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Genome-wide characterization and expression analysis of the *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 *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.

Genome-wide characterization and expression analysis of the *Dof* gene family related to abiotic stress 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

37 **Introduction**

38 DNA binding with one finger (Dof) proteins are a group of plant-specific transcription factors
39 present widely from green unicellular algae to higher angiosperms, while they are not reported in
40 other eukaryotes such as humans and yeast (*Azam et al.,* 2018; *Gupta et al.,* 2015). Genome-wide
41 surveys showed that the *Dof* family genes are widely distributed in the genomes of various plant
42 species. For example, as model plants, *Arabidopsis* and rice include 36 and 30 *Dof* genes in their
43 genomes, respectively (*Lijavetzky et al.,* 2003). In addition, it has been reported that there are 25
44 *Dof* genes in physic nut (*Jatropha curcas*) (*Wang et al.,* 2018; *Zou & Zhang,* 2019), 25 in peach
45 (*Prunus persica*) (*Chen et al.,* 2017), 29 in eggplant (*Solanum melongena*) (*Wei et al.,* 2018), 33
46 in pepper (*Capsicum annuum*) (*Kang et al.,* 2016; *Wu et al.,* 2016), 34 in tomato (*Solanum*
47 *lycopersicum*) (*Cai et al.,* 2013), 35 in potato (*Solanum tuberosum*) (*Venkatesh & Park,* 2015), 36
48 in cucumber (*Cucumis sativus*) (*Wen et al.,* 2016), 37 in chickpea (*Cicer arietinum*) (*Nasim et al.,*
49 2016), 38 in pigeonpea (*Cajanus cajan*) (*Malviya et al.,* 2015), 41 in poplar (*Populus trichocarpa*)
50 (*Wang et al.,* 2017), 45 in cassava (*Manihot esculenta*) (*Zou et al.,* 2019), 45 in pear (*Pyrus*
51 *bretschneideri*) (*Liu et al.,* 2019b), 46 in rubber tree (*Hevea brasiliensis*) (*Zou & Yang,* 2019), and
52 60 in apple (*Malus domestica*) (*Zhang et al.,* 2018). These reports revealed that the Dof proteins
53 are characterized by the highly conserved Dof domain in their N-terminal regions, which is
54 composed of about 52 amino acids with a Cys2/Cys2 zinc finger structure (*Umemura et al.,* 2004;
55 *Yanagisawa,* 2002). The Dof domain specifically recognizes and combines with a T/AAAAG core
56 sequence in the promoters of target genes (*Noguero et al.,* 2013; *Umemura et al.,* 2004). In
57 addition, the Dof proteins also contain a variable transcriptional activation domain at their C-
58 terminus. The N- and C-terminal regions of the Dof proteins contribute to their bi-functional roles
59 in DNA binding and protein-protein interactions to regulate the expression levels of the target

genes (*Gupta et al.,* 2015; *Noguero et al.,* 2013).

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

83 development of plants.

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

91

92 **Materials and methods**

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

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

103

104 **Sequence analyses and phylogenetic tree construction**

105 The MEME tool (http://meme-suite.org/tools/meme) was used to predict and analyze the

106 conserved motifs of watermelon Dof proteins with the maximum number of motifs set as 10. The
107 exon-intron structures of watermelon *Dof* genes were displayed by the GSDS tool (Gene Structure
108 Display Server, http://gsds.cbi.pku.edu.cn) based on the alignment of coding region sequences
109 (CDS) with the corresponding genomic DNA (gDNA) sequences. For phylogenetic tree
110 construction, the Dof proteins of watermelon, cucumber, rice and *Arabidopsis* were aligned by
111 Clustal Omega with default parameters. Then, the MEGA program (v7.0) was used to construct a
112 Neighbor-Joining (NJ) tree with parameters of 1,000 bootstrap replicates and pairwise deletion.
113

114 **Chromosomal location and duplication analysis**

115 The chromosomal location information of watermelon Dof genes was obtained from the
116 watermelon genome database, and MapChart was used to display the physical positions of all
117 *ClDof* genes along each chromosome. Gene duplications were examined using multiple collinear
118 scanning toolkits (MCScanX) software with the default parameters as previously reported (*You et*
119 *al.,* 2018).
120

121 **Plant materials and treatments**

122 Seeds of the watermelon cultivar "Xinong 8" (*Citrullus lanatus* L.) were first sterilized and
123 germinated in an incubator (28 °C). Then, the germinated seeds were sown in pots and cultivated
124 under a 12 h day/12 h night cycle (25 °C/19 °C, day/night temperature cycle) until the seedlings
125 developed four leaves. Uniformly developed plants were then exposed to NaCl (200 mM) and
126 ABA (100 μM) treatments for 0, 1, 3, 9, and 24 h. All leaves from watermelon plants were
127 collected and rapidly frozen in liquid nitrogen and stored at –80°C until RNA extraction.
128

129 **RNA extraction and quantitative real-time PCR (qRT-PCR)**

130 Total RNA was isolated using the total RNA Miniprep Kit (Axygen Biosciences, Union City,
131 CA, USA) according to the manufacturer's protocol. Then, RNase-free DNase I was added in RNA
132 solution to remove any contaminated genomic DNA. First-strand cDNA synthesis was carried out
133 following the manufacturer's procedure (ReverTra Ace qPCR-RT Ki, Toyobo, Japan). Primers
134 were designed using Primer Premier 5.0 software (Supplementary Table S1). The qRT-PCR was
135 performed on an CFX96 instrument (Bio-Rad, Alfred Nobel Drive Hercules, CA, USA) using
136 SYBR Green qPCR kits (Takara, Tokyo, Japan). The watermelon constitutive actin gene
137 (Cla007792) was used as the endogenous control (*Zhou et al.,* 2018b). The PCR amplification
138 conditions included an initial heat-denaturing step at 95°C for 3 min, followed by 40 cycles of 30
139 s at 95°C, 30 s at 58°C, and 1 min at 72°C. Relative expression levels were calculated using the 2^{-}
140 $^{\Delta\Delta Ct}$ method (*Livak & Schmittgen,* 2001), and each treatment included three independent biological
141 replicates and three technical replicates.

142

143 **Results**

144 **Genome-wide identification of *Dof* family genes in watermelon**

145 A total of 36 *Dof* genes were identified and named as *ClDof1–36* according to their order on the
146 chromosomes. Detailed information including the CDS length, protein length, predicted MW and
147 pI regarding each gene is listed in Table 1. These genes had CDS lengths ranging from 492 bp
148 (*ClDof1*) to 1575 bp (*ClDof33*), and encoded proteins ranging from 163 to 524 amino acid residues
149 with the predicted MW varying from 17.64 to 56.71 kDa. The pIs of the ClDof proteins ranged
150 from 5.00 (ClDof30) to 9.95 (ClDof13).

