Identification of the Tea Plant *DIR* Gene Family and Analysis of Its Expression Patterns under Anthracnose Stress

SUN Zhipeng¹, ZHAO Yichen^{1,2*}

2026-06-26

ChinaRxiv

Abstract

Dirigent (DIR) proteins participate in the biosynthesis of lignin and lignans in plants and play important roles in plant growth, development, and stress responses. In this study, 40 CsDIR family genes were identified from the whole genome of tea plant (Camellia sinensis), and the physicochemical properties, phylogenetic tree, conserved motifs, chromosomal localization, structural characteristics of cis-acting elements, and gene expression patterns under tea anthracnose stress were analyzed. The results showed that: (1) evolutionary analysis indicated that tea plant DIR genes could be divided into 3 subfamilies, and their promoter regions contained responsive elements related to auxin, abscisic acid, wounding, and pathogens. (2) Protein structure and conserved domain analyses showed that all 40 CsDIR proteins contained a β-barrel composed of 8 antiparallel β-sheets. 39 CsDIR proteins contained the Dirigent domain, and 1 CsDIR protein contained the Dirigent superfamily domain. (3) Chromosomal localization and collinearity analyses showed that the 40 CsDIR genes were distributed on 10 chromosomes and 5 different Contigs, with 12 pairs of tandemly duplicated genes and 4 pairs of collinear genes in the CsDIR gene family. (4) Analysis of the expression patterns of 12 CsDIR family genes under tea anthracnose stress showed that all 12 CsDIR family genes responded to tea anthracnose stress. These results provide a reference for further studies on the functions of the DIR gene family in the response of tea plant to biotic stress.

Full Text

DOI:10.11931/guihaia.gxzw202505020

Identification of the Tea Plant *DIR* Gene Family and Analysis of Its Expression Patterns under Anthracnose Stress

SUN Zhipeng1, ZHAO Yichen1,2*

(1. College of Life Sciences/Institute of Agro-Bioengineering/Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region, Ministry of Education, Guizhou University, Guiyang 550025; 2. College of Tea Sciences, Guizhou University, Guiyang 550025)

Abstract: Dirigent (DIR) proteins participate in the biosynthesis of lignin and lignans in plants and play important roles in plant growth, development, and stress responses. In this study, 40 *CsDIR* family genes were identified from the whole genome of tea plant (*Camellia sinensis*), and their physicochemical properties, phylogenetic tree, conserved motifs, chromosomal localization, structural characteristics of cis-acting elements, and gene expression patterns under tea anthracnose stress were analyzed. The results showed that: (1) phylogenetic

analysis indicated that tea plant *DIR* genes could be divided into three subfamilies, and their promoter regions contained response elements for auxin, abscisic acid, wounding, and pathogenic bacteria. (2) Analyses of protein structure and conserved domains showed that all 40 CsDIR proteins contained β-barrels composed of eight antiparallel β-strands. Thirty-nine CsDIR proteins contained a Dirigent structure, and one CsDIR protein contained a Dirigent superfamily domain. (3) Chromosomal localization and collinearity analyses showed that the 40 *CsDIR* genes were distributed on 10 chromosomes and five different contigs; within the *CsDIR* gene family, there were 12 pairs of tandemly duplicated genes and four pairs of collinear genes. (4) Analysis of the expression patterns of 12 *CsDIR* family genes under tea anthracnose stress showed that all 12 *CsDIR* family genes responded to tea anthracnose stress. These results provide a reference for further studies on the functions of the *DIR* gene family in tea plant responses to biotic stress.

Keywords: tea plant; *DIR* gene family; structural characteristics; physicochemical properties; expression analysis

Chinese Library Classification No.: Q943 **Document Code:** A

Identification and expression pattern of *DIR* gene family in *Camellia sinensis* under anthracnose stress

SUN Zhipeng1, ZHAO Yichen1,2*

(1. *Key laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), College of Life Sciences/Institute of Agro-Bioengineering, Guizhou University*, Guiyang 550025, China; 2. *College of Tea Sciences, Guizhou University*, Guiyang 550025, China)

Abstract: Dirigent (DIR) proteins play crucial roles in lignin and lignan biosynthesis, significantly influencing plant growth, development and stress responses. This study identified 40 *CsDIR* genes through genome-wide screening in *Camellia sinensis*, and analysed for physicochemical properties, phylogenetic tree, conserved motifs, chromosomal localisation, cis-acting element structural features, and gene expression patterns of *DIR* gene family under tea anthracnose stress.

Funding: National Natural Science Foundation of China (32160077); Guizhou Provincial Science and Technology Plan Project (Qian Academy of Agricultural Sciences Talent Special Project [2024]02).

First author: SUN Zhipeng (1998–), master' s student, research direction: plant genetic engineering, (E-mail) 1572612519@qq.com.

***Corresponding author:** ZHAO Yichen, Ph.D., professor; research interests include plant secondary signal transduction, plant physiology and biochem-

istry under abiotic/biotic stress, and biosynthetic pathways of plant secondary metabolites; (E-mail) yczhao@gzu.edu.cn.

The results were as follows: (1) Phylogenetic analysis classified the *CsDIR* genes into three subfamilies, and their promoter regions contained response elements for auxin, abscisic acid, wounding, and pathogens. (2) Analysis of protein structures and conserved domains confirmed that 40 CsDIRs contain a β-barrel composed of eight antiparallel β-strands. Thirty-nine CsDIRs possess a Dirigent domain, whereas one contains a Dirigent superfamily domain. (3) Chromosomal localization and collinearity analyses indicated that the 40 *CsDIR* genes are distributed across 10 chromosomes and 5 distinct contigs. Within the *CsDIR* gene family, we identified 12 pairs of tandemly duplicated genes and 4 pairs of collinear genes. (4) Expression-pattern analysis of 12 *CsDIR* genes under tea anthracnose stress demonstrated that all 12 *CsDIR* genes responded uniformly to tea anthracnose stress. This study provides a foundation for future research on the function of the *DIR* gene family in response to biotic stresses in tea plants.

Key words: *Camellia sinensis*, *DIR* gene family, structural features, physicochemical properties, expression analysis

Tea plant (*Camellia sinensis*) is a perennial woody plant of important economic value in China (Wu Yingqi et al., 2023). With the continued development of the tea industry, tea has become one of the major beverages consumed worldwide (Farag et al., 2023). Tea plants are rich in primary metabolites such as minerals, sugars, and amino acids, and they also contain abundant secondary metabolites such as catechins. These compounds endow tea plants with pronounced medicinal properties, including antibacterial activity, antioxidant capacity, and anti-inflammatory and sedative effects; they not only benefit human health but can also enhance plant resistance to biotic and abiotic stresses by activating defense mechanisms. As a typical leaf-harvested economic crop, fungal foliar diseases of tea plants are one of the main causes of quality deterioration and yield loss, imposing sustained pressure on the production of high-quality tea. Among them, tea anthracnose, as one of the important foliar diseases of tea plants, is characterized by widespread occurrence in tea-garden ecosystems and poses a significant threat to tea production capacity (Zhang Yudan et al., 2023). Tea plants are extremely sensitive to changes in the growing environment, and adverse conditions can seriously affect their growth and development as well as the yield and quality of tea leaves (Xu Xiaohan et al., 2022).

