Genome-wide characterization and expression analysis of the 
\textit{Dof} gene family related to abiotic stress in watermelon

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The plant DNA-binding with one finger (Dof) gene family is a class of plant-specific transcription factors that play vital roles in many biological processes and response to stresses. In the present study, a total of 36 \textit{ClDof} genes were identified in the watermelon genome, which were unevenly distributed on 10 chromosomes. Phylogenetic analysis showed that the ClDof proteins could be divided into nine groups, and the members in a particular group had similar motif arrangement and exon-intron structure. We then analyzed the expression patterns of nine selected \textit{ClDof} genes in eight specific tissues by qRT-PCR, and the results showed that they have tissue-specific expression patterns. We also evaluated the expression levels of the nine selected \textit{ClDof} genes under salt stress and ABA treatments using qRT-PCR, and they showed differential expression under these treatments, suggesting their important roles in stress response. Taken together, our results provide a basis for future research on the biological functions of \textit{Dof} genes in watermelon.
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Abstract

The plant DNA-binding with one finger (Dof) gene family is a class of plant-specific transcription factors that play vital roles in many biological processes and response to stresses. In the present study, a total of 36 ClDof genes were identified in the watermelon genome, which were unevenly distributed on 10 chromosomes. Phylogenetic analysis showed that the ClDof proteins could be divided into nine groups, and the members in a particular group had similar motif arrangement and exon-intron structure. We then analyzed the expression patterns of nine selected ClDof genes in eight specific tissues by qRT-PCR, and the results showed that they have-tissue-specific expression patterns. We also evaluated the expression levels of the nine selected ClDof genes under salt stress and ABA treatments using qRT-PCR, and they showed differential expression under these treatments, suggesting their important roles in stress response. Taken together, our results provide a basis for future research on the biological functions of Dof genes in watermelon.

Keywords: Watermelon; Dof; Phylogenetic analysis; Expression profile; Abiotic stress
Introduction

DNA binding with one finger (Dof) proteins are a group of plant-specific transcription factors present widely from green unicellular algae to higher angiosperms, while they are not reported in other eukaryotes such as humans and yeast (Azam et al., 2018; Gupta et al., 2015). Genome-wide surveys showed that the Dof family genes are widely distributed in the genomes of various plant species. For example, as model plants, Arabidopsis and rice include 36 and 30 Dof genes in their genomes, respectively (Lijavetzky et al., 2003). In addition, it has been reported that there are 25 Dof genes in physic nut (Jatropha curcas) (Wang et al., 2018; Zou & Zhang, 2019), 25 in peach (Prunus persica) (Chen et al., 2017), 29 in eggplant (Solanum melongena) (Wei et al., 2018), 33 in pepper (Capsicum annuum) (Kang et al., 2016; Wu et al., 2016), 34 in tomato (Solanum lycopersicum) (Cai et al., 2013), 35 in potato (Solanum tuberosum) (Venkatesh & Park, 2015), 36 in cucumber (Cucumis sativus) (Wen et al., 2016), 37 in chickpea (Cicer arietinum) (Nasim et al., 2016), 38 in pigeonpea (Cajanus cajan) (Malviya et al., 2015), 41 in poplar (Populus trichocarpa) (Wang et al., 2017), 45 in cassava (Manihot esculenta) (Zou et al., 2019), 45 in pear (Pyrus bretschneideri) (Liu et al., 2019b), 46 in rubber tree (Hevea brasiliensis) (Zou & Yang, 2019), and 60 in apple (Malus domestica) (Zhang et al., 2018). These reports revealed that the Dof proteins are characterized by the highly conserved Dof domain in their N-terminal regions, which is composed of about 52 amino acids with a Cys2/Cys2 zinc finger structure (Umemura et al., 2004; Yanagisawa, 2002). The Dof domain specifically recognizes and combines with a T/AAAAG core sequence in the promoters of target genes (Noguero et al., 2013; Umemura et al., 2004). In addition, the Dof proteins also contain a variable transcriptional activation domain at their C-terminus. The N- and C-terminal regions of the Dof proteins contribute to their bi-functional roles in DNA binding and protein-protein interactions to regulate the expression levels of the target
As the first identified Dof gene, ZmDof1 was found to play a role in light-regulated gene expression and affect light response and nitrogen assimilation (Yanagisawa & Izui, 1993; Yanagisawa & Sheen, 1998). Subsequently, a large number of Dof genes were reported to be involved in a variety of plant-specific biological processes, such as seed germination (Boccaccini et al., 2014; Gualberti et al., 2002; Santopolo et al., 2015), pollen development (Peng et al., 2017), endosperm development (Qi et al., 2017; Wu et al., 2019), fruit ripening (Feng et al., 2016), flowering time control (Li et al., 2009; Liu et al., 2019b; Wu et al., 2017), plant architecture (Wu et al., 2015; Zou et al., 2013), carbon and nitrogen metabolism (Kurai et al., 2011; Santos et al., 2012), and responses to plant hormones (Boccaccini et al., 2016; Lorrai et al., 2018; Qin et al., 2019; Rymen et al., 2017), as well as various stress responses (Su et al., 2017; Zang et al., 2017).

