Effects of shading on lignin biosynthesis in the leaf of tea plant (*Camellia sinensis* (L.) O. Kuntze)

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Research article

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Abstract

Background

Lignin is one of the most important secondary metabolite that play vital functions in plant growth and development. Shading is an effective method to improve tea quality. However, little is known about the relationship between shading and xylogenesis in tea plant.

Results

In this study, tea plant cultivar 'Longjing 43' was treated with no shading (S0), 40% (S1) and 80% (S2) shading treatments. The leaf area and lignin content of tea plant leaves decreased under shading treatments (especially S2). The lignin distribution and anatomical characteristics of the tea plant leaves showed that lignin is mainly distributed in the xylem. Promoter analysis indicated that the genes involved in lignin pathway contain several light recognition elements. The transcript abundances of 12 lignin-associated genes were altered under shading treatments. Correlation analysis indicated that the most genes showed strong positive correlation with lignin content, and *CsPAL*, *Cs4CL*, *CsF5H*, and *CsLAC* exhibited significant positively correlation under 40% and 80% shading treatments.

Conclusions

In this study, we evaluated the effect of shading on leaves growth, xylem development, and lignin accumulation in tea plant. Shading treatment inhibited leaves growth and lignin content. Safranin-O/fast green staining and autofluorescence indicated lignin was mostly deposited in the xylem. Shading may affect tea plant leaves lignification by regulating the transcript levels of lignin biosynthesis genes. This study provided potentially useful information for understanding the mechanism of lignin biosynthesis under shading treatments in tea plant.

Background

Light is an important factor that regulates plant growth and development. The light radiation could affects plant plant morphogenesis, photosynthesis, and phototropism [1, 2], which established itself as the driving force of the photosynthetic processes that convert carbon dioxide into organic products [3]. The physiological and molecular basis of light-induced process has been well elaborated [4]. The mechanism of light radiation regulates phenylpropanoid biosynthesis has been reported in some model plants [5-8]. Many metabolites are derived from downstream branches of the phenylpropanoid pathway, including lignin.

Lignin is a widely exist phenolic biopolymer in plants, which constitute the second most abundant organic compound after cellulose [9, 10]. Lignin and its related metabolism play critical roles in the growth and development of plants. Lignin plays an important role in promotes water transport, enhances plant cell wall rigidity, and also involves in plant pathogen defense and various environmental stresses

[11-14]. The synthesis of lignin monomers occurs through a sequence of aromatic hydroxylation and O-methylation reactions. Generally, the lignin polymers are primarily derived from mainly three monomers: coniferyl alcohol (G unit), p-coumaryl alcohol (H unit), and sinapyl alcohol (S unit) [15]. Lignin biosynthetic pathways have been elucidated in previous studies, and the corresponding enzymes and genes participating in the metabolic pathway of lignin biosynthesis have been elucidated (Fig. 1) [16, 17]. Phenylalanine ammonia lyase (PAL), 4-coumarate-CoA ligase (4CL), and cinnamate 4-hydroxylase (C4H) participated in general phenylpropanoid pathway [18, 19]. Hydroxycinnamoyl-CoA transferase (HCT), 4-coumarate 3-hydroxylase (C3'H), caffeoyl-CoA O-methyltransferase (CCoAOMT), ferulate 5-hydroxylase (F5H), caffeic acid O-methyltransferase (COMT), cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), peroxidase (PER) and laccase (LAC) participated in specific lignin pathways [20, 21].