To cope with biotic and abiotic stresses, plants have evolved a series of defense measures, including oxidative cleavage, lignan synthesis, lignin deposition, and signaling pathways such as abscisic acid and jasmonic acid, so as to respond rapidly to complex environments, reduce damage, and secure more resources for survival (Faisal & Muhammad, 2022). Proteins encoded by *Dirigent* (*DIR*) genes recognize specific substrates during lignin biosynthesis and guide the coupling of monolignol monomers to form dimers, thereby participating in lignin assembly. During lignan biosynthesis, DIR proteins can guide the coupling

of coniferyl alcohol enantiomers to produce lignans. *DIR* genes are found in almost all vascular plants and, as important regulatory factors in lignin and lignan biosynthesis, regulate plant stress responses (Mo et al., 2021). Shi et al. (2012) showed that overexpression of the cotton (*Gossypium hirsutum*) *DIR* family gene *GhDIR1* in cotton led to an increase in cotton lignin content and improved resistance to the Verticillium wilt fungus (*Verticillium dahliae*). The expression of *GmDIR22* was upregulated in soybean (*Glycine max*) after inoculation with *Phytophthora sojae*. Compared with the wild type, soybean overexpressing *GmDIR22* showed a significant increase in total lignan accumulation and enhanced resistance to *Phytophthora sojae* (Li et al., 2017). Overexpression of *PnDIR1* in notoginseng (*Panax notoginseng*) significantly increased the expression of genes related to lignin and lignan biosynthesis and enhanced the resistance of notoginseng to *Fusarium solani* (Guan Ruipan, 2018). Silencing pepper

(*Capsicum annuum*) *CaDIR7* reduced root activity in pepper, weakened the defensive capacity of pepper, and led to a significant decrease in pepper tolerance to *Phytophthora capsici* and salt stress (Khan et al., 2018). In maize (*Zea mays*), mutation of the *DIR* family gene *ZmESBL* caused damage to the Casparian strip, increased accumulation of Na^+ in shoots, and enhanced sensitivity to salt stress owing to reduced lignification of the endodermal Casparian strip (Wang et al., 2022). In the resurrection plant *Boea hygrometrica*, the *BhDIR1* gene can respond rapidly to stresses such as drought, chilling injury, and high temperature, and is also regulated by signaling molecules such as ethylene (ET), abscisic acid (ABA), and salicylic acid (SA) (Wu Renhua et al., 2008). Compared with untreated *Isatis indigotica*, the genes *IiDIR8*, *IiDIR9*, *IiDIR10*, and *IiDIR16* in roots of *I. indigotica* treated with methyl jasmonate (MeJA) were expressed to varying degrees, and *IiDIR8* and *IiDIR9* showed a trend of sustained up-regulation (Li et al., 2014). In summary, *DIR* genes play important roles in plant responses to biotic and abiotic stresses.

To understand the role of tea plant *DIR* genes in the response of tea plants to biotic stress, this study used the whole genome of tea plant as the material and employed bioinformatics methods and fluorescence quantitative PCR technology to analyze the tea plant *DIR* gene family in terms of genome-wide identification, phylogenetic tree, gene structure, conserved domains and motifs, promoter cis-acting elements, intra- and interspecific collinearity, and expression patterns during the response to biotic stress. The following questions were addressed: (1) the physicochemical properties of members of the tea plant *DIR* gene family; (2) the gene and protein structures, conserved protein domains, and conserved motifs of tea plant *DIR* family members; (3) the chromosomal localization of members of the tea plant *DIR* gene family and their collinearity within the species and among different species; and (4) the expression patterns of members of the tea plant *DIR* gene family under tea anthracnose stress. The aim was to provide a reference basis for in-depth study of the functions of tea plant *DIR* genes, and also to provide important gene resources for breeding new stress-resistant tea plant varieties.

1 Materials and Methods

1.1 Materials

The experimental material was one-year-old tea plants of 'Qiancha No. 1' grown in an artificial climate chamber at the College of Tea Science, Guizhou University (Guiyang, Guizhou). The culture conditions were as follows: light intensity 2,000 lx, culture temperature (24 ± 2) °C, photoperiod 16 h light and 8 h dark; the plants were healthy and free of diseases and insect pests. The tea anthracnose pathogen used was *Glomerella cingulata*, preserved at the College of Tea Science, Guizhou University.

1.2 Methods

1.2.1 Identification of members of the tea plant *DIR* gene family and analysis of physicochemical properties Tea plant genome data were obtained from TPIA (http://tpdbtmp.shengxin.ren:81/web/Download). The HMM model file (PF03018) of the *DIR* gene family was obtained from the Pfam database (http://pfam.xfam.org/) (Jaina et al., 2020), and HMMER 3.0 software was used to screen CsDIRs in the tea plant genome as preliminary candidate proteins (E-value $< 1e^{-5}$) (Finn et al., 2016). After redundant sequences were removed, the online tools Pfam (http://www.pfam.org/) and SMART (http://smart.embl-heidelberg.de/) were used to screen the candidate protein sequences, and the members of the *CsDIR* gene family were finally determined. The ExPASy online tool (http://web.expasy.org/protparam/) was used to analyze physicochemical properties of tea plant *DIR* family members, including protein molecular weight, isoelectric point, and hydrophilicity/hydrophobicity (Panu et al., 2012). Wolf psort (https://wolfpsort.hgc.jp/) was used to predict the subcellular localization of CsDIRs (Paul et al., 2007).

1.2.2 Construction of the phylogenetic tree Using MEGA 7.0 software, the neighbor-joining (NJ) method was used to construct a phylogenetic tree based on the protein sequences of *DIR* family members from tea plant, *Populus trichocarpa*, rice (*Oryza sativa*), and *Arabidopsis thaliana*, with Bootstrap set to 1,000 replicates. The generated Newick file was uploaded to the online tool Evolview

(https://evolgenius.info/evolview-v2/#login) was used for plotting.

1.2.3 Analysis of higher-order protein structure and GO enrichment

The SOPMA module for secondary structure prediction in the Prabi online analysis software (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma_f.html) was used to predict the secondary structures of tea plant DIR family proteins. The online tool SWISS-MODEL (https://swissmodel.expasy.org/) was used to model the tertiary structures of tea plant DIR family proteins. The EggNOG5.0 database (http://eggnog5.embl.de/#/app/home) was used to perform GO

functional enrichment analysis of *CsDIR* genes, and TBtools was used for visualization.

1.2.4 Analysis of gene structure, conserved domains, and conserved motifs

The online tool MEME (http://meme-suite.org/) was used to identify conserved motifs in tea plant DIR family protein sequences, with the number of motifs set to 10 (Bailey et al., 2006). NCBI Batch-CD-Search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) was used to export conserved-domain data for members of the tea plant DIR family. TBtools was used to visualize intron–exon structures, conserved domains, and conserved motifs of tea plant *DIR* genes (Chen et al., 2020).

1.2.5 Analysis of intra- and interspecific collinearity and chromosomal localization

MCScanX (Wang et al., 2012) was used, with default parameters, to analyze collinear blocks between Arabidopsis and tea plant as well as within tea plant itself. The collinearity files were then analyzed with TBtools, and the Advanced circos function in TBtools was used to draw intra- and interspecific collinearity maps for tea plant. TBtools was used to visualize the chromosomal distribution of the tea plant *DIR* gene family.