Moreover, some Dof genes can play multifaceted roles in the regulation of plant development and stress responses. For example, overexpression of Arabidopsis CDF3 contributed to higher tolerance of transgenic plants to drought, cold and osmotic stress and resulted in late flowering, suggesting that it is involved in both flowering time control and abiotic stress tolerance (Corrales et al., 2017). In tomato, overexpression of a Dof gene TDDF1 induced early flowering by increasing the expression of flowering-time control genes, and the transgenic plants also displayed higher resistance to drought, salt, and late blight caused by Phytophthora infestans (Ewas et al., 2017). In rice, salt stress repressed the expression of OsDOF15 in roots, and overexpression of OsDOF15 reduced the sensitivity of roots to salt stress via restricting ethylene biosynthesis, suggesting that OsDOF15-mediated ethylene biosynthesis plays a role in the inhibition of primary root elongation by salt stress (Qin et al., 2019). These findings demonstrate that the Dof proteins are involved in diverse biological processes and play important roles in the growth and
development of plants.

Although comprehensive analysis and functional characterization of the Dof gene family have been conducted in a number of plant species, little is known about this gene family in watermelon, an economically important fruit crop cultivated worldwide. In this study, we characterized the Dof family genes in watermelon by analysis of their phylogenetic relationships, conserved motifs, gene structures, and chromosomal localizations. In addition, the expression profiles of selected Dof genes in different tissues and under salt and ABA treatment conditions were also determined. Our findings provide a basis for future functional analysis of Dof genes in watermelon.

Materials and methods

Genome-wide identification and protein properties of Dof family in watermelon

To identify the watermelon Dof family genes, HHM profile of the Dof domain (PF02701) was used as a query to perform an HMMER search against the watermelon genome. A comprehensive search was also performed by using the amino acid sequences of Arabidopsis and rice Dof proteins from a previous study (Lijavetzky et al., 2003), which were obtained from the TIGR database (https://rice.plantbiology.msu.edu/) and the TAIR database (https://www.arabidopsis.org), respectively. The putative sequences were submitted to Pfam (http://pfam.sanger.ac.uk/) and SMART (http://smart.embl-heidelberg.de/) for checking the presence of the Dof domain. The biochemical features including molecular weight (MW) and isoelectric point (pI) of all Dof proteins were determined by ProtParam server (http://web.expasy.org/protparam/).

Sequence analyses and phylogenetic tree construction

The MEME tool (http://meme-suite.org/tools/meme) was used to predict and analyze the
conserved motifs of watermelon Dof proteins with the maximum number of motifs set as 10. The exon-intron structures of watermelon Dof genes were displayed by the GSDS tool (Gene Structure Display Server, http://gsds.cbi.pku.edu.cn) based on the alignment of coding region sequences (CDS) with the corresponding genomic DNA (gDNA) sequences. For phylogenetic tree construction, the Dof proteins of watermelon, cucumber, rice and Arabidopsis were aligned by Clustal Omega with default parameters. Then, the MEGA program (v7.0) was used to construct a Neighbor-Joining (NJ) tree with parameters of 1,000 bootstrap replicates and pairwise deletion.

Chromosomal location and duplication analysis

The chromosomal location information of watermelon Dof genes was obtained from the watermelon genome database, and MapChart was used to display the physical positions of all ClDof genes along each chromosome. Gene duplications were examined using multiple collinear scanning toolkits (MCScanX) software with the default parameters as previously reported (You et al., 2018).

