Rapid growth of Moso bamboo (Phyllostachys edulis): Cellular roadmaps, transcriptome dynamics, and environmental factors

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Abstract

Moso bamboo (Phyllostachys edulis) shows remarkably rapid growth (114.5 cm/day), but the underlying biological mechanisms remain unclear. After examining more than 12,750 internodes from more than 510 culms from 17 Moso populations, we identified internode 18 as a representative internode for rapid growth. This internode includes a 2-cm cell division zone (DZ), a cell elongation zone up to 12 cm, and a secondary cell wall (SCW) thickening zone. These zones elongated 11.8 cm, produced approximately 570,000,000 cells, and deposited ~28 mg g⁻¹ dry weight (DW) lignin and ~44 mg g⁻¹ DW cellulose daily, far exceeding vegetative growth observed in other plants. We used anatomical, mathematical, physiological, and genomic data to characterize development and transcriptional networks during rapid growth in internode 18. Our results suggest that (1) gibberellin may directly trigger the rapid growth of Moso shoots, (2) decreased cytokinin and increased auxin accumulation may trigger cell DZ elongation, and (3) abscisic acid and mechanical pressure may stimulate rapid SCW thickening via MYB83L. We conclude that internode length involves a possible tradeoff mediated by mechanical pressure caused by rapid growth, possibly influenced by environmental temperature and regulated by genes related to cell division and elongation. Our results provide insight into the rapid growth of Moso bamboo.
Introduction

Bamboo forests, comprising approximately 1,500 species, cover ~31.5 million hectares in tropical and subtropical regions worldwide (Guo et al., 2019b). They contribute to mitigating climate change by sequestering large amounts of carbon, which is a major contributor to global warming (Sohel et al., 2015). Bamboo is critical for environmental conservation and can be used to restore degraded lands, which has the potential to meet recently adopted global restoration targets, such as those in the New York Declaration on Forests (Paudyal et al., 2019). Bamboo is a sustainable nontimber forest product with myriad documented uses (INBAR, 2019; Paudyal et al., 2019). It is also the world’s most traded nontimber product, supporting a rapidly growing global industry with an estimated value of approximately $100 billion in 2025 (www.grandviewresearch.com/industry-analysis/bamboos-market).

Moso bamboo (Phyllostachys edulis) is a large, woody bamboo species widely distributed in East and Southeast Asia, covering ~4.43 million hectares in China (Song et al., 2020) and is an important raw material for the bamboo industry (Ramakrishnan et al., 2020). Moso bamboo is one of the fastest growing bamboo species on earth; during its rapid growth phase, culms can grow >20-m tall within 45–60 days (Peng et al., 2010). This rapid growth phase usually coincides with the beginning of spring (Peng et al., 2010). The rapid growth of Moso bamboo has long been a fascinating topic. About 1,000 years ago, during the Song Dynasty in China, the poet Lei Zhang wrote “Enjoying Bamboo Shoot,” in which he described the rapid growth of Moso bamboo after a spring rain: “Lush spring rain, moistening the barren hills and boosting the growth of bamboo shoots.”

The rapid growth of Moso bamboo is also a fascinating subject for scientific research to understand the mechanism of growth acceleration. Recent omics studies suggest that shoot growth is controlled at multiple levels and that hormones such as auxin (indole-3-acetic acid [IAA]) and gibberellins (GA), as well as their possible downstream target genes (e.g., those related to cell wall biogenesis), are closely linked to shoot growth (Cui et al., 2012; Peng et al., 2013a; Gamuyao et al., 2017; Tao et al., 2020; Wang et al., 2021). However, several fundamental aspects of the rapid growth of Moso bamboo remain unclear. First, the developmental roadmaps of the woody culm of Moso bamboo during rapid growth and the underlying transcriptional networks need to be elucidated. Second, the secondary cell wall (SCW) thickening that causes Moso bamboo shoot to lignify rapidly during rapid growth has not been well studied, although there are now some reports in the literature (Yang et al., 2021). Third, the factors that determine the length of culminating internodes during the rapid growth phase are poorly understood. Since bamboo is a commercial commodity, internode length is critical for the appropriate use of bamboo wood (Wei et al., 2018). Our preliminary observations suggest that internode length varies among geographically diverse Moso populations. However, the influence of environmental conditions on internode length in Moso bamboo populations remains to be investigated.

To address these knowledge gaps, we investigated the molecular mechanisms underlying rapid growth in a representative internode of Moso bamboo. We used an integrative approach combining anatomical, mathematical, physiological, and genomic insights. Detailed cellular and transcriptional roadmaps were generated, and critical developmental transitions and key candidate genes regulating cell division, primary cell wall elongation, and SCW thickening were identified. We also investigated the regulatory effects of environmental factors and corresponding genes on the rapid growth of Moso bamboo shoots in 17 different Moso bamboo populations in China. Our results provide insight into the rapid growth of this bamboo species and show how environmental signals may influence plant development and lead to substantial alterations in plant morphology.

Results

Internode 18 is the longest among the internodes of 17 P. edulis populations

We numbered the internodes of each of the 30 mature culms of P. edulis grown in the bamboo garden of Nanjing Forestry University in Xishau Town, Jurong City, China. Starting at the base of the plant, the first rootless internode was designated internode 1, up to internode 52 at the top of the plant (Figure 1, A and B). Each of the 30 culms had an average of approximately 50 internodes. Morphological analysis revealed that internode 18 had the greatest length, volume, fresh weight (FW), and dry weight (DW) among the approximately 50 internodes examined from each of the 30 culms (approximately 1,500 total) (Figure 1C). Internode 18 also had the lowest coefficients of variation for these indices (Figure 1D).

To determine whether internode 18 had similar characteristics in other P. edulis populations in China, we also measured approximately 12,000 internodes (internodes 1–25) from more than 480 P. edulis culms (30 culms each in the population) from 16 different P. edulis populations from 16 other regions in China (Figure 1E; Supplemental Table S1; Supplemental Data Set 1). Internode 18 was consistently the longest internode in all regions and populations (Figure 1E).

Internode 18 is a representative internode for rapid growth in P. edulis

We also examined the growth of internode 18 in shoots of P. edulis during fast growth (Figure 2A). In general, more internodes began to grow as shoot height increased, with internodes 15–18 showing the highest vertical growth rate (~15 cm every 2 days; Figure 2B). When shoot height reached ~463 cm, the number of internodes growing and the maximum growth rate of internodes decreased sharply (Figure 2, B and C). The absolute vertical growth rate of internode 18 was greatest when the internode was 6–18 cm long, reaching a maximum of ~11.8 cm/day (Figure 2C). This growth period, when the bamboo shoot was
Figure 1  Morphological identification of *P. edulis* internodes. A, Young *P. edulis* culms maintained in the bamboo garden of Nanjing Forestry University (Xiashu, China). Each number on the culm from bottom to top represents an internode. The rootless internode at the bottom of the shoot with respect to the ground is defined as the first internode of the shoot. B, Schematic representation of a *P. edulis* shoot with numbered internodes. C, Average internode lengths, volumes, FWs, and DWs of 30 *P. edulis* culms. Magenta line segments indicate internodes with the highest values, and arrows indicate internode 18. D, Coefficients of variation for the indices shown in C. Arrows indicate internode 18. E, Lengths of 25 individual internodes from 17 *P. edulis* populations. Detailed information on these regions is provided in Supplemental Table S1. Each population included 30 randomly selected *P. edulis* culms (510 culms in total). Each grey dot represents an internode, and each magenta dot represents an 18th internode.
340–463 cm tall, also corresponded to the fastest growth of the entire bamboo shoot, with a maximum value of 114.5 cm/day (Figure 2C).

Comparing the relative growth rates of the different internodes (internodes 1–22), the maximum relative growth rate of internode 18 was 5.0%/h—the highest value among all internodes (Figure 2D). The growth curve of internode 18 did not have the typical monotonic sigmoid shape (Figure 2E), but was a biphasic sigmoid curve that could be described by the biphasic dose–response equation ($r^2 = 0.999$) (Motulsky, 2016).

The morphological data showed that internode 18 was the longest among the different internodes and grew faster and more uniformly than all other internodes. The fastest growth of internode 18 was also responsible for the fastest growth of the $P. edulis$ shoot. Because it could be considered a representative fast-growing internode of the $P. edulis$ populations, subsequent analyses focused on this internode.

Cell division and cell elongation during the growth of internode 18
To better understand the growth of internode 18 at the cellular level, we analyzed samples of the internode at different lengths/stages (1–29 cm). Serial sections were taken from each sample every 1 cm, with the first 1 cm section being the lowest. The sampling strategy for the 18th internode at different growth stages is shown in Supplemental Figure S1. To better describe the developmental stages of these samples, we also created a figure showing the total growth of the 18th internodes at different time points (Supplemental Figure S2).

We examined 3- and 6-cm internodes at the stage with a relatively lower and higher growth rate, respectively (Figure 2, C and E). In internodes of both lengths, mitotic figures could be observed in the parenchyma cells of the first, second, and third sections (Figure 3A). However, in the fourth, fifth, and sixth sections of the 6-cm-long internode, no mitotic figures were observed, and the parenchyma cells were obviously larger and more elongated (Figure 3A). Consistent with this, significantly more nuclei were identified in the parenchyma cells of the first two sections of both the 3- and 6-cm internodes than in the fourth section of the 6-cm internode ($P < 0.05$; Figure 3B), but the cells in the fourth and fifth sections of the 6-cm internode were significantly longer than the cells in the first, second, and third sections of both the 3- and 6-cm internodes ($P < 0.05$;
Furthermore, flow cytometry analysis detected cells of the gap 2 (G2) or mitotic (M) phase only in the first (3i-1), second (3i-2), and third (3i-3) segments of the 3-cm-long 18th internode and the first (6i-1), second (6i-2), third (6i-3), fourth (6i-4), fifth (6i-5), and sixth (6i-6) segments of the 6-cm-long 18th internode. These data suggest that bamboo internode growth is divided into sections within the internodes, with the areas of cell division and cell elongation located at the lower and upper ends of the internode, respectively.

We further investigated cell growth in the two developmental zones in internode 18 during rapid growth. Remarkably, cell number stopped increasing early in the 14-cm length internode, and cells in the uppermost segment of the elongation zone (EZ) of the 14-cm internode also stopped growing longer (Figure 4A). The most rapid cell division and cell elongation occurred in the growth between...
the 6-cm internode and the 17.3-cm internode (Figure 4A). Like the growth curve of the internode, the rates of cell division and cell elongation could be accurately described by bi-phasic sigmoid curves ($r^2 > 0.99$) that matched the growth curve of the internode 18 (internode length versus cell number, Pearson $r = 0.96$; internode length versus cell length, Pearson $r = 0.95$) (Figure 4A).

