

# Identification of *Dof* Transcription Factors in the Genome of *Rosa chinensis*

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**ABSTRACT.** The DNA binding with one finger (*Dof*), as an important transcription factor, plays an important role in growth and development, primary and secondary metabolism, stress resistance, and plant hormone signal transduction. However, the identification and analysis of the *Dof* transcription factor family in *Rosa* is rarely reported. In this study, 28 *Rosa chinensis Dof* (*RcDof*) members were identified, which were located on seven chromosomes. The *RcDofs* were divided into 12 subfamilies according to evolutionary analysis. Through motif, gene structure, and *cis*-acting element analyses of the 12 subfamilies, the functions of *RcDofs* were analyzed and predicted. Furthermore, the *Dof* members in *R. chinensis* ‘Old Blush’ and another three species (*Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays*) were systematically analyzed. Twelve subfamilies were found in these four species and the motifs and gene structures of *Dof* members in each subfamily were similar, which further proves that the *RcDofs* analysis is accurate. Through an intra- and inter-species collinearity analysis, it was found that the collinearity between *A. thaliana* and *R. chinensis* is closer in comparison. Tissue expression analysis of *RcDofs* was by quantitative reverse-transcription polymerase chain reaction (PCR). Quantitative real-time PCR analysis showed expressions of the *RcDofs* are tissue specific. The *RcDofs* had higher expression in leaves, roots, and flowers than other tissues. Taken together, this study provides valuable information for future research on functional exploration of *RcDof* genes and molecular breeding in *Rosa*.

The *Dof* transcription factors contain a DNA binding single zinc finger, and the *Dof* proteins are one of the plant-specific transcription factor families with a single zinc finger conserved domain (*Dof* domain) rich in a unique cysteine residue that

belongs to the C2C2 single zinc finger protein superfamily (Diaz et al., 2002; Gupta et al., 2015). The *Dof* proteins usually contain only one copy of the conserved *Dof* domain, which consists of the nucleotide residues from 200 to 400, the N-terminal highly conserved DNA binding domain, and the C-terminal transcriptional regulatory domain (Yanagisawa, 2004). In the binding domain, it conserved the CX2CX21CX2C motif forms a single zinc finger structure, and four conserved cysteine residues in the single zinc finger structure covalently bind to one Zn<sup>2+</sup> (Yanagisawa and Schmidt, 1999).

There are similar DNA binding properties for all *Dof* proteins. The C-terminus contains a transcriptional regulatory domain with multiple functions, which can interact with a variety of regulatory proteins and activate gene expression. There is great

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variation among different *Dof* members because of the poor amino acid conservation of the *Dof* domains (Yanagisawa and Schmidt, 1999), which leads to the functional diversity of the *Dof* proteins (Kisu et al., 1998).

As an important transcription factor in plants, *Dofs* play essential roles in growth and development, primary and secondary metabolism, stress resistance, and plant hormone signal transduction (Gupta et al., 2015). Some *Dofs* are involved in regulation of fruit ripening (Khaksar et al., 2019). In terms of plant growth and development, the maize *ZmDof1* protein negatively regulates the expression of the pollen-specific gene *Zm401* to control pollen development (Yang et al., 2011). The overexpression of *ZmDof1* in *Arabidopsis thaliana* activated the expression of the phosphoenol/pyruvate carboxylase (PEPC) and pyruvate orthophosphate dikinase (PPDK) genes, which increased the amount of plant nitrogen in all of the examined organs in transgenic plants (Kurai et al., 2011). *AtDof4* (a *Dof* in *A. thaliana*) directly activates the cell wall relaxation factor *AtEXP49*, which regulates the development of the seed epidermis (Zou et al., 2013). *ZmDof3* regulates the formation of starch and aleurone grains during *Zea mays* endosperm development, which affects the starch content in grains (Qi et al., 2017). *AtDof2* and *AtDof3* could be direct targets by the odorant-binding proteins 1 (*OBP1*) in transgenic *A. thaliana*, which could regulate the number of cells that are decreased and cause plant dwarfing (Skirycz et al., 2008). *GmDof4* and *GmDof11* enhanced the lipid content in the seeds of transgenic *A. thaliana* (Wang et al., 2007). It was predicted that the *DzDof2.2* might have a role in regulating auxin biosynthesis based on its orthologue in *A. thaliana* (Khaksar et al., 2019). When *SlCDF1* and *SlCDF3*, which are *Dofs* in *Solanum lycopersicum*, were overexpressed in *A. thaliana*, the result was direct activation of the expression of *COR15*, *RD29A*, and *RD10*, which increased the resistance of transgenic *A. thaliana* under drought and salt stress (Corrales et al., 2014). The overexpression of *Dof* family gene *CDF3* in *A. thaliana* delayed flowering and increased the resistance to drought and cold stress (Corrales et al., 2017).

*Dof* has been identified in many plants, such as *Oryza sativa* (Lijavetzky et al., 2003), *A. thaliana* (Lijavetzky et al., 2003), *Jatropha curcas* (Wang et al., 2018), *Vitis vinifera* (Silva et al., 2016), *Medicago truncatula* (Shu et al., 2015), *Durio zibethinus* (Khaksar et al., 2019), and *Chrysanthemum morifolium* (Song et al., 2016), but the identification and analysis of *Dof* transcription factor family in *Rosa* is rarely reported. As one of the most important commercialized flower plants worldwide, the research on *Rosa* has been greatly promoted based on the *Rosa* genome (Raymond et al., 2018). In this study, we identified the *RcDof* family genes based on the *Rosa chinensis* genome, and their gene structures, conserved domains, and tissue-specific expression were comprehensively analyzed. Our findings provide a foundation for future functional analysis of *Dof* genes in *Rosa*.

## Materials and Methods

**GENOME-WIDE IDENTIFICATION AND ANALYSIS OF *RcDOFs*.** The protein and genome sequences of *R. chinensis* (PRJNA438537) were obtained from the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) database (Raymond et al.,

2018), and the domain of *Dof* (PF02701) originated from the Pfam database (El-Gebali et al., 2019), as an HMMER structure for an HMMER search (Robert et al., 2011), which the e-value was set for  $e^{-20}$ . After confirmation of the *Dof* domain of *RcDofs* by Interpro (Sarah et al., 2009), P<sup>3</sup>DB (Gao et al., 2012), and Ex-PASy (Elisabeth et al., 2003), and removing duplicates, unique names were given to each *RcDof* according to the positions on the reference chromosomes. The *Dof* protein and genome sequences for *A. thaliana*, *O. sativa*, and *Z. mays* were obtained from the phytozome database (Goodstein et al., 2012).

Clustal W (Thompson et al., 1994) was used to align the *Dof* multiple peptide sequences in *R. chinensis*. The MEGA X program (Kumar et al., 2018) was used to align the *Dof* protein sequences in *R. chinensis* ‘Old Blush’ and with *A. thaliana*, *O. sativa*, and *Z. mays*, which all contain *Dof* genes. The JTT+G+I model was the most optimal model predicted by MEGA X and produced bootstrap values in 1000 replicates.

The motifs of *RcDofs* were detected outside the domain of *Dof* protein sequences in *RcDofs*, with e-values less than  $1e^{-20}$  and lengths of 10 to 50 amino acids. The order of motif was numbered according to the protein sequences and predicted by MEME software (Timothy et al., 2009). The exon/intron structure of the *RcDofs* was analyzed and displayed by the GSDS program (Hu et al., 2015). The location of the *RcDofs* was according to the position in the DNA (containing the exon and intron together) sequence by the Genewise program (Chen et al., 2016).

The length of *RcDof* promoters was set in 1500 base pairs (bp), and the genome sequence from the NCBI database was extracted using PlantCARE (Magali et al., 2002) and TBtools (Chen et al., 2020). The collinearity analysis of *RcDofs* was defaulted by the MCScanX program (Baek et al., 2016) and graphed by Circos (Krzywinski et al., 2009).

**EXPRESSION ANALYSIS OF THE *RcDOFs*.** *rosa hybrid* ‘Month-pink’ was chosen as the plant material for analysis, which was a cultivar mainly cultivated in Heilongjiang. The stems, roots, leaves, flowers, stamens, and pistils of the *R. hybrid* ‘Month-pink’ were sampled during plant flowering, and  $\approx 0.2$  g of each sample was harvested. A Trelief RNAPrep Pure Plant Kit (TSP411; Tsingke Biotechnology Co., Beijing, China) was used to extract the RNA from the samples, and its purity was determined using a spectrophotometer (NanoDrop 2000C; Thermo Fisher Scientific, Waltham, MA, USA), with optical density (OD) 260/280 nm values between 1.8 and 2.1 and OD 260/230 nm values ranged from 2.0 to 2.2 for all RNA samples. Complementary DNA (cDNA) was generated from the RNA samples using a Goldenstar RT6 cDNA Synthesis Kit (TSK301; Tsingke Biotechnology Co.). The primers of *RcDofs* for use in quantitative real-time PCR (qRT-PCR) were designed by Primer Premier 5.0 (Singh et al., 1998) software (Supplemental Table 1). A qRT-PCR detection system (CFX96 Touch; Bio-Rad, Hercules, CA, USA) was used with the 2  $\times$  TSINGKE Master qPCR Mix (SYBR Green I) (TSE201, Tsingke Biotechnology Co.), and three biological replicates were processed for each sample. *RcUBR* was used as the reference gene based on the preliminary studies of laboratory (Dong et al., 2021; Klie and Debener, 2011). The relative expression of *RcDofs* was calculated according to the formula of  $2^{-\Delta Ct}$  (Zhang et al., 2020). The gene expression data were analyzed using Duncan’s test using statistical

Table 1. Identifying DNA binding with one-finger (*Dof*) genes from *Rosa chinensis* ‘Old Bush’.

