cross-section anatomical properties of *Pleioblastus chino* var. *hisauchii* before and after flowering; (a): the cross-section, (b, c): the parenchyma and fiber cells before flowering, and (d, e): the parenchyma and fiber cells after flowering

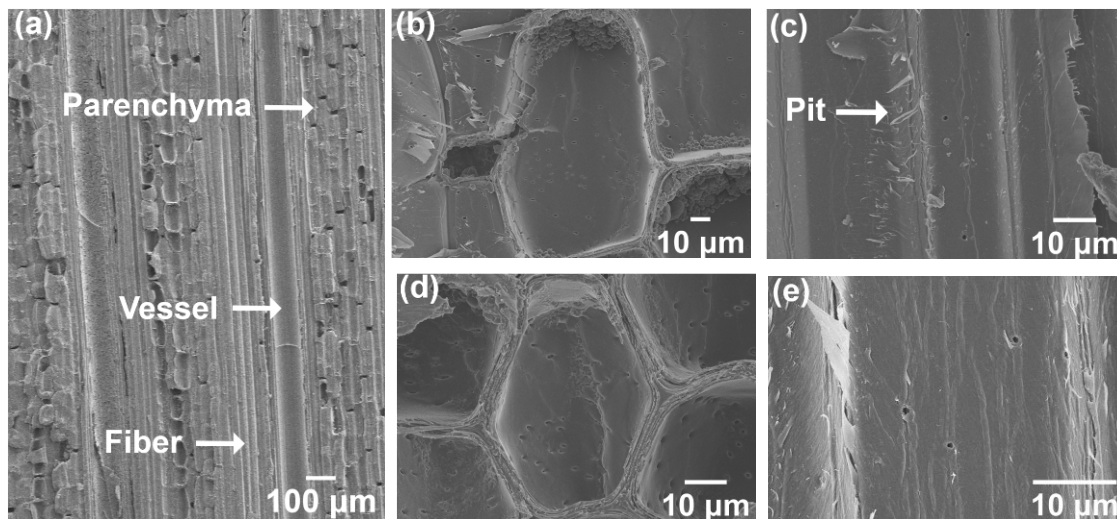


Fig. 3. The radial section anatomical properties of *Pleioblastus chino* var. *hisauchii* before and after flowering; (a): the radial section, (b, c): the parenchyma and fiber cells before flowering, and (d, e): the parenchyma and fiber cells after flowering

There were some changes in the anatomical properties of the culms before and after flowering. After flowering, the tangential width, radial width, frequency of the vascular bundle, and the vessel diameter decreased by 1.34% ($p < 0.05$), 1.70% ($p < 0.01$), 1.84% and 6.50% ($p < 0.001$), respectively (Fig. 4a); the ratio of the fiber, conducting tissue and vascular bundle dropped by 0.97%, 1.99% and 1.10%, while the ratio of the parenchyma rose by 1.99% (Fig. 4b); the length and double-wall thickness of the fiber fell by 5.22% and 3.12%, and the fiber width rose by 4.15% (Fig. 4c).

Only the differences in the vascular bundle diameter and vessel diameter were significant; this suggested that the reduction of the vessel diameter increased the rate of change of air pressure for water flow, thereby increasing the resistance to water flow and preserving some water for the flowering period. This structure promotes the exertion of the

advantages of bamboo groups (Song and Liu 2008). The insignificant difference for other variables could result from the fact that the growing environment and growth time of the bamboos of the two groups were similar, and the morphological development of the vascular bundles was nearly complete.