1.2.6 Analysis of cis-acting elements in promoters of *CsDIR* family genes

The 2 000 bp nucleic acid sequences upstream of *CsDIR* family genes were extracted, and the Plant care database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to analyze cis-acting elements in the promoter regions. The resulting files were visualized using TBtools.

1.2.7 Analysis of *CsDIR* gene family members during the disease-resistance process in tea plant

Newly emerged leaves were selected from tea plants with consistent growth vigor and healthy condition. After being wiped once with 75% alcohol, they were rinsed three times with sterile water. Once the surface moisture of the leaves had air-dried naturally, artificial wounds were made near the leaf margin in the central region of the leaves with a sterile blade. Mycelial blocks of the isolated tea anthracnose pathogen were inoculated onto the wound sites on the abaxial side of the leaves as the experimental group. The uninoculated wild type was used as the control group. Three biological replicates were set up for each treatment group. RNA was extracted from tea plant leaves using the Stesdy-Pure Plant RNA Extraction Kit (Changsha Aikerui Biotechnology, AG21019). Total RNA was reverse-transcribed into cDNA using a reverse transcription kit

(Takara, Dalian, RR037A). NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast) was used to design real-time quantitative PCR (qRT-PCR) primers (Table 1), with tea plant *GAPDH* used as the internal reference. qRT-PCR was performed using the SGExcel FastSYBR Mixture kit [Sangon Biotech (Shanghai) Co., Ltd., B532955] to detect the C_t values of the target genes and the internal reference gene, with three biological replicates. Relative expression levels were calculated and analyzed using $2^{-\Delta\Delta Ct}$. Data were organized using Excel 2019, statistical analysis was performed using SPSS 18, significance of differences was analyzed using a *t*-test, and figures were prepared with GraphPad Prism 9.0.

Table 1 qRT-PCR primer sequences

Table 1　Primer sequence for real time quantitative PCR (qRT-PCR)

Primer name | Primer sequence (5′$→3’)|*Primername*|*Primersequence*(5’ $$→3’ $) |

|–|–|–|–| | Primer name | Primer sequence (5′$→3’)|*Primername*|*Primersequence*(5’ $$→3’ $) |

Primer name	Sequence (5′-3′)	Primer name	Sequence (5′-3′)
CsDIR2-F	ACCATCTTCGCTTCCCTCAC	*CsDIR31*-F	TCGTTGTCGACTGTCATGGG
CsDIR2-R	CAGCGATCGAAATGGGACAG	*CsDIR31*-R	CGTCAACGCAAATGGGACAG
CsDIR10-F	GGGCTCCAACCCAGTTACTC	*CsDIR32*-F	GTGCCACTGGAGTTGAGTGA
CsDIR10-R	ACCCAAGGCAGCATTTCAA	*CsDIR32*-R	TGATGGACAACCCGCTCAC
CsDIR15-F	TACCCACTGTAACCCTCCTG	*CsDIR33*-F	AGTATCTGCGAGCCAGCCT
CsDIR15-R	ATTGACGGCTGGGTTTCTTA	*CsDIR33*-R	GCAAATACAATGGAAGCACCC
CsDIR20-F	CGGCATGCTCAATGTTTAGG	*CsDIR34*-F	CACTGTATGAGTCTTGGCCT
CsDIR20-R	AAGACCTGCCGCCATTTAA	*CsDIR34*-R	GGCCAAAGCCATCCCAAACGT
CsDIR21-F	CGTCTTTGAAACTCCTCCGC	*CsDIR36*-F	ATGTCGTGGAAGTAGAAGTG
CsDIR21-R	TGTGATTCTTTCTCCTTAACGG	*CsDIR40*-F	TCGTGCTTAGTTGTATGCGA
CsDIR25-F	TTGGGCGAGCACCAAGATTT	*CsDIR40*-R	GCCGCATTCCACAGTGTTTT
CsDIR25-R	TCAGCACCGGCAAACTTCAT		CACGGTCAATGGAAGCATCAT
CsDIR30-F	CCGCCTCAAGCTCATCTACC		GCAGCAGCCTTATCCTTATCAG

2 Results and Analysis

2.1 Basic information on members of the tea plant *DIR* gene family

A total of 40 *DIR* genes were identified from tea plant (Table 2). According to the positions of these genes on the chromosomes, they were sequentially named *CsDIR1*-*CsDIR40*. The proteins encoded by the 40 *CsDIR* genes ranged in amino-acid length from 123 aa (*CsDIR25*) to 381 aa (*CsDIR1*), and their molecular weights ranged from 14.40 kDa (*CsDIR25*) to 38.81 kDa (*CsDIR1*). The predicted isoelectric points ranged from 4.44 (*CsDIR1*) to 10.07 (*CsDIR34*). Except for *CsDIR34*, which was classified as an unstable protein (instability index greater than 40), the remaining CsDIRs were stable proteins. The aliphatic

index ranged from 60.24 (*CsDIR25*) to 103.72 (*CsDIR40*). Most CsDIRs were hydrophobic proteins, whereas *CsDIR25*, *CsDIR27*, *CsDIR28*, *CsDIR31*, and *CsDIR34* were hydrophilic proteins. Subcellular localization prediction indicated that most CsDIRs were localized to chloroplasts and the extracellular space, with a small number localized to the cytoplasm, vacuole, and endoplasmic reticulum.

Table 2 Information of *CsDIR* gene family

Gene name	Gene ID	Number of amino acids	Molecular weight (kD)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity	Subcellular localization prediction
CsDIR1	CSS0021743	381	38.81	4.44	35.85	93.12	0.171	Chloroplast
CsDIR2	CSS0010065	195	21.31	7.08	17.64	91.95	0.010	Chloroplast
CsDIR3	CSS0032670	181	19.72	6.65	29.44	92.15	0.050	Endoplasmic reticulum
CsDIR4	CSS0048417	192	20.57	7.92	32.82	95.05	0.118	Extracellular space
CsDIR5	CSS0020163	192	20.85	7.02	31.69	92.50	0.089	Extracellular space
CsDIR6	CSS0046097	170	18.82	9.16	33.87	86.53	0.119	Vacuole
CsDIR7	CSS0006406	192	20.48	6.58	31.51	98.59	0.183	Vacuole Extracellular Space
CsDIR8	CSS0006464	191	20.65	7.72	27.55	92.41	0.068	Extracellular Space
CsDIR9	CSS0048140	189	20.73	6.82	25.89	88.15	0.154	Vacuole
CsDIR10	CSS0000043	171	18.80	7.78	29.03	93.45	0.174	Vacuole
CsDIR11	CSS0030505	188	20.69	5.75	26.09	102.66	0.206	Extracellular Space
CsDIR12	CSS0021710	191	21.17	9.07	22.29	91.88	0.129	Vacuole
CsDIR13	CSS0046463	200	21.94	5.50	31.90	88.25	0.099	Chloroplast
CsDIR14	CSS0008829	201	22.01	5.50	32.75	87.81	0.094	Chloroplast
CsDIR15	CSS0008636	297	30.69	4.88	29.51	90.30	0.192	Cytoplasm
CsDIR16	CSS0023753	177	19.31	6.26	24.21	95.14	0.207	Chloroplast
CsDIR17	CSS0030481	177	19.22	5.84	21.31	96.78	0.238	Chloroplast
CsDIR18	CSS0049928	175	19.26	7.97	26.49	93.49	0.121	Chloroplast
CsDIR19	CSS0048755	191	20.89	9.28	10.43	95.92	0.157	Cytoplasm

