

Multi-omic analysis reveals formation mechanism of square stems of *Chimonobambusa utilis* Keng f.

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Abstract

Different from the normal circular-stem plants, the *Chimonobambusa utilis* presents an unusual square-like culm. Bamboo culms can alter its shape by adjusting the expression patterns of genes for appealing different living conditions. However, underlying mechanism for biomolecular function and regulation patterns of square-like culm is still absurd. Multi-omics analysis can be applied for revealing its molecular biological mechanism. In our study, phenotypic analysis suggested that change of stem wall thickness (STW) showed a trend of corner region (CR) > side region (SR), and fibre wall thickness (WT) showed a trend of SR > CR. It has been showed that there is an intrinsic correlation between Lignin content and cell wall thickness. Based on the anatomical features, FTIR spectroscopy analysis suggested that the relative lignin content at SR was higher than CR. It suggested that the deposition of lignin at CR and SR was different with each other. Therefore, the phenotypic analysis combined with transcriptome and metabolome were performed for identifying differential expressed genes and the relative metabolic pathways. Transcriptome results suggested that lignin biosynthesis related genes were up-regulated at SR. Metabolome results suggested that lignin precursors at SR accumulated more than CR and significantly enriched in phenylpropanoid biosynthesis KEGG pathway. These findings indicate that lignin accumulation that has a great possibility for change, leading to change of lignin content at different circumferential positions accordingly. It is suggested a coherence in phenotypic, chemical components, transcriptome, metabolome analysis. It is concluded that lignin biosynthesis related genes were up-regulated at SR which caused lignin biosynthesis precursors accumulated more at SR, leading to lignin content at SR was higher than at CR. After that, in position of the SR, cells have been enlarged. For those, the stem changed from its morphology from circle to square. The findings manifest the regulation mechanism of lignin related genes on the square-like stem. The analysis method provides an innovative means for revealing the molecular biological mechanism involved in the formation of non-circular plant.

Key Message

The expression of *HCT*, *CCR*, *F5H*, and *PAL* genes regulate lignin accumulation to change cell wall thickness of fibres, leading to the formation of square stems of *Chimonobambusa utilis*.

1. Introduction

Bamboo, like most of vascular plants, generally has cylinder trunk since it is essential to anti-interference of exterior harsh environments such as strong wind, the load of snow, and the severe competition of light. However, the *Chimonobambusa utilis* presents a different geometrical shape of stem, which closer to square or four-angle shape. Then, the species was assigned an informal nomenclature as 'square bamboo' for its "four-angled" culm (Dyer 1885). The underlying reason of this exception is attractive to biological scientists since how to control the geometrical shape of stem or trunk is an important and interesting research target. Besides that, a hyperelliptic formula have been applied for fitting the geometrical constructions of square-like culm to superellipse data (Gielis 2017; Huang et al. 2020). It was suggested that the geometrical character of the square-like culm was meaningful to optimization of

structure of thin-walled tube, which is a useful energy-absorbing component in constructing frame for preventing from collision (Chen et al. 2021). So, an understanding of the exception also has contribution to design protecting system with resisting impact damage function. Therefore, it is essential to explore the knowledge of formation of *Chimonobambusa utilis* stem, namely square-like stem.

As the first step toward understanding a species, anatomical characteristics represented important parameters that reflected the microstructure of natural biomass materials (Jiang, 2020). The anatomical structures of a bamboo culm can be characterized by vascular bundles and fibre morphology (Grosser and Liese, 1971; Lin et al. 2004). Arrangement of typical vascular bundles displayed as 'open' type (Li and Chin, 1960; Jiang et al. 2021), and morphology of fibre changed at different circumferential and radial position in square bamboo (Lin et al. 2004; Jiang et al. 2021). Meanwhile, since the biomolecular function and regulation mechanisms is the fundamental to understand the structure of plant, which started at nano-scale and ended at macro-scale, it is important to revealing the formation mechanism of square-like stem at a molecular level. However, previous work main focused on the phenotypic traits of square bamboo, for example, anatomical characteristics, geometrical structures, but its formation and development mechanism at molecular level remains to be discussed.

The exploration of the gene expression level and molecular mechanism on formation of square-like stems is meaningful to know how square-like stem form and reveal the morphogenetic process (Wang et al. 2018). The application of a multi-omics approach can be applied for investigating the molecular biological mechanism systematically and comprehensively (Hori et al. 2020). By considering the most effective method, analyses of phenotypic characters combined with transcriptome and metabolome has been extensively validated (Kasirajan et al. 2018; Hu et al. 2020a, 2020b). A transcriptome represents the intermediate state of gene expression and offered a relatively holistic approach to interpreting molecular mechanism of plant development (Shi, 2011; Wang et al. 2009). RNA-Seq experiment has been successfully applied to identify transcriptional levels of each expressed gene and drawing generalized conclusions about differential characteristics observed in plant groups (Costa et al. 2010). Metabolites, the final products of gene transcription, formed the material basis of all organismal phenotypes (Oliver et al. 1998; Fiehn et al. 2000; Fiehn 2002; Kopka et al. 2004). It can help us to characterize the activities of cells at the functional level, providing a great theoretical impact on our understanding of metabolic pathways in processes of plant development (Fiehn 2000; Oliver 2002; Cornish-Bowden and Cárdenas 2000). Combined analysis of transcriptome and metabolome can be applied to reveal internal relationship between the regulation mechanism and biomolecular function systematically (Jewett et al. 2005; Conesa et al. 2016). Such approach has been successfully employed in the exploration of stem growth and development of Bambusoideae (Yue 2017; Xu 2017; Wang 2018; Guo 2018; Hu et al. 2020a, 2020b; Yang et al. 2020; Hou et al. 2021). According to transcriptome analysis, abnormal thickening of stem wall in *Phyllostachys edulis* 'Pachyloen' and dwarfing and internode shortening traits in *Phyllostachys heterocycla* 'Pubescens' related candidate genes were identified (Yue 2017; Wang et al. 2018; Zhang 2016; Xu 2017). Nitrogen metabolism pathway and tyrosine metabolism metabolic pathways and their uniquely expressed genes were related to culm wall thickness in *Phyllostachys edulis* 'Pachyloen' (variant of moso bamboo) (Hu et al. 2020a, 2020b). These studies provide a reference for

detecting core genes related to bamboo morphology and anatomical characteristics, and exploring the gene regulation mechanism in the malformation of bamboo stem. However, unfortunately, these approaches haven't been applied to clarify the formation of square bamboo at present.

