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The Identification and Expression Analysis of the *Liriodendron chinense* (Hemsl.) Sarg. *SOD* Gene Family

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Abstract: Superoxide dismutase (oxide dismutase) is an enzyme synthesized via the expression of SOD-related genes. It is the first line of defense against oxygen free radicals, and it widely exists in the cytoplasm, chloroplasts, and mitochondria. However, the *SOD* gene family of woody plant *Liriodendron chinense* has not been studied. To reveal the potential role of *SOD* genes, we systematically identified and analyzed the *SOD* gene family of *L. chinense*, and investigated the transcriptional responses of *LcSOD* genes to several abiotic stresses, including cold, heat, and drought. A total of eight *SOD* genes were identified, namely, five Cu/Zn-SODs, two Fe-SODs, and one Mn-SOD, and they were divided into two subgroups according to phylogenetic analysis, gene structure, and conserved motifs. *Cis*-acting element analysis reveals various hormones and stress respond as *cis*-acting elements in the promoters of *LcSODs*. Gene expression analysis shows that most *LcSOD* genes were in response to abiotic stresses at the transcriptional level. These results help in clarifying the function of *LcSOD* genes under abiotic stresses, and provide potential targets for the improvement in abiotic stress tolerance in the endangered *L. chinense*.

Keywords: *Liriodendron chinense*; superoxide dismutase (SOD); gene family; abiotic stress

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1. Introduction

Reactive oxygen species (ROS) are produced in almost all types of cellular metabolism. Under extreme-temperature, drought, salinity, and other stresses, organisms produce a large number of ROS [1]. Excessive ROS affect various cell functions by destroying nucleic acid, protein, and lipid peroxidation, and eventually lead to cell death [2,3]. Abiotic stress is the main limiting factor affecting plant growth and development, and it leads to the accumulation of ROS, which, in turn, leads to oxidative damage [4,5]. However, evolution has equipped plants with a wider range of defenses, including morphological, metabolic, and genetic-level changes to adapt to adverse environmental conditions [6]. Superoxide dismutase (SOD), one of the main antioxidant enzymes, protects cells from toxicity by catalyzing the conversion of ROS into O_2 and H_2O_2, so that ROS reach a state of dynamic equilibrium in the cell [7–9].

Superoxide dismutase is synthesized by *SOD* genes, which can be classified into several categories according to different types of metal cofactors, i.e., Cu/Zn-SOD, Mn-SOD, Fe-SOD, and Ni-SOD [10,11]. Cu/Zn-SODs are commonly found in some prokaryotes, and the cytoplasm and chloroplasts of eukaryotic cells, while Mn-SODs are found in prokaryotes and mitochondria. Fe-SODs are commonly found in prokaryotes, algae, and the chloroplasts of some higher plants, and Ni-SOD is a unique SOD isoform in Streptomyces [12,13]. SOD, as the first line of the antioxidant system, is induced to be expressed when organisms are under stress and plays an important role in protecting cells [14]. For example, drought stress caused the downregulation of *SOD* genes in *Populus* [15]. The expression of the

SlSOD6 gene was significantly increased in tomatoes in response to drought stress [16].The expression of *BjuFSDs* in mustard *SOD* genes was upregulated under cold and salt stresses, but downregulated under heat and drought stresses [17].

Liriodendron chinense (*L. chinense*) is a precious tree species in China. It is an endangered species of the Magnoliaceae family because of its low natural seed-setting rate due to the different maturation times of the pistil and stamen. By analyzing the *Liriodendron* genome, we found that *Liriodendron* is a sister evolutionary clade of dicotyledonous and monocotyledonous plants, which is of great significance for understanding the phylogenetic position and evolution of Magnoliaceae [18]. It is a tall deciduous tree with a height of more than 40 m and a diameter at breast height of more than 1 m. These trees are mainly found south of the Yangtze River Basin, and prefer warm and humid conditions [19]. In order to achieve the good growth of the *L. chinense* tree species in cold and arid areas, it is necessary to solve its problem of poor stress resistance. Therefore, we studied the regulation effect of *SODs* on ROS. Then, we could produce resistant plants through transgenic technology to promote *L. chinense*. Although SOD plays a significant role in the plant response to abiotic stress, its genomewide identification and functional characterization in *L. chinensis* has not been reported [20].

In this study, the basic physical and chemical properties, gene structure, phylogeny, and expression pattern of *L. chinense* were analyzed with bioinformatics and quantitative real-time PCR (qRT-PCR). This study provides valuable gene resources for genetic engineering to improve the abiotic stress resistance of *L. chinense*.

2. Materials and Methods

2.1. Identification and Classification of LcSOD Gene Family

To obtain the potential *SOD* genes in *L. chinense*, the hidden horse models of Cu/ZnSOD (PF00080) and Fe-MnSOD (PF00081, PF02777) were used to search the *L. chinense* genome database using TBtools [21]. Next, the Blastp program was used to retrieve potential *SOD* genes from the genome of *L. chinense* on the basis of *SOD* genes in *Arabidopsis*, which were downloaded from phytozome v13 (https://phytozome-next.jgi.doe.gov/ (accessed on 20 March 2022)) [22]. The results of the two searches were combined, and CD-search tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi (accessed on 27 August 2022)) was used to further confirm the candidate *SOD* genes in *L. chinense* [23].

The physicochemical properties of SOD protein were predicted using the Protparam online tool (https://web.expasy.org/Protparam/ (accessed on 22 March 2022)) [24]. Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/ (accessed on 22 March 2022)) and TMHMM Server v. 2.0 (https://services.healthtech.dtu.dk/service.php?tmhmm-2.0 (accessed on 23 March 2022)) were used for the subcellular locations and transmembrane analysis, respectively [25,26].

2.2. Phylogenetic Analysis of LcSOD Genes

In order to reconstruct the phylogenetic tree, 42 SOD protein sequences were identified from five land plant species. The multiple sequence alignment was performed with the default parameters of ClustalW (http://www.clustal.org/clustal2/ (accessed on 28 March 2022)). Then, the maximal clade credibility tree was constructed with BEAST v2.6.6, and Figtree v1.4,3 was lastly used to view and beautify the tree [27,28].

2.3. Motifs, Gene Structure, Chromosomal Location, and Syntenic Analysis

The conserved motifs of LcSOD family members were analyzed using the MEME online tool (https://meme-suite.org/meme/doc/meme.html (accessed on 30 March 2022)) with the motif parameter set to 10 [29]. Gene structure was visualized using the mapping software of TBtools. The positions of *LcSODs* on the chromosomes were visualized with TBtools in accordance with the genomic annotation file of *L. chinense*. The syntenic analysis of *L. chinense*, *Arabidopsis*, and rice was performed using JCVI software (https://github.com/tanghaibao/jcvi/wiki/MCscan (accessed on 30 March 2022)) [30].