| Gene name      | Gene accession | Chr | Location              | Protein length | CDS length |
|----------------|----------------|-----|-----------------------|----------------|------------|
| <i>RcDof01</i> | XP_024191320   | 1   | 52,267,581:52,267,589 | 276            | 831        |
| <i>RcDof02</i> | XP_024191312   | 1   | 52,267,418:52,267,441 | 281            | 846        |
| <i>RcDof03</i> | XP_024178062   | 1   | 66,987,680:66,988,531 | 283            | 852        |
| <i>RcDof04</i> | XP_024178056   | 1   | 66,987,107:66,987,130 | 298            | 897        |
| <i>RcDof05</i> | XP_024191900   | 1   | 57,019,427:57,019,480 | 367            | 1,104      |
| <i>RcDof06</i> | XP_024181122   | 2   | 15,128,795:15,129,514 | 239            | 720        |
| <i>RcDof07</i> | XP_024186158   | 2   | 60,063,059:60,063,745 | 228            | 687        |
| <i>RcDof08</i> | XP_024184677   | 2   | 22,225,550:22,226,626 | 358            | 1,077      |
| <i>RcDof09</i> | XP_024188573   | 2   | 13,193,963:13,194,013 | 381            | 1,146      |
| <i>RcDof10</i> | XP_024185752   | 2   | 15,404,959:15,405,936 | 325            | 978        |
| <i>RcDof11</i> | XP_024181086   | 2   | 82,141,477:82,141,713 | 509            | 1,530      |
| <i>RcDof12</i> | XP_024190311   | 3   | 22,079,228:22,080,136 | 302            | 909        |
| <i>RcDof13</i> | XP_024189477   | 3   | 31,624,285:31,624,489 | 379            | 1,155      |
| <i>RcDof14</i> | XP_024189476   | 3   | 31,624,285:31,624,489 | 384            | 1,155      |
| <i>RcDof15</i> | XP_024194374   | 4   | 62,021,052:62,021,312 | 531            | 1,596      |
| <i>RcDof16</i> | XP_024161212   | 5   | 66,501,646:66,501,675 | 315            | 948        |
| <i>RcDof17</i> | XP_024158293   | 5   | 5,817,485:5,817,544   | 347            | 1,044      |
| <i>RcDof18</i> | XP_024159083   | 5   | 5,803,600:5,804,487   | 295            | 888        |
| <i>RcDof19</i> | XP_024158076   | 5   | 69,777,103:69,777,966 | 287            | 864        |
| <i>RcDof20</i> | XP_024158075   | 5   | 69,778,317:69,778,367 | 354            | 1,065      |
| <i>RcDof21</i> | XP_024159528   | 5   | 7,096,255:7,096,776   | 173            | 522        |
| <i>RcDof22</i> | XP_024199643   | 5   | 4,937,196:4,938,083   | 295            | 888        |
| <i>RcDof23</i> | XP_024166964   | 6   | 38,985,264:38,986,163 | 299            | 900        |
| <i>RcDof24</i> | XP_024165790   | 6   | 60,991,469:60,992,308 | 279            | 840        |
| <i>RcDof25</i> | XP_024174860   | 7   | 6,391,961:6,391,984   | 300            | 903        |
| <i>RcDof26</i> | XP_024173084   | 7   | 11,808,032:11,808,082 | 339            | 1,020      |
| <i>RcDof27</i> | XP_024174414   | 7   | 4,517,925:4,518,878   | 317            | 954        |
| <i>RcDof28</i> | XP_024172633   | 7   | 3,171,981:3,172,133   | 467            | 1,404      |

Chr = chromosome; CDS length = coding sequence length.

software (IBM SPSS Statistics ver. 19.0; IBM Corp., Armonk, NY, USA).

$$\text{Relative expression} = 2^{-\Delta Ct},$$

$$\{\Delta Ct = Ct(\text{Rc target genes}) - Ct(\text{RcUBR})\}$$

## Results

**IDENTIFICATION OF *R. CHINENSIS* DOF FAMILY MEMBERS.** After the hmmbuild analyses using the HMM profile analysis of *Dof* domains, and according to the retrieved protein sequences, a new hidden Markov model for the *Dof* protein conserved domain of *Rosa* was constructed. Twenty-eight protein sequences with *Dof* domains were identified and none of the duplicate values for the same gene number by genome-wide (Supplemental Fig. 1). They were designated as *RcDof01-28* from the *R. chinensis* ‘Old Blush’ genome. Full-length coding sequences of *RcDof01-28* ranged from 522 to 1596 bp, which encoded 28 putative proteins with 173 to 531 amino acid residues (Table 1, Supplemental Tables 2–4).

**PHYLOGENETIC ANALYSIS, CONSERVED MOTIFS, GENE STRUCTURE COMPOSITIONS AND CIS-REGULATORY ELEMENT ANALYSIS OF *RcDOFs*.** A phylogenetic tree comprising 28 *Dof* protein sequences from *R. chinensis* ‘Old Blush’ was constructed (Fig. 1). The phylogenetic tree of the *RcDof* sequences was generated with the maximum-likelihood method using a JTT+G+I

model, which was the optimal model as determined by MEGA X software. Using the phylogenetic tree and applying the classification method to the *RcDofs*, 12 subfamilies were separated as I–XII. The conserved motifs of the 28 *RcDofs* were analyzed using MEME software. Figure 2A shows the motifs and the composition of each *RcDof*. The motifs of each subfamily were relatively similar, which confirmed the close associations among the same subfamilies in the evolutionary tree. The gene structures and the phylogenetic trajectories of the 28 *RcDofs* were examined. The *Dof* domain was conserved in all subfamilies, and the exon and intron numbers, ranging from 0 to 6, were similar in each subfamily (Fig. 2B). The *RcDofs* *cis*-regulatory elements in promoters in the 1500-bp upstream region were analyzed using the online software PlantCARE and TBtools. Based on the *R. chinensis* genome database, nine *cis*-regulatory elements were found based on the PlantCARE predicted (Supplemental Table 5). Six of the *cis*-regulatory elements, which included abscisic acid responsive elements (ABRE), gibberellin-responsive elements (GARE-motif, P-box and TATC-box), and auxin-responsive elements (TGA-box and TGA-element) were directly related to hormones, and three were related to plant stress (Fig. 2C), such as salt and heat. These elements indicated that the *RcDofs* may have a very important role in abiotic stress, and may respond to hormones that are produced due to stress.

**COMPARATIVE ANALYSIS OF DOF GENES.** The *Dof* in *A. thaliana*, *O. sativa*, and *Z. mays* was retrieved by hmmpsearch, and the *RcDof* in *R. chinensis* was analyzed as a whole. The phylogenetic

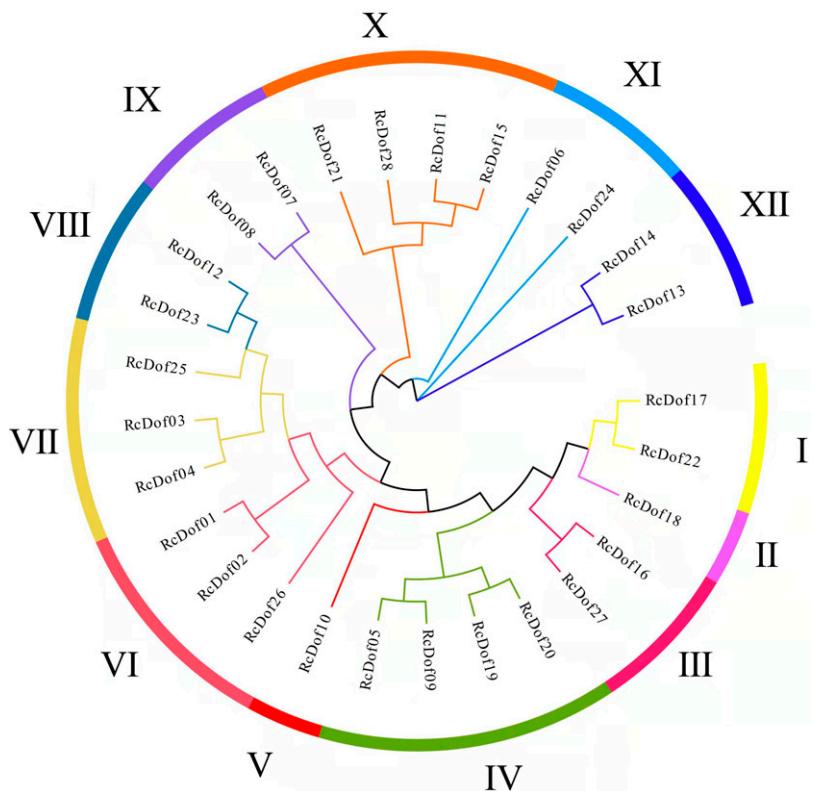


Fig. 1. Phylogenetic tree of the DNA binding with one-finger (*Dof*) genes in *Rosa chinensis*. The phylogenetic tree is constructed using MEGA X (Kumar et al., 2018) by the maximum likelihood (ML) method with 1000 bootstrap replicates. Twelve major phylogenetic groups designated from group I to group XII are indicated. Each *Dof* subgroup is indicated by a specific color.

tree still adopted the maximum-likelihood (ML) method, using the JTT+G+I model, which is the optimal model predicted by MEGA X. The gene structures of *Dof* in *R. chinensis* and others were examined for their relationship with the phylogenetic

trajectories. Similarly, the gene family of *Dofs* was divided into 12 subfamilies in these three species (Fig. 3), and the gene structure of *Dofs* in each subfamily was also very similar, indicating that their function may be similar.