Plant materials and treatments

Seeds of the watermelon cultivar “Xinong 8” (Citrullus lanatus L.) were first sterilized and germinated in an incubator (28 °C). Then, the germinated seeds were sown in pots and cultivated under a 12 h day/12 h night cycle (25 °C/19 °C, day/night temperature cycle) until the seedlings developed four leaves. Uniformly developed plants were then exposed to NaCl (200 mM) and ABA (100 µM) treatments for 0, 1, 3, 9, and 24 h. All leaves from watermelon plants were collected and rapidly frozen in liquid nitrogen and stored at –80°C until RNA extraction.
RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated using the total RNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer’s protocol. Then, RNase-free DNase I was added in RNA solution to remove any contaminated genomic DNA. First-strand cDNA synthesis was carried out following the manufacturer’s procedure (ReverTra Ace qPCR-RT Ki, Toyobo, Japan). Primers were designed using Primer Premier 5.0 software (Supplementary Table S1). The qRT-PCR was performed on an CFX96 instrument (Bio-Rad, Alfred Nobel Drive Hercules, CA, USA) using SYBR Green qPCR kits (Takara, Tokyo, Japan). The watermelon constitutive actin gene (Cla007792) was used as the endogenous control (Zhou et al., 2018b). The PCR amplification conditions included an initial heat-denaturing step at 95°C for 3 min, followed by 40 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C. Relative expression levels were calculated using the \(2^{-\Delta\Delta Ct}\) method (Livak & Schmittgen, 2001), and each treatment included three independent biological replicates and three technical replicates.

Results

Genome-wide identification of Dof family genes in watermelon

A total of 36 Dof genes were identified and named as CI_Dof1–36 according to their order on the chromosomes. Detailed information including the CDS length, protein length, predicted MW and pI regarding each gene is listed in Table 1. These genes had CDS lengths ranging from 492 bp (CI_Dof1) to 1575 bp (CI_Dof33), and encoded proteins ranging from 163 to 524 amino acid residues with the predicted MW varying from 17.64 to 56.71 kDa. The pI's of the CI_Dof proteins ranged from 5.00 (CI_Dof30) to 9.95 (CI_Dof13).
Phylogenetic characterization of the watermelon *Dof* gene family

To study the evolutionary relationship of *Dof* family genes between watermelon and other plants, a phylogenetic tree based on multiple sequence alignment was constructed by using the amino acid sequences of ClDofs together with those from cucumber (CsDofs) (*Wen et al.*, 2016), rice (OsDofs) and *Arabidopsis* (AtDofs) (*Lijavetzky et al.*, 2003). The phylogenetic tree showed that these Dof proteins could be classified into nine groups, namely A, B1, B2, C1, C2.1, C2.2, C3, D1, and D2, with well-supported bootstrap values (Fig. 1). Nearly all groups included ClDofs, CsDofs, OsDofs, and AtDofs, with the exception of group C3, which contained only dicotyledonous Dofs (ClDofs, CsDofs, and AtDofs). Besides, the numbers of ClDofs in groups A, B1, B2, C1, C2.1, C2.2, C3, D1, and D2 were 3, 7, 3, 3, 5, 2, 1, 8, and 4, respectively (Fig. 1).

Conserved motif analysis of ClDofs

By using the MEME program, a total of 10 conserved motifs were identified (Fig. 2). Amongst them, motif 1 was annotated as a Dof domain, which was widely present in all ClDof proteins, with the exception of ClDof4. Some other motifs were specifically present in individual groups. For example, motif 3, 4, 6, 7 and 10 were exclusively present in the ClDofs in group D1, while motif 2 was present in all ClDofs of group B1. Besides motif 2, nearly all group B1 ClDofs contained motif 9 (except for ClDof20). In addition, motif 8 was present in all group C1 ClDofs, as well as some ClDofs in group C2.1 and C2.2 (Fig. 2). It is worth noting that ClDof5 of group C2.1 possessed three motif 5 and one motif 9, implying that it may have a particular function.

To better understand the structural features of Dof domain, multiple sequence alignment of the Dof domain sequences of ClDofs was carried out. As a result, the Dof domain of ClDofs was highly conserved, and nearly all ClDof proteins harbored the four Cys residues associated with
zinc finger structure, with the exception of ClDof4 (Fig. 3), which may result in the divergence of its function from that of other ClDofs.

**Exon-intron arrangement analysis of Dof family genes in watermelon**

The CDS and gDNA sequences of the 36 ClDof genes were used to examine the distribution of exons and introns. As a result, most of the ClDof genes (20 out of 36) contained no introns, 11 ClDof genes (ClDof5, ClDof10, ClDof15, ClDof23, ClDof27, ClDof28, ClDof21, ClDof24, ClDof32, ClDof33, and ClDof34) had one intron each, whereas five ClDof genes (ClDof4, ClDof11, ClDof13, ClDof20, and ClDof36) possessed two introns.

