Genome-wide identification, phylogenetic and expression pattern analysis of Dof transcription factors in blueberry (*Vaccinium corymbosum* L.)

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Background

DNA binding with one finger (Dof) proteins are plant-specific transcription factor (TF) that plays a significant role in various biological processes such as plant growth and development, hormone regulation, and resistance to abiotic stress. The Dof genes have been identified and reported in multiple plants, but so far, the whole genome identification and analysis of Dof transcription factors in blueberry (*Vaccinium corymbosum* L.) have not been reported yet.

Methods

Using the Vaccinium genome, we have identified 51 *VcDof* genes in blueberry. We have further analyzed their physicochemical properties, phylogenetic relationships, gene structure, collinear analysis, selective evolutionary pressure, cis-acting promoter elements, and tissue and abiotic stress expression patterns.

Results

51 *VcDof* genes were divided into eight subfamilies, and the genes in each subfamily contained similar gene structure and motif ordering. A total of 24 pairs of colinear genes were screened; *VcDof* genes expanded mainly due to whole-genome duplication, which was subjected to strong purifying selection pressure during the evolution. The promoter of *VcDof* genes contains three types of cis-acting elements for plant growth and development, phytohormone and stress defense responsiveness. Expression profiles of *VcDof* genes in different tissues and fruit developmental stages of blueberry indicated that *VcDof2* and *VcDof45* might play a specific role in anthesis and fruit growth and development. Expression profiles of *VcDof* genes in different stress indicated that *VcDof1*, *VcDof11*, and *VcDof15* were highly sensitive to abiotic stress. This study provides a theoretical basis for further clarifying the biological function of *Dof* genes in blueberry.

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stress indicated that *VcDof1*, *VcDof11*, and *VcDof15* were highly sensitive to abiotic stress. This study provides a theoretical basis for further clarifying the biological function of *Dof* genes in blueberry.

Keywords

Dof transcription factor, Phylogeny, Expression pattern analysis, Blueberry

1 Introduction

Blueberry is a perennial woody plant that belongs to the genus Vaccinium. It is one of the five healthy fruits recommended by the Food and Agriculture Organization of the United Nations; it is rich in anthocyanins, vitamins, flavonoids, and other active ingredients. It has the functions of protecting eyesight, preventing type 2 diabetes, anti-aging, and anti-cancer. It is known as the 'king of berries' (Ma et al., 2018; Ono-Moore et al., 2016; Istek & Gurbuz, 2017). Due to various health benefits of blueberries and awareness of healthy diets, consumption of blueberries and their derivatives increased tremendously. So far, blueberry has grown in 71 countries (Gallardo et al., 2018). Rapidly changing climatic conditions may drastically affect the global production of blueberries in the coming years. So, understanding and identifying genetic factors' response to biotic and abiotic stress tolerance is imperative. Research shows that salt and drought stress alone in blueberry causes 25-30% yield losses (Wang et al., 2021).

A transcription factor (TF) is a DNA binding protein that can specifically interact with cis-acting elements of genes and regulate the specific expression of target genes. Understanding its structure and function is very important to recognize the biological role of the gene in the given context (Chen et al., 2017). DNA binding with one finger (Dof) TFs consist of 200-400 amino acids. Dof includes the DNA-binding domain and domain involved in transcriptional activation or repression and exhibits regulatory roles in nuclei. But, except for the highly conserved domain of Dof, the amino acid sequences are not well conserved, this creates a greater diversity across family members, and individual proteins exhibit very divergent sequences (Gupta et al., 2015; Chattha et al., 2020; Liu et al., 2020; Wu et al., 2016; Cominelli et al., 2011; Yang et al., 2011),

which may be an important reason for the functional diversity of Dof proteins and involved in varied plant physiological and biochemical processes (Yanagisawa, 2002).

In 1993, the first Dof transcription factor *ZmDof1* was identified and cloned in maize (Yanagisawa & Izui, 1993). With the recent developments in genome sequencing and bioinformatics capabilities, multiple Dof TFs have been identified in *Arabidopsis thaliana*, *Solanum tuberosum*, *Solanum lycopersicum*, *Oryza sativa*, and *Zea mays* (Lijavetzky, Carbonero & Vicente-Carbajosa, 2003; Venkatesh & Park, 2015; Cai et al.,2013; Jiang et al.,2012; Moreno-Risueno et al., 2007). Dof TFs have been shown to play an important role in plant growth and development, primary and secondary metabolism, hormone regulation, and abiotic stress resistance (Renau-Morata et al., 2017; Yanagisawa, 2004; Skirycz et al., 2007; Li et al., 2022). Specifically, inducible overexpression of *AtDOF5.4/OBP4* in Arabidopsis promoted early endocycle onset, inhibited cell expansion, and reduced cell size and number, resulting in dwarf plants (Xu et al., 2016). *AtDOF4.7* regulates the expression of cell wall hydrolysis enzymes; overexpression of *AtDOF4.7* causes an abscission-related polygalacturonase gene *PGAZAT* down-regulation, which affects the shedding of flower organs (Wei et al., 2010). *AtDof6* can regulate seed germination by interacting with TCP14 protein and affecting ABA anabolism (Rueda-Romero et al., 2012). Overexpression of *GmDof4* and *GmDof11* genes in *Glycine max* increased the content of total fatty acids and lipids of transgenic Arabidopsis seeds (Wang et al., 2007). Overexpression of tomato Dof gene family member *TDDF1* in plants can improve tomato tolerance to drought, salt, and various hormone treatments (Ewas et al., 2017). In *Tamarix hispida*, *ThDof1.4* enhances proline levels, reactive oxygen species (ROS) scavenging capacity,

and tolerance to salt and osmotic stress (Zang et al., 2017). Many studies have shown that the Dof transcription factor gene family plays an important role in plant growth, development, and resistance to abiotic stress.

The haplotype-phased genome assembly of blueberry was published in 2019 (Colle et al., 2019). So far, there are few reports on the role of Dof TFs in abiotic stress tolerance. Therefore, we aim to identify Dof TFs in blueberry using the whole genome of blueberry, and we further analyzed gene structure, phylogenetic relationships, collinear analysis, and the expression pattern of *VcDof* genes under abiotic stress in different tissue types was analyzed using RNA-Seq and qRT-PCR. In summary, the present research provides important information on the potential function of the blueberry Dof TFs in abiotic stress tolerance.

2 Materials & Methods

2.1 Identification and characterization of Dof transcription factors in blueberry

The blueberry genome and annotation data were downloaded using the Vaccinium database (GDV, https://www.vaccinium.org). The Dof TFs protein sequences of Arabidopsis and rice were used as query sequences (https://phytozome-next.jgi.doe.gov/). The blueberry genome was compared by Basic Local Alignment Search Tool Protein (BLASTP) in a Linux system, and the screening threshold E-value was 1e-5. The protein sequences of candidate genes were obtained. Furthermore, the Dof domain, PF02701, was used to search the blueberry genome using HMMER software (Potter et al., 2018). By integrating the results of the above two steps, the sequences of these genes were submitted to SMART (http://smart.embl.de/smart/batch.pl) and NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd/) to remove redundant and non-conservative

genes (Letunic & Bork., 2018; Lu et al., 2020). Then, the gene sequences of blueberry Dof TFs were obtained, named according to their position on the chromosome scaffold.

The amino acid number, theoretical isoelectric point (pI), and molecular weight (MW) of *VcDof* genes were analyzed online using the ProtParam tool (https://web.expasy.org/protparam). Subcellular localization information of *VcDof* genes was predicted using CELLO (http://cello.life.nctu.edu.tw) and WoLF PSORT (https://wolfpsort.hgc.jp).

2.2 Multiple sequence alignments and phylogenetic analysis

The protein sequences of Dof TFs in *Arabidopsis thaliana* and *Oryza sativa* were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) and phytozome (https://phytozome-next.jgi.doe.gov/). Multiple sequence alignments (MSA) were performed using MegaX software (Kumar et al., 2018). Based on multiple sequence alignment results, a phylogenetic tree was constructed using the maximum likelihood (ML) method; the optimal fitting model was selected as JTT+G, and the number of Bootstrap tests was adjusted to 1000. The resulting phylogenetic tree file was enhanced using iTol (https://itol.embl.de/) (Letunic &Bork, 2021).

2.3 Gene structure analysis of the Dof transcription factors

Based on the blueberry genome structure annotation information file, the number and location of exons/introns of *VcDof* genes were counted by Gene Structure Display Server (GSDS2.0, http://gsds.gao-lab.org, Hu et al., 2015). The conservative motif of blueberry Dof protein was analyzed online by Maximization for Motif Elicitation program (MEME, https://meme-suite.org/meme/tools/meme, Bailey et al., 2015). The TBtools were used for visualization (Chen et al., 2020).

126 **2.4 Gene duplication event and synteny analysis**

127 Replication events of *VcDof* genes in blueberry were analyzed using the multiple linear
128 analysis tool MCscanX (Tang et al., 2008) and visualized using the Advanced Circos tool,
129 TBtools. Using Ka/ks ratio to display evolutionary selection pressure between collinearity gene
130 pairs, Ka/ks>1, =1, <1 indicated positive selection, neutral evolution, and purifying selection,
131 respectively. Further, $T=Ks/2\lambda$ (T: calculates divergence time; Mya: million years; λ:
132 replacement rate, $\lambda=1.3x10^{-8}$) was used to compute the approximate date of duplication and
133 divergence events (Colle et al., 2019).