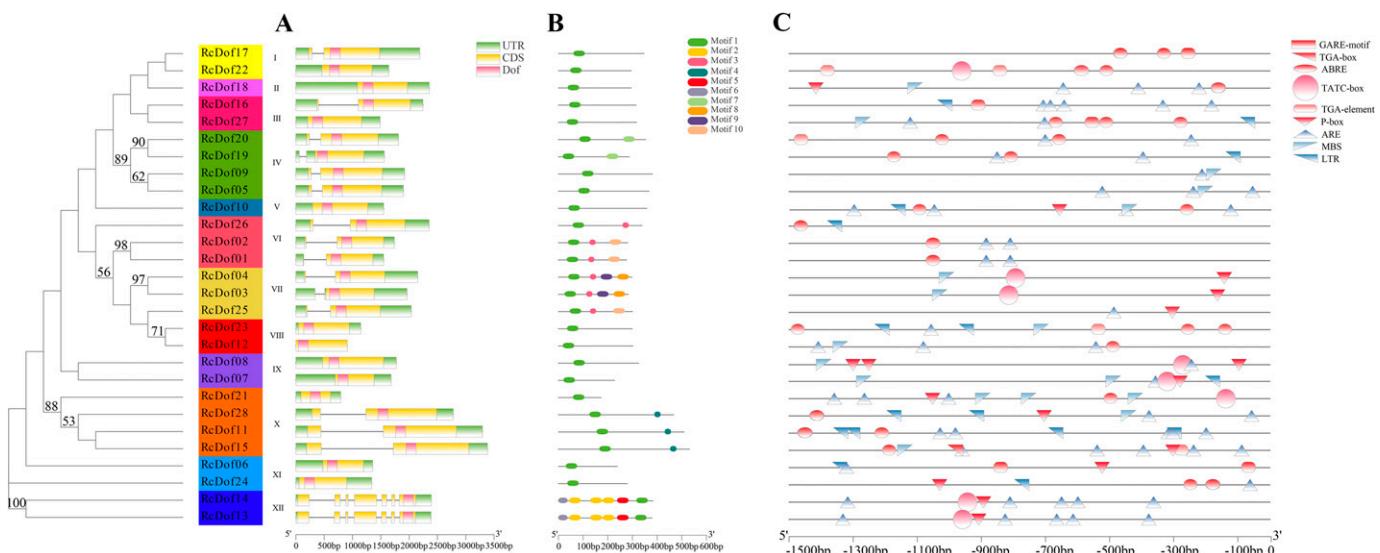


Fig. 2. The motifs and the gene structure analysis of the *Rosa chinensis* DNA binding with one-finger (*RcDof*) genes. (A) The motifs of *RcDof* members. (B) The gene structure of *RcDof* members. (C) The *RcDof* members *cis*-acting element analysis of the promoter regions. The motifs, numbered 1 to 10, are displayed in boxes with different colors. The hormone-related elements including abscisic acid responsive elements (ABRE), gibberellin-responsive elements (GARE-motif, P-box and TATC-box) and auxin-responsive elements (TGA-box and TGA-element) are red, whereas *cis*-acting regulatory elements essential for the anaerobic induction (ARE), drought-inducibility elements (MBS), and low-temperature responsive elements (LTR) connected with stress are blue.

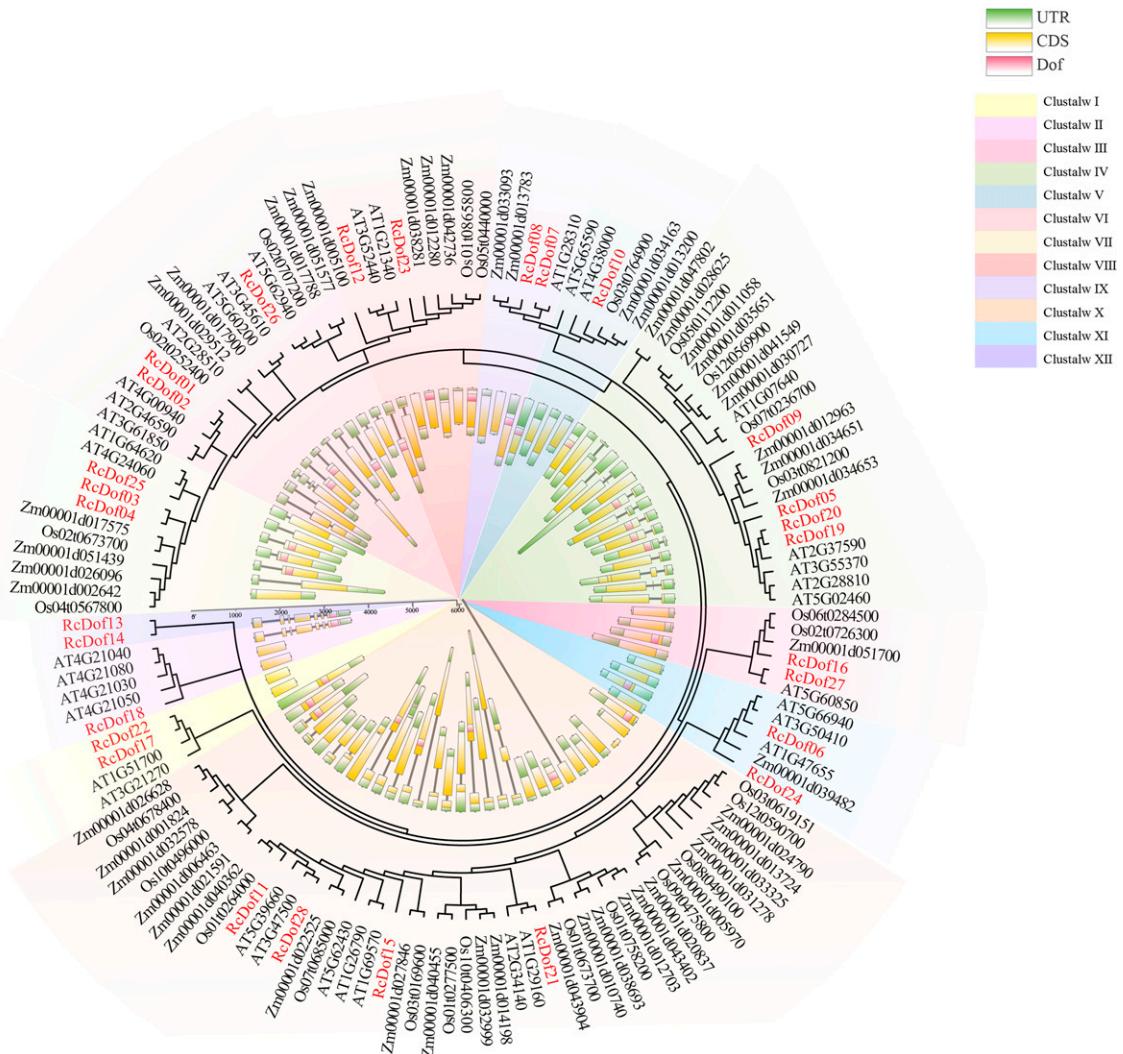


Fig. 3. Phylogenetic tree of the DNA binding with one-finger (*Dof*) members in *Rosa chinensis*, *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays*. Phylogenetic tree is constructed using the maximum likelihood (ML) method with 1000 bootstrap replicates. The inner ring shows the gene structure of *Dof* members in these four species. The squares in green, yellow, and pink represent untranslated region (UTR), coding sequence (CDS), and *Dof* sequence. The outer ring shows 12 major phylogenetic groups designated from group I to group XII. Each of the 12 clades is indicated by a specific color.

**THE LOCATION OF *RcDofs* AND COLLINEARITY ANALYSIS.** The 28 *RcDof* members were located on the seven chromosomes (Chrs) of the *R. chinensis* genome. Chr 5 had the largest number of *RcDofs*, with seven, followed by Chr2, with six *RcDofs*. Chr4 had the fewest *RcDofs*, with only one, followed by Chr6, with two *RcDofs*. At least one *RcDof* member was mapped to each chromosome of *R. chinensis* ‘Old Blush’. Among the 28 *RcDofs*, there was collinearity for eight pairs of *RcDofs*, and most of them were the *RcDofs* between different chromosomes that produced collinearity, indicating that there may be many close relationships between their *Dof* genes (Fig. 4).

The collinear analysis of *Rosa Dof* family genes with other species is shown in Fig. 5. *RcDofs* and *A. thaliana* genes produced more than nine pairs of collinearity, and there are only two sets of genes in *O. sativa* and *Glycine max*, respectively, and only one pair of genes in *Z. mays*. In comparison, there was a similarity in the relationship between *Rosa* and *A. thaliana*.

**SPECIFIC-TISSUE EXPRESSION ANALYSIS OF *RcDofs*.** Here, 16 *RcDofs* randomly distributed in all subgroups were selected for specific-tissue expression analysis by qRT-PCR in leaves, stems, roots, flowers, stamens, and pistils. *RcDofs* had the expression in all those tissues (leaves, stems, roots, flowers, stamens, and pistils) and the data expression in different tissues showed obvious difference, as shown in Fig. 6. The expression data of *RcDofs* by qRT-PCR analysis showed that *RcDofs* had a higher expression in the roots, flowers, or leaves compared with other tissues, which indicated that the roots, leaves, and flowers might be target tissues for future research on the function of *RcDofs*.