**Chromosome distribution and gene duplication events of ClDof genes**

Using the MapInspect program, a total of 34 ClDof genes were mapped on 10 out of the 12 chromosomes in watermelon genome, while ClDof1 and ClDof2 were located on chromosome 0 (Fig. 5). In detail, there were 10, 2, 5, 2, 3, 3, 2, 2, 1 and 4 ClDof genes on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively. Moreover, the gene duplication events were analyzed through MCScanX program, and a total of 20 ClDof genes exhibited segmental duplication, which made up 21 pairs of segmental duplication genes (Fig. 5).

**Tissue-specific expression profiles of the ClDof genes**

To access the functions of ClDof genes in the growth and development of watermelon, the expression of nine selected ClDof genes in different tissues (mature and expanding leaves, roots, stems, stem apexes, flowers, and fruits) was examined with qRT-PCR. Most ClDof genes were highly expressed in flowers and/or fruits, such as ClDof11, ClDof21, ClDof27, ClDof29, ClDof35,
and ClDof36 (Fig. 6), suggesting that they may function in flower and fruit development of watermelon. In addition, ClDof2, ClDof5, ClDof8, ClDof21, and ClDof35 displayed the highest expression in leaves, and relatively lower expression in other tissues, especially roots, stems, and tendrils (Fig. 6). Besides expanding leaves, ClDof5 also showed relatively higher expression in fruits as compared with other tissues, while its expression was extremely low in flowers. Finally, nearly all ClDof genes exhibited moderate transcript abundance in stem apexes (Fig. 6), implying their possible roles in stem apex development of watermelon.

**Expression profiles of ClDof genes in response to salt stress and ABA treatment**

To reveal the possible roles of ClDof genes in response to abiotic stress, we investigated the expression levels of the nine selected ClDof genes under salt stress and ABA treatments using qRT-PCR. Under salt stress, five ClDof genes (ClDof5, ClDof8, ClDof29, ClDof35, and ClDof36) were up-regulated at certain time points (Fig. 7). Amongst them, ClDof5 was induced gradually and reached the highest transcript abundance at 24 h, while the expression of ClDof36 showed a decrease at early time point (1 h) and then gradually increased until 24 h (Fig. 7). In addition, three ClDof genes (ClDof2, ClDof11 and ClDof21) were down-regulated across all time points under salt stress, indicating their negative roles in response to salt stress. It should be noted that the expression levels of ClDof8, ClDof27 and ClDof35 were significantly decreased at some time points (Fig. 7). We also determined whether these ClDof genes are regulated by ABA. As shown in Fig. 8, the expression of all detected ClDof genes was significantly altered by ABA treatment, and the expression of most ClDof genes showed a decreasing tendency at early time points (1 h and 3 h) and finally increased at the late time points (24 h). It is worth noting that the expression of ClDof5 was dramatically induced at 1 h, followed by sharp decreases subsequently. These
results indicated that the ClDof genes may play crucial roles in stress responses.

Discussion

In the present study, we systematically predicted and identified 36 Dof genes in the watermelon genome (Table 1). The number of ClDof genes is similar to that in many other plant species, such as pepper (33 genes) (Kang et al., 2016; Wu et al., 2016), tomato (34 genes) (Cai et al., 2013), potato (35 genes) (Venkatesh & Park, 2015), foxtail millet (35 genes) (Zhang et al., 2017), cucumber (36 genes) (Wen et al., 2016), 37 in chickpea (37 genes) (Nasim et al., 2016), and pigeonpea (38 genes) (Malviya et al., 2015), suggesting that Dof genes usually form multigene families in plants. Duplication events were found to be the primary driving force for the evolution of Dof genes. For example, two pairs of tandemly duplicated genes and six pairs of segmentally duplicated genes were identified in the cucumber genome (Wen et al., 2016). In poplar, up to 49% (20 out of 41) of PtrDof genes were found to be located in both segmental and tandem duplicated regions (Wang et al., 2017). In apple, a total of 57 and 18 MdDof genes were located in segmental and tandem duplicated regions, respectively, and 13 MdDof genes were both segmentally and tandemly duplicated genes (Hong et al., 2019). In this study, more than half of the ClDof genes (20 out of 36) exhibited segmental duplications, while no tandem duplication was identified in the watermelon chromosomes, suggesting that segmental duplication has been predominant in the expansion of the Dof genes in watermelon. Similar results have also been reported in other plants such as cotton (Li et al., 2018).