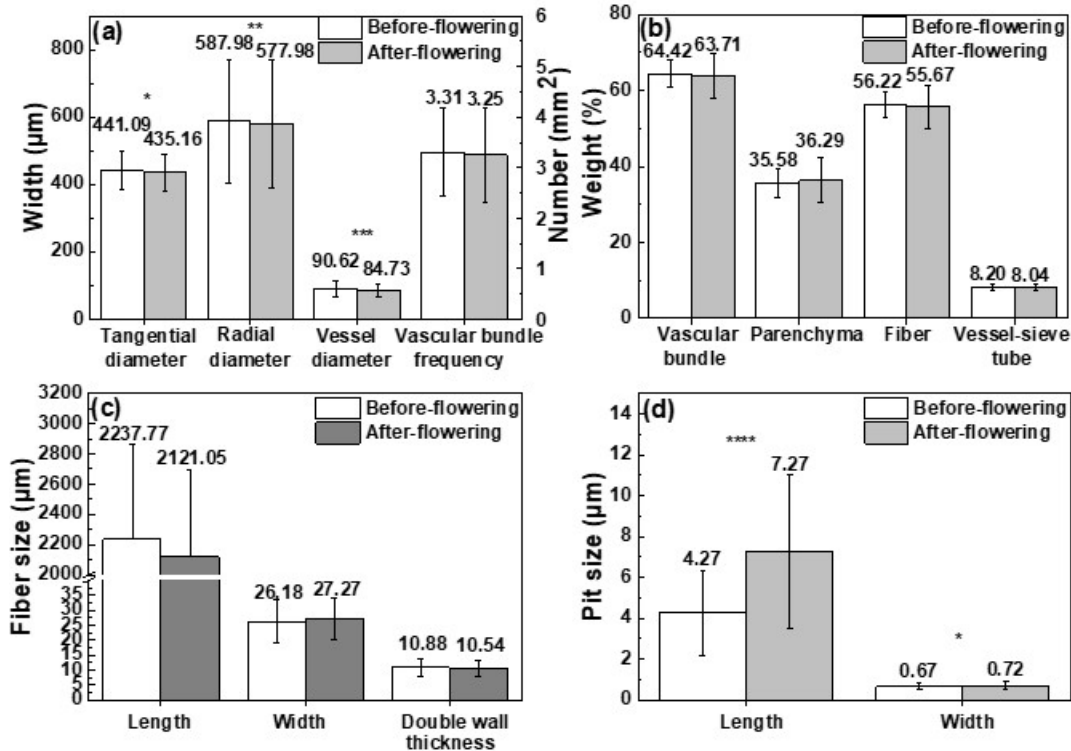


Fig. 4. The tissue and cell morphology of *Pleioblastus chino* var. *hisauchii* before and after flowering; (a): the vascular bundle and vessel morphology; (b): tissue ratio; (c): the fiber morphology, and (d): the pit morphology in the vessel wall. Significance: * = 0.05, ** = 0.01, *** = 0.001, **** = 0.0001, no mark means insignificant, the same below

There were notable differences in the pits in the vessel wall of *P. chino* var. *hisauchii* before and after flowering (Fig. 5). The pits in the vessel wall were all alternate to opposite, and before flowering, the pits were mostly small, alternate, with short and oblate ellipses closely arranged and distributed disorderly (Figs. 5a and 5b). After flowering, the pits were mostly large, opposite, with oblate ellipses uniformly and distributed regularly (Figs. 5c and 5d).

Openings in flowering bamboo pits were much larger than in flowering bamboo pits. The length and width of pits (Fig. 4d) went up by 70.15% ($p < 0.0001$) and 7.79% ($p < 0.05$), respectively, which might be a result of the enhanced metabolism. Transport of nutrients occurs more frequently during bamboo flowering, which leads to more efficient transport channels, especially transverse channels, consisting mainly of pits (Carlquist 2012). Thus, the pits had evolved to have appropriate structures and distributions, enlarged for material transport.

