

Article

The Effect of Guttation on the Growth of Bamboo Shoots

Huifang Zheng , Miaomiao Cai, Yucong Bai, Junlei Xu, Yali Xie, Huajian Song, Juan Li and Jian Gao * 

Key Laboratory of National Forestry and Grassland Administration/Beijing for Bamboo & Rattan Science and Technology, International Center for Bamboo and Rattan, State Forestry and Grassland Administration, Beijing 100102, China; zhenghuifang@icbr.ac.cn (H.Z.); cmm@icbr.ac.cn (M.C.); bai.yucong@icbr.ac.cn (Y.B.); xjl@icbr.ac.cn (J.X.); xylyn@163.com (Y.X.); songhja@163.com (H.S.); lijuan@icbr.ac.cn (J.L.)

* Correspondence: gaojian@icbr.ac.cn or gaojianicbr@163.com; Tel.: +86-010-8478-9801

Abstract: Guttation is the process of exuding droplets from the tips, edges, and adaxial and abaxial surfaces of the undamaged leaves. Guttation is a natural and spontaneous biological phenomenon that occurs in a wide variety of plants. Despite its generally positive effect on plant growth, many aspects of this cryptic process are unknown. In this study, the guttation phenomenon characteristic of bamboo shoots and the anatomical feature of these and culm sheaths were systematically observed. In addition, the water transport pathway and the compounds in guttation droplets of bamboo shoots were analyzed, and the effect of bamboo sheaths' guttation on the growth of bamboo shoots was assessed. The results revealed that bamboo shoots began to exude liquid in the evening through to the next morning, during which period the volume of guttation liquid gradually increases and then decreases before sunrise. Many vascular bundles are in bamboo shoots and culm sheaths to facilitate this water transport. The exudate liquid contains organic acids, sugars, and hormones, among other compounds. Our findings suggest that the regular guttation of the sheath blade is crucial to maintain the normal growth of bamboo shoots.

Keywords: anatomy; bamboo shoot; guttation; sheath blade; water transport



Citation: Zheng, H.; Cai, M.; Bai, Y.; Xu, J.; Xie, Y.; Song, H.; Li, J.; Gao, J. The Effect of Guttation on the Growth of Bamboo Shoots. *Forests* **2022**, *13*, 31. <https://doi.org/10.3390/f13010031>

Academic Editor: Cate Macinnis-Ng

Received: 21 November 2021

Accepted: 24 December 2021

Published: 28 December 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Guttation is a very common natural phenomenon in the plant kingdom. Many plant species reportedly engage in guttation, such as rice, wheat, barley, maize, tomato, potatoes, tobacco [1–5]. Guttation refers to the process of liquid seeping from the tips, edges, and adaxial and abaxial surfaces of the undamaged leaves of plant species in the form of droplets [6]. This usually occurs during the early morning or late hours of the day and is a complex phenomenon affected by many internal and external factors. Some factors affecting guttation include a plant's nutritional condition, its water balance and root activity, local soil moisture, air temperature and humidity, and wind speed [7]. It is generally thought that the continual absorption of water by the root system causes water to accumulate in roots, which creates hydrostatic pressure; this pressure can move water upward to the leaves of the plant via the xylem duct network in the stem, forcing some water to exit through hydathodes located at surfaces of leaves to form the characteristic drops of guttation [7]. Guttation plays an important role in the transport of nutrients, water, proteins, enzymes, hormones, and metabolites as a plant develops and grows in size [6]. The study showed that plant guttation can provide a continuous non-destructive system for producing recombinant proteins, greatly increasing yield, eliminating extraction, and simplifying downstream processing [8]. The guttation status of plants can be used as a rapid screening technology for rice germplasm resources [9]. However, we do not yet fully understand the internal linkage between the guttation phenomenon and the growth dynamics of plants.

Moso bamboo (*Phyllostachys edulis*) is the most important bamboo species in China because of its paramount ecological, economic, and cultural value among all bamboo types. Within the last decade, considerable research has investigated the mechanism

underpinning the rapid growth of bamboo shoots, including the morphological anatomy aspects, physiological responses, and molecular regulation [10–13]. Compared with trees, bamboo is a grass that has a unique vascular system that is likely linked to its special growth and development characteristics [13]. The shoot stage has an astounding growth rate, capable of reaching a maximum daily growth of 1 m [10]. Water dynamics figure prominently in the facilitation of the rapid growth of bamboo shoots, and guttation is a direct indicator of a plant's water status. However, the relationship between bamboo's guttation and its shoots' rapid growth is understudied, and therefore this phenomenon and its implications remain unclear.

To fill this knowledge gap, the guttation phenomenon of culm sheaths was observed in detail in this study. Specifically, the guttation liquid was collected, and its volume and contents measured, while the ambient temperature and humidity were recorded. The anatomical structure of bamboo shoots and culm sheaths were examined to analyze the transport of water through the plant. The purpose of this work was to better understand the effect of guttation on the rapid growth of bamboo shoots.

2. Materials and Methods

2.1. Study Area

The study site was located in Lucun town of Guangde city (30°49'13" N; 119°25'28" E), Anhui Province, China, where bamboo samples were collected in April and May 2020. All field observations and data collection were completed in Guangde, with subsequent plant physiology experiments were carried out in the laboratory. Guangde belongs to the humid climate area of the north subtropical zone, with an average annual temperature of 15.4 °C, annual precipitation of 1328 mm, and a frost-free period of nearly 300 days. Lucun town is rich in natural resources of moso bamboo, which has earned it the reputation and moniker of being the "hometown of the bamboo sea".

2.2. Observation and Collection of Guttation Samples

We observed and recorded the start/end time (on an hourly basis) of exudation on consecutive days, based on visible exudate on the surface of sheaths blades. Sheath blades that did not guttate in the daytime and those that were exuding droplets at night were selected ($n = 5$) and their fresh weight and dry weight were recorded, respectively. The water content was calculated as $(\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100\%$. Six bamboo shoots (about 1.3 m in height) were randomly selected in a bamboo forest. At the same time, the air temperature and humidity of the bamboo forest were recorded daily. For collecting guttation liquid with a syringe, the bamboo shoots were wrapped in foil, and the volume of the liquid was recorded (every four hours). In addition, about 25 mL of guttation liquid of six bamboo shoots was collected with a syringe and centrifuge tube on sheath blades and frozen immediately in liquid nitrogen. Solid carbon dioxide was used to transport the samples to the laboratory, where they were stored in a $-80\text{ }^{\circ}\text{C}$ refrigerator until analysis. Qualitative analysis of the chemical substances present in guttation liquid ('exudate water') was carried out by liquid chromatograph–mass spectrometer (LC–MS). The composition of the guttation was determined by Shanghai Applied protein technology Co., Ltd. (Shanghai, China) The samples were separated on an Agilent 1290 Infinity LC Ultra Performance Liquid Chromatography (UHPLC) HILIC column [14]. Column temperature $25\text{ }^{\circ}\text{C}$; flow rate of 0.3 mL/min ; Mobile phase composition A: water +25 mM ammonium acetate +25 mM ammonia, B: acetonitrile; gradient elution; The sample was placed in a $4\text{ }^{\circ}\text{C}$ automatic sampler during the whole analysis process.

2.3. Water Transport Path Tracer Experiment

A dye tracer experiment was conducted in vitro, as described by Xie [15]. Each bamboo shoot (about 1.5 m in height, $n = 3$) was cut at the base and the culm sheaths were cut from the top of bamboo shoots and were immersed into an aqueous solution containing 8% acid

magenta. After 1 h of soaking, the staining condition of the samples was observed by the freehand section.