We also examined the cell division zone (DZ) of internode 18 by comparing cell morphologies during fast growth. There were significantly more nuclei in the cell DZ of the 1-, 3-, and 6-cm internodes than in the cell DZs of the 9-, 14-, and 21-cm internodes ($P < 0.05$; Figure 4B; Supplemental Figure S3). Cells in the cell DZs of the 1- and 3-cm internodes were also significantly shorter than cells in the cell division regions of the 6-, 9-, 14-, and 21-cm internodes ($P < 0.05$; Figure 4C; Supplemental Figure S3). In addition, although flow cytometry analysis detected cells of G2/M phase in the first 1-cm segment of the internode at the 9-cm stage (Figure 4D), no cells of G2/M phase were observed in the second 1-cm segment of the 9-cm internode or in the first 1-cm segment of the 14-cm internode (Figure 4D).

We examined the cell patterns in the regions of cell elongation of the 14-cm internode once cell division ceased. Cell length increased linearly from the lowest section to the 12th section, after which cell length stagnated (Figure 4C). In addition, exam-ination of 14-cm-long internodes by transmission electron microscopy (TEM) showed that the cell wall had fewer wrinkles from the lowermost section toward the top of the internode (Figure 4F). The mat-tery pit field could be observed in the first, third, and sixth sections, and was fully formed in the 12th section (Figure 4F). Possible SCW thickening was observed in the 10th and 12th sections (Figure 4F).

Cell elongation was most rapid when internode 18 grew from 6 cm in length to 14 cm (Figure 4A). However, unlike other monocotyledonous plants such as maize (Zea mays) and deepwater rice (Oryza sativa “Habiganj Aman II”), which usually have only one culm sheath covering their internodes (Métraux and Kendé, 1984), the young internode 18 of Moso bamboo was covered by many sheaths. Therefore, it is difficult to determine internode growth in real time. We then developed a method to determine the corresponding parts between 6- and 14-cm internodes based on the number of cells and cell lengths in each part to investigate the cell elongation potential of P. edulis (Figure 4G; Supplemental Figure S5). We found that both the absolute cell growth rate and the relative cell elongation rate first increased and then decreased along the EZ from the bottom to the top (Figure 4H). In our study, the fastest cell elongation occurred in a 0.2 cm segment between 4 and 4.2 cm of the 6 cm internode and a corresponding 0.5 cm segment between 10 and 10.5 cm of the 14 cm internode within ~0.7 days (Figure 4, G and H). During this growth, parenchyma cells ranging in size from 13.9 to 33.1 $\mu$m could grow up to 127.4 $\mu$m in length (Figure 4I).

Based on the above studies, we summarized the parameters of cell growth distributed among the cell division and EZs (Table 1). Accordingly, we found a maximum cell division rate of ~0.058 cells cell$^{-1}$ h$^{-1}$. Because of the large number of dividing cells, the internode DZ of P. edulis was able to produce ~67.33 cells/h in vertical increase and more than $2.4 \times 10^7$ cells/h in total (Table 1). The maximum relative cell elongation rate in the EZ was 0.17–0.49 $\mu$m $\mu$m$^{-1}$ h$^{-1}$, which allowed the cell to grow 5.6–6.8 $\mu$m in 1 h (Table 1).

Cell wall thickening during the growth of internode 18

Next, we examined the process of SCW thickening. After completion of cell elongation, cell wall thickening increased steadily (Figure 5A). TEM showed significant cell wall thickening in the middle lamella of the corner of the cell wall in the uppermost section of the 23-cm internode (stage where the rapid growth decreases) (Figure 5B). Significant cell wall thickening was also observed in the uppermost section of the mature internode (~29 cm long) 20 and 50 days after completion of rapid growth (Figure 5B). Consistent with this, lignin content in the uppermost sections of all internodes tested increased rapidly until 50 days after completion of rapid growth and then increased slowly (Figure 5C). Cellulose content also increased rapidly in the uppermost 1 cm sections of the 23- and 28-cm internodes, but reached a plateau after the rapid growth phase (Figure 5D). The maximum lignin and cellulose deposition rates were ~28.04 and ~44.05 mg g$^{-1}$ DW per day, respectively (Figure 5, C and D).

Model of internode 18 growth during the rapid growth phase

Based on our histological and physiological results, we proposed a model for the growth of internode 18. The growing internode includes a cell DZ of ~2 cm in length and a cell EZ with a maximum length of 12 cm. The remaining part of the internode is a mature zone for SCW thickening (Figure 6A). The size of the zones and the number of cells in the EZ, DZ, and maturation zone depend on the stage of internode development (Figure 6, B and C).

The functions that take place in each zone change as the internode grows. For example, in the lowest sections of the 1-, 3-, and 6-cm internodes, cell division is the primary growth mode, whereas in this section of the 14-cm internodes, cell elongation is the primary growth mode (Figure 6A). Once the internode reached ~29 cm, SCW thickening was also observed in the lowermost section (Figure 6A). Based on the portions of the internode that were capable of growth during rapid growth (Figure 6A), we determined the adjusted relative growth rates of internode 18 during the rapid growth phase (Figure 6D). The maximum growth rate of ~0.08 cm$^{-1}$ h$^{-1}$ was found when internode 18 grew from 6 cm in length to 17.3 cm in length (from growth day 15–16) (Figure 6D).
Parenchyma cell growth in the cell division and EZ of internode 18 during rapid growth. A, Changes in relative cell number and cell length during the rapid growth of internode 18 show biphasic sigmoid curves. Data are mean values. Cells in the uppermost 1-cm internode segment are used to plot cell length changes. When internode length was less than 1 cm, the entire internode was used to examine cell length. The cell numbers of each length internode were the sum of the cell numbers in each 1-cm section of each length internode. The cell number of each 1-cm section of a given length internode was calculated by dividing the average cell length in each 1-cm section of internodes by 1 cm. The numbers next to the dots indicate the absolute length of the internode. The table at the bottom of the figure shows the correlations between relative cell number and relative internode length, and relative cell length and relative internode length. LN on the Y-axis means Napierian logarithm.
Sequencing, assembly, and annotation of the transcriptome of internode 18 throughout its development

To explore the molecular basis of the rapid growth of internode 18, we selected 14 developmental stages (Supplemental Figure S6) representing critical transition points in cell division, cell elongation, and SCW thickening in internode 18 for transcriptome sequencing. To generate highly accurate transcriptome profiles, we performed both full-length transcriptome sequencing (Pacific Biosciences [PacBio] Iso-Seq) and strand-specific paired-end Illumina sequencing. We obtained ~50 Gbp of Iso-Seq reads (Supplemental Table S2) and ~674 Gbp of cleaned Illumina reads (Supplemental Table S3).

In total, 46,511 genes were identified that were expressed in at least one developmental stage (Supplemental Figure S6). A total of 43,963 of these genes had been previously annotated in the *P. edulis* genome and were associated with various metabolic and cellular processes (Supplemental Figure S7). Notably, ~81.3% and ~88.0% of all *P. edulis* genes associated with cell wall (Supplemental Figure S8A) and lignin biosynthesis, respectively, were expressed in at least one developmental stage (Supplemental Figure S8B). The remaining 2,548 genes had not been previously identified in the *P. edulis* genome (Peng et al., 2013b; Zhao et al., 2018) (Supplemental Figure S6; Suplemental Data Set 2). These genes were associated with various cellular processes and metabolic pathways, including cell wall assembly (Supplemental Figure S9). Notably, 112 of the genes belonged to 33 transcription factor families (Supplemental Figure S10). In addition, six of the genes were possibly related to cell wall lignification (Supplemental Figure S10): four genes encoded phenylalanine ammonia lyase (PAL), one encoded caffeic acid O-methyltransferase (COMT), and one encoded caffeoyl-CoA O-methyltransferase. These data characterize the transcriptome profiles of Moso bamboo during rapid growth and improve the annotation of the Moso genome.

Candidate genes associated with primary cell wall growth and SCW thickening in internode 18

We used weighted gene coexpression network analysis (WGCNA) (Langfelder and Horvath, 2008) to investigate candidate genes associated with cell wall growth during rapid growth of internode 18 (Figure 7A). Four coexpression modules closely related to cell wall growth were identified: (1) genes that were upregulated in internode sections with actively dividing cells; (2) genes in internode sections with actively elongating cells; (3) genes that were upregulated in internode sections where cell elongation was reduced and SCW thickening was increased; and (4) genes in internode sections where SCW thickening was slightly increased (Figure 7B).

Cytoscape (Shannon et al., 2003) and CytoHubba (Chin et al., 2014) were used to visualize the interaction networks of these four modules associated with cell wall growth. Several genes associated with cell wall biogenesis, cell division, and the cell cycle were identified in internode sections with active cell division (Figure 8A). Several genes of interest have been identified in internode sections characterized by rapid cell elongation, including four in GA pathways, GA 3-oxidase1, gibberellin insensitive dwarf1, GAST1 protein homolog2, and GASA14; 18 transcription factors, some of which may promote cell wall growth (e.g. HOMEBOX1) (Capella et al., 2015); 9 genes associated with cell wall growth; 3 genes associated with sugar metabolism (two for sucrose invertases and one for a starch-degrading enzyme); 11 genes encoding vacuolar transmembrane transporters; 2 genes for cell cytoskeleton organization; 5 genes encoding Rab GTPases, which may be involved in cell wall growth (Lyczett, 2008); and 2 aquaporin genes (Figure 8B). In internode sections characterized by a decrease in cell wall elongation associated with rapid SCW thickening, a number of genes related to SCW thickening were discovered, including COUMARATE 3-HYDROXYLASE (C3H), CAOMT, 4-COUMARATE-COA LIGASE1, HYDROXYCINNAMOYL-COA SHIKIMATE/QUINATE HYDROXYCINNAMOYL TRANSFERASE (HCT), PAL1, LACCASE (LAC), IRREGULAR XYLEM1 (IRX1),
IRX3, IRX6, IRX9, IRX14 LIKE (IRX14L), KORRIGAN1 (KOR1), and CELLULOSE SYNTHASE A4 (CESA4) (Figure 8C). A total of 26 transcription factor genes, including BLH6 (Liu et al., 2014), C3H14 (Kim et al., 2014), KNAT7 (Li et al., 2011), MYB4 (Shen et al., 2012), MYB83 (McCarthy et al., 2009), and MYB86 (Taylor-Teeples et al., 2015), which may contribute to the regulation of SCW thickening, were also detected in this module (Figure 8C). However, only nine genes