The phylogenetic results revealed that ClDofs could be clearly divided into nine groups: A, B1, B2, C1, C2.1, C2.2, C3, D1 and D2 (Fig. 1), which is consistent with the results in eggplant (Wei et al., 2018), pear (Liu et al., 2019b), Arabidopsis and rice (Lijavetzky et al., 2003). Besides, each
of the watermelon *Dof* gene has at least one homologous gene in *Arabidopsis* (Fig. 1), implying that *Dof* genes might play similar roles in watermelon as their homologues in *Arabidopsis*. In addition, nearly all ClDofs had a common Dof motif (motif 1), but there were also some unique motifs in certain groups with nearly conserved motif compositions (Fig. 2). However, gain or loss of certain motifs was observed between several duplicate pairs, such as ClDof3/ClDof23, ClDof13/ClDof15, ClDof14/ClDof16, ClDof13/ClDof20, and ClDof20/ClDof36 (Figs. 2 and 5), suggesting that these motifs might be involved in the functional divergence of ClDofs. The organization of exon-intron structures can provide insights into the evolutionary relationships within certain gene families (Zhou et al., 2018a). In this study, the number of introns of ClDof genes varied from 0 to a maximum of 2, and most of them contained one intron or no intron at all (Fig. 4). Similar results were obtained in many other plant species, such as cucumber (Wen et al., 2016), poplar (Wang et al., 2017), eggplant (Wei et al., 2018), pear (Liu et al., 2019b), *Arabidopsis* and rice (Lijavetzky et al., 2003), revealing that the exon-intron structure of *Dof* genes is highly conserved in plants, which may be related to their similar functions.

The specificity of gene expression in plant tissues and developmental stages can provide important information about the possible functions of genes, and previous reports have revealed that some *Dof* genes usually have tissue-specific expression patterns (Ma et al., 2015; Venkatesh & Park, 2015; Zou & Yang, 2019). For example, ZmDof3 was found to be exclusively expressed in the endosperm of maize kernel and participate in the regulation of starch accumulation and aleurone development in maize endosperm (Qi et al., 2017). Another maize *Dof* gene ZmDof36 was also reported to be highly expressed in maize endosperm and function in starch synthesis by regulating the expression of starch synthesis genes (Wu et al., 2019). In this study, ClDof2, ClDof5, ClDof8, ClDof21, and ClDof35 showed much higher expression in leaves than in other tissues,
suggesting that they play essential roles in leaf development. Similarly, seven potato Dof genes 
(StDof15a, StDof22, StDof26, StDof29a, StDof32, and StDof34) exhibited higher expression in leaf 
tissues than in other tissues (Venkatesh & Park, 2015). In addition, ClDof11, ClDof27, ClDof29, 
and ClDof36 were predominantly expressed in fruits (Fig. 6), suggesting that they may be 
associated with fruit development of watermelon. In a previous report, a number of MaDof genes 
were markedly regulated throughout the fruit development in banana (Dong et al., 2016), and 
MaDof23 can act as a transcriptional repressor and interact with MaERF9 to regulate the fruit 
ripening by controlling specific ripening-related genes (Feng et al., 2016). Besides fruits, ClDof11, 
ClDof21, ClDof27, ClDof29, ClDof35, and ClDof36 also showed high expression in flowers, 
which was also observed in other plants. For example, all PheDof genes displayed differential 
expression patterns during the flower development stage of moso bamboo (Cheng et al., 2018; 
Wang et al., 2016), and overexpression of PheDof12-1 in Arabidopsis resulted in early flowering 
under long-day conditions (Liu et al., 2019a). In rubber tree, the HbDof genes in Cluster III and 
Cluster VI are typically expressed in male and female flowers, respectively (Zou & Yang, 2019). 
The tissue-specific expression patterns revealed that ClDof genes play vital and seemingly 
redundant roles in watermelon growth and development.

