BMC Genomics

RESEARCH

Open Access

Transcriptome analyses for revealing leaf abscission of *Cyclocarya paliurus* stem segments in vitro

Gaoyin Wu[1,2†], Zhongcheng Peng[1,2†], Qiuying Li[1,2], Xiang Zhang[1,2], Shuanggui Geng[1,2], Shuang Wang[1,2], Enrong Lu[1,2], Yingying Liu[3], Congjun Yuan[4], Xiaoli Wei[5] and Yingliang Liu[1,2*]

Abstract

Leaf abscission of *Cyclocarya paliurus* stem segments in vitro is very serious, and more than 90% of the leaves gradually fall off with prolonged culture time, which hinders breeding. This study investigated the molecular mechanism of leaf abscission. The emerged leaves of *C. paliurus* stem segments were cultured for 22 days (T0) in vitro; leaves at 27 days (T1) and leaves that had fallen after ≥ 32 days (T2) were used as materials for analysis of the physiological characteristics and transcriptome data. During the leaf abscission process of *C. paliurus*, the Indole-3-acetic acid (IAA) content gradually decreased, whereas the carotenoid, 1-aminocyclopropane-1-carboxylic acid (ACC) and lignin contents and pectinase and cellulase activities significantly increased; 1807 and 10,908 DEGs were obtained in T0 vs T1 and T1 vs T2, respectively. The plant hormone signal transduction pathway, phenylpropanoid biosynthesis pathway and flavonoid biosynthesis pathway were significantly enriched in the KEGG metabolic pathway analysis. The differential expression of related genes affected AUX and Ethylene (ETH) biosynthesis and signal transduction, lignin synthesis, ROS metabolism, leaf color changes. Weighted gene coexpression network analysis (WGCNA) identified 10 hub genes (U-box protein, ERF5, ERF109, ERF4, SAUR36, CML19, MYC2-like,SPHK1, TOE3, POD55) that interact to activate abscission signaling, which subsequently influences the genes expression involved in the biosynthesis and signal transduction of auxin and ethylene; this resulted in an imbalance of endogenous hormone levels in the leaves, leading to the upregulation of pectinase, cellulase, and lignin biosynthesis genes and acceleration of the rupture of the abscission zone (AZ) cell and vascular cell wall, which ultimately led to leaf abscission. The present study illustrates a regulatory mechanism of leaf abscission of *C. paliurus* stem segments in vitro, which provides potential application value for guiding the inhibition of leaf abscission in vitro.

Keywords *Cyclocarya paliurus*, Stem segments, Leaf abscission, Mechanism

†Gaoyin Wu and Zhongcheng Peng contributed equally to this work.

*Correspondence:
Yingliang Liu
liuyl-23@126.com
Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Introduction

Plant organ abscission is a common biological phenomenon of senescence and is a physiological process in which the leaves, flowers, fruits, seeds, or branches of plants are separated from their parents. The process occurs at a specific site, and the site and its neighboring cells are collectively referred to as the abscission zone (AZ) [1]. The initiation of organ abscission in plants such as flowers, fruits, and leaves in nature is triggered by developmental and environmental factors [2, 3]. Furthermore, organ abscission represents a sophisticated regulatory mechanism enabling plants to respond effectively to biotic and abiotic stresses, as well as nutrient and hormone imbalances [4], which is highly important for the reproduction and spread of species [5, 6]. The abscission of fruit from crops such as *Oryza sativa*, *Triticum aestivum*, *Prunus avium*, and *Litchi chinensis* results in considerable losses in crop productivity. Consequently, during their domestication process, our ancestors selected germplasms with reduced fruit or grain abscission to increase yield and facilitate harvesting [7]. Therefore, an in-depth understanding of the mechanism of organ abscission is important for guiding practical production activities. However, the molecular mechanism of leaf, flower, fruit and grain AZ development in different plants is regulated by different genes or regulatory networks. Research has indicated that the IDA-HAE/HSL2 module, which is responsible for the transcriptional activation of genes associated with cell wall degradation and cell wall relaxation, is highly conserved in the regulation of organ abscission in angiosperms. Furthermore, in the Gramineae family, Shattering4 (SH4) [8], qSH1 [9], and Shattering abortion 1 (SHAT1) [10, 11] positively regulate AZ development in grains. As a key gene in auxin biosynthesis, YUCCA overexpression can increase the auxin content, inhibit the expression of age-related genes, and delay organ aging and abscission [12]. Conversely, ethylene is one of the key factors in the regulation of plant organ abscission as an intermediate in response to the regulation of upstream abscission signals and the transmission of abscission signals [13]. The abscission signal can increase pectinase (polygalacturonase (PG) and pectin methylesterase (PME)) and cellulase (endoglucanase (EG) and β-glucosidase (BGL)) activities, which degrade AZ cell walls and accelerate organ abscission [14, 15]. Additionally, the upregulation of POD genes increases the AZ lignin content of *Prunus pseudocerasus* fruits and promotes the formation of a protective layer during abscission [14]. Nevertheless, there is a paucity of comprehensive research on organ abscission during in vitro plant culture, and few in-depth studies on organ abscission during plant culture in vitro have been reported.

C. paliurus is a plant of the Juglandaceae family, the only species in this genus; it is a rare species unique to China and an endangered plant under key protection. The leaves are rich in polysaccharides, flavonoids, terpenoids, organic acids, and alkaloids, as well as other chemical components. The trunk is straight and hard, and the fruit is similar to a coin. It is a multifunctional tree species that integrates medicinal, timber, ornamental and ecological value, with a wide range of uses and high potential for market development. In recent years, the *C. paliurus* industry has gradually developed, with a positive effect on the alleviation and treatment of type 2 diabetic symptoms [16–18]. However, the existing wild resources of *C. paliurus* are scarce, and previous researchers have conducted propagation and cultivation technology studies on *C. paliurus* through sexual and asexual reproduction to meet the increasing demand for this species. However, owing to their intrinsic biological characteristics, including the deep dormancy of seeds, low inflorescence formation, low rooting rate of cuttings and human overuse [19, 20], natural populations are rapidly declining, which severely hinders their development and utilization. Consequently, research on efficient breeding technology for *C. paliurus* is urgently needed to solve the above problems.

Our research group reported that the phenomenon of leaf abscission was extremely serious in the process of optimizing the tissue culture of *C. paliurus* stem segments and that more than 90% of the leaves gradually fell off with prolonged culture time. The germinated and well-grown stem segments were transferred to fresh medium in advance, and the stem segments from different provenances and from different seasons were used as explants. After the inorganic salt composition of the medium or the type and concentration of plant growth regulators were adjusted, the leaf abscission rates did not significantly decrease, and the lateral buds of the secondary branches grew slowly and exhibited weak growth potential after the leaves fell off [21]. This technical obstacle has significantly impeded the rapid propagation of *C. paliurus*. Reducing the rate of leaf abscission, promoting the growth of lateral shoots, and increasing the proliferation rate of lateral shoots represent significant challenges. Previous research has demonstrated that the stem segments of *C. paliurus* are subjected to some stresses in vitro, resulting in the vigorous metabolism of AZ cells in leaves; excessive production of reactive oxygen species; stimulation of the activities of antistress enzymes, pectinases, and cellulases; and accelerated rupture of the AZ cells and the vascular cell wall [22]. Concurrently, the imbalance of endogenous hormones in the leaves, with a

reduction in IAA levels and an increase in ACC content, was reported to promote the abscission of *C. paliurus* leaves [23]. However, the molecular mechanism regulating leaf abscission in *C. paliurus* has not been reported.

Therefore, to elucidate the causes of leaf abscission in *C. paliurus* stem segments, the emerged leaves of *C. paliurus* stem segments were cultured for 22 days (T0) in vitro; leaves that had germinated for 27 days (T1) and those that had been exfoliated for ≥ 32 days (T2) were used as materials. The transcriptome data were analyzed via WGCNA package with physiological and biochemical indices to screen the hub genes that regulate leaf abscission, with the aim of revealing the leaf abscission mechanism of *C. paliurus* stem segments. This study provides a theoretical and technical reference for solving the problem of leaf abscission in *C. paliurus* and has great significance for guiding the breeding of *C. paliurus* in vitro.

Materials and methods

Plant materials

The six-year-old tender stem segments of *C. paliurus*, collected in May 2023 from the nursery of the Guizhou Institute of Biology, were observed to be in good condition and free from pests and diseases. Each node was trimmed to a length of approximately 10 cm, with an axillary bud retained. The impurities on the stem segments were then removed with soft brushes and Tween-20. Subsequently, the samples were rinsed for 30 min under tap water and transferred to an ultraclean bench. The stem segments were sterilized in 0.2% $HgCl_2$ for 8 min, shaken continuously to ensure full contact with the sterilization solution, and washed with sterile water five times. The extremities of the stem segments in contact with the sterilization solution were bisected at the superior end, while the inferior end was incised at a 45° angle. The stem segments, along with their axillary buds, measuring approximately 5 cm in length, were then inoculated into a 1/2 MS basic medium. The medium was augmented with 6-BA (0.5 mg/L), IBA (0.1 mg/L), sucrose (30 g/L), agar (6 g/L), and PVP (0.25 g/L), and the pH was calibrated to 5.90 ± 0.1 prior to sterilization (121 °C for 20 min). A single stem segment was inoculated into each bottle, with 300 segments inoculated in total each time. This process was repeated three times. The plants were placed in an incubation chamber at a temperature of 25 ± 2 °C and a light intensity of 2000–2500 lx for a period of 16 h per day, in 8-h cycles. The process of collecting samples is described below. The fully extended normal-growing leaves of the stem segments were collected and cultured for 22 days (T0) or 27 days (T1) in vitro. Subsequently, the leaves were detached from the lateral buds of the stem segments and cultured for a minimum of 32 days (T2) (Fig. 1). The samples were rapidly frozen in liquid nitrogen and stored at −80 °C [22]

Transcriptome sequencing and data analysis

The procedures of RNA extraction, detection, library construction, sequencing and bioinformatics analysis were conducted at the facilities of Shanghai Meiji Biotechnology Co., Ltd. (Shanghai, China; www.majorbio.com). Total RNA was extracted using the MJZol total RNA extraction kit (Meiji, Shanghai, China), following the manufacturer's instructions. The quality of the RNA was determined via a 5300 Bioanalyzer (Agilent) and quantified via the ND-2000 (NanoDrop Technologies). Only high-quality RNA samples (OD260/280 = 1.8 ~ 2.2, OD260/230 ≥ 2.0, RQN ≥ 6.5, 28S:18S ≥ 1.0, > 1 μg) were used to construct sequencing libraries. The mRNA was enriched using magnetic beads with oligo (dT), the mRNA was fragmented, and the resulting fragments were employed as templates for cDNA synthesis. The sequencing library was constructed and subsequently sequenced via the Illumina HiSeq platform, thereby obtaining the raw transcriptome sequencing data. The raw data were subjected to filtration using the fastp tool (https://github.com/OpenGene/fastp). The clear reads obtained from the samples were then subjected to de novo assembly using Trinity (https://github.com/trinityrnaseq/trini

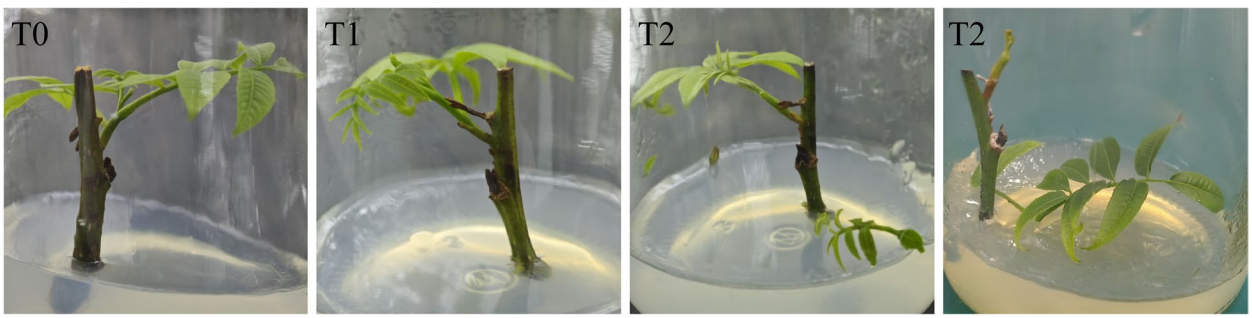

Fig. 1 The process of leaf abscission during in vitro cultivation and germination of stem segments of *C. paliurus*

tyrnaseq/wiki). In order to enhance the quality of the assembly, all assembled sequences were subjected to filtration through the CD-HIT and TransRate software, and subsequently evaluated with the BUSCO (Benchmarking Universal Single-Copy Orthologs) tool. All transcripts obtained from the transcriptome sequencing were compared with those from six major databases (the NR, Swiss-Prot, Pfam, COG, GO and KEGG databases) via the use of the Diamond software (https://github.com/bbuchfink/diamond). The annotation information for each database was obtained and the annotation status of each database was determined. The expression levels of genes and transcripts were quantified using RSEM software (http://deweylab.github.io/RSEM/). The DEGs were identified using the DESeq2 software (http://bioconductor.org/packages/stats/bioc/DESeq2/). Genes with a log2 (fold change) value of ≥ 1 and a p-value of ≤ 0.05 were defined as differentially expressed.