151

152 **Phylogenetic characterization of the watermelon *Dof* gene family**

153 To study the evolutionary relationship of *Dof* family genes between watermelon and other
154 plants, a phylogenetic tree based on multiple sequence alignment was constructed by using the
155 amino acid sequences of ClDofs together with those from cucumber (CsDofs) (*Wen et al.,* 2016),
156 rice (OsDofs) and *Arabidopsis* (AtDofs) (*Lijavetzky et al.,* 2003). The phylogenetic tree showed
157 that these Dof proteins could be classified into nine groups, namely A, B1, B2, C1, C2.1, C2.2,
158 C3, D1, and D2, with well-supported bootstrap values (Fig. 1). Nearly all groups included ClDofs,
159 CsDofs, OsDofs, and AtDofs, with the exception of group C3, which contained only
160 dicotyledonous Dofs (ClDofs, CsDofs, and AtDofs). Besides, the numbers of ClDofs in groups A,
161 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).

162

163 **Conserved motif analysis of ClDofs**

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

172 To better understand the structural features of Dof domain, multiple sequence alignment of the
173 Dof domain sequences of ClDofs was carried out. As a result, the Dof domain of ClDofs was
174 highly conserved, and nearly all ClDof proteins harbored the four Cys residues associated with

175 zinc finger structure, with the exception of ClDof4 (Fig. 3), which may result in the divergence of
176 its function from that of other ClDofs.
177

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

179 The CDS and gDNA sequences of the 36 *ClDof* genes were used to examine the distribution of
180 exons and introns. As a result, most of the *ClDof* genes (20 out of 36) contained no introns, 11
181 *ClDof* genes (*ClDof5*, *ClDof10*, *ClDof15*, *ClDof23*, *ClDof27*, *ClDof28*, *ClDof21*, *ClDof24*,
182 *ClDof32*, *ClDof33*, and *ClDof34*) had one intron each, whereas five *ClDof* genes (*ClDof4*,
183 *ClDof11*, *ClDof13*, *ClDof20*, and *ClDof36*) possessed two introns.
184

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

186 Using the MapInspect program, a total of 34 *ClDof* genes were mapped on 10 out of the 12
187 chromosomes in watermelon genome, while *ClDof1* and *ClDof2* were located on chromosome 0
188 (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,
189 5, 6, 7, 8, 9 and 10, respectively. Moreover, the gene duplication events were analyzed through
190 MCScanX program, and a total of 20 *ClDof* genes exhibited segmental duplication, which made
191 up 21 pairs of segmental duplication genes (Fig. 5).
192

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

194 To access the functions of *ClDof* genes in the growth and development of watermelon, the
195 expression of nine selected *ClDof* genes in different tissues (mature and expanding leaves, roots,
196 stems, stem apexes, flowers, and fruits) was examined with qRT-PCR. Most *ClDof* genes were
197 highly expressed in flowers and/or fruits, such as *ClDof11*, *ClDof21*, *ClDof27*, *ClDof29*, *ClDof35*,

198 and *ClDof36* (Fig. 6), suggesting that they may function in flower and fruit development of
199 watermelon. In addition, *ClDof2*, *ClDof5*, *ClDof8*, *ClDof21*, and *ClDof35* displayed the highest
200 expression in leaves, and relatively lower expression in other tissues, especially roots, stems, and
201 tendrils (Fig. 6). Besides expanding leaves, *ClDof5* also showed relatively higher expression in
202 fruits as compared with other tissues, while its expression was extremely low in flowers. Finally,
203 nearly all *ClDof* genes exhibited moderate transcript abundance in stem apexes (Fig. 6), implying
204 their possible roles in stem apex development of watermelon.
205

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

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

221 results indicated that the *ClDof* genes may play crucial roles in stress responses.

222

223 **Discussion**

224 In the present study, we systematically predicted and identified 36 *Dof* genes in the watermelon
225 genome (Table 1). The number of *ClDof* genes is similar to that in many other plant species, such
226 as pepper (33 genes) (*Kang et al.,* 2016; *Wu et al.,* 2016), tomato (34 genes) (*Cai et al.,* 2013),
227 potato (35 genes) (*Venkatesh & Park,* 2015), foxtail millet (35 genes) (*Zhang et al.,* 2017),
228 cucumber (36 genes) (*Wen et al.,* 2016), 37 in chickpea (37 genes) (*Nasim et al.,* 2016), and
229 pigeonpea (38 genes) (*Malviya et al.,* 2015), suggesting that *Dof* genes usually form multigene
230 families in plants. Duplication events were found to be the primary driving force for the evolution
231 of *Dof* genes. For example, two pairs of tandemly duplicated genes and six pairs of segmentally
232 duplicated genes were identified in the cucumber genome (*Wen et al.,* 2016). In poplar, up to 49%
233 (20 out of 41) of *PtrDof* genes were found to be located in both segmental and tandem duplicated
234 regions (*Wang et al.,* 2017). In apple, a total of 57 and 18 *MdDof* genes were located in segmental
235 and tandem duplicated regions, respectively, and 13 *MdDof* genes were both segmentally and
236 tandemly duplicated genes (*Hong et al.,* 2019). In this study, more than half of the *ClDof* genes
237 (20 out of 36) exhibited segmental duplications, while no tandem duplication was identified in the
238 watermelon chromosomes, suggesting that segmental duplication has been predominant in the
239 expansion of the *Dof* genes in watermelon. Similar results have also been reported in other plants
240 such as cotton (*Li et al.,* 2018).

241 The phylogenetic results revealed that ClDofs could be clearly divided into nine groups: A, B1,
242 B2, C1, C2.1, C2.2, C3, D1 and D2 (Fig. 1), which is consistent with the results in eggplant (*Wei*
243 *et al.,* 2018), pear (*Liu et al.,* 2019b), *Arabidopsis* and rice (*Lijavetzky et al.,* 2003). Besides, each

244 of the watermelon *Dof* gene has at least one homologous gene in *Arabidopsis* (Fig. 1), implying
245 that *Dof* genes might play similar roles in watermelon as their homologues in *Arabidopsis*. In
246 addition, nearly all ClDofs had a common Dof motif (motif 1), but there were also some unique
247 motifs in certain groups with nearly conserved motif compositions (Fig. 2). However, gain or loss
248 of certain motifs was observed between several duplicate pairs, such as ClDof3/ClDof23,
249 ClDof13/ClDof15, ClDof14/ClDof16, ClDof13/ClDof20, and ClDof20/ClDof36 (Figs. 2 and 5),
250 suggesting that these motifs might be involved in the functional divergence of ClDofs. The
251 organization of exon-intron structures can provide insights into the evolutionary relationships
252 within certain gene families (*Zhou et al.,* 2018a). In this study, the number of introns of *ClDof*
253 genes varied from 0 to a maximum of 2, and most of them contained one intron or no intron at all
254 (Fig. 4). Similar results were obtained in many other plant species, such as cucumber (*Wen et al.,*
255 2016), poplar (*Wang et al.,* 2017), eggplant (*Wei et al.,* 2018), pear (*Liu et al.,* 2019b), *Arabidopsis*
256 and rice (*Lijavetzky et al.,* 2003), revealing that the exon-intron structure of *Dof* genes is highly
257 conserved in plants, which may be related to their similar functions.

