Increasing Lignin Accumulation in Arabidopsis and Poplar by Overexpressing a CCoAOMT Gene from the Dove Tree (Davidia involucrata Baill.)

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Abstract
Rapid lignification and high lignin accumulation occur in the endocarps of the dove tree (Davidia involucrata) during a short developmental phase. Through transcriptome analysis, we identified a gene named DiCCoAOMT1 that plays a vital role in the rapid lignification process. The expression profile of the DiCCoAOMT1 gene was endocarp-specific, and its encoding product showed strong O-methyltransferase activity in vitro. Here, we overexpressed the DiCCoAOMT1 gene in both Arabidopsis and poplar (Populus tomentosa) to verify its function of lignin biosynthesis and accumulation. Increased plant height and lengthened pods arose in transgenic Arabidopsis lines, while elongated petioles were observed in transgenic poplar lines. Moreover, the stems exhibited enlarged xylem area, reduced pith area, and more compact cell architecture in both transgenic Arabidopsis and poplar lines. The lignin content was elevated by 26% and 20% on average in the stems of transgenic Arabidopsis and poplar lines, respectively. Furthermore, the lignin composition was altered in the transgenic lines indicated by the elevated S/G ratio. Taken together, we proposed that overexpressing the DiCCoAOMT1 gene can effectively increase lignin biosynthesis and change lignin monomer composition in both herb and woody plants. The endocarp-specific expression pattern of the DiCCoAOMT1 gene is assumed to be a key point to form the highly lignified structure in a short period, thus causing the long-period dormancy of Davidia seeds.

Keywords CCoAOMT · Lignin · Poplar (Populus tomentosa) · Arabidopsis thaliana · Dove tree (Davidia involucrata Baill.)

Introduction
Lignin is one of the most important macromolecular organics in plants with multiple biological functions (Liu et al. 2018). Lignin enhances the mechanical hardness and strength of the plant body by filling the cellulose framework in cells. Besides supporting functions, lignin also plays a role in the defense of plants against biotic and abiotic stress, such as avoiding biological invasion and water erosion, anti-bacterial, anti-oxidation, anti-ultraviolet rays, and flame-retardant. Therefore, lignin is extremely essential for plants to adapt to the complex natural environment. (Qiao 2016; Weng and Chapple 2010). The lignin biosynthesis pathway has been extensively studied, and the genes and enzymes involved in this process have also been well identified. Lignin is a biopolymer composed of three monomers linked by chemical bonds. Phenylalanine forms three kinds of lignin monomer through the phenylpropane metabolism pathway, and the monomer finally forms lignin through a series of reactions. The lignin monomers derived from monolignols are classified into guaiacyl (G-), syringyl (S-), and p-hydroxyphenyl (H-) type units, which are eventually incorporated into the lignin polymer (Shigeto et al. 2016). During the biosynthesis of lignin monolignols, PAL (phenylalanine ammonia-lyase), C4H (cinnamate 4-hydroxylase), C3H (p-coumarate 3-hydroxylase), F5H (ferulate 5-hydroxylase), CAD (cinnamyl alcohol dehydrogenase), 4CL (4-coumarate: CoA reductase), CCR (cinnamoyl-CoA reductase), HCT...
(shikimate hydroxycinnamoyl transferase), COMT (caffeic-acid-methyltransferase) and CCoAOMT (caffeoyl-CoA-methyltransferase) have been identified as key enzymes, and their encoding genes were often used as the targets of genetic modification for regulating lignin amount or alter the lignin composition in different species (Simon et al. 2017). Besides these regular units, there are some other monomer components found in some specific tissues. For example, a novel lignin monomer called hydrostilbenes was found in the endocarp of palm (Río et al. 2017), indicating the specificity of lignin composition in different tissues.

Lignin is widely distributed in plant cells, while mainly accumulated in the secondary wall of fiber cells. Wood fiber cells are concentrated in the xylem of plants, thus most related studies were focused on the changes in lignin content and composition in the xylem. However, lignification is not limited just to the xylem. In many woody plants, lignification can also be observed in roots, endocarps, and pulps (Simon et al. 2017). Different from that in the xylem, the lignification process in these organs was constricted to be completed in a quite limited period to form some specific structures, such as the shell of walnut and the endocarp of almond. The enzymes and genes involved in this process are supposed to be tissue-specific and high efficiency due to the spatiotemporal constraints. Therefore, the regulatory mechanism of lignin biosynthesis in these specific tissues might be quite different from that in the xylem. However, the studies regarding lignification in non-xylem tissues are scarce to our knowledge.