To investigate the biomolecular function and regulation mechanisms associated with the formation of square stem, *Chimonobambusa utilis* was selected as study material. The stem was divided into eight micro areas in circumferential direction at first. Comprehensive analysis of anatomy, spectroscopy, transcriptome, and metabolome was performed simultaneously at each individual micro area later then. Morphology of fibre and vascular bundles was observed by using the anatomical method, the relative lignin content was detected using Fourier transform infrared (FTIR) spectroscopy, and transcriptomic and metabolomic responses in different circumferential position of the bamboo shoots were analyzed. Finally, the formation mechanism of square-like stems was revealed comprehensively and systematically, through analyzing the link between macro and micro structures, chemical components, expression level of genes, and metabolites. This work identified square-like stem related candidate genes and revealed its formation mechanism from macro-scale to nano-scale, furthermore, it is meaningful to reference to other stem mutant species in vascular plants.

2. Materials And Methods

2.1 Plant materials

Three bamboo shoots with heights of 30cm, 50cm, and 70cm were harvested from Tongzi district, Guizhou, China (106°26'E, 27°57'N), where *Chimonobambusa utilis* is naturally distributed. The bamboo, used in our work, grows at heights between 1200 ~ 2800 m of altitude, with slope of 67.8°~83.5°, annual average temperature of 10.4 °C, annual average rainfall of 1434 mm, and annual sunshine duration of 1092 h in subtropical humid monsoon climate zone. Samples were collected from the sixth internode of three individuals as shown in Fig. 1a. Each sample was set for CR_vs_SR as a comparison group, and CR was set as control group, SR was set as treated group for comparative analysis. Accordingly, samples at height 30 cm, 50 cm, and 70 cm were marked as 3CR_vs_3SR, 5CR_vs_5SR, and 7CR_vs_7SR, respectively.

2.2 Circumferential differential method

Cross-sections of bamboo shoots presented like a square for its four-angled structure (Fig. 1b). According to our previous studies, cross-sections were divided into 'corner region' (CR) and 'side region' (SR) as shown in Fig. 1c. (Jiang et al. 2021). Moreover, each CR and SR position was separated into micro zones for three biological replicates (Fig. 1c).

2.3 Microscopy observation and image analysis

Samples were fixed with formaldehyde 24 hours, embedded in paraffin, and then cut into 4µm-thick slices for determining differences of anatomical structures (Leica RM2265). After that, their microstructures were examined under an HD Stereo Digital Microscope (KEYENCE VHX-600E) in detail (Guerreiro 2013).

Then, 120 fibres were measured for its wall thickness (WT) from each micro zone using Image J software.

2.4 The relative lignin content determination

Samples were oven dried at 60°C for 36 h, then ground into powder for spectrum collection using Fourier transform infrared spectroscopy to detect difference of relative lignin content between the corner and side region (Jayamani et al. 2020).

2.5 RNA extraction, Illumine sequencing, and Quantitative real-time PCR analysis

Each sample was pulverized by Retsch GmbH (42781 Haan, Retsch-Allee 1–5, Germany), and then total RNA was extracted by using TRIzol reagent (Invitrogen) following the manufacturer's instructions preparing for RNA-Seq. Total RNA was then extracted for RNA-Seq. Raw data (raw reads) was filtered to obtain a high-quality read dataset. Q20, Q30, GC-content, and error rate were calculated, and the clean data was then assembled using Trinity software. For q-PCR, the PrimeScript™ RT reagent kit with g DNA Eraser (TaKaRa, Japan) was used to reverse transcribe mRNA into cDNA, and Primer Premier 5 software was used to design the gene-specific primers. The TB Green Premix EX Taq™ (TaKaRa) was used for qRT-PCR reactions on a BioRad CFX96 Real-time PCR System (Bio-Rad, USA). The β -actin gene was used as an internal reference and the gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Primers used in this study are listed in supplementary table S4.

2.6 Non-targeted metabolic profiling

Histologic sections from each group were freeze-dried by a vacuum freeze-dryer (Scientz-100F). Freeze-dried samples were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. Dissolve 100 mg of lyophilized powder with 1.2 ml 70% methanol solution, vortex 30 seconds every 30 minutes for 6 times in total, place the sample in a refrigerator at 4°C overnight. Following centrifugation at 12000 rpm for 10 min, the extracts were filtrated (SCAA-104, 0.22 μ m pore size; ANPEL) before UPLC-MS/MS analysis preparing for metabolites detection. Measurements were taken three times by ultra-high-performance liquid chromatography (Shim-pack UPLC SHIMADZU CBM30A) and tandem mass spectrometry (MS/MS, Applied Biosystems 6500 QTRAP) to obtain mass spectrometric data for metabolite profiling (Metware). MS data for all samples was then qualitatively and quantitatively analyzed using the Metware database (MWDB), a public database of metabolite information.

2.7 Statistical analysis

A one-way analysis of variance (ANOVA) was performed for exploring variation patterns. Pearson correlation coefficients between samples were calculated by the corfunction in R, the probability of hypothesis testing (p -value) is corrected by multiple hypothesis testing using by Benjamini-Hochberg. A OPLS-DA test was examined based on a VIP value ≥ 1 and univariate statistical analysis at $p < 0.05$ were applied for metabolomic profiling. The P value threshold was corrected for all statistical tests using the

FDR, a threshold of $FDR \leq 0.05$ and an absolute fold change ≥ 2.0 were used to signify the significance of gene expression.

3. Results

3.1 Difference of structural characteristics between CR and SR

The bamboo shoots stem displayed a square-like shape, it was found that stem wall thickness (SWT) seems non-uniform at various circumferential regions. SWT was measured for analyzing the difference between the CR and SR. The results showed that SWT at CR and SR was 4.13 mm and 3.68 mm from 3CR_vs_3SR, 3.11 mm and 2.40 mm from 5CR_vs_5SR, and 2.58 mm and 1.96 mm from 7CR_vs_7SR (Table S1). It indicates that SWT showed a trend of $CR > SR$. (Fig. 2a).

Besides that, fibre wall thickness (FWT) was examined for analyzing the difference of fibre morphology between CR and SR. The results showed that FWT at CR and SR was 0.9 μm and 0.7 μm from 3CR_vs_3SR, 0.9 μm and 0.7 μm from 5CR_vs_5SR, 0.8 μm and 0.7 μm from 7CR_vs_7SR (Table S1). It indicates that FWT at the corner and side region was different with each other, and showed a trend of $CR < SR$ (Fig. 2b). Furthermore, a one way ANOVA analysis was applied for comparing its statistical significance. The results suggested that the difference of FWT between CR and SR was significant at 0.05 level from all groups (Fig. 2b and Table S1). The chemical component is another physiological traits, which closely associated with cell wall thickness, so, it gives us a clue to investigate its difference between CR and SR.