134 **2.5 Promoter cis-acting element analysis**

135 Extract the sequence of the promoter region 2000 bp upstream of the start site of each
136 *VcDof* gene using Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) to
137 predict the cis-acting elements of *VcDof* genes and speculate their functions.

138 **2.6 Expression profile of Dof transcription factors in different tissues**

139 Using the RNA-Seq data, the expression level of *VcDof* genes in different tissues and fruit
140 development stages were identified (https://www.vaccinium.org/; and NCBI accession number:
141 PRJNA494180). The transcript abundance was estimated using FPKM (fragments per kilobase
142 per million measure) after $\log_2$ conversion (Colle et al., 2019). Hierarchical clustered heatmaps
143 and visualization were done using the TBtools.

144 **2.7 Plant material and experimental treatments**

145 In this study, the saplings of the northern highbush blueberry 'Bluecrop' preserved in the
146 laboratory with relatively consistent growth potential and no pests and diseases were used as

147 experimental materials, and the mixed nutrient soil and vermiculite were used as cultivation soil
148 (mixed ratio 1:1). The effective components of nutrient soil were nitrogen 140 mg/kg^{-1},
149 phosphorus 100mg/kg^{-1}, potassium 180mg/kg^{-1}, organic matter content 91.3%, PH 5.5. The
150 biennial sapling plants were placed in the greenhouse of Tianjin Agricultural University. The
151 deionized water and 1/2 Hoagland nutrient solution were regularly irrigated to make the plants
152 grow under the optimal growth conditions.

153 This experimental design was a completely randomized design with three treatments. The
154 specific treatments were as follows: 150 mMol/L NaCl was used to irrigate blueberry plants to
155 simulate salt stress; 20% PEG 6000 was used to irrigate the plants to simulate drought stress; and
156 ABA hormone 100 μMol/L was used to spray blueberry leaves to simulate adversity stress. After
157 0(control), 3, 6, 12, and 24 h of each treatment, leaf samples were collected. Triplicate leaf
158 samples were collected for each time point and treatment and frozen in liquid nitrogen. The
159 samples were stored in an ultra-low temperature freezer at -80℃ (Bano et al., 2021; Han, Wu &
160 Li, 2021).

161 **2.8 RNA isolation, cDNA preparation, and quantitative RT-PCR analysis**

162 The RNA extraction was done using the Easypure Plant RNA Kit (TransGen Biotech,
163 China). After gel electrophoresis detected clear RNA bands and apparent separation, cDNA was
164 synthesized using the kit PrimeScript TMRT Master Mix (TaKaRa BIO INC., Japan). The
165 blueberry EIF *(VcEIF)* gene was used as the reference (Deng, Li & Sun, 2020), and the specific
166 primers for the target gene and the reference gene were designed using Premier 5.0 software. The
167 instrument used for qRT-PCR was qTOWER 2.2 (Analytik Jena, Germany), and the qRT-PCR

reaction was performed using the iTaq Universal SYBR® Green Supermix (Bio-Rad INC., USA). The relative expression levels were calculated as $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen, 2001). Statistical analysis was conducted using SPSS 26.0 (SPSS Inc., USA). Each treatment was compared to the control (0h) using parametric one-way ANOVA. Error bars indicate standard deviation, and asterisks indicate significant differences between the treatments and control, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

3 Results

3.1 Genome-wide identification of Dof transcription factors in blueberry

The 51 blueberry Dof TFs were identified and named *VcDof1* to *VcDof51* according to the position of the genes on the chromosome scaffold (Table 1). The length of amino acids varied greatly, with VcDof40 the longest, containing 493 amino acids, and VcDof36 the shortest, containing 118 amino acids. The analysis of physicochemical properties using ProtParam showed that the molecular weight of *VcDof* genes was between 13621.8 and 53701.66, with an average of 33638.36. The theoretical isoelectric point was 4.56-10.56 for both acidic and alkaline proteins. The theoretical isoelectric point of *VcDof15* is the smallest, showing a higher precipitation coefficient. The theoretical isoelectric point of *VcDof36* is the largest, suggesting that it has strong solubility and weak precipitation ability. Subcellular localization prediction showed that most *VcDof* genes were located in the nucleus.

3.2 Phylogenetic tree of the DOF transcription factors in blueberry

To further understand the evolutionary relationship of Dof TFs, the amino acid sequences of Dof genes in blueberry, Arabidopsis, and rice was compared using the MegaX software. The

results showed that (Fig. 1), all Dof genes were divided into four main subfamilies (subfamilies A–D), which could be divided into multiple subfamilies, A, B1, B2, C1, C2.1, C2.2, C3, D1, and D2 with supported bootstrap values. Analysis of each subfamily found that the D1 subfamily contained the largest number of Dof TFs, consistent with Wei's finding that the D1 subfamily had the most members in the eggplant phylogenetic tree (Wei et al., 2018). The Dof TFs of dicotyledonous blueberry, Arabidopsis, and monocotyledonous rice did not show apparent separation in the phylogenetic tree, indicating that in the long evolutionary process, Dof TFs did not appear obvious differentiation between monocotyledonous and dicotyledonous plants, may contain similar functions. Therefore, the Dof TFs are relatively conserved in the evolution of plants. Interestingly, *VcDof* and *OsDof* genes did not appear in the C3 subfamily.

3.3 Gene structure and conserved motifs analysis of the DOF transcription factors

In order to clarify the genetic structure of the *VcDof* genes, the conserved motifs of *VcDof* genes were analyzed by MEME. The results showed that ten motifs (motif1-motif10) were obtained (Supplementary Fig.S1). Motif1 was the conserved motif of the Dof transcription factor, and motif1 was contained in each identified *VcDof* gene, proving the identification results reliability. The arrangement and number of motifs in each subfamily were the same(Fig. 2A). Studies have shown that the gene's distribution of exons and introns is closely related to the evolution of the gene family. Gene structure analysis results show that *VcDof* genes contain coding sequence (CDS) and untranslated region (UTR) with different numbers and lengths(Fig. 2B). The number of introns in *VcDof* genes was 0-3, 66.7% of *VcDof* genes contained only one intron, *VcDof4, VcDof26,* and *VcDof33* had three introns, and members of the same subfamily

had similar numbers of introns. There are differences in intron length between different subfamilies, possibly due to the absence or increase of introns in the long-term evolution process. *VcDof* genes belong to the exon-poor subgroup, indicating that blueberry Dof TFs are relatively conservative in the evolutionary process (Hu et al., 2015).

3.4 Collinearity gene pair and divergence time analysis of the Dof transcription factors in blueberry

Due to the incomplete splicing and chromosomal assembly of the blueberry genome, the genes can only be located on the chromosomal scaffold of the blueberry. The results showed that (Fig. 3), 24 pairs of collinearity genes were found on 21 chromosome scaffolds, showing uneven distribution; VaccDscaff19 and VaccDscaff11 contained the most collinearity genes, which were four pairs. We have found only a pair of collinearity genes in the chromosome 9 scaffold. The non-synonymous/synonymous mutation ratio (Ka/ks) is a common tool to study the selection pressure of gene evolution. This study calculated the evolutionary selection pressure of *VcDof* collinearity gene pairs. The results showed that the ka/ks ratio of 95.83% collinear gene pairs were all less than 1, subjected to purifying selection during the evolution process (Table 2), while *VcDof7-VcDof24* this pair of genes was subjected to positive selection. Overall, the blueberry Dof TFs were subjected to intense purifying selection pressure during the evolution process. Whole-genome duplication (WGD) events provided the main driving force for the evolution of the *VcDof* genes. The collinear gene pairs divergence events time were meristems 0.9644-105.5069 Mya ago.

3.5 Promoters analysis of the Dof transcription factors in blueberry

Transcription factors bind to cis-acting elements of promoters to initiate gene transcription, and promoters are key factors that determine the spatiotemporal expression and transcription levels of genes (Brázda, Bartas & Bowater, 2021). In this study, PlantCARE was used to analyze the cis-acting promoter elements of 2000 bp upstream of the start site of the blueberry Dof TFs (Fig. 4). All cis-acting promoters were classified into three categories according to their functions: plant growth and development, phytohormone responsiveness, and stress defense responsiveness. Plant growth and development-related elements analysis showed that the 'as-1 promoter' was most distributed in *VcDof* genes, accounting for 32%; and 12% of *VcDof* genes contain an 'O2-site' promoter related to cis-acting regulatory element involved in zein metabolism regulation. Among the phytohormone responsiveness elements, ABRE (abscisic acid responsiveness) was the most numerous promote (30%), which was the ABA-responsive element. 86.3 % of the *VcDof* genes promoter contained ABRE elements, suggesting that the blueberry Dof TF played an important role in regulating abscisic acid. STRE (thermal stress responsive element) was the promoter with the highest proportion (28%) in the stress defense responsiveness elements, and it also contained TC-rich repeats (6%), LTR (long terminal repeats, 9%), and MBS (MYB binding site, 3%) promoters. Studies have shown that these promoters are involved in defense and stress tolerance (Wang et al., 2018). *VcDof11* gene contains 25 abiotic stress-related promoters, one of the most stress tolerance elements in *VcDof* genes. It is speculated that *VcDof11* plays an important role in the response of blueberry to abiotic stress.