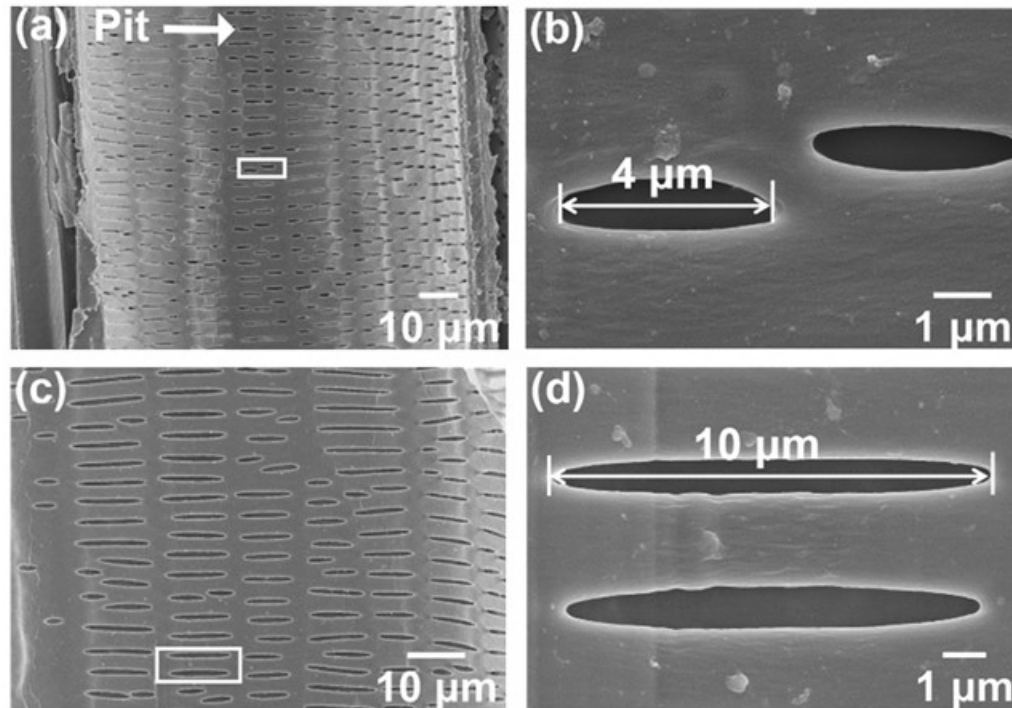


Fig. 5. The pit anatomical properties in the vessel wall of *Pleioblastus chino* var. *hisauchii*; (a, b): vessels and pits before flowering and (c, d): vessels and pits after flowering

Chemical Composition

Similar to wood, the primary chemical components of bamboo are cellulose, hemicellulose, and lignin (Nishiwaki-Akine *et al.* 2020), which have pronounced effects on the properties of bamboo. The stretching vibrations of the main functional groups of the three major components can be observed by infrared spectroscopy (1730 cm^{-1} : stretching vibration of the non-conjugated carbonyl group in xylan; 1240 cm^{-1} : C-O bond vibration of the syringa ring in lignin and C-O bond vibration of xylan; and 1048 cm^{-1} : stretching vibration of C-O bonds in cellulose and hemicellulose) (Fig. 6a).

The results showed that the contents of extractives, lignin, holocellulose, cellulose, and pentosan in flowering *P. chino* var. *hisauchii* samples increased by 38.51%, 7.93%, 4.57% ($p < 0.01$), 11.07% ($p < 0.001$), and 7.37%, respectively, in comparison to those in the non-flowering samples, while the ash content fell by 16.76% ($p < 0.001$) (Fig. 6b). Results from previous studies indicate that flowering consumed the nutrients of bamboo, leading to lower mineral content and relatively elevated levels of chemical components (Bisht *et al.* 2017). Meanwhile, flowering had accelerated the aging of bamboo and promoted fibrillation and lignification; the results of this study were consistent with previous findings (Wang *et al.* 2016a).