3.2 Difference of the relative lignin content between CR and SR

Lignin, one of the main chemical composition of plant tissues, can be detected by FTIR spectroscopy was applied for detecting the relative lignin content, comparing the difference between CR and SR. In the FTIR spectra, the peak located at 1424 cm^{-1} was assigned to the shear vibration of CH_2 in cellulose (Li 2003), which can be used for normalizing the spectra in the analysis of the relative lignin content (Guo et al. 2015; Lei et al. 2021). As shown in Fig. 3, the intensity of lignin-related peak (1600 cm^{-1}) was higher at SR than that of the CR in all groups. It indicates that difference of the relative lignin content existed in circumferential regions. Besides that, by comparing with micro analysis results, a coherence of variation pattern was determined between the microstructure and chemical component, laying on a clue for further analyzing the molecular mechanism of square bamboo shoots.

3.3 Transcriptome profiling

3.3.1 Illumina sequencing and de novo assembly

RNA-Seq analysis was performed for exploring the possible reasons related to square-like stem at the transcriptional level. As a result, a total of 16,102,942,190 raw reads were generated, 1,516,567,890 clean reads after removing low quality sequences (Table S2). Assembly of clean reads produced 142,724 unigenes ranging in sequence length from 200 to 2k bp, 74,056 unigenes with a sequence lengths ranging from 200 to 500 bp, 43,460 unigenes with sequence lengths ranging from 500 to 1k bp, 25,208 unigenes with sequence lengths ranging from 1k to 2k bp, and 12,424 unigenes with sequence lengths greater than 2k bp. The N50 length of these unigenes was 1192 bp (Table S3). It indicates that the sequencing results were of high quality and thus can be used in subsequent analysis.

3.3.2 Quantitative Real-Time PCR (qRT-PCR) validation of differential expression

The expression profiles of 10 randomly chosen genes are provided in Supplementary File table 4. The relative gene expression was calculated using $2^{-\Delta\Delta C_t}$ and amplified using a LightCycler®96 fluorescence quantitative PCR instrument for three biological replicates. Correlation analysis was conducted on the relevance of Log2 FC between the q-PCR and RNA-seq. The result showed that R^2 was 0.9196. These findings indicated that the qRT-PCR verification of the 10 selected genes was consistent with the sequencing results (Fig. 4).

3.3.3 Functional annotation and classification of unigenes

The function of all unigenes was analyzed by annotating and examining their predicted function. The differentially expressed genes (DEGs) between CR and SR were identified. Results showed that 69, 413, and 32 DEGs were differentially transcribed in comparison groups 3CR_vs_3SR, 5CR_vs_5SR, and 7CR_vs_7SR, to maintain the morphologic of square-like stem (Fig. 5a). 52 DEGs were upregulated, whereas 17 were down-regulated in SR from 3CR_vs_3SR. 283 DEGs were upregulated, whereas 130 were down-regulated in SR from 5CR_vs_5SR. 25 DEGs were upregulated, whereas 7 were down-regulated in SR from 7CR_vs_7SR (Table S5).

3.3.4 GO and KEGG enrichment analysis

Gene ontology (GO) enrichment analysis revealed that carboxylic acid catabolic process, organic acid catabolic process, and small molecule catabolic process were highly enriched under biological process, oxidoreductase activity (acting on peroxide as acceptor), phenylalanine ammonia-lyase activity and peroxidase activity were highly enriched under molecular function from 3CR_vs_3SR. Hydrogen peroxide catabolic process, hydrogen peroxide metabolic process, and lignin metabolic process were highly enriched under biological process, protein-DNA complex was highly enriched under cellular component, oxidoreductase activity (acting on peroxide as acceptor) and peroxidase activity were highly enriched under molecular function from 5CR_vs_5SR. Antibiotic catabolic process, cofactor catabolic process, hydrogen peroxide catabolic process, hydrogen peroxide metabolic process were highly enriched under biological process, plant-type vacuole and plant-type vacuole membrane were highly enriched under cellular component, toxin activity, oxidoreductase activity (acting on peroxide as acceptor), and peroxidase activity were highly enriched under molecular function from 7CR_vs_7SR (Fig. 5b, Fig. S1).

Furthermore, KEGG enrichment analyses was applied for elucidating the mechanisms responsible in DEGs between CR and SR. The results showed that DEGs were significantly enriched in phenylpropanoid biosynthesis, phenylalanine metabolism, and flavonoid biosynthesis pathway from 3CR_vs_3SR, in phenylpropanoid biosynthesis pathway and starch and sucrose metabolism from 5CR_vs_5SR, and in phenylpropanoid biosynthesis pathway from 7CR_vs_7SR (Fig. 5c and Fig. S2). Notably, DEGs were significantly enriched in phenylpropanoid biosynthesis from all comparison groups, promoting us to conduct lignin-related metabolic pathway in depth analysis.

3.4 Metabolome profiling

Metabolome analysis showed that a total of 79 compounds were significantly changed in total between CR and SR (Fig. 6a). 9 DAMs were upregulated and 36 were down-regulated in SR from 3CR_vs_3SR, 10 DAMs were upregulated and 8 were down regulated in SR from 5CR_vs_5SR, and 11 DAMs were upregulated and 5 were down regulated in SR from 7CR_vs_7SR (Fig. 6b, Fig. S3). Taking 5CR_vs_5SR as an example, Lmhp007598 (LysoPC 19:2 (2n isomer)), Lmhp007840 (LysoPC 19:2), Zmgn000447 (3-Phospho-D-glyceric acid), mws1350 (Syringaldehyde), pmf0400 (Docosanoic acid), Lmmp003042 (Protocatechuic Acid Methyl Ester), Lmmn001643 (2-Hydroxycinnamic acid), Hmbp001276 (Gallacetophenone), pme3443 (Sinapinaldehyde), and Zmhn001883 (Vanillic acid-4-O-glucoside) were upregulated, pmb0880 (LysoPE 18:0(2n isomer)), HJN037 (4-O-Methylgallic Acid), pmd0160 (LysoPE 16:0(2n isomer)), pme1109 (Guanine), Lmhp010040 (LysoPE 20:3), Lmhp008924 (LysoPC 19:3), pme1021 (D-Glucono-1,5-lactone), and pmd0142 (LysoPC 20:0(2n isomer)) were downregulated in SR from 5CR_vs_5SR (Fig. 6c). KEGG analysis showed that DAMs were significantly enriched in linoleic acid metabolism, purine metabolism, alpha-Linolenic acid metabolism, and phenylpropanoid biosynthesis from 3CR_vs_3SR, DAMs were significantly enriched in phenylpropanoid biosynthesis, pentose phosphate pathway, and carbon metabolism from 5CR_vs_5SR, and DAMs were significantly enriched in linoleic acid metabolism, alpha-Linolenic acid metabolism, flavonoid biosynthesis, and phenylpropanoid biosynthesis pathway from 7CR_vs_7SR (Fig. 6d, Fig. S4). Notably, the phenylpropanoid biosynthesis pathway, involved in lignin biosynthesis, has been enriched from all comparison groups. The results suggest a coherence of enrichment pathway between DEGs and DAMs, serving as a reminder that combing analysis of transcriptome and metabolome is essential.