2.4. Anatomical Structure of Bamboo Shoot and Sheath

Paraffin sectioning of plant tissue was implemented according to the modified methodology of Li [10]. The bamboo shoot (the same position of the different internode), culm sheath, and bamboo leaf samples (Supplementary Figure S2) were cut into approximately 1-cm × 0.5-cm sections and fixed in an FAA liquid (Formaldehyde: acetic acid: ethanol = 5:5:90) and air was extracted from the samples by placing them into a vessel equipped with a vacuum pump. Each sample section was embedded in paraffin after a gradient series of alcohol dehydration. Serial transverse and longitudinal sections (8 µm thick) were then sliced and sequentially stained with safranin O for 10 min and Fast Green for 5–10 s, sealed with neutral gum, and observed under an Olympus BX51 microscope equipped with a digital image acquisition system.

2.5. Bamboo Sheath Treatment

Bamboo shoots (about 1 m in height) were selected in the field and allocated to five treatment groups: (1) normal growth conditions, without any treatment applied, to serve as the control; (2) complete removal of sheath blade, from bottom to top of the shoot; (3) partial removal of the expanded sheath blades only; (4) removal of the sheath blades from one side of the shoot; and (5) entire bamboo sheath stripped, from bottom to top (Figure 1A–E). We observed the guttation liquid and measured the height of bamboo shoots every day (9–20 April 2020, $n = 3$).

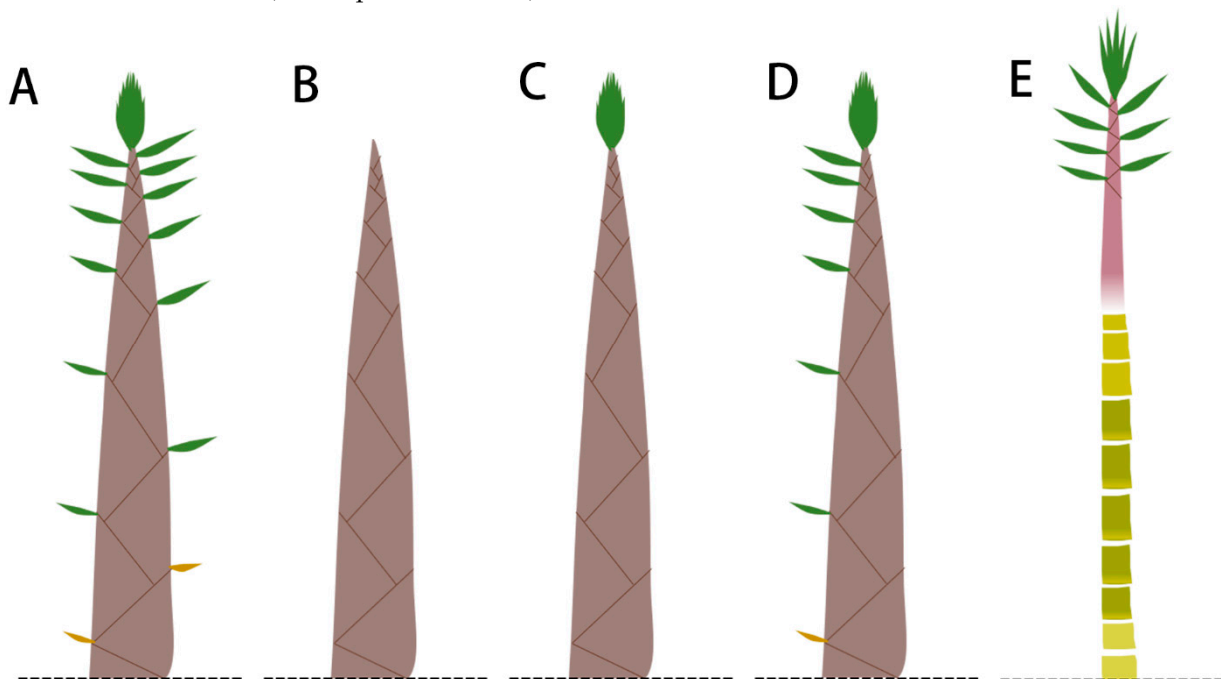


Figure 1. (A) No treatment was imposed, to serve as the experiment’s control. (B) Removal of all sheath blades. (C) Removal of all expanded sheath blades. (D) Removal of the sheath blades spread on one side only. (E) Removal of the entire intact bamboo sheath.

2.6. Statistical Analysis

The data presented are the means of at least three replicates for each experiment (means ± SE). For all response variables, one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was used to determine the statistical significance of pairwise comparisons. These analyses were conducted using the SPSS 20.0 software for Windows. Differences among or between means were considered significant at $p < 0.05$.

3. Results

3.1. Guttation of Sheath Blades

The bamboo shoots began to exudate liquid as soon as they sprout from the soil. Guttation continued throughout the growth period of bamboo shoots, beginning in the evening until sunrise the next morning with no interruptions (Figure 2A). According to the results, an important factor affecting the start and end time of guttation was the weather conditions, namely temperature and humidity (Figure 2B). When the weather was clear, the temperature was high and the humidity was low, the guttation time started late, and its overnight duration was short. We observed that guttation occurred from 9:30 p.m. to 10:00 p.m. and lasted until 6:00 a.m. to 7:00 a.m. of the following day under clear weather. In contrast, under low temperature and high humidity conditions, the onset of guttation was sooner, and its duration was longer. In this respect, guttation began at 5:30 p.m. and ceased at 8:30 a.m. to 9:00 a.m. the next day. In addition, soil moisture also considerably influenced the timing and duration of guttation in bamboo. After rainfall events, soil moisture increased and guttation started earlier and lasted longer, whereas it happened later when the soil water content was in a deficient state, leading to marked variation among different bamboo shoots in their guttation.

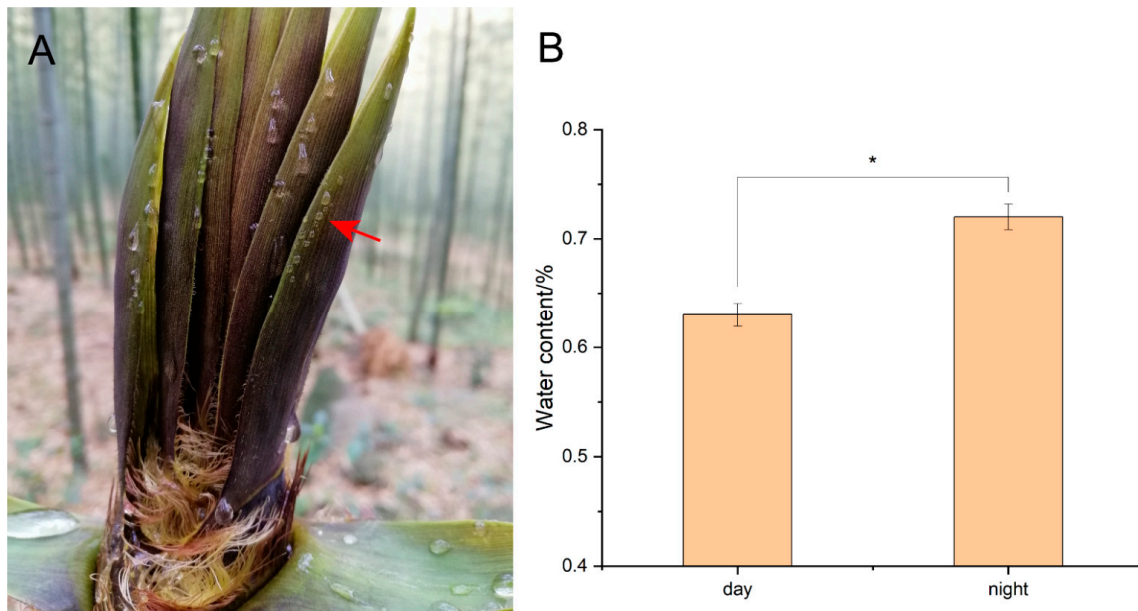


Figure 2. (A) Guttation liquid on edge of a bamboo sheath blade. The red arrow points to the location of guttation droplets; (B) Sheath blade water content during the day and night; the asterisk indicate a different significance, * $p < 0.05$.