The dove tree (Davidia involucrata Baill., Davidia hereafter) is an endangered species and the sole member of Davidiaceae. It is a relic species of Paleotropical flora in the Tertiary period with many ancient and unique characteristics (Li et al. 2016). The highly lignified structure of Davidia endocarp is considered as one of the main reasons leading to the long period of seed dormancy. The formation of this distinctive structure was triggered by a rapid lignification process occurring at the initiation stage of fruit development and was completed in approximately 20 days. We previously performed transcriptome analysis in Davidia endocarps at different developmental stages and identified a gene called DiCCoAOMT1 from the Davidia genome. The DiCCoAOMT1 gene showed a high expression level specifically in the endocarp, and its expression level has a positive correlation with the lignin content in Davidia endocarp. Moreover, in vitro enzymatic assessment demonstrated that the DiCCoAOMT1 protein has super high O-methyltransferase activity, which is critical for monolignol biosynthesis (Wu et al. 2019).

CCoAOMT is an S-adenosyl-l-methionine (SAM) methyltransferase. It can transfer the methyl from S-adenosylmethionine to the benzene ring carbon 3 position of caffeoyl-CoA, to form feruloyl-CoA. Feruloyl-CoA is catalyzed by CCR, F5H, COMT and other enzymes, and finally forms three lignin monomers (Walker et al. 2016). Therefore, CCoAOMT plays an important role in lignin synthesis as a key enzyme in the process of lignin monomer formation. The CCoAOMT genes have been identified in many plant species, including Arabidopsis, rice (Oryza sativa) and poplar (Populus tomentosa), etc. (Ns et al. 2020; Sakamoto et al. 2020). Suppressing the CCoAOMT gene expression resulted in a 12% decrease in lignin content and an increase in the S/G ratio by 11% in Populus trichocarpa (Chiang 2010). Similarly, inhibition of the expression level of a CCoAOMT gene in tobacco (Nicotiana benthamiana) resulted in the decrease of lignin content and the alternation of lignin components (Pang et al. 2014). While, down-regulation of the CCoAOMT gene leads to a decrease of both lignin and G-unit content, but an unchanged S-unit content in transgenic alfalfa (Chen et al. 2010). Most studies have proved that the CCoAOMT gene mainly functions in the precursor synthesis of the G-unit (Guo et al. 2001). However, a poplar CCoAOMT gene was verified to affect the biosynthesis of both S- and G-unit (Zhong 2000). Suppression or knock-out of the CCoAOMT gene was usually adopted to reduce the lignin content of timber, which is an urgent concern for the paper making industry (Li et al. 2013). On the contrary, overexpression of the CCoAOMT gene always results in the improvement of lignin content. For instance, overexpression of a CcCCoAOMT gene from jute (Corchorus capsularis L.) and a PaCCoAOMT gene from Polypodiodes amoena both led to increased lignin content in transgenic Arabidopsis (Zhang et al. 2014; Zhang et al. 2019).

We have verified that the DiCCoAOMT1 protein is a hyperactive O-methyltransferase via in vitro assessment (Wu et al. 2019). However, the expression of the DiCCoAOMT1 gene in Davidia is endocarp-specific, whether it can affect lignin biosynthesis in other tissues is unknown. We searched against the genome database of Arabidopsis and poplar for the homologs of the DiCCoAOMT1 protein and found that the similarity between the DiCCoAOMT1 protein and its homologs in these species was relatively low (36.16% in Arabidopsis and 52.13% in poplar), indicating that the DiCCoAOMT1 gene is foreign to these species. Whether the DiCCoAOMT1 gene can ectopically function in different plant species will be investigated in this study.

Materials and Methods

Plant Material

The samples of the endocarp of Davidia were collected from the Badagong Mountain National Nature Reserve in Sangzhi County, Zhangjiajie City, Hunan Province (110° 5′ 30″ E, 28° 46′ 60″ N, 1383 m altitude). The seeds of Arabidopsis
ecotype Columbia-0 and the tissue culture seedlings of poplar (Populus tomentosa Carr. Clone 741) are preserved in our lab. The tissue culture materials were grown at 22 °C under a 16 h light/8 h dark photoperiod. The illumination was from the cool-white fluorescent lights (100–150 μmol m⁻² s⁻¹). Wild type (WT) and transgenic Arabidopsis plants, and WT and transgenic poplar plants were grown in pots containing mixed medium (vermiculite: high-quality soil = 1: 3), respectively. All plants were grown in controlled environment chambers at 20 °C under a 16 h light/8 h dark photoperiod.