2.4. Cis-Regulatory Element Analysis

The 2000 bp upstream sequence of *LcSOD* transcription start sites (TSSs) was extracted to search the *cis*-acting element using the PlantCare website (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 30 March 2022)), and visualized with Tbtools [31].

2.5. Expression Analysis of LcSOD Genes

To explore the potential functions of *SOD* genes of *L. chinense*, their expression patterns were analyzed. Gene expression data from the transcriptomes of different tissues were collected: bud, leaf, bark, phloem, xylem, stigma, stamen, and sepal. In addition, gene expression patterns under various abiotic stresses (heat, cold, and drought) were examined. In order to simulate heat, cold, and drought stresses, 3-month-old tissue culture seedlings were treated with 4 or 40 °C, or 15% PEG 6000 for 1, 3, 6, and 12 h, and 1 and and 3 days (Figure 1). Then, the leaf samples were selected for relative expression analysis. The transcriptome data used in this study were archived and can be found on the NCBI website; cold and heat stress accession number is PRJNA679089 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA679089/ (accessed on 25 November 2022)), and drought stress accession number is PRJNA679101 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA679101/ (accessed on 25 November 2022)). Relative expression analysis was performed using kallisto [32,33].

Figure 1. Three-month-old tissue culture seedlings. Scale, 1 cm.

2.6. Plant Material and Cold Treatment

For cold stress simulation, seedlings were treated with 4 or 28 °C (control) for 3, 12, and 48 h. The materials at each time point were collected into liquid nitrogen and then stored at −80 °C for RNA extraction.

2.7. RNA Isolation and qRT-PCR

RNA extraction was performed using the Eastep®Super Total RNA Extraction Kit (Promega, Shanghai, China). Then, the RNA was reverse-transcribed into cDNA using the HiScript® III 1st Strand cDNA Synthesis Kit. Specific primers for qRT-PCR analysis were designed at https://primer3.ut.ee/ (Table 1). The gene expression of 8 *LcSOD* gene family members was analyzed with qRT-PCR, which was performed using the Roche lightcycler 480 II real-time fluorescent quantitative PCR instrument (Applied Biosystems, American). Three independent technical replicates and three biological replicates were performed for each *LcSOD* gene. The PCR procedure consisted of an initial denaturation at 95 °C for 15 min, followed by 34 cycles at 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 30 s. The relative expression levels were calculated using the $2^{-\triangle\triangle Ct}$ method [34]. The 18S RNA and

GAPDH were the reference genes. IBM SPSS Statistics 22 and GraphPad Prism 8 were used for one-way ANOVA and histogram representation [35,36].

Table 1. qRT-PCR primers used to quantify *LcSOD* gene expression.

Gene Name	qRT-PCR Primers (5′-3′)	
LcMSD1	F	ATCATGAAGCTCCACCACCA
	R	GTGATTGACATGACCTCCGC
LcFSD2	F	CATGGGGCTGGAAAATCACC
	R	GTGCCTTCCCCAGTGATACT
LcFSD3	F	TCCTGCCTAGTTCCTCCTCT
	R	AAGTCCTTTGGGTCCGACAT
LcCSD1a	F	ATGCTGGTGATCTCGGGAAT
	R	TCCGCCCTTTCCAAGATCAT
LcCSD1b	F	GGAGATGGCCCAACTACAGT
	R	CCATGTTCTTTCCCAGCAGG
LcCSD2a	F	TCCAGGGAAGTGCTGATCAA
	R	CTGCTCCGACGAATATCCCT
LcCSD2b	F	TCCCATGGATCATCGCCTAC
	R	GTTGGTCGGGCAGTTGATTT
LcCSD3	F	AGGCTCTCTTCAGTTCGTCC
	R	GAATTGCATCCGTTGGTGGT

3. Results

3.1. Identification of SODs in L. chinense

According to the search results of blastp and hmmer, and domain identification, we obtained eight *LcSOD* genes, which we named *LcMSD1*, *LcFSD2*, *LcCSD2b*, *LcCSD1a*, *LcCSD1b*, *LcFSD3*, *LcCSD2a*, and *LcCSD3* according to their homology with *AtSOD*.

Then, the LcSOD gene characteristics of amino acid length, molecular weight, aliphatic index, GRAVY values, isoelectric point, subcellular location and transmembrane domain were analyzed (Table 2). The results show that the LcSOD length was between 103 and 309 (aa), the molecular weight (MWs) varied from 1.32 to 3.52 kDa, and the predicted pI was between 4.8 and 6.71. The aliphatic index ranged from 71.68 to 93.63. The GRAVY values were all negative, indicating that the proteins encoded by the *LcSOD* family were hydrophilic. In addition, most LcSOD proteins are localized in chloroplasts, followed by mitochondria and cytoplasm. These LcSOD proteins also did not have transmembrane domains.

Table 2. Details of *SOD* family members in *L. chinense*.

Gene ID	Number of Amino Acids (aa)	Molecular Weight (Da)	Aliphatic Index	GRAVY	pI	Subcellular Localization	Transmembrane Domain
LcCSD2b	257	27,288.8	79.3	−0.153	4.8	Chloroplast	NO
LcCSD1b	134	13,299.7	80.75	−0.096	5.07	Chloroplast	NO
LcFSD2	309	35,287.44	71.68	−0.559	5.24	Chloroplast	NO
LcCSD2a	211	22,096.94	92.42	−0.085	5.41	Chloroplast	NO
LcCSD1a	178	18,166.16	78.88	−0.166	5.44	Chloroplast	NO
LcMSD1	226	25,121.64	93.63	−0.358	6.55	Mitochondrion	NO
LcFSD3	271	31,106.47	75.9	−0.409	6.71	Mitochondrion	NO
LcCSD3	150	15,125.9	87.2	−0.173	7.16	Chloroplast/Cytoplasm	NO

3.2. Phylogenetic Analysis of LcSODs in L. chinense

A phylogenetic tree was constructed using the SOD protein sequences of *Liriodendron chinense* (8 *LcSODs*), *Arabidopsis thaliana* (8 *AtSODs*), *Oryza sativa* (8 *OsSODs*), *Amborella trichopoda* (7 *ArSODs*), and *Vitis vinifera* (11 *VvSODs*) with the Bayesian method. The

42 sequences were divided into 2 groups, Cu/Zn-SODs and Fe/Mn-SODs, which were consistent with their metal cofactors. Cu/ZnSOD members clustered into one group, while MnSOD and FeSOD members clustered into another group, which was consistent with the clustering results of three types of SODs in other plants (Figure 2).