3.6 Expression profiles of blueberry Dof transcription factors in different tissues and fruit development stages

Using the RNA-seq data, we have explored the expression profile of different tissues at different time points. A heatmap of Dof TFs for root, shoot, leaf (day and night), flower (bud, anthesis, and petal), and fruit (green, pink, and ripe) is available in Fig. 5 (Zong et al., 2021). It was found that, except for *VcDof24*, *VcDof35*, and *VcDof36*, other blueberry Dof TFs were detected in various blueberry tissues. Overall, all blueberry Dof TFs were highly expressed in the root. Among them, a total of 15 *VcDof* genes, *VcDof8, VcDof9, VcDof14, VcDof17, VcDof19, VcDof20, VcDof25, VcDof31, VcDof34, VcDof38, VcDof40, VcDof45, VcDof47, VcDof48*, and *VcDof49* were highly expressed in different tissues and fruit development stages of blueberry, suggesting that they may play an important positive regulatory role in blueberry growth and development. However, the expression levels of 7 *VcDof* genes, including *VcDof1, VcDof2, VcDof5, VcDof10, VcDof13, VcDof23*, and *VcDof42* were relatively low in different tissues and fruit development stages of blueberry. Therefore, they may play a negative feedback regulation role in blueberry growth and fruit ripening. Interestingly, the expression level of *VcDof45* gradually increased during the process of blueberry fruit ripening (green to ripe), while the expression level of *VcDof2* gradually decreased. It is speculated that these two genes play important regulatory roles in the development of blueberry fruit. The remaining blueberry Dof TFs showed different expression levels in different tissues, which may have different biological functions.

3.7 Expression profiles of blueberry Dof transcription factors in response to salt, drought and abscisic acid

Previous studies have shown that Dof TFs are widely involved in the biological processes of plants responding to abiotic stresses. To clarify the possible biological functions of blueberry Dof TFs under abiotic stress, we selected eight genes with stress defense response elements in promoters. We performed qRT-PCR to analyze their expression profiles under the abiotic stress .

The results showed that the expression of *VcDof* genes was regulated early by salt stress (Fig. 6A). With the prolongation of stress time, *VcDof1, VcDof11*, and *VcDof15* showed an up-regulated expression trend, and their relative expression levels were the highest at 24h, 8.54, 3.26, and 9.07 times that of the control group, significant differences have been noticed. *VcDof2* showed a down-regulated expression trend, and the relative expression level was the lowest at 24h, which was significantly lower than the control group. Short-term drought stress also caused changes in the relative expression levels of *VcDof* genes (Fig. 6B). The relative expression levels of *VcDof5, VcDof11*, and *VcDof15* at 24 h of drought stress were 7.35, 18.47, and 14.48 times higher than those in the control group. The expression trend was down-regulated at 0-12 hours of stress and up-regulated at 24 hours. In general, the eight blueberry Dof TFs responded positively to drought stress in the early stage and were mainly up-regulated. Under ABA stress (Fig. 6C), the relative expression levels of *VcDof* genes changed significantly, and *VcDof1* and *VcDof2* were the highest at 24h stress, which was 9.59 and 25.28 times that of the control group. The relative expression levels of *VcDof5, VcDof14, VcDof15*, and *VcDof49* were the highest at 3h of stress, which were 20.40, 4.30, 5.13, and 29.26 times that of the control group. Blueberry Dof

TFs responded positively to ABA stress, and the relative expression changed significantly.

4 Discussion

4.1 Identification and characterization of *VcDof* genes

As a class of transcription factors with C2C2-Dof zinc finger structure in plants, Dof plays an important role in plant growth and development and stress resistance (Venkatesh & Park, 2015). In this study, we have identified 51 *VcDof* genes in blueberry, which was lower than the number of Dof TFs in Chinese cabbage (76 *BraDof* genes, Ma et al., 2015). The number of Dof TFs was similar to that contained in maize (46 *Zndof* genes, Chen & Cao, 2015), higher than that of Dof TFs in Arabidopsis (36 *Atdof* genes, Liu et al., 2020), tomato (34 *Sldof* genes, Cai et al., 2013), and pepper (33 *Cadof* genes, Wu et al., 2016). The results of multiple sequence alignment (Supplementary Fig.S2) showed that all blueberry Dof TFs contained Zinc-finger Dof conserved domains, and the results of gene structure and motif analysis showed that the *VcDof* genes in the same subfamily had similar exon or intron structure and motif ordering prove that blueberry Dof TFs are highly conserved (Wang et al., 2021).

4.2 Collinearity and duplication events analysis of *VcDof* genes

The Dof TFs of Arabidopsis, rice, and blueberry were constructed using MegaX to construct a phylogenetic tree, and the results showed that all Dof TFs were divided into 4 families (A-D) and nine subfamilies (A, B1, B2, C1, C2. 1. C2.2, C3, D1, and D2), in which the blueberry Dof gene was not found in the C3 subfamily. This is consistent with the results of studies by Wen and Lijavetzky found that cucumber *CsDofs* and rice *OsDofs* were lost in the C3

311 subfamily (Lijavetzky, Carbonero & Vicente-Carbajosa, 2003; Wen et al., 2016).

312 Gene duplication often occurs among gene family members, making gene function-specific
313 and diverse, which is one of the main driving forces for plant genome evolution. Whole-genome
314 duplication (WGD) promotes chromosomal recombination, gene doubling, and diversification of
315 gene functions (Van de Peer, Maere & Meyer, 2009). Bowers's study showed two recent WGD
316 events occurred in Arabidopsis: the β whole-genome duplication event and the α whole-genome
317 duplication event (Bowers et al., 2003). The amplification sources of 36 Arabidopsis Dof TFs are
318 mainly β-genome duplication events and tandem duplication events (Wang, Tan & Paterson,
319 2013). The results of collinearity analysis showed that there were 24 pairs of collinearity gene
320 pairs in 51 blueberry Dof TFs, all of which belonged to WGD. The Ka/ks ratio of genes except
321 *VcDof7*-*VcDof24* was less than 1, indicating that the WGD event of *VcDof* genes was the result
322 of purifying selection. The repeated divergence events of monocotyledonous and dicotyledonous
323 plants occurred before 170-235 Mya (Blanc & Wolfe, 2004). In this study, the divergence time
324 of the collinearity gene of blueberry Dof TFs was before 0.9644-105.5069 Mya and later than
325 that of monocotyledonous plants and dicotyledonous plants, which also explained why the Dof
326 TFs of dicotyledonous plants Arabidopsis, blueberry and monocotyledonous plants rice in the
327 evolutionary tree of this study did not have obvious segregation in clustering.

328 **4.3 Tissue specific expression of *VcDof* genes**

329 The analysis of gene expression patterns can reflect the function of genes to a certain extent.
330 The tissue expression analysis results of this study showed that blueberry Dof TFs were
331 differentially expressed in different tissues and developmental stages, but the overall expression

was higher in the root. This is consistent with the experimental results that the Dof transcription factors were highly expressed in cucumber and pepper root tissues (Wu et al., 2016; Wen et al., 2016). The expression levels of *VcDof2* and *VcDof45* continued to change during blueberry flowering and fruit development. Previous studies have shown that *AtDof4.1*, as a transcription inhibitor, delays the flowering of Arabidopsis and inhibits the development of reproductive organs, resulting in smaller leaves, flowers, and siliques (Ahmad et al., 2013). In rice, under dark conditions, the *OsDof12* (*Rdd1*) gene was inhibited, while the expression was up-regulated under light conditions. Over-expression of *OsDof12* (*Rdd1*) significantly delayed the flowering time of transgenic rice under long-day conditions, and the downstream genes *Hd3a* and *OsMADS14* were up-regulated. After interfering with *OsDof12* (*Rdd1*) gene expression, the flowering time of rice was delayed, and rice's grain size and thousand-grain weight were significantly reduced (Li et al., 2009). It is speculated that *VcDof45* and *VcDof2* play important roles in flowering regulation and fruit development of blueberries.

4.4 Potential role of *VcDof* genes in response to abiotic stress

Dof TFs play an important role in abiotic stress in plants. This study selected eight *VcDof* genes for expression pattern analysis under abiotic stress. The results showed that the relative expression levels of *VcDof1, VcDof11*, and *VcDof15* under salt, drought, and ABA short-term induction stress were significantly higher than those in the control group, showing an upward expression trend. The *VcDof* gene expression differed among the three stresses, but they could respond positively to stress. According to previous studies, *StDof4, StDof5*, and *StDof11b* were up-regulated under salt and drought stress in potatoes and positively responded (Venkatesh &

353 Park, 2015). Most *BraDof* genes were rapidly up-regulated under salt, drought, heat, and cold
354 stress in Chinese cabbage (Ma et al., 2015). *TaDof2, TaDof3*, and *TaDof6* were up-regulated, and
355 soluble protein synthesis increased under drought stress in wheat (Liu et al., 2020).