DNA Extraction, RNA Extraction, and cDNA Synthesis

The samples were rapidly frozen in liquid nitrogen for nucleic acid extraction. Genomic DNA was extracted through the CTAB method (Stewart and Via 1993). Total RNA was extracted with an RNeasy Plant Kit (Qiagen). RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked with the NanoPhotometer spectrophotometer (IMPLEN). The first strand of cDNA was synthesized by reverse transcription with the Evo M-MLV reverse transcription kit (Takara). The cDNA was then diluted and used as a template for gene cloning and qPCR analysis, respectively.

Genetic Transformation in Arabidopsis and Poplar

DNA fragment of the DiCCoAOMT1 gene (GeneBank ID: KY243330) was inserted into the pRI101-AN vector with the cauliflower mosaic virus 35S promoter. This vector is linearized by restriction endonucleases BamHI and SalI and ligated with the target fragment using T₄ DNA ligase. Then the vector was transferred into Escherichia coli DH5α for sequencing. The successfully constructed vector was transferred into Agrobacterium tumefaciens strain EHA105 for genetic transformation.

The floral dip method (Zhang et al. 2006) was used for genetic transformation of Arabidopsis. Transformed Arabidopsis seeds were sowed on the MS medium (Murashige and Skoog 2006) containing 50 mg/L kanamycin and 200 mg/L cefotaxime, and the seedlings with resistance to antibiotics were selected. Homozygous transgenic lines were obtained by serial passage until no trait separation was observed in progenies. More than 20 individual transgenic lines were confirmed by PCR using gene-specific primers, and the lines used for further study were selected according to the expression level detected by qPCR of the target gene. The information on primers used in this study is listed in Table S1.

Detecting Gene Expression Levels by qPCR

The cDNA samples of leaves collected from 30-day-old Arabidopsis plants and leaves collected from 90-day-old poplar plants were used as templates for qPCR analysis. The plants are placed in a controlled incubator (MGC-250HP-2, Shanghai Gaozhi Precision Instrument Co., Ltd.) at 20°C and 70% humidity under 16 h illumination from cool-white fluorescent lights (100–150 μmol m⁻² s⁻¹). An Arabidopsis gene, AtUBQ5 (GeneBank ID: AT3G62250), and a poplar gene, PtActin (GeneBank ID: JN986590) were used as reference genes for data normalization. Real-time PCR was performed with the 2 × SYBR Premix Green Pro Taq HS Premix (Biotools, USA) and the ABI StepOne Plus-Type qPCR instrument (Applied Biosystems, USA). The 20.0 μL qPCR reaction system contained 10 μL of 2 × SYBR Green Pro Taq HS Premix (Biotools, USA), 2.0 μL cDNA, 0.5 μL of the forward primer (10 μM), 0.5 μL of the reverse primer (10 μM), 0.4 μL of ROX Reference Dye (Biotools, USA) (4 μM) and 6.6 μL of RNase-free water. The PCR reaction program was as follows: pre-denaturation at 95 °C for 30 s, then a cycle of denaturation at 95 °C for 15 s and annealing at 60 °C for 40 s, repeated for 40 cycles. The relative expression levels were calculated according to the 2⁻ΔΔCt value (Derveaux et al. 2010). Three independent biological replicates of each line with internal repeats were used for qPCR analysis.

Analysis of Lignin Content and Lignin Monomer Composition

Lignin content was detected using the acetyl bromide method described by Fukushima and Hatfield (2004) with adjustments. The stems of 30-day-old Arabidopsis plants and 90-day-old poplar plants were collected for lignin content measuring. Glacial acetic acid was used as the blank solution to measure the absorbance at a wavelength of 280 nm. Each measurement was repeated three times for each sample. The lignin content was calculated according to the absorbance at 280 nm. Each line contains at least three biological replicates with internal repeats.

The lignin monomer composition was analyzed using the method described by Lapierre et al. (1995). The stems were collected from 30-day-old Arabidopsis plants and 90-day-old poplar plants. The samples were dried at 55 °C for 24 h and...
then crushed by a crusher for measurement. Each line contains at least three biological replicates with internal repeats.