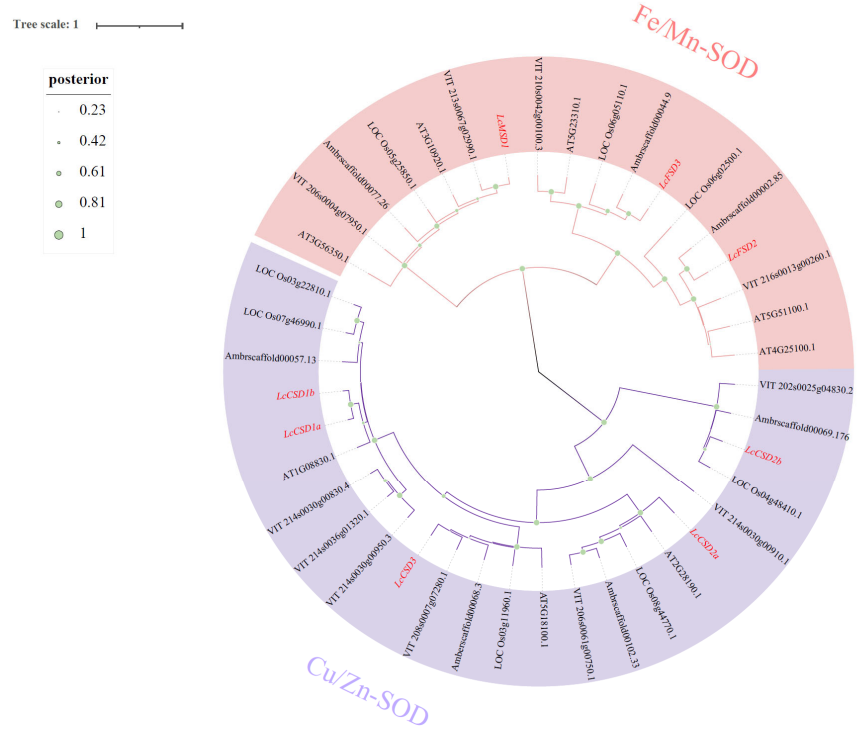

Figure 2. Phylogenetic relationships of the *SOD* family genes from *L. chinense* (Lc), *Arabidopsis thaliana* (At), rice (Os), *A. trichopoda* (Ambr), and *V. vinifera* (VIT). Fe/Mn-SODs and Cu/Zn-SODs are indicated with pink and purple, respectively, and the LcSOD protein is in red. The size of the green circle represents the posterior value of the phylogenetic tree.

3.3. Chromosome Distribution and Syntenic Analysis of LcSODs

In terms of the genomic annotation document, we analyzed the relative position of eight *LcSODs* on the genome. The results verified that the eight genes were evenly distributed on seven different chromosomes, two genes, *LcCSD2b* and *LcFSD2*, were located on chromosome 11, and the other genes were located on chromosomes 4–6 and 8–10 (Figure 3A).

We used JCVI software to analyze the syntenic relationship of SOD genes in *L. chinense*, *Arabidopsis* and rice, and found that *L. chinense* and *Arabidopsis* had three collinear gene pairs, namely, *LchMSD1/AT3G10920.1*, *LchMSD1/AT3G56350.1*, and *LcCSD3/AT5G18100.1*. *L. chinense*, and rice had five collinear gene pairs: *LcCSD1a/LOC_Os07g46990.1*, *LcCSD1a/LOC_Os07g46990.1*, *LcCSD2b/LOC_Os04g48410.1*, *LcCSD1b/LOC_Os07g46990.1*, *LcCSD1b/LOC_Os03g22810.1*, and *LcCSD3/LOC_Os03g11960.1* (Figure 3B).

3.4. Analysis of the SOD Family Gene Structure in L. chinense

To further study the information of *SOD* genes in *L. chinense*, we analyzed their structure, and the results show that all *LcSOD* genes contained 2 to 8 introns. Some *LcSOD* genes contained a large number of introns: *LcFSD2* (8 introns), *LcCSD1a* (7 introns), *LcCSD1b* (7 introns), *LcCSD2a* (7 introns), and *LcCSD3* (5 introns); the other *LcSOD* genes had 2 to 3 introns (Figure 4A).

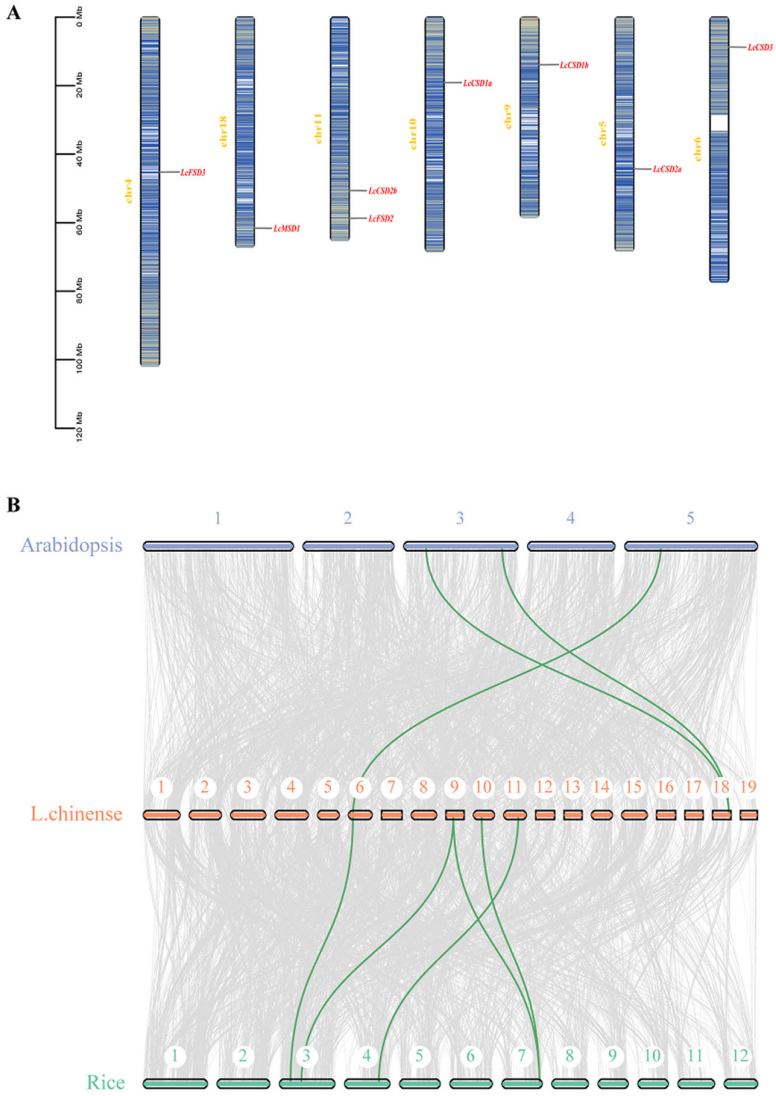

Figure 3. (**A**) Location of eight *SOD* genes on the chromosome of *L. chinense*. Gray lines, positions of each member of the *SOD* gene family on the chromosome. The orange font on the left of the chromosome is the chromosome number. The color distribution on the chromosome represents the distribution of the gene density on the chromosome. The vertical scale indicates the chromosome size. (**B**) Syntenic analysis of *LcSOD* genes among *L. chinense*, *Arabidopsis*, *and* rice. *AtSOD*, *LcSOD*, and *OsSOD* are represented by haze blue, orange, and green, respectively. Grass-green lines connect the genes with their collinear relationship.