Table 1 Growth summary of the 18th internode of *P. edulis* during the fast-growth phase

| Kinematic parameter                                      | Value                                      |
|----------------------------------------------------------|--------------------------------------------|
| Absolute internode elongation rate (cm d⁻¹)              | Up to 11.8 (~0.5 cm h⁻¹)                   |
| Relative internode elongation rate (cm cm⁻¹ h⁻¹)         | Up to 0.08                                 |
| Length of DZ (cm)                                        | 2.0                                        |
| Length of EZ (cm)                                        | Up to 12.0                                 |
| Number of cells in the cell DZ                           | ~5.2 × 10⁵ cells; ~1,512 cells in the vertical dimension |
| Number of cells in the EZ                                | Up to 1.2 × 10⁹ cells; up to 3,419 cells in the vertical dimension |
| Maximum cell production (cells h⁻¹)                      | 2.4 × 10⁷, 67.33 in the vertical increment |
| Maximum cell division rate (cell cell⁻¹ h⁻¹)             | ~0.058                                     |
| Maximum absolute cell elongation rate (µm h⁻¹)           | 5.6–6.8                                    |
| Maximum relative cell elongation rates (µm µm⁻¹ h⁻¹)     | 0.17–0.49                                  |
| Maximum cellulose and lignin deposition rate in the mature zone (mg g⁻¹ DW/day) | Cellulose: ~44.0; Lignin: ~28.0 |

Figure 5 SCW thickening in the mature zone of internode 18 during and after rapid growth. A and B, Light microscopy (A) and TEM images (B) showing increasing cell wall thickening in the 14th (14i-14), 23rd (23i-23), and 29th (29i-29) segments (uppermost section) of 14-, 23-, and 29-cm-long 18th internodes. Scale bars in (A) and (B) = 100 and 1 µm, respectively. The line in B indicates presumed thickening in the middle lamella of the corner of the cell wall. C and D, Acid-insoluble lignin (C) and cellulose (D) contents in the uppermost 1-cm segments of 14-, 23-, 28-, and 29-cm-long 18th internodes during the rapid growth stage and up to 140 days after the rapid growth stage. The numbers ~5.3 d, ~4 d, ~2 d, 20 d, 50 d, 80 d, 110 d, and 140 d represent the days before or after internode 18 completes its rapid growth. Internode 18 begins to elongate when it reaches a length of ~0.13 cm (1 day) and completes its rapid growth at 29 cm (23 days). Data shown are means ± sd (n = 3).
Figure 6 Proposed spatiotemporal growth model for the 18th internode of *P. edulis* during or after the rapid growth stage. A, During the rapid growth phase, different regions of internode 18 perform different functions. Stacked cylinders show the lengths of internode 18 at different growth stages (1, 3, 6, 9, 14, 18, 21, and 29 cm). Each cylinder represents a 1-cm segment, and the segments are labeled in order from bottom to top with respect to the bottom. The remaining bottom segments are labeled to indicate the number of days after the rapid growth stage. White segments represent the cell DZ, yellow-green segments represent the cell EZ, gold segments represent the cell MZ with SCW thickening, and purple segments represent stable SCW thickening after rapid growth. A black spot indicates retrograde cell division, a red spot indicates the end of cell division, black triangles indicate retrograde cell elongation, and the red triangle indicates the end of cell elongation. B and C, Three zone sizes (B) and cell numbers (C) in the 18th internode with different lengths during rapid growth. D, Adjusted relative growth rates of internode 18 corresponding to internode parts with growth ability during rapid growth.
potentially playing a role in SCW thickening were identified in the internode at 20 and 110 days after the fast-growing phase (Figure 8D).

**Molecular regulation of functional transitions in internode 18**

We compared transcriptome profiles between the stages of cell division and cell elongation and between the stages of cell elongation and SCW thickening to identify key molecular transitions based on the 14 developmental stages of internode 18, as described above. Several differentially expressed genes (DEGs) were found at the different developmental stages. The transcriptome profiles were similar in the cell division regions of the 1-, 3-, and 9-cm-long 18th internode, respectively; 14i-1, 14i-2, 14i-3, 14i-6, 14i-10, and 14i-12 represent the first, second, third, sixth, tenth, and twelfth 1-cm segments of the 14-cm-long 18th internode, respectively; 23i-23 represents the 23rd segment of the 23-cm-long 18th internode; and 29i-29-20d and 29i-29-110d represent the 29th segment of the 29-cm-long internode collected on the 20th and 110th day after the rapid growth stage, respectively. B, Expression profiles of eigengenes in the four expression modules closely associated with cell wall growth of the 18th internode. Shown are modules of coexpressed genes associated with rapid cell division (turquoise), rapid cell elongation (green), rapid SCW thickening (yellow), and stable SCW thickening (brown). Numbers 1–12 in the modules correspond to 1-cm segments of internodes at different stages, as indicated in (A).

In contrast, genes related to DNA synthesis, cell division, and the cell cycle were downregulated (Figure 9A).

During the transition from cell elongation to SCW formation in internode 18, genes related to lignin synthesis, such as PALs and HCTs, were significantly upregulated in the 10th 1-cm section of the 14-cm internode (the site where the SCW thickening began, Figures 4, E and 9, C). MYB (myeloblastosis) transcription factor genes, including MYB83 and MYB46, were also strongly upregulated in this section (Figure 9, C and D). These genes may contribute to triggering SCW thickening (McCarthy et al., 2009). Genes of the abscisic acid (ABA) pathway, including ABA biosynthesis genes such as NINE-CIS-EPOXYCAROTENOID DIOXYGENASE3 and ALDEHYDE OXIDASE1, as well as signal transduction genes such as PROTEIN PHOSPHATASE 2CA, HIGHLY ABA-INDUCED PP2C GENE2 (HAII2), HAII3, and ABA-RESPONSIVE ELEMENT BINDING PROTEIN3, were also strongly upregulated in this section (Figure 9, C and E). Genes related to cell wall growth were downregulated in the uppermost
section of the 23-cm-long internode, which was in the decreasing growth stage (Figure 2C), and in the uppermost sections of the internodes, which completed rapid growth for 20 and 100 days, respectively (Figure 9F). However, genes related to transport and secondary metabolism, such as lignin synthesis, remained significantly upregulated in these sections (Figure 9F).

Hormonal regulation of functional transitions in internode 18
Transcriptome analysis revealed that GA, auxin, and ABA signaling pathways may play a role in regulating cell growth changes in internode 18. To further verify the transcriptome data and investigate phytohormone-related changes during growth, we quantified phytohormone levels in the different sections. Notably, high levels of GA₄ were detected in the EZ and increased significantly in the EZ of the internode with the highest growth potential (14-cm internode) (Figures 2, C and 10, A). Small amounts of GA₁ and GA₇ were also detected, whereas GA₃ was almost undetectable (Figure 10A). To better understand the role of GA in the rapid growth of the internode of *P. edulis*, we also detected its content in the internode DZ with different cell division intensities and recorded cytokinin to mark the cell division intensity. GA was higher in the internode DZ with a relatively higher cell division intensity, which correlated well with cytokinin content (CK) (Pearson r = 0.99) (Figure 10B; Supplemental Table S4).

Molecular analysis revealed that auxin signaling was enhanced in the DZ of the 9-cm internode (Figure 9, A and B). We then detected the auxin content in the cell DZ of the internodes at different growth stages. We also determined the CK to characterize the intensity of cell division. Of note, CK was significantly lower in the cell DZ of the

Figure 8 Major *P. edulis* candidate genes and their networks regulating the growth of the 18th internode. A–D, Networks of major candidate genes at different stages including active cell division (A), rapid cell elongation (B), rapid SCW thickening (C), and stable SCW thickening (D).
Figure 9 Key molecular events during cell division, cell elongation, and SCW thickening in internode 18. A, Pairwise comparisons of transcriptome profiles at the six developmental stages of the cell DZ. 1i-1, 3i-1, 6i-1, 9i-1, 14i-1, and 21i-1 represent the first segments of 1-, 3-, 6-, 9-, 14-, and 21-cm internodes, respectively. B, Relative expression levels of six auxin transport genes identified in the modules of the first segments of 6- (6i-1) and 9-cm-long (9i-1) internodes. Data are means ± SD (n = 6). *P < 0.05. Each point represents the measured data. C, Pairwise comparisons of gene expression profiles at the six developmental stages of cell elongation and lignification. 14i-1, 14i-2, 14i-3, 14i-6, 14i-10, and 14i-12 represent the first, second, third, sixth, tenth, and twelfth 1-cm segments of the 14-cm-long 18th internode, respectively. D, Relative expression levels of representative MYB transcription factors in 14i-6 and 14i-10. Shown are log2 changes, and gene abundances in 14i-6 were set as controls and normalized to 0 (log2). E, Expression of ABA synthesis and signaling pathway genes in 14i-6 and 14i-10. Data are means ± SD (n = 6). *P < 0.05. Each point represents the measured data. F, Pairwise comparisons of transcriptome profiles between the four developmental stages of SCW lignification. 23i-23 represents the 23rd segment of the 23-cm internode, and 20 d and 110 d represent the 29th segments of the 29-cm internodes collected at 20 and 110 days after the rapid growth stage, respectively. The rows in A, C, and F correspond to the categories, and the colors of the bands correspond to the average change in expression of all DEGs in that category.
14-cm internode than in the 6- and 9-cm internodes (Figure 10C; Supplemental Table S4), but IAA content was not significantly different between the cell DZ of the 6-, 9-, and 14-cm internodes (Figure 10C). However, compared with the DZ, IAA content in the EZ increased significantly in the 3- and 6-cm internodes, whereas it decreased significantly in the 14-cm internodes (Figure 10D). In contrast to GA, auxin content decreased significantly with increasing cell elongation rate (i.e. in the longer internodes; Figure 10D).

Indeed, the ABA content was significantly higher in the 10th 1-cm section of the 14-cm internode than in the first section (an increase of ~60%; Figure 10E), which correlated well with the transcriptome finding that ABA signaling was enhanced in the 10th 1-cm section of the 14-cm internode (Figure 9, C and E).
GA may stimulate rapid growth of *P. edulis* shoots

Genes of the GA pathway were identified as possible players in internode sections characterized by cell elongation (Figure 8B), and indeed high levels of GA were detected in internode EZ (Figure 10A) and even in cell DZ (Figure 10B). Concentrations of GA were also significantly elevated in the EZ and in the cell DZ of the internode with the highest cell elongation potential (14-cm internode) and cell division potential (6-cm internode) (Figure 10, A and B). We then investigated how GA promotes cell growth in the internodes of *P. edulis*.

In growth experiments, the growth increased dramatically in *P. edulis* seedlings treated with different GA$_4$ concentrations (Figure 11A). In fact, the average slopes of the growth curve for GA$_4$-treated seedlings ranged from 0.78 to 1.36, whereas they were 0.35 for the untreated seedlings (Figure 11B). Thus, GA$_4$ treatment increased seedling growth rate by ~2.23- to ~3.89-fold, which was positively dependent on GA$_4$ concentration. This type of growth promotion was similar to or even much higher than the increase in shoot growth rate between the normal growth phase and the rapid growth phase (a ~2.81-fold increase; Figure 28; Supplemental Figure S11).