CsDIR26	CSS0042357	187	20.54	9.04	37.99	90.16	0.116	Chloroplast
CsDIR27	CSS0043959	180	19.69	7.86	20.52	95.72	0.156	Chloroplast
CsDIR28	CSS0025951	191	20.83	9.28	13.63	92.88	0.165	Cytoplasm
CsDIR29	CSS0013337	249	19.69	5.47	33.67	93.29	0.272	Extracellular Space
CsDIR24	CSS0028211	191	21.07	9.66	18.49	87.28	0.112	Chloroplast
CsDIR25	CSS0018205	123	14.40	5.78	36.96	60.24	-0.365	Cytoplasm
CsDIR26	CSS0033203	188	20.83	7.84	22.19	84.63	0.088	Chloroplast
CsDIR27	CSS0004240	181	20.21	7.72	23.41	75.41	-0.014	Chloroplast
CsDIR28	CSS0039034	181	20.21	7.72	23.88	75.41	-0.018	Chloroplast
CsDIR29	CSS0020889	188	20.86	7.84	22.19	84.63	0.073	Chloroplast
CsDIR30	CSS0046546	182	20.58	5.07	19.28	73.41	0.101	Chloroplast
CsDIR31	CSS0024173	185	20.77	6.82	17.71	68.00	-0.128	Chloroplast
CsDIR32	CSS0024616	159	17.80	9.47	31.21	90.19	0.051	Extracellular Space
CsDIR33	CSS0019864	177	19.29	9.07	33.28	85.99	0.157	Extracellular Space
CsDIR34	CSS0033209	185	20.20	10.07	43.14	90.65	-0.048	Chloroplast
CsDIR35	CSS0026090	198	21.66	8.69	34.51	96.06	0.148	Extracellular Space
CsDIR36	CSS0002646	202	22.65	9.64	39.69	95.59	0.115	Extracellular Space
CsDIR37	CSS0010713	249	25.37	5.47	33.33	92.89	0.262	Extracellular Space
CsDIR38	CSS0009270	191	20.98	9.55	21.60	88.32	0.074	Chloroplast
CsDIR39	CSS0010013	191	21.03	9.95	30.06	83.72	0.020	Chloroplast
CsDIR40	CSS0018582	188	20.57	6.07	39.58	103.72	0.245	Extracellular Space

2.2 Phylogenetic Analysis of the Tea Plant *DIR* Family

To investigate the phylogenetic relationships among members of the tea plant *DIR* gene family, a phylogenetic tree was constructed using a total of 144 *DIR* protein sequences from *Arabidopsis thaliana* (27), rice (37), *Populus trichocarpa* (40), and tea plant (40) (Figure 1). The results showed that *DIR* proteins could be divided into five subfamilies (DIR-a, DIR-b/d, DIR-c, DIR-e, and DIR-g). Tea plant *DIR* proteins were distributed among three subfamilies—DIR-a, DIR-b/d, and DIR-e—with the DIR-b/d subfamily containing the largest number of CsDIRs (27), the DIR-a subfamily containing 7 CsDIRs, and the DIR-e subfamily containing 6 CsDIRs. Analysis of the evolutionary relationships of *DIR* family proteins from the four species revealed that the *DIR* proteins of tea plant, *Arabidopsis thaliana*, and *Populus trichocarpa* were distributed

only in the DIR-a, DIR-b/d, and DIR-e subfamilies, whereas DIR-c and DIR-g contained only *DIR* proteins from the monocotyledonous plant rice. These results indicate that the evolution of the plant *DIR* gene family underwent marked divergence accompanying the differentiation of monocotyledonous and dicotyledonous plants.

Fig. 1 Phylogenetic tree of DIR proteins from *Camellia sinensis* (Cs), *Populus trichocarpa* (Pt), *Oryza sativa* (Os), and *Arabidopsis thaliana* (At)

2.3 Analysis of the Higher-Order Structure of Tea Plant DIR Proteins

To investigate the structural characteristics of tea plant DIR proteins, the secondary structures of the DIR proteins were predicted (Table 3). Tea plant DIR proteins contain four structural forms: α-helix, extended strand, β-turn, and random coil. The proportion of α-helix ranges from 11.28% (CsDIR2) to 29.26% (CsDIR11); the proportion of β-turn ranges from 3.00% (CsDIR13) to 6.67% (CsDIR21); the proportion of extended strand ranges from 19.68% (CsDIR23) to 38.22% (CsDIR24); and the proportion of random coil ranges from 34.04% (CsDIR11) to 62.47% (CsDIR1). Among these, the proportions of extended strand and random coil are relatively high, together accounting for more than 65% of the sequence. The tertiary-structure modeling of the proteins is shown in Fig. 2; all 40 DIR proteins contain a β-barrel composed of eight antiparallel β-strands.

Table 3 Secondary structure of CsDIRs

Protein name	α-helix (%)	Extended strand (%)	β-turn (%)	Random coil (%)
CsDIR1	13.65	19.95	3.94	62.47
CsDIR2	11.28	31.28	5.64	51.79
CsDIR3	27.07	30.94	4.97	37.02
CsDIR4	22.92	32.29	4.69	40.10
CsDIR5	26.04	30.73	3.12	40.10
CsDIR6	28.24	29.41	4.71	37.65
CsDIR7	26.04	31.77	3.65	38.54
CsDIR8	26.18	31.41	4.71	37.70
CsDIR9	21.16	31.22	5.29	42.33
CsDIR10	23.39	32.75	4.68	39.18
CsDIR11	29.26	31.38	5.32	34.04
CsDIR12	24.61	32.98	4.19	38.22
CsDIR13	24.00	30.00	3.00	43.00
CsDIR14	24.88	29.85	4.98	40.30
CsDIR15	15.82	24.58	5.72	53.87

CsDIR16	25.42	32.20	4.52	37.85
CsDIR17	22.60	35.03	5.65	36.72
CsDIR18	23.43	33.71	5.71	37.14
CsDIR19	24.61	33.51	4.71	37.17
CsDIR20	19.79	35.83	3.74	40.64
CsDIR21	21.67	36.11	6.67	35.56
CsDIR22	25.65	34.55	5.24	34.55
CsDIR23	22.89	19.68	5.22	52.21
CsDIR24	12.57	38.22	4.71	44.50
CsDIR25	21.95	37.40	5.69	34.96
CsDIR26	23.94	29.79	5.85	40.43
CsDIR27	15.47	34.25	6.08	44.20
CsDIR28	16.02	34.81	4.42	44.75
CsDIR29	24.47	28.72	4.79	42.02
CsDIR30	14.84	33.52	5.49	46.15
CsDIR31	19.46	34.59	4.86	41.08
CsDIR32	20.75	31.45	3.77	44.03
CsDIR33	18.64	33.33	3.39	44.63
CsDIR34	22.16	26.49	3.24	48.11
CsDIR35	23.23	32.83	5.05	38.89
CsDIR36	25.25	32.67	3.96	38.12
CsDIR37	22.09	21.29	3.61	53.01
CsDIR38	18.32	34.03	4.71	42.93
CsDIR39	20.42	33.51	5.76	40.31
CsDIR40	28.19	31.91	5.32	34.57