258 The specificity of gene expression in plant tissues and developmental stages can provide
259 important information about the possible functions of genes, and previous reports have revealed
260 that some *Dof* genes usually have tissue-specific expression patterns (*Ma et al.,* 2015; *Venkatesh*
261 *& Park,* 2015; *Zou & Yang,* 2019). For example, *ZmDof3* was found to be exclusively expressed
262 in the endosperm of maize kernel and participate in the regulation of starch accumulation and
263 aleurone development in maize endosperm (*Qi et al.,* 2017). Another maize *Dof* gene *ZmDof36*
264 was also reported to be highly expressed in maize endosperm and function in starch synthesis by
265 regulating the expression of starch synthesis genes (*Wu et al.,* 2019). In this study, *ClDof2*, *ClDof5*,
266 *ClDof8*, *ClDof21*, and *ClDof35* showed much higher expression in leaves than in other tissues,

267 suggesting that they play essential roles in leaf development. Similarly, seven potato *Dof* genes
268 (*StDof15a*, *StDof22*, *StDof26*, *StDof29a*, *StDof32*, and *StDof34*) exhibited higher expression in leaf
269 tissues than in other tissues (*Venkatesh & Park,* 2015). In addition, *ClDof11*, *ClDof27*, *ClDof29*,
270 and *ClDof36* were predominantly expressed in fruits (Fig. 6), suggesting that they may be
271 associated with fruit development of watermelon. In a previous report, a number of *MaDof* genes
272 were markedly regulated throughout the fruit development in banana (*Dong et al.,* 2016), and
273 MaDof23 can act as a transcriptional repressor and interact with MaERF9 to regulate the fruit
274 ripening by controlling specific ripening-related genes (*Feng et al.,* 2016). Besides fruits, *ClDof11*,
275 *ClDof21*, *ClDof27*, *ClDof29*, *ClDof35*, and *ClDof36* also showed high expression in flowers,
276 which was also observed in other plants. For example, all *PheDof* genes displayed differential
277 expression patterns during the flower development stage of moso bamboo (*Cheng et al.,* 2018;
278 *Wang et al.,* 2016), and overexpression of *PheDof12-1* in *Arabidopsis* resulted in early flowering
279 under long-day conditions (*Liu et al.,* 2019a). In rubber tree, the *HbDof* genes in Cluster III and
280 Cluster VI are typically expressed in male and female flowers, respectively (*Zou & Yang,* 2019).
281 The tissue-specific expression patterns revealed that *ClDof* genes play vital and seemingly
282 redundant roles in watermelon growth and development.

283 *Dof* genes are known to play a crucial role in stress responses. For example, tomato *SlCDF1–5*
284 genes were differentially up-regulated by osmotic, salt, heat, and cold stresses, and transgenic
285 *Arabidopsis* plants overexpressing *SlCDF1* or *SlCDF3* displayed higher drought and salt tolerance
286 (*Corrales et al.,* 2014). Another *Dof* gene *SlDof22* was shown to control the ascorbate
287 accumulation and salt stress in tomato (*Cai et al.,* 2016). In this study, all of the detected *ClDof*
288 genes showed differential expression under salt stress (Fig. 7), suggesting their regulatory roles in
289 salt stress response. It should be noted that *ClDof5* was induced gradually by salt stress (Fig. 7),

290 and its expression was the highest in leaves (Fig. 6). Similarly, *GhDof1* also showed the highest
291 expression in leaves as compared with any other tissues, and salt treatment induced its transcript
292 accumulation. Overexpression of *GhDof1* in cotton resulted in significantly higher salt and cold
293 tolerance (*Su et al.,* 2017). Moreover, all of the detected *ClDof* genes exhibited an ABA-dependent
294 expression pattern (Fig. 8). In castor bean, a large number of RcDof genes were regulated (13 up-
295 regulated and 2 down-regulated) in response to ABA treatment (*Jin et al.,* 2014). In *Arabidopsis*,
296 the expression of *AtCDF3* was induced by cold, drought, salt, and ABA treatment, and *AtCDF3*
297 overexpression could promote tolerance to drought, cold and osmotic stress (*Corrales et al.,* 2017).
298 These results indicate that the *ClDof* genes may play important roles in plant adaptation to salt
299 stress through ABA-dependent pathways.
300

301 **Conclusions**

302 In this study, we performed a comprehensive analysis of the phylogenetic relationships,
303 conserved motifs, gene structures, chromosome distributions, and gene duplications of 36 *Dof*
304 genes in watermelon. In addition, qRT-PCR was employed to examine the expression profiles of
305 the *ClDof* genes in different tissues and in responses to salt and ABA treatments. All of the detected
306 *ClDof* genes were regulated by salt and ABA treatments. Our findings may help the functional
307 research of *ClDof* genes for dissecting their roles in the growth, development and stress responses
308 of watermelon.
309

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Conflict of interest

The authors declare that they have no conflict of interest.

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501 **Figure legends**

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

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

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

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

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

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

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

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

519

Figure 1(on next page)