Treatment with GA$_4$ promoted the growth rate of *P. edulis* seedlings and resulted in longer internode cells (Figure 11C). For example, the average cell length in the maturing internode increased by 53.5% after treatment with 50 μM GA$_4$ (Figure 11D). The maximum length of parenchyma cells in the internode treated with GA$_4$ reached as high as 172.5 μm, 2.1 times the longest parenchyma cell (~82 μm) in the untreated seedlings (Figure 11D). Because the increase in cell elongation (up to a 2.1-fold increase) was less than the growth promotion after GA$_4$ treatment (a ~3.38-fold increase), we assumed that the cell number of the *P. edulis* internode must also increase to achieve this growth promotion. As expected, the relative cell number in the internode of GA$_4$-treated seedlings increased by 37.7% compared with normally growing seedlings (Figure 11, E and F), suggesting that GA$_4$ is also important for promoting cell division.

Because auxin has been found to promote cell elongation in plants (Gray et al., 1998) and may promote the transition from cell division to cell elongation in the DZ of *P. edulis* internodes (Figure 9, A and B), we also investigated the role of auxin in *P. edulis* seedling growth. However, in the growth experiments, treatment with different concentrations of IAA had almost no effect on promoting growth of *P. edulis* seedlings (Figure 11, G and H). We also used different amounts of 2,4-dichlorophenoxyacetic acid (2,4-D), which has a similar function to IAA and is more stable than IAA, to treat *P. edulis* seedlings. Like auxin, treatment with different amounts of 2,4-D had almost no effect on promoting growth of *P. edulis* seedlings (Supplemental Figure S12).

Close relationship between mechanical pressure and SCW thickening of *P. edulis* shoots during rapid growth

Normally, the internodes of *P. edulis* are subjected to two types of mechanical pressure during rapid growth: One is weight pressure, and the other is “bending pressure,” by which we meant the tension and compression due to bending that occurs during rapid growth as result of lifting a tall culm (Thompson, 1917; Figure 12A). These two types of pressure are thought to cause the bamboo shoots to bend during rapid growth (Figure 12B), and result in some of the growing *P. edulis* shoots suddenly bending during the rapid growth phase (Supplemental Figure S13). This usually occurs after a sudden rapid growth of the shoot and usually causes growth to slow down for a few days (Supplemental Figure S13, A and B), which was thought to strengthen the kinked part, and then the shoot straightens and accelerates the growth again (Supplemental Figure S13, A and C). This phenomenon is thought to be related to the bending caused by a sudden rapid growth of the bamboo shoot, which cannot be overcome by the secondary thickening of the cell walls and the support of the culm sheath (Figure 12C; Supplemental Figure S13).

The culm sheath covers the internode and provides mechanical support to the internode during rapid growth to allow the shoot to withstand rapidly increasing bending pressure (Chen et al., 2020) (Figure 12C). However, with the rapid growth of the internodes, the covering bamboo sheath is quickly lost (Figure 12E) and eventually falls off (Figure 12D). This process would cause bending stress on the internode, which may trigger rapid lignification in the exposed internode.

We also investigated the effects of weight pressure on the growing internode during rapid growth. In our previous work on internode growth of *Bambusa multiplex*, we found that internode length directly reflects weight pressure (Wei et al., 2019). However, we found that internode diameter varies greatly in the culm of Moso bamboo (Supplemental Figure S14). Therefore, length may not be an appropriate parameter to reflect pressure. Because volume can account for variation in both length and diameter, we chose volume to represent pressure (Figure 12F). Our measurements of the growth curve of all lower internodes (internodes 1–17) and their above-shoot volumes during rapid growth showed a relationship between shoot volume and internode growth behavior. We found that shoot volume initially increased explosively in all 17 internodes tested, but all 17 internodes stopped growing when their above-shoot volumes reached ~3,915 cm$^3$ (Figure 12G).

Based on the above study, we then tested whether upregulation of the ABA pathway and activation of SCW thickening (Figures 9, C, E and 10, E) were closely related to loss of sheath cover and increasing weight pressure. First, we examined whether the number of sheaths covering the
GA may stimulate rapid growth of internodes of *P. edulis*. A–F, Effects of GA₄ treatment on *P. edulis* seedlings. A, Phyllostachys edulis seedlings treated for 11 days with different GA₄ concentrations (10, 30, 50, 70, and 90 μM) and untreated (control, 0 μM). Scale bar = 10 cm. B, Growth curves of untreated (control) and GA₄-treated *P. edulis* seedlings (*n ≥ 10* per group). Each dot represents one seedling. GA₄-10 to GA₄-90 represent 10–90 μM GA₄ treatments, respectively. C, Comparison of internode cell length of *P. edulis* seedlings treated with 50 μM GA₄ and control after 11 days. The enclosed lines indicate cell morphologies. Scale bar = 50 μm. D, Internode cell lengths of untreated (control) and GA₄-treated *P. edulis* seedlings (*n ≥ 150* per group). Each dot represents a cell. Numbers indicate average cell lengths. **** P < 0.0001. E, The relative cell number in the GA₄ treated internode and in the normally growing internode. F, The increase in cell size and cell number in the internode after GA₄ treatment. G, *P. edulis* seedlings after treatment with 25, 50, and 100 μM IAA for 11 days and control seedlings (0 μM). H, Growth curves of IAA-treated *P. edulis* seedlings (*n ≥ 10* per group). Each point represents one seedling. IAA-0.0625 to IAA-100 represent treatments with 0.0625–100 μM IAA.
uppermost 1-cm section of internode 18 varied during the rapid growth period. As expected, the number of covering leaf sheaths decreased from 18 to 0 as internode 18 grew from 0.1 to 10.7 cm (Figure 12H). Further examination of internode 18 growth versus shoot volume increase revealed a similar pattern to the lower internodes: Internode 18 stopped growing when the above-shoot volume reached ~3,735 cm³ (Figure 12I). Only 9% of the total shoot volume was formed when the internode grew from 0.1 to 3 cm in length, and this 9% occurred within 17 days. Surprisingly, the remaining 91% increase in shoot volume occurred over the next 6 days, during which internodes elongated from 6 to
29 cm (Figure 12I). Remarkably, significant lignification of the internode occurred during the rapid elongation of the 6-cm internode to the 29-cm internode (Figure 5C).

Functional genes for mechanical pressure and ABA, which may promote SCW thickening of internodes of *P. edulis*

To further test the relationship between mechanical pressure and SCW thickening, we subjected the growing internodes of *P. edulis* seedlings to a curvature treatment representing bending force (Supplemental Figure S15). We collected the whole parts, including the tensile and compressive parts of the internodes, for the next gene expression analysis. Mechanical pressure directly increased the expression of genes related to SCW thickening, such as *IRX9*, *LAC17*, *KOR1*, and *PAL1* (Figure 13A). These genes were also strongly expressed in the rapid SCW thickening module (Figure 8C). In addition, the expression of a gene encoding an AtMYB83/OsMYB46-like transcription factor, PeMYB83L (PH02Gene47557.t1) (Zhao et al., 2018), which shares 40% and 59% amino acid sequence identity with AtMYB83 (At3g08500) and OsMYB46 (Os12g33070), respectively (Figure 13B), increased with the rapid SCW thickening of internode 18 (Figure 13C). Treatment with mechanical pressure also resulted in a marked upregulation of this transcription factor gene (Figure 13D).

Recently, ABA was found to affect SCW formation and lignin deposition in Arabidopsis (*Arabidopsis thaliana*) (Liu et al., 2021). Several regulators of SCW thickening, such as AtMYB83, were dramatically downregulated in the ABA-defective mutants (Liu et al., 2021). Because ABA was enhanced in the area of rapid SCW formation in internode 18 (Figures 9, C, E and 10, E), we selected three genes related to SCW formation, *MYB83L, C3H14* (Chai et al., 2015), and *KNAT7* (Wang et al., 2020), and characterized their expression in response to ABA treatment. These three transcription factors could also be upregulated by treatment with ABA (Figure 13E).

Because MYB83L could be induced by both mechanical pressure and treatment with ABA, we selected MYB83L for further functional characterization in Arabidopsis. In particular, overexpression of MYB83L resulted in smaller plants (Figure 13F) with greater SCW thickening (Figure 13G).

Interactions between environment and factors for rapid growth may lead to variation in internode length in *P. edulis* populations

We have previously found that internode length in bamboo culms is mainly determined by rapid growth (Wei et al., 2018; Gao et al., 2022). Here, we further investigated how internode length differs in 16 *P. edulis* populations and how environmental factors influence the rapid growth of the *P. edulis* shoot. The number of fibers (Pearson r = 0.72) and parenchyma cells (Pearson r = 0.87) (Figure 14, A and B), but not the size of fibers and parenchyma cells (Figure 14, C and D), were strongly correlated with internode length in all *P. edulis* populations. We also examined the correlations between environmental conditions and internode length in the different populations. During the rapid growth phase (March and April), precipitation (Figure 15, A–C) and humidity (Figure 15, D–F) showed a low correlation with internode length, fiber cell number, and parenchyma cell number. Air temperature, on the other hand, correlated negatively with internode length (Pearson r = −0.72), fiber cell number (Pearson r = −0.81), and parenchyma cell number (Pearson r = −0.88) (Figure 15, I).

To determine how temperature regulates the rapid growth of *P. edulis* shoots and affects internode length, we measured the growth rates of *P. edulis* shoots over a 48-h period during the rapid growth phase. Unexpectedly, *P. edulis* shoots grew faster under warm conditions than under cold conditions (Figure 16A). To investigate the temperature-dependent genes related to cell growth, we also checked the expression of six randomly selected genes related to cell division that were strongly upregulated in the internode sections with active cell division (Figure 16B), and seven genes related to cell elongation that were strongly upregulated in the internode sections with active cell elongation (Figure 16D). The expression levels of most genes related to cell division were upregulated with increasing temperature (Figure 16C), whereas the expression levels of most genes related to cell elongation were constitutively expressed at different temperatures (Figure 16E). An exception was the cell elongation gene *EXPANSIN A1* (*EXP1*), which was strongly upregulated upon an increase in temperature (Figure 16E). *EXP1* is orthologous to rice (*O. sativa*) *OsEXPA4*, which promotes cell growth by loosening the cell wall (Choi et al., 2003). Our results suggest that an increase in ambient temperature promotes growth mainly by upregulating cell division genes and partially by upregulating cell growth genes during the rapid growth phase.

The acceleration of shoot growth may have a negative effect on internode growth

The increase in internode length during rapid growth was strongly correlated with the increase in shoot volume but negatively correlated with the number of covering sheaths (Figure 17A). Therefore, we hypothesized that the faster growth of *P. edulis* shoots under warm conditions might increase shoot volume and reduce the number of sheath covers more rapidly. As a result, mechanical pressure, such as bending pressure and weight pressure, would develop more rapidly than it could be alleviated by thickening of the SCW s of the internodes. In this scenario, the increased pressure on the cell DZ of the internode could lead to early inhibition of genes associated with cell division, reducing the total number of cells in the internodes.