Tea plant (*Camellia sinensis* (L.) O. Ktze), an important economic crop species of theaceae, is widely grown in the world, which provides raw materials to produce non-alcoholic beverage "tea". Tea leaf is rich in flavonoid, theanine, vitamins, polysaccharides, and minerals [22, 23]. A large number of studies have proved that tea flavonoids (especially catechins) are beneficial to human health. Studies have shown that shading treatment can effectively reduce photoinhibition and improve the quality of tea beverages [24, 25]. Tea plants grown in the shading conditions show higher amino acid contents but lower catechins [26]. Theanine is a main and special kind of amino acid components in tea, which mainly contributes to sweetness and umami, whereas catechins and caffeine contribute to astringency. In the past years, studies on the effects of light conditions on the synthesis of tea plant secondary metabolites mainly focus on the biosynthesis of flavonoids [27-30]. However, few researches have been reported on the effect of shading treatment on the molecular mechanisms of lignin synthesis in tea plant.

'Longjing 43', originating in Hangzhou city, Zhejiang Province, China, is a well-known diploid tea plant cultivar ($2n=30$) due to its high yield as well as for its high quality. This study aims to investigate the effects of shading treatments on morphological, lignin accumulation, anatomical characteristics, and expression profiles of lignin-related genes in the leaves of tea plant cultivar 'Longjing 43'. The result showed that shading treatments reduce lignin content and leaf area of the leaves in tea plant. The transcripts of lignin biosynthesis genes were altered under shading treatments. This study may serve as a guide for further elucidating of the molecular mechanisms of light influences the lignin biosynthetic pathways in the tea plant.

Results

Phenotypic changes of tea plant leaves

Tea shoots of 'Longjing 43' at 3, 6, and 9 days after shading treatments (S0, S1, and S2) were harvested and investigated (Fig. 2). Under shading treatments, the color of leaves seems more green, and the leaves are softer, especially in the S2 treatment. Leaf area increased during leaf development, and leaf areas of

S0 are obvious larger than that in shading treatments, S1 and S2 (Fig. 3). The leaf areas at 3, 6, and 9 days of S0 are 1.2-, 1.5-, and 1.5-folds of S1, respectively, and 1.5-, 2.2-, and 2.5-folds of S2.

Lignin content levels in tea plant leaves under shading treatments

Tea plant leaves with shading treatments were collected and analyzed for lignin content (Fig. 4). With the growth and development of tea plant, lignin accumulation of the leaves showed an upward trend in S0 and S1, while, the change was not significant in S2. The lignin contents were obvious higher in S0 than that in S1 and S2 at 9 days. The content of lignin decreased with increasing shading ($S0 > S1 > S2$). The results showed that shading might have a negative influence on lignin accumulation in tea plant leaves.

Anatomical structure analysis of tea plant leaves under shading treatments

To further understand the effect of shading on lignin levels in tea plant, the anatomical structure of tea plant leaves was investigated. The transverse sections of leaves were obtained and stained with safranin-*O* and fast green to highlight the basic anatomical structure of tea plant leaves (Fig. 5). The lignin in the tea plant leaves was mainly distributed in the secondary walls of the xylem region. Compared with control, the lignification of the xylem of leaves under 80% shading treatment appeared to be declining at 9d. The transverse sections were placed under UV excitation to determine the effect of shading treatments on tea plant leaves (Fig. 6). Lignin was also found in the xylem region of tea plant leaves, which was consistent with the observation in Fig. 5.

Analysis of the promoter regions of the genes involved in the lignin pathway in tea plant

The sequences of *CsPAL*, *CsC4H*, *Cs4CL*, *CsHCT*, *CsC3'H*, *CsCCoAOMT*, *CsF5H*, *CsCOMT*, *CsCCR*, *CsCAD*, *CsPER*, and *CsLAC*, 2000 bp upstream of the transcription start site were analyzed to understand the regulatory mechanisms that control the expression of these genes.

As shown in Table 2 and Supplementary Table 1, each gene involved in the lignin pathway contain several light-responsive elements. For example, Box 4 elements appear at the promoters of all lignin pathway genes. G-box, GT1-motif, and TCT-motif are also light-responsive elements, and present in most lignin pathway genes. Those results suggested that the genes involved in the lignin pathway might be regulated by light.