Figure 1

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

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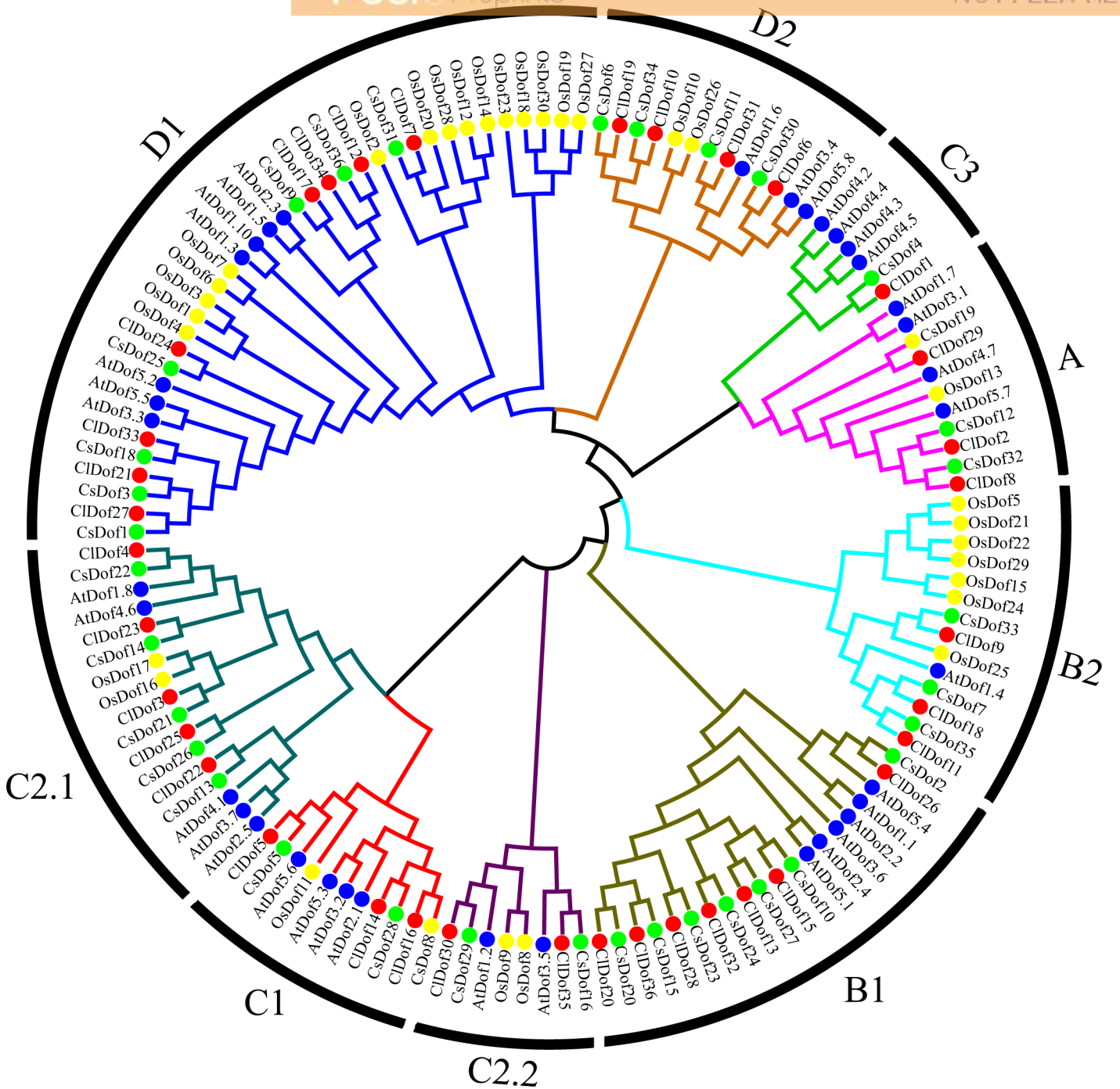

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Figure 2(on next page)

Figure 2

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

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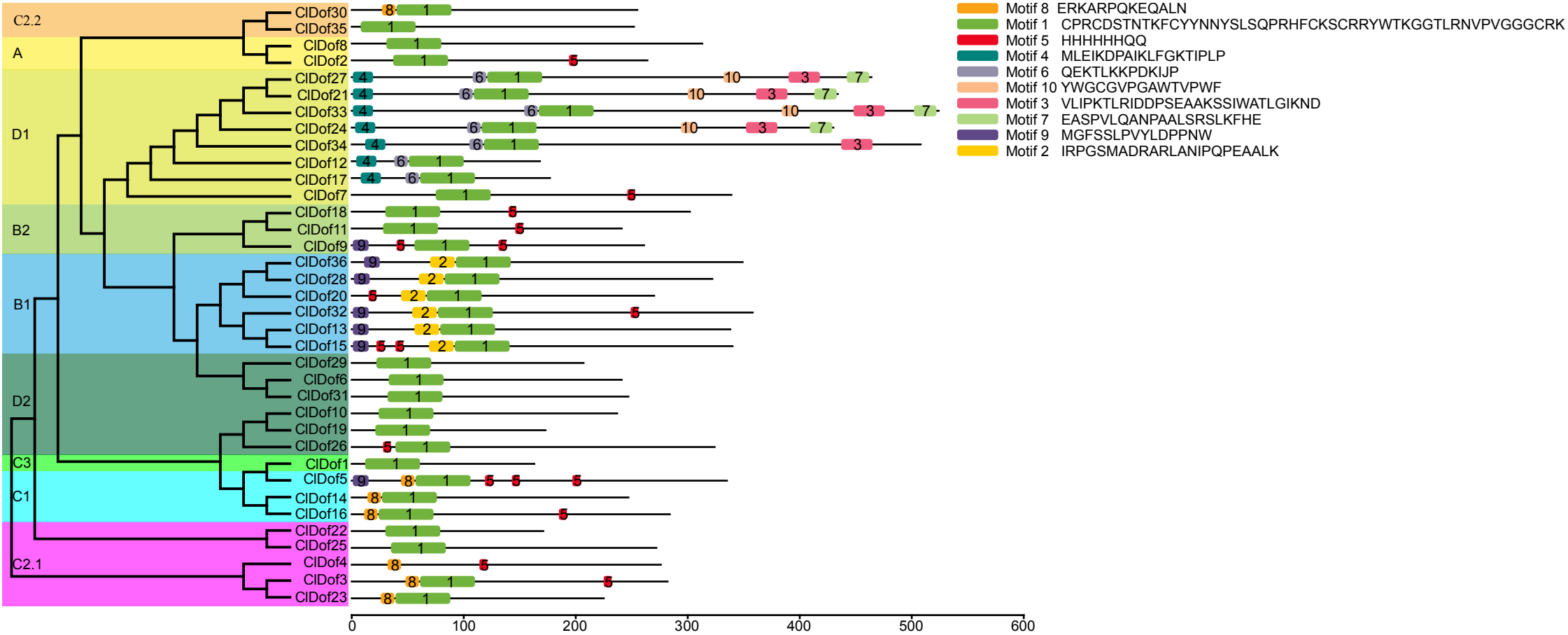

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Figure 3

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 ClDofs are colored in red.