Motif analysis shows that LcSODs contained different motif numbers. Among them, LcCSD2b contained only motif 1; LcCSD1a, LcCSD1b, LcCSD2a, and LcCSD3 contained motifs 1, 2, and 6, indicating that motif 1 was the only conserved domain of Cu/Zn-SOD subfamily members. Besides LcCSD3, the other three proteins all contained motif 8, while LcFSD2 and LcFSD3 all contained motifs 3, 4, 5, 7, and 10. The LcCSD1a, LcCSD1b, and LcCSD2a proteins all contained motif 8, while the LcCSD1a and LcCSD3 proteins both contained motif 9 (Figure 3B).

The comparison of the *LcFSD3* gene structure with four other plant species showed that the intron length of *LcFSD3* was abnormal. To verify the existence of this abnormality, we designed specific primers on the basis of the predicted CDS, and the results of electrophoresis and sequencing show that *LcFSD3* was normal despite the long intron (Figure 4D,E). Some error may have occurred when the genome was assembled.

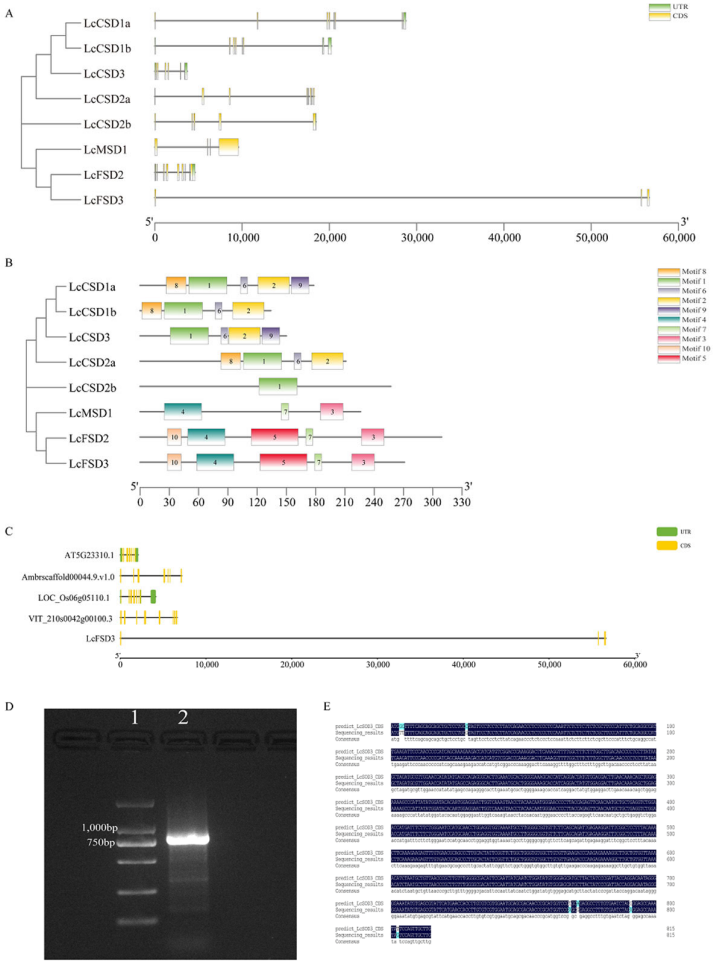

Figure 4. (**A**) Domain distribution of *LcSOD* genes. Introns and exons are represented by horizontal lines and yellow boxes, respectively. UTRs are indicated by green boxes. (**B**) Conservative motif distribution of *LcSOD* genes. The different colors represent different motifs. (**C**) Structure of *FSD3* from *L. chinense* (Lc), *A. thaliana* (At), rice (Os), *A. trichopoda* (Ambr), and *V. vinifera* (VIT). (**D**) Cloning results of the *LcFSD3* gene. Lane 1 is the 2000 maker and Lane 2 is the amplification products. The amplification products were identified at 750–1000 bp. (**E**) Sequence comparison between the *LcFSD3* reference sequence and Sanger sequencing results. Different colors represent different comparison conditions.

3.5. Cis-Regulatory Element Analysis

In this study, we predicted the 2000 bp upstream *cis*-elements of eight *LcSOD* genes, which helped in exploring the potential mechanism of the gene response to multiple stresses. Promoter prediction results show that the *cis*-regulatory element contained a variety of response elements associated with environmental stress and plant hormone responses: the MYB binding site, the defense and stress response element, the cryogenic response element, the anaerobic induction element, the ABA response element, the MeJA response element, the gibberellin (GA) response element, the salicylic acid (SA) response element, the auxin response element, and the circadian control element.

Among these eight members, seven *LcSOD* gene promoters contained anaerobic induction elements, three *LcSOD* gene promoters contained defense and stress response elements, and four *LcSOD* gene promoters contained low-temperature response elements. We identified seven *LcSOD* gene promoters containing ABA response elements, six *LcSOD* gene promoters containing MeJA response elements, five *LcSOD* gene promoters containing SA response elements, eight *LcSOD* gene promoters containing auxin response elements.

These results suggest that SODs may be regulated by these elements, which may influence the expression of *SODs* under stress (Figure 5).