Other *cis*-regulatory elements, such as the TGA-element, CGTCA-motif, ABRE, ERE, STRE, W box, ARE, are present in most of genes involved in the lignin pathway (Supplementary Table 1). These elements are related to the signal pathways of methyl jasmonic acid (MeJA), ethylene (Eth), abscisic acid (ABA), anaerobic, and other biotic and abiotic stresses. The results indicated that expression of those genes in lignin pathway may also involved in the response to hormones, biotic and abiotic stresses.

Interaction network of the proteins involved in the lignin pathway in tea plant

To better understand the interactions among the structural genes involved in the lignin pathway in tea plant, an interaction network was predicted using STRING software basis on the orthologs in Arabidopsis (Fig. 7). Most of the proteins could interacted with more than eight proteins, and OPCL1 (Cs4CL) interacted with all of the other members. LAC3 (CsLAC) and RCI3 (CsPER) are the necessary enzymes for lignin polymerization, which interacted with the least proteins. LAC3 (CsLAC) only interacted with CYP84A1 (CsF5H), CYP98A3 (CsC3'H), RCI3 (CsPER), and CCoAOMT1 (CsCCoAOMT); RCI3 (CsPER) interacted with CYP84A1 (CsF5H), OMT1 (CsCOMT), ATCAD4 (CsCAD), LAC3 (CsLAC) and CCoAOMT1 (CsCCoAOMT).

Expression patterns of the genes involved in the lignin pathway under shading treatments

To further investigate the effect of shading treatments on lignin pathway, the expression profiles of the 12 lignin biosynthetic related genes were selected for RT-qPCR. *CsPAL*, *CsC4H*, and *Cs4CL*, participated in the general phenylpropanoid pathway. *CsHCT*, *CsC3'H*, *CsCCoAOMT*, *CsF5H*, *CsCOMT*, *CsCCR*, *CsCAD*, *CsPER*, and *CsLAC*, were involved in the specific lignin pathway.

General phenylpropanoid pathway The expression levels of *CsPAL*, *CsC4H*, and *Cs4CL* were gradually increased in leaves during tea plant growth under S0 and S1 treatments (Fig. 8). The trends of *Cs4CL* was decreased with increasing shading (S0 > S1 > S2). The expression profiles of *CsPAL* and *CsC4H* were obvious decreased in S2 compared with S0 (control), while they were increased at 9 d in S1.

Specific lignin pathway With the growth and development of the leaves in tea plant, the expression profiles of all specific lignin related genes showed an upward trend in S0 and S1, whereas *CsCCoAOMT*, *CsCOMT*, and *CsLAC* were reduced significantly in S2 treatment (Fig. 9). Most genes showed slightly higher expression levels in S0 compared with S1 treated group. The expression profiles of *CsHCT*, *CsF5H*, *CsCCR*, *CsPER*, and *CsLAC* were obvious decreased in S2 compared with that in S0. *CsC3'H*, and *CsCAD*, showed a similar trend, which is, declining in 3 d and 6 d, while increasing in 9 d with increasing shading.

Correlation analysis of lignin content and expression levels of related genes

The correlation coefficients were calculated by correlating lignin content with expression levels of genes in the lignin pathway by Pearson analysis (Fig. 10 and Table 3). The correlations were calculated from the 40% shading treatment (S0&S1), 80% shading treatment (S0&S2), and the whole shading treatments (S0&S1&S2). The expression levels of all lignin related genes were positively correlated with the lignin content in S0&S1, S0&S2, and S0&S1&S2.

Among the genes, the expression profiles of *CsPAL*, *Cs4CL*, *CsF5H*, and *CsLAC* exhibited significant positively correlation with lignin content in S0&S1, S0&S2, and S0&S1&S2. *CsHCT* expression was significant correlated with lignin content in S0&S2 and S0&S1&S2; while, *CsCOMT* was significant correlated with lignin content in S0&S1 and S0&S1&S2. In addition, the gene expression profiles of *CsCCoAOMT* showed significant positive correlation with the lignin content in S0&S1, whereas *Cs4CL* was significant correlated in S0&S2, and *CsCCR* and *CsPER* were significant correlated in S0&S1&S2.