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                        Zn
ClDof1    --AFKCPRCHSLHTKFCYYNNYNYSQPRHLCKTCCRYWTLGGLLCNVSIGGGTHRSKK
ClDof2    ---LKCPRCDSTNTKFCYYNNYSLTQPRHFCKTCRRYWTKGGALRNVPIGGGCRKTKK
ClDof3    -QALNCPRCNSTNTKFCYYNNYSLSQPRYFCKACRRYWTEGGSLRNVPVGGGSRKNKR
ClDof4    -----CP----------------------------RRYWTEGGSLRNIPVGGGSRKNKR
ClDof5    DQALKCPRCDSTHTKFCYYNNYSLSQPRYFCKTCRRYWTKGGTLRNIPVGGGCRKNKK
ClDof6    -EHLPCPRCDSTNTKFCYYNNYNFSQPRHFCKSCRRYWTHGGTLRDIPVGGGSRKNAK
ClDof7    -QNLRCPRCDSSNTKFCYYNNYNLTQPRHFCKTCRRYWTKGGALRNVPIGGGCRKNKS
ClDof8    -QALKCPRCDSPNTKFCYYNNYSLTQPRYFCKTCRRYWTKGGALRNVPVGGGCRKNKK
ClDof9    --QLKCPRCDSTNTKFCYYNNYNKSQPRHFCRACKRHWTKGGTLRNVPVGGG-RKNK-
ClDof10   --PQKCPRCDSLNTKFCYYNNYSLSQPRYLCKACRRYWTQGGILRNVPVGGGCRKGKR
ClDof11   --TLKCPRCDSSNTKFCYYNNYSLSQPRHFCKACKRYWTRGGTLRNVPVGGGCRKNKR
ClDof12   -KIIPCPRCKSMDTKFCYFNNYNVNQPRHFCKGCQRYWTAGGALRNVPIGAGRRRTK-
ClDof13   --ALKCPRCDSTNTKFCYFNNYSLSQPRHFCKACRRYWTRGGALRNVPVGGGFRKNKK
ClDof14   -QALRCPRCDSTNTKFCYYNNYSLSQPRYFCKSCRRYWTKGGTLRNVPVGGGCRKNKR
ClDof15   --ALKCPRCDSTNTKFCYFNNYSLSQPRHFCKSCRRYWTRGGALRNVPVGGGCRRNKK
ClDof16   -QALKCPRCDSTNTKFCYYNNYSLSQPRYFCKSCRRYWTQGGTLRNVPVGGGCRKNKR
ClDof17   -KIIPCPRCKSMDTKFCYFNNYNVNQPRHFCKGCQRYWTAGGALRNVPVGAGRRKTK-
ClDof18   -EQLRCPRCDSSNTKFCYYNNYSLSQPRHFCKACKRYWTRGGTLRNVPVGGGCRKNKR
ClDof19   -QKQKCPRCESSNTKFCYYNNYSLSQPRYFCKSCRRYWTHGGTLRNVPIGGGSRKSKR
ClDof20   --ALKCPRCESTNTKFCYFNNYSLSQPRHFCKTCRRYWTRGGALRSVPVGGGYRRNTK
ClDof21   -KLLPCPRCKSMETKFCYYNNYNVNQPRHFCKACQRYWTAGGTMRNVPVGAGRRKSKN
ClDof22   -EQLNCPRCKSSNTKFCYYNNYSLTQPRYFCKSCRRYWTEGGSLRNIPVGGGSRKNRK
ClDof23   DQILNCPRCNSNNTKFCYYNNYSLSQPRYFCKSCRRYWTAGGSLRNIPVGGASRKNKR
ClDof24   -KILPCPRCNSMDTKFCYYNNYNVNQPRHFCKNCQRYWTAGGTMRNVPVGAGRRKNKS
ClDof25   --QLNCPRCKSNNTKFCYYNNYSLTQPRYFCKSCRRYWTEGGSLRNVPIGGGSRKNRS
ClDof26   -QALKCPRCDSLNTKFCYYNNYNLSQPRHFCKSCRRYWTKGGVLRNVPVGGGCRKTKR
ClDof27   -KILPCPRCNSMETKFCYYNNYNVNQPRHFCKACQRYWTEGGTMRNVPVGAGRRKNKN
ClDof28   --GLKCPRCESTNTKFCYFNNYNLSQPRHFCKTCRRYWTRGGALRNVPVGGGCRRNKR
ClDof29   -EQLKCPRCDSTNTKFCYYNNYNLSQPRHFCKNCRRYWTKGGALRNIPVGGGTRKNSK
ClDof30   --APNCPRCASANTKFCYYNNYSLSQPRYFCKSCRRYWTKGGSLRNVPVGGGCRKSRR
ClDof31   --PLPCPRCDSLNTKFCYYNNYNLSQPRHFCKSCRRYWTHGGTLRDVPVGGGSRKNSK
ClDof32   --GLKCPRCESTNTKFCYFNNYSLTQPRHFCKTCRRYWTRGGAMRNVPVGGGCRRSSK
ClDof33   -KILPCPRCNSMDTKFCYYNNYNVNQPRHFCKNCQRYWTAGGTMRNVPVGAGRRKNKS
ClDof34   -KIIPCPRCNSLETKFCYFNNYNVNQPRHFCKNCQRYWTAGGTMRNVPIGAGRRRNKQ
ClDof35   ---PNCPRCGSSNTKFCYYNNYSLTQPRYFCKGCRRYWTKGGSLRNVPVGGGCRKNRR
ClDof36   --ALKCPRCESTNTKFCYFNNYSLTQPRHFCKTCRRYWTRGGALRNVPVGGGCRRNKR
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Figure 4

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

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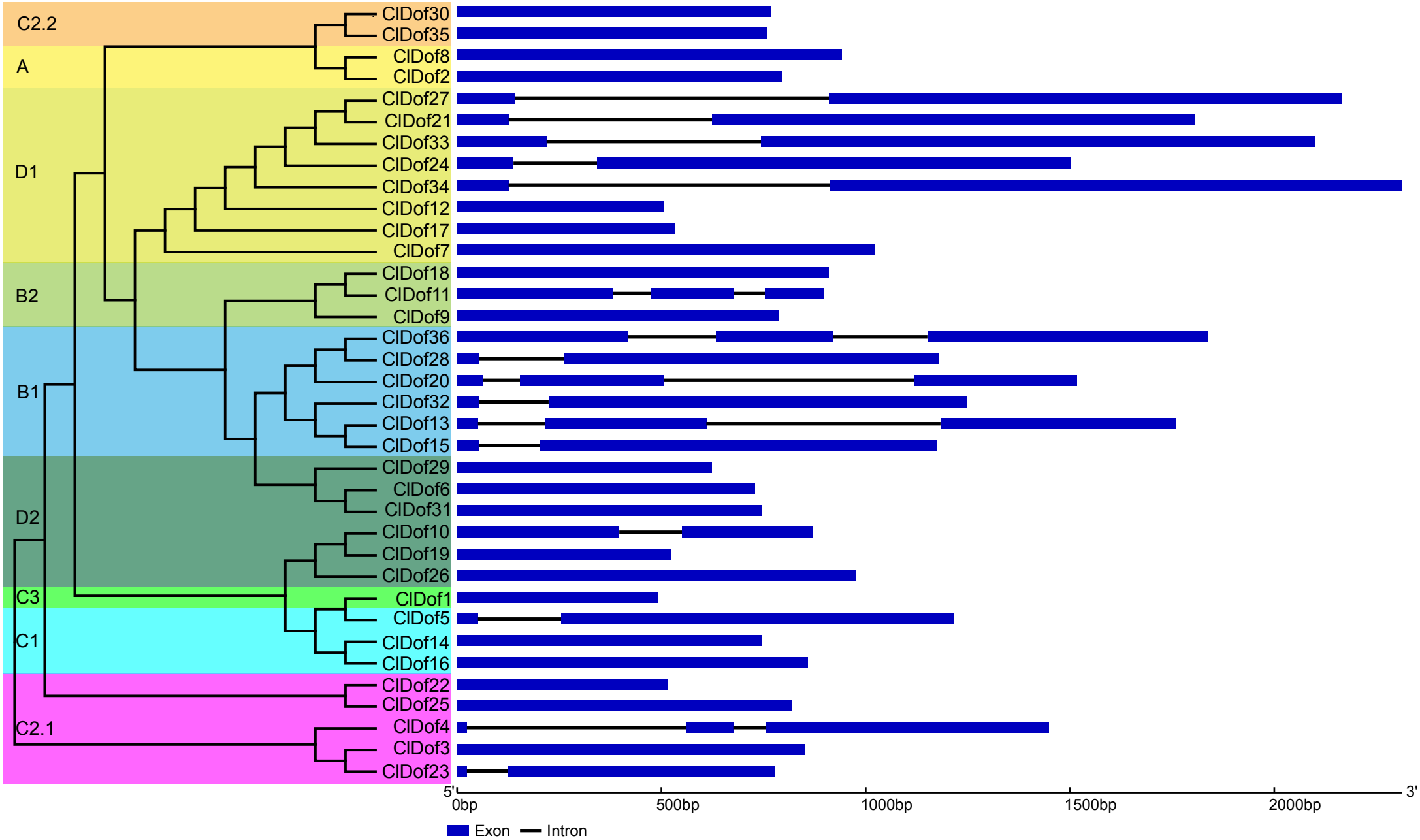