Among different parts of the sheath, there was inconsistent guttation. In almost all sheaths observed early in bamboo shoot development, there was obvious guttation; in the middle and upper parts of the bamboo sheaths, the exudate was especially obvious. However, as bamboo shoots grew larger, the basal sheath started to undergo senescence, causing the tips of the sheath blade to change from green to yellow, with sheath guttation evidently waning until it ceased altogether. At the beginning of guttation, water slowly seeped out of the pore, gradually forming visible droplets that rapidly enlarged and merged with surrounding droplets to form larger ones that slid down along the sheath blade. According to our observations and data, the guttation volume of bamboo shoots was not constant with a 24-h period, as it initially increased and then decreased (Figure 2B). The water content of sheath blades over a 24-h period was significantly higher in the day than at night (Table 1).

Table 1. Changes the average of guttation volume with temperature and humidity.

Time	Guttation/mL	Temperature/°C	Humidity/%
22:00	14.962 a	14.4	64.5
2:00	24.044 b	16.6	43.8
6:00	18.148 ab	13.7	60.1

Different letters indicate significant differences between the average values of the same column (\pm SE, $n = 5$), $p < 0.05$.

3.2. Composition Analysis of the Guttation Fluid

A variety of substances was detected, including amino acids, sugars, and hormones. In the anion state, 54 substances were detected, mainly various organic acids, hormones, and sugars (Figure 3A). Specifically, six sugars were detected: trehalose, raffinose, D-mannose, L-rhamnose, sucrose, and sedoheptulose. Raffinose was the most abundant, followed by L-rhamnose, with D-mannose present in the lowest relative amount. Absciscic acid, as a stress response hormone, was also detected in the guttation. Four nucleotides and various organic acids, such as oleic acid, linoleic acid, and glyceric acid, were also detected. In the cation state, 72 substances were detected, consisting of amino acids, sugars, hormones, and dipeptides (Figure 3B,C). More Specifically, four sugars were detected, namely stachyose, raffinose, sucrose, and maltotriose. The highest relative content was for raffinose. In all, fifteen dipeptides were detected in the guttation, with relatively high levels of Lys-Pro, Arg-Ile, and Ile-Leu.

3.3. Water Transport in Bamboo Sheaths

Xylem vessels are long-distance transport channels for water in plants. It was observed that magenta was transported from the bottom to the top of the vascular bundles, and it also extended to the vascular bundles connected with the culm sheath at the nodes (Figure 4A,B). This indicated that the water of bamboo shoots could be transported to the culm sheath along the vascular bundles, which were also observed in the bamboo nodal diaphragm (Figure 4A). Almost all vascular bundles were stained magenta in the bamboo sheath (Figure 4C,D). Examination of the paraffin sections showed that the vascular bundles at different internodes had developed differently (Figure 4E,F). The vascular bundles at the bottom of the bamboo shoot were relatively well developed, and their cell structure was clear, and the cell was thick (Figure 4E). Many nuclei were observed in the upper part of the bamboo shoot, where the cells were smaller (Figure 4F), indicating they were still in the stage of differentiating.

When compared with the vascular bundles of bamboo shoots, the sheath blades also included phloem, xylem vessels (metaxylema and protoxylema in the primary xylem), parenchyma cells, and fiber cells. The difference between the bundles, as compared to bamboo shoots, was that in the sheath blades the fibers were turned and concentrated towards the epidermis (Figure 5A). We also observed the hydathodes in the upper and lower epidermis of sheath blades, resembling stomatal consisting of loosely connected cells and many intercellular spaces (Figure 5A). Further, in comparison with the leaf structure of moso bamboo, the parenchymatous sheath cells tightly surrounded the outside of the vascular bundle (Figure 5B). Bulliform cells were easily observed in the adaxial epidermis of the bamboo leaf, but they did not appear in culm sheath blades and leaf sheaths (Figure 5A,B). Fusoid cells appeared on both sides of the vascular bundle in the bamboo leaves, and their cell walls were very thin and easy to observe. This structure was not observed in culm sheaths or leaf sheaths. In general, the structure of the culm sheath was similar to that of the leaf sheath (Figure 5C–E), which was composed of large and small-spaced vascular bundles arranged near the distal axial surface, whose fiber cap was situated near the outer side of the phloem. The transverse conduits connecting the two longitudinal vascular bundles were also observed in the longitudinal section of the sheath (Figure 5F).

