SUPEROXIDE DISMUTASE FAMILY GENES IN WATERMELON AND THEIR RESPONSES TO DIFFERENT ABIOTIC STRESSES

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KEYWORDS
abiotic stress, expression analysis, phylogeny, SOD, superoxide dismutase, watermelon

HIGHLIGHTS
- A total of 8 SOD genes from watermelon were identified and bioinformatically analyzed.
- The SOD proteins from watermelon and other different plant species can be classified into five groups consistent with their metal cofactors.
- ClSOD genes exhibited distinctive tissue-specific and abiotic stress responsive expression patterns.

GRAPHICAL ABSTRACT

ABSTRACT
Superoxide dismutase (SOD) is an important enzyme in the antioxidant system of plants and plays a vital role in stress responses by maintaining the dynamic balance of reactive oxygen species (ROS) concentrations. Genome-wide analysis of the SOD gene family in various plant species has been conducted but little is known about this gene family in watermelon (Citrullus lanatus). Here, eight SOD genes were identified in the watermelon genome and are designated ClCSD1-5, ClFSD1-2 and ClMSD according to their metal cofactors. Phylogenetic analysis shows that SOD proteins from various plant species can be classified into five groups and members in the same group possess the same metal cofactor and similar subcellular localizations. Expression analysis of the ClSOD genes indicates that they had tissue-specific expression patterns with high expression in different tissues including the leaves, flowers and fruits. In addition, the expression of ClSOD genes differed appreciably under salinity, drought and abscisic acid (ABA) treatments, indicating that they may be involved in ROS scavenging under different abiotic stresses via an ABA-dependent signaling pathway. These results lay the foundation for elucidating the function of ClSOD genes in stress tolerance and fruit development in watermelon.

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Plants are constantly exposed to numerous abiotic and biotic stresses including high and low temperatures, drought, salinity, high solar radiation, metal toxicity, UV radiation and pathogen infection\textsuperscript{[4–2]}. These environmental stimuli can lead to the generation of a number of reactive oxygen species (ROS) such as singlet oxygen (\( \text{O}_2^* \)), superoxide anion radicals (\( \text{O}_2^- \)) and hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) which can cause oxidative damage to cells impairing cellular components and ultimately affecting the growth and development of plants\textsuperscript{[14]}. A range of enzymatic and nonenzymatic antioxidant defense systems have evolved in plants to mitigate ROS toxicity and maintain the dynamic balance of ROS concentrations\textsuperscript{[5–8]}. Superoxide dismutase (SOD, EC 1.15.1.1) is the first line of defense against ROS by catalyzing the decomposition of toxic \( \text{O}_2^- \) to \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \), which can be further degraded by peroxidase and catalase\textsuperscript{[8]}.

Plant SODs can be classified into three types based on prosthetic metals, namely copper-zinc SOD (Cu/ZnSOD), iron SOD (FeSOD) and manganese SOD (MnSOD)\textsuperscript{[6,10]}. The SOD genes often constitute a multigene family and all three types of SOD are present in plants with different subcellular localizations, including the cytoplasm, peroxisome, chloroplasts, mitochondria, nuclei or extracellular spaces\textsuperscript{[10–12]}. Genome-wide identification of the SOD gene family has been conducted in recent years in a range of plant species such as tomato (\textit{Solanum lycopersicum})\textsuperscript{[13]}, Gossypium species\textsuperscript{[6,14]}, cucumber (\textit{Cucumis sativus})\textsuperscript{[15]}, \textit{Medicago truncatula}\textsuperscript{[9]}, foxtail millet (\textit{Setaria italica})\textsuperscript{[16]}, pear (\textit{Pyrus bretschneideri})\textsuperscript{[17]}, Japanese larch (\textit{Larix kaempferi})\textsuperscript{[18]}, tea (\textit{Camellia sinensis})\textsuperscript{[19]} and wheat (\textit{Triticum aestivum})\textsuperscript{[20]}. These studies report the basic characteristics and spatiotemporal expression profiles of the SOD family genes, demonstrating that the family members are pivotal for multiple developmental plant processes. For example, \textit{PbrCSD5} and \textit{PbrFSD1} might be closely associated with the postharvest ripening process of Fengshui pear\textsuperscript{[17]} and \textit{Xanthoceras sorbifolium} SOD genes have been found to be involved in ovule development after fertilization\textsuperscript{[21]}.