Construction of a gene coexpression network

Using the WGCNA package (v 1.47) in R software [24], the physiological indicators during leaf abscission in *C. paliurus* in vitro, such as the contents of soluble sugar (SS), soluble protein (SP), chlorophyll, H_2O_2, lignin, indole-3-acetic acid (IAA), cytokinins (CKs), gibberellins (GAs), abscisic acid (ABA), ethylene, jasmonic acid (JA), salicylic acid (SA) and strigolactones (SL), and the activities of pectinase, cellulase, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) [22, 23] and the genes in each module were screened for phenotypically related modules. Coexpression network analysis was performed on the screened module genes, and the hub genes were screened via Cytoscape_3.7.2 [25]. Each node in the network represents a gene, and edges represent relationships between genes. Graphs and charts were plotted via the R packages ggplot2 and pheatmap.

Quantitative real-time PCR validation

Total RNA was extracted using a Qiagen kit (Germany). cDNA synthesis was performed using a StarScript Pro reverse transcription kit (www.gene-star.com), with the specific steps carried out in accordance with the instructions provided with the kit. The reaction procedure was as follows: pre-denaturation at 95 °C for 2 min, denaturation at 95 °C for 15 s for 40 cycles, and annealing/extension at 60 °C for 30 s. The relative expression of genes was calculated via the $2^{-\Delta\Delta Ct}$ method. The 18S rRNA gene was employed as the internal reference gene, and primers were designed using the Primer 3.0 software (Supplementary file 1). The primer sequence was synthesized by Sangon Biotech (Shanghai) Co., Ltd. (https://www.sangon.com/). All reactions were conducted in triplicate, with three biological and technical replicates for each sample.

Results

Transcriptome data and DEG analysis

Transcriptome sequencing was performed via the Illumina HiSeqTM 4000 sequencing platform, and a total of 71.56 Gb of clean data were obtained from 9 samples. The amount of clean data for each sample was greater than 6.57 Gb. The Principal Component Analysis (PCA) between samples revealed good intragroup clustering and obvious intergroup differences (Fig. 2a). The GC percentages ranged from 46.34% to 46.81%, and the percentage of Q30 bases was greater than 93.71% (Supplementary file 2). The number of unigenes was 74,610, the number of transcripts was 122,213, the average length of the unigenes was 855.85 bp, and the N50 length was 1,539 bp (Supplementary file 3). Unigenes were annotated to the NR, Swiss-Prot, Pfam, EggNOG, GO, and KEGG databases, the assembled unigenes were annotated to at least one of the databases, and the percentage of annotated genes reached 100%. As illustrated in Supplementary file 4, the unigenes were annotated to 41,824 (56.06%) in NR, 25,371 (34.00%) in Swiss-Prot, 23,367 (31.32%) in Pfam, 30,071 (40.30%) in EggNOG, 30,357 (40.69%) in GO, and 13,255 (13.69%) in KEGG. These findings indicate that the results are highly reliable.

A total of 11,842 DEGs were obtained, and the clustering heatmap of the DEGs between the 9 samples revealed significant differences in different stages (Fig. 2b), with 1,807 DEGs in T0 vs. T1 (893 upregulated and 914 downregulated) and 10,908 DEGs in T1 vs. T2 (6,079 upregulated and 4,829 downregulated). Notably, the number of DEGs increased significantly during leaf abscission (Fig. 2c), and the number of common DEGs was 873 from T0–T2 (Fig. 2d).

Enrichment analysis of DEGs

GO enrichment analysis revealed that the DEGs were enriched in three categories: biological process (BP), cellular component (CC), and molecular function (MF). A total of 246 and 603 terms were enriched in T0 vs. T1 and T1 vs. T2, respectively (Supplementary files 5, 6). The top 20 GO terms with the most significant enrichment were selected for GO enrichment bubble plots (Fig. 3a, b). In T0 vs. T1, response to auxin (GO: 0009733), response to hormone (GO: 0009725), response to endogenous stimulus (GO: 0009719) and response to stimulus (GO: 0050896) were enriched. In T1 vs. T2, several terms related to the cell membrane were enriched, including anchoring component of membrane (GO: 0031225), intrinsic component of membrane (GO: 0031224), and integral component of membrane (GO: 0016021).

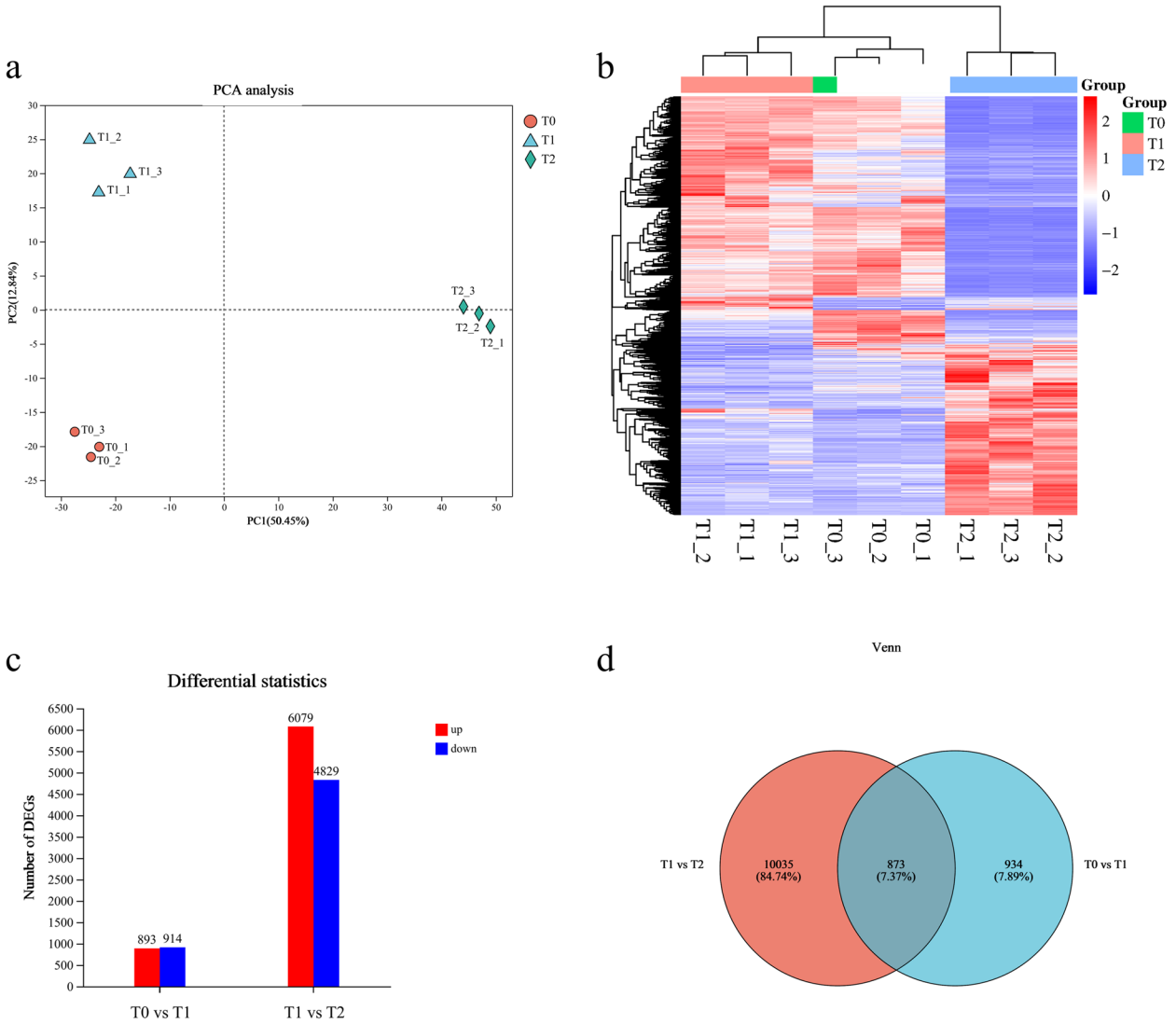

Fig. 2 RNA-Seq results, (**a**) PCA among transcriptome sequencing samples, (**b**) Heatmaps of DEGs between different groups, (**c**) The total number of upregulated and downregulated differential genes (**d**) Venn diagram of the DEGs

KEGG enrichment analysis was performed on the DEGs, and a total of 84 and 133 metabolic pathways were enriched in T0 vs. T1 and T1 vs. T2, respectively (Supplementary files 7, 8). These genes are significantly enriched in plant hormone signal transduction, phenylpropanoid biosynthesis, and flavonoid biosynthesis, suggesting that they play important roles in regulation of leaf abscission.

Analysis of DEGs involved in plant hormone biosynthesis and signal transduction pathways

The plant hormone signal transduction pathway was significantly enriched during leaf abscission. In terms of auxin synthesis, 1 TAA1 and 6 YUCCA genes were downregulated in T2. Sixty-one DEGs related to the auxin signal transduction pathway were screened, among which 2 AUX1, 1 TIR1, 23 AUX/IAA, 2 ARF, 16 SAUR, and 4 GH3 genes were upregulated in T0 and T1 and significantly downregulated in T2 (Fig. 4a).

In the ethylene biosynthesis and signal transduction pathway, the expression of 4 ACO, 1 ACS, 1 ETR, 1 EBF1/2, and 3 ERF1/2 genes was upregulated in T2 (Fig. 4b). The IAA content gradually decreased during the leaf abscission process [23]; in contrast, the ACC content gradually increased [23]. These DEGs affect leaf endogenous hormone levels and play important roles in regulating the leaf abscission process.