356 ## 5 Conclusions

357 In general, 51 conserved blueberry Dof TFs in the blueberry whole genome were identified
358 in the present study, distributed in eight subfamilies. Colinearity and evolution analysis showed
359 that the main driving force of gene duplication of the *VcDof* gene was WGD, which was purify
360 selected in the evolution process. The gene divergence event occurred after the divergence
361 between monocotyledonous and dicotyledonous plants. The results of tissue expression analysis
362 showed that *VcDof2* and *VcDof45* might play important roles in blueberry flowering and fruit
363 development. *VcDof1, VcDof11*, and *VcDof15* can respond positively and up-regulate expression
364 under abiotic stress, which may play an important role in blueberry defense against abiotic stress.
365

Reference

Ahmad M, Rim Y, Chen H, Kim JY. 2013. Functional characterization of *Arabidopsis* Dof transcription factor AtDof4.1. *Russian journal of plant physiology* **60(1)**:116-123 DOI 10.1134/S1021443712060027.

Bailey TL, Johnson J, Grant CE, Noble WS. 2015. The MEME Suite. *Nucleic acids research* **43(W1)**:W39–W49 DOI 10.1093/nar/gkv416.

Bano N, Fakhrah S, Mohanty CS, Bag SK. 2021. Genome-Wide Identification and Evolutionary Analysis of *Gossypium* Tubby-Like Protein (TLP) Gene Family and Expression Analyses During Salt and Drought Stress. *Frontiers in plant science* **12**:667929 DOI 10.3389/fpls.2021.667929.

Blanc G, Wolfe KH. 2004. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *Plant Cell* **16(7)**:1667-78 DOI 10.1105/tpc.021345.

Bowers JE, Chapman BA, Rong J, Paterson AH. 2003. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422(6930)**:433-8 DOI 10.1038/nature01521.

Brázda V, Bartas M, Bowater RP. 2021. Evolution of Diverse Strategies for Promoter Regulation. *Trends in genetics* **37(8)**:730-744 DOI 10.1016/j.tig.2021.04.003.

Cai X, Zhang Y, Zhang C, Zhang T, Hu T, Ye J, Zhang J, Wang T, Li H, Ye Z. 2013. Genome-wide analysis of plant-specific Dof transcription factor family in tomato. *Journal of Integrative Plant Biology* **55(6)**:552-566 DOI 10.1111/jipb.12043.

Chattha WS, Atif RM, Iqbal M, Shafqat W, Farooq MA, Shakeel A. 2020. Genome-wide identification and evolution of Dof transcription factor family in cultivated and ancestral cotton species. *Genomics* **112(6)**:4155-4170. DOI 10.1016/j.ygeno.2020.07.006

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. 2020. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Molecular plant* **13(8)**:1194–1202 DOI 10.1016/j.molp.2020.06.009.

Chen F, Hu Y, Vannozzi A, Wu K, Cai H, Qin Y, Mullis A, Zhen G, Zhang L. 2017. The

WRKY transcription factor family in model plants and crops. *Critical Reviews in Plant Sciences* **36(5-6)**:311-335 DOI 10.1080/07352689.2018.1441103.

Chen Y, Cao J. 2015. Comparative analysis of Dof transcription factor family in maize. *Plant Molecular Biology Reporter* **33(5)**:1245-1258 DOI 10.1007/s11105-014-0835-9.

Colle M, Leisner CP, Wai CM, Ou S, Bird KA, Wang J, Wisecaver JH, Yocca AE, Alger EI, Tang H +15 more. 2019. Haplotype-phased genome and evolution of phytonutrient pathways of tetraploid blueberry. *Gigascience* **8(3)**:giz012 DOI 10.1093/gigascience/giz012.

Cominelli E, Galbiati M, Albertini A, Fornara F, Conti L, Coupland G, Tonelli C. 2011. DOF-binding sites additively contribute to guard cell-specificity of AtMYB60promoter. *BMC plant biology* **11(1)**:1-13 DOI 10.1186/1471-2229-11-162.

Deng Y, Li Y, Sun H. 2020. Selection of reference genes for RT-qPCR normalization in blueberry *(Vaccinium corymbosum×angustifolium)* under various abiotic stresses. *FEBS open bio* **10(8)**:1418-1435 DOI 10.1002/2211-5463.12903.

Ewas M, Khames E, Ziaf K, Shahzad R, Nishawy E, Ali F, Subthain H, Amar MH, Ayaad M, Ghaly O +1 more. 2017. The Tomato DOF Daily Fluctuations 1, *TDDF1* acts as flowering accelerator and protector against various stresses. *Scientific reports* **7(1)**:10299 DOI 10.1038/s41598-017-10399-7.

Gallardo RK, Zhang Q, Dossett M, Polashock JJ, Rodriguez-Saona C, Vorsa N, PEdger P, Ashrafi H, Babiker E, EFinn C +1 more. 2018. Breeding trait priorities of the blueberry industry in the United States and Canada. *American Society for Horticultural Science* **53(7)**:1021-1028 DOI 10.21273/HORTSCI12964-18.

Gupta S, Malviya N, Kushwaha H, Nasim J, Bisht NC, Singh VK, Yadav D. 2015. Insights into structural and functional diversity of Dof (DNA binding with one finger) transcription factor. *Planta* **241(3)**:549-562 DOI 10.1007/s00425-014-2239-3.

Han T, Wu W, Li W. 2021. Transcriptome Analysis Revealed the Mechanism by Which Exogenous ABA Increases Anthocyanins in Blueberry Fruit During Veraison. *Frontiers in plant science* **12**:758215 DOI 10.3389/fpls.2021.758215.

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G. 2015. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* **31(8)**:1296-7 DOI 10.1093/bioinformatics/btu817.

Hu W, Xia Z, Yan Y, Ding Z, Tie W, Wang L, Zou M, Wei Y, Lu C, Hou X +2 more. 2015. Genome-wide gene phylogeny of CIPK family in cassava and expression analysis of partial drought-induced genes. *Frontiers in plant science* **6**:914 DOI 10.3389/fpls.2015.00914.

Istek N, Gurbuz O. 2017. Investigation of the impact of blueberries on metabolic factors influencing health. *Journal of Functional Foods* **38**:298-307 DOI 10.1016/j.jff.2017.09.039.

Jiang Y, Zeng B, Zhao H, Zhang M, Xie S, Lai J. 2012. Genome-wide Transcription Factor Gene Prediction and their Expressional Tissue-Specificities in Maize. *Journal of integrative plant biology* **54(9)**:616-630 DOI 10.1111/j.1744-7909.2012.01149.x.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution* **35(6)**:1547–1549 DOI 10.1093/molbev/msy096.

Letunic I, Bork P. 2018. 20 years of the SMART protein domain annotation resource. *Nucleic acids research* **46(D1)**:D493–D496 DOI 10.1093/nar/gkx922.

Letunic I, Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic acids research* **49(W1)**:W293-W296 DOI 10.1093/nar/gkab301.

Li D, Yang C, Li X, Gan Q, Zhao X, Zhu L. 2009. Functional characterization of rice OsDof12. *Planta* **229(6)**:1159-69 DOI 10.1007/s00425-009-0893-7.

Li J, Zhang Y, Xu L, Wang C, Luo Y, Feng S, Yuan Y, Yang Q, Feng B. 2022. Genome-Wide Identification of DNA Binding with One Finger (Dof) Gene Family in Tartary Buckwheat *(Fagopyrum tataricum)* and Analysis of Its Expression Pattern after Exogenous Hormone Stimulation. *Biology* **11(2)**:173 DOI 10.3390/biology11020173.

Lijavetzky D, Carbonero P, Vicente-Carbajosa J. 2003. Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis Dof gene families. *BMC evolutionary biology* **3(1)**:1-11 DOI 10.1186/1471-2148-3-17.

Liu Y, Liu N, Deng X, Liu D, Li M, Cui D, Hu Y, Yan Y. (2020). Genome-wide analysis of wheat DNA-binding with one finger (Dof) transcription factor genes: evolutionary characteristics and diverse abiotic stress responses. *BMC genomics* **21(1)**:1-18 DOI 10.1186/s12864-020-6691-0.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25(4)**:402-408 DOI 10.1006/meth.2001.1262.

Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS +7 more. 2020. CDD/SPARCLE: the conserved domain database in 2020. *Nucleic acids research* **48(D1)**:D265–D268 DOI 10.1093/nar/gkz991.

Ma J, Li MY, Wang F, Tang J, Xiong AS. 2015. Genome-wide analysis of Dof family transcription factors and their responses to abiotic stresses in Chinese cabbage. *BMC Genomics* **16(1)**:33 DOI 10.1186/s12864-015-1242-9.

Ma L, Sun Z, Zeng Y, Luo M, Yang J. 2018. Molecular Mechanism and Health Role of Functional Ingredients in Blueberry for Chronic Disease in Human Beings. *International Journal of Molecular Sciences* **19(9)**:2785 DOI 10.3390/ijms19092785.