Numerous studies show that plant SOD genes are important regulators of responses to various environmental stimuli by protecting cells from oxidative damage by ROS\textsuperscript{[8,11,22]}. For example, overexpression of \textit{TmMnSOD} from \textit{Triticum monococcum} in yeast and \textit{Escherichia coli} cells increased tolerance to abiotic stresses and metal toxicity\textsuperscript{[23]}. Overexpression of the \textit{Oxya chinensis \textit{OcMnSOD}} was recently found to enhance the tolerance of \textit{E. coli} to chlorpyrifos-induced oxidative stress\textsuperscript{[24]}. Also, overexpression of different SOD genes may lead to enhanced tolerance to single or multiple abiotic stresses in transgenic plants. For example, overexpression of a \textit{Puccinellia tenuiflora Cu/ZnSOD} gene in transgenic yeast and \textit{Arabidopsis} enhanced their tolerance to saline-sodic stress\textsuperscript{[25]}. Higher NaCl and NaHCO\(_3\) stress tolerance was also observed in transgenic rice plants overexpressing \textit{OsCu/Zn-SOD}\textsuperscript{[26]}. Overexpression of \textit{CsCSD1} from cucumber was demonstrated to enhance salinity tolerance via an ABA-dependent signaling pathway in transgenic \textit{Arabidopsis}\textsuperscript{[11]}. Overexpression of three \textit{Larix SOD} genes (\textit{LkSOD2}, \textit{LkSOD4} and \textit{LkSOD6}) conferred tolerance to salt stress in transformed \textit{Arabidopsis}\textsuperscript{[18]}.

Although the SOD gene family has been widely studied in many plant species this family has not been systematically investigated in watermelon (\textit{Citrullus lanatus cv. Xinong 8}), an economically important crop worldwide. The growth and development of watermelon can be adversely affected by various abiotic and biotic stresses, particularly drought and salinity\textsuperscript{[27–29]}. Here, we have systematically analyzed the SOD gene family in watermelon using phylogenetic analysis, multiple sequence alignment, and analysis of the conserved motifs, gene structures and promoter sequences. Expression analysis was conducted to examine the expression of watermelon SOD genes in different tissues, during fruit development, and under drought, salinity and abscisic acid (ABA) treatments. The results will provide valuable information in further dissecting the potential role of watermelon SOD genes in stress tolerance.

## 2. MATERIALS AND METHODS

### 2.1 Genome-wide identification of the SOD gene family in watermelon

The watermelon SOD gene family members were first identified by a BLAST\textit{p} search against the watermelon (97103) \( v1 \) proteome, with all known \textit{Arabidopsis} and rice SOD proteins as queries\textsuperscript{[30,31]}. In addition, Hidden Markov Model profiles of \textit{Cu/ZnSOD} ([PF00080]) and \textit{Fe-MnSOD} ([PF00081 and PF02777]) were downloaded from the Pfam tool and used as queries to conduct an HMMER search with HMMER 3.0 software against the watermelon proteome with an E-value setting of \( \text{E}^{-5} \). All the watermelon SOD proteins were subjected to Pfam and SMART tools to check that they contained the SOD domains (PF00080, PF00081 and PF02777). Sequences without the SOD domains were removed.