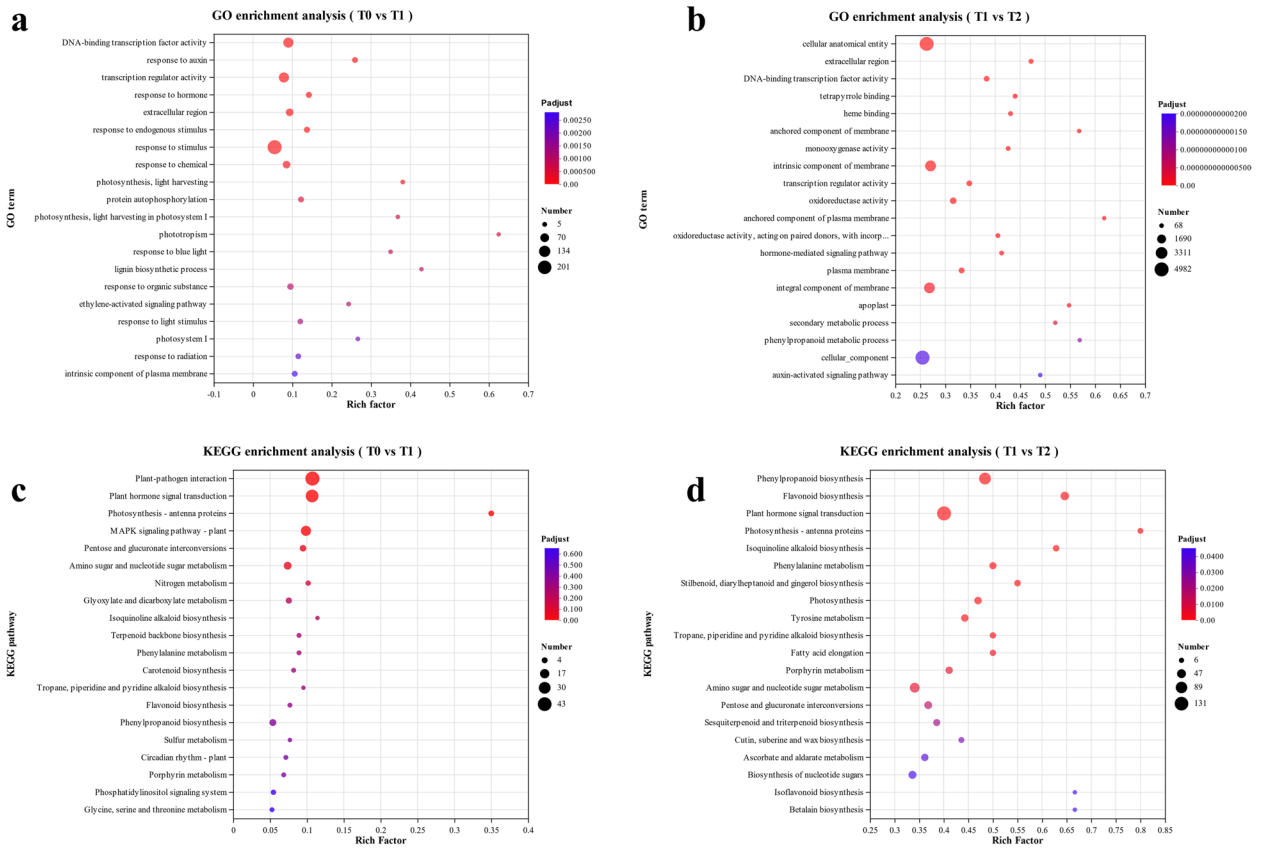

Fig. 3 GO and KEGG enrichment analyses of DEGs during leaf abscission in *C. paliurus*. (**a**) GO enrichment of DEGs in T0 vs. T1, (**b**) GO enrichment of DEGs in T1 vs. T2, (**c**) KEGG enrichment of DEGs in T0 vs. T1, (d) KEGG enrichment of DEGs in T1 vs. T2

Phenylpropanoid and flavonoid biosynthesis

Phenylpropanoid biosynthesis is the main pathway for lignin synthesis, and phenylalanine lyase (PAL), peroxidase (POD), and 4-carboxymethyl coenzyme A ligase (4CL) are the key enzymes regulating lignin synthesis. Chalcone synthase (CHS) is a key enzyme in the regulation of flavonoid biosynthesis. With respect to phenylpropanoid biosynthesis, the genes encoding 1 PAL, 2 4CL and 7 POD were significantly upregulated in T2 (Fig. 5), and the lignin content in T2 was significantly greater than that in T0 and T1 [22]. In terms of flavonoid biosynthesis, 5 CHSs were significantly upregulated in T2 (Fig. 5), while the carotenoid content gradually increased with leaf abscission [22]. This may be related to the gradual yellowing of leaves during leaf abscission in *C. paliurus*.

Pectinase and cellulase activities and related genes

During leaf abscission, 2 PMEs were significantly upregulated in T1, and 7 PGs, 4 EGs and 5 BGLs were significantly upregulated in T1 and T2 (Fig. 6), whereas pectinase and cellulase activities gradually increased and significantly increased in T1 and T2 [22].

Weighted gene coexpression network analysis (WGCNA)

The initial set of genes (74,610) was subjected to a filtration process based on the criteria of expression mean < 1 and coefficient of variation < 0.1, resulting in the selection of 21,101 genes for subsequent analysis. The weights were calculated using the pickSoftThreshold function in R, and the optimal soft threshold $\beta = 20$ was obtained (Supplementary file 9a). The network connectivity under different soft thresholds is illustrated in Supplementary File 9b, which was employed for the construction of the co-expression network. The DEGs were clustered according to their expression levels. Modules with eigenvector values below 0.25 were merged, and modules with correlations below 0.3 between module members and vector genes were eliminated. In conclusion, 44 modules were identified (see Supplementary file 10). The grey module comprised 328 genes that could not be assigned to any module. The turquoise module had the greatest number of genes (11,266), while the cream-coloured module had the fewest genes (32) (Fig. 7).

Correlation analysis was conducted between modules and phenotypic traits (Fig. 7). The implementation of correlation analysis between module eigenvalues and

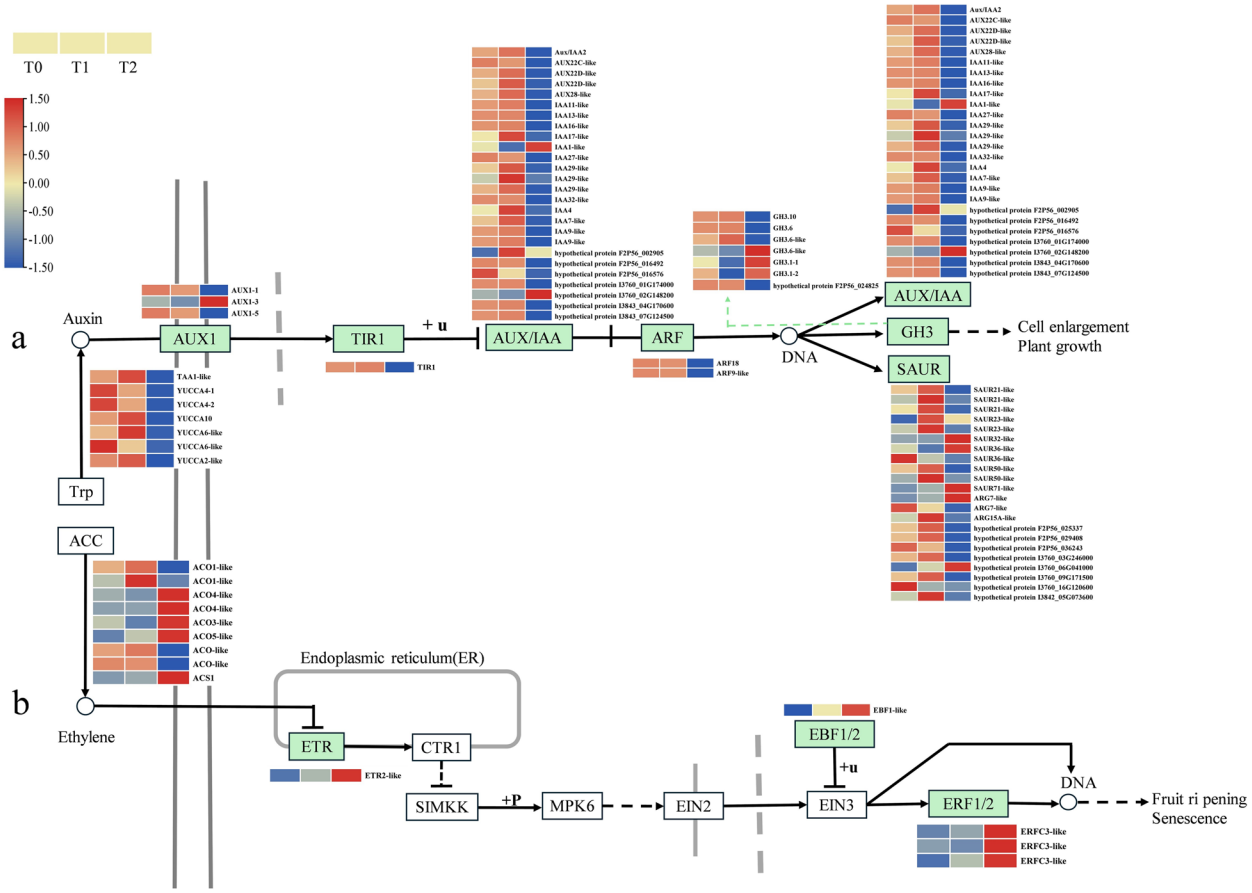

Fig. 4 Analysis of DEGs in auxin and ethylene biosynthesis and signal transduction pathways during leaf abscission in *C. paliurus*, (**a**) Auxin biosynthesis and signal transduction pathways, (**b**) Ethylene biosynthesis and signal transduction pathways

trait data demonstrated that nine modules exhibited a significant correlation with physiological indicators. Of these, the brown module (867 DEGs) was negatively correlated with CKs (r = −0.933, p = 0.00024), SOD (r = −0.967, p = 0.00002), pectinase (r = −0.983, p = 0) and lignin (r = −0.824, p = 0.00631), and the blue module (4064 DEGs) was significant correlated with IAA (r = −0.85, p = 0.0037), CKs (r = 0.833, p = 0.0053), SOD (r = 0.867, p = 0.00247), pectinase (r = 0.883, p = 0.00161) and lignin (r = 0.857, p = 0.00316), and the yellow module (858 DEGs) was positively correlated with Chlorophy (r = 0.833, p = 0.0053), and the pink module (171 DEGs) was negatively correlated with POD(r = −0.867, p = 0.00247), and the darkgrey module (62 DEGs) was positively correlated with SA (r = 0.817, p = 0.00718) and CAT (r = 0.833, p = 0.0053), and the darkorange module (62 DEGs) was positively correlated with SS (r = 0.828, p = 0.00585) and Chlorophy (r = 0.883, p = 0.00161), and the red module (274 DEGs) was negatively correlated with JA (r = −0.9, p = 0.00094), and the grey60 module (76 DEGs) was positively correlated with SOD (r = 0.833, p = 0.0053) and pectinase (r = 0.817, p = 0.00718), and the green module (649 DEGs) was positively correlated with JA (r = 0.867, p = 0.00247). Combining the DGEs of the modules and the number of physiological indicators associated with the modules, the brown and blue modules were selected for further analysis.

GO and KEGG enrichment analyses of module genes

To explore the gene functions in specific modules, GO and KEGG enrichment analyses were performed on the genes of the brown and blue modules (Fig. 8a-d). The top 20 terms with the highest enrichment are shown in the bar charts of the GO classification statistics. The brown module included 5 terms for biological processes and 15 terms for molecular functions; the blue module included 7 terms for biological processes, 2 terms for cellular components and 11 terms for molecular functions.