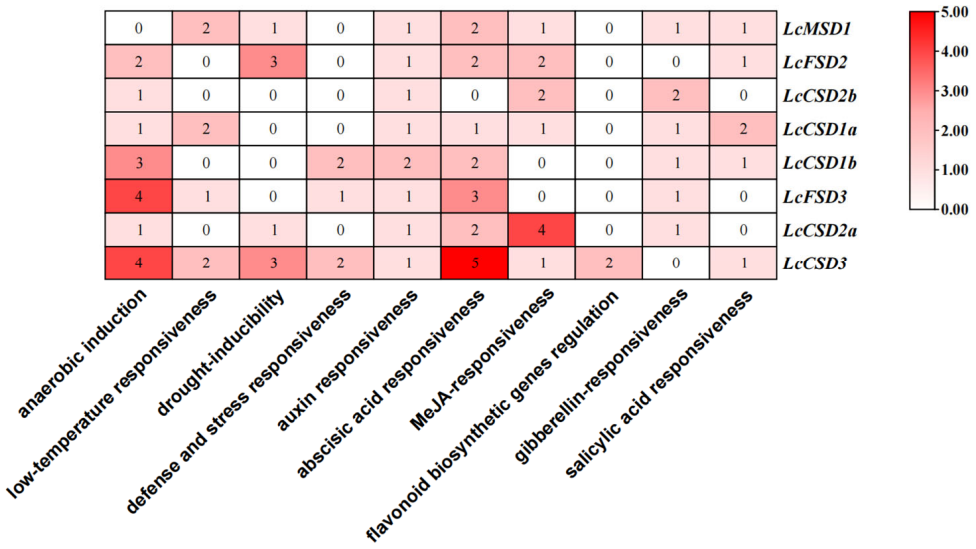

Figure 5. Summary of cis-acting elements' number and function of *SOD* genes in *L. chinense.* Shading represents quantity.

3.6. Expression Analysis of LcSOD Genes across Different Tissues and Abiotic Stresses

On the basis of available transcriptome data, we studied the expression patterns of *SOD* genes in yjr bark, bud, phloem, sepal, stamen, stigma, xylem, and leaves, and found that most *LcSOD* genes were relatively highly expressed in the leaves, while *LcCDS1b* peaked in the bark, and *LcMSD1* and *LcFSD3* peaked in the sepal (Figure 6A). The expression levels of *LcCSD1b*, *2b,* and *1a* in bark were higher, while the expression levels of other *LcSOD* genes were lower. The expression of most *LchSOD* genes in the xylem and phloem was generally not high.

In addition, we examined the expression patterns of *LcSODs* under the abiotic stresses of heat, drought, and cold (Figure 6B–D). Under high-temperature stress, *LcSOD2b* and *LcSOD1b* responded quickly and peaked at 1 h, while *LcSOD3* increased to the peak value at 6 h (Figure 6B). In response to drought stress, most *LcSOD* genes first increased and then decreased with a peak value at around 3–12 h (Figure 6C). Interestingly, most *LcSOD* genes showed identical expression patterns in response to cold stress that first decreased and was then restored to the original expression level at 12 h, followed by a marked decrease into the valley (Figure 6D).

3.7. Expression Analysis of LcSOD Genes under Cold Stress

To further confirm the transcriptional responses of *LcSODs* to cold stress, we analyzed their expression patterns under cold stress using qRT-PCR experiments. The results show that only three *LcSODs* were significantly induced by cold stress (Figure 7). Among them, *LcMSD1* decreased first into the valley at 12 h, and was then restored at 48 h, and *LcCSD2a* maintained a downward trend. *LcFSD3,* on the other hand, increased and then decreased with a peak value at 12 h.

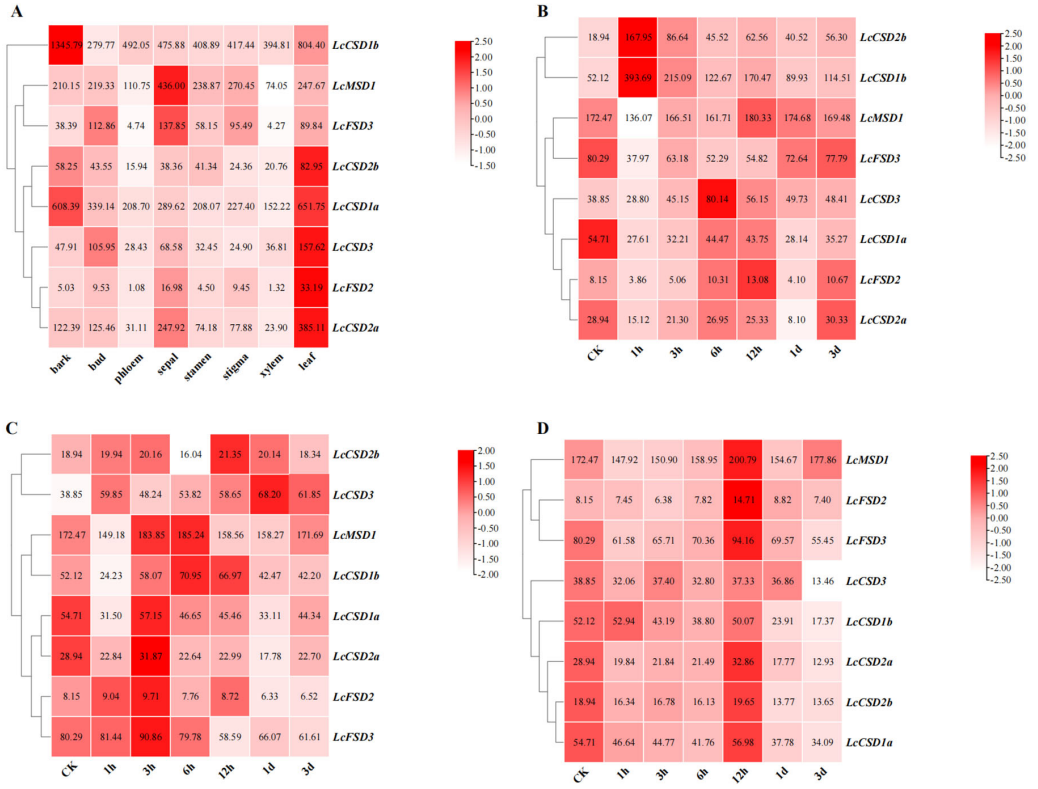

Figure 6. Heat map of the differential expression of *LcSOD* genes in different tissues and under different abiotic stresses. (**A**) Heat map of the differential expression of *LcSOD* genes in different tissues. (**B**) Heat map of the differential expression of *LcSOD* genes under heat stress. (**C**) Heat map of the differential expression of *LcSOD* genes under drought stress. (**D**) Heat map of the differential expression of *LcSOD* genes under cold stress. Darker red in the expression map indicates a higher expression level. CK, untreated control group. Row data were standardized by using $\log_2^{(x+1)}$. X, relative expression quantity.