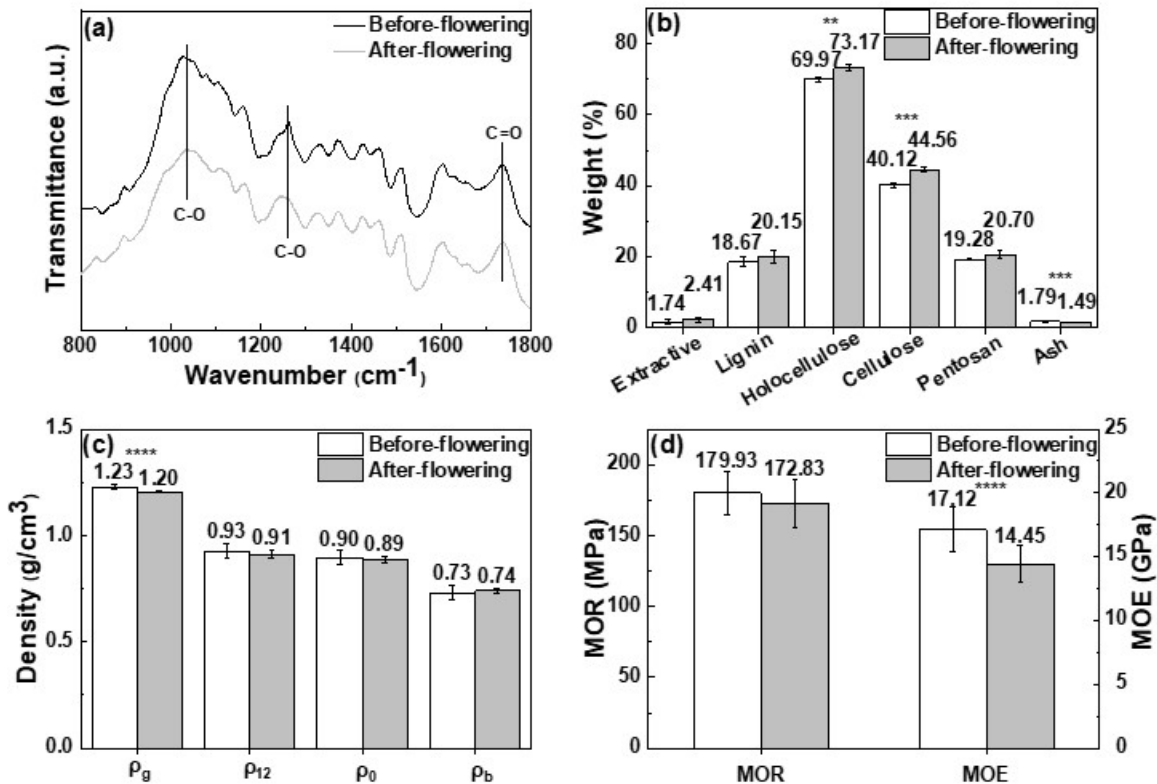


Fig. 6. The chemical composition, density parameters, and bending performance of *Pleioblastus chino* var. *hisauchii* before and after flowering; (a): infrared spectrum, (b): chemical composition, (c): density, ρ_g is green density, ρ_{12} is the density when the moisture content is 12% (air-dried density), ρ_0 is oven-dried density, and ρ_b is basic density, and (d): modulus of rupture (MOR) and modulus of elasticity (MOE)

Mechanical Performance

Bamboo is a natural biocomposite with unique multilevel structures that endow it with excellent mechanical performance (Wani and Shitole 2017). After the flowering of *P. chino* var. *hisauchii*, both the density and the mechanical strength of the bamboo decreased (Figs. 6c and 6d).

The density of green, air-dried, and oven-dried decline were respectively 1.97% ($p < 0.0001$), 1.65%, and 1.13%, but the basic density went up by 1.35% in flowering bamboo. While the volume of the bamboo remained the same, the consumption of nutrients such as starch during flowering reduced the weight of the bamboo and reduced its density. At the same time, the fiber ratio, vascular bundle ratio and vascular bundle density of *P. chino* var. *hisauchii* decreased after flowering, which was also a reason for the decrease in density (Liu *et al.* 2014a). Bamboo was more lignified after flowering, and the cellulose molecular chains were more compactly arranged to expose fewer free hydroxyl groups, thus leading to decreased hygroscopicity and increased basic density (Kubicki *et al.* 2014).