In the KEGG enrichment analysis, plant–pathogen interactions were the most significantly enriched pathway in the brown module, and the MAPK signaling pathway-plant pathway was significantly enriched in the brown

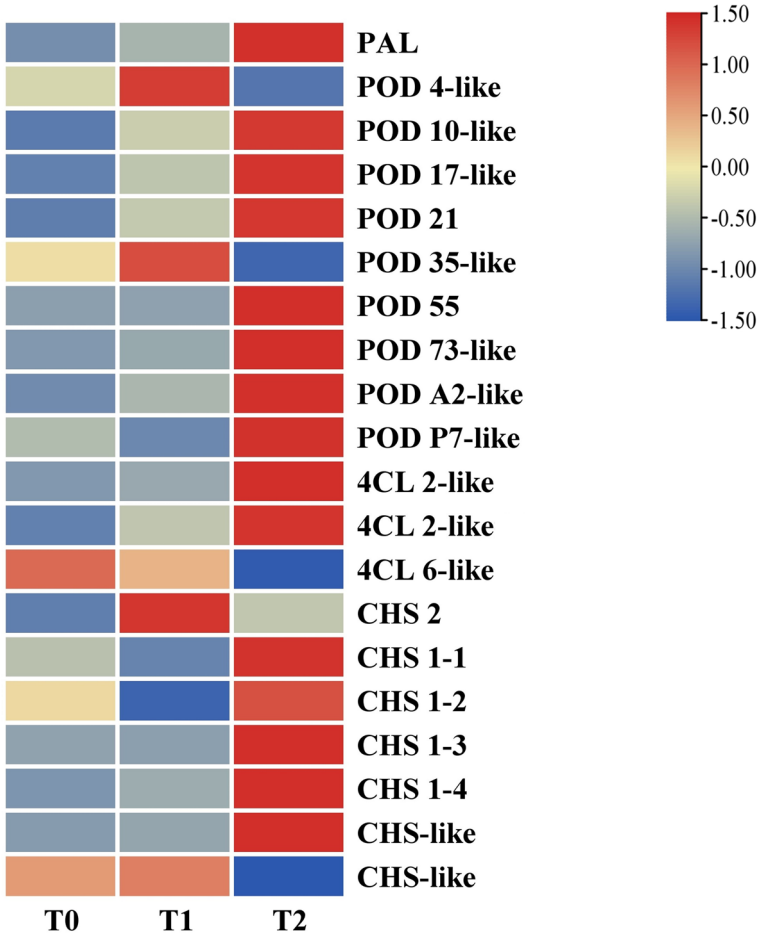

Fig. 5 Heatmap of DEGs related to phenylpropanoid and flavonoid biosynthesis

and blue modules. In addition, the phenylpropanoid biosynthesis pathway and the phytohormone signaling pathway were significantly enriched in the blue module.

Construction of the gene coexpression network

Thirty genes with high connectivity and variable weight values in the brown and blue modules were used as central genes, and the central genes and their related genes were visualized via Cytoscape software (Fig. 9). A total of 10 hub genes were identified, among which were 7 hub genes in the brown module: TRINITY_DN5591_c0_g1 (U-box protein), TRINITY_DN4918_c0_g1 (ERF 5), TRINITY_DN20400_c0_g1 (ERF 109), TRINITY_DN5350_c0_g2 (ERF 4), TRINITY_DN9681_c0_g1 (SAUR36), TRINITY_DN5625_c0_g1 (CML19), TRINITY_DN698_c0_g1 (MYC2-like), and 3 hub genes in the blue module: TRINITY_DN21724_c0_g2 (SPHK1), TRINITY_DN31560_c0_g1 (TOE3) and TRINITY_DN12399_c0_g1 (POD 55). These genes play important roles in regulating leaf abscission in *C. paliurus*.

Quantitative real-time PCR validation

To verify the reliability of the RNA sequencing results, 8 DEGs were selected and amplified via qRT−PCR, and their relative expression was calculated. These results were consistent with the gene expression trend in the transcriptome sequencing results (Fig. 10), indicating that the sequencing results were reliable.

Discussion

DEGs related to plant hormones biosynthesis and signal transduction pathways on leaf abscission in *C. paliurus*

Plant hormones are effectors of organ abscission, and their complex signals of synthesis, metabolism and transport directly or indirectly affect AZ development and plant organ abscission. Among these factors, the balance of ethylene and auxin levels is decisive for plant organ abscission [26, 27]. The indole-3-pyruvate (IPA) pathway is the main auxin biosynthesis pathway in the plant kingdom. The IPA pathway consists of two steps: Trp is converted to IPA by tryptophan aminotransferase/

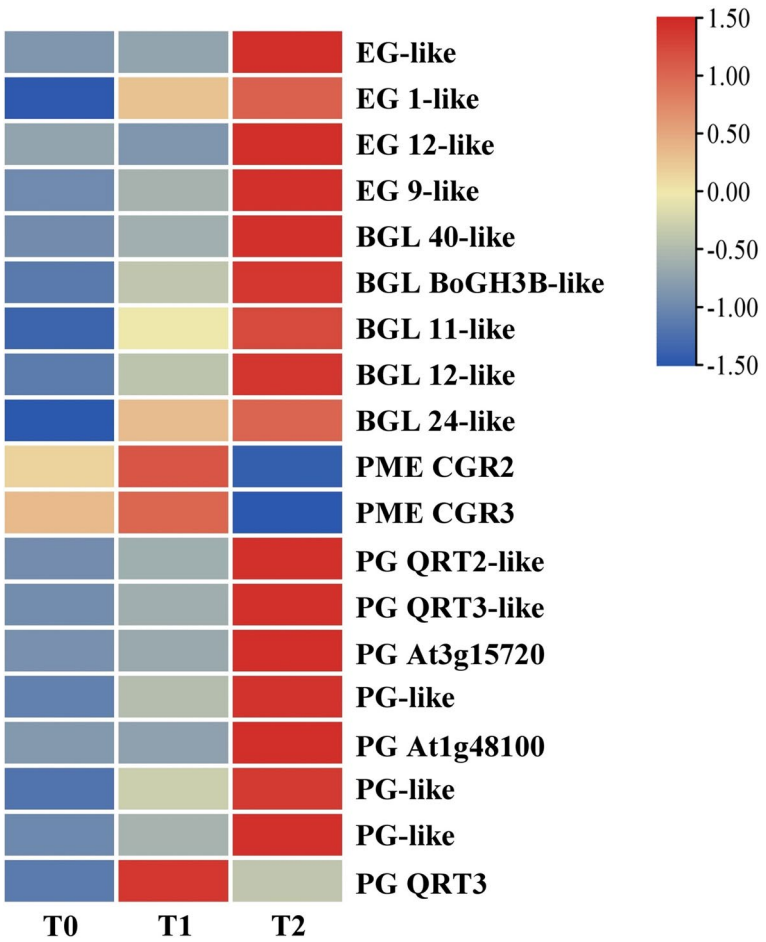

Fig. 6 Heatmap of DEGs related to pectinase and cellulase biosynthesis

tryptophan aminotransferase-associated proteins (TAA1/TARs), and IPA is then converted to IAA via YUCCAs [28, 29]. The downregulated expression of TAA1 and YUCCA reduced the IAA content in T2. This finding is consistent with previous studies on premature abscission of *Lycopersicon esculentum* pedicels, whereas YUCCA overexpression increases the auxin content, which binds to the receptor TIR1 and promotes hydrolysis of the AUX/IAA ubiquitination pathway and dissociation of AUX/IAA-ARF, thus activating ARF, which inhibits the expression of senescence-associated genes and delays organ senescence and abscission [12]. We found that the expression of most of the AUX1, AUX/IAA, and SAUR genes was downregulated in T2; similarly, the expression of the AUX1 and TIR1 proteins was downregulated in *P. avium* stalk abscission, the expression of auxin synthesis precursors and auxin transport carriers was reduced [14], and the suppression of SlPIN1 expression reduced the auxin content of the AZ in tomato [30], leading to the significant upregulation of ethylene and abscisic acid-related genes, which disrupted the hormonal imbalance and stimulated the abscission process [31]. In addition, SAUR acts as an early auxin responsive gene involved in various developmental and physiological processes, such as leaf senescence, fruitlet abscission and hypocotyl development [32]. SAUR was downregulated in T2, whereas *PavSAUR13/16/55/61* are differentially expressed during *P. avium* fruit abscission and are used as candidate genes for physiological fruit drop in *P. avium* [33]. These results suggest that the downregulation of auxin biosynthesis and signal transduction genes activates the expression of organ abscission signals and promotes the process of organ abscission.

However, IAA depletion activates ETH production in AZ cells [26]. In ethylene biosynthesis, s-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) are used as intermediates to synthesize ethylene through three steps, and ACS and ACO are essential for synthesis [34]. During leaf abscission in *C. paliurus*, ACS and ACO were inhibited in T0 and T1 but upregulated in T2. Similarly, these genes were upregulated before fruit abscission in *Pyrus sinkiangensis* [35], *Delphinium grandiflorum* [36],

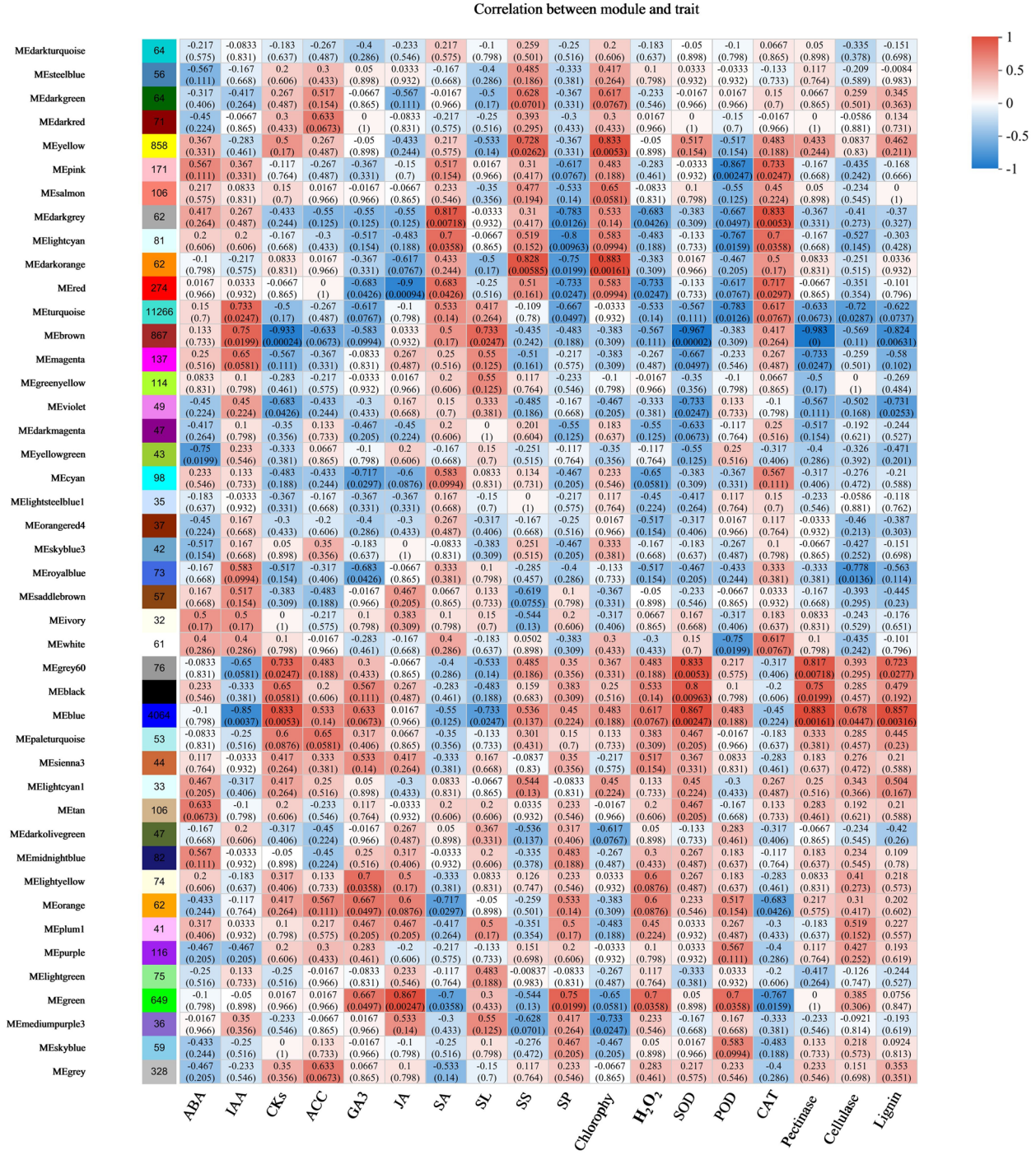

Fig. 7 Heatmap of gene coexpression network modules and phenotype correlation

Malus domestica [15] *L. chinensis* [37], and *P. avium* [14], thereby increasing ethylene biosynthesis. We found that 3 ERFs were significantly upregulated in T2, *AcERF116* played a key role in ethylene crosstalk in *Acacia catechu* fruitlet abscission, and overexpression of the gene accelerated cell separation in the AZ and promoted pedicel abscission in transgenic tomato lines [38]. In addition, SlERF52 is a positive regulator of tomato pedicel abscission, and its silencing delays pedicel abscission because its expression further induces SITIP1;1 expression, which mediates abscission by gating cytoplasmic H_2O_2 concentrations and osmotic water permeability [26], and can