### 2.2 Analysis of physicochemical properties and conserved motifs

Based on the ProtParam tool, selected physicochemical
properties of watermelon SOD proteins, specifically protein length, molecular weight (MW), and isoelectric point (pI), were determined. Subcellular localization was predicted by the ProtComp 9.0 server. The conserved motifs of the watermelon SOD proteins were analyzed with the MEME program using the parameters of 10 motifs and the motif analysis results have been displayed using the TBtools software[32].

2.3 Sequence alignment and evolutionary analysis
Sequence alignment was conducted with Clustal Omega with default parameters[33], using full-length SOD protein sequences from different plants. The alignment was then imported into MEGA 7.0 to create a neighbor-joining phylogenetic tree with bootstrap tests repeated 1000 times[34].

2.4 Chromosomal location, gene structure and promoter sequence analysis
The positions of watermelon SOD genes were obtained in the watermelon (97103) v1 genome and the illustration was drawn using the MapInspect software. Duplication analysis (segmental and tandem duplications) of watermelon SOD genes was conducted with the MCScanX software[35]. Gene structure analysis was conducted by retrieving the CDS and gDNA sequences of each watermelon SOD gene from the watermelon genome and their exon-intron arrangements were analyzed using the GSDS program. Promoter sequence analysis was done by obtaining the 1-kb upstream region from the translation start code (ATG) of each watermelon SOD gene as the promoter region from the watermelon genome and the phytohormone- and stress-responsive cis-elements were analyzed using the PlantCARE program.

2.5 Transcriptome analysis of the watermelon SOD genes
The raw RNA-seq data of both the flesh and rind at the four critical fruit development stages 10, 18, 26 and 34 days after pollination (DAP) were retrieved from the NCBI SRA database under BioProject SRP012849 and then the expression of watermelon SOD genes was estimated as RPKM values following a previous study[36]. The raw RPKM values were logarithmically transformed and heat maps were generated using TBtools[32].

2.6 Expression analysis of watermelon SOD genes by quantitative RT-PCR (qRT-PCR)
Watermelon cv. Xinong 8 seeds were germinated and grown in plastic pots containing fertilized soil. The pots were then placed in a greenhouse at 25/19°C day/night, 12:12 h light:dark photoperiod, 200 µmol·m⁻²·s⁻¹ light intensity and 70% RH. Tissue expression pattern analysis of the watermelon SOD genes was conducted by collecting different plant parts (roots, leaves, stems, flowers and fruits) from two-month-old plants. Three abiotic stress treatments (drought, salinity and ABA) were applied as described previously[27]. In brief, four-leaf-stage watermelon plants were transferred into Hoagland solution containing 200 mmol·L⁻¹ NaCl or 20% PEG-6000 (w/v) to induce salt stress and drought stress, respectively. The ABA treatment was set up by spraying 100 µmol·L⁻¹ ABA on the leaves of four-leaf-stage plants. The leaves were collected after different durations of treatment (0, 1, 3, 9 and 24 h). Total RNA was isolated using a total RNA Miniprep Kit (Axygen Biosciences, Corning, NY) and about 1 µg RNA was used for first-strand cDNA synthesis with a ReverTra Ace qPCR-RT kit (Toyobo, Osaka, Japan) according to the manufacturers’ protocols. qRT-PCR analysis was conducted by assaying each gene in three biological replicates and three technical replicates under the experimental conditions described in our previous study[27]. The relative expressions of detected genes were analyzed by the 2⁻ΔΔCt method using β-actin gene (Cla007792) for normalization[27] and the expression at 0 h was set at a value of 1.0. The relative expressions of the genes were compared with those of the control (0 h) using Tukey’s test and P<0.05 was assumed as significantly different. The sequences of primer pairs for qRT-PCR analysis are listed in Table S1.