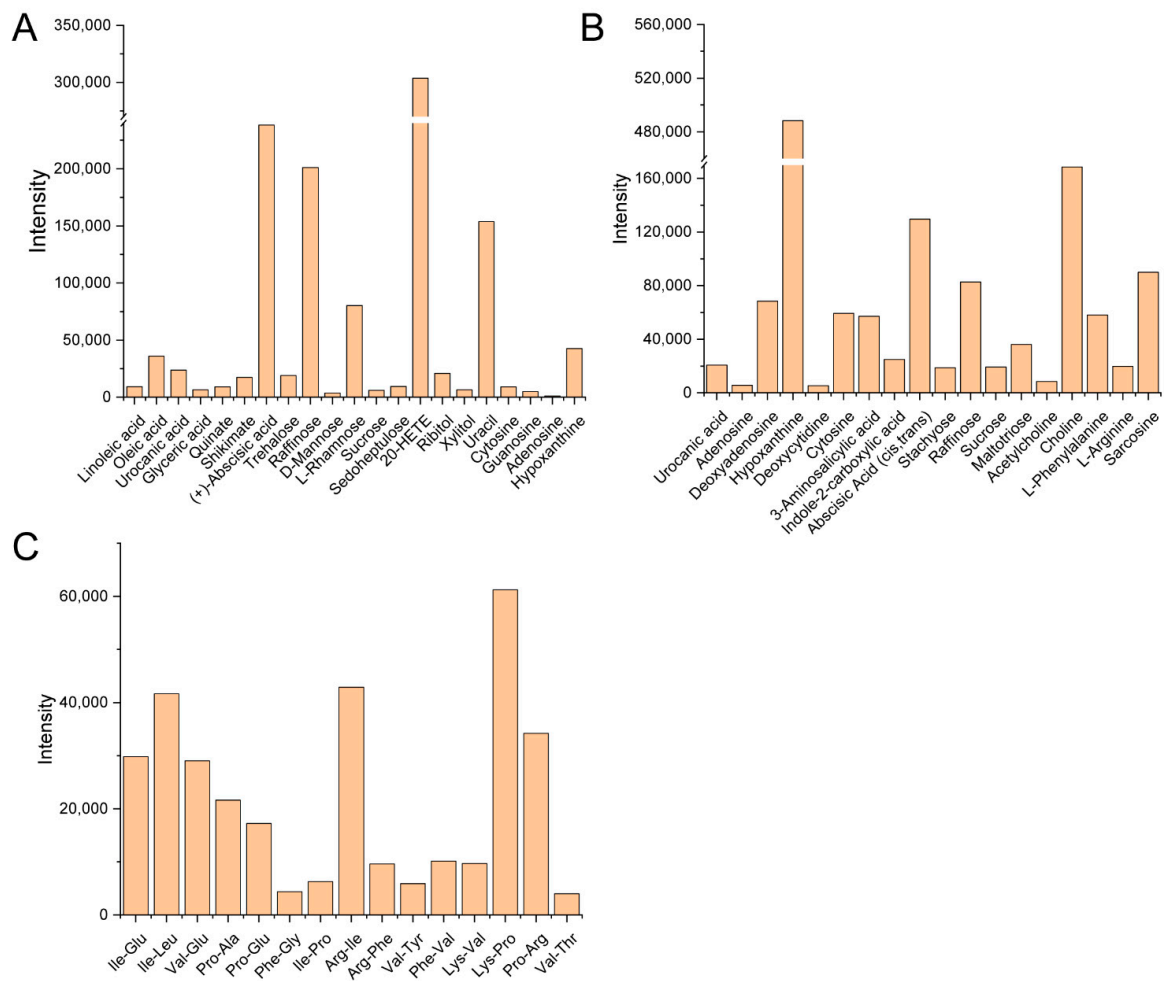


Figure 3. The relative content of the components in the guttation. (A) Components identified in a cationic state; (B,C) Components identified in an anionic state.

3.4. Effect of Sheath Blades and Culm Sheaths on the Growth of Bamboo Shoots

In the treatment groups where culm sheaths and sheath blades were removed in full or in part, liquid outflow from the wound sites was always observed (Figure 6A,B). When a single complete culm sheath was removed from the node, small droplets were observed to exude from the vascular bundle at the wound site at night (Figure 6C); however, this exudation rate was slow, and the amount of liquid was less than that produced by normal guttation. Removal of sheath blades alone barely affected the height growth of bamboo shoots (Figure 6D). Eventually, however, the bamboo shoots achieved a high stature. We noted, however, that peeling off a single complete culm sheath of the bamboo shoot, but retaining the upper sheath enclosing the tip, led to slower growth after which the upper part of the bamboo shoot bent and broke within 2 to 3 days (Figure 7A,B). The internodes of the bamboo shoot were shorter when the culm sheath was removed, and its growth rate was slower, and its upper part bent to one side. In the subsequent growth period, the exposed intermodal lengths gradually became fixed, and the upper part of the bamboo shoot sheath was restored to its normal growth (Figure 7C–F). Eventually, the bamboo shoot continued to growth, but it was still dwarfed by normally growing plants in the control group (Figure 7F). To study the effect of water supply interruption on bamboo shoots, we tried to cut the bamboo whip, finding that 7 days after this broken rhizome treatment, the upper part of the bamboo shoot displayed yellowing and shrinkage, along with signs of growth stagnation. Subsequently, the bamboo sheath began to shrink and eventually fell off.

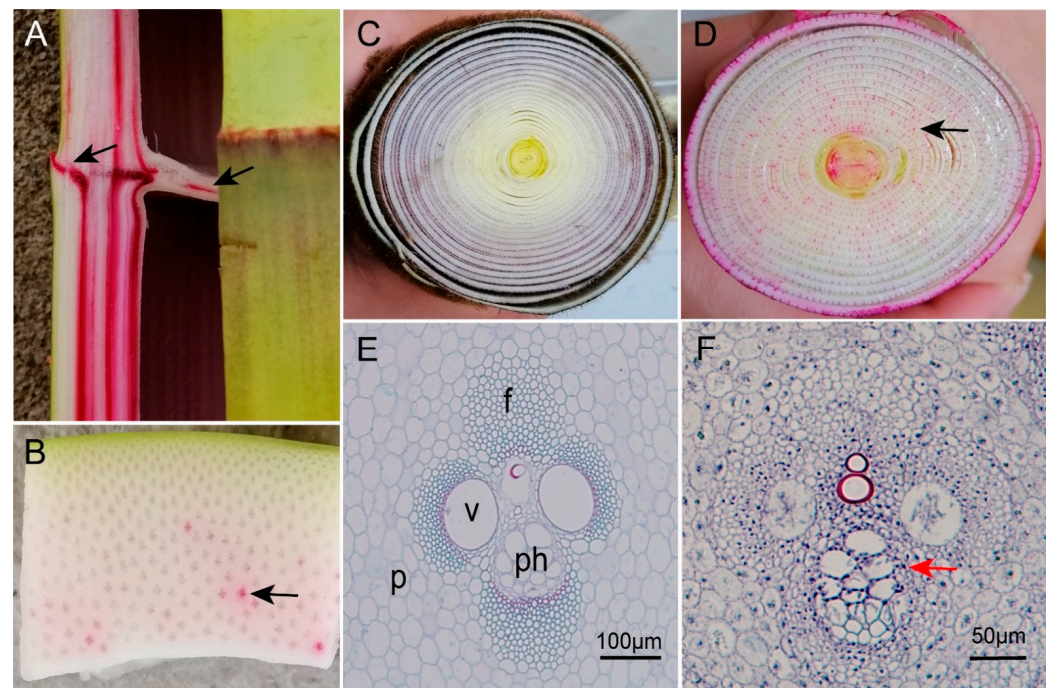


Figure 4. (A) Bamboo shoot stained with magenta; (B) Cross-cut section view of the culm in panel A. (C) Unstained bamboo sheaths. (D) Bamboo sheaths stained with magenta. (E) The vascular bundles at the base of a bamboo shoot; (F) The vascular bundles at the upper of a bamboo shoot; the black arrow indicates vascular bundles stained with magenta; the red arrow indicates nucleus. v, vessel element; ph, phloem; P, parenchyma; f, fiber cells.

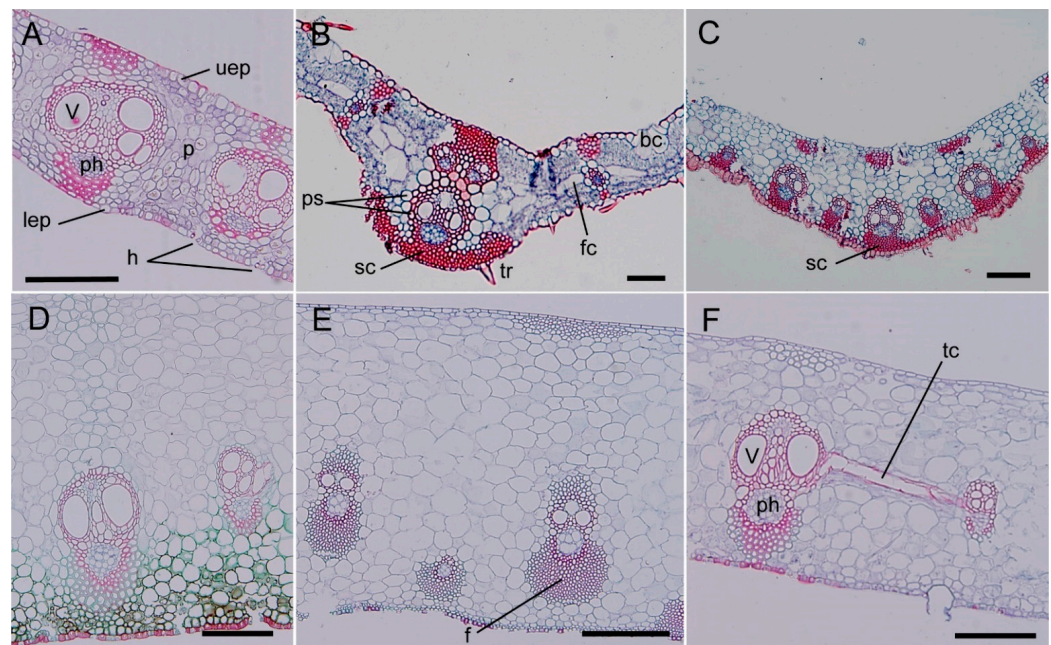


Figure 5. Transverse section of the culm sheath and bamboo leaf. (A) The sheath blade of moso bamboo. (B) The leaf of moso bamboo. (C) The leaf sheath of moso bamboo. (D,E) Base and upper part of culm sheath proper. (F) Cross-section of culm sheath proper. v, vessel element; ph, phloem; h, hydathode; p, parenchyma; bc, bulliform cells; fc, fusoid cells; sc, sclerenchyma cells; tr, trichomes; ps, parenchymatous sheath cells; uep, upper epidermis; lep, lower epidermis; f, fiber cells; tc transverse catheters. Scale bar = 100 μm.