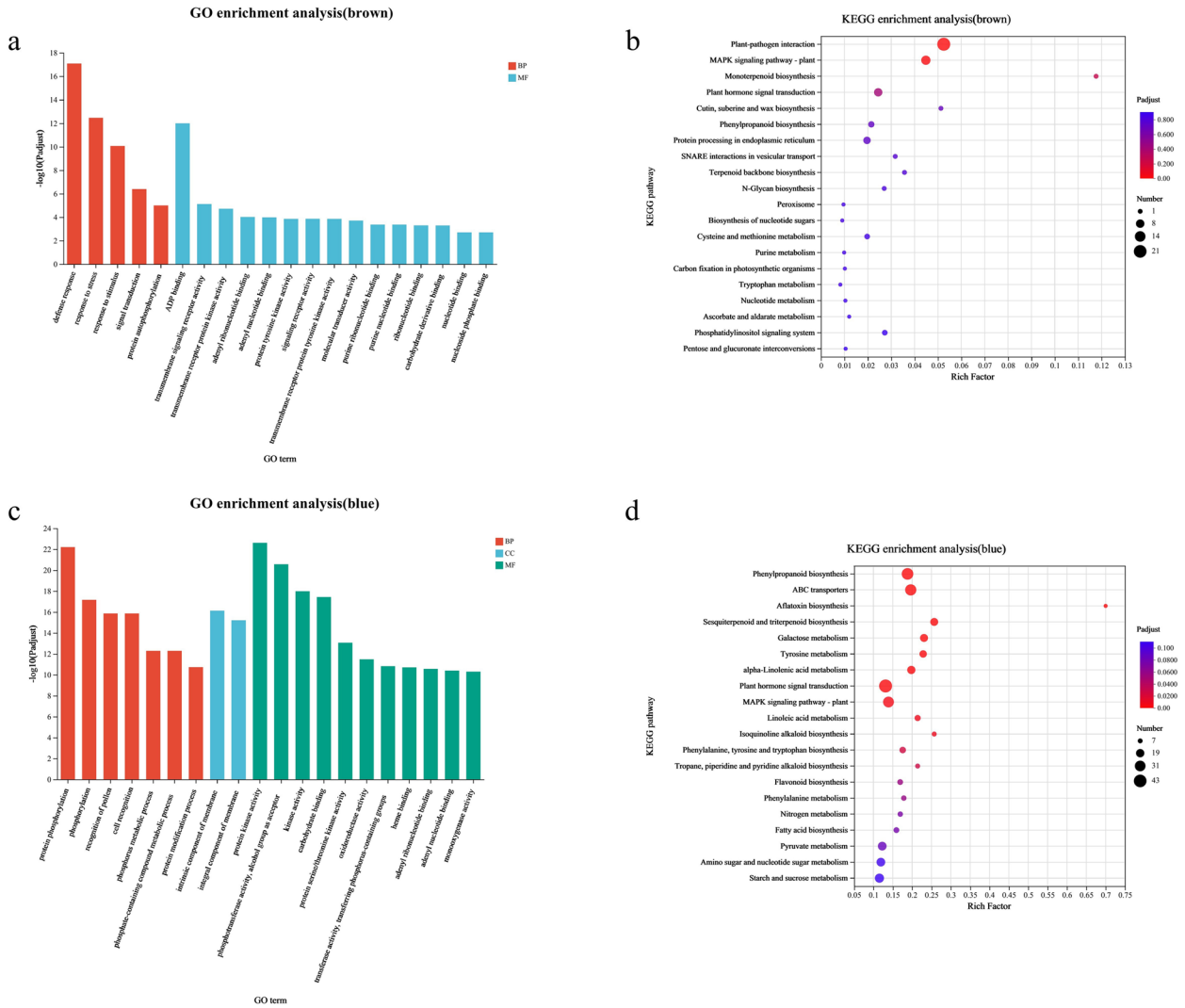

Fig. 8 Enrichment analyses of genes in brown and blue modules, (**a**) GO enrichment in the brown module, (**b**) KEGG enrichment in the brown module, (**c**) GO enrichment in the blue module, (**d**) KEGG enrichment in the blue module

activate cell wall-degrading enzymes, which trigger abscission [39]. In contrast, we identified 3 hub genes (ERF4, ERF5, and ERF109) in the brown module that were significantly downregulated in T2, and Gao et al. [2] reported that reduced expression of *RhERF1 or RhERF4* accelerated petal abscission in *Rosa hybrida*, which regulated petal abscission by affecting the expression of pectin-metabolizing enzyme-encoding genes [2]. These results suggest that ethylene accumulation and related gene expression play decisive roles in the process of leaf abscission in *C. paliurus* (Fig. 11).

DEGs related to phenylpropanoid and flavonoid biosynthesis on leaf abscission in *C. paliurus*

Lignin is the second most abundant component of plant cell walls, which not only improves plant cell wall strength and stem bending resistance but is also associated with the formation of a protective layer in AZ cells during the process of abscission [40, 41]. Lignin is a complex phenolic polymer, and its monomers are synthesized via the shikimate pathway, the phenylpropane pathway, and the lignin-specific pathway [42]. PAL and 4CL are key enzymes in the phenylpropane metabolic

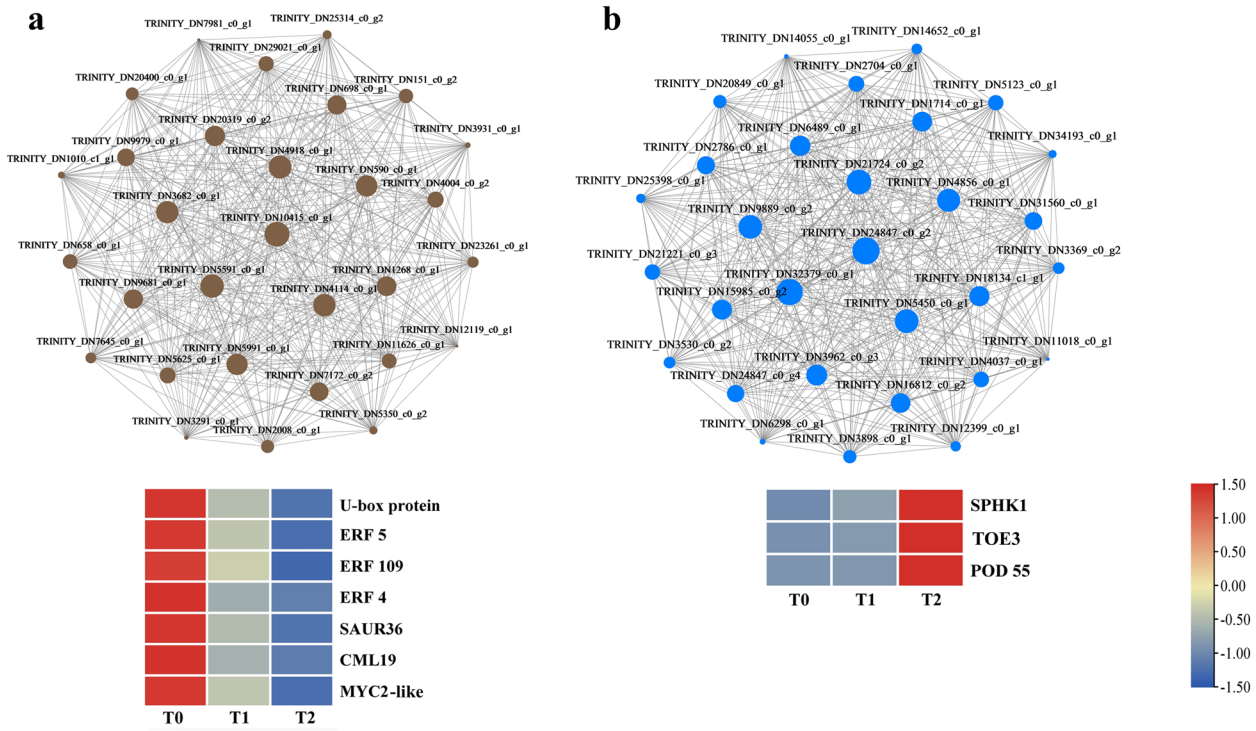

Fig. 9 Coexpression regulatory network, (**a**) Coexpression network and hub gene heatmap in the brown module, (**b**) Coexpression network and hub gene heatmap in the blue module

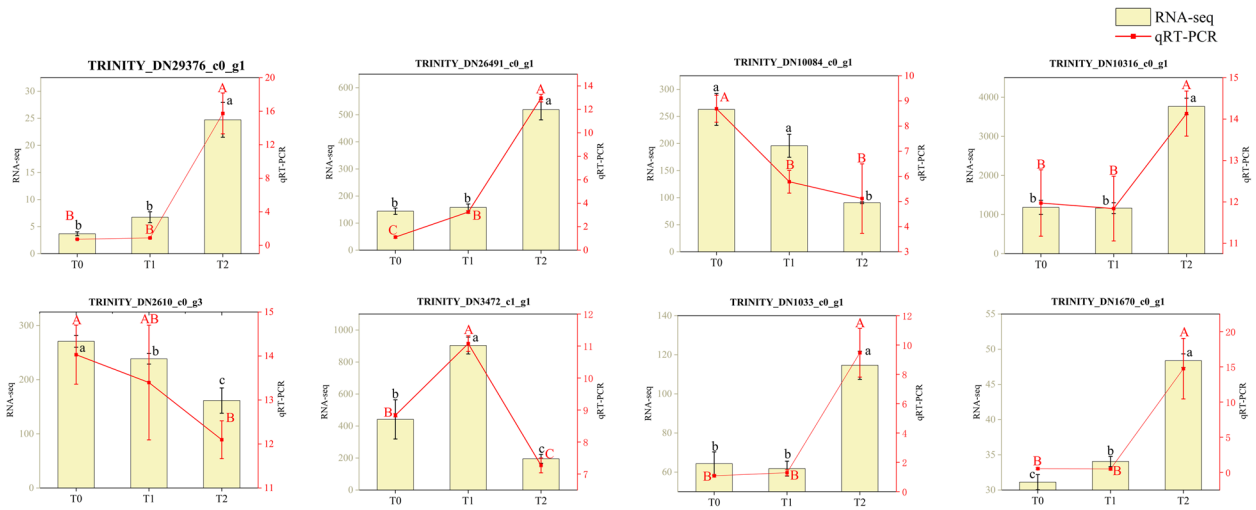

Fig. 10 Comparison of the RNA-Seq and qRT–PCR expression levels. Different letters indicate significant differences ($p < 0.05$)

pathway. PAL converts phenylalanine to cinnamic acid, whereas 4CL catalyzes the conversion of 4-coumaric acid, ferulic acid, and other acids to generate corresponding CoA thioesters, providing a substrate for lignin synthesis [43]. PAL inhibition decreases the lignin content, which affects plant growth and development [44, 45]. We found that the lignin content was significantly greater in T2 than in T0 and that the PAL and 4CL genes were significantly upregulated in T2. Similarly, PAL overexpression increased lignin content in Arabidopsis [46] and promoted *P. avium* fruit abscission [47]. The upregulated expression of the 4CL gene promoted lignin biosynthesis in *Boehmeria nivea* [48] and *Ginkgo biloba* [43]. In addition, POD can catalyze the production of lignin from