3.5 Combined analysis of transcriptome and metabolome

The obtained DEGs and DAMs related to phenylpropanoid biosynthesis pathway were analyzed for elucidating the mechanism responsible to the observed differences between the CR and SR (Fig. 7). The results were shown in Fig. 7. The expression level of lignin biosynthesis related genes, such as β -D-Glucosidase (*bglB*), ferulate 5-hydroxylase (*F5H*), cinnamoyl-CoA Reductase (*CCR*), shikimate O-hydroxycinnamoyl transferase (*HCT*) were all upregulated in SR from all comparison groups. Phenylalanine ammonia-lyase (*PAL*) was up-regulated in SR from 3CR_vs_3SR, and down-regulated in SR from 5CR_vs_5SR and 7CR_vs_7SR. Peroxidase (*POD*) was up-regulated in SR from 3CR_vs_3SR and 5CR_vs_5SR, and down-regulated in SR from 7CR_vs_7SR. Lignin biosynthesis related compounds, such as Caffeoyl quinic acid, trans-2-Hydroxy-cinnamate, and sinapaldehyde at SR accumulated higher than

CR from 5CR_vs_5SR. The results suggested that the up-regulated expression of *bglB*, *F5H*, *CCR*, and *HCT* in SR, and highly accumulation of Caffeoyl quinic acid, trans-2-Hydroxy-cinnamate, and sinapaldehyde at SR showed an intrinsic link with square-like stem morphogenesis.

4. Discussion

It is commonly known that plant cell walls can tolerate large variations in lignin content and structure, playing a crucial role in maintaining the stem morphology during the growth and development of bamboo (Zhao et al. 2010; Wang et al. 2010; Wang et al. 2016). Previous studies told that change of cell wall thickness was significantly correlated with the difference of stem morphology (Guo et al. 1999; Xu 2017; Guo et al. 2018; Yang et al. 2020). And lignin deposition, one of the final stages of xylem cell differentiation, will last the whole period during the thickening of the cell wall (Sonbol et al. 2009; Gan et al. 2006; Zhang et al. 2006; Donaldson 2001). So, there is an inherent, positive link between lignin content and cell wall structure. Moreover, previous studies told that lignin biosynthesis related genes, such as expression of the phenylalanine ammonia-lyase (*PAL*), 4-coumarate-CoA ligase (*4CL*), coumarate-3-hydroxylase (*C3H*), ferulate 5-hydroxylase (*F5H*), cinnamoyl-CoA reductase (*CCR*), and *p*-hydroxycinnamoyl-CoA:quinate/shikimate O-hydroxycinnamoyltransferase (*HCT*) genes, which are involved in monolignols biosynthesis and are thought to play critical roles in regulating lignin accumulation, related to variant culm in *Phyllostachys heterocycla* 'Pubescens' (variant of moso bamboo)(Yue 2017; Wang et al. 2018). So, the interactions between genes and phenotypic characters are worth of investigating, and it helps us to reveal the formation mechanism of square-like stem from *Chimonobambusa utilis*.

Therefore, based on the macro and micro structures of *Chimonobambusa utilis*, transcriptomic and metabolomic analysis were performed for comparing differential gene expression on transcriptional level and statistically significant difference on metabolic level between CR and SR from square-like stem here. Transcriptome analysis indicated that *bglB*, *F5H*, *CCR*, and *HCT* genes were upregulated in SR from all comparison groups (Fig. 7). Since lignin biogenesis depends on the expression of genes in phenylpropanoid biosynthesis pathway (Hamberger et al. 2007). Among them, *CCR*, is considered a committed step into monolignol, lignin, and lignin biosynthesis (Costa 2003), knockout of the *CCR*-genes showed a reduced in lignin formation and sinapoylmalate accumulation (Mir Derikvand et al. 2008). In addition to the entry enzymes into lignin biosynthesis, overexpression of *F5H* displayed a critical role for the structure and physical properties of lignin, and knock-down of *f5h* gene revealed lignin changes (Stewart et al. 2009). so up-regulated *CCR* and *F5H* may cause SR accumulated higher lignin than CR. Metabolome analysis indicated that relative quantity of sinapaldehyde showed a trend of SR > CR (Fig. 7). The accumulation of sinapaldehyde affects lignin biosynthesis directly, ultimately the lignin content (Mir Derikvand et al. 2008). Furthermore, FTIR spectroscopy analysis has shown that the relative contents of lignin at SR was higher than those of CR (Fig. 3). Besides that, lignin plays an important role in maintaining normal cell morphology in cell wall structure (Nolin 2009), lack of lignin deposition can lead to less thickening of fibre wall (Liu et al. 2017; Su et al. 2018). Difference of lignin content in our work may cause fibre cell wall at CR and SR become different with each other. Anatomical analysis

suggested that fibre cell wall at SR was thicker than that of the CR (Fig. 2). These results corresponds with the previous studies (Mir Derikvand et al. 2008; Stewart et al. 2009; Guo et al. 1999; Xu 2017; Guo et al. 2018; Yang et al. 2020).

Therefore, it can be predicted that square bamboo accumulated intermediated product-sinapaldehyde and end product-lignin at SR in response to biotic and harmful environmental stresses, making great changes in cell walls between CR and SR along with the formation and lignification of the secondary wall. These may cause stem wall thickness change between CR and SR accordingly, and can ultimately lead to a mutation in the square-like stem of *Chimonobambusa utilis* (Fig. 8).

5. Conclusion

Chimonobambusa utilis has a special square-like stems. Macro and micro structures were rigorously investigated in order to compare the difference of phenotypic traits from CR and SR. The results suggested a significant difference in stem wall thickness and fibre wall thickness between CR and SR. Besides that, the relative content of lignin, closely related to cell wall structure, was detected by FTIR spectroscopy. The results indicated that lignin at SR accumulated higher than that of CR. Moreover, transcriptome and metabolite profiling were performed for revealing the mechanism underlying biological processes that cause the square-like stem. The results showed that lignin biosynthesis related DEGs were up-regulated in SR, and sinapaldehyde, syringyl lignin precursors, also accumulated higher at SR than those of CR. Both of DEGs and DAMs were enriched in lignin biosynthesis pathway. These results suggested that DEGs may influence the lignin accumulation, causing the difference of fibre wall thickness, and ultimately lead to the formation of square-like stems. Taken together, our findings allowed us to refine the understanding of the mechanisms responsible for the square-like stem morphology on the molecular level.