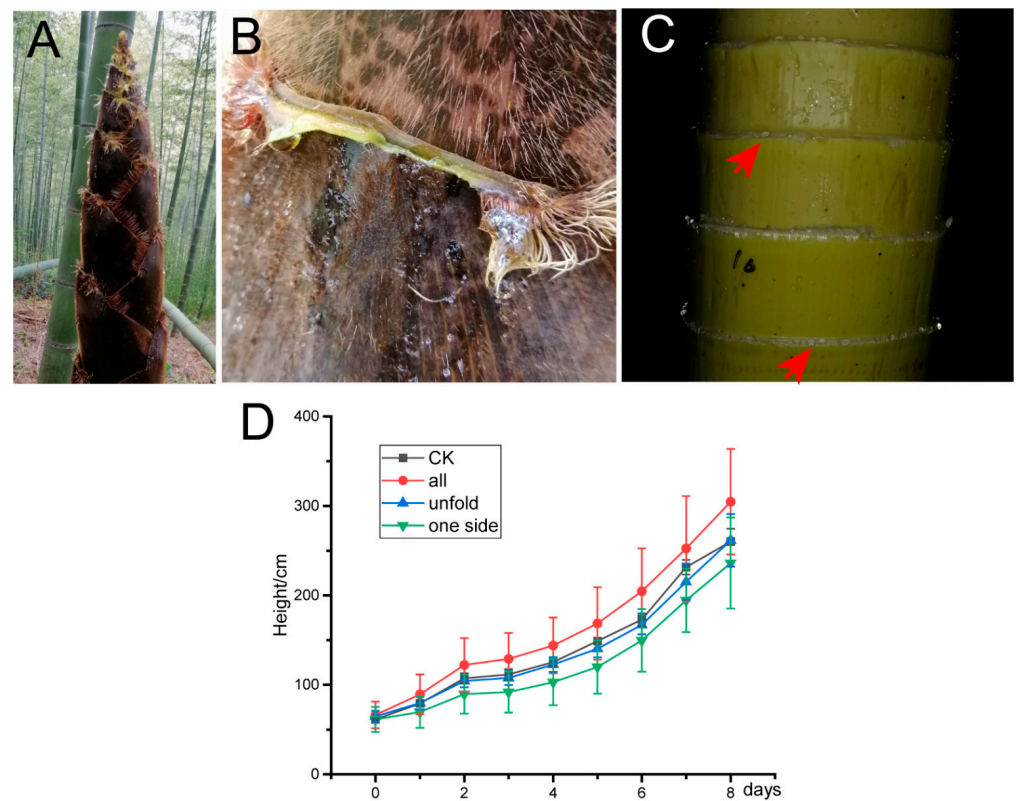


Figure 6. (A) All sheath blades removed from the bamboo shoot. (B) Guttation after removal of the sheath blades; (C) guttation after removal of the bamboo sheaths; the red arrow points to where the guttation liquid is emerging. (D) Changes in the height of bamboo shoots over eight days after culling the sheath. $n = 3$. The data were the mean height of the three plants. “CK” represents unprocessed bamboo shoots, “all” represents shoots with all culm sheaths removed, “unfold” represents only culm sheaths expanded, and “one side” represents shoots with only one culm sheaths removed.

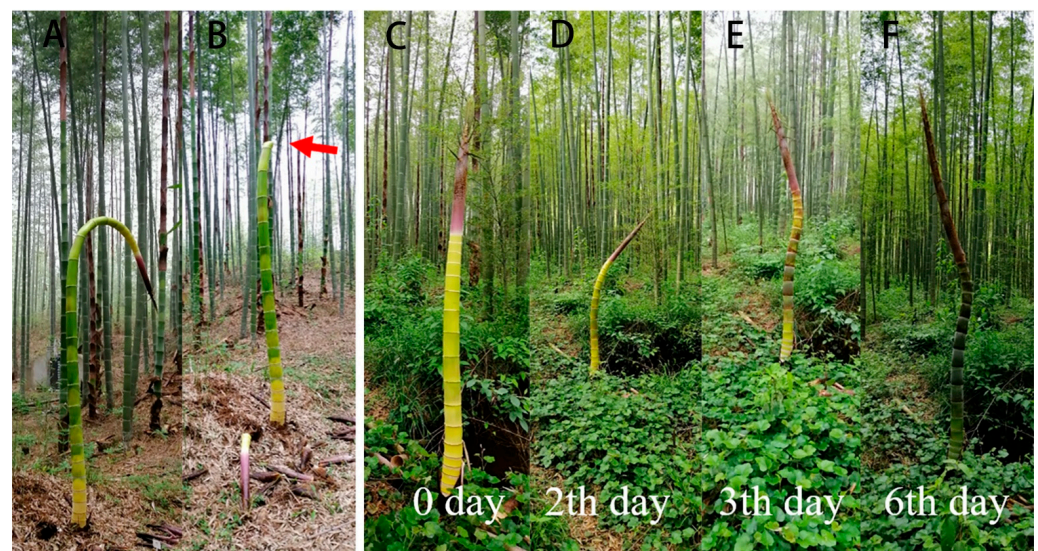


Figure 7. Stripped culm sheaths of bamboo shoots. (A,B) The upper part of the bamboo shoot after stripping was bent and broken. (C–F) Ensuing structural changes of a bamboo shoot over a 1-week period after stripping the sheath. The red arrow shows where the bamboo shoot has broken off.

4. Discussion

4.1. Guttation Phenomenon of Sheath Blades of Bamboo Shoots

Guttation is a common phenomenon in many plants when conditions favor absorbing water through their roots but limiting transpiration from their leaves. The phenomenon has been reported not only for crop plants, such as barley, wheat, rice, and corn but also for other plant species [1,5,16,17]. Research on water exudation phenomena includes the structure of hydathode, the composition of guttation, the factors influencing the formation of guttation, and the application of guttation [3,6–8,16,18]. A typical hydathode is composed of terminal tracheids, epithem cells, and a stomata-like pore and is responsible for exuding guttation fluid [6]. According to the structure, location, and manner of function of these hydathodes, they can be divided into two types: epidermal and epithemal. Most hydathodes are termed epidermal or active hydathodes and versus epithemal or passive hydathodes [6]. Epidermal hydathodes are simple and similar to the stomatal, lacking a connection with the water-conducting system known as the water pore active drainer. By contrast, epithemal hydathodes are a more complex type of passive drainer, consisting of a water pore and a group of parenchyma cells that are related to the water-conducting tissue and communicate directly with the conduit at the end of the water-conducting system of the plant [6]. We observed many vascular bundles in the culm sheath, especially in the sheath blade, where there were more xylem ducts apparent in a vascular bundle and their cross-sectional area exceeded that of the phloem (Figure S1). This suggests that sheaths may have a efficient water transport capacity.