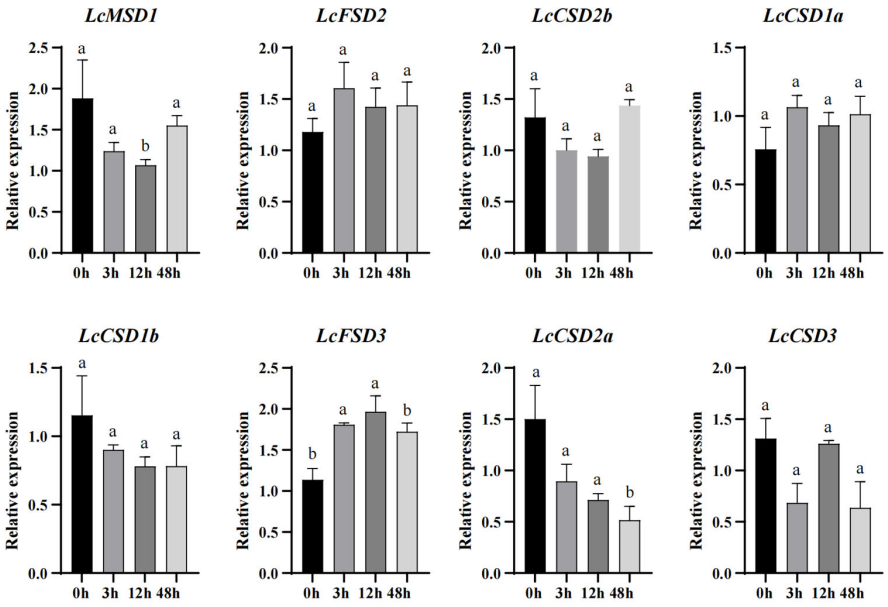

Figure 7. Expression profiles of *LcSODs* under cold stress. Experimental results are expressed in terms of the relative expression quantity. The reference genes were 18S RNA and *GAPDH* of *L. chinense*. The results of multiple comparisons are represented by letters.

4. Discussion

L. chinense, as a rare and endangered species in China, has high economic, appreciation, and medicinal value. It also has high scientific research value because it is an ancient Cretaceous relict plant. Stress often induces the excessive accumulation of reactive oxygen species (ROS), which leads to oxidative stress and negatively affects *L. chinense* growth and development. However, plants have formed a self-protective mechanism in the process of evolution to reduce harm caused by adverse environments. SOD is a key antioxidant enzyme that participates in the plant resistance to ROS damage. *SOD* is an important gene family encoding the SOD protein, which plays an indispensable role in plants in responding to all kinds of environmental stimuli (such as droughts, salt stress, and low and high temperatures) [37]. Enhanced *SOD* activity in plants can increase the plant resistance to a variety of stresses [38]. The plant *SOD* gene family was identified genomewide in *Arabidopsis*, *Brassica rapa*, barley, grapes, *Zea mays*, *Sorghum bicolor*, cotton, cucumber and *Salvia miltiorrhiza*, indicating a regulating role when plants are subjected to low and high temperatures, droughts, and other environmental stresses [39–47].

Genomic size varies considerably between plant species, but the number of *SOD* genes does not change proportionally with genomic size [48]. Although there are differences in the genome among *L. chinense*, rice, *A.trichopoda*, *V.vinifera,* and *Arabidopsis*, there is no significant difference in the number of SOD genes. The 8 members identified in the *L. chinense* genome in this study are distributed on 7 chromosomes, namely, 5 *Cu/Zn-SOD* members, 2 *Fe-SOD* members, and 1 *Mn-SOD* member, which constitute 3 main types of plant *SOD* genes. The number and proportion of the genes of *Cu/Zn-SOD*, Fe-SOD, and *Mn-SOD* are similar in different species. The number of *Cu/Zn-SOD* and *Fe-SOD* is greater, but the number of Mn-SOD is smaller. The 9 tomato *SISOD* genes are distributed on 6 chromosomes: 4 *Cu/Zn-SOD* members, 4 *Fe-SOD* members, and 1 *Mn-SOD* member. In this study, no tandem repeat events were identified in *LcSOD* genes through collinearity analysis, but tandem repeats from the same chromosome were identified in tomatoes (*SlSOD5* and *SlSOD8*) [16]. The results of the syntenic analysis of *SODs* among three species show that there were three collinear gene pairs in *Arabidopsis* and *L. chinense*, and five collinear gene pairs in *L. chinense* and rice. According to the analysis of physical and chemical properties, the three different types of LcSOD proteins were predicted to localize in the cytoplasm, chloroplast, and mitochondria, which is consistent with the localization results in tobacco [49].

The analysis of the gene structure shows that the number of the introns of *LcSOD* was in the range of 2–8. Most of *LcSOD* genes contained 7–8 introns, and only a few contained 2–3 introns. In addition, the two groups of *LcSOD* genes contained similar intron–exon structures, which indicates that *LcSOD* genes were highly conserved during evolution. This is consistent with the similar intron/exon organizational pattern of *SOD* genes in tomatoes. *LcSOD* genes were clustered into different clades, each of which possessing similar motifs in regard to the number and sequence. The results show that different *LcSOD* gene members of the same subfamily were more conserved in evolution, and these genes may have also had similar biological functions. *Cu/Zn-SOD* contains motif 1, while *Fe/Mn-SOD* contains motifs 3 and 4. These results are similar to those in *A. thaliana* and tomatoes, indicating that *SOD* genes were highly conserved across different plant species during evolution [16,50].

To better understand the phylogenetic relationship of *LcSOD*, we combined the *SOD* genes of several species to construct a phylogenetic tree. The results show that the subfamily classification was closely related to the type of the functional domains of the SOD protein. The eight *LcSOD* genes were homologous with the *SOD* genes of rice and grapes, suggesting that *LcSOD* genes might have a similar abiotic stress resistance function as that of rice and grape *SOD* genes. In addition, six LcSOD proteins were predicted to be localized in chloroplasts, indicating that these *LcSOD* genes might be involved in scavenging photosynthate-generated ROS, which is similar to the high expression of five *SOD* genes in watermelon leaves [51]. There are substantially more members in the Cu/Zn-SOD subfamily than those of the Fe-SOD or Mn-SOD subfamily, suggesting that the *SOD* gene in

this subfamily is an indispensable part of plant growth, development, and stress response. In addition, the closest evolutionary distance between *A. trichopoda* and *L. chinense SOD* genes indicates the basal evolutionary position of *L. chinense* in the angiosperm.