## Discussion

The *Dof* genes are plant-specific transcription factors. They are components that play important roles in plant growth, plant hormone response, and environmental stress (Noguero et al.,

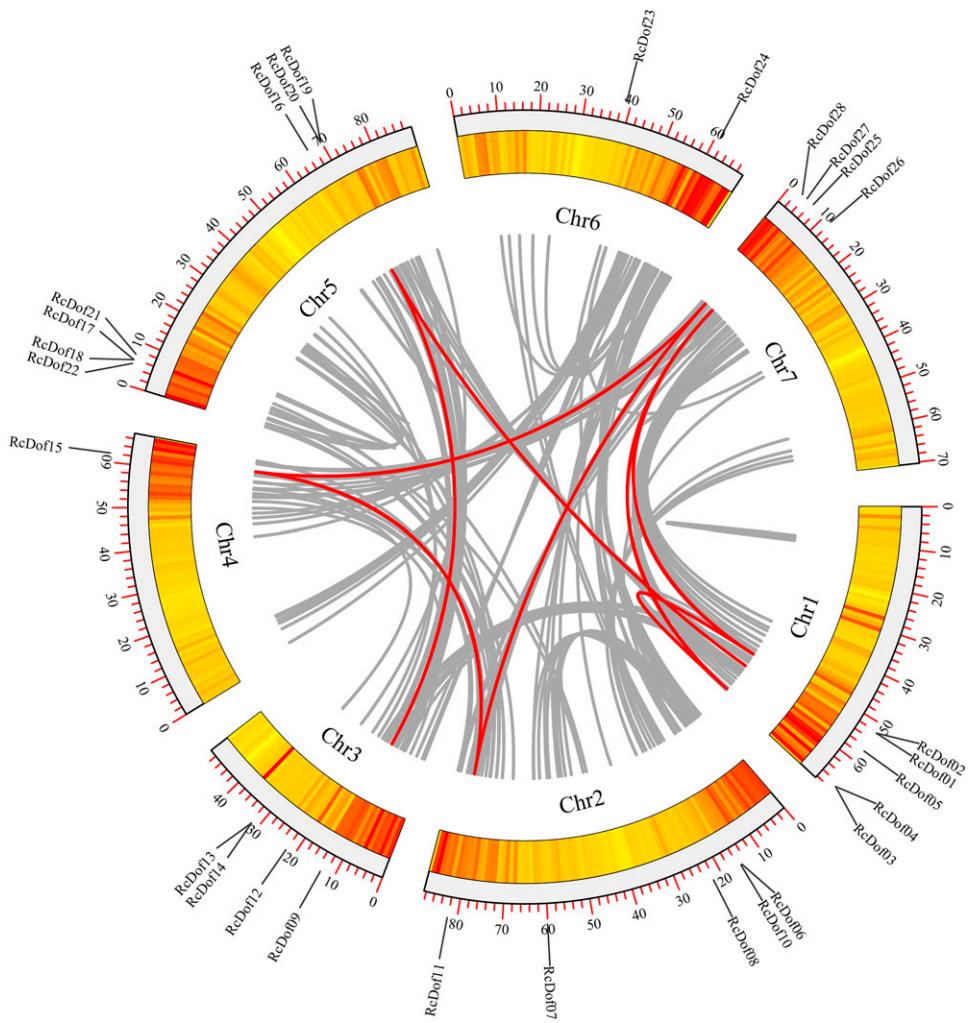


Fig. 4. The location and collinearity analysis of the *Rosa chinensis* DNA binding with one-finger (*RcDof*) genes. The outer ring represents the chromosomes of the location of *RcDofs*. The denser the red line, the greater the number of genes in this part. The inner ring denotes the collinearity analysis of *RcDofs*. The bright line represents collinearity, and the gray line represents all isomorphic blocks in the genome.

2013). In this study, a total of 28 *RcDof* genes were identified from the *R. chinensis* reference genome. The number of *Dof* genes is similar to *Hordeum vulgare* [26 (Moreno et al., 2007)], *V. vinifera* [25 (Silva et al., 2016)], *Sorghum bicolor* [28 (Kushwaha et al., 2011)], and *O. sativa* [30 (Lijavetzky et al., 2003)]. *Dof* genes in *G. max* [78 (Guo et al., 2013)] are much larger than those in *R. chinensis*. These *RcDofs* were distributed in 12 subfamilies in the phylogenetic relationship analysis, and similar conclusions have been reported for *Camellia sinensis* (Yu et al., 2020), *V. vinifera* (Silva et al., 2016), *Manihot esculenta* (Zou et al., 2019a), *Solanum melongena* (Wei et al., 2018), and *A. thaliana* (Lijavetzky et al., 2003). After analysis of the *cis*-regulatory elements of *RcDof* members, which are related to plant hormones and plant stress tolerance, similar findings were obtained for *Pyrus bretschneideri* (Liu et al., 2020), *Daucus carota* spp. *sativus* (Huang et al., 2016), *A. thaliana* (Le and Bellini, 2013), *O. sativa* (Gaur et al., 2011), *Brassica rapa* (Ma et al., 2015), and *C. sinensis* (Li et al., 2016). Similar multispecies analyses have also been reported for *S. bicolor* with *O. sativa* and *A. thaliana* (Kushwaha et al., 2011), and *Populus trichocarpa* with *O. sativa* and *A. thaliana* (Yang et al., 2006).

There are eight pairs of *RcDofs* with collinearity among the 28 *RcDofs*, indicating that these *RcDofs* may have many close relationships. *RcDofs* had nine pairs of collinearity with the genes in *A. thaliana*, which had more collinearity pairs compared with *O. sativa* (2) and *G. max* (2). This phenomenon might be caused by the distance of the genetic relationship and the size of chromosomes of different plants. The functions of these collinear genes can be used as references when studying the functions of *RcDofs*. *AT5G62940* (*HCA2*) has the function of DNA-binding transcription factor activity in phloem, xylem, and root (Guo et al., 2009; Miyashima et al., 2019). *AT5G39660* (*CDF2*) may have an important role in the timing of the transition from the vegetative to the reproductive phase (Imaiizumi et al., 2005), with a similar function for *AT1G28310* (*DOF1.4*) (Riechmann et al., 2000), whereas *AT2G46590* (*DAG2*) may have the function of responding to red light and water stimulus, or in seed germination (Gualberti et al., 2002; Santopolo et al., 2015). *AT1G69570* (*CDF5*) may have the function of regulation of photoperiodism and flowering (Henriques et al., 2017). These gene functions can be studied as potential targets of *RcDofs*, especially the *RcDofs* that produce collinearity.

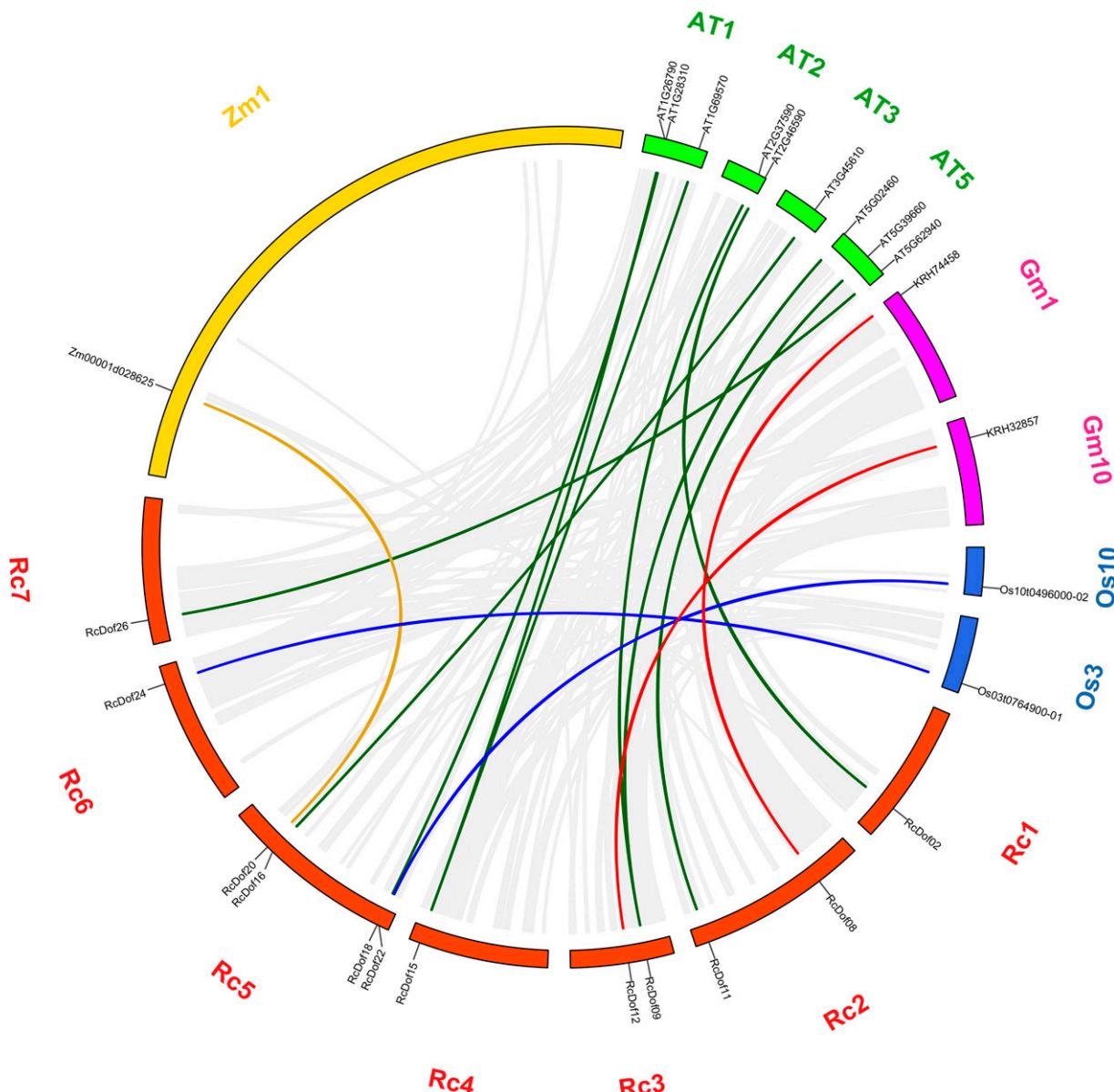


Fig. 5. The collinearity analysis of the *Rosa chinensis* DNA binding with one-finger (*RcDof*) genes with *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Glycine max*. The bright line represents collinearity with *RcDof*, and the gray line represents all isomorphic blocks in the genomes of these five species. The yellow line represents the collinearity *RcDofs* with *Z. mays*, and the blue line represents the collinearity *RcDofs* with *O. sativa*. The green line represents the collinearity *RcDofs* with *A. thaliana*, and the orange line represents the collinearity *RcDofs* with *G. max*.