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Figure 5

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

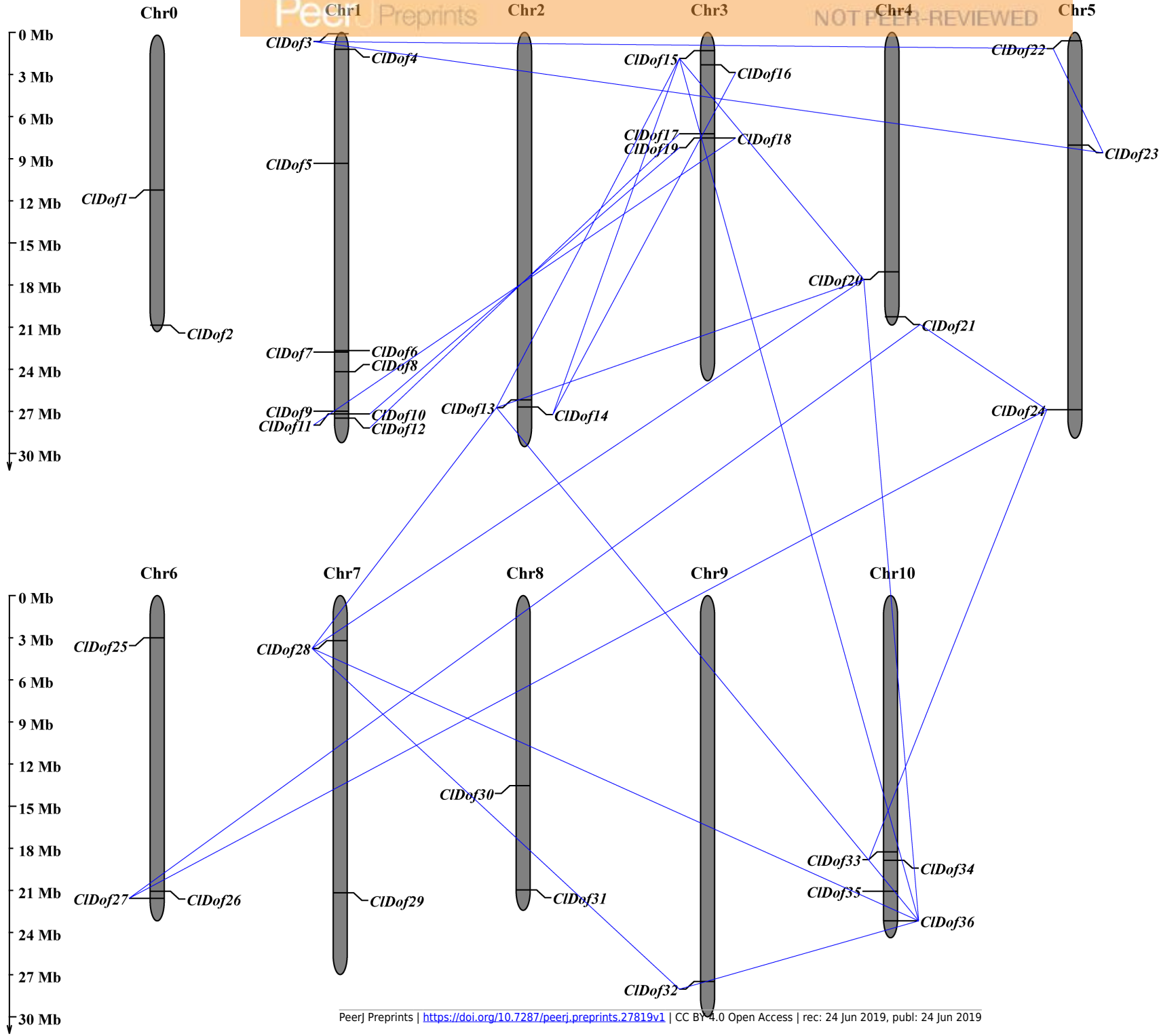

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Figure 6(on next page)

Figure 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.