To test this hypothesis, we quantified the expression of five genes associated with cell division at different shoot volumes. We also examined the expression of these five genes at different sheath coverages, as the internode differs in the number of sheath coverages during the rapid growth phase.
Figure 13  Mechanical pressure and treatment with ABA increase expression of genes associated with SCW thickening in *P. edulis*. A, Expression of four genes associated with SCW thickening under bending force treatment. Data are means ± SD (*n* = 3). *P* < 0.05, **P** < 0.01. Details of the bending force treatment of *P. edulis* seedlings are given in Supplemental Figure S15. B, Similarity of amino acid sequences of *P. edulis* MYB83L (PeMYB83L) with Arabidopsis AtMYB83 and rice OsMYB46. C, fragments per kilobase of transcript per million mapped reads (FPKM) values of *P. edulis* MYB83L during growth of internode 18. Each pink spot represents an FPKM value. 1i-1, 3i-1, and 9i-1 denote the first segments of the 1-, 3-, and 9-cm-long 18th internode, respectively; 14i-1, 14i-2, 14i-3, 14i-6, 14i-10, and 14i-12 represent the first, second, third, sixth, tenth, and twelfth 1-cm segments of the 14-cm-long 18th internode, respectively; 23i-23 represents the 23rd segment of the 23-cm-long 18th internode; and
The expression of all five genes related to cell division correlated negatively with shoot volume (average Pearson $r = -0.86$) but positively with the number of sheath (average Pearson $r = -0.81$) (Figure 17, B and C). When *P. edulis* seedlings were subjected to curvature treatment (Supplemental Figure S15), the observed relative expression levels of all five of these genes were significantly downregulated ($P < 0.05$; Figure 17D), consistent with our hypothesis.

The number of sheaths correlated strongly with the expression levels of cell division genes (average Pearson $r = -0.81$) (Figure 17C), and internodes differed in length along the culm (Figure 17E). Each internode also differed in the number of covering sheaths during the fast-growing phase (Figure 17F). Therefore, we examined the correlation between the number of sheaths and internode length to further test the above hypothesis of a negative feedback of shoot growth on internode growth. Cell number (Figure 17G), but not cell size (Figure 17H), was strongly positively correlated (Pearson $r = 0.94$) with internode length within the culm (internodes 1–18 below the first branch of the culm). As expected, the number of internode cells was strongly correlated (Pearson $r = 0.97$) with the number of sheaths during the rapid growth phase (Figure 17I). Thus, these correlations suggest that an increase in ambient temperature might promote the growth of bamboo shoots, increasing shoot volume and reducing the number of sheaths more rapidly, and this might lead to bending pressure and weight pressure, causing cell division-associated genes to be downregulated more rapidly and decreasing the number of internode cells during the rapid growth phase.

**The mechanical pressure associated with shoot growth may also negatively affect internode growth of *B. multiplex***

We used another small bamboo species, *B. multiplex*, to further test whether there is a relationship between temperature and mechanical pressure associated with shoot growth that affects internode growth of bamboo. As with *P. edulis* shoots, *B. multiplex* shoots grew faster under warm temperature conditions (Figure 18A). Internode growth of *B. multiplex* was also closely related to shoot volume. When the above-shoot volume reached $\sim 34.5\, \text{cm}^3$, the four internodes detected stopped growing (Figure 18B). In this volume, the average shoot weight was $\sim 62.5\, \text{g}$ (Figure 18C). We applied this weight to the third internode with a length of $\sim 8.5\, \text{cm}$ (Figure 18D). At this length, the third internode was in the fastest growth phase (Wei et al., 2019) (Figure 18D).

Direct weight pressure treatment inhibited internode growth, shortened the growth period, and eventually resulted in shorter internodes (Figure 18E). Anatomical analysis revealed that weight pressure treatment not only resulted in shorter cells, but more importantly resulted in fewer cells (Figure 18, F and G). Compared with the reduction in cell size, cell number decreased by $\sim 60\%$, much more than the reduction in cell size ($\sim 21\%$) (Figure 18H). To characterize the possible molecular basis for the reduction in cell number after weight treatment, we examined the expression levels of five genes, *CYC1BAT*, *MAD2*, *CDC20.1*, *TPX2*, and *CYCB2.4*, associated with cell division underweight treatment or under different shoot volumes. These five genes are homologs of *P. edulis* genes and are important candidate genes for cell division (Figure 8A). A total of 11, 9, 6, 14, and 11 transcripts of *CYC1BAT*, *MAD2*, *CDC20.1*, *TPX2*, and *CYCB2.4*, respectively, were found in the transcriptome of the internode of *B. multiplex* (Wei et al., 2019) (Figure 18I). As in *P. edulis*, expression of the tested cell division genes in *B. multiplex* correlated negatively with shoot volume (average Pearson $r = -0.83$) (Figure 18I). Direct application of weight pressure significantly decreased the expression of these five genes in the cell DZs of the third internode of *B. multiplex* (Figure 18I). Thus, the feedback regulation of mechanical pressure on internode growth relative to shoot growth may be universal in bamboo.

**Discussion**

Although rapid growth of woody bamboo plants has been widely studied, the developmental dynamics of this process and the underlying regulatory mechanisms remain poorly understood. In this study, we investigated the development of a representative internode of Moso bamboo (*P. edulis*) during the rapid growth phase and characterized several important anatomical, physiological, and molecular aspects of this growth phase.

**Perspective on rapid growth of Moso bamboo using a representative fast-growing internode**

After an extensive morphological study of Moso bamboo populations throughout China, we selected internode 18 as a representative internode to study the rapid growth of Moso bamboo. This internode was selected because it had the best morphological indices, the fastest growth, and low interindividual variation (Figures 1 and 2). As with other grasses (Bleecker et al., 1986; Wei et al., 2019), analysis of internode 18 revealed that 1-cm sections of different lengths of the same internode performed different functions during rapid growth (Figure 3). However, in the internode of Moso bamboo, the cell DZ and especially the cell EZ were much longer than in other plants (Bleecker et al., 1986; Wei et al.,...
In absolute terms, cell division and elongation activities in these zones were also greater than those previously reported (Wei et al., 2019) (Table 1). Extrapolating the cell production of the entire internode, we estimate a maximum cell production of \( \frac{2.4}{24} \times 10^7 \) cells/h \( \left( \frac{5.7}{24} \times 10^8 \right) \) cells/day in the cell DZ of internode 18 (Table 1). This high cell production and elongation rate could explain the rapid vertical growth we observed on the internodes of Moso bamboo, which grew \( 11.8 \) cm in the vertical direction within 24 h. This cell growth rate surpasses previous reports of vegetative organ growth, even in other bamboo species (Sprangers et al., 2016; Phyo et al., 2017; Youssef et al., 2018; Wei et al., 2019). However, when we compared the relative growth rate of Moso bamboo shoots with other plants, Moso bamboo did not exhibit the fastest rate. For example, the maximum relative internode elongation rate of Moso shoot was 0.08 cm cm\(^{-1}\) h\(^{-1}\), which was lower than the internode elongation rate of deepwater rice \( (\sim 0.11 \text{ cm cm}\^{-1}\text{ h}\^{-1}) \) (Métraux and Kende, 1984) and maize roots \( (0.4 \text{ mm mm}\^{-1}\text{ h}\^{-1}) \) (Erickson and Sax, 1956). Using a natural logarithm to calculate the relative elongation rate, the maximum internode growth rate of the Moso bamboo shoot was \( \sim 5.0\%/h \), which was even lower than that of the Arabidopsis root with a growth rate of \( > 30\% /h \) (Beemster and Baskin, 1998). In addition, the maximum cell division rate of Moso internode was \( \sim 0.06 \text{ cells cell}\^{-1}\text{ h}\^{-1} \), which was higher than the cell division rates of most known plants, but still not the fastest. For example, a cell division rate of \( \sim 0.29 \text{ cells cell}\^{-1}\text{ h}\^{-1} \) was found in the internode of oats, which is \( \sim 5 \) times higher than that of the internode of Moso bamboo (Kaufman et al., 1965). The maximum cell elongation rate of \( 0.49 \mu \text{m } \mu \text{m}\^{-1}\text{ h}\^{-1} \) of the Moso bamboo internode was even lower than that of Arabidopsis root cells, which reached \( \sim 1.44 \mu \text{m } \mu \text{m}\^{-1}\text{ h}\^{-1} \) (Band et al., 2012). Thus, contrary to previous assumptions, the rapid growth of the internode of Moso bamboo is not due to particularly rapid cell growth or a particularly high division rate, but rather to the large number of dividing and elongating cells within the division and elongation zones.

Unlike other bamboo species in which fewer internodes elongate simultaneously during the rapid growth phase, Moso bamboo has more than 40 elongating internodes during its rapid growth phase (Figure 2B). In B. multiplex, for example, only 10 internodes per shoot were found to elongate (Supplemental Figure S16) (Wei et al., 2019), and in maize only four internodes per shoot elongate (Morrison et al., 1994a, 1994b). Thus, similar to DNA replication bubbles that can effectively accelerate DNA synthesis during cell division,
the unusually rapid growth of Moso bamboo shoots is due to the simultaneous growth of a large number of internodes as well as long cell division and elongation zones with a large number of dividing and elongating cells within each internode.

During rapid growth, the shoot of Moso bamboo expands very rapidly, exerting great mechanical pressure (e.g. weight pressure and bending pressure) on the lower internodes (Figure 12). The mechanical pressure in the growing internodes (e.g. internode 18) is further increased by the loss of sheath cover and the increasing above-shoot weight (Figure 12). To counteract this sudden increase in mechanical pressure, the corresponding internodes must lignify rapidly to support the rapid growth of the internodes above.

The maximum deposition rate of lignin and cellulose in the SCW thickening region of internode 18 occurred during the rapid growth phase, reaching ~28.04 and ~44.05 mg g⁻¹ DW per day, respectively. This deposition rate far exceeds previous reports of SCW thickening in other plants, such as maize (Morrison et al., 1994a, 1994b; Zhang et al., 2014).

Thus, the interaction of simultaneous elongation of a large number of internodes with enormous cell production and elongation of many cells at the same time, as well as rapid SCW thickening, supports Moso shoot growth of up to 114.5 cm/day. This surpasses the Guinness World Record for daily plant growth of 91 cm/day for certain bamboo species. (https://www.guinnessworldrecords.com/world-records/fastest-growing-plant).
Molecular regulation of rapid growth of Moso bamboo shoots

Our cellular, physiological, and molecular analyzes indicate that GA, rather than auxin signaling, is the primary driver of rapid growth of Moso bamboo shoots (Figures 8, B, 10, and 11). This is not surprising, because GA is a key factor for internode elongation in other monocots, such as deepwater rice (Nagai et al., 2020; Ayano et al., 2014). Exogenous application of 100 µM GA₃ also resulted in longer internodes in Moso seedlings (Zhang et al., 2018).