Discussion

Light is the basis of plant photosynthesis and plays an important role in plant growth and morphogenesis [31]. A large number of studies have shown that shading conditions can significantly change the plant height stem thickness, leaf area, leaf structure and other biological character, but also affect the change of photosynthetic efficiency, and secondary metabolites content [32, 33]. The present study showed that leaf area decreased with increasing shading. The leaf area reduced 2.5 times in 80% shading treatment (S2) compared with control (S0). Shading also changed leaf color. The color of tea plant leaves seems more green with increasing shading. That's mainly because shading treatment improved the chlorophyll concentration [27, 34]. Lignin content of leaves in tea plant reduced under shading treatment, indicating shading might have a negative influence on lignin accumulation in tea plant leaves. A similar study was found. Shading causes the decrease of acid detergent lignin in perennial herbaceous Texas legumes [35].

Lignin distribution was qualitatively measured by histochemistry and autofluorescence microscopy. In this study, lignin is mainly distributed in the xylem of leaves in tea plant. Previous research shown that lignin mainly distributed in the cell walls of xylem and vascular bundle sheath of main leaf vein in tea plant [36]. That is mainly due to the leaves used in this research are too young, and lignin may preferentially deposited in xylem and second vascular bundle sheath, or that is mainly determined by the characteristics of the tea plant cultivar 'Longjing 43'. As far as we know, there is no report on the anatomy of lignin by shading. Histochemistry and autofluorescence microscopy studies indicated that 80% shading reduced the degree of lignification of xylem. These results were consistent with that lignin accumulation was reduced with increasing shading.

Previous studies have shown that changes in lignin content are accompanied with changes of the expression of genes involved in lignin biosynthesis [37-39]. Here, 12 genes (*CsPAL*, *CsC4H*, *Cs4CL*, *CsHCT*, *CsC3'H*, *CsCCoAOMT*, *CsF5H*, *CsCOMT*, *CsCCR*, *CsCAD*, *CsPER*, and *CsLAC*) involved in lignin synthesis were selected to investigate the regulation mechanism under shading treatment at the transcript level. Promoter analysis indicated that all of those genes contain several light responsive elements, indicating that the expression of those genes might be regulated by light.

The lignin biosynthetic related genes showed an upward trend in control (S0) with the growth and development of the leaves of tea plant, indicating those genes play an important role in the development of lignin in tea plant. The expressions of most lignin biosynthetic related genes were obviously decreased in 80% shading treatment than that in no shading, which is consistent with lignin content decreased in 80% shading treatment. *PAL* is the first enzyme in the lignin biosynthesis pathway, catalyzing the conversion of phenylalanine to cinnamic acid [40, 41]. Previous research has shown that *PAL* was notably reduced in the shade-treated leaves of tea plant, 'Shuchazao'[27]. *4CL* is another enzyme in lignin pathway and also thought to catalyzes the third step of phenylpropanoid pathway [26]. The lignin content in plants was significantly decreased by the down-regulation of *4CL* [42, 43]. In the present study, *CsPAL* and *Cs4CL* showed strong positive correlation with lignin concentration, and reduced in shading

treatments. *PAL*, *C4H*, and *4CL* are involved in the general phenylpropanoid pathway and also shared by the formation of other important secondary metabolites, such as flavonoids and coumarin [19, 44, 45]. Therefore, the expression of these genes may not only affects the lignin content, but also affects other secondary metabolites.