3 RESULTS

3.1 Identification of SOD family genes in the watermelon genome
BLASTp and HMMER searches were used to identify SOD family members in the watermelon genome. After the removal of redundant sequences all protein sequences were confirmed by Pfam and SMART programs for the presence of a SOD domain and a total of eight SOD genes were identified, comprising five Cu/ZnSOD genes, two FeSOD genes and one MnSOD gene (Table 1). Details of the gene designations, chromosomal position, CDS length, protein length, MW, pI, and subcellular localization of each SOD family member are given in Table 1. The watermelon SOD genes ranged from 411 to 888 bp in CDS length and encoded proteins ranged from 136 (CICSD5) to 295 (CIFSD2) aa in length. The MW of the watermelon SOD proteins ranged from 14.27 (ClFSD5) to 33.29 (ClFSD2) kDa, and the pI from 4.49 (CICSD5) to 8.72 (CIMSD). According to the subcellular localization, CICSD proteins were predicted to be in the chloroplast or cytoplasm. All CIFSD proteins were in the...
chloroplasts and ClMSD was located in the mitochondria (Table 1).

3.2 Phylogenetic characterization of SOD family genes in watermelon and other plant species

Insights into the evolutionary relationships of SOD family genes in watermelon with those in other plant species were gained by constructing a phylogenetic tree based on the alignment of SOD protein sequences from C. lanatus, Arabidopsis thaliana [30], Oryza sativa [31], Brachypodium distachyon [37], S. lycopersicum [13], Sorghum bicolor [38], C. sativus [15] and M. truncatula [9]. The phylogenetic results show that the SOD proteins could all be divided into five groups (Groups a–e) (Fig. 1). Among these groups, members from Groups a–c were Cu/ZnSODs while those from Groups d and e were MnSODs and FeSODs, respectively. As in other plant species the ClSODs were distributed in each group. ClCSD1, ClCSD2 and ClCSD3 were clustered in Group a, while ClCSD4 and ClCSD5 fell into Groups b and c, respectively. All SOD members in the three groups, a, b and c, were Cu/Zn-SOD proteins (Fig. 1). In addition, ClMSD was clustered together with other MnSOD proteins in Group d, whereas ClFSD1 and ClFSD2 together with other FeSOD proteins were placed in Group e (Fig. 1).

3.3 Characterization and conserved motif analysis of ClSOD proteins

Analysis of putative ClSOD protein sequences using the Pfam database predicted that all ClCSD proteins contained the Cu-ZnSOD domain (Sod_Cu, PF00080), whereas the Fe-MnSOD alpha-hairpin domain (Sod_Fe_N, PF00081) and Fe-MnSOD C-terminal domain (Sod_Fe_C, PF02777) were present in the ClMSD and ClFSD proteins (Fig. 2(a)).

Using MEME a total of 10 conserved motifs were identified and are shown in Fig. 2(b) and Table S3. Motifs 1, 2 and 4, which were widely present in ClCSD proteins, were annotated as the Cu-ZnSOD domain (Table S3). However, ClCSD5 was lacking in motifs 2 and 4. In addition, motifs 3, 5, 6, 8 and 9 were conserved domains of Fe-SODs and Mn-SODs. Motif 10 was found only in ClCSD5 and ClFSD1 (Fig. 2(b)).

The conserved SOD signatures and residues of ClSOD proteins were further analyzed. The multiple sequence alignment results show that two conserved Cu/ZnSOD signatures (GFH[VLI][AES][LY]GDTT and GNAG[EGA]R[ILV][CAG]CG) were present in nearly all ClCSDs with the exception of ClCSD5 (Fig. 3). ClFSD1 and ClFSD2 contained the conserved FeSOD signature (AQ[VI]WNHDF[FL]WES) and metal binding domain (D[MV]WEHAYY) whereas ClMSD had only the metal binding domain. In addition, nearly all ClSODs possessed conserved residues, especially His, which is necessary for metal binding (Fig. 3).