The *RcDofs* expression data showed a difference in different tissues, which indicated that *RcDofs* might play an important role in plant growth and development. Similarly, *Dof* transcription factors in some species also showed a tissue-specific expression: In *V. vinifera*, the expression data of *Dof* family members showed a significant difference in berry, flower, rachis, tendril, bud, and seed. The rachis, tendril, and bud had a higher expression (Silva et al., 2016). In *S. bicolor*, the *Dof* genes had tissue-specific expression in roots, seedlings, leaves, and stems. The roots, seedlings, and leaves could be used as target tissues because the *Dof* genes had a higher expression in these tissues (Gupta et al., 2016). In *Musa nana*, *MaDof* genes had specific expression at different development stages (Fr1, Fr2, Fr3, and Fr4) (Dong et al., 2016). In *Brachypodium distachyon*, *BdDof* members had a specific expression in four organs (leaf, root, ear, and seed) by

qRT-PCR analysis (Hernando-Amado et al., 2012). All of these results showed *Dof* members had a specific expression in different tissues. In this study, *RcDofs* had a higher expression in roots, flowers, and leaves than other tissues, similar to these species, which showed a specific-tissue expression. Furthermore, some tissues could be used as target tissues because of the higher expression of *Dof* genes. In *J. curcas*, flower buds could be used as target tissue because of the high gene expression of *Dof* members (Zou and Zhang, 2019). In *Citrullus lanatus*, leaves and roots could be used as target tissues because *CIDofs* had a high expression (Zhou et al., 2020). It had been reported that *AtDof4.2* was specifically expressed in flower, which inferred that flower could serve as the target tissue for this gene (Skirycz et al., 2007). In this study, *RcDofs* had a higher expression level in roots, leaves, and flowers. Our findings suggest that roots, leaves,

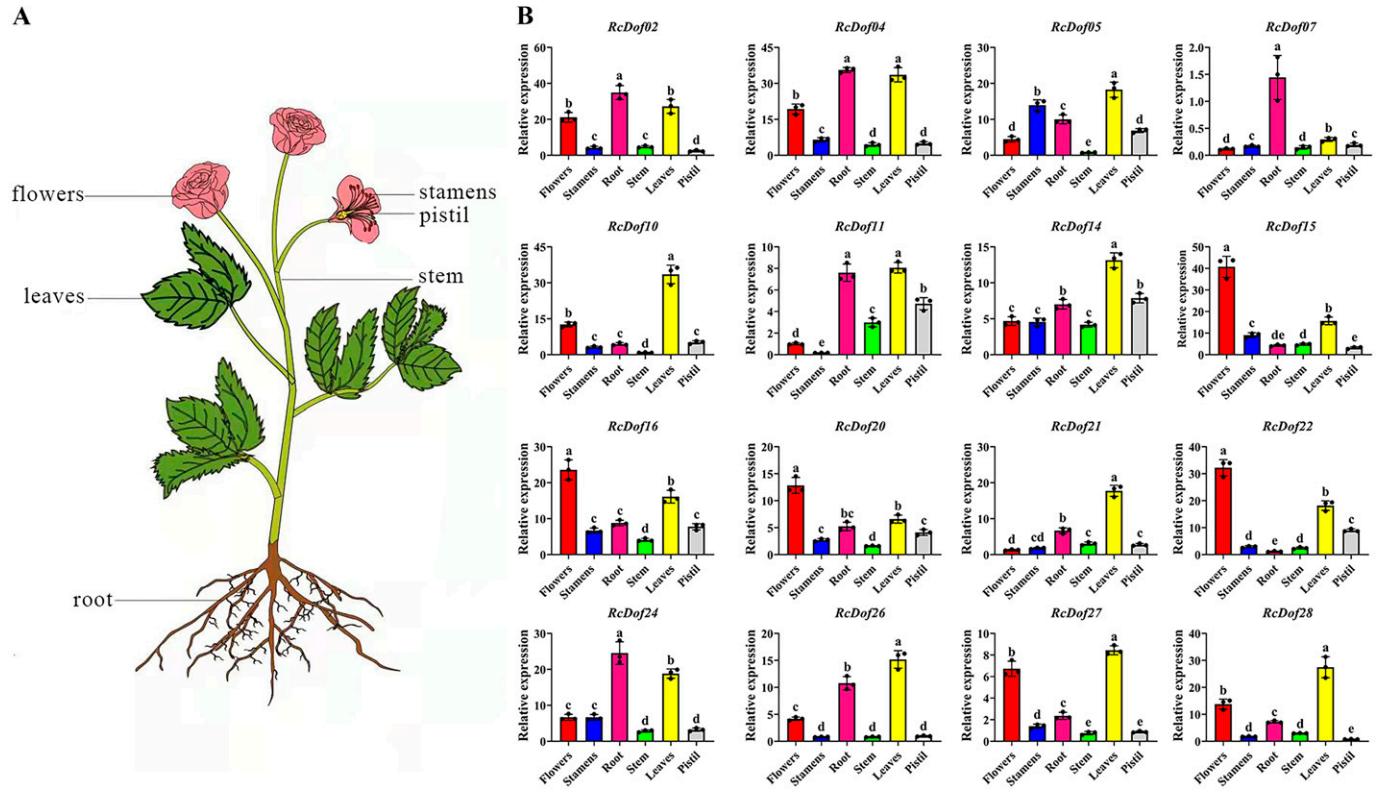


Fig. 6. The expression analysis of 16 *Rosa chinensis* DNA binding with one-finger (*RcDof*) genes in different tissues (roots, stems, leaves, flowers, stamens, and pistils). (A) The organizational diagram of each part of *R. chinensis*. Each part of the tissue is painted in a different color. (B) The bar chart of 16 *RcDofs* expression in different tissues. Each *RcDof*'s name is the title on each small image. Different lowercase letters in the same column indicate significant differences between different treatments using Duncan's test ( $P < 0.05$ ).

and flowers could be used as target tissues for studying the function of *RcDofs*.

## Conclusions

In this study, 28 *RcDofs* were identified using the published genome of *R. chinensis*, and a comprehensive analysis of *RcDofs* was performed for their detailed information. The evolutionary tree, gene structure motifs, *cis*-elements, and collinearity had been analyzed. The *RcDofs* were divided into 12 subfamilies and the gene structure motifs and *cis*-elements of *RcDofs* in each subfamily were similar. In collinear analysis, *RcDofs* had nine pair collinear genes with *A. thaliana*, which these nine *RcDofs* might have had a similar function with these *A. thaliana* genes. Also, the *RcDofs* showed the tissue-specific expression in qRT-PCR analysis. The expression of *RcDofs* was higher in roots, leaves, and flowers compared with other tissues, which showed roots, leaves, and flowers could be used as target tissues for studying the function of *RcDofs*. These results provide a theoretical basis for the follow-up exploration of the functions of *RcDofs* in *Rosa*.

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## Clustal W (Thompson et al., 1994).



Supplemental Fig. 1. The multiple peptide sequence alignment of *RcDofs* using Clustal W (Thompson et al., 1994).

Supplemental Table 1. Primer sequences used for quantitative real-time polymerase chain reaction (qRT-PCR).

| No. | Primer name     | Primer sequence         |
|-----|-----------------|-------------------------|
| 1   | <i>RcDof02F</i> | AAGGTGGAACACTCTCAGAAATG |
| 2   | <i>RcDof02R</i> | TACCTACTTCCAACCCCATCA   |
| 3   | <i>RcDof04F</i> | ATCCCCCTCATATCTCTCGGC   |
| 4   | <i>RcDof04R</i> | AGAATGGTCTCCTTCCCCTCC   |
| 5   | <i>RcDof05F</i> | AAGGAGCAAAGAGTCAAAGT    |
| 6   | <i>RcDof05R</i> | TGATGTAAGAGGGCAGAAA     |
| 7   | <i>RcDof07F</i> | CTACAATCTGTCGCAGCCTC    |
| 8   | <i>RcDof07R</i> | TTGACAGGGTTGATGATGAG    |
| 9   | <i>RcDof10F</i> | GGTGGTGTGGAGGGTTGAACG   |
| 10  | <i>RcDof10R</i> | GAAGGAAGAGGGGGGAATGA    |
| 11  | <i>RcDof11F</i> | TTTGCTCAGACACGCCCT      |
| 12  | <i>RcDof11F</i> | TCCATTTACTCCGCCCAT      |
| 13  | <i>RcDof14F</i> | AACCAACTTACCAACCCGCC    |
| 14  | <i>RcDof14R</i> | GCTTACGAAACCCACTCTA     |
| 15  | <i>RcDof15F</i> | ACTGGAGATAATGGGGATGA    |
| 16  | <i>RcDof15R</i> | TAAGATTGGAGGATGGTGA     |
| 17  | <i>RcDof16F</i> | AGCAACACAGACCAGAGAAA    |
| 18  | <i>RcDof16R</i> | GTCATTGGAGTCGTAGGAGA    |
| 19  | <i>RcDof20F</i> | AGAAACAAGAAGAGCAAAAC    |
| 20  | <i>RcDof20R</i> | ACAAATGACTAATCAAACCG    |
| 21  | <i>RcDof21F</i> | ATCATCGTAACCCAAGAAA     |
| 22  | <i>RcDof21R</i> | CTCTTCATCTCACCGTTG      |
| 23  | <i>RcDof22F</i> | CGAGGTGTGAATCAACAAAC    |
| 24  | <i>RcDof22R</i> | GGAAGAAGAACCTCCGTATG    |
| 25  | <i>RcDof24F</i> | GGAACACTCGCAACAAAGAAACC |
| 26  | <i>RcDof24R</i> | CGTCAGAAACGAAAACCTC     |
| 27  | <i>RcDof26F</i> | AATAACTACAGCCTCTCA      |
| 28  | <i>RcDof26R</i> | CTTGTCTTCTACAAACCAC     |
| 29  | <i>RcDof27F</i> | TGCCTCCTCGAACGACGTGC    |
| 30  | <i>RcDof27R</i> | CAATCCAGCGACCCAAACCC    |
| 31  | <i>RcDof28F</i> | CCTATACCAGTTTCCCTGC     |
| 32  | <i>RcDof28R</i> | CTGAACATCTCCCTCCCT      |