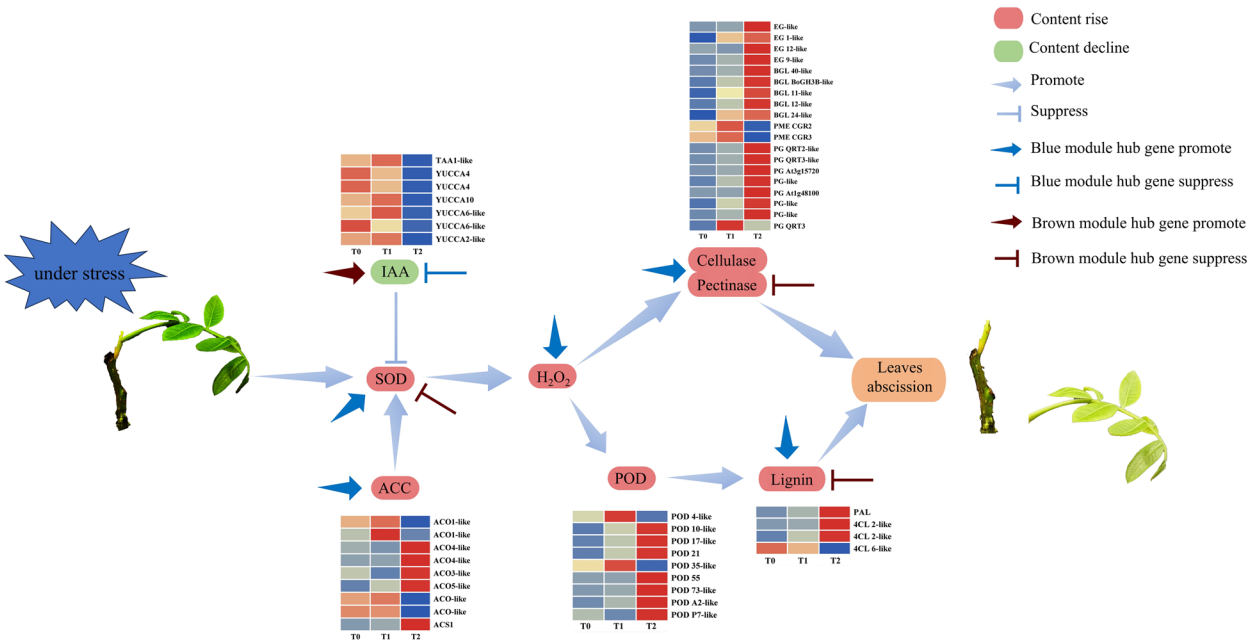

Fig. 11 Modeling of key genes regulating leaf abscission. The stem segments of C. paliurus are subjected to some stresses in vitro, resulting in excessive production of reactive oxygen species; stimulation of the activities of antistress enzymes, pectinases, and cellulases; and accelerated rupture of the AZ cells and the vascular cell wall. Concurrently, the imbalance of endogenous hormones in the leaves, with a reduction in IAA levels and an increase in ACC content [23], was promote the abscission of *C. paliurus* leaves. However the brown/blue module hub genes were positively/negatively correlated with IAA, SOD, pectinase and lignin, which played an important role in regulating leaf abscission of *C. paliurus*

alcohols [46, 49]. In a previous study, POD activity was significantly increased in T2 [22], and 9 POD genes were significantly upregulated in T2, which may be related to the removal of excessive H_2O_2 and lignin synthesis during leaf abscission in *C. paliurus*. These results suggest that the upregulation of lignin synthesis-related genes leads to lignin accumulation, which plays an important role in promoting leaf abscission and the formation of the protective layer in *C. paliurus*.

Flavonoids promote the expression of enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase in cells [50], which can eliminate and mitigate damage caused by abiotic stresses [51]. Flavonoid synthesis is regulated by a complex network; CHS is the first key enzyme that catalyzes flavonoid biosynthesis, and its overexpression increases anthocyanin biosynthesis, which in turn increases plant adaptation to adversity, maintains the highest photosynthetic capacity and causes the least damage [52]. Our study revealed that CHS genes were significantly upregulated in T2, possibly because H_2O_2 accumulation during leaf abscission activated flavonoid biosynthesis, which stimulated the expression of enzymatic antioxidants to scavenge excess ROS. In addition, CHS plays an important role in regulating flavonoid content and leaf color changes in plants [53], and its differential expression affects color changes in *Hosta plantaginea* flowers [54], soybean seed coats [55] and *Alternanthera bettzickiana* leaves [56]. Previous studies revealed that the color of leaves gradually changed from green to yellow during leaf abscission in *C. paliurus* [22], and the CHS gene was significantly upregulated in T2, which was consistent with the results of upregulated expression of the CHS gene, causing the leaves of *Gleditsia sinensis* to turn yellow [57].

DEGs related to cell wall modifying enzymes on leaf abscission in *C. paliurus*

AZ cells are closely connected to each other through growth and differentiation, and their middle layer is rich in pectin, while plant organ abscission is accompanied by cell wall remodeling. AZ cell walls are degraded under the catalysis of pectinases, cellulases and peroxidases [6, 31]. PME and PG are involved in pectin hydrolysis [58, 59]. Cellulase is a complex mixture of extracellular enzymes consisting of three main enzymes that synergistically degrade cellulose: endoglucanase (EG), exoglucanase (or cellobiose hydrolase, CBH), and β-glucosidase (BGL) [60]. PG generates oligogalacturonides and galacturonides by hydrolyzing β−1,4 glycosidic bonds in cell wall polygalacturonides, which are key substances involved in the loss of adhesion between AZ cells [15]. β−1,4-glucanase is associated with abscission [61], which

is involved in the degradation of the AZ cell wall and middle layer [62]. During leaf abscission in *C. paliurus*, pectinase and cellulase activities gradually increased, and the PG, EG, and BGL genes were highly expressed in T2. Similarly, the PG and PME genes are significantly upregulated during fruit abscission in *P. avium* and *L. esculentum* [14, 63], whereas the HD-Zip transcription factor LcHB2 regulates litchi fruit abscission by activating two cellulase genes [64]. Therefore, the AZ cells of *C. paliurus* leaves received abscission signals and activated the expression of the PG, EG and BGL genes, which affected their enzymatic activities and promoted the degradation of the AZ cell wall, leading to leaf abscission (Fig. 11).

Effects of the hub genes on leaf abscission in *C. paliurus*

Among the hub genes in the brown module, the U-box domain protein, as an E3 ubiquitin ligase, is involved in abiotic stress responses such as salt, drought, and low-temperature stress [65, 66], and its significantly downregulated expression in T2 may play an important role in the response to stresses during leaf abscission. The ERF subfamily has specific functions in regulating AZ development [26], and many ERF subfamily transcription factors are involved in the regulation of leaf abscission in *Manihot esculenta* [67]. The Arabidopsis genome contains 8 ERF repressors, namely, *AtERF3*, *AtERF4*, *AtERF7* and *AtERF12*, which have differential expression, suggesting that they may have different functions; *AtERF4* has been shown to be a negative regulator capable of modulating ethylene and abscisic acid responses [68]. As hub genes regulating leaf abscission, ERF4, ERF5 and ERF109 are highly expressed in the early stage of leaf abscission and expressed at low levels in the late stage, suggesting that they are negative regulatory factors for leaf abscission. Mo et al. (2022) reported that ERF5 regulated mulberry anthocyanin biosynthesis by interacting with the MYBA and F3H genes [69]. The SAUR gene family is large, and SAUR36 plays an important role in the regulation of hypocotyl development [70], adventitious root growth [71], and cellular senescence [72]. SAUR36 knockdown in Arabidopsis delayed leaf senescence and was a positive regulator of leaf senescence, mediating the induction of auxin for the process of leaf senescence [73], whereas SAUR36 was downregulated during leaf abscission in *C. paliurus*, which may be related to the complexity of SAUR36 regulation of plant growth and development in different plants. In addition, CMI1 is a Ca^{2+}-dependent transducer of auxin-regulated gene expression, which can function in a cell-specific fashion at steady state as well as at elevated cellular Ca^{2+} levels to regulate auxin responses, and CMI1 is regulated by TIR1/AFB-AUX/IAAs and can rapidly transduce nontranscriptional responses after its expression [74]. Its high expression in the early stage of leaf abscission may be related to the inhibition of the early auxin response in *C. paliurus*. The MYC2 transcription factor has a wide range of biological functions during plant growth, and promoting the expression of MYC2 enhances leaf senescence [75]. In addition, MYC2 is a major regulator of JA-dependent responses [76], which can induce the upregulation of flavonoid and terpenoid biosynthesis genes through JA [77], and its low expression in the leaf abscission of *C. paliurus* plays an important role in leaf color change, leaf development, and leaf abscission.

Among the hub genes of the blue module, sphingosine (Sph) normally inhibits proliferation and promotes apoptosis, whereas the further metabolite sphingosine-1-phosphate (S1P) stimulates growth and inhibits apoptosis; their relative levels are important for the regulation of cell proliferation, survival, and cell death, and sphingosine kinase (SPHK1) phosphorylates Sph to form S1P [78]. In Arabidopsis, SPHK1 modulates FB1-triggered cell death via the SA pathway, ROS accumulation and JA pathway interactions [79]. These findings suggest that SPHK1 may promote AZ cell apoptosis and leaf abscission in *C. paliurus* through the regulation of phytohormones and ROS. The AP2-like ethylene-responsive transcription factor TOE3 (TOE3) has been shown to play an important role in plant responses to multiple abiotic stresses [80–82]. POD is one of the respiratory terminal oxidases involved in plant respiration; it participates in the auxin oxidation process, passivates IAA and decreases IAA levels in AZ cells, and can act not only as an antioxidant to convert H_2O_2 to H_2O but also as a key synthase for lignin biosynthesis [83]. We found that POD55 was significantly upregulated during leaf abscission in *C. paliurus*, suggesting that it plays an important role in regulating ROS metabolism, auxin oxidation, lignin synthesis and protective layer formation (Fig. 11).

Modeling of key genes regulating leaf abscission

In contrast to our previous research [22, 23], we employed a more integrated bioinformatics analysis pipeline. In addition to the standard identification of differentially expressed genes, we constructed a gene co—expression network using WGCNA. This allowed us to explore the complex relationships among genes and identify gene modules with coordinated functions related to leaf abscission. A comprehensive correlation analysis of the hub genes and physiological and biochemical indices revealed that the stem segments of *C. paliurus* were subjected to some stress in vitro, which led to excessive ROS production and stimulated anti-reverse enzyme reactions (Supplementary file 11). In this process, 3 hub genes (SPHK1, TOE3, POD 55) in the blue module played important roles in regulating ROS metabolism,

cell wall-modifying enzyme activity, lignin synthesis and protective layer formation. The 7 hub genes (U-box protein, ERF 5, ERF 109, ERF 4, SAUR36, CML19, and MYC2-like) in the brown module negatively regulated leaf abscission change by affecting AUX and ETH gene expression. In addition, the DEGs in yelliw and darkorange modules were involved in color change of *C. paliurus* during leaves abscission.

Conclusion

A comprehensive correlation analysis of the hub genes and physiological and biochemical indices revealed that the stem segments of *C. paliurus* were subjected to some stress in vitro, which led to excessive ROS production and stimulated anti-reverse enzyme reactions. In this process, 3 hub genes (SPHK1, TOE3, POD 55) in the blue module and 7 hub genes (U-box protein, ERF 5, ERF 109, ERF 4, SAUR36, CML19, and MYC2-like) in the brown module were positively/negatively correlated with IAA, SOD, pectinase and lignin, which played an important role in regulating leaf abscission of *C. paliurus.* The proposed model offers insights into the regulatory mechanisms of leaf abscission in *C. paliurus* stem segments in vitro. By targeting the key genes and pathways identified in the model, it may be possible to develop strategies to reduce leaf abscission, thereby improving the propagation efficiency of *C. paliurus*. Further studies are needed to validate and apply these findings in practical tissue culture propagation systems.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11394-3.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.
Additional file 5.
Additional file 6.
Additional file 7.
Additional file 8.
Additional file 9.
Additional file 10.
Additional file 11.