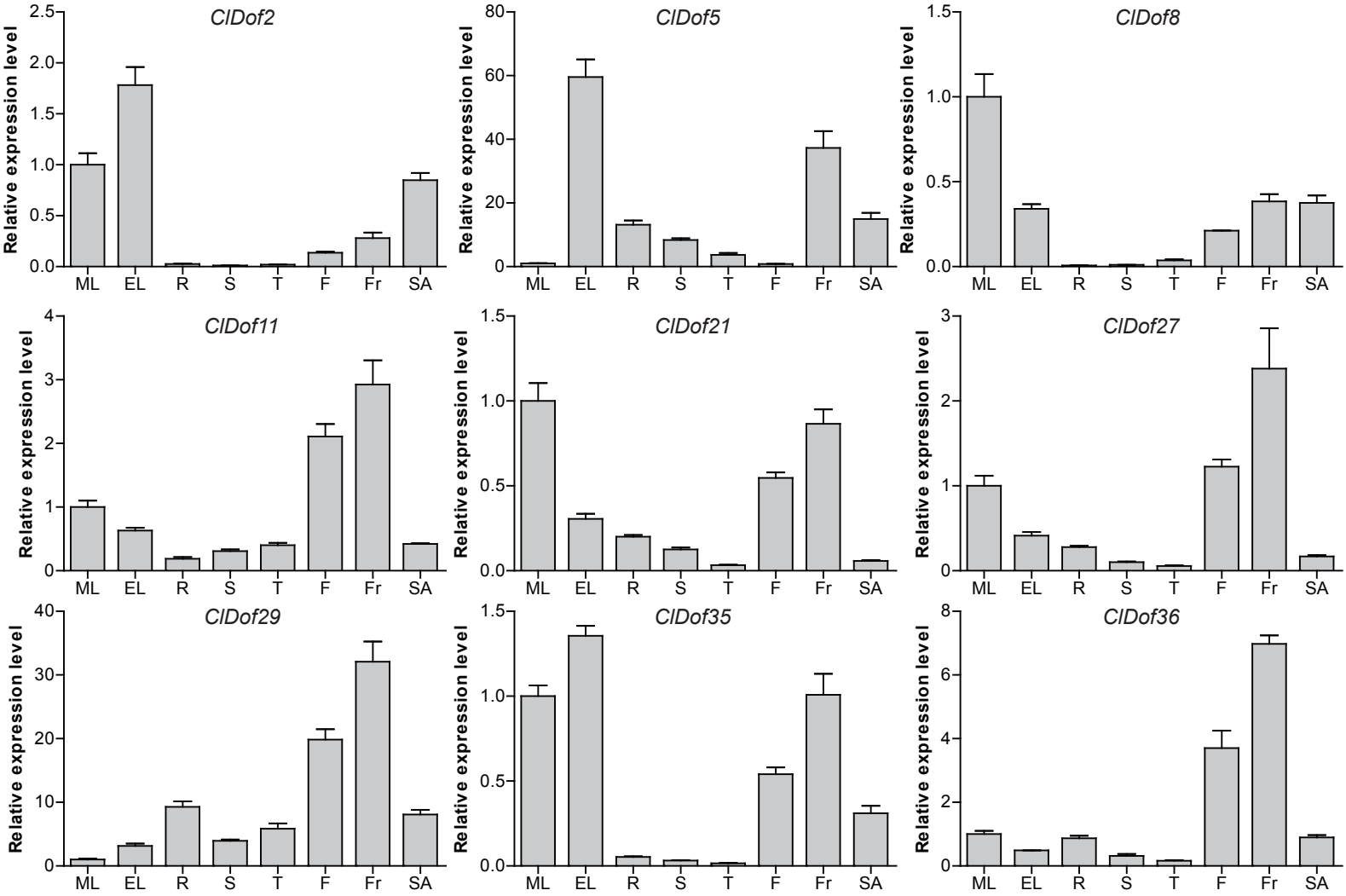

Figure 7 (on next page)

Figure 7

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

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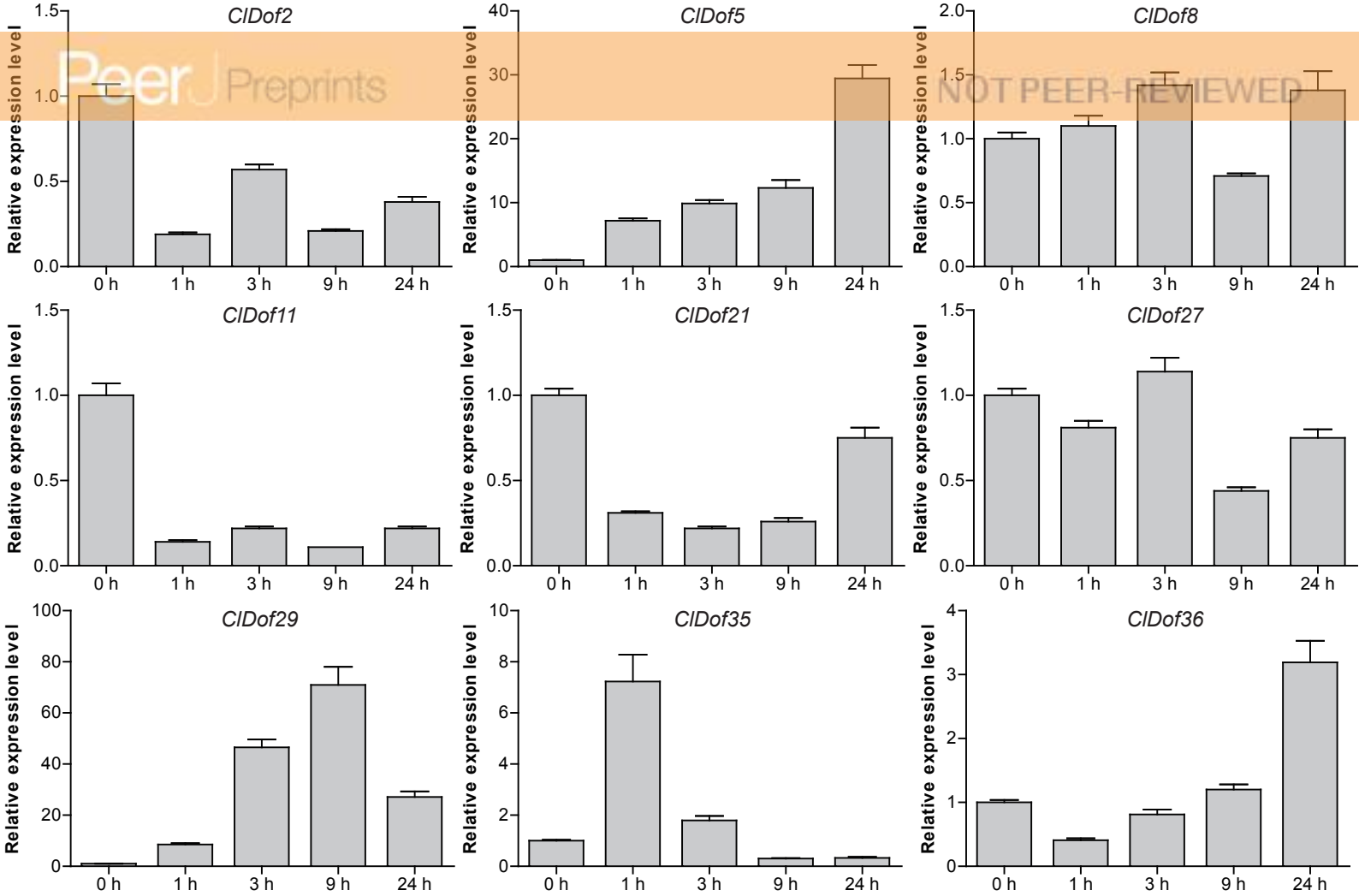

Figure 8(on next page)