3.4 Gene structure analysis of ClSOD genes

The structural features of the ClSOD genes were determined by comparing their CDS and gDNA sequences. This comparison shows that ClSOD genes had different intron numbers ranging from one (ClCSD5) to seven (ClFSD1) (Fig. 4), with most genes containing five or six introns. For example, ClCSD4 and ClMSD possessed five introns, and ClCSD1, ClCSD2, ClCSD3 and ClFSD2 had six introns each (Fig. 4).

3.5 Chromosomal distribution and duplication analysis of watermelon SOD genes

The eight ClSOD genes were differently and unevenly distributed
Fig. 1  Phylogenetic analysis of SOD proteins in watermelon and other plant species. The phylogenetic tree was constructed using Clustal Omega and MEGA 7.0 by the neighbor-joining method with 1000 bootstrap repeats. The protein sequences used for creation of the phylogenetic tree are listed in Table S2. Cl, Citrullus lanatus; At, Arabidopsis thaliana; Sl, Solanum lycopersicum; Cs, Cucumis sativus; Mt, Medicago truncatula; Sb, Sorghum bicolor; Bd, Brachypodium distachyon.

Fig. 2  (a) Phylogenetic relationships, (b) structures and (c) conserved motifs of watermelon SOD proteins. Multiple alignments were conducted with Clustal Omega using full-length watermelon SOD protein sequences, and the phylogenetic tree was created with MEGA 7.0 using the NJ method with bootstrap tests repeated 1000 times. The colored boxes indicate different conserved motifs, and their positions in each watermelon SOD protein sequence are displayed proportionally.
on five chromosomes in the watermelon genome. Chromosome 3 had the most genes (ClMSD, ClFSD1, and ClCSD3); chromosome 2 had two genes; and chromosomes 4, 7 and 10 each had one gene (Fig. 5). No tandem duplications were found in these genes. However, a pair of ClSOD genes, ClCSD1 and ClCSD3, were found as segmental duplication genes (Fig. 5).

3.6 Analysis of cis-elements in ClSOD genes

The potential functions of ClSOD genes were clarified by extracting a 1-kb genomic sequence upstream of the translation start code (ATG) in each ClSOD gene and analyzing using the PlantCARE database. A total of six types of stress-responsive cis-elements were found, and the promoters of seven ClSOD genes were found to possess one or more AREs (anaerobic induction elements), which comprised the most cis-elements (Fig. 6). In addition, five other stress-responsive cis-elements were found in the promoters of several ClSOD genes such as LTR, MBS, W-box, WUN-motif and TC-rich repeats, suggesting that ClSOD genes are associated with the stress response (Fig. 6). Seven types of phytohormone-responsive cis-elements were also found in the ClSOD gene promoters including ABRE, ERE, CGTCA-motif, TCA-element, TGA-element, P-box and TATC-box, which are related to ABA, ethylene, methyl jasmonate, salicylic acid, auxin and gibberellin responses, respectively. It was noted that ClFSD2 had the largest number of ERE elements in its promoter region, indicating that this gene may have a primary function in the ethylene response.
3.7 Expression patterns of ClSOD genes in watermelon plant parts

The possible functions of ClSOD genes were analyzed by examining the expression patterns of selected ClSOD genes in five major plant parts (roots, stems, leaves, flowers and fruits) using qRT-PCR. ClCSD2 and ClCSD4 had higher expression in leaves than in other parts, and a relatively higher transcript level of ClCSD5 was also found in leaves (Fig. 7(a)). In addition, ClCSD1, ClCSD3 and ClCSD5 were mostly (and at the highest level of expression) in fruits (Fig. 7(a)), suggesting that these
genes may be involved in fruit development. The expression patterns of the ClSOD genes during fruit development were also examined based on the RNA-seq data, but no ClSOD genes had observably upregulated expression during fruit development (Fig. 7(b); Table S4). ClCSD4 and ClMSD expression was significantly lower at 26 and 34 DAP in the flesh; ClCSD1 expression clearly decreased at 26 and 34 DAP in the rind and substantially lower at the later stages of both flesh and rind development (Fig. 7(b)). These results indicate possible functions of these genes in watermelon fruit development.