**Microscopic Observation**

The paraffin sections of plant stems were prepared according to the method described by He et al. (2014). The stem segments were collected from 90-day-old transgenic poplar and 30-day-old Arabidopsis. The stem segments were collected from 90-day-old transgenic poplar and 30-day-old Arabidopsis. The paraffin sections were stained with 1% TBO (Toluidine Blue O) dye solution for observation by microscope (Leica DM2000 LED). The paraffin sections were stained with 5% phloroglucinol dye solution for observation by microscope.

**Statistical Analysis**

One-way ANOVA was performed on the mean of the experimental data by SPSS ver. 25.0 for Windows (SPSS Inc.) with the honestly significant difference test of LSD, Duncan’s test, and Dunnett’s test. The level of significance was set to $P < 0.05$.

**Results**

**Overexpression of the DiCCoAOMT1 Gene Caused Morphological Changes in Transgenic Arabidopsis**

Three transgenic Arabidopsis lines (P$_{35S}$::DiCCoAOMT1) with different expression levels of the DiCCoAOMT1 gene were selected for further analysis. The expression profiles in the stems and leaves of transgenic lines have the same trend (Fig. 1a). The transgenic Arabidopsis plants exhibited larger organs (Fig. 1b, c). The plant height of transgenic lines was elevated by 12.70% on average compared with the WT plants (Fig. 1d). In addition, significantly longer length of pods was observed in the transgenic plants (Fig. 1c). The length of the transgenic pods was 1.11-fold on average of that of WT ones (Fig. 1e).

**Lignin Content Increased in Transgenic Arabidopsis**

Lignin content was compared between WT and transgenic Arabidopsis plants. The lignin content of different transgenic lines increased by 23.22 to 33.85% compared to that of WT plants (Fig. 2a). There was a positive correlation between the relative expression levels of the DiCCoAOMT1 gene and lignin content, indicating that the high lignin content was determined by the overexpression of the DiCCoAOMT1 gene (Fig. 2b). Then histochemical staining in the cross-sections confirmed that lignin accumulation was increased in the stems of transgenic plants (Fig. 2c, d). Moreover, we observed that the stem diameter was extended by 7.14% on average, and the cortex area was thickened in the transgenic lines (Fig. 2c, d). The proportion of pith area dropped by 8.08%, the size of pith cells significantly increased, while the number of pith cells decreased by 26.38% (Fig. 2f–h).
Morphological Changes in Transgenic Poplar Plants

The *DiCCoAOMT1* gene was then introduced into poplar to reveal its functions in woody plants. Three transgenic lines with different expression levels of the *DiCCoAOMT1* gene were selected for further analysis (Fig. 3a). Different from the case in *Arabidopsis*, the plant height of transgenic poplar lines was not improved by overexpressing the *DiCCoAOMT1* gene (Fig. 3b). Instead, we noticed that the length of the petiole was obviously increased in the transgenic plants (Fig. 3b, c).

Lignin Accumulation Increased in Transgenic Poplar Plants

Lignin content was detected in the stems of transgenic poplar lines. The lignin content of P3, P8 and P12 lines increased by 11.47%, 21.54%, and 13.63% compared to that of WT plants, respectively (Fig. 4a). There was a positive correlation between the relative expression level of the *DiCCoAOMT1* gene and lignin content (Fig. 4b). Histochemical staining demonstrated that lignin accumulation was significantly increased in the primary and secondary xylem cells of the transgenic plants (Fig. 4c, d). Microscopic observation showed that the intra-fascicular cambium and primary xylem were thickened, the pith rays were lengthened, and the pith cells were enlarged in the stems of transgenic lines. There was no obvious change in the parenchyma and primary phloem cells of the cortex (Fig. 4c, d).