Figure 16 Air temperature positively correlates with rapid growth of internodes of P. edulis. A, Growth rates of P. edulis shoots correlated with daily air temperature measured over 48 h. Each colored circle represents the average growth rate of ≥5 shoots of the respective length. Triangles represent ambient air temperatures in degree Celsius. B and C Expression of six randomly selected genes associated with cell division in different internode segments (B) and under daily temperature cycles (C). D and E, Expression of seven randomly selected genes associated with cell elongation in different internode segments (D) and under daily temperature cycles (E). Log₂ fold changes are shown in the heat maps (B and D), and transcript abundances in the first 1-cm segment of the 1-cm-long internode (1i-1) were set as controls and normalized to 0 (log₂). Fold changes were used to draw line plots (C and E), and transcript abundance at 12.4°C were set as controls and normalized to 1. The terms 1i-1, 3i-1, and 9i-1 represent the first segments of the 1-, 3-, and 9-cm-long 18th internodes, respectively; 14i-1, 14i-6, 14i-10, and 14i-12 represent the first, sixth, tenth, and twelfth 1-cm segments of the 14-cm-long 18th internode, respectively; 23i-23 represents the 23rd segment of the 23-cm-long 18th internode; and 29i-29-20d represents the 29th segment of the 29-cm-long internode collected on the 20th day after the rapid growth stage.
Figure 17 Internode cell number correlates with both the shoot volume factor, which is positively associated with shoot growth, and the sheath cover number factor, which is negatively associated with shoot growth. A, Pearson coefficients of internode length of internodes 3–18 with AV, and number of sheaths during rapid growth. B and C, The expression levels of five genes associated with cell division in the DZs of internode 18 at different AV (B) and different numbers of sheaths (C), and their correlations. Data are mean values (n ≥ 6). D, Expression of genes associated with cell division under bending pressure treatment. Data are means ± SD (n = 3). **P < 0.01. E and F, Internode length (30 culms) (E) and number of sheaths (F) increased linearly from lower internodes to upper internodes below the first culm branch. G and H, Correlation of internode length (internodes 1–18 from bottom to top of culm of P. edulis) with parenchyma cell number (G) and cell length (H). Parenchyma cell length represents the average cell length of more than 160 parenchyma cells of each internode. I, The number of sheaths as a function of the number of parenchyma cells in the internode.
Mechanical pressure associated with shoot growth may affect internode growth of *B. multiplex*. A. Growth rates of *B. multiplex* shoots positively correlated with daily air temperature measured over one month. Each green circle represents the average growth rate of ≥15 shoots of the respective length. Pink circles represent average daily air temperatures in °C. B. Growth pattern of four internodes compared to their AV during rapid growth. The dashed pink line on the right Y-axis represents the average AV when the internodes stopped growing. The terms 1i–4i represent first through fourth internodes, respectively. C. Weight of the shoot above the third internode when it stopped growing. The green dashed line represents the average shoots weight above the third internode when it stopped growing. Application (weight pressure) of 40 g of iron beads (continued)
high concentrations of GA were found in internodes with strong elongation ability (Figure 10A) and in the DZ of internodes with high cell division intensity (Figure 10B). This suggests that GA-mediated growth stimulation depends on both cell division and cell elongation. Indeed, GA treatment leads to both increased cell number and increased cell length (Figure 11, C–F). Moreover, GA treatment at different doses significantly increased the growth rates of Moso bamboo seedlings by 2.23- to 3.89-fold. This is similar to and even higher than the 2.81-fold increase in growth rate between the normal and rapid growth stages (Figure 11B; Supplemental Figure S11). These results suggest that GA may promote the transition between normal growth and rapid growth in Moso bamboo shoots by promoting both cell elongation and cell division.

In the seedling internodes treated with GA, the increase in cell length (up to 2.1-fold) was much greater than the increase in cell number (1.37-fold increase) (Figure 11F). This implies that GA-mediated growth depends mainly on cell elongation. GA-mediated cell elongation might be related to the induction of downstream functional genes such as CWINV2, EXPA1/EXPA4, CELLULOSE SYNTHASE-LIKE D3 (CLSD3), ACTIN2, and TIP1;1 (Figure 8B), which are related to sugar metabolism (Tang et al., 1999), cytoskeletal organization (McDowell et al., 1996), cell wall assembly (Choi et al., 2003), and water transport (Schussler et al., 2008) (Figure 8B). Remarkably, many genes related to the vacuolar proton pump were also strongly upregulated in the EZ of internode 18 (Figure 8B). The vacuolar proton pump could provide energy for secondary active transport systems such as Na\(^+\)/H\(^+\) antiporters. These active transport systems may be critical for maintaining the osmotic pressure required for vacuum enlargement and for maintaining the turgor pressure required for cell growth (Supplemental Figure S17) (Muto et al., 2011).

Prior to rapid growth, cell division sets the stage for rapid cell elongation in Moso bamboo shoots. Here we document a possible gene network that could maintain cell division in the internode during the rapid growth phase (Figure 8A). As in other bamboo internodes (Wei et al., 2019), cells in the cell DZ of the Moso bamboo internode eventually stop dividing and begin to elongate. To elucidate the mechanisms underlying this transition, we carefully examined specific sections of internode 18 (i.e. the lowest 1-cm-long sections of the 1-, 3-, 6-, 9-, and 14-cm internodes). During the transition from cell division to cell elongation, polar auxin transport genes were significantly upregulated in these sections, auxin levels were stably high, and cytokinin levels decreased when cell elongation began to dominate (Figures 9, A, B, 10, C, and D). Thus, the transition from cell division to cell elongation in the DZ of internode 18 may be triggered by the accumulation of auxin via polar auxin transport. Mechanical pressure can induce the expression of auxin transporter genes (Ko et al., 2004), which triggers the translocation of PIN1 to the most stressed membrane and promotes cell growth via polar auxin transport (Nakayama et al., 2012). Shoot volume above the cell DZ transitioning to cell elongation increased sharply (Figure 12I). In our previous work, mechanical pressure associated with shoot height was important for the growth change in the internode of B. multiplex during rapid growth (Wei et al., 2019). We hypothesized that mechanical stress might also stimulate polar auxin transport, leading to auxin accumulation and promoting cell elongation in the DZ of the internodes of Moso bamboo.

We also identified several functional genes (37) that were simultaneously and significantly upregulated in the area of rapid SCW thickening in the internode (Figure 8C). Potential upstream regulators of these genes include C3H14 (Chai et al., 2015) and MYB83 (McCarthy et al., 2009). These interaction networks may help elucidate the molecular mechanisms underlying the rapid SCW thickening of the internode of Moso bamboo during rapid growth. More genes associated with SCW thickening in the fast-growing internodes were more highly expressed than SCW thickening genes identified after the fast growth stage (Figure 8, C and D). This suggests that rapid cell wall reinforcement was particularly important to support rapid growth. This accelerated SCW thickening may have been triggered by the dramatic increase in weight pressure within the explosively growing shoot (Figure 12, G and I).

Of note, the regular decrease in the number of sheaths could also increase the mechanical bending pressure on the exposed internode (Figure 12, E and H). During this process, the growing internode is also exposed to air, which might activate ABA signaling and subsequently inhibit cell elongation at the exposed site (Figures 9, C, E, 10, E, and 12, H) (Liu et al., 2016). Indeed, direct application of mechanical stress could upregulate the expression of genes related to SCW thickening, such as KOR1, PAL1, LAC17, LAC2, and IRX9, as well as their possible regulator, a MYB83L gene (MYB83L) transcription factor. MYB83L enhanced SCW thickening when overexpressed in Arabidopsis stems (Figure 13G). Treatment with ABA upregulated potential regulators such as C3H14, MYB83L, and KNAT7 in SCW formation. Recently, ABA was also reported to promote SCW formation and lignin deposition in Arabidopsis (Liu et al., 2021) via key regulators such as AtMYB83 (Liu et al., 2021).

**Figure 18** (Continued)
to the rapidly growing third internode (D) resulted in growth inhibition (E). Data are means ± SD (n = 10). F–H, Parenchyma cell lengths (F), cell numbers (G), and reduction ratios of cell size and cell number (H) of internode 3 under the 40 g weight treatment. Each circle in F represents a parenchyma cell. \(^*P < 0.0001, I\), The expression levels of transcripts of five genes related to cell division, CDC201, CYCB2/4, CYC1BAT, MAD2, and TPX2, in the DZs of internode 3 at different AV. Data are mean values (n = 5). Each green line represents the expression trend of a transcript at different shoot volumes. J, Expression of genes associated with cell division underweight pressure treatment (D). Data are means ± SD (n = 3). \(^*P < 0.01\).
Thus, loss of sheath cover may not only increase bending pressure but also enhance ABA signals in the exposed part of the internode. Both loss of sheath cover and signals from ABA appear to promote SCW thickening in the exposed part of the internode 18.

**Moso internode size as a function of temperature**

Although internode growth of Moso bamboo occurs mainly during the rapid growth phase, the effects of environmental conditions on this phase remain to be explored. Internode length is positively correlated with the number of cells in the internode, but not with the length of these cells (Figure 14). Unexpectedly, air temperature was negatively correlated with internode length and cell number (Figure 15, G–I), but positively correlated with overall growth of Moso bamboo shoots (Figure 16A). This is consistent with the temperature-size rule (TSR) (Atkinson and Sibly, 1997), a universal, phenotypically plastic response of body size to ambient temperature that is considered a fundamental law of biology (van der Have, 2008). Atkinson (1994) reported that over 80% of 109 studies found larger body sizes at cooler temperatures, although developmental rates were faster at warmer temperatures. Species studied include bacteria, protists, plants, and animals. To date, scientists have paid considerable attention to the TSR life history puzzle; in fact, it has generated considerable controversy.

To elucidate the mechanisms underlying the plastic response of Moso internode size to growth temperature, we analyzed the expression of cell growth genes at different temperatures. This temperature-mediated growth response may be due, in part, to the temperature-dependent upregulation of genes related to cell growth and division (Figure 16, B–E). Consistent with this, Shi et al. (2019) have shown that elevated temperatures within a certain range effectively accelerate the growth and development of Moso bamboo seedlings. Based on our results, we hypothesize that growth rates of Moso bamboo shoots increase in response to elevated temperatures, which may lead to a sudden increase in mechanical pressure (due to rapid increase in shoot volume and rapid loss of sheath cover) on the cell DZ and an early termination of cell division. Indeed, we found that the expression of genes related to cell division was negatively correlated with shoot volume (Figure 17B) but positively correlated with the number of covering sheaths (Figure 17C). Direct application of a bending force to the internodes of Moso seedlings also downregulated genes related to cell division (Figure 17D). In addition, the number of sheaths on the internodes during rapid growth correlated strongly with the number of internodal cells in the culm of Moso bamboo (Figure 17I), and direct application of weight pressure to the growing internodes of B. multiplex resulted in downregulation of genes associated with cell division, leading to shorter internodes with fewer cells (Figure 18). These results support the notion of feedback regulation of mechanical pressure induced by shoot growth.