Declarations

Declaration of Competing Interest

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

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Author's contributions

The corresponding authors contributed equally to this article. We acknowledge all authors for their helpful works. S.Q.L. participated in the original screening and research plans; L.Z. supervised the writing. Q.Q.J and W.L.G. performed the experiments based on molecular biology methods, designed the experiments and analyzed the data, and conceived the project and wrote the article with contributions of all the authors. Z.C.D. provided sample assistance to Q.Q.J; C.Q.L. and Y.P.Z. assisted the experiments; M.Y. and J.L.G. supervised the experiments; S.Q.L. agrees to serve as the author responsible for contact and ensures communication. All authors approved the final version of the manuscript for publication.

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References

1. Barros J, Serrani-Yarce J, Chen F et al (2016) Role of bifunctional ammonia-lyase in grass cell wall biosynthesis. *Nat Plants* 2:16050. <https://doi.org/10.1038/nplants.2016.50>
2. Boerjan W, Ralph J, Baucher M (2003) Lignin Biosynthesis. *Plant Biol* 54:519–546. <https://doi.org/10.1146/annurev.arplant.54.031902.134938>
3. Chen L-G, Guo X-J, Cui Y-Z, Zheng X-G, Yang H-Q (2018) Comparative Transcriptome Analysis Reveals Hormone Signaling Genes Involved in the Launch of Culm-Shape Differentiation in *Dendrocalamus sinicus*. *Genes* 9(1):1–14. [10.3390/genes9010004](https://doi.org/10.3390/genes9010004)
4. Chen M, Zheng L, Zheng Y, Lin Z (2017) Research on flexural behaviour of curved concrete filled steel tubular members. *Steel Constr* 32(09):52–56. [10.13206/j.gjg201709010](https://doi.org/10.13206/j.gjg201709010)
5. Chen W, Gong L, Guo Z-L, Wang W-S, Zhang H-Y, Liu X-Q, Yu S-B, Xiong L-Z, Luo J (2013) A Novel Integrated Method for Large-Scale Detection, Identification, and Quantification of Widely Targeted Metabolites: Application in the Study of Rice Metabolomics. *Mol Plant* 6(6):1769–1780
6. <https://doi.org/10.1093/mp/sst080>
7. Chen X-W, Ma B, Chen Y (2021) Analysis and optimization of crashworthiness of the thin-walled tube based on the square bamboo structure. *J Mach Des* 38(5). [10.13841/j.cnki.jxsj.2021.05.008](https://doi.org/10.13841/j.cnki.jxsj.2021.05.008)
8. Conesa A, Pedro M, Sonia T, Gomez C-D, Alejandra C, McPherson M, Wojciech S-M et al (2016) A Survey of Best Practices for RNA-Seq Data Analysis. *Genome Biol* 17(1):13. <https://doi.org/10.1186/s13059-016-0881-8>

9. Cornish B-A, Cárdenas M-L (2000) From genome to cellular phenotype-a role for metabolic flux analysis? *Nat Biotechnol* 18:267–268. 10.1038/73696
10. Costa M-A, Collins R-E, Anterola A-M, Cochrane Laurence F-C, Davin L-B, Lewis N-G (2003) An in silico assessment of gene function and organization of the phenylpropanoid pathway metabolic networks in *Arabidopsis thaliana* and limitations thereof. *Phytochemistry* 64(6):1097–1112
11. [https://doi.org/10.1016/S0031-9422\(03\)00517-X](https://doi.org/10.1016/S0031-9422(03)00517-X)
12. Costa V, Angelini C-D et al (2010) Uncovering the complexity of transcriptomes with RNA-Seq. *Biomed Res Int*. <https://doi.org/10.1155/2010/853916>
13. D'Auria J-C (2006) Acyltransferases in plants: a good time to be BAHD. *Plant Biol* 9(3):331–340. <https://doi.org/10.1016/j.pbi.2006.03.016>
14. Donaldson L-A (2001) Lignification and lignin topochemistry-an ultrastructural view. *Phytochemistry* 57(6):859–873. [https://doi.org/10.1016/S0031-9422\(01\)00049-8](https://doi.org/10.1016/S0031-9422(01)00049-8)
15. Dyer T-W (1885) The Square Bamboo. *Nature* 32:391–392. <https://doi.org/10.1038/032391d0>
16. Fiehn O (2002) Metabolomics – the link between genotypes and phenotypes. In: Town C. (eds) *Functional Genomics* 155–171. https://doi.org/10.1007/978-94-010-0448-0_11
17. Fiehn O, Kopka J, Dörmann P et al (2000) Metabolite profiling for plant functional genomics. *Nat Biotechnol* 18:1157–1161. <https://doi.org/10.1038/81137>
18. Gan X-G, Ding Y-L (2006) Investigation on the variation of fiber wall in *Phyllostachys edulis* culms. *For Res* 19(4):6
19. Gielis J (2017) *Lamé Curves and Surfaces [M]*. Atlantis Press
20. Grosser D, Liese W (1971) On the anatomy of Asian bamboos, with special reference to their vascular bundles. *Wood Sci Technol* 5(4):290–312. DOI: 10.1007/BF00365061
21. Guerreiro C, Rodríguez M-F, de Agrasar Z-E-R (2013) Culm anatomy: a contribution to the identification of vegetative Andean woody bamboos in southernmost America. *Kew Bull* 68:209–218. <https://doi.org/10.1007/s12225-013-9444-2>
22. Guo J, Yin Y-F et al (2015) Changes of wood cell walls in response to hygro-mechanical steam treatment. *Carbohydr Polym* 115:207–214. 10.1016/j.carbpol.2014.08.040
23. Guo Q-R, Yang G-Y, Hu Y-B et al (1999) The fiber morphology of *Phyllostachys heterocycla* cv. *Pachyloen* *Acta Agriculturae Universitatis Jiangxiensis* 21(2):3
24. Guo X (2018) Primary study on molecular mechanism of the culm-shape differentiation in *Dendrocalamus sinicus*. *Chinese academy of forestry*
25. Hamberger B, Ellis M, Friedmann M, Souza C-D-A, Barbazuk B, Douglas C-J (2007) Genome-wide analyses of phenylpropanoid-related genes in *Populus trichocarpa*, *Arabidopsis thaliana*, and *Oryza sativa*: the *Populus* lignin toolbox and conservation and diversification of angiosperm gene families. *Can J Bot* 85(12):1182–1201. <https://doi.org/10.1139/B07-098>
26. Hoffmann L, Besseau S, Geoffroy P, Ritzenthaler C, Meyer D, Catherine L, Pollet B, Legrand M (2004) Silencing of Hydroxycinnamoyl-Coenzyme A Shikimate/Quinate Hydroxycinnamoyltransferase