The bamboo's sheath blade was the primary site of guttation and the main organ of exudation; hydathodes also were observed on its surface (Figure 5A). As the main transpiration organ of the bamboo shoot, many stomata are expected to be distributed on the surface of the bamboo sheath as well as the sheath blade [19]. The effect of plant structural damage as imposed here by snapping off the sheath blade or removing the culm sheath showed that following sheath removal, a large amount of liquid emerged from the wound site; this result suggests the main reason for guttation lies with the inherent physiology of the plant. This same phenomenon has been observed in *Fargesia yunnanensis* [19]. The damage permits the rapid leakage of much tissue fluid that disrupts the hydraulic balance in the injured plant. This may be why bamboo's growth was inhibited after stripping its sheath; this process evidently did not prevent water exudation from happening. When the hydrostatic pressure in the water guide system reaches a certain level, water can be promoted to exude from the water holes on the leaf surface to form exudate [7].

Guttation can reflect the dynamics of water metabolism in bamboo shoots and local changes in soil moisture. Vigorous exudation is the expression of robust plant growth and normal water metabolism in the plant body, whereas non-exudation implies an imbalance or stagnation of water in the body. The intensity of exudation is a good physiological indicator of water availability to a plant, which is convenient for observation and does not damage its tissues [2]. In the bamboo forest we studied, degraded shoots accounted for a large proportion of the stands, which seriously restricted their yield and quality of bamboo shoots. Whether the degraded shoot can be found as early as possible and dug out to improve the yield of the bamboo forest is the current focus of both bamboo farmers and researchers. The guttation condition of sheath blades can be used as an index to judge whether bamboo shoots are degraded.

4.2. Various Components of the Guttation Fluid from Bamboo Sheath Blades

Some studies have shown that compared with the xylem liquid phase, the guttation fluid contains fewer amino acids [3], indicating that spouted water is not simply transported directly from the catheter to the hydathodes. Substances are reabsorbed and reused as they are transported through the xylem, and only a small amount is excreted through the hydathodes, which contain substances harmful to the plant [16,20]. However, little is known about the dynamics of substance reabsorption, transport proteins, and energy

supply in the guttation of plants. As a healthy and delicious food, the composition of bamboo shoots at different developmental stages has been studied extensively [21–23]. Yet, surprisingly, the determination of bamboo's exudate composition has yet to be reported. Here, based on LC-MS analysis of the bamboo exudate, we found that although the liquid appeared clear, it contained a variety of organic acids, sugars, and hormones. Sugar has a central regulatory function in the coordination of plant growth. Sugars not only fuel cellular carbon and energy metabolism but also play a key role as signaling molecules. Sucrose was transported over long distances in the xylem, produced a series of sugar signaling molecules through its own metabolism, such as sucrose itself, glucose, and trehalose-6 phosphate [24]. The hormone abscisic acid (ABA), which is the most important phytohormone involved in plant growth, development, and adaptation to various stress conditions [25–27]. A variety of chemical components in guttation liquid indicated that guttation is a physiological performance of plants to balance water and transport substances in vivo. Recent studies have shown that the guttation droplets not only serve as a source of water for insects but also that the carbohydrates and proteins in the guttation can serve as a food source for insects [28]. As an important plant trait, guttation plays a significant role in the interaction between polytrophic insects and plants. Plant exudation, especially crop exudation, is a potential route for insect exposure to pesticides, such as nicotinamide residue in exudate from corn seeds [2,17]. The complex components in the exudate may therefore have ecological significance for the growth of the plant itself, an intriguing aspect serving further study.

4.3. Water and Substance Transport Pathways in Bamboo Shoots

Long-distance transport of water and solutes through the xylem and along vascular bundles into cells is a long-recognized concept in plant physiology [18,29]. Moso bamboo is connected by an intricate underground rhizome system. Bamboo's ability to integrate resources allows the bamboo culm to connect belowground in a small forest and redistribute water among bamboo through the rhizome system [30,31]. Bamboo shoots obtain their water from the mature bamboo, via the rhizome root and base root. Studies have shown that after severing these rhizomes, the water utilization ratio between adjacent stems is decreased by 20% [32]. One study on the diurnal variation of sap flux density in culms and subsurface flow of bamboo shoots demonstrated the stem flow of leafless bamboo shoots was dominant at night. After leaf development, the sap flux density is mainly at day, which is similar to the mature stem [30]. The freshly sprouted culms had high sap flux during the night and low sap flux during the daytime [30]. This result is consistent with the observed water exudation and the changed considerable leaf water content of bamboo mature stalks lose considerable water via transpiration during the day, but the transpiration rate at night is greatly diminished. Roots will continue to absorb water to generate high root pressure, and as bamboo mature, the need for water is greatly reduced. Compared with mature bamboo, new bamboo shoots still have higher water requirements.

The scattered distribution of vascular bundles is a common characteristic of Gramineae plants (grass). Compared with the vascular bundles of the C3 plant rice and C4 plant maize, the distribution of vascular bundles of bamboo stems more closely resembles that of maize [33–35]. The vascular bundle is mainly divided into phloem and xylem structural components; the former includes a sieve tube and companion cells, which are mainly responsible for the transport of assimilates, and the latter functions mainly as a conduit, responsible for moving moisture and inorganic salts. Vascular bundles also play an important role in the long-distance transportation and mechanical support of crops [36]. From our observations, the development of vascular bundles in the different internodes of bamboo shoots was variable (Figure 4D–F). Two large vessels in the single vascular bundle are responsible for water transport within the culm [13]. The results of the magenta staining also revealed that water could be transported up the xylem vessels, as well as from the inside to outside of the xylem vessels at the nodes. Staining was also observed in the ducts of the bamboo nodal diaphragm, suggesting that these interconnecting ducts are the basis

of an efficient water transport system in bamboo. Parenchyma cells within and between the vascular bundles in the bamboo nodes featuring rapid elongation play a role in glucose metabolism and transport of substances and water [37]. Water is transferred from the node to the connected bamboo sheath along the vascular bundle and then from the bamboo sheath to the sheath blade. Under the upward push of root pressure, this liquid is exuded from the water pore on the surface of the sheath blade.

Aquaporins (AQPs) are known as water-channel proteins, and they are associated with energy-dependent cells and intercellular and long-distance water transport (and of other small solutes) [38]. Accordingly, AQPs are crucial for the maintenance of plant water physiological functions, which include many aspects of plant development. Plant leaves can rapidly change their hydraulic conductivity by the regulation of plasma membrane AQPs [39]. Furthermore, light intensity can affect the activity of an aquaporin [40], and the expression patterns of several AQP genes in stomatal complexes have been well documented [41]. Further, there is evidence of their involvement in conferring immunity to pathogen infections [42]. Therefore, given our results, we think the in-depth study of the distribution and physiological activity of aquaporin at each cell layer along the vascular bundle is necessary. During the growth process of bamboo, especially bamboo shoots, many phenomena related to water dynamics at the physiological level have yet to be fully understood. Since the underlying mechanism of water transport from the mature stem to the bamboo shoots as well as its relationship with carbohydrate transport remains unclear, further investigation is warranted. Future research should pay more attention to the molecular mechanisms of guttation, water transport, and aquaporin regulation.