Moreno-Risueno MA, Martínez M, Vicente-Carbajosa J, Carbonero P. 2007. The family of DOF transcription factors: from green unicellular algae to vascular plants. *Mol Genet Genomics* **277(4)**:379-390 DOI 10.1007/s00438-006-0186-9.

Ono-Moore KD, Snodgrass RG, Huang S, Singh S, Freytag TL, Burnett DJ, Bonnel EL, Woodhouse LR, Zunino SJ, Peerson JM +3 more. 2016. Postprandial Inflammatory Responses and Free Fatty Acids in Plasma of Adults Who Consumed a Moderately High-Fat Breakfast with and without Blueberry Powder in a Randomized Placebo-Controlled Trial. *The Journal of nutrition* **146(7)**:1411–1419 DOI 10.3945/jn.115.223909.

Potter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD. 2018. HMMER web server: 2018 update. *Nucleic acids research* **46(W1)**:W200–W204 DOI 10.1093/nar/gky448.

Renau-Morata B, Molina RV, Carrillo L, Cebolla-Cornejo J, Sánchez-Perales M, Pollmann

S, Domínguez-Figueroa J, Corrales AR, Flexas J, Vicente-Carbajosa J +2 more. 2017. Ectopic Expression of *CDF3* Genes in Tomato Enhances Biomass Production and Yield under Salinity Stress Conditions. *Frontiers in plant science* **8**:660 DOI 10.3389/fpls.2017.00660.

Rueda-Romero P, Barrero-Sicilia C, Gómez-Cadenas A, Carbonero P, Oñate-Sánchez L. 2012. Arabidopsis thaliana DOF6 negatively affects germination in non-after-ripened seeds and interacts with TCP14. *Journal of experimental botany* **63(5)**:1937–1949 DOI 10.1093/jxb/err388.

Skirycz A, Jozefczuk S, Stobiecki M, Muth D, Zanor MI, Witt I, Mueller-Roeber B. 2007. Transcription factor AtDOF4.2 affects phenylpropanoid metabolism in Arabidopsis thaliana. *The New phytologist* **175(3)**:425–438 DOI 10.1111/j.1469-8137.2007.02129.x.

Tang H, Bowers JE, Wang X, Ming R, Alam M, Paterson AH. 2008. Synteny and collinearity in plant genomes. *Science* **320(5875)**:486-488 DOI 10.1126/science.1153917.

Van de Peer Y, Maere S, Meyer A. 2009. The evolutionary significance of ancient genome duplications. *Nature reviews. Genetics* **10(10)**:725-732 DOI 10.1038/nrg2600.

Venkatesh J, Park SW. 2015. Genome-wide analysis and expression profiling of DNA-binding with one zinc finger (Dof) transcription factor family in potato. *Plant Physiology and Biochemistry* **94**:73-85 DOI 10.1016/j.plaphy.2015.05.010.

Wang A, Liang K, Yang S, Cao Y, Wang L, Zhang M, Zhou J, Zhang L. 2021. Genome-wide analysis of MYB transcription factors of *Vaccinium corymbosum* and their positive responses to drought stress. *BMC genomics* **22(1)**:1-17 DOI 10.1186/s12864-021-07850-5.

Wang CT, Ru JN, Liu YW, Li M, Zhao D, Yang JF, Fu JD, Xu ZS. 2018. Maize WRKY Transcription Factor ZmWRKY106 Confers Drought and Heat Tolerance in Transgenic Plants. *International journal of molecular sciences* **19(10)**:3046 DOI 10.3390/ijms19103046.

Wang HW, Zhang B, Hao YJ, Huang J, Tian AG, Liao Y, Zhang JS, Chen SY. 2007. The soybean Dof-type transcription factor genes, GmDof4 and GmDof11, enhance lipid content

in the seeds of transgenic Arabidopsis plants. *The Plant journal:for cell and molecular biology* **52(4)**:716–729 DOI 10.1111/j.1365-313X.2007.03268.x.

Wang X, Liu X, An YC, Zhang H, Meng D, Jin Y, Huo H, Yu L, Zhang J. 2021. Identification of Glutathione Peroxidase Gene Family in *Ricinus communis* and Functional Characterization of RcGPX4 in Cold Tolerance. *Frontiers in plant science* **12**:707127 DOI 10.3389/fpls.2021.707127.

Wang Y, Tan X, Paterson AH. 2013. Different patterns of gene structure divergence following gene duplication in Arabidopsis. *BMC Genomics* **14**:652 DOI 10.1186/1471-2164-14-652.

Wei PC, Tan F, Gao XQ, Zhang XQ, Wang GQ, Heng X, Li LJ, Chen J, Wang XC. 2010. Overexpression of AtDOF4.7, an Arabidopsis DOF family transcription factor, induces floral organ abscission deficiency in Arabidopsis. *Plant physiology* **153(3)**:1031–1045 DOI 10.1104/pp.110.153247.

Wei Q, Wang W, Hu T, Hu H, Mao W, Zhu Q, Bao C. 2018. Genome-wide identification and characterization of Dof transcription factors in eggplant *(Solanum melongena L.)*. *PeerJ* **6**:e4481 DOI 10.7717/peerj.4481.

Wen CL, Cheng Q, Zhao L, Mao A, Yang J, Yu S, Weng Y, Xu Y. 2016. Identification and characterisation of Dof transcription factors in the cucumber genome. *Scientific reports* **6**:23072 DOI 10.1038/srep23072.

Wu Z, Cheng J, Cui J, Xu X, Liang G, Luo X, Chen X, Tang X, Hu K, Qin C. 2016. Genome-wide identification and expression profile of Dof transcription factor gene family in pepper (*Capsicum annuum* L.). *Frontiers in Plant Science* **7**:574 DOI 10.3389/fpls.2016.00574.

Xu P, Chen H, Ying L, Cai W. 2016. AtDOF5.4/OBP4, a DOF Transcription Factor Gene that Negatively Regulates Cell Cycle Progression and Cell Expansion in Arabidopsis thaliana. *Scientific reports* **6**:27705 DOI 10.1038/srep27705.

Yanagisawa S, Izui K. 1993. Molecular cloning of two DNA-binding proteins of maize that are structurally different but interact with the same sequence motif. *Journal of Biological*

Chemistry **268(21)**:16028-16036 DOI 10.1016/S0021-9258(18)82353-5.

Yanagisawa S. 2002. The Dof family of plant transcription factors. *Trends in plant science* **7(12):**555-560 DOI 10.1016/s1360-1385(02)02362-2.

Yanagisawa S. 2004. Dof domain proteins: plant-specific transcription factors associated with diverse phenomena unique to plants. *Plant cell physiology* **45(4)**:386–391 DOI 10.1093/pcp/pch055.

Yang J, Yang MF, Zhang WP, Chen F, Shen SH. 2011. A putative flowering-time-related Dof transcription factor gene, JcDof3, is controlled by the circadian clock in Jatropha curcas. *Plant Science* **181(6)**:667-674 DOI 10.1016/j.plantsci.2011.05.003.

Zang D, Wang L, Zhang Y, Zhao H, Wang Y. 2017. ThDof1.4 and ThZFP1 constitute a transcriptional regulatory cascade involved in salt or osmotic stress in Tamarix hispida. *Plant molecular biology* **94(4-5)**:495-507 DOI 10.1007/s11103-017-0620-x.

Zong Y, Gu L, Shen Z, Kang H, Li Y, Liao F, Xu L, Guo W. 2021. Genome-Wide Identification and Bioinformatics Analysis of Auxin Response Factor Genes in Highbush Blueberry. *Horticulturae* **7(10)**:403 DOI10.3390/horticulturae7100403.

Table 1(on next page)

Table 1: The basic information of Dof TFs in blueberry.