CsDIR1 CsDIR2 CsDIR3 CsDIR4 CsDIR5

CsDIR6 CsDIR7 CsDIR8 CsDIR9 CsDIR10

CsDIR11 CsDIR12 CsDIR13 CsDIR14 CsDIR15

CsDIR16 CsDIR17 CsDIR18 CsDIR19 CsDIR20

CsDIR21 CsDIR22 CsDIR23 CsDIR24 CsDIR25

CsDIR26 CsDIR27 CsDIR28 CsDIR29 CsDIR30

CsDIR31 CsDIR32 CsDIR33 CsDIR34 CsDIR35

CsDIR36 CsDIR37 CsDIR38 CsDIR39 CsDIR40

Fig. 2 Tertiary structures of CsDIRs

2.4 GO Functional Enrichment of *CsDIR* Genes

To more comprehensively reveal the potential functions of *CsDIR* genes, the EggNOG 5.0 tool was used to perform detailed functional annotation and clas-

sification of them (Fig. 3). *CsDIR* participates in biological processes such as pinoresinol biosynthesis, defense responses, regulation of catalytic activity, acetyl-CoA metabolic processes, brassinosteroid biosynthesis, sterol biosynthesis, and lignin biosynthesis; its principal molecular functions involve mediating stereospecific synthesis and metal-ion binding. In the cellular component category, the obtained

The number of genes related to cellular components was relatively small, and they were located in the extracellular region.

Fig. 3 GO function enrichment analysis of *CsDIR* genes

2.5 Analysis of gene structure, conserved protein domains, and conserved motifs of tea plant *DIR* family members

To further investigate the structural characteristics of the tea plant *DIR* gene family, analysis of the conserved motifs of CsDIR proteins showed that members of the *DIR* gene family within the same subfamily had relatively similar conserved motifs (Fig. 4B). CsDIRs contained 3–7 conserved motifs, and the vast majority of CsDIRs contained Motif 1 (38 members). Domain analysis of the protein sequences encoded by *CsDIR* genes showed (Fig. 4C) that, except for CsDIR34, which contained one Dirigent superfamily domain, all other CsDIR protein sequences contained one conserved Dirigent domain; among them, CsDIR1 also contained one PHA03247 superfamily domain. Analysis of the gene structures of the *CsDIR* family showed (Fig. 4D) that most *CsDIR* genes (29) had no introns and only one exon; 9 *CsDIR* genes (*CsDIR3*, *CsDIR6*, *CsDIR10*, *CsDIR16*, *CsDIR17*, *CsDIR18*, *CsDIR20*, *CsDIR21*, and *CsDIR32*) contained 1 intron and 2 exons; 2 *CsDIR* genes (*CsDIR25* and *CsDIR40*) contained 2 introns and 3 exons; and *CsDIR11* contained 3 introns and 4 exons.

A. Phylogenetic tree of CsDIRs; B. Conserved motifs of CsDIRs; C. Conserved domains of CsDIRs; D. Gene structure of *CsDIRs*.

Fig. 4 Gene structures, conserved domains, and conserved motif analysis of *CsDIR*

2.6 Chromosomal localization and collinearity analysis of *DIR* gene-family genes in tea plant

The chromosomal locations of the *CsDIR* family genes are shown in Fig. 5A. Thirty-three *CsDIR* family genes were unevenly distributed across 10 chromosomes of tea plant, and 7 *CsDIR* family genes were distributed on 5 different contigs. Chromosome 3 contained the largest number of genes, with 12 genes; chromosome 6 contained 7 genes; chromosome 12 contained 5 genes; chromosomes 14 and 15 each contained 2 genes; and chromosomes 1, 2, 4, 9, and 10 each contained 1 gene. Contig514 and Contig866 each contained 2 *DIR* genes, while each of the remaining 3 contigs contained 1 *DIR* gene. In the *CsDIR* gene family, there were 12 pairs of tandemly duplicated genes, comprising 21 genes in total:

CsDIR4 and *CsDIR15*, *CsDIR6* and *CsDIR7*, *CsDIR7* and *CsDIR8*, *CsDIR8* and *CsDIR9*, *CsDIR11* and *CsDIR12*, *CsDIR13* and *CsDIR14*, *CsDIR16* and *CsDIR17*, *CsDIR25* and *CsDIR26*, *CsDIR26* and *CsDIR27*, *CsDIR28* and *CsDIR29*, *CsDIR30* and *CsDIR31*, and *CsDIR35* and *CsDIR36*. These accounted for $21/40 = 52.5\%$ of the *CsDIR* genes, indicating that tandem duplication may have played an important role during the evolution of *CsDIR* genes.

Intraspecific collinearity analysis identified 4 pairs of collinear genes: *CsDIR1* and *CsDIR15*, *CsDIR3* and *CsDIR22*, *CsDIR13* and *CsDIR14*, and *CsDIR25* and *CsDIR30* (Fig. 5B). These 8 *CsDIR* genes are the result of gene duplication, indicating that whole-genome segmental duplication played an important role in increasing the number of members of the *DIR* gene family in the tea plant genome.

To further analyze the phylogenetic history of the *CsDIR* gene family among different species, a homology relationship map between tea plant and *Arabidopsis thaliana* was constructed (Fig. 5C). Seventeen pairs of genes in tea plant and *Arabidopsis thaliana* showed collinearity. Among them, *CsDIR1* exhibited collinearity with 4 *DIR* genes in *Arabidopsis thaliana*; *CsDIR25* and *CsDIR30* each exhibited collinearity with 3 *DIR* genes in *Arabidopsis thaliana*; and *CsDIR15* and *CsDIR22* each exhibited collinearity with 2 *DIR* genes in *Arabidopsis thaliana*. These results indicate that the 17 pairs of genes may have played important roles during the evolution of the *DIR* gene family.

A. Chromosomal localization of *CsDIRs*; **B.** Intraspecific collinearity analysis of *CsDIRs*; **C.** Interspecific collinearity analysis of *DIR* gene family members between *Camellia sinensis* and *Arabidopsis thaliana*.

Fig. 5 Chromosomal localization analysis and interspecific synteny analysis of tea plant *DIR* gene family members.

Fig. 5 Chromosomal localization analysis and the syntenic relationship of *CsDIR* gene family members between different species.

2.7 Analysis of cis-acting elements in promoters of tea plant *DIR* family genes

To investigate the regulatory roles of the *CsDIR* family genes, cis-acting elements in the 2,000 bp promoter regions upstream of the *CsDIR* genes were predicted and analyzed (Fig. 6). A total of 15 types of promoter cis-acting elements were identified across the promoters of the 40 *CsDIR* family genes. Plant hormone- and stress-responsive elements were widely distributed in the promoter regions of this gene family. Among them, the promoter regions of 28 *CsDIR* genes contained ethylene-responsive elements (ERE), 26 contained abscisic acid-responsive elements (ABRE), 25 contained jasmonic acid-responsive elements (CGTCA-motif), 22 contained wound-responsive elements (WUN-motif), 20 contained pathogen-responsive elements (W-box), 18 contained gibberellin-responsive elements (GARE-motif, P-box, and TATC-

box), 11 contained low-temperature-responsive elements (LTR), 9 contained salicylic acid-responsive elements (TCA-element), 9 contained defense- and stress-responsive elements (TC-rich), 8 contained drought-responsive elements (MBS), and 7 contained auxin-responsive elements (TGA-element and AuxRR-core), indicating that most *CsDIR* genes possess cis-acting elements responsive to hormones and environmental stresses.