Figure 8

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

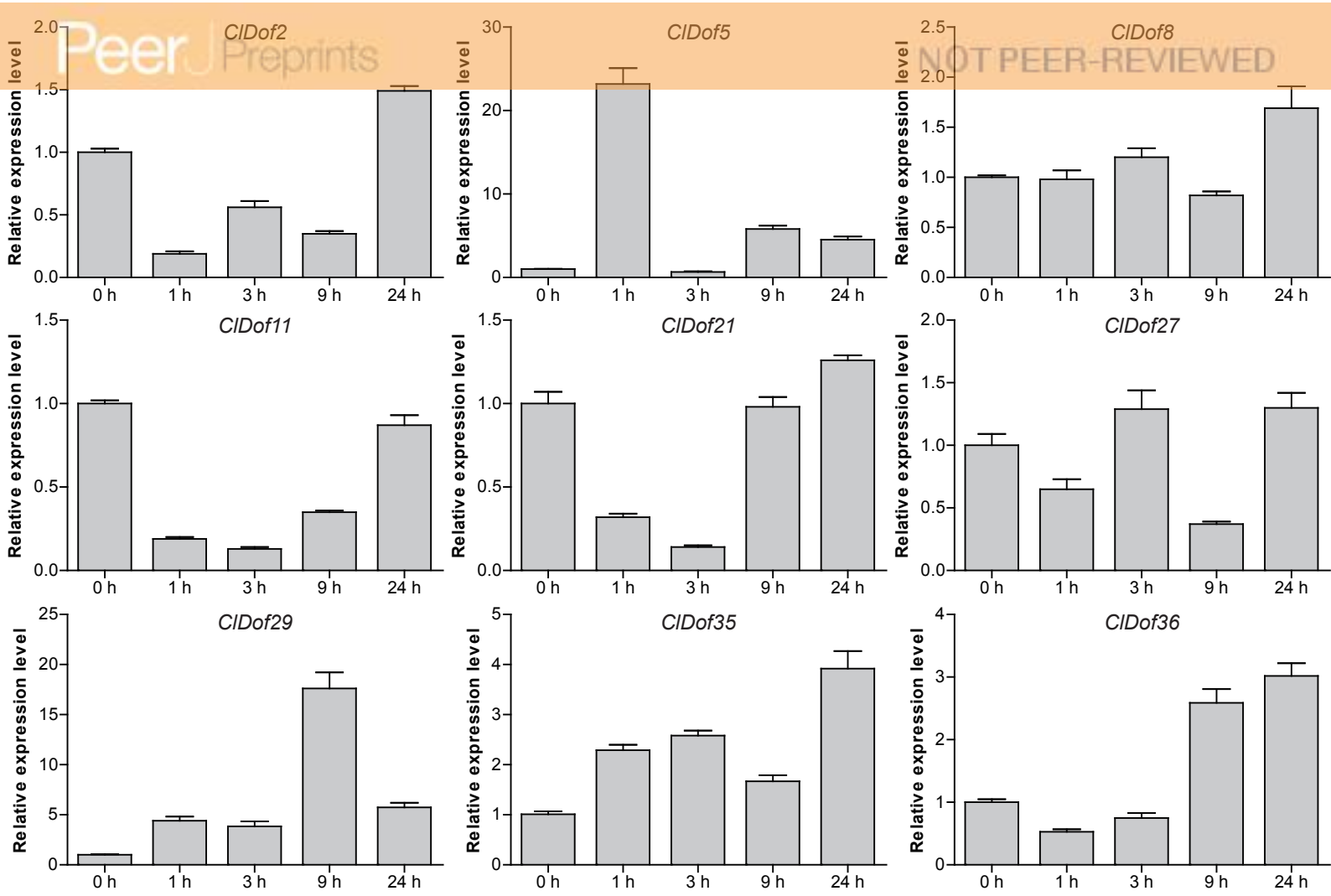

Table 1(on next page)

Table 1

Table 1. Members of *Dof* family genes identified in watermelon.

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Gene name	Gene ID	Map Position (bp)	CDS length (bp)	Protein length (aa)	MW (kDa)	pI
ClDof1	Cla000091	Chr0:12921851-12922342	492	163	17.64	8.21
ClDof2	Cla000604	Chr0:24087372-24088166	795	264	29.22	8.41
ClDof3	Cla004880	Chr1:83833-84684	852	283	30.46	8.4
ClDof4	Cla011343	Chr1:1447591-1449038	831	276	29.57	7.72
ClDof5	Cla000975	Chr1:10830770-10831984	1011	336	37.74	7.31
ClDof6	Cla001812	Chr1:26447800-26448528	729	242	24.73	8.34
ClDof7	Cla001818	Chr1:26513973-26514995	1023	340	35.45	9.21
ClDof8	Cla014094	Chr1:28161694-28162635	942	313	33.83	8.26
ClDof9	Cla001373	Chr1:31447086-31447871	786	261	29.33	8.84
ClDof10	Cla009627	Chr1:31658215-31659085	717	238	25.84	8.84
ClDof11	Cla009628	Chr1:31665641-31666539	729	242	26.88	9.49
ClDof12	Cla009692	Chr1:32112455-32112961	507	168	19.04	8.81
ClDof13	Cla013297	Chr2:30590643-30592400	1020	339	37.52	9.95
ClDof14	Cla000540	Chr2:31118585-31119331	747	248	27.29	8.73
ClDof15	Cla008250	Chr3:1516113-1517286	1026	341	37.25	9.31
ClDof16	Cla005059	Chr3:2677903-2678760	858	285	31.75	8.39
ClDof17	Cla019672	Chr3:8389380-8389913	534	177	20.21	7.13
ClDof18	Cla019705	Chr3:8782843-8783751	909	302	33.57	7.46
ClDof19	Cla019706	Chr3:8791610-8792131	522	173	18.69	9.22
ClDof20	Cla018219	Chr4:19894774-19896290	813	270	29.93	9.9
ClDof21	Cla018604	Chr4:23659963-23661769	1308	435	47.56	7.04
ClDof22	Cla021140	Chr5:723346-723861	516	171	18.06	8.99
ClDof23	Cla004274	Chr5:9417748-9418525	678	225	24.96	8.32
ClDof24	Cla010192	Chr5:31339279-31340779	1296	431	47.33	8.11
ClDof25	Cla006705	Chr6:3496040-3496858	819	272	29.94	8.26
ClDof26	Cla019034	Chr6:24515454-24516428	975	324	34.96	8.08
ClDof27	Cla019107	Chr6:25139609-25141772	1395	464	50.63	6.19
ClDof28	Cla004013	Chr7:3742674-3743851	969	322	34.24	9.24
ClDof29	Cla012621	Chr7:24693545-24694168	624	207	22.36	8.36
ClDof30	Cla013851	Chr8:15842719-15843486	768	255	28.77	5
ClDof31	Cla022532	Chr8:24427298-24428044	747	248	25.77	8.12
ClDof32	Cla004676	Chr9:32014839-32016085	1077	358	39.08	8.43
ClDof33	Cla016993	Chr10:21239053-21241153	1575	524	56.71	5.07
ClDof34	Cla002907	Chr10:21961596-21963908	1527	508	54.74	6.06
ClDof35	Cla017622	Chr10:24621093-24621851	759	252	27.81	6.76
ClDof36	Cla017890	Chr10:27032680-27034515	1053	350	37.19	9.85