- Affects Phenylpropanoid Biosynthesis. *Plant Cell* 16:1446–1465. 10.1105/tpc.020297
27. Hori C, Takata N, Lam P-Y et al (2020) Identifying transcription factors that reduce wood recalcitrance and improve enzymatic degradation of xylem cell wall in *Populus*. *Sci Rep* 10:22043. <https://doi.org/10.1038/s41598-020-78781-6>
 28. Hou D, Lu H-W, Zhao Z-Y, Pei J-L, Yu X-J, Lin X-C et al (2021) Integrative transcriptomic and metabolomic data provide insights into gene networks associated with lignification in postharvest lei bamboo shoots under low temperature. *Food Chemistry*
 29. <https://doi.org/10.1016/j.foodchem.2021.130822>
 30. Hu Y-P et al (2020a) Transcriptome Reveals the Specificity of *Phyllostachys edulis* ‘Pachyloen’ shoots at different Development Stages, *Forests*
 31. Hu Y-P et al (2020b) Gen Set Subtraction Reveals 633 Candidate Genes for Bamboo Culm Wall Thickening, *Forests*
 32. Huang W-W, Li Y, Niklas K-J, Gielis J, Ding YL, Cao L, Shi P-J (2020) A Superellipse with Deformation and Its Application in Describing the Cross-Sectional Shapes of a Square Bamboo. *Symmetry* 12(12). 10.3390/SYM12122073
 33. Jayamani E, Loong T-G, Bakri M-K-B (2020) Comparative study of Fourier transform infrared spectroscopy (FTIR) analysis of natural fibres treated with chemical, physical and biological methods. *Polym Bull* 77:1605–1629. <https://doi.org/10.1007/s00289-019-02824-w>
 34. Jewett M-C, Oliveira A-P, Patil K-R et al (2005) The role of high-throughput transcriptome analysis in metabolic engineering. *Biotechnol Bioprocess Eng* 10:385. <https://doi.org/10.1007/BF02989821>
 35. Jiang Q-Q, Ding Z-C, Lu C-Q, Gao J-L, Yan Y, Liu S-Q (2021) Anatomical characterization of *Chimonobambusa quadrangularis* based on circumferential and radial variation patterns in cross-sections. *IAWA J*. <https://doi.org/10.1163/22941932-bja10071>
 36. Jiang Z-H (2020) Research advances in bamboo anatomy. *World Forestry Research* 33(03):1–6. 10.13348/j.cnki.sjllyj.2020.0036.y
 37. Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 27(1):29–34. <https://doi.org/10.1093/nar/27.1.29>
 38. Kasirajan L, Hoang N-V, Furtado A et al (2018) Transcriptome analysis highlights key differentially expressed genes involved in cellulose and lignin biosynthesis of sugarcane genotypes varying in fiber content. *Sci Rep* 8:11612. <https://doi.org/10.1038/s41598-018-30033-4>
 39. Kopka J, Fernie A, Weckwerth W et al (2004) Metabolite profiling in plant biology: platforms and destinations. *Genome Biol* 5:109. <https://doi.org/10.1186/gb-2004-5-6-109>
 40. Lei W, Zhang Y, Yu W et al (2021) The adsorption and desorption characteristics of moso bamboo induced by heat treatment. *J Forestry Eng* 6(3):41–46. Doi:10.13360/j.issn.2096-1359.202010008
 41. Li J (2003) Wood spectroscopy [M]. Science Press, Beijing
 42. Li Z, Chin Z (1960) Anatomical studies of some Chinese bamboos. *Acta Bot Sin* 9(1):76–97. <https://www.jipb.net/EN/Y1960/V9/I1/>

43. Lin J, Lin Y, Lai G, Dong J (2004) A study on the law of variation in fiber morphology of *Chimonobambusa quadrangularis* Culm-wood. J Acta Agriculturae Universitatis Jiangxiensis 26(01):56–58. 10.1109/JLT.2003.821766
44. Liu C-W, Su M-L, Wang Y-R, Zhao R-J (2018) Advances in the effects of genetic regulation of lignin on chemical components and microstructures of wood cell walls. J Northwest Sci Tech Univ Agric Forestry 46(2):8
45. Liu W, Zhang L, Cui X, Li W, Zhang W, Wang Y (2018) Pyrolysis of *Chimonobambusa utilis* (Keng) Keng f. and characteristics of its carbonization products. J Bamboo Res 37(3):85–92. 10.19560/j.cnki.issn1000-6567.2018.03.014
46. Mir Derikvand M, Sierra J-B, Ruel K, Pollet B, Do C-T, Thévenin J, Buffard D, Jouanin L, Lapierre C (2008) Redirection of the phenylpropanoid pathway to feruloyl malate in Arabidopsis mutants deficient for cinnamoyl-CoA reductase 1. Planta 227:943–956. <https://doi.org/10.1007/s00425-007-0669-x>
47. Nolin F (2009) Caffeoyl coenzyme A O-methyltransferase down-regulation is associated with modifications in lignin and cell-wall architecture in flax secondary xylem. Plant Physiol Biochem 47(1):9–19
48. Ogita S, Nomura T, Kato Y et al (2018) Transcriptional alterations during proliferation and lignification in *Phyllostachys nigra* cells. Sci Rep 8:11347. <https://doi.org/10.1038/s41598-018-29645-7>
49. Oliver S-G, Winson M-K, Kell D-B, Baganz F (1998) Systematic functional analysis of the yeast genome. Trends Biotechnol 16(9):373–378. [https://doi.org/10.1016/S0167-7799\(98\)01214-1](https://doi.org/10.1016/S0167-7799(98)01214-1)
50. Orangi S, Abrinia K, Bihamta R (2011) Process Parameter Investigations of Backward Extrusion for Various Aluminum Shaped Section Tubes Using FEM Analysis. Materi Eng and Perform 20:40–47. <https://doi.org/10.1007/s11665-010-9655-8>
51. Sewalt V-J-H, Ni W, Blount J-W, Jung H-G, Masoud S-A, Howles P-A, Lamb C, Dixon R-A (1997) Reduced lignin content and altered lignin composition in transgenic tobacco down-regulated in expression of L-phenylalanine Ammonia-Lyase or Cinnamate 4-Hydroxylase. Plant Physiol 115(1):41–50. <https://doi.org/10.1104/pp.115.1.41>
52. Shi J, Zhang Y, Zhou D, Melissa, Yao J (2017) A review of bamboo cultivar from *Chimonobambusa* in the world. J World Bamboo and Rattan 15(06):41–48. DOI: 10.13640/j.cnki.wbr.2017.06.012
53. Shi S-B (2011) Advancing transcriptome platforms. *Nature Proceedings*. Doi: 10.1038/npre.2011.5894
54. Sonbol F-M, Fornalé S, Capellades M et al (2009) The maize ZmMYB42 represses the phenylpropanoid pathway and affects the cell wall structure, composition and degradability in Arabidopsis thaliana. Plant Mol Biol 70:283. <https://doi.org/10.1007/s11103-009-9473-2>
55. Jaclyn J, Stewart, Akiyama T, Chapple C, Ralph J, Mansfield S-D (2009) The Effects on Lignin Structure of Overexpression of Ferulate 5-Hydroxylase in Hybrid Poplar1. Plant Physiol 150(2):621–635. <https://doi.org/10.1104/pp.109.137059>