The modulus of rupture and modulus of elasticity dropped by 3.95% and 15.59% ($p < 0.0001$), respectively, in flowering bamboos. After flowering, the bending strength of bamboo decreased as the density decreased, and the ash content of flowering bamboo was lower (Fig. 6b), which also lowered the mechanical strength of bamboo (Fig. 6b) (Rowell 1984). The density and the microfibril angle are the two main factors determining the

modulus of elastic. The higher the density, the smaller the microfibril angle and the more proportion of microfibrils, resulting in a larger elastic module (Tanabe *et al.* 2018). The larger angle of the bamboo microfibrils after flowering meant that the fibrils deviated from the longitudinal axis of the bamboo or bamboo fibers, which made the bamboo have poor resistance to bending. At the same time, the reduction of fiber ratio in anatomical structure also reduced the proportion of microfibrils in the cell wall (Abdullah *et al.* 2010).

Microfibril Angle and Crystallinity

The value of the microfibril angle determines the microscopic and macroscopic performance of plant materials and is an important indicator affecting the properties of bamboo and other biomass materials (Sun *et al.* 2016). It can be observed in the figure that the change curve has a high degree of fit, and the microfibril angle after flowering is larger than that before flowering, which is increased by 3.33% (Figs. 7a and 7b). According to the theory of external causes of bamboo flowering, the environmental stress is an important cause. As bamboo ages, its capacity for water transport is weakened and the consumption of its internal nutrients and minerals is accelerated, leading to the formation of gaps between microfibrils in cell wall that widen the microfibril angle (Fang *et al.* 2004).