### 3.8 Expression pattern analysis of ClSOD genes in response to different abiotic stresses

The potential roles of ClSOD genes in response to various abiotic stresses were examined by observing the expression of ClSOD genes under salinity, drought and ABA treatments using qRT-PCR. Nearly all ClSOD genes were significantly upregulated or downregulated under the salinity treatment with the exception of ClCSD5 (Fig. 8). Of these, five genes were significantly upregulated and the expression of ClFSD1, ClFSD2 and ClMSD reached a maximum after 1 h, whereas expression of ClCSD1 and ClCSD3 was highest after at 3 and 24 h, respectively. However, ClCSD2 and ClCSD4 were clearly downregulated in response to salinity stress (Fig. 8). Under the drought treatment the expression of ClCSD1, ClCSD3, ClCSD5 and ClMSD was significantly upregulated whereas that of ClCSD2, ClCSD4, ClFSD1 and ClFSD2 declined strongly at all elapsed times (Fig. 9). The expression of all ClSOD genes was induced by the ABA treatment and reached a peak after 24 h (Fig. 10). These results indicate that the ClSOD genes may participate in the responses to a number of different stresses.
Here, a comprehensive genome-wide identification of the SOD gene family in watermelon was conducted. Eight SOD genes comprising five Cu/ZnSOD genes, two FeSOD genes and one MnSOD gene were identified in the plant genome (Table 1), corresponding to three dominant plant SOD gene types. The number of SOD genes in each type was comparable to that in other plant species such as rice (5 Cu/ZnSODs, 2 FeSODs and 1 MnSOD)\textsuperscript{[31]}, cucumber (5 Cu/ZnSODs, 3 FeSODs and 1 MnSOD)\textsuperscript{[15]}, and M. truncatula (4 Cu/ZnSODs, 3 FeSODs and 1 MnSOD)\textsuperscript{[9]}, and tea (7 Cu/ZnSODs, 2 FeSODs and 1 MnSOD)\textsuperscript{[19]}. In addition, phylogenetic analysis allows the SOD proteins to be classified into five groups consistent with their
metal cofactors (Fig. 1). Groups a-c and d-e constitute the Cu/ZnSODs and Fe-MnSODs, respectively, and were separated by a high bootstrap value (Fig. 1). Watermelon Cu/ZnSOD and Fe-MnSOD proteins had similar arrangement of conserved motifs such as Cu/ZnSOD and FeSOD signatures, and metal binding domains (Fig. 2; Fig. 3). In addition, the SODs that are evolutionarily clustered together always have the same subcellular localizations. For example, ClCSD1, ClCSD2 and ClCSD3, together with SlSOD1, MtSOD4, cCuZn-SOD2, CsCSD1 and CsCSD2, were all in Group a (Fig. 1; Table 1), and all were predicted to be cytoplasmic CSDs [9,13,15,31]. ClMSD was in Group d with other mitochondrial MnSODs such as MnSOD1 [31], SbSOD6 [38], SlsOD9 [13], CsMSD [15] and MtMSD [4]. Moreover, most ClSOD genes had five or six introns.
(Fig. 4), and this is consistent with the results of previous studies, for example on *B. distachyon* [37], sorghum [38], tomato [13] and wheat [20]. These SOD members in different plant species share common gene structure and domain architecture, demonstrating that plant SOD family genes have been fairly conserved during evolution.