Lignin Monomer Composition Was Changed in Transgenic Plants

To further reveal the function of the *DiCCoAOMT1* gene, the lignin monomer composition in transgenic poplar stems was analyzed using GC–MS technology (Fig. S1). The results showed that the G-unit content was significantly increased in P8, but not in P3. Similarly, the S-unit content was also increased in P8. Remarkably, the P8 line with the highest expression level of the *DiCCoAOMT1* gene showed 7.32-fold and 8.68-fold G-unit and S-unit content of that in WT plants, respectively. The H-type lignin monomer content was low in both WT and transgenic plants, while there was an increase in H-unit in P8 and P12 (Fig. 5a). The S/G ratio ranged from 0.78 to 1.03 in transgenic lines, which was significantly higher than that in WT plants (0.66) (Fig. 5b). Under the same experimental conditions, we also detected the content of lignin monomer components in *Arabidopsis* (Fig. S2). The results showed that the G-unit content was significantly increased in the transgenic plants. Similar to the results in poplar, the composition of S-type monomers in transgenic *Arabidopsis* also increased. The H-type lignin monomer content was low in both WT and transgenic plants with no significant differences (Fig. 5c). The increased S/G ratio in transgenic plants indicated that overexpression of the *DiCCoAOMT1* gene contributed more to the biosynthesis of the S-unit (Fig. 5d). This evidences confirmed that the *DiCCoAOMT1* gene plays a role in lignin monomer, especially S-unit synthesis, and consequently improved lignin accumulation.

Discussion

The process of lignin biosynthesis is highly conserved in most plants, and its regulatory mechanism is complicated (Chen et al. 2021; Gui et al. 2020; Teng et al. 2021). As one of the key regulators, the *CCoAOMT* gene family has been a research hotspot for a long time (Díaz et al. 2010; Zhao et al. 2021). We have identified 14 *CCoAOMT* genes from the transcriptome data of *Davidia*. However, only one gene, *DiCCoAOMT1*, showed an endocarp-specific expression pattern. This finding indicated that fine division in the *DiCCoAOMT* gene family determines lignin biosynthesis in different tissues (Wu et al. 2019). Similar results were reported in other species. Among 7 *CCoAOMT* genes in *Arabidopsis*, *AtCCoAOMT1* was expressed in all tissues with the highest expression level. The expression levels of *AtCCoAOMT4*, *AtCCoAOMT5* and *AtCCoAOMT7* increased along with stem development. However, *AtCCoAOMT2*, *AtCCoAOMT3*, and *AtCCoAOMT6* were only expressed in the late stages of stem development (Raes et al. 2003). In the study on giant bamboo, the expression of a *DsCCoAOMT* gene was up-regulated with the development of bamboo shoots and maintained a high level in the metaphase of bamboo shoot development (Zhao et al. 2016). Therefore, although lignin biosynthesis in different tissues shares the same pathway, the selection of tissue-specific genes might be an important regulation mode to realize lignification in designated regions.

Endocarp is essential for the adaptations in seed protection and dispersal strategies in plants. Lignification in endocarp is a very different developmental program that requires unique sets of genes or enzymes (Dardick and Callahan 2014). The enzymatic activity of PAL, C4H, 4CL, CAD, and POD increased rapidly in 30 days in the endocarps of peach (*Prunus persica*) (Zhao et al. 2016). Similar to our results, phenylpropanoid (PAL and C4H) and lignin (*CCoAOMT, peroxidase*, and *laccase*) pathway genes were specifically induced in the endocarp layer over a 10 days (Wu et al. 2019). However, in walnut (*Juglans regia*), the enzymatic activity of both POD and PAL was decreased in endocarp during fruit development (Zhao et al. 2016). This evidences indicated that lignification in endocarps or other non-xylem tissues was under different regulations, which might be the reason for the lengthened pods we observed in transgenic *Arabidopsis* lines.
There was a positive correlation between expression levels of the DiCCoAOMT1 gene and lignin content in both transgenic Arabidopsis and poplar lines (Figs. 2b and 4b), indicating the dosage effect of the function of the DiCCoAOMT1 gene. We observed that the cambium area was thickened or replaced by the primary xylem cells in the
stem cross-section of transgenic poplar lines. However, this variation was not obvious in line P3 (Fig. 4c), in which the expression level of the DiCCoAOMT1 gene was relatively low. Similarly, the results of lignin monomer composition also showed that lignin monomer content was not altered in line P3 (Fig. 5a). These findings showed that high expression of the DiCCoAOMT1 gene is necessary for its function of lignin biosynthesis.

Consistent with our results, overexpression of a MIC-CoAOMT1 gene from Miscanthus lutarioriparius also resulted in the thickened secondary cell wall in transgenic Arabidopsis (Yu et al. 2013). However, different results have been found in the study of a LjCCoAOMT1 gene from Lonicera japonica. Overexpression of the LjCCoAOMT1 gene did not cause variations in plant height and leaves in transgenic rice (Oryza sativa L.) lines, but there were significant differences in the number of tillers and the number of grains per ear (Jiang et al. 2014). Although the transgenic lines of Arabidopsis and poplar both exhibited elevated vigor, they have different morphological variations (Figs. 1a and 3a). Higher lignin content might provide stronger mechanical support to the transgenic lines, meanwhile promote the growth rate of stems and leaves.