Supplemental Table 2. The protein sequence information of *RcDof* members.

| No. | Name           | Protein sequence   |
|-----|----------------|--|
| 1   | <i>RcDof01</i> | MFKEIGLEKTMEENMGSEARLASEKRARPQEQLNCPRCNSTNTKFCYYNNYSLTQPRYFCKTCRRYWTEG<br>GTLRNPVGGGSRKNNKSTSSTASSSAISSSKNIIPDLNPPNLSHFSSQNPRMTHEGQDNLNLAFSANVEHYNG<br>TSTAHYVDHHQVPKMESNNSALELLRGTDIASRGVNSYIPNSIQDSNAVLYSSSGFSLQEFKPSHLGFCVDG<br>VGSRYGDHPQENGNGRLLFPFGDLKQISSTTGHELDQNKGQGNPNSGYWNGLMGGGSW  |
| 2   | <i>RcDof02</i> | MDTAQWPQEIGLEKTMEENMGSEARLASEKRARPQEQLNCPRCNSTNTKFCYYNNYSLTQPRYFCKTCRR<br>YWTEGGTLRNPVGGGSRKNNKSTSSTASSSAISSSKNIIPDLNPPNLSHFSSQNPRMTHEGQDNLNLA<br>VEHYNGTSTAHYVDHHQVPKMESNNSALELLRGTDIASRGVNSYIPNSIQDSNAVLYSSSGFSLQEFKPSHL<br>GFCVDGVGSRYGDHPQENGNGRLLFPFGDLKQISSTTGHELDQNKGQGNPNSGYWNGLMGGGSW   |
| 3   | <i>RcDof03</i> | MGEVEVSDSRPILERRGRPQKDQALNCPRCNSTTKFCYYNNYLSQPRYFCKTCRRYWTEGGSLRNPVGG<br>GSRKNNKRSSTSSSSPSTSNSKKLAHHDLVMTQQSADSQNPKIHQGQDNLAYPPTSDQDYDHHSIFNPS<br>SSRISTSSPHNLSAMELLKSTGINASRGLMNSFMAPISVADSNTITMFSTGNHGFPLNQEFKPRLSFSLDGYES<br>GYGSLQGVQDGTSHHHARLLFPTDQDLKQIPSGTTNTTSTEFEQNGGEHDHSGVYWGMLGGGSW   |
| 4   | <i>RcDof04</i> | MDTAHWPQGIGVVKSMSGVEVSDSRPILERRGRPQKDQALNCPRCNSTTKFCYYNNYLSQPRYFCKTCRR<br>YWTEGGSLRNPVGGGSRKNNKRSSTSSSSPSTSNSKKLAHHDLVMTQQSADSQNPKIHQGQDNLAYP<br>PTSDQDYDHHSIFNPPSSRISTSSPHNLSAMELLKSTGINASRGLMNSFMAPISVADSNTITMFSTGNHGFPLN<br>QEFKPRLSFSLDGYESGYGSLQGVQDGTSHHHARLLFPTDQDLKQIPSGTTNTTSTEFEQNGGEHDHSGV<br>YWNGMLGGGSW   |
| 5   | <i>RcDof05</i> | MVFSSIPGYLDPPNWQQQSTHHQQQGGGTDQNPPLLPPPPPSGGPGDAGNGGAGTIRPGSMSDLAR<br>QAKLPQPETALKCPCRESTNTKFCYFNNSLTQPRHFCKTCRRYWTRGGALRSVPVGGCRRNKRSKRSKS<br>PASASAADHHHQRQGGAGSSSTVSNSNCSTSENILGHЛАAPPHQFPFLPSLHLDYSSGGLSFGIQQP<br>SGVEFHQSQNIAGNSGGVGSIESTGMAEHWRSSLQHHQVQQQQFPFLANLEPPSSHGLYQFDDNQNN<br>AHTDASYGKGAGQLSKPLVDSSIGATQVANVKMEESQALNMSRNFLGSLGNDHHQYWGSTTAAGGINN<br>AWTDLSGFTSSTSHLL  |
| 6   | <i>RcDof06</i> | MPSDSDGPNNRRTKAQSLGAPPPEQEHLPCPRCESTNTKFCYYNNYNSQPRHFCKSCRRYWTHGGTLR<br>DIPVGGSRKNAKRSRTSSTGSMVSSASDHALPATPVFPVPSAGQGGAGGGAGQFGGCGVKGNVNGGSF<br>TSLLNTQPGFLALGGFGLGLATGFEEMFGFGGRAMWPFPVGSDGGAGIGANGVSHGMNTWQFENGET<br>GGLVNVGLGGGEFCSWPELAISTPGNGLK   |
| 7   | <i>RcDof07</i> | MQDPTTFQSMKAQFPEQEQLKCPRCDSNTKFCYYNNYNSQPRHFCKNCRKYWTKGGLSRNIPIGGGSRK<br>NTKRSSSGSKRSSSATNSSSTLSSAAVAAAAAAQNQDPQPDTRLYGPKMDPDNPMLDISGSFSSLTS<br>NDQFGSLMEGLNPNGSLKLMQMGEFGENVMDDSSGHGLNSDPGLEVQSSGNPGSYMGLQSGDSSCWNG<br>SNGWPDLAIYTPGSSFQ  |
| 8   | <i>RcDof08</i> | MMSSDNTPAKKPADDTQQGGGGSGNSRKTTSSKAPPEQALKCPCRDSPNTKFCYYNNYSLTQPRHFCKTC<br>RRYWTKGALRNPVPIGGGCRKNKVRSSSSRLCSLDSDKDGSASDHMGLKFFHGISPAMDFQLGGLSLPR<br>LNHHPSSSSAVVTALYNNNNSFSPYGDVSVTSGLTLDPSGGASGNSSFMGFNYPLTLGSGSGGGVGFQQ<br>NNMNSSMSVHSSLASSIESLSSINQDLHWKLQQQRLSMLFGTTIAGSDTQPSHKDMQMNDLEKNQTAPILF<br>QNLEISKPKESTGGDTAATEWWFGNSYAPPPVTPPTPTNSGGNGGGNENNAGNWNTHGVVQAWGDLQ<br>QYNALP   |
| 9   | <i>RcDof09</i> | MVFSSIPAYLDPANWQQQSPHHHHHLLSGSSNNNNNNSQLHPLPPPPPPSNPGGGSGPGG<br>AGSIRPGSMADRARMANIPMPEPALKCPRESTNTKFCYFNNSLTQPRHFCKTCRRYWTRGGALRNPVPG<br>GGCRRNKRSSKGSSRSKPVSSDRPTGSASSSGQAIPSNSTGTVDMIAAGLSPQIPPLRFINPFNDHHFA<br>ASENLGLNYGMNYGGGVGDLGFQIGSLGGGITSTPTGSLFEQQWRFQQQTPSFPFLSGGLDPSPGLFEG<br>SGVEFPGYNQVRPRLSSGSSVGVSSQMASVKTEDPHHQNHQQQELNLSRQLLGQGGNDSQYWINSGGN<br>GNGTSTTAWTDLSGFSSSTATNHP  |
| 10  | <i>RcDof10</i> | MIQELFGGAGFNTGGGGGERKISSSSTSPLSPSPSPSSSTTTTTAAAATTNSENLRCPCRCDSANAKFC<br>YYNNYNLTQPRHFCKTCRRYWTKGGALRNPVPIGGGCRKNKNGTMSTSISKTAVAGKLKTMAEIGRSGFGLG<br>FDHEVQASPLWLGPQNHSILALLRSSTPNPNPNPVQLCTNSVKEEGGMNMIGSHMMSESSGAGNDAMT<br>ARTLGLDPSSFWRNNQNQVPPHHQQQNGNLLGHEVQSNQGIQELFQRLRSSSSSGYYSHDLNNGVS<br>SSSTSSILEAAPAVGGEGLGYNWNPAFTWSDQLPTTNGAYP   |
| 11  | <i>RcDof11</i> | MSEGIRDPAIKLFGTIPLTEVPPLLSETVPAAEVVAAPDPGSDADRVSSSSNNSSPGGGGEVNDNKDGE<br>EEKEVNKDLSLGEKAIQNKEGDEVLPLSSKELMNPDAASRIGESPQKADPKETAALKTLKAEEQSETSNSQE<br>KTLKKPDKIIPCPRCNMSMDTKFCYYNNVNQPRHFCKNCQRYWTAGGTMRNPVGAQRRKNNTPSQYR<br>HITVSEAHNPTRPDVHPSVQSNGTVLTFCSDTPLCESMTSVLQLADKNLQNRTPNGFKSEGLVVPASYG<br>GGVNGDDHSNRSTVDSNSKDETSKPGPQEVMQNYPGFPQIPCFPGAPWPYPWPAQWSSPVSPPAF<br>CPPGYPMPFYAAYWCAVQGTWNMPWLPQPSSPNQTAGSSGSNSPTLGKHSRDENPLKQTSEEET<br>TKEKKAERCLWIPKTLRIDDPGEARSSIWATLGKNDKADSISSGGLFKAFRSKGDEKNHIAEASPVLQANPAA<br>LSRSLKFQESS |