Fig. 6 Promoter *cis*-acting elements of the *CsDIRs* family

Legend: TGA-element; TCA-element; TC-rich; CGTCA-motif; ERE; LTR; ABRE; MBS; W box; P-box; WUN-motif; GARE-motif; TATC-box; AuxRR-core; G-Box.

2.8 Expression patterns of tea plant *DIR* family genes under anthracnose stress

To investigate the roles of *CsDIR* genes in the response of tea plants to tea anthracnose stress, this study selected 12 genes in total: *CsDIR25*, *CsDIR30*, and *CsDIR31* from the DIR-a subfamily; *CsDIR10*, *CsDIR20*, *CsDIR21*, *CsDIR32*, *CsDIR35*, *CsDIR38*, and *CsDIR40* from the DIR-b/d subfamilies; and *CsDIR2* and *CsDIR15* from the DIR-e subfamily. The expression patterns of these 12 *CsDIR* genes at different time points after tea plants were inoculated with the tea anthracnose pathogen were analyzed by qRT-PCR (Fig. 7). The results showed that, except for *CsDIR2*, the relative expression levels of the remaining *CsDIR* genes all first increased and then decreased with increasing inoculation time, reaching a peak on day 3. Among them, the relative expression levels of *CsDIR10*, *CsDIR20*, *CsDIR21*, *CsDIR30*, and *CsDIR40* were upregulated by more than 100-fold after 3 d of inoculation. Specifically, after 3 d of inoculation, the relative expression level of *CsDIR10* was 345.68 times that at 0 d; that of *CsDIR20* was 263.19 times that at 0 d; that of *CsDIR21* was 328.73 times that at 0 d; that of *CsDIR30* was 100.32 times that at 0 d; and that of *CsDIR40* was 121.65 times that at 0 d. These results indicate that these five genes may play very important roles in tea plant resistance to tea anthracnose. The relative expression level of *CsDIR2* decreased with increasing inoculation time, suggesting that *CsDIR2* may play a negative regulatory role in tea plant resistance to tea anthracnose.

Figure panels: CsDIR2, *CsDIR10*, *CsDIR15*, *CsDIR20*; *CsDIR21*, *CsDIR25*, *CsDIR30*, *CsDIR31*; *CsDIR32*, *CsDIR35*, *CsDIR38*, *CsDIR40*.
Y-axis: Relative expression.
X-axis: Treatment time (d): 0, 1, 3, 5, 7.
Significance annotations shown in the panels include ns, *, **, ***, and ****.

Values are based on the mean ± standard deviation of three repeated experiments. Each point was compared with the 0 d group, and significance was calculated using the *t*-test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.000\,1$).

Fig. 7 Quantitative expression analysis of some members of the *Cs-DIR* gene family under tea anthracnose treatment

3 Discussion and Conclusion

DIR family genes regulate the stereoselectivity and regioselectivity of oxidative coupling reactions during the biosynthesis of lignin and lignans, and play important roles in plant responses to biotic and abiotic stresses (Chen Yu et al., 2022). At present, *DIR* genes have been identified in a variety of plants, such as alfalfa (*Medicago sativa*) (52) (Ma Yonglong et al., 2025), foxtail millet (*Setaria italica*) (41) (Zhao Yuxue et al., 2024), *Arabidopsis thaliana* (27) (Kim et al., 2012), pepper (24) (Khan et al., 2018), and woad (*Isatis indigotica*) (19) (Li et al., 2014). In this study, 40 *DIR* genes were screened and obtained from the tea plant genome, indicating that the number of *DIR* genes varies considerably among different species. Analysis of physicochemical properties showed that 97.5% of CsDIR proteins are stable proteins, suggesting that they may play important roles in coping with long-term stress. In addition, 87.5% of CsDIR proteins are hydrophobic proteins, indicating that they may be localized in cellular membrane systems, such as chloroplast or endoplasmic reticulum membranes; this is similar to related findings in maize (Qin Tao et al., 2025). Subcellular localization results showed that CsDIRs are localized in chloroplasts, the extracellular space, cytoplasm, vacuoles, and the endoplasmic reticulum, indicating that the CsDIR family may function in different envir-

perform different biological functions under environmental conditions; similar conclusions have also been reached in related studies of alfalfa (Ma Yonglong et al., 2025) and potato (*Solanum tuberosum*) (Zhang Yixuan et al., 2025). This study found that the tea plant *DIR* family contains 12 pairs of tandemly duplicated genes, accounting for 52.5% of all genes. In *Arabidopsis*, tandemly duplicated genes account for 60%, indicating that the expansion of the *DIR* gene family during evolution differs among species. Tandem duplication has played an important role in the expansion of the *DIR* family in both tea plant and *Arabidopsis*.

Tea plant DIRs are distributed only in three subfamilies (DIR-a, DIR-b/d, and DIR-e), among which the DIR-b/d subfamily contains the largest number of members. This result is similar to the phylogenetic grouping of DIRs in the dicotyledonous plants *Arabidopsis* and black cottonwood, whereas the DIR-c and DIR-g subfamilies contain only rice DIRs. This indicates that the monocotyledonous plant rice and the dicotyledonous plants tea plant, black cottonwood, and *Arabidopsis* may have followed different evolutionary patterns after divergence. Liu et al. (2021) showed that, in cotton, genes in the DIR-b/d subfamily account for more than 75%, far higher than in spruce (*Picea asperata*) (34.3%) (Ralph et al., 2007), rice (20.4%), and pepper (50.0%), suggesting that the DIR-b/d subfamily evolved more rapidly in cotton and may play an important role in cotton responses to adverse stress. In tea plant, the proportion of DIR-b/d subfamily genes is 67.5%, also higher than that in spruce and rice, indicating

that the DIR-b/d subfamily may play an important role in the response of tea plant to adverse stress. The distribution and arrangement of conserved motifs in CsDIRs within the same subfamily are similar, and some conserved motifs occur specifically in certain *DIR* subfamilies, leading to differentiation among the DIR-a, DIR-b/d, and DIR-e subfamilies. The specificity of conserved motifs may be related to functional conservation among members of the same subfamily. These results are similar to the distribution and arrangement of conserved motifs in black cottonwood and lopseed (*Phryma leptostachya*) (Pei et al., 2023). Most members of the tea plant *DIR* family contain only one exon and no introns, which is similar to the gene structure of the *DIR* families in potato (Zhang Yixuan et al., 2025), alfalfa (Ma Yonglong et al., 2025), and foxtail millet (Zhao Yuxue et al., 2024). CsDIR proteins have relatively high proportions of extended strands and random coils, consistent with predictions of the secondary structures of DIR proteins in *Houpoea officinalis* (Zhang Bainian et al., 2024) and *Zanthoxylum piperitum* (Chen Bingzhu et al., 2020). Extended strands and random coils confer high flexibility and conformational variability on proteins and improve spatial utilization efficiency, enabling them to bind multiple substrates and participate in diverse biological processes. CsDIR proteins all contain a conserved β-barrel composed of eight antiparallel β-sheets, which is consistent with tertiary-structure modeling results for DIR proteins in lopseed and pigeon pea (*Cajanus cajan*) (Dokka et al., 2024). Most CsDIR proteins also possess an N-terminal tail structure, which may allow them to perform substrate-guiding functions more efficiently.