Thus, our results suggest a possible causal mechanism in which lower temperatures slow the increase in shoot height above the cell DZ of internodes and reduce the number of sheaths by decreasing the growth rate of internodes; this in turn would prolong cell division activity and result in internodes with more cells. Therefore, the differences in internode length among Moso populations may be due to a tradeoff mediated by mechanical stress: Faster growth is rapidly inhibited, while slower growth tends to be elongated. Our work helps us understand how temperature may affect plant development, leading to significant changes in mature plant body size. It also helps clarify the TSR, which remains a mystery in biology.

**Materials and methods**

**Plant materials**

Moso bamboo (P. edulis) samples were harvested from the bamboo garden of Nanjing Forestry University in Xishui Town, Jurong City, Jiangsu Province, China (32°07’N, 119°13’E, 160 m elevation). Here, the soils are Alfisols, the average annual temperature is 15.5°C, and the average annual precipitation is 1,099 mm. To investigate the growth relationships among P. edulis populations during rapid growth, we collected P. edulis culms from 16 different populations grown in the major centers of bamboo diversity in China (Supplemental Table S1). A total of 17 P. edulis populations were included in this study, and each population comprised 30 mature bamboo culms.

**Morphological analysis of P. edulis internodes**

To study the morphology of the internodes of culms, 30 mature bamboo culms were randomly selected from the bamboo population in the bamboo garden of Nanjing Forestry University. The diameters were measured at breast height and the average diameter was calculated. Another 30 mature culms with the same average diameter as the first were cut, and the length, diameter, and wall thickness of all internodes of each culm were recorded. In addition, 10 1-year-old culms were selected to measure the FW and DW of each internode.

To investigate the differences in internode growth (length) among P. edulis populations during rapid growth, 30 mature bamboo culms from each of the 16 populations were randomly selected and their average diameters at breast height were determined. Another 30 mature culms with the same mean diameter from the corresponding populations were harvested, and the lengths of internodes 1–25 (from bottom to top) of each culm were recorded. About 12,000 internodes from 480 culms from 16 P. edulis populations were used.
Investigation of the growth pattern of shoots and internodes of *P. edulis*

Thirty bamboo shoots with a height of 18 cm from the *P. edulis* population of Nanjing Forestry University were selected. Their height was recorded every 2 days until the end of the rapid growth period. Meanwhile, three other bamboo shoots with the same average height as these 30 bamboo shoots were cut and the length of each internode of each bamboo shoot was measured. From these data, the growth curves of all 30 bamboo shoots and the 18th internode were derived.

To investigate the response of internode growth to mechanical pressure (above-shoot volume) and to determine growth curves, the lengths of 18 internodes (internodes 1–18) and their above-shoot volumes were recorded every 2 days during rapid growth. The length (L) of every other internode was also recorded. Based on the average diameter (D) of each of the 30 internodes calculated from 30 culms in the morphological study described above, the formula $3.14 \times (D/2)^2 \times L$ was used to calculate the volume of each internode. The sum of the volumes of all the above internodes was considered as the shoot volume of the internodes studied above (Figure 12F).

**Sampling of the 18th internode segments**

Based on previous observations of culm elongation, spatial tissue samples were collected from the 18th internodes ranging from 1 to 29 cm in length. The sampling strategy for the 18th internode at different growth stages is shown in Supplemental Figure S1. To better describe the developmental stages of these samples, we also created a figure showing the entire growth of internode 18 at different time points (Supplemental Figure S2). For this purpose, two opposite sections of the entire internode, each 1 cm wide, were taken and sawed into 1 cm sections from bottom to top. Their green outer skins and yellow inner skins were removed, these 1 cm sections were immediately fixed in formalin–acetic acid–50% alcohol (FAA, v/v) buffer for at least 48 h, dehydrated in an ethanol series, and infiltrated with xylene. Paraffin processing, sectioning, and observation were performed according to our previous method using a Leica DM2500 light microscope (Leica, Wetzlar, Germany) (Wei et al., 2018).

For cell length observation, the lengths of approximately 150 parenchyma cells (long cells) (He et al., 2002) were measured in each 1 cm section. To count cell nuclei, more than three sections, each with 10 random observation areas, were analyzed for each section that was observed.

**TEM**

To characterize cell wall growth in different parts of internode 18 of *P. edulis* with a length of 14 cm, each 1 cm section was taken from the bottom to the top. The uppermost 1 cm section of a 23 cm-long internode 18 and the uppermost 1 cm sections of an internode 18 with a length of 29 cm at 0, 20, and 50 days after the rapid growth stage were also sampled. The green outer skins and yellow inner skins of these sections were removed, and the sections were immediately fixed in 2.5% glutaraldehyde for 20 h at 4°C. Samples were then sectioned and observed using an H-600 transmission electron microscope (Hitachi, Tokyo, Japan) as described by Wang et al. (2019).

**Flow cytometry**

The first, second, and third 1 cm sections (from bottom to top) of 3-, 6-, and 9 cm-long 18th internodes and the first 1 cm section of 14 cm-long 18th internodes were dissected in LB01 solution. After filtration, the extracted sections were kept in an ice bath for 5 min and then stained with propidium iodide (Biofroxx, Einhausen, Germany) for another 5 min. The number of nuclei in each of these sections was then counted and analyzed as described by Zou et al. (2020) using a BD Influx cell sorter (BD Company, Franklin Lakes, NJ, USA).

**Quantification of cellulose and lignin**

The 14th, 23rd, 28th, and 29th 1 cm-long parts of the 18th internodes of lengths 14, 23, 28, and 29 cm, and the 29th 1 cm-long sections (uppermost 1 cm-long sections) of the 18th internodes, 20, 50, 80, 110, and 140 days after the rapid growth stage, were collected and immediately frozen in liquid nitrogen. These were ground to powder in liquid nitrogen, and 200 mg of the powder from each sample was used to quantify cellulose and lignin content. Cellulose and lignin contents were measured according to the methods described by Wang et al. (2011) and Kristensen et al. (2008), respectively.

**RNA extraction, transcriptome sequencing, and data analysis**

During rapid growth, the following segments of internode 18 were collected and used for transcriptome sequencing: The 1st 1 cm segments of the 1-, 3-, 6-, 9-, 14-, and 21 cm-long 18th internodes; the 2nd, 3rd, 6th, 10th, and 12th 1 cm...
segments of the 14-cm-long 18th internodes; the 23rd 1-cm segment of the 23-cm-long internode 18; and the 29th 1-cm segment of the 29-cm-long internode 18 (Supplemental Figure S6). Similarly, the 29th 1-cm segment of the 29-cm-long 18th internode was collected 20 and 110 days after the rapid growth stage and used for transcriptome sequencing (Supplemental Figure S6). Fourteen samples were collected with at least three biological replicates of at least three internodes from at least three independent culms; however, most had at least five biological replicates, depending on sample availability (Supplemental Table S3).

Total RNA was extracted using the RNAprep Pure Kit (DP441) (TIANGEN Biotechnology Company, Beijing, China) according to the manufacturer’s protocol. Evaluation of the quality of the extracted RNA and examination of the RNA integrity number were performed as described by Wei et al. (2017). Strand-specific RNA-Seq library construction and sequencing were performed at Novogene Biotech (Beijing, China) according to Illumina standard protocols. Illumina sequencing data have been deposited in the Sequence Read Archive of the National Center for Biotechnology Information (accession number PRJNA694793). The full-length PacBio sequencing data were deposited in the China National Gene Bank Sequence Archive Database under the accession number CNP0001577.

Transcriptome sequence processing, assembly, and annotation were performed using methods described by Guo et al. (2019a). Transcripts with a P < 0.05 and an associated log2 fold change of 0.67 or greater were identified as DEGs. DEG expression patterns were visualized using MapMan (version 3.5.1. R2) (Thimm et al., 2004). Mercator was used to obtain the mapping file of the assembled transcripts (Lohse et al., 2014). Coexpression networks based on RNA-Seq expression data (Supplemental Data Set 3) were constructed using the WGCNA package in R (Langfelder and Horvath, 2008). Gene interaction networks were visualized using Cytoscape (version 3.7.2) (Shannon et al., 2003).

Quantification of hormones
To quantify GA and auxin (indole-3-acetic acid, IAA) in the EZ of 18th internodes with different growth potential, the following segments were harvested: Three 1-cm segments from the third centimeter of 3-, 6- and 9-cm-long 18th internodes and three 1-cm parts from the sixth centimeter of 14-cm-long 18th internodes.

To quantify GA and IAA in the cell DZ of 18th internodes with different cell division potentials, the following segments were harvested: Three 1-cm segments from the first centimeter of 3-, 6-, and 14-cm-long 18th internodes. An additional segment from the first centimeter of 9-cm-long 18th internodes was collected for quantification of IAA.

For quantification of CK, segments from the first 1 cm of 3-, 6-, 9-, and 14-cm-long 18th internodes were collected.

For quantification of ABA content, 1-cm segments were collected from the 1st, 10th, and 12th centimeters of the 14-cm-long 18th internodes.

The above samples were immediately ground to powder using liquid nitrogen. GA₄, GA₃, GA₆, GA₇, and GA₉ were quantified by high-performance liquid chromatography according to the method described by Liu et al. (2018). Endogenous IAA, ABA, and CKs (zeatin, cis-zeatin, trans-zeatin, cis-zeatin riboside, trans-zeatin riboside, dihydrozeatin, dihydrozeatin riboside, N6-isopentenyladenosine, and N6-isopentenyladenosine) were extracted, purified, and measured for each sample as previously described by You et al. (2016).

Investigation of the expression of SCW thickening genes under mechanical pressure
To investigate the effect of bending pressure on the expression of genes related to SCW thickening, a curvature treatment was applied to 10-cm-tall P. edulis seedlings (Supplemental Figure S15). After treatment for 1 day, the lower 0.5−1.0-cm segment of the third internode (~2 cm in length) was collected. Total RNA was extracted as described above, and first-strand cDNA synthesis and reverse transcription–quantitative PCR (RT-qPCR) were performed as described by Guo et al. (2019a). The relative abundance of each gene was calculated from the $2^{-ΔΔCq}$ values between the control and treated samples (three replicates for each gene) (Livak and Schmittgen, 2001). The nucleotide tract-binding protein gene was used as an internal control (Fan et al., 2013). Gene-specific primers used for RT-qPCR are listed in Supplemental Table S5.

Heterologous overexpression of PeMYB83L in A. thaliana
The coding sequence of PeMYB83L (PH02Gene47557.t1) was directly synthesized by BIOGLE GenenTech Company (Nanjing, China) and cloned into a pCAMBIA-1301 vector. After transformation into Agrobacterium, pCAMBIA-1301-PeMYB83L was transferred into Arabidopsis (Col-0) using the floral dip method. Transgenic plants grown on a plate supplemented with 25 mg L⁻¹ hygromycin were selected and verified by RT-qPCR as described above. Five transgenic lines were obtained, two of which were used for the next observation of cell wall thickening.