(Continued on next page)

Supplemental Table 2. (Continued)

| No. | Name           | Protein sequence   |
|-----|----------------|--|
| 12  | <i>RcDof12</i> | MERSGWKPNDHHHICPNCPRCGSNTKFCYYNNYSLTQPRYFCKGCRRYWTKGGSLRNVPVGGGCRKN<br>RRGSRSRLRSTNTTTATGSNNVNSGTIGHNSYGISSSLPDSTSSGESNQDAPRIDLAVVYANFLNQPKVSR<br>SSGSDQLPEFPGEGLNPGLDFSSCIANMNIAGSGSSIQLVEENGLNGSIGCHSTLSENCGTINDHMYFGGLDP<br>LIDHHQKHQDHSSDLVQYASSTHESTDHGLPPLPDQEVLWSSHDQMMASPIMQPTPVLGSEAHDPNLLN<br>WSPLFDLPCNGTFSRT   |
| 13  | <i>RcDof13</i> | MQMVADSFSSKTKAKQESCLRLKGADCLFHPPFHQISYLYLHLIQQIHYSPTDFTLIDSETIEASRGTSRTR<br>VNMAEGSSIEGPQGKRLLQHLKDLHEENKTLMKLVADKEKRIESYMIDFEELNGLKIVNKLEKQNFVLTQANS<br>QMLQELRSLRNHVQKVEQPTLPPAQEINQVQEAQQPNLQPQEIIVQVQEAQQPNLQPAQARVQVQELEPPS<br>LQAAQASSQMEEELDLENSQILLELKSHDHMLSSTDEAVRECIDMIEKLQALGPPTNEAIQQVKAKLQALGP<br>LTIDA VRQIRVGFRKLQKLQCPRCESNNTKFSVLKAKRISAPRYHCKNCKMEWTLGGEIRKPIVLGGVNRRDLL<br>QQRIQRVQRRRMKT  |
| 14  | <i>RcDof14</i> | MQMVADSFSSKTKAKQESCLRLKGADCLFHPPFHQISYLYLHLIQQIHYSPTDFTLIDSETIEASRGTSRTR<br>VNMAEGSSIEGPQGKRLLQHLKDLHEENKTLMKLVADKEKRIESYMIDFEELNGLKIVNKLEKQNFVLTQANS<br>QMLQELRSLRNHVQKVEQPTLPPAQEINQVQEAQQPNLQPQEIIVQVQEAQQPNLQPAQARVQVQELEPPS<br>LQAAQASSQMEEELDLENSQILLELKSHDHMLSSTDEAVRECIDMIEKLQALGPPTNEAIQQVKAKLQAYFP<br>AALGPLTIDA VRQIRVGFRKLQKLQCPRCESNNTKFSVLKAKRISAPRYHCKNCKMEWTLGGEIRKPIVLGGVN<br>RRDLLQQRVQRRRMKT  |
| 15  | <i>RcDof15</i> | MSENEISPTIKLFGTIPLASAPHNGHEVPTNDDPPCGASDSA VDSKISAAAAAAAASGGGA VEEEDCSVKLP<br>CCSETTSPEDQENKETISGKEFTADKEEDNPLDQFTEDLKPPPTSSGISENPKTPSADRETSVSLKSSKNGEQ<br>SDETSCSQDKTLKKPDKILPCPRCNSMDTKFCYYNNYNVNQPRHFCKNCQRYWTAGGIMRNVPVGAGRK<br>NKNASSASHYRHMLMSDALQTAHATAAAAASNGAHNPTLGRNGTVLTFGSDSPLCESMASVNLNAEKTQNS<br>LQNGFHVPDQQRILVPSITGDNGDDQSSGSSNVTASNNSSEGGCKAGIQEPAVHCNCQSFSPHQVPCFPGPSPW<br>PYPWNSPQWTSAMPPPAAFFHSGFPISFYAPPYWGCTPGPSWNVPCNSPSSNLSHCGTSSGPNSTLGK<br>HPRGDILSQASSQKEESSRDNRSDRCVLIPTLRIDDPEAAKSSIWETLGKNEKGNSINEGGIFKSFSKGH<br>EKRVVQEQQSVLQANPAAFSRSLNFREIS |
| 16  | <i>RcDof16</i> | MDPSSGQQNQDMQAHNSLDMLVSSKSQQQTDRQKPRPQPEQALKCPCRDSTNTKFCYYNNYSLTQPRYF<br>CKSCRRYWTKGGLRNPVVGCCRKNKRSSKRSSQDPQPLTPNSNPLPSLSDYDSNDSLAFARLQRSC<br>GQLGYDDHLSMLGNPTTNHSIDLNGSSASA SPGFLDALRTGFLETHNSNSFQNNLYYFGSSGGNAMVG<br>EAEDMNGVCDEMGLHPYEELSNVATTAVT VTMKQELS RDIE TNNSRVLWGFPWQMNGDHHNLMNG<br>GGGGGGGAHHDQYDNGRESWNGLANPSWHGLLPLV  |
| 17  | <i>RcDof17</i> | MLMDQQKVMLISTTNDWPQIDDDDDQNHKGVMGSTAVGASRLMEKPGQEQLQQQQALKCPCRDSSN<br>TKFCYYNNYSLSPRHFCKACKRYWTRGTLRNPVVGCCRKNKRVKRPSSSAVDNGASSSASSAPNSG<br>NSNSNPNAQQNHPQIDLASNSNHMNQLFYGLGSNQPDHMGINPFTTSGFDQFSALGLGFDQRFNPAAT<br>SKQIQDVLVAANSLHSSDHISIFNSTSYSTAPTMASV LASKFINGGFKDVRGNNNQYFQNLLPFEDLQIAAGNG<br>GGQAVKEVKVEDGGQSRMGWSTNHQANTEQ MGLSSDPTLYWSSNLGANNWHDPANLGSSVSSLI   |
| 18  | <i>RcDof18</i> | MQGERDQQQQNQERKVNQQQQQQQQQQHHEPQKCPRCESLNTKFCYYNNYSLSPRHFCKAC<br>RRYWTQGGTLRNPVVGCCRKGKRAKT MSSSSATSSRSVLQPQQPQDPDHQLQQRPLMMSTGPAA LNS<br>LAGSQYYPGGTGYLTSMMPFAQSLNHLGGSQLGS GGASNLG LHFNAISSY GQRQMQLFKGNMEPP<br>PPYPSEDHRLQLQPARPSGSMTDWPESFINKSHASV ASAAAASAGQSA WTTTMGHNTSTSGAGPSLN<br>SAQWPDDIAGSFAAGPPQ  |
| 19  | <i>RcDof19</i> | MADRARLAKMPQPETALKCPRCESTNTKFCYFNNYSLSPRHFCKTCRRYWTRGGALRNVPVGCCRNNK<br>KS KTS SSSRSKSPVSASSTENQQMRQ TGSNPTGLISHLSQ PQT QSLP FLESIQNLSRYGGAGNMALNSFNEI<br>QQAQAQST DHMGFQIGAGNNLMNLSGGLGM DWQRNLQ QIPFLG STGF DLS STT GLY P FQNTTEGV DQD QLS<br>QLRTANSRV TTHQVPPVKVEDHNHGVLTRPFLGISGNNNNNNNNNNNQFWGGNAWT DISGTLNSSTS<br>SSTS HLL  |
| 20  | <i>RcDof20</i> | MVFSSIPVYLDPPNWHQQPQHPLGSGSTENHPQLPPLPRQFGTHVGGCGGT DGS GSGTGSIRPNSMAD<br>RARLAKMPQPETALKCPRCESTNTKFCYFNNYSLSPRHFCKTCRRYWTRGGALRNVPVGCCRNNKKS<br>TSSSSRSKSPVSASSTENQQMRQ TGSNPTGLISHLSQ PQT QSLP FLESIQNLSRYGGAGNMALNSFNEI QQA<br>QAQST DHMGFQIGAGNNLMNLSGGLGM DWQRNLQ QIPFLG STGF DLS STT GLY P FQNTTEGV DQD QLS<br>ANSRV TTHQVPPVKVEDHNHGVLTRPFLGISGNNNNNNNNNNNQFWGGNAWT DISGTLNSSTS<br>HLL  |
| 21  | <i>RcDof21</i> | MAEVSSGQDVPGI KLF GTT ITLQNKQVVLKDDHHHRQPKKADDVHDQT VEMKRPEKIIPCRC KSMETKFCY<br>FNNY NVNQPRHFCKGCQRYWTAGGALRNVPVGAGRRKT KPPGREL AFPEG CLYDASE VVQQFELDGVV<br>EEWHVAAVQGDFRHVFPVVKRRRGSPGQTC S  |

(Continued on next page)