Plants under stress need to increase the activity of the SOD enzyme to enhance the ability of scavenging ROS, which is the premise of improving the expression of *SOD* genes [52]. On this basis, we studied and analyzed the expression patterns of *LcSODs* in different tissues and under different abiotic stresses, and found that *LcSOD* genes were especially highly expressed in the leaves, and involved in the response to different abiotic stresses. Almost all *LcSOD* genes were highly expressed in the leaves, the main photosynthetic organ in plants, suggesting that SOD is a crucial enzyme in scavenging ROS generated from photosynthesis [53]. Expression analysis shows that each *LcSOD* gene responded to at least one type of abiotic stress (heat, cold, and drought) examined in this study. In addition, according to the analysis of putative *LcSOD* promoters, *LcSOD* gene family promoters contain a number of abiotic stress *cis*-elements that are involved in the low-temperature, drought-response, defense and stress response elements, etc. This could explain why *LcSOD* showed an obvious response to the four abiotic stresses. Under drought stress, the expression levels of *LcCSD1b* and *2b* were upregulated at 1 h, and *LcCSD3* was upregulated at 6 h, while the changes of other genes were not obvious, which was similar to the upregulated expression levels of *BjuCSD8* and *BjuFSD3d* in *Brassica juncea* under drought stress [17]. Studies have shown that heat stress can lead to the photosuppression of Photosystem II, thus causing ROS to accumulate in cells. Under heat stress, almost all *LcSOD* genes were upregulated. Therefore, we speculate that *LcSOD* genes may contribute to ROS clearance and protect cells from damage [54]. However, *LcMSD1* was downregulated under cold stress, and *MSD* was also downregulated in *Medicago truncatula*, tomatoes, and cotton for a period of time [16,45,53]. In addition, we identified that *LcFSD3* may participate in recovery from cold stress by scavenging excessive ROS caused by low-temperature stress in the leaves (Figure 7), thus providing a prospective target gene to enhance cold resistance, particularly in valuable woody plant *L. chinense*.

5. Conclusions

In this study, the *SOD* gene family of *L. chinense* was analyzed with bioinformatics for the first time. A total of 8 *LcSOD* genes were identified from *L. chinense*. *LcSODs* could be divided into two groups according to phylogenetic and structural analyses. Members of the same group had similar structural characteristics. The promoter region of *LcSOD* genes also identified *cis*-regulatory elements in response to plant hormone and abiotic stress. In addition, transcriptome data and qRT-PCR analysis showed that *LcSOD* genes could respond to temperature and drought stresses. These results provide insight for further research on the biological function of single genes of and genetic improvements in *L. chinense*.

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References

1. Zhang, H.; Guo, C. Cloning, characterization and expression analysis of two superoxide dismutase (SOD) genes in wheat (*Triticum aestivum* L.). *Front. Agric. China* **2008**, *2*, 141–149. [CrossRef]
2. Mhamdi, A.; Van Breusegem, F. Reactive oxygen species in plant development. *Development* **2018**, *145*, dev164376. [CrossRef]
3. Gill, S.S.; Peter Singh, L. Generation and scavenging of reactive oxygen species in plants under stress. In *Improving Crop Resistance to Abiotic Stress*; Wiley Online Library: Hoboken, NJ, USA, 2012; pp. 49–70. [CrossRef]
4. Hasanuzzaman, M.; Bhuyan, M.B. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* **2020**, *9*, 681. [CrossRef] [PubMed]
5. Feng, X.; Lai, Z. Genome-wide identification and characterization of the superoxide dismutase gene family in *Musa acuminata* cv. Tianbaojiao (AAA group). *BMC Genom.* **2015**, *16*, 823. [CrossRef] [PubMed]
6. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
7. Gill, S.S.; Anjum, N.A. Superoxide dismutase—Mentor of abiotic stress tolerance in crop plants. *Environ. Sci. Pollut. Res.* **2015**, *22*, 10375–10394. [CrossRef]
8. Miao, L.; Clair, D.K.S. Regulation of superoxide dismutase genes: Implications in disease. *Free. Radic. Biol. Med.* **2009**, *47*, 344–356. [CrossRef]
9. Wang, M.; Zhao, X. A wheat superoxide dismutase gene $TaSOD_2$ enhances salt resistance through modulating redox homeostasis by promoting NADPH oxidase activity. *Plant Mol. Biol.* **2016**, *91*, 115–130. [CrossRef]
10. Zelko, I.N.; Mariani, T.J. Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD_1), Mn-SOD (SOD_2), and EC-SOD (SOD_3) gene structures, evolution, and expression. *Free. Radic. Biol. Med.* **2002**, *33*, 337–349. [CrossRef]
11. Alscher, R.G.; Erturk, N. Role of superoxide dismutases (SOD_s) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [CrossRef]
12. Han, X.M.; Chen, Q.X. Genome-wide analysis of superoxide dismutase genes in *Larix kaempferi*. *Gene* **2019**, *686*, 29–36. [CrossRef] [PubMed]
13. Dupont, C.L.; Neupane, K. Diversity, function and evolution of genes coding for putative Ni-containing superoxide dismutases. *Environ. Microbiol.* **2008**, *10*, 1831–1843. [CrossRef]
14. Sen, R.S.; Deng, X.W. The role of superoxide dismutase in combating oxidative stress in higher plants. *Bot. Rev.* **2000**, *66*, 89–98. [CrossRef]
15. Molina-Rueda, J.J.; Tsai, C.J. The Populus superoxide dismutase gene family and its responses to drought stress in transgenic poplar overexpressing a pine cytosolic glutamine synthetase (GS1a). *PLoS ONE* **2013**, *8*, e56421. [CrossRef] [PubMed]
16. Feng, K.; Yu, J. The SOD gene family in tomato: Identification, phylogenetic relationships, and expression patterns. *Front. Plant Sci.* **2016**, *7*, 1279. [CrossRef] [PubMed]
17. Verma, D.; Lakhanpal, N.; Singh, K. Genome-wide identification and characterization of abiotic-stress responsive *SOD* (*superoxide dismutase*) gene family in *Brassica juncea* and *B. rapa*. *BMC Genom.* **2019**, *20*, 227. [CrossRef]
18. Chen, J.; Hao, Z.; Guang, X.; Zhao, C.; Wang, P.; Xue, L.; Zhu, Q.; Yang, L.; Sheng, Y.; Zhou, Y.; et al. *Liriodendron* genome sheds light on angiosperm phylogeny and species–pair differentiation. *Nat. Plants* **2019**, *5*, 18–25. [CrossRef]
19. Cao, Y.; Feng, J. Alterations in Population Distribution of *Liriodendron chinense* (Hemsl.) Sarg. and *Liriodendron tulipifera* Linn. Caused by Climate Change. *Forests* **2022**, *13*, 488. [CrossRef]
20. Yu, W.; Kong, G.; Chao, J.; Yin, T.; Tian, H.; Ya, H.; He, L.; Zhang, H. Genome-wide identification of the rubber tree superoxide dismutase (*SOD*) gene family and analysis of its expression under abiotic stress. *PeerJ* **2022**, *10*, e14251. [CrossRef] [PubMed]
21. Chen, C.; Chen, H. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
22. Goodstein, D.M.; Shu, S.; Howson, R.; Neupane, R.; Hayes, R.D.; Fazo, J.; Mitros, T.; Dirks, W.; Hellsten, U.; Putnam, N.; et al. Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Res.* **2012**, *40*, D1178–D1186. [CrossRef]
23. Marchler-Bauer, A.; Bryant, S.H. CD-Search: Protein domain annotations on the fly. *Nucleic Acids Res.* **2004**, *32* (Suppl. S2), W327–W331. [CrossRef]
24. Garg, V.K.; Avashthi, H.; Tiwari, A.; Jain, P.A.; Ramkete, P.W.; Kayastha, A.M.; Singh, V.K. MFPPI–multi FASTA ProtParam interface. *Bio. Inf.* **2016**, *12*, 74–77. [CrossRef]
25. Chou, K.C.; Shen, H.B. Plant-mPLoc: A top-down strategy to augment the power for predicting plant protein subcellular localization. *PLoS ONE* **2010**, *5*, e11335. [CrossRef]
26. Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E.L. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J. Mol. Biol.* **2001**, *305*, 567–580. Available online: https://pubmed.ncbi.nlm.nih.gov/11152613/ (accessed on 1 January 2023). [CrossRef]
27. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [CrossRef]
28. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kühnert, D.; Maio, N.D.; et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef]

29. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37* (Suppl. S2), W202–W208. [CrossRef]
30. Tang, H.; Bowers, J.E.; Wang, X.; Ming, R.; Alam, M.; Paterson, A.H. Synteny and collinearity in plant genomes. *Science* **2008**, *320*, 486–488. [CrossRef]
31. Lescot, M.; Déhais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouzé, P.; Rombauts, S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327. [CrossRef] [PubMed]
32. Bray, N.L.; Pimentel, H.; Melsted, P.; Pachter, L. Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.* **2016**, *34*, 525–527. [CrossRef]
33. Wu, W.; Zhu, S.; Xu, L.; Zhu, L.; Wang, D.; Liu, Y.; Liu, S.; Hao, Z.; Lu, Y.; Yang, L.; et al. Genome-wide identification of the *Liriodendron chinense WRKY* gene family and its diverse roles in response to multiple abiotic stress. *BMC Plant Biol.* **2022**, *22*, 25. [CrossRef] [PubMed]
34. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
35. Field, A. *Discovering Statistics Using IBM SPSS Statistics*; Sage Publications Ltd.: Thousand Oaks, CA, USA, 2013.
36. Swift, M.L. GraphPad prism, data analysis, and scientific graphing. *J. Chem. Inf. Comput. Sci.* **1997**, *37*, 411–412. [CrossRef]
37. Wang, W.; Xia, M.X. Gene expression characteristics and regulation mechanisms of superoxide dismutase and its physiological roles in plants under stress. *Biochemistry* **2016**, *81*, 465–480. [CrossRef] [PubMed]
38. Jiang, W.; Yang, L. Genome-wide identification and transcriptional expression analysis of superoxide dismutase (SOD) family in wheat (*Triticum aestivum*). *PeerJ* **2019**, *7*, e8062. [CrossRef] [PubMed]
39. Kliebenstein, D.J.; Monde, R.A. Superoxide dismutase in *Arabidopsis*: An eclectic enzyme family with disparate regulation and protein localization. *Plant Physiol.* **1998**, *118*, 637–650. [CrossRef]
40. Filiz, E.; Tombuloğlu, H. Genome-wide distribution of superoxide dismutase (*SOD*) gene families in *Sorghum bicolor*. *Turk. J. Biol.* **2015**, *39*, 49–59. [CrossRef]
41. Qureshi, A.M.I.; Sofi, M.U. Insilco identification and characterization of superoxide dismutase gene family in *Brassica rapa*. *Saudi J. Biol. Sci.* **2021**, *28*, 5526–5537. [CrossRef]
42. Aleem, M.; Aleem, S. Characterization of *SOD* and *GPX* Gene Families in the Soybeans in Response to Drought and Salinity Stresses. *Antioxidants* **2022**, *11*, 460. [CrossRef]
43. Zhang, X.; Zhang, L. Genome-wide identification of the *SOD* gene family and expression analysis under drought and salt stress in barley. *Plant Growth Regul.* **2021**, *94*, 49–60. [CrossRef]
44. Hu, X.; Hao, C. Genome-wide identification, characterization, and expression analysis of the grapevine superoxide dismutase (SOD) family. *Int. J. Genom.* **2019**, *2019*, 7350414. [CrossRef] [PubMed]
45. Wang, W.; Zhang, X. Genome-wide characterization and expression analyses of superoxide dismutase (*SOD*) genes in *Gossypium hirsutum*. *BMC Genom.* **2017**, *18*, 376. [CrossRef] [PubMed]
46. Han, L.; Hua, W. Genome-wide identification and expression analysis of the superoxide dismutase (*SOD*) gene family in *Salvia miltiorrhiza*. *Gene* **2020**, *742*, 144603. [CrossRef]
47. Zhang, J.; Li, B. Genome-wide characterization and expression profiles of the superoxide dismutase gene family in *Gossypium*. *Int. J. Genom.* **2016**, *2016*, 8740901. [CrossRef]
48. Zhou, Y.; Hu, L. Genome-wide identification and transcriptional expression analysis of cucumber superoxide dismutase (SOD) family in response to various abiotic stresses. *Int. J. Genom.* **2017**, *2017*, 7243973. [CrossRef]
49. Huo, C.; He, L. The Superoxide Dismutase Gene Family in *Nicotiana tabacum*, Genome-Wide Identification, Characterization, Expression Profiling and Functional Analysis in Response to Heavy Metal Stress. *Front. Plant Sci.* **2022**, *13*, 904105. [CrossRef]
50. Fink, R.C.; Scandalios, J.G. Molecular evolution and structure–function relationships of the superoxide dismutase gene families in angiosperms and their relationship to other eukaryotic and prokaryotic superoxide dismutases. *Arch. Biochem. Biophys.* **2002**, *399*, 19–36. [CrossRef]
51. Zhou, Y.; Ouang, L. Superoxide dismutase family genes in watermelon and their responses to different abiotic stresses. *Front. Agric. Sci. Eng.* **2021**, *8*, 645–658. [CrossRef]
52. Bowler, C.; Van Camp, W. Superoxide dismutase in plants. *Crit. Rev. Plant Sci.* **1994**, *13*, 199–218. [CrossRef]
53. Song, J.; Zeng, L. In silico identification and expression analysis of superoxide dismutase (*SOD*) gene family in *Medicago truncatula*. *3 Biotech* **2018**, *8*, 348. [CrossRef] [PubMed]
54. Lu, T.; Meng, Z.; Zhang, G.; Qi, M.; Sun, Z.; Liu, Y.; Li, T. Sub-high temperature and high light intensity induced irreversible inhibition on photosynthesis system of tomato plant (*Solanum lycopersicum* L.). *Front. Plant Sci.* **2017**, *8*, 365. [CrossRef] [PubMed]