1 **Table 1: The basic information of Dof TFs in blueberry.**

Gene name	Gene ID	Chromosome location	CDS (bp)	Length (aa)	pI	Molecular weight (Da)	Subcellular location
VcDof1	VaccDscaff3-snap-gene-159.14	VaccDscaff3:15954245-15956961	1200	399	8.31	44,373.94	Nuclear
VcDof2	VaccDscaff4-augustus-gene-345.25	VaccDscaff4:34550915-34552763	876	291	8.1	31,800.02	Nuclear
VcDof3	VaccDscaff8-augustus-gene-261.30	VaccDscaff8:26158034-26160021	918	305	9.26	33,049.91	Nuclear
VcDof4	VaccDscaff9-augustus-gene-161.15	VaccDscaff9:16127607-16130475	801	266	9.18	29,743.08	Nuclear
VcDof5	VaccDscaff10-snap-gene-247.18	VaccDscaff10:24688121-24689836	906	301	9.26	32,614.39	Nuclear
VcDof6	VaccDscaff11-processed-gene-136.2	VaccDscaff11:13655066-13655560	495	164	9.43	18,177.54	Nuclear
VcDof7	VaccDscaff11-snap-gene-158.12	VaccDscaff11:15805973-15808315	1071	356	8.96	39,331.62	Nuclear
VcDof8	VaccDscaff11-processed-gene-168.11	VaccDscaff11:16838900-16843433	612	203	9.85	22,095.71	Nuclear
VcDof9	VaccDscaff11-processed-gene-307.12	VaccDscaff11:30754783-30756949	951	316	7.63	34,553.12	Nuclear
VcDof10	VaccDscaff11-augustus-gene-330.45	VaccDscaff11:33025388-33027124	867	288	9.43	30,770.94	Nuclear
VcDof11	VaccDscaff12-processed-gene-81.3	VaccDscaff12:8170002-8170682	681	226	6.37	24,042.46	Nuclear
VcDof12	VaccDscaff13-augustus-gene-79.30	VaccDscaff13:7890522-7894462	1470	489	5.74	53,351.37	Nuclear
VcDof13	VaccDscaff13-augustus-gene-135.30	VaccDscaff13:13508244-13511349	957	318	9.04	34,095.62	Nuclear
VcDof14	VaccDscaff13-augustus-gene-254.20	VaccDscaff13:25461888-25465267	1407	468	5.57	51,450.94	Nuclear
VcDof15	VaccDscaff14-processed-gene-266.8	VaccDscaff14:26675412-26676173	762	253	4.56	28,167.22	Nuclear
VcDof16	VaccDscaff15-snap-gene-139.15	VaccDscaff15:13899895-13902343	1062	353	9.07	39,109.51	Nuclear
VcDof17	VaccDscaff15-processed-gene-145.13	VaccDscaff15:14528524-14530344	795	264	9.3	28,571.85	Nuclear
VcDof18	VaccDscaff15-augustus-gene-292.25	VaccDscaff15:29225641-29227947	954	317	7.21	34,696.27	Nuclear
VcDof19	VaccDscaff15-processed-gene-320.11	VaccDscaff15:32030644-32032478	1281	426	9.88	46,664.59	Nuclear
VcDof20	VaccDscaff16-augustus-gene-64.37	VaccDscaff16:6408947-6412982	1395	464	5.93	50,683.97	Nuclear

VcDof21	VaccDscaff17-augustus-gene-280.24	VaccDscaff17:28047716-28050042	963	320	6.52	35,155.14	Nuclear
VcDof22	VaccDscaff17-processed-gene-380.10	VaccDscaff17:38033194-38035650	885	294	8.11	32,152.67	Nuclear
VcDof23	VaccDscaff19-augustus-gene-7.37	VaccDscaff19:722925-724763	873	290	8.81	32,131.57	Nuclear
VcDof24	VaccDscaff19-processed-gene-223.3	VaccDscaff19:22351441-22352415	975	324	9.20	35,667.63	Nuclear
VcDof25	VaccDscaff19-processed-gene-236.11	VaccDscaff19:23614873-23616681	789	262	9.30	28,419.66	Nuclear
VcDof26	VaccDscaff19-snap-gene-338.49	VaccDscaff19:33815798-33819654	1182	393	6.00	43,431.08	Nuclear
VcDof27	VaccDscaff19-augustus-gene-349.24	VaccDscaff19:34887825-34889658	1239	412	9.93	45,154.72	Nuclear
VcDof28	VaccDscaff20-augustus-gene-371.28	VaccDscaff20:37159924-37162529	990	329	8.13	35,787.13	Nuclear
VcDof29	VaccDscaff21-processed-gene-152.13	VaccDscaff21:15189887-15192636	1014	337	8.91	36,226.51	Nuclear
VcDof30	VaccDscaff21-augustus-gene-306.24	VaccDscaff21:30639419-30644218	1245	414	6.47	45,615.07	Nuclear
VcDof31	VaccDscaff22-augustus-gene-267.28	VaccDscaff22:26762373-26763954	753	250	8.34	27,207.32	Nuclear
VcDof32	VaccDscaff22-processed-gene-275.2	VaccDscaff22:27553163-27555116	939	312	8.81	33,950.99	Nuclear/Peroxisome
VcDof33	VaccDscaff24-snap-gene-94.38	VaccDscaff24:9471913-9475742	1197	398	5.76	44,173.03	Nuclear
VcDof34	VaccDscaff24-processed-gene-229.1	VaccDscaff24:22899664-22900548	885	294	8.13	32,341.95	Nuclear
VcDof35	VaccDscaff24-processed-gene-290.7	VaccDscaff24:29059975-29060412	438	145	10.48	16,520.86	Nuclear
VcDof36	VaccDscaff27-snap-gene-20.34	VaccDscaff27:2024971-2025485	357	118	10.56	13,621.80	Nuclear
VcDof37	VaccDscaff27-augustus-gene-35.18	VaccDscaff27:3516664-3518800	1026	341	9.14	35,675.48	Nuclear/Peroxisome
VcDof38	VaccDscaff28-augustus-gene-4.38	VaccDscaff28:457494-460214	993	330	8.13	35,901.23	Nuclear
VcDof39	VaccDscaff29-snap-gene-153.37	VaccDscaff29:15276983-15278775	960	319	9.16	34,455.72	Nuclear
VcDof40	VaccDscaff30-augustus-gene-326.23	VaccDscaff30:32673932-32678040	1482	493	5.83	53,701.66	Nuclear/mitochondrial
VcDof41	VaccDscaff31-processed-gene-100.12	VaccDscaff31:10070463-10071110	648	215	9.05	22,434.25	Nuclear
VcDof42	VaccDscaff33-augustus-gene-185.34	VaccDscaff33:18572264-18573956	966	321	9.14	34,766.97	Nuclear

VcDof43	VaccDscaff34-augustus-gene-312.13	VaccDscaff34:31205413-31207358	969	322	9.20	33,725.28	Nuclear/Peroxisome
VcDof44	VaccDscaff35-processed-gene-123.5	VaccDscaff35:12329415-12329894	480	159	9.27	17,650.15	Nuclear
VcDof45	VaccDscaff35-augustus-gene-253.36	VaccDscaff35:25292011-25293987	879	292	8.10	31,873.07	Nuclear
VcDof46	VaccDscaff37-augustus-gene-143.22	VaccDscaff37:14293905-14295828	837	278	9.40	30,154.54	Nuclear
VcDof47	VaccDscaff40-processed-gene-12.4	VaccDscaff40:1275799-1276467	669	222	6.64	23,658.98	Nuclear
VcDof48	VaccDscaff41-processed-gene-58.8	VaccDscaff41:5852050-5852715	666	221	6.64	23,614.92	Nuclear
VcDof49	VaccDscaff46-augustus-gene-100.28	VaccDscaff46:10034713-10036487	747	248	8.34	27,093.22	Nuclear
VcDof50	VaccDscaff46-snap-gene-129.47	VaccDscaff46:12897112-12899008	936	311	8.58	33,975.09	Nuclear/Peroxisome
VcDof51	VaccDscaff48-augustus-gene-102.22	VaccDscaff48:10277144-10279070	864	287	8.81	31,974.36	Nuclear

Table 2(on next page)

Table 2: The evolution selection pressure and divergence time of Dof TFs in blueberry

Table 2: The evolution selection pressure and divergence time of Dof TFs in blueberry

Duplicated pair	Duplicated model	Ka	Ks	Ka/ks	Selection pressure	Divergence time (Mya)
VcDof1-VcDof12	WGD	2.486705939	0.146874625	0.146874625	purify selection	5.649024038
VcDof2-VcDof45	WGD	0.060032031	0.02507443	0.02507443	purify selection	0.964401154
VcDof3-VcDof5	WGD	0.033338822	0.087321976	0.087321976	purify selection	3.358537538
VcDof5-VcDof27	WGD	1.723444937	0.208458308	0.208458308	purify selection	8.017627231
VcDof5-VcDof29	WGD	1.517602966	0.274965217	0.274965217	purify selection	10.57558527
VcDof7-VcDof24	WGD	0.00899561	1.504986721	1.504986721	positive selection	57.88410465
VcDof8-VcDof17	WGD	0.144930931	0.439208611	0.439208611	purify selection	16.89263888
VcDof9-VcDof18	WGD	0.009646435	0.140341064	0.140341064	purify selection	5.397733231
VcDof10-VcDof19	WGD	0.025426164	0.178231206	0.178231206	purify selection	6.855046385
VcDof12-VcDof20	WGD	0.709859291	0.21128658	0.21128658	purify selection	8.126406923
VcDof12-VcDof40	WGD	0.040226191	0.176036186	0.176036186	purify selection	6.770622538
VcDof17-VcDof25	WGD	0.027627433	0.181080807	0.181080807	purify selection	6.964646423
VcDof18-VcDof26	WGD	0.036268486	0.899055024	0.899055024	purify selection	34.57903938
VcDof19-VcDof27	WGD	0.007017595	0.451105363	0.451105363	purify selection	17.35020627
VcDof20-VcDof40	WGD	0.708639164	0.204062825	0.204062825	purify selection	7.848570192
VcDof25-VcDof34	WGD	0.010969117	0.608108789	0.608108789	purify selection	23.38879958
VcDof26-VcDof31	WGD	0.565207401	0.397165382	0.397165382	purify selection	15.27559162
VcDof27-VcDof32	WGD	0.906127367	0.324096428	0.324096428	purify selection	12.46524723
VcDof28-VcDof38	WGD	0.018377642	0.071036498	0.071036498	purify selection	2.732173
VcDof29-VcDof39	WGD	0.036550818	0.495327557	0.495327557	purify selection	19.05105988
VcDof32-VcDof50	WGD	0.009371461	2.743181808	2.743181808	purify selection	105.5069926
VcDof33-VcDof49	WGD	0.542497777	0.407042967	0.407042967	purify selection	15.65549873
VcDof37-VcDof43	WGD	0.038690527	0.214393966	0.214393966	purify selection	8.245921769
VcDof39-VcDof42	WGD	0.034226471	0.467221286	0.467221286	purify selection	17.97004946

Figure 1

Figure 1: Phylogenetic tree of the Arabidopsis, rice and blueberry Dof transcription factors.