CsHCT, *CsC3'H*, *CsCCoAOMT*, *CsF5H*, *CsCOMT*, *CsCCR*, *CsCAD*, *CsPER*, and *CsLAC* were involved in the specific lignin pathway. Wang and his colleagues found that lignin content was increased compared to darkness in light-induced calli of tea plant, and several lignin-related genes (*CCoAOMT*, *HCT* and *CCR*) were identified in the light-induced SSH library [44]. Here, *CsF5H*, and *CsLAC* exhibited significant positively correlation both in the 40% and 80% shading treatments, indicating the importance of these two genes for lignin synthesis under shading conditions. Some genes (*CsHCT*, *CsCCoAOMT* and *CsCOMT*) are highly correlated with 40% or 80% shading treatments. Among them, *CsCCoAOMT* and *CsCOMT* had similar expression patterns, indicating these genes might have similar functions in lignin metabolism.

Lignin is the main component of the plant secondary cell walls that providing mechanical support and water transport for the plants [46, 47]. In tea plant, lignification of leaf tissues is closely related to its quality and stress resistance ability. Suitable lignification is essential for tea quality. Shading affects the growth of tea plants, wherea it also increases the satisfactory amino acid contents. Strong shading seriously affected the development of tea plant leaves, and reduce tea yield. Tea flavonoids (especially catechins) are the main biologically active components in tea plant and shared the phenylalanine metabolism branch pathway with lignin. Shading reduced the flavonoids, and also lignin content [27]. That may mainly due to shading reduced the carbon source upstream of phenylpropane.

Conclusions

In this study, we evaluated the effect of shading on leaves growth, xylem development, and lignin accumulation in tea plant. Shading treatment inhibited leaves growth and lignin content. Safranin-O/fast green staining and autofluorescence indicated lignin was mostly deposited in the xylem. Shading may affect tea plant leaves lignification by regulating the transcript levels of lignin biosynthesis genes. This study provided potentially useful information for understanding the mechanism of lignin biosynthesis under shading treatments in tea plant.

Materials And Methods

Preparation of plant materials

Two-year-old 'Longjing 43' tea plant cutting seedlings were grown in a controlled-environment growth chamber at the Nanjing Agricultural University (Nanjing, China). The chamber conditions were set at 25 °C temperature, 70% relative humidity, 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and 16 h light/8 h dark cycle. Shade nettings with the corresponding light transmittance coefficients were used to provide shading treatments

with approximately 40% (S1) and 80% (S2) shading rates. Tea plants maintained under no shading condition (0% shading rate) were used as controls (S0). After shading treatment, tea shoots containing first and second leaves, were collected at 3, 6, and 9 days. Each tea plant sample was prepared with three copies. The collected tea plant samples were quickly immersed in liquid nitrogen and then stored at -80°C .

Measurement of lignin content

The lignin of tea plant samples was purified and measured according to the previously described procedure with some modifications [48, 49]. In brief, approximately 1 g of frozen tea plant samples were ground in liquid nitrogen, then immediately homogenized in 99.5% ethanol and centrifuged for 20 min in a refrigerated centrifuge at $12,000\times g$. The sediment was collected and spread in a clean petri dish for 24 h to air dry at room temperature. About 10 mg of dried sediment was transferred to a 2 mL plastic tube and then added with 0.1 mL of thioglycolic acid and 1 mL of 2 M HCl. The mixture sample was incubated at 100°C for 8 h in a thermostatic water bath, and then cooled on ice. After centrifuged at $14,000\times g$ for 20 min at 4°C , the sediment was washed with 1 mL of distilled water, centrifuged again and then resuspended with 1 mL of 1 M NaOH. The mixed solution was incubated at 25°C for 18 h, and then centrifuged at $14,000\times g$ for 20 min. The water phase on the top was transferred into a new plastic tube and added with 1 mL of concentrated HCl. The mixture was exposed at 4°C for 6 h and then centrifugation at $14,000\times g$ for 20 min. The remaining sediment was redissolved with 1 mL of 1 M NaOH. The sample was determined by spectrophotometer at 280 nm with 1 M NaOH as the control. The extraction and determination of lignin from each sample were conducted with three biological replicates.