Unexpectedly, overexpression of the DiCCoAOMT1 gene increased the S/G rate in transgenic lines. There are several reports proved that inhibiting the CCoAOMT enzymatic activity reduced the lignin content and G-unit content in transgenic plants, but the S-unit content was not affected, thus increasing the S/G ratio (Eom et al. 2010; Guo et al. 2001). This evidence indicated that the CCoAOMT protein is mainly involved in the biosynthesis of G-unit. However, some studies found that the CCoAOMT gene is not only involved in the synthesis of G-unit but also in the synthesis of S-unit (Ye et al. 2001). Conifer aldehyde is located at the
shunt node of the G- and S-unit. It comes from two sources, including caffeic acid-CoA and ferulyl-CoA. We speculate that the high expression level of the DiCCoAOMT1 gene might cause massive accumulation of conifer aldehydes, and catalyze the conversion from F5'H to S-unit. In addition, the dove tree is a relic plant left over from the Tertiary in the Cenozoic era, which may result in a functional difference between the DiCCoAOMT1 gene and its homologs in currently known plants. In Davidia, the enhancement of the DiCCoAOMT1 enzymatic activity on both G- and S-unit might accelerate the lignin biosynthesis, thus facilitating the rapid lignification process in a limited period.

Taken together, we identified an endocarp-specific CCoAOMT gene and verified its function in both herb and woody plants. The target gene significantly increased lignin content and altered lignin composition in transgenic lines, indicating its critical role in improving lignin accumulation.

Fig. 4 Lignin accumulation increased and the development of stem in transgenic poplar plants. a Lignin content in the stems of WT and transgenic poplar plants; w/w, weight per weight of cell wall residue. b Correlation analysis between lignin content of all transgenic plants and relative expression levels of the DiCCoAOMT1 gene. c The cross-section of the stems of poplar stained by phloroglucinol. d The cross-section of the stems of poplar stained by toluidine blue; ca, cambium; co, cortex; ph, phloem; pi, pith; pr, pith ray; xy, xylem. Data were collected at 90-day-old poplars. Each bar is the mean ± standard error of n = 3 plants. Different letters represent significant differences at P < 0.05 according to Duncan’s multiple range tests.
Fig. 5 Lignin monomer composition was changed in transgenic plants. 

a Lignin monomer content in the stems of poplar lines. G-unit, Guaiacyl unit; S-unit, Syringyl unit; H-unit, Hydroxyphenyl unit. 

b The S/G ratio in poplar lines. Data were collected at 90-day-old poplars. 

c Lignin monomer content in the stems of Arabidopsis lines. 

d The S/G ratio in Arabidopsis lines. The content of G-, S- and H-unit of lignin monomer detected by GC–MS. 

e Poplars WT, f Line P8, g Arabidopsis WT, h Line A5. Data were collected at 30-day-old Arabidopsis lines and 90-day-old poplars. Each bar is the mean ± standard error of n = 3 plants with three technical replicates. Different letters represent significant differences at P < 0.05 according to Duncan’s multiple range tests.
Conclusion

In this study, we verified the function of a Davidia endocarp-specific gene named DiCCoAOMT1 in Arabidopsis and poplar. Overexpression of the DiCCoAOMT1 gene resulted in transgenic plants with enlarged organs and improved vigor. Overexpression of the DiCCoAOMT1 gene increased lignin content by 26% and 20% in transgenic Arabidopsis and poplar lines, respectively. The DiCCoAOMT1 gene affected the biosynthesis of both G- and S-unit and contributed more to S-unit. Thus, the S/G rate was increased and the lignin composition was altered in transgenic lines. The spatiotemporal expression of the DiCCoAOMT1 gene is important for the lignification of Davidia endocarp. Our results not only demonstrated the potential of the DiCCoAOMT1 gene to improve lignin accumulation, but also provided new insights for understanding the molecular mechanism of lignification in specific organs.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00344-022-10872-2.

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Author Contributions ML and FC designed the experimental scheme; RM provided the endocarp materials and transcriptome data of Davidia; JL and XJ performed experiments and data analysis; ML and JL wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare that there is no conflict of interest in submitting this manuscript.

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