In this study, analysis of the expression patterns of members of the *CsDIR* family after inoculation with the tea anthracnose pathogen showed that 11 *CsDIRs* were induced as early as 1 d after inoculation and reached their maximum values at 3 d after inoculation, overall exhibiting a trend of first increasing and then decreasing. Similar to this study, *GmDIR22* in soybean (Li et al., 2017), *CaDIR7* in pepper (Khan et al., 2018), and *PnDIR1* in *Panax notoginseng* (Guan Ruipan, 2018), among others, likewise play positive regulatory roles in plant resistance to pathogen invasion. Sha Jinlan et al. (2025) found that the expression of genes related to lignin synthesis (such as *CaLAC1*, *CaLAC3*, *CaLAC13*, *CaLAC14*, and *CaLAC18*) was significantly upregulated in pepper infected with anthracnose. Most DIR family proteins participate in the synthesis of lignans and lignin (Ma & Liu, 2015). This study likewise found that, in tea plants infected with anthracnose, the expression levels of genes related to lignin synthesis (such as *CsDIR10*, *CsDIR20*, *CsDIR21*, *CsDIR30*, and *CsDIR40*) were significantly upregulated. It is inferred that these genes may be closely associated with the disease-resistance stress response of tea plant; during anthracnose infection, these genes may have a certain synergistic effect and jointly resist anthracnose infection. GO enrichment analysis of *CsDIR* genes showed that these family genes are involved in defense responses and in the biosynthesis of brassinolide, sterols, lignin, and pinoresinol. Studies have shown that, after inoculation with *Ralstonia solanacearum*, the chlorophyll content in leaves of plants with silenced *NbDIR27* was significantly lower than that of the wild type, whereas leaves of

overexpression plants showed an increase in chlorophyll (Wang Ruping, 2024). Increasing the chlorophyll content of tea plant leaves,

laid a sound material foundation for enhancing the disease resistance of tea plants (Xu Chao et al., 2024). Subcellular localization prediction showed that most CsDIRs are localized in chloroplasts, suggesting that CsDIRs may play important roles in photosynthesis to resist infection by anthracnose. The promoters of *CsDIR* family genes also contain numerous cis-acting elements responsive to hormones and involved in biotic and abiotic stress, indicating that *CsDIR* genes play important roles in tea-plant growth and development as well as in abiotic stress responses. To comprehensively elucidate the expression patterns of tea-plant *DIR* genes in response to adversity, further correlation analysis and identification may be conducted in combination with the biological functions of their promoters.

In this study, 40 tea-plant *DIR* genes were screened and obtained, and their phylogenetic relationships, gene and protein structures, subcellular localization, and expression patterns under tea anthracnose stress were predicted and analyzed, providing a certain theoretical basis for further exploration of the functional characteristics of the tea-plant *DIR* gene family.

References:

BAILEY TL, WILLIAMS N, MISLEH C, et al., 2006. MEME: discovering and analyzing DNA and protein sequence motifs [J]. Nucleic Acids Research, 34(Web Server issue): W369-W373.

CHEN BZ, WANG RY, ZHAO YC, et al., 2020. Identification and analysis of *DIR* Gene Family in *Zanthoxylum piperitum* DC. var. *inerme* Makino [J]. Journal of Mountain Agriculture and Biology, 39(2): 21-26. [Chen Bingzhu, Wang Runying, Zhao YC, et al., 2020. Identification and analysis of the *DIR* gene family in Asakura pepper [J]. Journal of Mountain Agriculture and Biology, 39(2): 21-26.]

CHEN C, CHEN H, ZHANG Y, et al., 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data [J]. Molecular Plant, 13(8): 1194-1202.

CHEN Y, LIU JS, MU XL, et al., 2022. Research progress in *DIR* gene of lignan biosynthesis in medicinal plant [J]. China Journal of Chinese Materia Medica, 47(11): 2890-2898. [Chen Yu, Liu Jiushi, Mu Xinlu, et al., 2022. Research progress on *DIR* genes in lignan biosynthesis in medicinal plants [J]. China Journal of Chinese Materia Medica, 47(11): 2890-2898.]

Dokka N, Tyagi S, Ramkumar KM, et al., 2024. Genome-wide identification and characterization of DIRIGENT gene family (*CcDIR*) in pigeonpea (*Cajanus cajan* L.) provide insights on their spatial expression pattern and relevance to stress response [J]. Gene, 914: 148417-148429.

FAISAL Z, MUHAMMAD A, 2022. Proline Alleviates abiotic stress induced oxidative stress in plants [J]. Journal of Plant Growth Regulation, 42(8): 4629-4651.

FARAG MA, ELMETWALLY F, ELGHANAM R, et al., 2023. Metabolomics in tea products; a compile of applications for enhancing agricultural traits and quality control analysis of *Camellia sinensis* [J]. Food Chemistry, 404(PB): 134628-134644.

FINN DR, COGGILL P, EBERHARDT YR, et al., 2016. The Pfam protein families database: towards a more sustainable future [J]. Nucleic Acids Research, 44(D1): D279-D285.

GUAN RP, 2018. Molecular mechanism of *Dirigent* genes involved in the interaction of *Panaxnotoginseng-Fusarium solani* [D]. Kunming: Kunming University of Science and Technology. [Guan Ruipan, 2018. Study on the molecular mechanism by which *Dirigent* genes participate in the interaction between *Panax notoginseng* and *Fusarium solani* [D]. Kunming: Kunming University of Science and Technology.]

JAINA M, SARA C, LOWRI W, et al., 2020. Pfam: The protein families database in 2021 [J]. Nucleic Acids Research, 49(D1): D412-D419.

KHAN A, LI RJ, SUN JT, et al., 2018. Genome-wide analysis of dirigent gene family in pepper (*Capsicum annuum* L.) and characterization of *CaDIR7* in biotic and abiotic stresses [J]. Scientific Reports, 8(1): 5500-5520.

KIM KW, MOINUDDIN SG, ATWELL KM, et al., 2012. Opposite stereoselectivities of dirigent proteins in *Arabidopsis* and *Schizandra* species [J]. The Journal of Biological Chemistry, 287(41): 33957-33972.

LI NH, ZHAO M, LIU TF, et al., 2017. A novel soybean dirigent gene *GmDIR22* contributes to promotion of lignan biosynthesis and enhances resistance to *Phytophthora sojae* [J]. Frontiers in Plant Science, 8:1185-1196.

LI Q, CHEN J, XIAO Y, et al., 2014. The dirigent multigene family in *Isatis indigotica*: gene discovery and differential transcript abundance [J]. BMC Genomics, 15(1): 388-400.

LIU ZW, WANG XF, SUN ZW, et al., 2021. Evolution, expression and functional analysis of cultivated allotetraploid cotton *DIR* genes [J]. BMC Plant Biology, 21(1): 89-104.

MA QH, LIU YC, 2015. TaDIR13 a dirigent protein from wheat, promotes lignan biosynthesis and enhances pathogen resistance [J]. Plant Molecular Biology Reporter, 33(1): 143-152.

MA YL, WANG XT, LI XH, et al., 2025. Identification and analysis of members of the *DIR* gene family in alfalfa [J]. Pratacultural Science: 1-20[2025-07-31]. https://link.cnki.net/urlid/62.1069.s.20250311.1151.006. [MA Yonglong,

WANG Xiaotong, LI Xiaohong, et al., 2025. Identification and analysis of members of the *DIR* gene family in alfalfa [J]. Pratacultural Science: 1-20[2025-07-31]. https://link.cnki.net/urlid/62.1069.s.20250311.1151.006.]