Acknowledgements
The authors are grateful to all the reviewers for their valuable suggestions regarding the improvement of the manuscript.

Accession numbers
Raw RNA-Seq data files were deposited in SRA (https://www.ncbi.nlm.nih.gov/sra/) under the accession numbers PRJNA1171194.

Authors' contributions
Gaoyin Wu: Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. Zhongcheng Peng: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. Qiuying Li: Investigation, Resources, Supervision. Xiang Zhang: Data curation, Investigation, Validation. Shuanggui Geng: Data curation, Investigation. Shuang Wang: Investigation, Methodology. Enrong Lu: Investigation, Resources. Yingying Liu: Project administration, Resources. Congjun Yuan: Methodology, Resources. Xiaoli Wei: Funding acquisition, Methodology, Resources. Yingliang Liu: Funding acquisition, Resources, Writing – review & editing.

Funding
This manuscript was funded and supported by the National Natural Science Foundation of China–Guizhou Provincial People's Government Karst Science Research Center Project (U1812401), the National Natural Science Foundation of China (31760124), the Department of Education of Guizhou Province [grant code qianjiaoji (2022) 136], the New Seedling Program of Guizhou Normal University [grant code 2021-B05], and the Innovative Talent Team Project of Planting Breeding and Planting Cultivation for Precious Tree Species in Guizhou (Qiankehe Platform Talents -CXTD[2023]006).

Data availability
Raw RNA-Seq data files were deposited in SRA (https://www.ncbi.nlm.nih.gov/sra/) under the accession numbers PRJNA1171194.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]School of Life Sciences, Guizhou Normal University, Guiyang, Guizhou Province 550025, China. [2]Guizhou Key Laboratory of Forest Cultivation in Plateau Mountain, Guiyang, Guizhou Province 550025, China. [3]Guizhou Institute of Biology, Guiyang City, Guizhou Province 550027, China. [4]Guizhou Academy of Forestry, Guiyang, Guizhou Province 550005, China. [5]College of Forestry, Guizhou University, Guiyang, Guizhou 550025, China.

Received: 29 October 2024 Accepted: 21 February 2025
Published online: 03 March 2025

References

1. Sundaresan S, Philosoph-Hadas S, Riov J, et al. Expression Kinetics of Regulatory Genes Involved in the Vesicle Trafficking Processes Operating in Tomato Flower Abscission Zone Cells during Pedicel Abscission [J]. Life. 2020;10(11):273. https://doi.org/10.3390/life10110273.
2. Gao Y, Liu Y, Liang Y, et al. Rosa hybrida RhERF1 and RhERF4 mediate ethylene- and auxin-regulated petal abscission by influencing pectin degradation [J]. Plant J. 2019;99(6):1159–71. https://doi.org/10.1111/tpj.14412.
3. Xu J, Chen L, Sun H, et al. Crosstalk between cytokinin and ethylene signaling pathways regulates leaf abscission in cotton in response to chemical defoliants [J]. J Experiment Botany. 2019;70(5):1525–38. https://doi.org/10.1093/jxb/erz036.
4. Zhao ML, Li JG. Molecular Events Involved in Fruitlet Abscission in Litchi [J]. Plants-Basel. 2020:9(2):151. https://doi.org/10.3390/plants9020151.
5. Liu YT, Xiao WJ. Research progress on square and boll shed ding and high temperature tolerance breeding of cotton under heat stress [J]. Mol Plant Breed. 2019;17(15):5089–96. https://doi.org/10.13271/j.mpb.017.005089.

6. Yu Y, Leyva P, Tavares RL, et al. The anatomy of abscission zones is diverse among grass species [J]. Am J Bot. 2020;107(4):549–61. https://doi.org/10.1002/ajb2.1454.
7. Nakano T, Ito Y. Molecular mechanisms controlling plant organ abscission [J]. Plant Biotechnol. 2013;30(3):209–16. https://doi.org/10.5511/plantbiotechnology.13.0318a.
8. Li C, Zhou A, Sang T. Rice domestication by reducing shattering [J]. Science. 2006;311(5769):1936–9. https://doi.org/10.1126/science.1123604.
9. Konishi S, Izawa T, Lin SY, et al. An SNP caused loss of seed shattering during rice domestication [J]. Science. 2006;312(5778):1392–6. https://doi.org/10.1126/science.1126410.
10. Hofmann NR. SHAT1, A new player in seed shattering of rice [J]. Plant Cell. 2012;24(3):839. https://doi.org/10.1105/tpc.112.240310.
11. Zhou Y, Lu D, Li C, et al. Genetic control of seed shattering in rice by the APETALA2 transcription factor shattering abortion1[J]. Plant Cell. 2012;24(3):1034–48. https://doi.org/10.1105/tpc.111.094383.
12. Nakano T, Fujisawa M, Shima Y, et al. Expression profiling of tomato pre-abscission pedicels provides insights into abscission zone properties including competence to respond to abscission signals [J]. BMC Plant Biol. 2013:13(1). https://doi.org/10.1186/1471-2229-13-40.
13. Wilmowicz E, Kucko A, Tranbarger TJ, et al. EPIP as an abscission promoting agent in the phytohormonal pathway [J]. Plant Physiol Biochem. 2022;178:137–45. https://doi.org/10.1016/j.plaphy.2022.03.008.
14. Qiu ZL, Wen Z, Yang K, et al. Comparative Proteomics Profiling Illuminates the Fruitlet Abscission Mechanism of Sweet Cherry as Induced by Embryo Abortion [J]. Int J Mol Sci. 2020; 21(4). https://doi.org/10.3390/ijms21041200.
15. Qi X, Wu J, Wang L, et al. Identifying the candidate genes involved in the calyx abscission process of "Kuerlexiangli" (Pyrus sinkiangensis Yu) by digital transcript abundance measurements[J]. BMC Genomics. 2013;14(1):727. https://doi.org/10.1186/1471-2164-14-727.
16. Yao Y, Yan LJ, Chen H, et al. Polysaccharides alleviate type 2 diabetic symptoms by modulating gut microbiota and short-chain fatty acids [J]. Phytomedicine. 2020:77(0):153268. https://doi.org/10.1016/j.phymed.2020.153268.
17. Wang H, Tang C, Gao ZZ, et al. Potential Role of Natural Plant Medicine in the Treatment of Type 2 Diabetes Mellitus [J]. J Diabet Res. 2021:2021(0):1–12.
18. Wang X, Tang L, Ping W, et al. Progress in Research on the Alleviation of Glucose Metabolism Disorders in Type 2 Diabetes Using Cyclocarya paliurus [J]. Nutrients. 2022:14(15). https://doi.org/10.3390/nu14153169.
19. Fang S, Wang J, Wei Z, et al. Methods to break seed dormancy in Cyclocarya paliurus (Batal)Iljinskaja[J]. Scientia Horticulturae. 2006;110(3):305–9. https://doi.org/10.1016/j.scienta.2006.06.031.
20. Mao X, Fu XX, Huang P, et al. Heterodichogamy, Pollen Viability, and Seed Set in a Population of Polyploidy Cyclocarya Paliurus (Batal) Iljinskaja (Juglandaceae) [J]. Forests. 2019;10(4):347. https://doi.org/10.3390/f10040347.
21. Liu YY, Wu GY, Zhang ZM, et al. A method for inducing germination of Cyclocarya paliurus stem tissue culture by a bacteriostatic agent [P]. China: ZL202111340555.7,2022-05-27.
22. Li QY, Wang S, Wu GY, et al. Physiological and biochemical changes in leaf abscission of stem segments in vitro [J]. Plant Cell Tissue and Organ Culture. 2023;155(3):773–83. https://doi.org/10.1007/s11240-023-02598-0.
23. Wu GY, Li QY, Wang L, et al. Endogenous hormone levels regulate the leaf abscission process of Cyclocarya paliurus stem segments in vitro [J]. Scientia Horticulturae. 2024; 329. https://doi.org/10.1016/j.scienta.2024.113010.
24. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis [J]. BMC Bioinformatics. 2008;9:559. https://doi.org/10.1186/1471-2105-9-559.
25. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks [J]. Genome Res. 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303.
26. Wang R, Li R, Cheng L, et al. SlERF52 regulates SlTIP1;1 expression to accelerate tomato pedicel abscission [J]. Plant Physiol. 2021;185(4):1829–46. https://doi.org/10.1093/plphys/kiab026.
27. Shi CL, Butenko MA. Visualizing Morphological Changes of Abscission Zone Cells in Arabidopsis by Scanning Electron Microscope [J]. Methods Mol Biol. 2018;1744:321–8. https://doi.org/10.1007/978-1-4939-7672-0_26.
28. Luo P, Di DW. Precise Regulation of the TAA1/TAR-YUCCA Auxin Biosynthesis Pathway in Plants [J]. Int J Mol Sci. 2023:24(10). https://doi.org/10.3390/ijms24108514.
29. Sato A, Soeno K, Kikuchi R, et al. Indole-3-pyruvic acid regulates TAA1 activity, which plays a key role in coordinating the two steps of auxin biosynthesis [J]. Proc Natl Acad Sci U S A. 2022;119(25):e2203633119. https://doi.org/10.1073/pnas.2203633119.
30. Shi Z, Jiang Y, Han X, et al. SlPIN1 regulates auxin efflux to affect flower abscission process [J]. Sci Rep. 2017;7(1):14919. https://doi.org/10.1038/s41598-017-15072-7.
31. Basu MM, González-Carranza ZH, Azam-Ali S, et al. The manipulation of auxin in the abscission zone cells of Arabidopsis flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding[J]. Plant Physiol. 2013;162(1):96–106. https://doi.org/10.1104/pp.113.216234.
32. Xie R, Dong C, Ma Y, et al. Comprehensive analysis of SAUR gene family in citrus and its transcriptional correlation with fruitlet drop from abscission zone A [J]. Funct Integr Genomics. 2015;15(6):729–40. https://doi.org/10.1007/s10142-015-0450-3.
33. Hou QD, Hong Y, Wen Z, et al. Molecular characterization of the gene family in sweet cherry and functional analysis of in the process of abscission [J]. J Integrat Agricult. 2023;22(6):1720–39. https://doi.org/10.1016/j.jia.2023.04.031.
34. Polko JK, Kieber JJ. 1-Aminocyclopropane 1-Carboxylic Acid and Its Emerging Role as an Ethylene-Independent Growth Regulator [J]. Front Plant Sci. 2019;10:1602. https://doi.org/10.3389/fpls.2019.01602.
35. Dal Cin V, Barbaro E, Danesin M, et al. Fruitlet abscission: A cDNA-AFLP approach to study genes differentially expressed during shedding of immature fruits reveals the involvement of a putative auxin hydrogen symporter in apple (Malus domestica L. Borkh) [J]. Gene. 2009:442(1–2):26–36. https://doi.org/10.1016/j.gene.2009.04.009.
36. Okamoto M, Niki T, Azuma M, et al. Expression of ethylene biosynthesis genes in the gynoecium and receptacle associated with sepal abscission during senescence in Delphinium grandiflorum [J]. Plant Growth Regulation. 2022;97(3):593–609. https://doi.org/10.1007/s10725-022-00822-z.
37. Ma X, Yuan Y, Li C, et al. Brassinosteroids suppress ethylene-induced fruitlet abscission through LcBZR1/2-mediated transcriptional repression of LcACS1/4 and LcACO2/3 in litchi [J]. Hortic Res. 2021;8(1):105. https://doi.org/10.1038/s41438-021-00540-z.
38. Li J, Chen Y, Wang L, et al. An ethylene response factor AcERF116 identified from A. catechu is involved in fruitlet abscission [J]. Plant Sci. 2024:344:112091. https://doi.org/10.1016/j.plantsci.2024.112091.
39. Nakano T, Fujisawa M, Shima Y, et al. The AP2/ERF transcription factor SlERF52 functions in flower pedicel abscission in tomato [J]. J Exp Bot. 2014;65(12):3111–9. https://doi.org/10.1093/jxb/eru154.
40. Hu WJ, Harding SA, Lung J, et al. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees [J]. Nat Biotechnol. 1999;17(8):808–12. https://doi.org/10.1038/11758.
41. Agustí J, Merelo P, Cercós M, et al. Ethylene-induced differential gene expression during abscission of citrus leaves [J]. J Exp Bot. 2008;59(10):2717–33. https://doi.org/10.1093/jxb/ern138.
42. Bonawitz ND, Chapple C. The genetics of lignin biosynthesis: connecting genotype to phenotype [J]. Annu Rev Genet. 2010;44:337–63. https://doi.org/10.1146/annurev-genet-102209-163508.
43. Zhou X, Cao J, Liu XM, et al. Cloning and functional analysis of Gb4CL1 and Gb4CL2 from Ginkgo biloba [J]. Plant Genome. 2024;17(2):e20440. https://doi.org/10.1002/tpg2.20440.
44. Zhang X, Liu CJ. Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids [J]. Mol Plant. 2014. https://doi.org/10.1093/mp/ssu134.
45. Zhang W, Zhang S, Lu X, et al. Tissue-specific Transcriptome analysis reveals lignocellulose synthesis regulation in elephant grass (Pennisetum purpureum Schum) [J]. BMC Plant Biol. 2020;20(1):528. https://doi.org/10.1186/s12870-020-02735-3.
46. Qin Y, Li Q, An Q, et al. A phenylalanine ammonia lyase from Fritillaria unibracteata promotes drought tolerance by regulating lignin biosynthesis and SA signaling pathway [J]. Int J Biol Macromol. 2022;213:574–88. https://doi.org/10.1016/j.ijbiomac.2022.05.161.
47. Qiu Z, Wen Z, Hou Q, et al. Cross-talk between transcriptome, phytohormone and HD-ZIP gene family analysis illuminates the molecular mechanism underlying fruitlet abscission in sweet cherry (Prunus