Supplemental Table 2. (Continued)

| No. | Name           | Protein sequence  |
|-----|----------------|---|
| 22  | <i>RcDof22</i> | MGLSTKQVSSDGLDWSQTLLQAQSFEKPCKPPVARRQQQQSEQEQLKCPCRESTNTKFCYYNNYNKTQ<br>RHFCRACKRHWTNGTTLRNPVGGHGRKNKRTRKPNSASAAKTTNGACTIATTRVNNTNLVFDRDG<br>VQRLLNSHLAIDGEQRLNSHTGVLLPQQNLITSSELDNHIFSSSSGLSSTTMPFNFLQAGRAQVLSFPFSSST<br>SSSFDAISTSFQSPNVCNYPQEFSMEEPTITSIMPSTTIPQANNSSVGMYTSNDWNWEDIETLVSTDLNVP<br>WDDSDMN  |
| 23  | <i>RcDof23</i> | MFTAPVLEQMLHSQSGQLVMNQPKSWKPHVEAPNCPRCASSNTKFCYYNNYLSQPRYFCKGCRRYWTK<br>GGSLRNVPVGGSRKNRGRSRVLSQADRVSLSYRHNSSSGSGDLSEQSMEGGANGSDIDLAAVFAK<br>FLNQDSSNEHDPNLVGESIPDDVHEANALSDHSQISSNPEEDLLESVELLEGVLVDQDHQLHEEDNIQN<br>MGNQHHHDLIHEFGLQGLLGDEDVFWTDTTASLTSFTWQELDNSFPSNDDDHMKISTTTNLCSDN<br>WSSFDMSGLEVFGSGS   |
| 24  | <i>RcDof24</i> | MPASTTPPEVSGIKRPRLIQSMPAAYPPIPSSADPLPCPRCNSTQTKFCYYNNYLAQPRHFCKGCRRYWTRG<br>GALRNVPVGGSRKNRGRSRVLSQADRVSLSYRHNSSSGSGDLSEQSMEGGANGSDIDLAAVFAK<br>DNVTSEGGFSFLTMMAQEQPGLSLGGYYGYGSGFAHGFQQGYGYGARAAGVDFPTEMVGAFAGVYGV<br>GAAASSGFDVNTWQMAVADHEGGGGLVDWPEVQILDSPAVSGGGVKEEKVAADTPDCLNFP<br>MDTAQWPQGIVVKPIIEIVTNTCPKPSSANNLERKLARPQKESALNCPRCNSTNTKFCYYNNYSLTQPRYFC  |
| 25  | <i>RcDof25</i> | KTCRRYWTEGGSLRNIPVGGSRKNKRSSSSSSTTSNISNNSKRLPDLIQPQGNQGNQGQDNLNGFPSN<br>QVFAQHQIPNIDQNSDKNNNSSTTTASHLSALELLTGLTSRGLNSFMPMPVPNSDPNSSNSASVYTSGF<br>PMSELMIKPTLNFSLDGLGSGYGSALQGHVQENSGRLLFPFEDLKQVSGSGSIDHHHQNNKEHHGGDS<br>TGWTGMLGGTW  |
| 26  | <i>RcDof26</i> | MGHNSLQVCMDSSDWLQGSIHEDQSPMDSSPLSGDILTCRPLIERRLRPPHDQSLKCPCRDSTHTKFCYY<br>NNYSLSQPRYFCKTCRRYWTKGTLRNIPVGGSRKNNKVASKKPNEDHSPSMNPNLGSSSSSQHNPTD<br>LQLSPDQMQLFQSHLNLMGAQGTGNPNFMDGRYGNINSMLENSNQLRPIDFMESKLEAIVGNARNYDFMG<br>SGGDGLGMVGGLSNMGAHGLESGFHGFCSSSGSMSLDHHGNNNGTNFMDTCQRLMLPYEANEDHHHH<br>QNEIHDVKPNTKLLSLEWDQGCSDAGKESSYGYLNGLGTWTGVMNNYGPSTTNSLV  |
| 27  | <i>RcDof27</i> | MQDIQSIGGRFFGGAGGGDRRLRPHHNQNQHQLKCPRCDINTKFCYYNNYLSQPRHFCKSCRRYWTKG<br>GVLRNVPVGGSRKTKRSKPSSSSSPASPPQPNNSERRASSNSESSESSLNTATATAEAVSAPSSTSS<br>ASNLLNIPRPESKFFVSPSTNFEAAGATMLEQSATEIGMFSDIGSFSLITASSNDVPGFTNINDISVSPFRPN<br>QPPAGHLNQVQQNQWEQQHNQNQELKMPEITGGMLDQTAQVNLSVFQTKINGGGGGFGSLDWQPAG<br>DQGLFDLPNTVDQAYWSQQQWSDQDPPSYLP   |
| 28  | <i>RcDof28</i> | MLQETKDPAIKLFGMKIPPLAAGDYDDLGFMPGKRVVDQEHEEEEEEEKDSPSTKDTEIESIEQDDSPKTE<br>DSTSMEMLPDCNVNPKTPSIDEESAKSKAPEKEQNDATNLQEKTLPDKILPCPRCNMSMTKFCYYNNY<br>VNQPRHFCKACQRYWTAGGTMRNVPVGAGRRKNKNSASHYRQITISEALQVAQMDAPNGAQHPGLKSNG<br>RVLNFGVDALVCDMASVLHLADKKGLNGTTRNGFHKSEELVFPAPCKGRESGDDCSIASSVTASKPQIQNA<br>NGFSAQIPCLPGVPWPYQWNSAVPPPAGPPGMPIPVFPAAYWNCGVPFQVPWFSPQAASPNQSPNS<br>ASSSPTLGKHSREGEMFRPEVHLEKEEPSKKKNGFVLVPKTLRIDDPSEAAKSSIWTTLGIKNDCISSGNGLFK<br>AFQSQSKGDQKKHVPETSPALLANPAALSRSLNFHERT |

Supplemental Table 3. The coding sequence (CDS) information of *RcDof* members.

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### Supplemental Table 3. (Continued)

*(Continued on next page)*

Supplemental Table 3. (Continued)

*(Continued on next page)*

Supplemental Table 3. (Continued)

*(Continued on next page)*

Supplemental Table 3. (Continued)

Supplemental Table 4. Information for *RcDof* genes in the *Rosa chinensis* genome.

| Gene name      | Gene_id        | MW        | Instability index | Aliphatic index | pI   |
|----------------|----------------|-----------|-------------------|-----------------|------|
| <i>RcDof01</i> | XP_024191320.1 | 30,330.41 | 40.53             | 53.01           | 8.68 |
| <i>RcDof02</i> | XP_024191312.1 | 30,881.92 | 41.35             | 52.42           | 8.18 |
| <i>RcDof03</i> | XP_024178062.1 | 31,047.02 | 48.98             | 49.96           | 8.56 |
| <i>RcDof04</i> | XP_024178056.1 | 32,654.87 | 46.39             | 51.04           | 8.55 |
| <i>RcDof05</i> | XP_024191900.1 | 39,093.84 | 55.67             | 52.7            | 8.84 |
| <i>RcDof06</i> | XP_024181122.1 | 24,530.25 | 38.73             | 47.36           | 8.73 |
| <i>RcDof07</i> | XP_024186158.1 | 24,435.83 | 52.43             | 43.29           | 8.19 |
| <i>RcDof08</i> | XP_024184677.1 | 38,109.93 | 50.14             | 50.17           | 8.77 |
| <i>RcDof09</i> | XP_024188573.1 | 40,270.28 | 54.37             | 51.73           | 9.36 |
| <i>RcDof10</i> | XP_024185752.1 | 34,536.11 | 53.36             | 56.18           | 8.98 |
| <i>RcDof11</i> | XP_024181086.1 | 55,193.15 | 57.94             | 52.93           | 5.84 |
| <i>RcDof12</i> | XP_024190311.1 | 32,938.2  | 47.84             | 7.78            | 6.02 |
| <i>RcDof13</i> | XP_024189477.1 | 43,583.13 | 63.72             | 88.52           | 8.72 |
| <i>RcDof14</i> | XP_024189476.1 | 44,132.76 | 64.02             | 87.89           | 8.72 |
| <i>RcDof15</i> | XP_024194374.1 | 56,830.46 | 52.28             | 51.69           | 5.73 |
| <i>RcDof16</i> | XP_024161212.1 | 34,539.9  | 50.69             | 51.71           | 6.78 |
| <i>RcDof17</i> | XP_024158293.1 | 37,542.15 | 42.01             | 54.87           | 7.68 |
| <i>RcDof18</i> | XP_024159083.1 | 31,843.12 | 65.05             | 44.44           | 9.48 |
| <i>RcDof19</i> | XP_024158076.1 | 31,481.68 | 49.36             | 54.39           | 9.62 |
| <i>RcDof20</i> | XP_024158075.1 | 38,379.23 | 48.32             | 53.16           | 9.39 |
| <i>RcDof21</i> | XP_024159528.1 | 19,522.23 | 49.93             | 60.17           | 9.12 |
| <i>RcDof22</i> | XP_024199643.1 | 32,750.17 | 62.95             | 56.85           | 8.16 |
| <i>RcDof23</i> | XP_024166964.1 | 33,218.16 | 44.75             | 62.91           | 4.82 |
| <i>RcDof24</i> | XP_024165790.1 | 29,458.66 | 45.43             | 53.19           | 6.51 |
| <i>RcDof25</i> | XP_024174860.1 | 32,619.89 | 43.96             | 55.9            | 8.87 |
| <i>RcDof26</i> | XP_024173084.1 | 37,471.48 | 41.41             | 52.63           | 6.36 |
| <i>RcDof27</i> | XP_024174414.1 | 34,330.7  | 67.92             | 51.74           | 8.52 |
| <i>RcDof28</i> | XP_024172633.1 | 51,227.54 | 56.28             | 56.45           | 6.58 |

MW = molecular weight, pI = isoelectric point.

Supplemental Table 5. The *cis*-regulatory elements of *RcDofs*.

| Cis-acting element | Sequence | The function notes  |
|--------------------|----------|---|
| ARE                | AAACCA   | <i>cis</i> -acting regulatory element essential for the anaerobic induction |
| ABRE               | ACGTG    | <i>cis</i> -acting element involved in the abscisic acid responsiveness     |
| TGA-element        | AACGAC   | auxin-responsive element  |
| LTR                | CCGAAA   | <i>cis</i> -acting element involved in low-temperature responsiveness       |
| GARE-motif         | TCTGTTG  | gibberellin-responsive element  |
| P-box              | CCTTTG   | gibberellin-responsive element  |
| MBS                | CAACTG   | MYB binding site involved in drought-inducibility                           |
| TATC-box           | TATCCA   | <i>cis</i> -acting element involved in gibberellin-responsiveness           |
| TGA-box            | TGACGTAA | part of an auxin-responsive element   |