Dof genes are known to play a crucial role in stress responses. For example, tomato SlCDF1–5 
genes were differentially up-regulated by osmotic, salt, heat, and cold stresses, and transgenic 
Arabidopsis plants overexpressing SlCDF1 or SlCDF3 displayed higher drought and salt tolerance 
(Corrales et al., 2014). Another Dof gene SlDof22 was shown to control the ascorbate 
accumulation and salt stress in tomato (Cai et al., 2016). In this study, all of the detected ClDof 
genes showed differential expression under salt stress (Fig. 7), suggesting their regulatory roles in 
salt stress response. It should be noted that ClDof5 was induced gradually by salt stress (Fig. 7),
and its expression was the highest in leaves (Fig. 6). Similarly, \textit{GhDof1} also showed the highest expression in leaves as compared with any other tissues, and salt treatment induced its transcript accumulation. Overexpression of \textit{GhDof1} in cotton resulted in significantly higher salt and cold tolerance (\textit{Su et al.}, 2017). Moreover, all of the detected \textit{ClDof} genes exhibited an ABA-dependent expression pattern (Fig. 8). In castor bean, a large number of \textit{RcDof} genes were regulated (13 up-regulated and 2 down-regulated) in response to ABA treatment (\textit{Jin et al.}, 2014). In \textit{Arabidopsis}, the expression of \textit{AtCDF3} was induced by cold, drought, salt, and ABA treatment, and \textit{AtCDF3} overexpression could promote tolerance to drought, cold and osmotic stress (\textit{Corrales et al.}, 2017). These results indicate that the \textit{ClDof} genes may play important roles in plant adaptation to salt stress through ABA-dependent pathways.

### Conclusions

In this study, we performed a comprehensive analysis of the phylogenetic relationships, conserved motifs, gene structures, chromosome distributions, and gene duplications of 36 \textit{Dof} genes in watermelon. In addition, qRT-PCR was employed to examine the expression profiles of the \textit{ClDof} genes in different tissues and in responses to salt and ABA treatments. All of the detected \textit{ClDof} genes were regulated by salt and ABA treatments. Our findings may help the functional research of \textit{ClDof} genes for dissecting their roles in the growth, development and stress responses of watermelon.

### Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1. Phylogenetic relationships of Dof family proteins in watermelon, cucumber, rice, and *Arabidopsis*.

Fig. 2. Conserved domains of ClDofs based on the evolutionary relationship. Distribution of conserved motifs in the ClDof proteins.

Fig. 3. Dof domain sequence alignment of watermelon Dof proteins. The Dof DNA-binding domains among watermelon Dof proteins were aligned and the four Cys residues associated with zinc finger structure of the ClDofs are colored in red.

Fig. 4. Exon-intron structure of *ClDof* genes based on the evolutionary relationship.

Fig. 5. Chromosomal distribution of *ClDof* genes in watermelon genome. The segmental duplication genes are connected by lines.

Fig. 6. Expression profiles of nine selected *ClDof* genes in various tissues determined by qRT-PCR. ML, mature leaves; EL, expanding leaves; R, roots; S, stems; T, tendrils; F, flowers; Fr, fruits; SA, stem apexes.

Fig. 7. Expression profiles of nine selected *ClDof* genes in response to salt stress determined by qRT-PCR.

Fig. 8. Expression profiles of nine selected *ClDof* genes under ABA treatment determined by qRT-PCR.
Figure 1

Phylogenetic relationships of Dof family proteins in watermelon, cucumber, rice, and Arabidopsis.
**Figure 2** (on next page)

Figure 2

Conserved domains of ClDofs based on the evolutionary relationship. Distribution of conserved motifs in the ClDof proteins.
Figure 3

Dof domain sequence alignment of watermelon Dof proteins. The Dof DNA-binding domains among watermelon Dof proteins were aligned and the four Cys residues associated with zinc finger structure of the CI-Dofs are colored in red.
Figure 4

Exon-intron structure of ClDof genes based on the evolutionary relationship.
Figure 5

Chromosomal distribution of *ClDof* genes in watermelon genome. The segmental duplication genes are connected by lines.
**Figure 6** (on next page)

Expression profiles of nine selected *ClDof* genes in various tissues determined by qRT-PCR. ML, mature leaves; EL, expanding leaves; R, roots; S, stems; T, tendrils; F, flowers; Fr, fruits; SA, stem apexes.
Figure 7 (on next page)

Figure 7

Expression profiles of nine selected *CIDof* genes in response to salt stress determined by qRT-PCR.
Expression profiles of nine selected *CIDof* genes under ABA treatment determined by qRT-PCR.
Table 1 (on next page)

Table 1

Table 1. Members of *Dof* family genes identified in watermelon.
<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>Map Position (bp)</th>
<th>CDS length (bp)</th>
<th>Protein length (aa)</th>
<th>MW (kDa)</th>
<th>pI</th>
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<tbody>
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<td>ClDof1</td>
<td>Cla000091</td>
<td>Chr0:12921851-12922342</td>
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