Histochemical staining and UV microscopy

Histochemistry staining and UV microscopy were performed to observe lignin distribution. The leaf specimens were cut from healthy tea plant with shading treatments using a razor blade. The first leaves, including major veins, were cut into section with small pieces and immediately stored in 2.5% glutaraldehyde at 4°C . For safranin-*O*/fast green staining, the leaf sections were first deparaffinized in xylene and then washed and dehydrated with ethanol. Subsequently, the sections were stained for 2 h in 1% safranin-*O* and then counterstained for 15 s with 0.5 % fast green. The sections were washed with ethanol to remove the extra stain and then mounted using neutral balsam. Safranin-*O* stained the lignified cell walls with red, whereas fast green stained the cellulosic tissues with green.

Lignified cell walls exhibit autofluorescence under UV excitation [50, 51]. For autofluorescence observation, the lignin autofluorescence of the leaf specimens were observed by fluorescence microscopy under UV excitation.

Leaf area calculation

First leaf was selected as materials to calculate the leaf area. The formula for leaf area is as follows: leaf area (cm^2) = leaf width (cm) $\times$ leaf length (cm) $\times$ 0.7.

Selection and analysis of gene involved in lignin biosynthesis

The genes involved in lignin biosynthesis and polymerization were selected from the Tea Plant Information Archive (TPIA) (<http://tpia.teaplant.org/>), a genomic database for tea plant [52]. A total of 12 genes related to lignin pathway in tea plant, namely *CsPAL*, *CsC4H*, *Cs4CL*, *CsHCT*, *CsC3'H*, *CsCCoAOMT*, *CsF5H*, *CsCOMT*, *CsCCR*, *CsCAD*, *CsPER*, and *CsLAC*, were selected.

The functional interaction networks of these genes involved in the lignin biosynthesis pathway were constructed using STRING software. The upstream 2000 bp regions of the genes involved in the lignin pathway were analyzed to search for putative *cis*-acting elements using the plant database PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [53].

RNA isolation, reverse transcription, and qRT-PCR detection

Total RNA was extracted from tea plant leaves by using an RNA extraction kit (Huayueyang Biotech Co., Ltd., Beijing, China) following the manufacturer's instruction. The RNA concentration was assessed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). One microgram of each RNA sample was reverse transcribed into cDNAs by using the PrimeScript RT reagent kit (TaKaRa, Dalian, China) in accordance with the manufacturer's instructions.

RT-qPCR was carried out using a Bio-Rad CFX96 fluorescence quantitative PCR platform with TaKaRa SYBR Premix *Ex Taq* (TaKaRa, Dalian, China). The primers used in this study were designed using Primer Premier 5.0 software and listed at Table 1. The *Actin* gene of tea plant was chosen to normalize the expression levels of lignin related genes [54]. The reaction conditions were set as follows: 95 °C for 3 min, 40 cycles of 95 °C for 5 s, 58 °C for 20 s, and followed with a melting curve analysis ranging from 65 to 95 °C. The reaction of each sample was conducted with three biological and technical replicates. The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method [55].

Statistical analysis

Statistical analysis was assessed by One-way ANOVA using SPSS18.0 software. Significant differences were detected by Duncan's multiple-range test at significance levels $P < 0.05$. A pearson correlation coefficient (PCC) analysis was performed to evaluate the correlations between lignin contents and expression levels of genes in the lignin pathway using SPSS software at significance levels $p < 0.01$ and $p < 0.05$.