56. Stewart JJ, Kadla J-F, Mansfield S-D (2006) The influence of lignin chemistry and ultrastructure on the pulping efficiency of clonal aspen (*Populus tremuloides* Michx.). *Holzforschung* 60(2):111–122. <https://doi.org/10.1515/HF.2006.019>
57. Su M-L, Sun H-Y, Jia R, Wang Y-R (2019) The effects of genetic engineering on growth phenotypes, cell wall components and structures of forest trees. *For Prod Ind* 46(03):1–5
58. Taylor-Teeples M, Lin L, de Lucas M et al (2015) An Arabidopsis gene regulatory network for secondary cell wall synthesis. *Nature* 517:571. <https://doi.org/10.1038/nature14099>
59. Vogt T (2010) Phenylpropanoid biosynthesis. *Mol Plant* 3(1):2–20
60. <https://doi.org/10.1093/mp/ssp106>
61. Wang T, Liu L, Wang X, Liang L, Yue J, Li L (2018) Comparative Analyses of Anatomical Structure, Phytohormone Levels, and Gene Expression Profiles Reveal Potential Dwarfing Mechanisms in Shengyin Bamboo (*Phyllostachys edulis* f. *tubaeformis*). *Int J Mol Sci* 19(6):1697. <https://doi.org/10.3390/ijms19061697>
62. Wang H-Z, Yang J-H, Chen F, Torres-Jerez I (2016) Transcriptome analysis of secondary cell wall development in *Medicago truncatula*. *BMC Genomics* 17(1):23. <https://doi.org/10.1186/s12864-015-2330-6>
63. Wang X, Zhang R, Shi Z et al (2019) Multi-omics analysis of the development and fracture resistance for maize internode. *Sci Rep* 9:8183. <https://doi.org/10.1038/s41598-019-44690-6>
64. Wang X-X, Cao F, Xue Y-C (2009) Cloning and sequence analysis of lignin biosynthetic enzyme hct gene in Poplar. *Journal of Liaoning Forestry Science & Technology*
65. http://en.cnki.com.cn/Article_en/CJFDTOTAL-LNLK200906005.htm
66. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: A Revolutionary Tool for Transcriptomics. *Nat Rev Genet* 10(1):57–63. <https://doi.org/10.1038/nrg2484>
67. Xu T-T (2017) The cytological mechanism of internode elongation during the rapid elongation growth in *Phyllostachys edulis* ‘Pachyloen’ culms [D]. Jiangxi Agricultural University
68. Yang B, Shen Z, Li Z, Li Y, Shen X, Chen Y (2020) A Study on the Stalk Factor and Fiber Traits and Their Correlation of *Phyllostachys edulis* ‘Pachyloen’. *Acta Agriculturae Universitatis Jiangxiensis* 42(1)
69. Yue J-J (2017) Study on the Regulation of Culm Type in *Phyllostachys edulis* f. *tubaeformis*. *Chinese academy of forestry*
70. Zhang H, Ying Y-Q, Wang J et al (2018) Transcriptome analysis provides insights into xylogenesis formation in Moso bamboo (*Phyllostachys edulis*) shoot. *Sci Rep* 8:3951. <https://doi.org/10.1038/s41598-018-21766-3>
71. Zhang X-Y, Guo A-P, He L-K, Kong H (2006) Advances in study of lignin biosynthesis and its genetic manipulation. *Mol Plant Breed* 4(3):7
72. Zhang Y (2016) Transcriptome analysis of *Phyllostachys edulis* ‘Pachyloen’ from shoot to young culm [D]. Chinese Academy of Forestry Sciences

73. Zhang Y, Tian G, Lu H-P, Ran H, Hu T, Guo Q-R (2015) Transcriptome Characterization of *Phyllostachys edulis* 'Pachyloen' Shoots in Different Solar Terms. *Acta Agriculturae Universitatis Jiangxiensis* 37(3):466–474. 10.13836/j.jjau.2015073
74. Zhao Q et al (2010) Syringyl lignin biosynthesis is directly regulated by a secondary cell wall master switch. *Proc Natl Acad Sci* 107:14496–14501. <https://doi.org/10.1073/pnas.1009170107>
75. Zheng Y, He C, Zheng L (2016) Flexural behaviour and practical calculation of load bearing capacity for composite sectioned square concrete filled steel Tube reinforced by inner circular steel tube. *Build Sci* 32(05):34–38. 10.13614/j.cnki.11-1962/tu.2016.05.006
76. Zhu H-F, Yao X-S (1964) Studies on the fiber structure of 33 Chinese bamboos available for pulp manufacture. *J Sci Forestry J* 4:33–53

Figures

Figure 1

Samples of bamboo shoots at different height

Note: CR: Corner regions, SR: Side regions, Star: sampling position.