4.4. Bamboo Sheath Affects Internode Growth

Studies have shown that culm sheath plays an important role in controlling water and assimilative transport [43]. We also observed in the bamboo forest that after culm sheaths were removed, the internodes of bamboo shoots could not develop normally, internodes became short and brown, and the younger the internodes were affected more (Figure 7). In addition to the apical meristem [44], there was an intermediate meristem in each segment of bamboo. The division and elongation of a large number of cells in a short period is the main reason for the rapid growth of bamboo [10]. The anatomic study showed that the developing internodes were divided into meristem, elongate, and mature regions. The meristem and elongate regions were near the base of internodes [45]. The rapid expansion of basal internode meristem cells in bamboo shoots requires intracellular turgor pressure to maintain growth [46]. Culm sheath growing at the base of internodes provides strongly mechanical support for tender internodes. Bamboo shoots have vigorous cell division, strong metabolic activity, and generate a lot of heat [10]. Sheaths wrapped in layers can reduce heat loss and maintain a high temperature, especially at night with low temperature, which may be the reason why bamboo shoots can maintain a fast growth rate at night.

5. Conclusions

The sheath blade was the main organ of guttation in bamboo shoots, whose guttation droplets contain organic acids, sugars, hormones, and other compounds. The abundant conduit in the culm sheath was connected with that for the node of the bamboo shoot, which channels water and carries other substances to the culm sheath. The guttation process was jointly influenced by the local environment and physiological conditions of the bamboo plants. Removing their culm sheath damaged the internal homeostasis of water and material transport, thereby impairing the normal growth of bamboo shoots.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13010031/s1>, Figure S1. The average values of xylem ducts and phloem area, an asterisk indicates significant difference between data, $n = 16$, $p < 0.05$. Figure S2. Culm sheath and bamboo leaf anatomical sampling sites. (A) Culm sheath; (B) Bamboo leaves; white arrows indicate sampling sites.

Author Contributions: H.Z. and J.G. designed the experiments; H.Z. performed the field observation and recording; M.C., Y.B. and J.X. performed the culm sheath treatment experiments; Y.X. and H.S. assisted in equipment maintenance and sample collection; H.Z. analyze data and write papers; J.L. provided experimental instruments; J.G. review and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (Grant No. 2018YFD0600100) and the Fundamental Research Funds of ICBR (Grant No. 1632020005).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Raleigh, G.J. The effect of various ions on guttation of the tomato. *Plant Physiol.* **1946**, *21*, 194–200. [\[CrossRef\]](#)
2. Schenke, D.; Wirtz, I.P.; Lorenz, S.; Pistorius, J.; Heimbach, U. Two-year field data on neonicotinoid concentrations in guttation drops of seed treated maize (*Zea mays*). *Data Brief.* **2018**, *21*, 299–306. [\[CrossRef\]](#)
3. Bailey, K.J.; Leegood, R.C. Nitrogen recycling from the xylem in rice leaves: Dependence upon metabolism and associated changes in xylem hydraulics. *J. Exp. Bot.* **2016**, *67*, 2901–2911. [\[CrossRef\]](#)
4. Kovalskaya, N.; Owens, R.; Baker, C.J.; Deahl, K.; Hammond, R.W. Application of a modified EDTA-mediated exudation technique and guttation fluid analysis for Potato spindle tuber viroid RNA detection in tomato plants (*Solanum lycopersicum*). *J. Virol. Methods* **2014**, *198*, 75–81. [\[CrossRef\]](#)
5. Goatley, J.L.; Lewis, R.W. Composition of guttation fluid from rye, wheat, and barley seedlings. *Plant Physiol.* **1966**, *41*, 373–375. [\[CrossRef\]](#)
6. Singh, S. Guttation: Path, principles and functions. *Aust. J. Bot.* **2013**, *61*, 497–515. [\[CrossRef\]](#)
7. Singh, S. Guttation: Mechanism, momentum and modulation. *Bot. Rev.* **2016**, *82*, 149–182. [\[CrossRef\]](#)
8. Komarnytsky, S.; Borisjuk, N.V.; Borisjuk, L.G.; Alam, M.Z.; Raskin, I. Production of recombinant proteins in tobacco guttation fluid. *Plant Physiol.* **2000**, *124*, 927–934. [\[CrossRef\]](#)
9. Singh, S.; Singh, T.N.; Chauhan, J.S. Guttation in rice: Occurrence, regulation, and significance in varietal improvement. *J. Crop Improv.* **2009**, *23*, 351–365. [\[CrossRef\]](#)
10. Li, L.; Cheng, Z.; Ma, Y.; Bai, Q.; Li, X.; Cao, Z.; Wu, Z.; Gao, J. The association of hormone signalling genes, transcription and changes in shoot anatomy during moso bamboo growth. *Plant Biotechnol. J.* **2017**, *16*, 72–85. [\[CrossRef\]](#)
11. Li, L.; Mu, S.; Cheng, Z.; Cheng, Y.; Zhang, Y.; Miao, Y.; Hou, C.; Li, X.; Gao, J. Characterization and expression analysis of the WRKY gene family in moso bamboo. *Sci. Rep.* **2017**, *7*, 6675. [\[CrossRef\]](#)
12. Peng, Z.; Zhang, C.; Zhang, Y.; Hu, T.; Mu, S.; Li, X.; Gao, J. Transcriptome sequencing and analysis of the fast growing shoots of moso bamboo (*Phyllostachys edulis*). *PLoS ONE* **2013**, *8*, e78944. [\[CrossRef\]](#)
13. Liese, W.; Tang, T.K.H. Properties of the bamboo culm. In *Bamboo*; Springer: Cham, Switzerland, 2015; pp. 227–256.
14. Plumb, R.S.; Granger, J.H.; Stumpf, C.L.; Johnson, K.A.; Smith, B.W.; Gaulitz, S.; Wilson, I.D.; Castro-Perez, J. A rapid screening approach to metabonomics using UPLC and oa-TOF mass spectrometry: Application to age, gender and diurnal variation in normal/Zucker obese rats and black, white and nude mice. *Analyst* **2005**, *130*, 844–849. [\[CrossRef\]](#)
15. Xie, Z.; Bondada, B.; Li, B.; Ding, J. Visualization of axial water transport pathways in grapevines using dye-tracing technique. *Plant Physiol. J.* **2016**, *52*, 1159–1168. [\[CrossRef\]](#)
16. Nagai, M.; Ohnishi, M.; Uehara, T.; Yamagami, M.; Miura, E.; Kamakura, M.; Kitamura, A.; Sakaguchi, S.; Sakamoto, W.; Shimmen, T.; et al. Ion gradients in xylem exudate and guttation fluid related to tissue ion levels along primary leaves of barley. *Plant Cell Environ.* **2013**, *36*, 1826–1837. [\[CrossRef\]](#)
17. Mortl, M.; Takacs, E.; Klatyik, S.; Szekacs, A. Appearance of thiacloprid in the guttation liquid of coated maize seeds. *Int. J. Environ. Res. Public Health* **2020**, *17*, 3290. [\[CrossRef\]](#)
18. Hoffmann, E.J.; Castle, S.J. Imidacloprid in melon guttation fluid: A potential mode of exposure for pest and beneficial organisms. *J. Econ. Entomol.* **2012**, *105*, 67–71. [\[CrossRef\]](#)
19. Wang, S.; He, W.; Zhan, H. Culm sheaths affect height growth of bamboo shoots in *Fargesia yunnanensis*. *Braz. J. Bot.* **2018**, *41*, 255–266. [\[CrossRef\]](#)
20. Shapira, O.R.; Israeli, Y.; Shani, U.; Schwartz, A. Salt stress aggravates boron toxicity symptoms in banana leaves by impairing guttation. *Plant Cell Environ.* **2013**, *36*, 275–287. [\[CrossRef\]](#)
21. Li, D.; Sun, D.; Sun, W.; Yu, C. Nutrient components in fresh bamboo shoots of eight *Phyllostachys* species. *J. Bamboo Res.* **2018**, *37*, 14–19. [\[CrossRef\]](#)
22. Zhou, W.; He, Q.; Ye, C.; Xu, R.; Tong, X.; Wang, B.; Hua, X.; Zhou, L.; Lu, J. Comparative analysis of nutrients in bamboo shoot at different seasons. *J. Zhejiang For. Sci. Technol.* **2013**, *33*, 64–67.
23. Huang, X.; Li, J.; Zhang, W.; Peng, S.; Zhu, Y.; Lin, L. Nutritional components and active ingredient analysis of different parts of winter moso bamboo shoots. *Food Sci. Technol.* **2014**, *39*, 59–63. [\[CrossRef\]](#)