MO FL, ZHANG N, QU YW, et al., 2021. Molecular characterization, gene evolution and expression analysis of the F-Box gene family in tomato (*Solanum lycopersicum*) [J]. Genes, 12(3): 417-435.

PANU A, MANOHAR J, KONSTANTIN A, et al., 2012. ExPASy: SIB bioinformatics resource portal [J]. Nucleic Acids Research, 40(Web Server issue): W597-W603.

PAUL H, KEUN-JOON P, TAKESHI O, et al., 2007. WoLF PSORT: protein localization predictor [J]. Nucleic Acids Research, 35(Web Server issue): W585-W587.

PEI YK, CAO WH, YU WW, et al., 2023. Identification and functional characterization of the dirigent gene family in *Phryma leptostachya* and the contribution of *PlDIR1* in lignan biosynthesis [J]. BMC Plant Biology, 23(1): 291-311.

QIN T, WANG H, ZHAO ZX, et al., 2025. Genome-wide identification of the *DIR* gene family in maize and functional characterization of *ZmDIR17* gene in grain development [J]. Acta Agriculturae Shanghai, 41 (3): 1-15. [QIN Tao, WANG Hui, ZHAO Zhixiong, et al., 2025. Genome-wide identification of the maize *DIR* gene family and functional analysis of the *ZmDIR17* gene in grain development [J]. Acta Agriculturae Shanghai, 41(3): 1-15.]

RALPH GS, JANCSIK S, BOHLMANN J, et al., 2007. Dirigent proteins in conifer defense II:

Extended gene discovery, phylogeny, and constitutive and stress-induced gene expression in spruce (*Picea* spp.) [J]. *Phytochemistry*, 68(14): 1975–1991.

SHA JL, SHUAI L, YIN FL, et al., 2025. Genome-wide identification of the *laccase* gene family and responses to *Colletotrichum* infection in postharvest pepper [J]. *Journal of Nuclear Agricultural Sciences*: 1-15 [2025-07-31]. https://link.cnki.net/urlid/11.2265.s.20250604.1812.002. [Sha Jinlan, Shuai Liang, Yin Feilong, et al., 2025. Identification of the pepper laccase gene family and its response to *Colletotrichum* infection [J]. *Journal of Nuclear Agricultural Sciences*: 1-15 [2025-07-31]. https://link.cnki.net/urlid/11.2265.s.20250604.1812.002.]

SHI HY, LIU ZH, ZHU L, et al., 2012. Overexpression of cotton (*Gossypium hirsutum*) *dirigent1* gene enhances lignification that blocks the spread of *Verticillium dahliae* [J]. *Acta Biochimica et Biophysica Sinica*, 44(7): 555–564.

WANG RP, 2024. Expression of *StDIR27* regulated by transcription factor *StTCP14* enhances resistance to *Ralstonia solanacearum* [D]. Taiyuan: Shanxi Normal University. [Wang Ruping, 2024. The transcription factor *StTCP14* regulates *StDIR27* expression to enhance resistance to *Ralstonia solanacearum* [D]. Taiyuan: Shanxi Normal University.]

WANG YP, TANG HB, D J D, et al., 2012. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity [J]. *Nucleic Acids Research*, 40(7): e49–e62.

WANG YY, CAO YB, LIANG XY, et al., 2022. A dirigent family protein confers variation of Casparian strip thickness and salt tolerance in maize [J]. *Nature Communications*, 13(1): 2222–2235.

WU RH, WANG LL, WANG Z, et al., 2008. Cloning and expression of the guidance protein gene of the resurrection plant *Boea hygrometrica* [J]. *Progress in Natural Science*, 10(18): 1111–1118. [Wu Renhua, Wang Lili, Wang Zhi, et al., 2008. Cloning and expression of the guidance protein gene from the resurrection plant *Boea hygrometrica* [J]. *Progress in Natural Science*, 10(18): 1111–1118.]

WU YQ, SONG XF, DENG GTY, et al., 2023. Isolation and transcription activation analysis of *CsbHLH71* gene in *Camellia sinensis* [J]. *Acta Botanica Boreali-Occidentalia Sinica*, 43(1): 37–44. [Wu Yingqi, Song Xiaofeng, Deng Jiantianye, et al., 2023. Cloning and transcriptional activity analysis of the tea plant *CsbHLH71* gene [J]. *Acta Botanica Boreali-Occidentalia Sinica*, 43(1): 37–44.]

XU C, ZHANG HY, WU MZ, et al., 2024. Effects of high-quality biocontrol agents on *Camellia sinensis* infection and their underlying disease resistance mechanisms [J]. *Journal of Agricultural Catastrophology*, 14(10): 34–36. [Xu Chao, Zhang Hongyan, Wu Maozhen, et al., 2024. Effects of high-quality biocontrol agents on tea anthracnose and their disease-resistance mechanisms [J]. *Journal of Agricultural Catastrophology*, 14(10): 34–36.]

XU XH, YE XL, XING AQ, et al., 2022. Functional analysis of small GTPase gene *CsRAC5* in *Camellia sinensis* under abiotic stresses [J]. *Journal of Nanjing Agricultural University*, 45(3): 503–510. [Xu Xiaohan, Ye Xiaoli, Xing Anqi, et al., 2022. Functional analysis of the tea plant small G-protein gene *CsRAC5* under abiotic stress [J]. *Journal of Nanjing Agricultural University*, 45(3): 503–510.]

ZHANG BN, ZHAO MH, TIAN H, et al., 2024. Mining and bioinformatics analysis of Houpo (*Houpoea officinalis*) *Dirigent* gene families [J]. *Chinese Archives of Traditional Chinese*

Medicine, 42(1): 189–193, 275–277. [ZHANG Bainian, ZHAO Minghai, TIAN Hui, et al., 2024. Mining and bioinformatics analysis of the *Dirigent* gene family in *Magnolia officinalis* [J]. Chinese Archives of Traditional Chinese Medicine, 42(1): 189–193, 275–277.]

ZHANG YD, TAN L, REN ZH, et al., 2023. Screening, identification and determination of antagonistic actinomycetes strain against tea anthracnose [J]. Chinese Journal of Biological Control, 39(3): 646–656. [ZHANG Yudan, TAN Lin, REN Zuohua, et al., 2023. Screening and identification of antagonistic *Streptomyces* against tea anthracnose and determination of antagonistic ability [J]. Chinese Journal of Biological Control, 39(3): 646–656.]

ZHANG YX, MA Y, WANG TT, et al., 2025. Genome-wide identification and expression profiles of *DIR* gene family in potato [J]. Biotechnology Bulletin, 41(3): 123–136. [ZHANG Yixuan, MA Yu, WANG Tongtong, et al., 2025. Genome-wide identification and expression-pattern analysis of the potato *DIR* family [J]. Biotechnology Bulletin, 41(3): 123–136.]

ZHAO YX, REN XJ, LI Y, et al., 2024. Genome-wide identification of *Dirigent* gene family and analysis in *Setaria italica* [J]. Journal of Nuclear Agricultural Sciences, 38(9): 1647–1660. [ZHAO Yuxue, REN Xuejun, LI Yun, et al., 2024. Identification and analysis of *Dirigent* family genes in foxtail millet [J]. Journal of Nuclear Agricultural Sciences, 38(9): 1647–1660.]

Note: Figure translations are in progress. See original paper for figures.