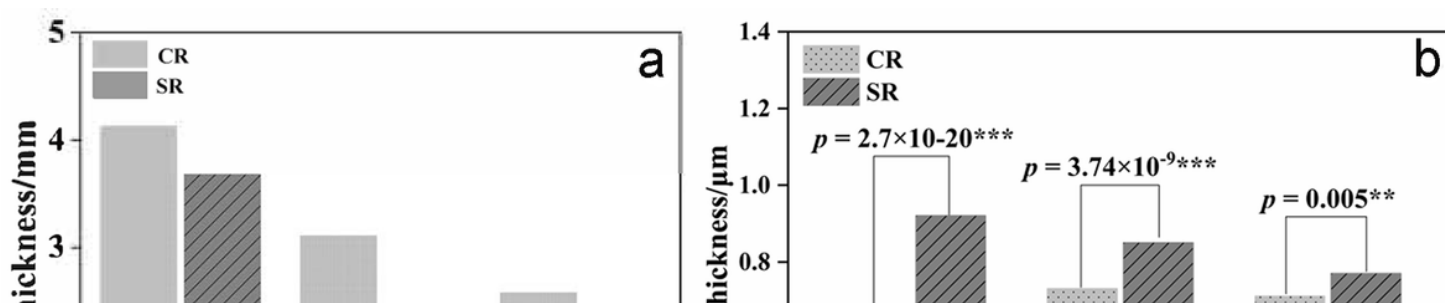


Figure 2

Bar graphs showing difference of SWT and FWT between CR and SR

Note: A, difference of SWT. B, difference of FWT. SWT: stem wall thickness, FWT: fibre wall thickness. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

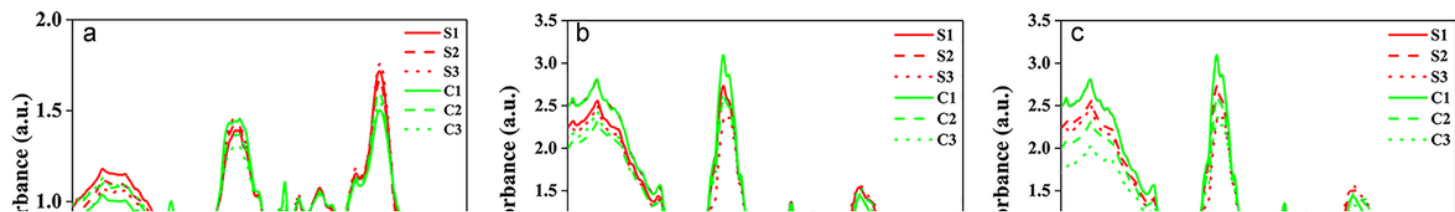


Figure 3

FTIR spectra from the corner and side regions of the square bamboo shoots

Note: A, FTIR spectra from 3CR_vs_3SR. B, FTIR spectra from 5CR_vs_5SR. C, FTIR spectra from 7CR_vs_7SR. C1, C2, C3: micro corner zones, S1, S2, S3: micro side zones.

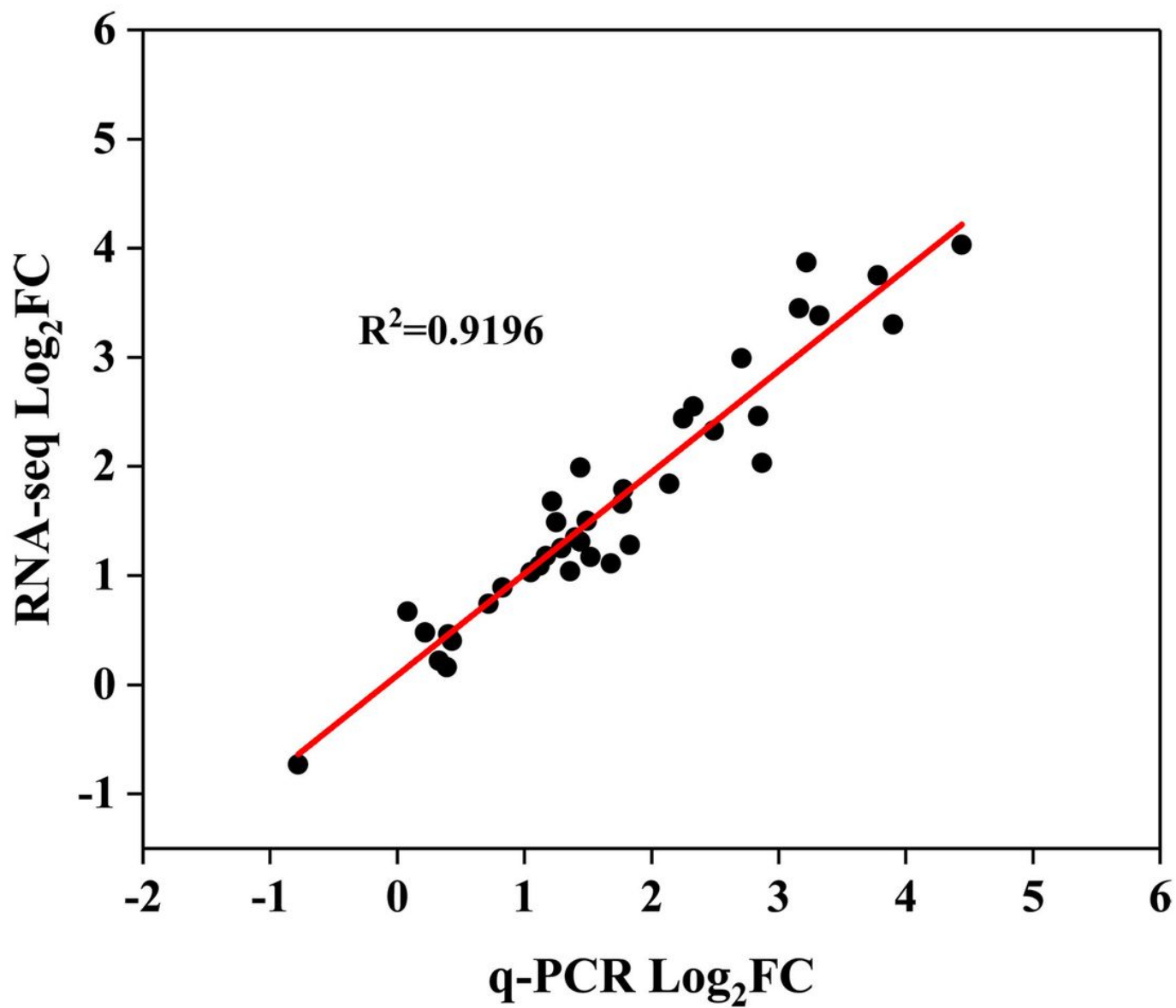


Figure 4

The correlation plot of Fold change of relative gene expression levels between q-PCR and RNA-Seq.

Figure 7

KEGG Co-annotation pathway and correlation network of DAMs and DEGs

Note: *PAL*: phenylalanine ammonia lyase; *bglB*: β -D-Glucosidase; *CCR*: cinnamate-4-hydroxylase; *HCT*: shikimate O-hydroxycinnamoyl transferase; *POD*: peroxidase; *F5H*: ferulate 5 hydroxylase.

Figure 8

Prediction of the developmental regulation process of square-like stems

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