24. Dahiya, A.; Saini, R.; Saini, H.S.; Devi, A. Sucrose metabolism: Controls the sugar sensing and generation of signalling molecules in plants. *J. Pharmacogn. Phytochem.* **2017**, *6*, 1563–1572.
25. Calvo-Polanco, M.; Armada, E.; Zamarreno, A.M.; Garcia-Mina, J.M.; Aroca, R. Local root ABA/cytokinin status and aquaporins regulate poplar responses to mild drought stress independently of the ectomycorrhizal fungus *Laccaria bicolor*. *J. Exp. Bot.* **2019**, *70*, 6437–6446. [[CrossRef](#)]
26. Yunta, C.; Martinez-Ripoll, M.; Zhu, J.K.; Albert, A. The structure of *Arabidopsis thaliana* OST1 provides insights into the kinase regulation mechanism in response to osmotic stress. *J. Mol. Biol.* **2011**, *414*, 135–144. [[CrossRef](#)]
27. Gamuyao, R.; Nagai, K.; Ayano, M.; Mori, Y.; Minami, A.; Kojima, M.; Suzuki, T.; Sakakibara, H.; Higashiyama, T.; Ashikari, M.; et al. Hormone distribution and transcriptome profiles in bamboo shoots provide insights on bamboo stem emergence and growth. *Plant Cell Physiol.* **2017**, *58*, 702–716. [[CrossRef](#)] [[PubMed](#)]
28. Urbaneja-Bernat, P.; Tena, A.; Gonzalez-Cabrera, J.; Rodriguez-Saona, C. Plant guttation provides nutrient-rich food for insects. *Proc. Biol. Sci.* **2020**, *287*, 20201080. [[CrossRef](#)]
29. Pospíšilová, J.; Zimmermann, M.H. Xylem structure and the ascent of sap. *Struct. Chem.* **2012**, *23*, 1831–1836.
30. Fang, D.; Mei, T.; Roll, A.; Holscher, D. Water transfer between bamboo culms in the period of sprouting. *Front. Plant Sci.* **2019**, *10*, 786. [[CrossRef](#)]
31. Mei, T.; Fang, D.; Röhl, A.; Holscher, D. Bamboo water transport assessed with deuterium tracing. *Forests* **2019**, *10*, 623. [[CrossRef](#)]
32. Zhao, X.H.; Zhao, P.; Zhang, Z.Z.; Zhu, L.W.; Niu, J.F.; Ni, G.Y.; Hu, Y.T.; Lei, O.Y. Sap flow-based transpiration in *Phyllostachys pubescens*: Applicability of the TDP methodology, age effect and rhizome role. *Trees* **2016**, *31*, 765–779. [[CrossRef](#)]
33. Suzuki, S.; Burnell, J.N. The pck1 promoter from *Urochloa panicoides* (a C4 plant) directs expression differently in rice (a C3 plant) and maize (a C4 plant). *Plant Sci.* **2003**, *165*, 603–611. [[CrossRef](#)]
34. Zhang, Y.; Wang, J.; Du, J.; Zhao, Y.; Lu, X.; Wen, W.; Gu, S.; Fan, J.; Wang, C.; Wu, S.; et al. Dissecting the phenotypic components and genetic architecture of maize stem vascular bundles using high-throughput phenotypic analysis. *Plant Biotechnol. J.* **2020**, *19*, 35–50. [[CrossRef](#)] [[PubMed](#)]
35. Fei, C.; Geng, X.; Xu, Z.; Xu, Q. Multiple areas investigation reveals the genes related to vascular bundles in rice. *Rice* **2019**, *12*, 17. [[CrossRef](#)]
36. Zhang, Y.; Ma, L.; Pan, X.; Wang, J.; Guo, X.; Du, J. Micron-scale phenotyping techniques of maize vascular bundles based on X-ray microcomputed tomography. *J. Vis. Exp.* **2018**, *140*, e58501. [[CrossRef](#)]
37. Deng, L.; Li, P.; Chu, C.; Ding, Y.; Wang, S. Symplasmic phloem unloading and post-phloem transport during bamboo internode elongation. *Tree Physiol.* **2020**, *40*, 391–412. [[CrossRef](#)] [[PubMed](#)]
38. Canessa Fortuna, A.; Zerbetto De Palma, G.; Aliperti Car, L.; Armentia, L.; Vitali, V.; Zeida, A.; Estrin, D.A.; Alleva, K. Gating in plant plasma membrane aquaporins: The involvement of leucine in the formation of a pore constriction in the closed state. *FEBS J.* **2019**, *286*, 3473–3487. [[CrossRef](#)]
39. Cochard, H.; Venisse, J.S.; Barigah, T.S.; Brunel, N.; Herbette, S.; Guillot, A.; Tyree, M.T.; Sakr, S. Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiol.* **2007**, *143*, 122–133. [[CrossRef](#)] [[PubMed](#)]
40. Kim, Y.X.; Steudle, E. Gating of aquaporins by light and reactive oxygen species in leaf parenchyma cells of the midrib of *Zea mays*. *J. Exp. Bot.* **2009**, *60*, 547–556. [[CrossRef](#)]
41. Chen, Z.H.; Chen, G.; Dai, F.; Wang, Y.; Hills, A.; Ruan, Y.L.; Zhang, G.; Franks, P.J.; Nevo, E.; Blatt, M.R. Molecular evolution of grass stomata. *Trends Plant Sci.* **2017**, *22*, 124–139. [[CrossRef](#)]
42. Zhang, L.; Chen, L.; Dong, H. Plant aquaporins in infection by and immunity against pathogens—A critical review. *Front. Plant Sci.* **2019**, *10*, 632. [[CrossRef](#)]
43. Wang, S.; Zhan, H.; Li, P.; Chu, C.; Li, J.; Wang, C. Physiological mechanism of internode bending growth after the excision of shoot sheath in *Fargesia yunnanensis* and its implications for understanding the rapid growth of bamboos. *Front. Plant Sci.* **2020**, *11*, 418. [[CrossRef](#)]
44. Wang, Y.; Sun, X.; Ding, Y.; Fei, Z.; Jiao, C.; Fan, M.; Yao, B.; Xin, P.; Chu, J.; Wei, Q. Cellular and molecular characterization of a thick-walled variant reveal a pivotal role of shoot apical meristem in transverse development of bamboo culm. *J. Exp. Bot.* **2019**, *70*, 3911–3926. [[CrossRef](#)]
45. Wei, Q.; Guo, L.; Jiao, C.; Fei, Z.; Chen, M.; Cao, J.; Ding, Y.; Yuan, Q. Characterization of the developmental dynamics of the elongation of a bamboo internode during the fast growth stage. *Tree Physiol.* **2019**, *39*, 1201–1214. [[CrossRef](#)]
46. Cao, K.F.; Yang, S.J.; Zhang, Y.J.; Brodribb, T.J. The maximum height of grasses is determined by roots. *Ecol. Lett.* **2012**, *15*, 666–672. [[CrossRef](#)]