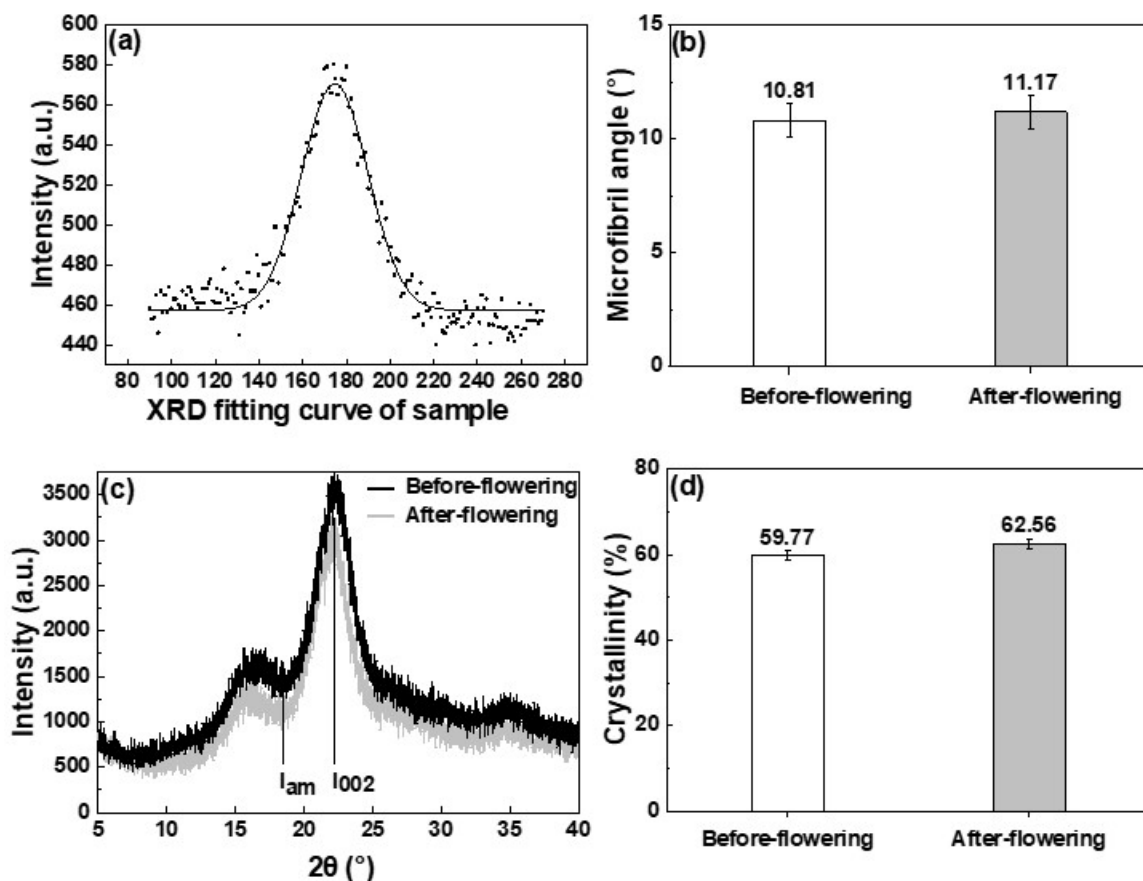


Fig. 7. The microfibril angle and crystallinity of *Pleioblastus chino* var. *hisauchii* before and after flowering, (a): microfibril angle fitting curve; (b): microfibril angle; (c): crystallinity curve; (d): crystallinity the ability to transport materials

Crystallinity represents the proportion of crystalline regions in polymers. The molecular chain arrangement of cellulose in crystalline regions is regular, while it is disordered and loosely structured in amorphous regions (Jun *et al.* 2019). The X-ray diffraction variation curve shows that the crystallinity after flowering is greater than that before flowering, increasing by 4.68% (Figs. 7c and 7d). When samples were dried, the cell walls were dehydrated and the gaps between microfibrils tended to aggregate, leading to the expansion of crystalline regions and increased crystallinity overall (Toba *et al.* 2013; Lv *et al.* 2019). Therefore, bamboo showed a high degree of lignification after flowering, and the cellulose molecules were tightly arranged to form higher proportions of crystalline regions that elevated the overall cellulose crystallinity (Tabet and Aziz 2013).

CONCLUSIONS

1. After flowering, the strength of *P. chino* var. *hisauichii* culms decreased and the ability to transport nutrients increased, which were closely related to the changes in internal structure and properties.
2. There were some changes in the properties of *P. chino* var. *hisauichii* culms after flowering. For anatomical properties, the length and double-wall thickness of the fiber fell by 5.22%, 3.12%, and the width rose by 4.15%; the tangential width, radial width, frequency of the vascular bundle, and the vessel diameter decreased by 1.34% ($p < 0.05$), 1.70% ($p < 0.01$), 1.84% and 6.50% ($p < 0.001$), respectively. The ratios of the fiber, conducting tissue and vascular bundle dropped by 0.97%, 1.99% and 1.10%, but the parenchyma ratio rose by 1.99%; moreover, the length and width of the pits in the vessel wall went up by 70.15% ($p < 0.0001$) and 7.79% ($p < 0.05$). For chemical components, the contents of extractives, lignin, holocellulose, cellulose and pentosan increased by 38.51%, 7.93%, 4.57% ($p < 0.01$), 11.07% ($p < 0.001$) and 7.37%, while the ash content fell by 16.76% ($p < 0.001$). For mechanical performance, the density of green, air-dried, and oven-dried declined by 1.97% ($p < 0.0001$), 1.65%, and 1.13%, respectively, but the basic density went up 1.35%; the modulus of rupture and modulus of elasticity dropped by 3.95% and 15.59% ($p < 0.0001$), respectively. The microfibril angle and crystallinity rose by 3.33% and 4.68%, respectively.
3. A comprehensive and systematic study on the chemical, physical, and anatomical properties of bamboo culms before and after flowering was carried out to explore the changing rules of the corresponding characteristics, which not only can provide a theoretical reference for exploring the flowering mechanism, but it may also be possible to monitor or predict the flowering of bamboo plants through some internal structure or property changes, and this may provide a novel approach for the investigation of bamboo flowering.

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