Numerous studies show that the expression of plant SOD genes always has a certain degree of temporal and spatial specificity. For example, four *M. truncatula* genes (*MtCSD1, MtCSD2, MtCSD4* and *MtMSD*) had the highest expression in seeds [9]. Likewise, the expression of *PbrCSD2-3, PbrCSD5-6* and *PbrFSD1-3* was found to be maximum in seeds [17]. In addition, *MtCSD3* and *MtFSD1* had relatively high expression in leaves compare with other plant parts [9]. Here, all five *ClCSD* genes were highly expressed in leaves, especially *ClCSD2, ClCSD4* and
ClCSD5 (Fig. 7(a)), and similar results have been found in other plant species including tomato[13], cotton[6], and cucumber[15]. Leaves have a fundamental role in maintaining the life of plants through photosynthesis[14] and therefore these SOD genes may participate in scavenging the ROS generated from photosynthesis. In addition, ClCSD1, ClCSD3 and ClCSD5 have considerably higher mRNA contents in the fruits and flowers (Fig. 7(a)), common reproductive organs, and large amounts of ROS are also produced in these tissues[14]. It was noted that four genes (ClCSD1, ClCSD4, ClFSD1 and ClMSD) exhibited significant declines in transcripts during the development of flesh and/or rind (Fig. 7(b)), consistent with previous reports showing that SODs contribute to fruit ripening processes[17,39]. In summary, the ClSOD genes also have tissue-specific expression patterns, indicating their important functions in specific tissues and developmental stages of watermelon.

SOD is an efficient ROS scavenger that has a vital regulatory role in responses to different abiotic stresses in plants, and many SOD genes have been found to have differential expression patterns in response to abiotic stresses[6,15,16,20]. In the present study a number of hormone- and stress-responsive cis-elements were identified in the promoters of ClSOD genes (Fig. 6), indicating the importance of these genes in hormone and stress responses. Hence, we further examined their levels of expression under three stress treatments comprising drought, salinity and ABA. The qRT-PCR shows that under salinity and drought stress the expression of three ClSOD genes (ClCSD1, ClCSD3 and ClMSD) was significantly induced (Fig. 8; Fig. 9), suggesting that they contribute positively in response to these stresses. Generally, the gene expression and enzyme activities of SOD may be upregulated under stress conditions to enhance the tolerance of plants to different stresses. However, the expression of ClCSD2 and ClCSD4 was conspicuously downregulated under salinity and drought stress (Fig. 8; Fig. 9), indicating their negative functions in response to abiotic stresses. Downregulation of the SOD gene has also been reported in other plant species such as Gossypium hirsutum[14] and wheat[20]. In addition, the expression of ClFSD1 and ClFSD2 was significantly upregulated by salinity stress but downregulated by drought stress (Fig. 8; Fig. 9), suggesting their different roles in response to the two stresses, and watermelon may have developed diverse regulatory mechanisms to tolerate salinity and drought stress. ABA is important in plant response to abiotic stresses, and overexpression of some SOD genes can confer tolerance to diverse stresses via an ABA-dependent pathway such as GtCSD1[1], AtSOD or CmSOD[40]. Here, the expression of all ClSOD genes was affected by ABA treatment (Fig. 10), indicating that these genes are involved in the abiotic stress response, possibly via an ABA-dependent signaling pathway.

5 CONCLUSIONS

Here, the SOD gene family in watermelon was comprehensively analyzed. A total of eight SOD family genes were identified comprising five Cu/ZnSOD genes, two FeSOD genes and one MnSOD gene. Their phylogenetic relationships, conserved motifs and residues, gene structures, and stress- and hormone-responsive cis-elements in the promoter regions were examined. In addition, RNA-Seq data and qRT-PCR were used to examine the expression patterns of the ClSOD genes in different tissues and during fruit development, as well as under different abiotic stresses. Our findings provide a theoretical basis for further functional identification of the ClSOD genes in stress tolerance and fruit development in watermelon.

Supplementary materials
The online version of this article at https://doi.org/10.15302/J-FASE-2020350 contains supplementary materials (Tables S1–S4).

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Compliance with ethics guidelines
Yong Zhou, Linjuan Ouyang, Dahu Zhou, Yicong Cai, and Haohua He declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.
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