avium L) [J]. BMC Plant Biol. 2021;21(1):173. https://doi.org/10.1186/s12870-021-02940-8.
48. Tang Y, Liu F, Xing H, et al. Correlation Analysis of Lignin Accumulation and Expression of Key Genes Involved in Lignin Biosynthesis of Ramie (Boehmeria nivea) [J]. Genes (Basel). 2019:10(5). https://doi.org/10.3390/genes10050389.
49. Poovaiah BW. Peroxidase Activity in the Abscission Zone of Bean Leaves during Abscission [J]. Plant Physiol. 1973;52(3):263–7. https://doi.org/10.1104/pp.52.3.263.
50. Kejík Z, Kaplánek R, Masařík M, et al. Iron Complexes of Flavonoids-Antioxidant Capacity and Beyond [J]. Int J Mol Sci. 2021:22(2). https://doi.org/10.3390/ijms22020646.
51. Wheeler LC, Walker JF, Ng J, et al. Transcription Factors Evolve Faster Than Their Structural Gene Targets in the Flavonoid Pigment Pathway [J]. Mol Biol Evol. 2022:39(3). https://doi.org/10.1093/molbev/msac044.
52. Zhang XH, Zheng XT, Sun BY, et al. Over-expression of the gene enhances resistance of leaves to high light [J]. Environment Experiment Botany. 2018;154:33–43. https://doi.org/10.1016/j.envexpbot.2017.12.011.
53. Zhang Z, Qu P, Hao S, et al. Characterization and Functional Analysis of Chalcone Synthase Genes in Highbush Blueberry (Vaccinium corymbosum) [J]. Int J Mol Sci. 2023:24(18). https://doi.org/10.3390/ijms241813882.
54. Zhang J, Sui C, Wang Y, et al. Transcriptome-Wide Analysis Reveals Key DEGs in Flower Color Regulation of Hosta plantaginea (Lam.) Aschers [J]. Genes (Basel). 2019:11(1). https://doi.org/10.3390/genes11010031.
55. Yuan B, Yuan C, Wang Y, et al. Identification of genetic loci conferring seed coat color based on a high-density map in soybean [J]. Front Plant Sci. 2022;13:968618. https://doi.org/10.3389/fpls.2022.968618.
56. Li W, Li H, Shi L, et al. Leaf color formation mechanisms in Alternanthera bettzickiana elucidated by metabolite and transcriptome analyses [J]. Planta. 2022;255(3):59. https://doi.org/10.1007/s00425-022-03840-3.
57. Wu C, Yang XM, Feng LJ, et al. Identification of key leaf color-associated genes in using bioinformatics [J]. Horticulture Environ Biotechnol. 2019;60(5):711–20. https://doi.org/10.1007/s13580-019-00161-5.
58. Carrasco M, Rozas JM, Alcaíno J, et al. Pectinase secreted by psychrotolerant fungi: identification, molecular characterization and heterologous expression of a cold-active polygalacturonase from Tetracladium sp [J]. Microb Cell Fact. 2019;18(1):45. https://doi.org/10.1186/s12934-019-1092-2.
59. Tu T, Bai Y, Luo H, et al. A novel bifunctional pectinase from Penicillium oxalicum SX6 with separate pectin methylesterase and polygalacturonase catalytic domains [J]. Appl Microbiol Biotechnol. 2014;98(11):5019–28. https://doi.org/10.1007/s00253-014-5533-x.
60. Kubicek CP, Mikus M, Schuster A, et al. Metabolic engineering strategies for the improvement of cellulase production by Hypocrea jecorina [J]. Biotechnol Biofuels. 2009;2:19. https://doi.org/10.1186/1754-6834-2-19.
61. Wang Q, Zhao D, Shen L, et al. Regulation of plant organs abscission by abscission regulating substances[J]. Acta Bot Boreali-Occidentalia Sinica. 2009;29(11):2352–9. https://doi.org/10.3321/j.issn:1000-4025.2009.11.030.
62. Hao Z, Wu X, Liu Z, et al. Research progress on organ abscission of fruit trees [J]. Jiangsu Agricultural Sciences. 2020;48(16):58–66. https://doi.org/10.15889/j.issn.1002-1302.2020.16.010.
63. Nakano T, Kimbara J, Fujisawa M, et al. MACROCALYX and JOINTLESS interact in the transcriptional regulation of tomato fruit abscission zone development [J]. Plant Physiol. 2012;158(1):439–50. https://doi.org/10.1104/pp.111.183731.
64. Li C, Zhao M, Ma X, et al. The HD-Zip transcription factor LcHB2 regulates litchi fruit abscission through the activation of two cellulase genes [J]. J Exp Bot. 2019;70(19):5189–203. https://doi.org/10.1093/jxb/erz276.
65. Hu H, Dong C, Sun D, et al. Genome-Wide Identification and Analysis of U-Box E3 Ubiquitin-Protein Ligase Gene Family in Banana [J]. Int J Mol Sci. 2018:19(12). https://doi.org/10.3390/ijms19123874.
66. Tang X, Ghimire S, Liu W, et al. Genome-wide identification of U-box genes and protein ubiquitination under PEG-induced drought stress in potato [J]. Physiol Plant. 2022;174(1):e1347. https://doi.org/10.1111/ppl.13475.
67. Liao W, Li Y, Yang Y, et al. Exposure to various abscission-promoting treatments suggests substantial ERF subfamily transcription factors involvement in the regulation of cassava leaf abscission [J]. BMC Genomics. 2016;17:538. https://doi.org/10.1186/s12864-016-2845-5.
68. Yang Z, Tian L, Latoszek-Green M, et al. Arabidopsis ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses [J]. Plant Mol Biol. 2005;58(4):585–96. https://doi.org/10.1007/s11103-005-7294-5.
69. Mo R, Han G, Zhu Z, et al. The Ethylene Response Factor ERF5 Regulates Anthocyanin Biosynthesis in 'Zijin' Mulberry Fruits by Interacting with MYBA and F3H Genes [J]. Int J Mol Sci. 2022:23(14). https://doi.org/10.3390/ijms23147615.
70. Stamm P, Kumar PP. Auxin and gibberellin responsive Arabidopsis SMALL AUXIN UP RNA36 regulates hypocotyl elongation in the light [J]. Plant Cell Rep. 2013:32(6):759–769. https://doi.org/10.1007/s00299-013-1406-5.
71. Liu R, Wen SS, Sun TT, et al. PagWOX11/12a positively regulates the PagSAUR36 gene that enhances adventitious root development in poplar [J]. J Exp Bot. 2022;73(22):7298–311. https://doi.org/10.1093/jxb/erac345.
72. Ghimire U, Abeli P, Brecht JK, et al. Unique molecular mechanisms revealed for the effects of temperature, CA, ethylene exposure, and 1-MCP on postharvest senescence of broccoli [J]. Postharvest Biol Technol. 2024:213. http://ARTN 11291910.1016/j.postharvbio.2024.112919
73. Hou K, Wu W, Gan SS. SAUR36, a small auxin up RNA gene, is involved in the promotion of leaf senescence in Arabidopsis [J]. Plant Physiol. 2013;161(2):1002–9. https://doi.org/10.1104/pp.112.212787.
74. Hazak O, Mamon E, Lavy M, et al. A novel Ca2+-binding protein that can rapidly transduce auxin responses during root growth [J]. PLoS Biol. 2019;17(7):e3000085. https://doi.org/10.1371/journal.pbio.3000085.
75. Zhuo M, Sakuraba Y, Yanagisawa S. A Jasmonate-Activated MYC2-Dof2.1-MYC2 Transcriptional Loop Promotes Leaf Senescence in Arabidopsis [J]. Plant Cell. 2020:32(1):242–262. https://doi.org/10.1105/tpc.19.00297.
76. Wu S, Hu C, Zhu C, et al. The MYC2-PUB22-JAZ4 module plays a crucial role in jasmonate signaling in tomato [J]. Mol Plant. 2024;17(4):598–613. https://doi.org/10.1016/j.molp.2024.02.006.
77. Adolfsson L, Nziengui H, Abreu IN, et al. Enhanced Secondary- and Hormone Metabolism in Leaves of Arbuscular Mycorrhizal Medicago truncatula [J]. Plant Physiol. 2017;175(1):392–411. https://doi.org/10.1104/pp.16.01509.
78. Maceyka M, Payne SG, Milstien S, et al. Sphingosine kinase, sphingosine-1-phosphate, and apoptosis [J]. Biochim Biophys Acta. 2002;1585(2–3):193–201. https://doi.org/10.1016/s1388-1981(02)00341-4.
79. Qin X, Zhang RX, Ge S, et al. Sphingosine kinase AtSPHK1 functions in fumonisin B1-triggered cell death in Arabidopsis [J]. Plant Physiol Biochem. 2017;119:70–80. https://doi.org/10.1016/j.plaphy.2017.08.008.
80. Yin F, Zeng Y, Ji J, et al. The Halophyte Halostachys caspica AP2/ERF Transcription Factor HcTOE3 Positively Regulates Freezing Tolerance in Arabidopsis [J]. Front Plant Sci. 2021;12:638788. https://doi.org/10.3389/fpls.2021.638788.
81. Zhu J, Wei X, Yin C, et al. ZmEREB57 regulates OPDA synthesis and enhances salt stress tolerance through two distinct signalling pathways in Zea mays [J]. Plant Cell Environ. 2023;46(9):2867–83. https://doi.org/10.1111/pce.14644.
82. Xie Z, Nolan T, Jiang H, et al. The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Responses in Arabidopsis [J]. Plant Cell. 2019;31(8):1788–806. https://doi.org/10.1105/tpc.18.00918.
83. Liu J, Lv Y, Li M, et al. Peroxidase in plant defense: Novel insights for cadmium accumulation in rice (Oryza sativa L.) [J]. J Hazard Mater. 2024:474:134826. https://doi.org/10.1016/j.jhazmat.2024.134826.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.