Abbreviations

G unit: coniferyl alcohol; H unit: p-coumaryl alcohol; S unit: sinapyl alcohol; PAL: Phenylalanine ammonia lyase; 4CL: 4-coumarate-CoA ligase; C4H: cinnamate 4-hydroxylase; HCT: Hydroxycinnamoyl-CoA transferase; C3'H: 4-coumarate 3-hydroxylase; CCoAOMT: caffeoyl-CoA O-methyltransferase; F5H: ferulate 5-hydroxylase; COMT: caffeic acid O-methyltransferase; CCR: cinnamoyl-CoA reductase, CAD:

cinnamyl alcohol dehydrogenase; PER: peroxidase; LAC: laccase; MeJA: methyl jasmonic acid, Eth: ethylene; ABA: abscisic acid

Declarations

Availability of data and materials

The data sets supporting the conclusions of this article are included within the article.

Consent to publish

Not applicable.

Ethics approval and consent to participate

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

Contributions

ZJ, WYX and TRM designed this research. WYX, TMR, LH, LH,WY and LSJ performed the experiments. WYX conducted the data analysis. TRM and WYX wrote the manuscript. ZJ and WYX revised the paper. All the authors contributed to improving the paper and approved the final manuscript.

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Figures

Figure 1

Simplified diagram of lignin biosynthesis pathways in plants in tea plant. The enzymes are as follows: phenylalanine ammonia lyase (PAL); cinnamate 4-hydroxylase (C4H); 4-coumarate-CoA ligase (4CL); cinnamoyl-CoA reductase (CCR); cinnamyl alcohol dehydrogenase (CAD); hydroxycinnamoyl-CoA shikimate/quinic acid hydroxycinnamoyltransferase (HCT); p-coumaroyl shikimate/quinic acid 3' -hydroxylase (C3'H); caffeoyl-CoA O-methyltransferase (CCoAOMT); ferulate 5-hydroxylase (F5H); caffeic acid O-methyltransferase (COMT); peroxidase (PER), and laccase (LAC).

Figure 2

Photograph of the leaves of tea plant cultivar 'Longjing43' with shading treatments. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Scale bars are 1 cm in length.

Figure 3

Effect of shading treatments on leaves area in tea plant leaves. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Error bars represent standard deviation among three independent replicates. Different lowercase letters indicate significant differences at $P < 0.05$.

Figure 4

Effect of shading treatments on lignin accumulation in tea plant leaves. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Error bars represent standard deviation among three independent replicates. Different lowercase letters indicate significant differences at $P < 0.05$.

Figure 5

Safranin-O/fast green staining of transverse sections of tea plant leaves with shading treatments. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Tea plant leaves were harvested at 3 (a, d, g), 6 (b, e, h), and 9 (c, f, i) day, respectively. Ue upper epidermis, De lower epidermis, Cu cuticula, P phloem, X xylem. Scale bars are equivalent to 50 μm in length.

Figure 6

Fluorescence micrographs of transverse sections of tea plant leaves with shading treatments. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Tea plant leaves were harvested at 3 (a, d, g), 6 (b, e, h), and 9 (c, f, i) day, respectively. Cu cuticula, X xylem. Scale bars are equivalent to 50 μm in length.

Figure 7

Interaction network of the proteins involved in lignin pathway.

Figure 8

Expression profiles of genes involved in the general phenylpropanoid pathway under shading treatments in tea plant leaves. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Error bars represent standard deviation among three independent replicates. Different lowercase letters indicate significant differences at $P < 0.05$.

Figure 9

Expression profiles of genes involved in the specific lignin pathway under shading treatments in tea plant leaves. S0, no shading; S1, approximately 40% shading rate; S2, approximately 80% shading rate. Error bars represent standard deviation among three independent replicates. Different lowercase letters indicate significant differences at $P < 0.05$.

Figure 10

Correlation analyses between lignin content and expression levels of related genes. The genes from left to right in abscissa were followed by CsPAL, CsC4H, Cs4CL, CsHCT, CsC3'H, CsCCoAOMT, CsF5H, CsCOMT, CsCCR, CsCAD, CsPER, and CsLAC.

Supplementary Files

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