The nine subfamilies were shown in different colors. The blue filled pentagram denoted *VcDof* genes; The green-filled pentagram denoted *AtDof* genes; The yellow-filled pentagram denoted *OsDof* genes.

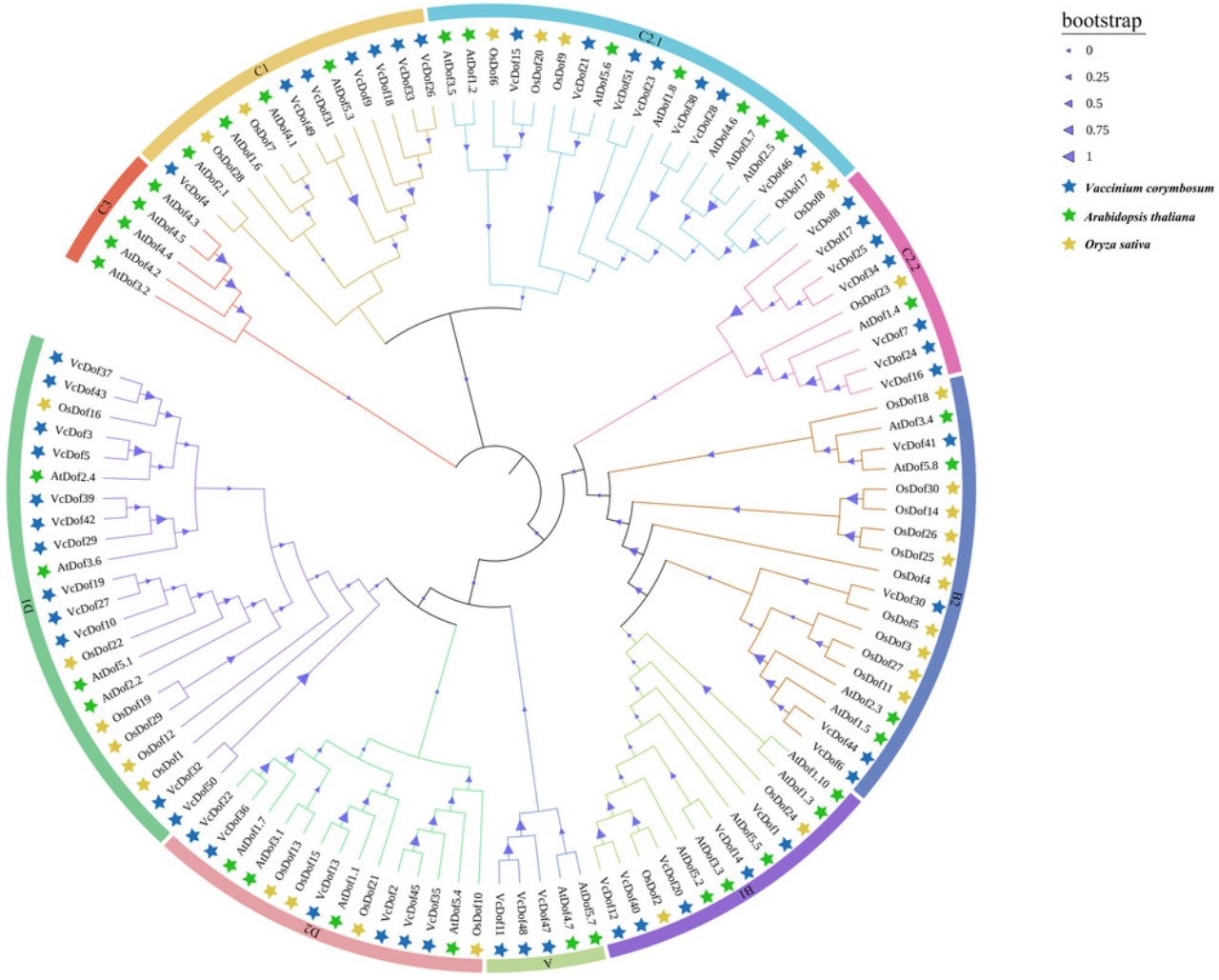


Figure 2

Figure 2: Conserved protein motifs and gene structure analysis of Dof transcription factors in blueberry.

(A) Identified conserved protein motifs in the *VcDof* genes. Each motif was indicated with a specific color, different colors of lines denoted the different subfamilies; (B) Gene structure of the *VcDof* genes. The yellow box represented the CDS region and green box represented the UTR, and the grey lines represented introns.

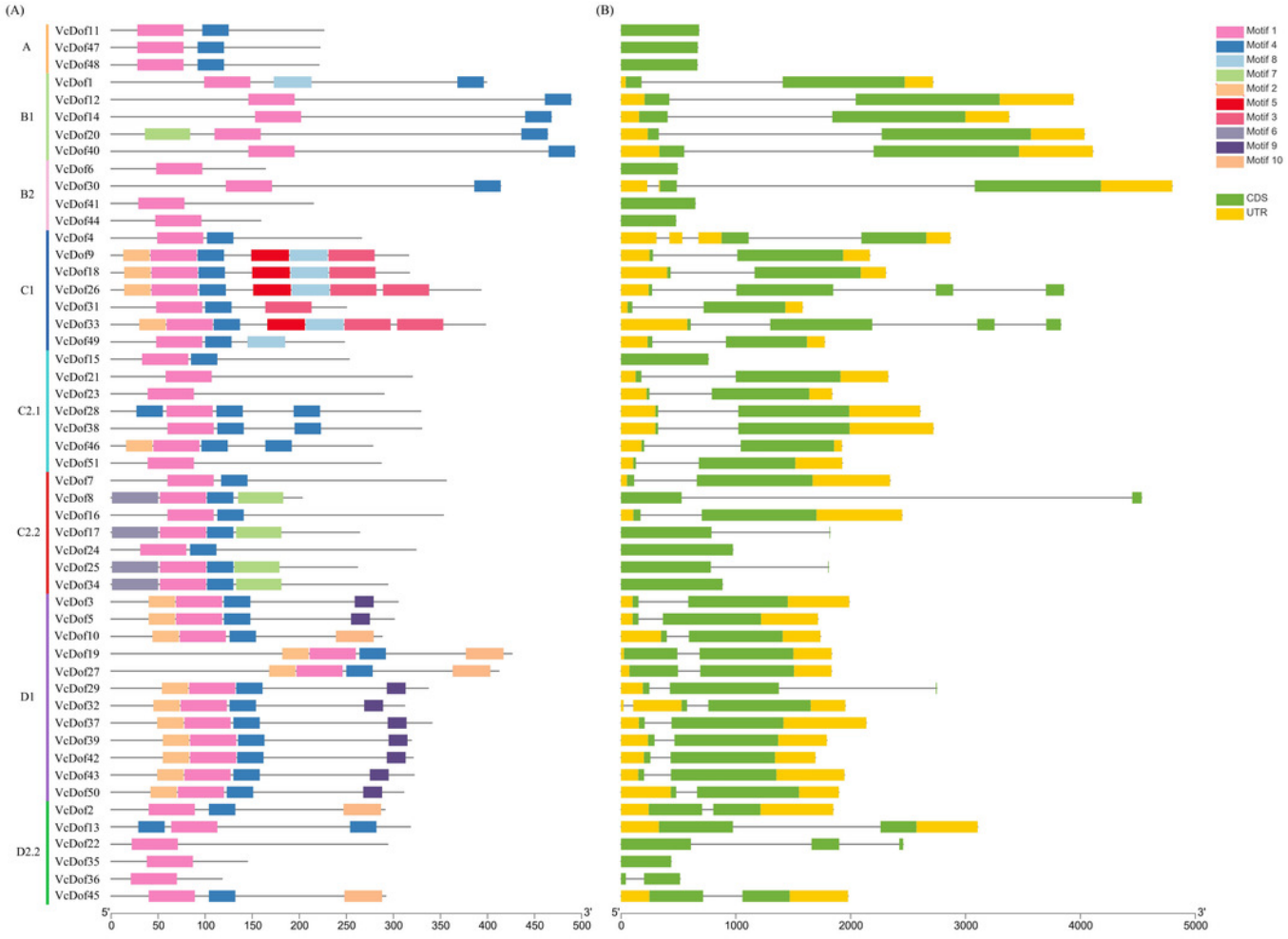


Figure 3

Figure 3: Chromosomal location and collinearity analysis of Dof TFs in blueberry.