To study SCW thickening in transgenic Arabidopsis, flower stems of 6-week-old wild-type Arabidopsis plants and PeMYB83L transgenic plants (T₂ generation) were harvested and fixed in FAA, v/v buffer for at least 48 h. Paraffin sections were prepared and observed as described above.

Hormone treatment
To understand the effect of exogenous application of GA and auxins, at least 10 P. edulis seedlings with a height of ~2.0 cm were selected, and different concentrations of GA₄ (product number, G7276, Sigma-Aldrich Trading Co. Ltd., Shanghai, China) (10, 30, 50, 70, and 90 μM), IAA (product number, I2,886, Sigma-Aldrich Trading Co. Ltd., Shanghai, China) (0.0625, 0.12, 0.5, 1, 10, 25, 50, and 100 μM), 2,4-D (product number, 31,518, Sigma-Aldrich Trading Co. Ltd., Shanghai, China) (0.0625, 0.12, 0.5, 1, 10, 25, 50, and 100 μM), and ABA (product number, 90,769, Sigma-Aldrich
We examined the internodal cell length of the GA₄-treated seedlings. After 11 days of treatment, the third internodes (from bottom to top) of GA-tREATED seedlings and control seedlings were collected and fixed in FAA, v/v buffer for at least 48 h. Paraffin-embedded sections for observation were prepared according to Wei et al. (2018). A total of approximately 150 parenchyma cells were measured for each group (long parenchyma cells).

After treatment with 50-μM ABA for 6 days, the lower 0.5–1.0 cm sections of internode 3 of ABA-treated P. edulis seedlings and normally growing seedlings were used to determine the expression levels of three transcription factor genes (Figure 13E). RT–qPCR was performed as described above. Gene-specific primers used for RT–qPCR are listed in Supplemental Table S5.

Study of internode cell number and environmental factors in different P. edulis populations

Four internodes from each population were selected at breast height (~1.5 m) of the culm of P. edulis. Sections (2 × 2 cm) were taken from the center of each internode, and the green outer skins and yellow inner skins were removed. The remaining sections were cut into 2-cm long matchstick-like pieces. After culturing with Jeffrey segregated liquid at 50°C for 36–72 h, the sticks were washed with ddH₂O and then stained with 1% (w/v) safranin solution. Conventional cell sections were prepared, and parenchyma cell lengths (approximately 150 for each sample) were measured under a Leica DM2500 light microscope (Leica, Wetzlar, Germany). Fiber cell length data from previous work were used (Chen et al., 2018). The number of parenchyma and fiber cells was calculated by dividing the average internode length by the average length of the parenchyma and fiber cells, respectively.

Data on environmental factors, including temperature, humidity, and precipitation, that might be associated with internode length in March and April were downloaded from the China Meteorological Data Service Center (http://data.cma.cn/data/weatherBk.html). Weather data from 1981 to 2010 were used to calculate the average temperature, humidity, and precipitation in March and April in different regions.

Investigation of growth dynamics of P. edulis shoots within 48 h

More than 24 P. edulis shoots were divided into four groups according to their height: 30, 60, 90, and 100 cm. Each group consisted of at least six shoots. Ambient temperatures and height of each P. edulis shoot were recorded every 2 h.

Growth was calculated every 2 h and used to create a growth curve.

The expression of genes related to cell division under curvature treatment

To investigate the effect of bending pressure on genes related to cell division, a bending treatment was applied to 10-cm-tall P. edulis seedlings, as described above. After treatment for 2 days, the lower 0.5-cm segment of the third internode (~2 cm in length) was collected. Total RNA was extracted as described above, and first-strand cDNA synthesis and RT–qPCR were performed as described above. Gene-specific primers used for RT–qPCR are listed in Supplemental Table S5.

Investigation of internode cell number and cell size in the culm of P. edulis

Three bamboo culms were collected from the bamboo garden of Nanjing Forestry University. All three culms had the average ground diameter (8 cm) of P. edulis culms in the bamboo garden of Nanjing Forestry University. Sections (2 × 2 cm) were taken from the center of each internode (from bottom to top, internodes 1–18), and conventional cell sections were prepared as described above. Approximately 450 parenchyma cells were examined for each sample. The number of parenchyma cells was calculated by dividing the average internode length by the average parenchyma cell length.

Investigation of growth dynamics and weight pressure treatment in B. multiplex shoots

Daily growth of fifteen 95-cm-long B. multiplex shoots, and air temperatures, were recorded every day for 32 days and used to generate growth and temperature curves.

The lengths of internodes 1–4 (from bottom to top) of B. multiplex shoots at the rapid growth stage were obtained from our previously published work (Wei et al., 2019). The volumes of shoots above internodes 1–4 during growth were the sum of the volumes of each internode above. The volume of each internode was calculated using the following formula: \( V = \pi R^2 L \), where \( R \) is the radius of the internode, and \( L \) is the internode length. Ten shoots were used to measure shoot weight above the third internode when it terminates rapid growth.

Iron beads with a total mass of 40 g were attached to the upper part of the third internode with a length of 8.5 cm to increase the weight pressure. The iron beads and the weight of the internode above the growth zone of the 8.5-cm-long third internode together correspond to the average shoot weight (62.5 g) above the third internode when it had just finished its rapid growth. The third internode was in its fastest growth phase at this length (Wei et al., 2019). The lengths of internodes with weight treatment and internodes with normal growth were recorded each day and used to create a growth curve. After growth stopped, the newly formed parts of the internode after weight treatment and
the corresponding controls of the internodes with normal growth were harvested and used to examine the lengths of the parenchyma cells. A total of 500 cells were used to measure the cell length of the internodes treated with weight pressure and the internodes with normal growth. Cell number was calculated by dividing the average length of the newly formed internode by the average cell length of the newly formed internode.

The fragments per kilobase of transcript per million mapped reads of five genes associated with cell division during rapid growth of the third internode of B. multiplex were obtained from our previously published work (Wei et al., 2019). To investigate how weight pressure affects the expression of these five cell division genes, 40 g of iron beads were applied to the third internode with a length of 8.5 cm. After 1 day of treatment, the DZ of the weight treatment and the internodes of normal growth were collected and used for RNA extraction. RT–qPCR was performed as described above. Gene-specific primers used for RT–qPCR are listed in Supplemental Table S5.

Statistical analysis
Mean difference was performed with IBM SPSS Statistics version 22 (IBM, Armonk, NA, USA) using Duncan’s multiple range test or with GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA) using the integrated t-test analysis. All linear regression analyses or nonlinear regression analyses were performed with GraphPad Prism version 8.0 using the integrated formula with the default parameters. All statistical results are included in Supplemental Data Set 4.

Accession numbers
The sequence data of P. edulis from this article can be found in the GenBank/EMBL libraries under the following accession numbers: PeCDC20.1 (PH02Gene08773.t1), PeCIC1BAT (PH02Gene02703.t1), PeCYCB24 (PH02Gene35175.t1), PeMAD2 (PH02Gene03239.t1), PeTCX2 (PH02Gene12177.t1), PeTPX2 (PH02Gene32394.t1), PeCICLD3 (PH02Gene29050.t1), PeEXPA1 (PH02Gene28974.t1), PeEXPB2 (PH02Gene01650.t1), PeFLA7 (PH02Gene40552.t1), PeXTH22 (PH02Gene05411.t1), PeXTH30 (PH02Gene32672.t1), PeXTH31 (PH02Gene21576.t1), PeIRX9 (PH02Gene04386.t1), PeKOR1 (PH02Gene19289.t1), PePAL1 (PH02Gene30509.t1), PeLAC17 (PH02Gene00184.t1), PeMYB83L (PH02Gene47557.t1), PeCIC14 (PH02Gene32013.t1), PeKAT7 (PH02Gene0891.t2), and PeNTB (PH02Gene24776.t1). The sequence data of the B. multiplex genes from this article can be found in Supplemental Data Set 5. In addition, the sequences in supplemental data sets 2 and 5 can be found in the China National Gene Bank Sequence Archive Database under accession number CNP0003163.

Supplemental data
The following materials are available in the online version of this article.

Supplemental Figure S1. Sampling strategy of the 18th internode of P. edulis (Moso bamboo) shoots during different growth stages.
Supplemental Figure S2. Internode length and growth activities corresponding to the growth day of internode 18 during rapid growth and after rapid growth phase.
Supplemental Figure S3. Cell morphologies of the first 1-cm parts in internode 18 with different lengths.
Supplemental Figure S4. Cell morphologies in different parts of the 14-cm-long internode 18.
Supplemental Figure S5. The corresponding parts between 6- and 14-cm internodes, based on the number of cells in each part.
Supplemental Figure S6. Samples (1–14) used for transcriptome analysis.
Supplemental Figure S7. MapMan annotation analysis of the expressed genes and genes annotated in the P. edulis genome.
Supplemental Figure S8. Cell wall biosynthesis (A) and lignification-related genes (B) in the transcriptome and P. edulis genome.
Supplemental Figure S9. MapMan annotation of the identified 2,548 new genes.
Supplemental Figure S10. Lignin synthesis genes and transcription factors identified in the 2,548 new genes.
Supplemental Figure S11. Growth curves of P. edulis shoots during the rapid growth stage and during normal growth.
Supplemental Figure S12. Treatment with 2,4-D has almost no effect on the promotion of P. edulis seedlings.
Supplemental Figure S13. Some of the growing P. edulis shoots suddenly bend during the rapid growth phase.
Supplemental Figure S14. Internode diameter of P. edulis.
Supplemental Figure S15. Bending force treatment of the 10 cm tall P. edulis seedlings.
Supplemental Figure S16. Average growth rates of individual internodes of B. multiplex shoots (more than three shoots) in the fastest growth stage.
Supplemental Figure S17. Cell morphology in the first (lowermost; 1i-1) and sixth (1i-6) sections of internode 18 with a length of 14 cm.

Supplemental Table S1. Sixteen sampling regions for P. edulis.
Supplemental Table S2. Summary of PacBio Iso-Seq sequences.
Supplemental Table S3. Summary of Illumina RNA sequencing data.
Supplemental Table S4. CKs in the bottom 1-cm segments of 18th internodes of different lengths.
Supplemental Table S5. Genes and primer sets used for RT–qPCR.
Supplemental Data Set 1. Internode length data (1–25) of 510 culms from 17 P. edulis populations.
Supplemental Data Set 2. Nucleotide sequences of the genes that had not been previously identified in the P. edulis genome.
**Supplemental Data Set 3.** Expression data of each gene at different developmental stages.

**Supplemental Data Set 4.** Statistical results.

**Supplemental Data Set 5.** Nucleotide sequences of five cell division-associated genes of *B. multiplex*.

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**Conflict of interest statement.** The authors declare no competing interests.

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