The outer color ring box in the circle represented the chromosome scaffold, the color part inside the circle was the blueberry genome collinear region, and the red line was the blueberry Dof TFs collinear gene pair.

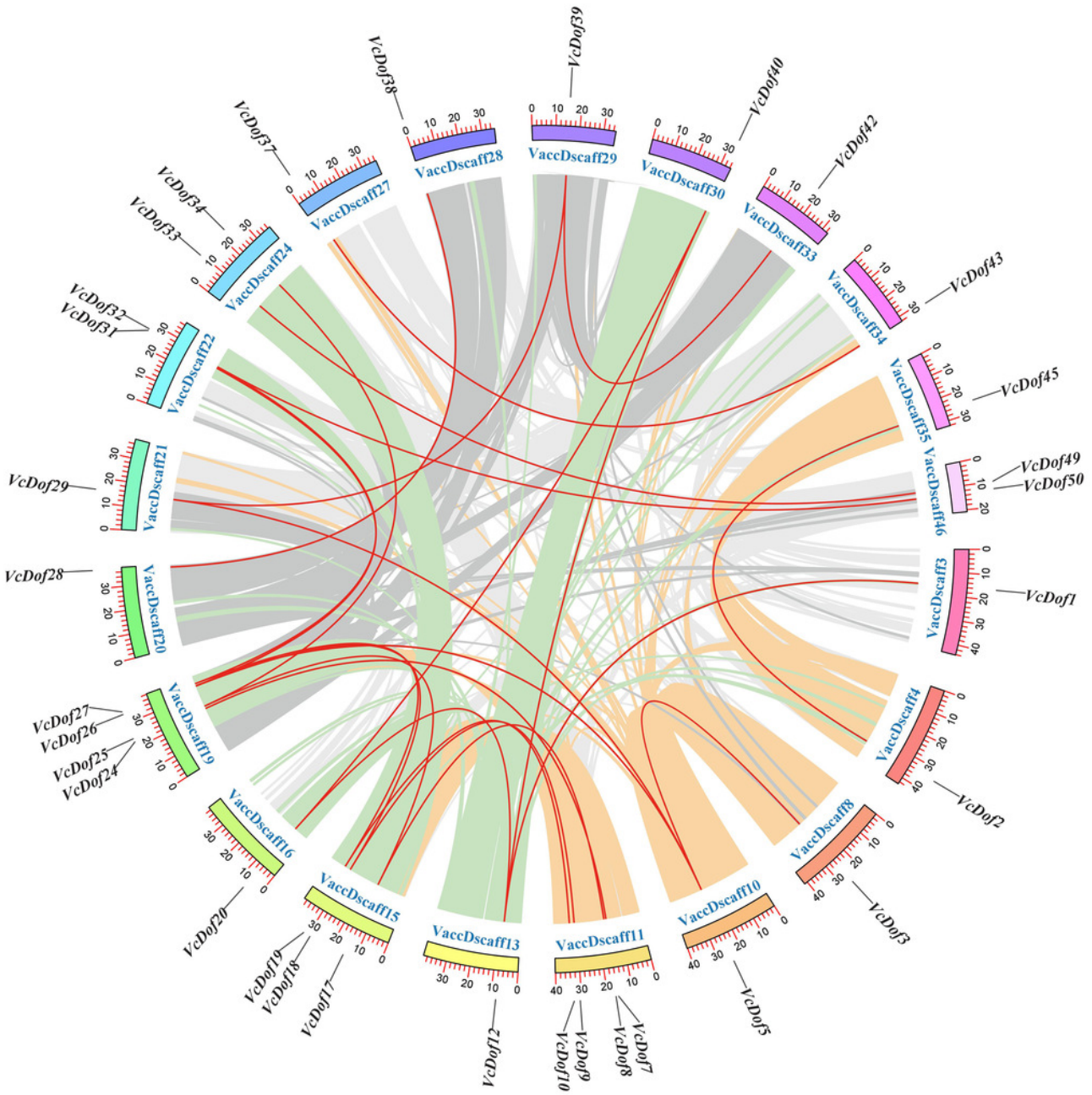
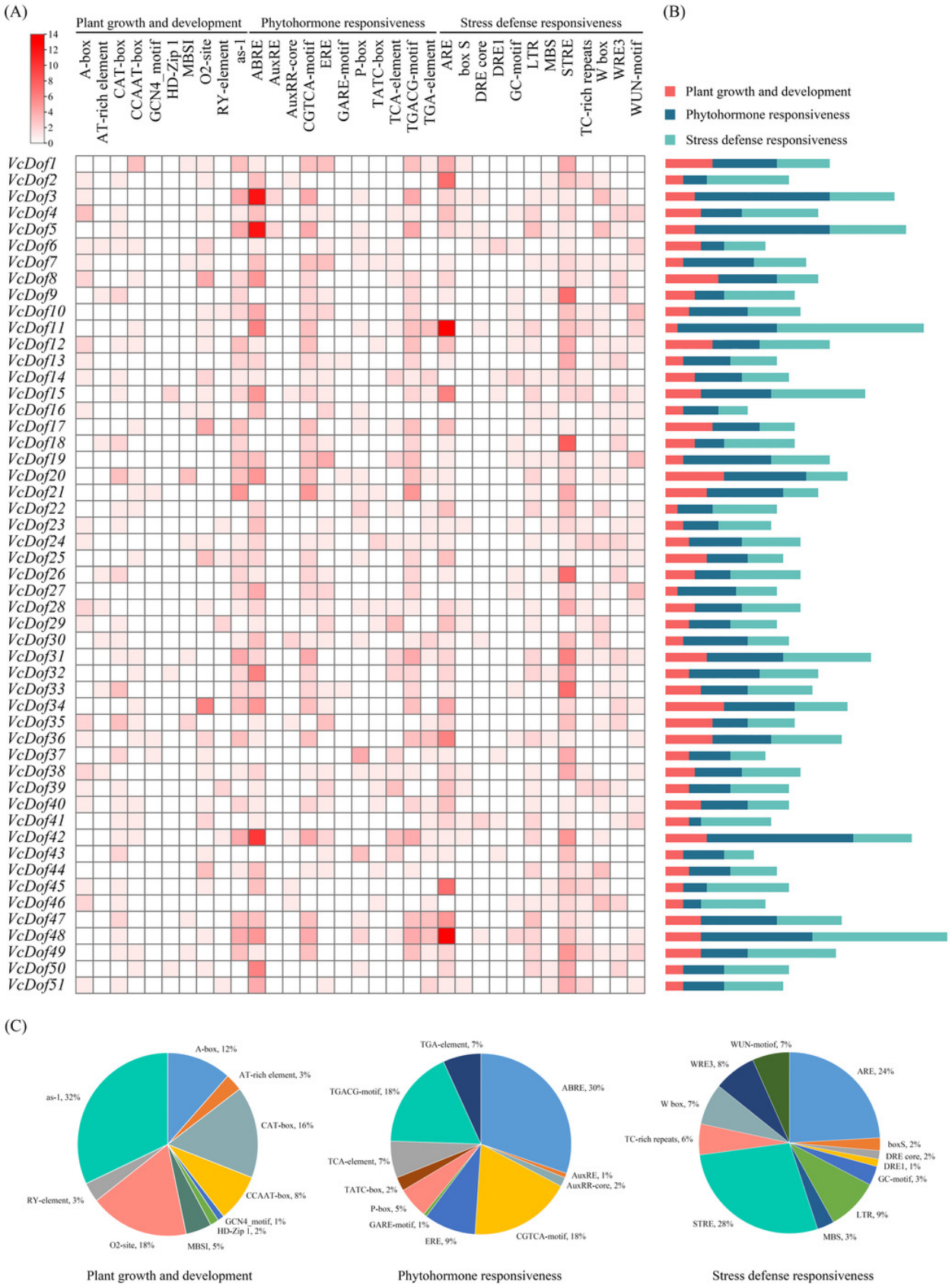

VcDof39
VcDof38
VcDof40
VcDof37
VcDof42
VcDof34
VcDof33
VcDof43
VcDof32
VcDof31
VcDof45
VcDof49
VcDof50
VcDof29
VcDof1
VcDof28
VcDof2
VcDof27
VcDof26
VcDof25
VcDof24
VcDof3
VcDof20
VcDof19
VcDof18
VcDof17
VcDof12
VcDof5
VcDof10
VcDof9
VcDof8
VcDof7
VaccDscaff28
VaccDscaff29
VaccDscaff30
VaccDscaff33
VaccDscaff34
VaccDscaff35
VaccDscaff46
VaccDscaff3
VaccDscaff4
VaccDscaff8
VaccDscaff10
VaccDscaff11
VaccDscaff13
VaccDscaff15
VaccDscaff16
VaccDscaff19
VaccDscaff20
VaccDscaff21
VaccDscaff22
VaccDscaff24
VaccDscaff27

Figure 4

Figure 4: The cis-acting elements of Dof TFs in blueberry.

(A) The gradient red colors indicate the number of cis-acting elements; (B) Color-coded histograms indicate the number of cis-acting elements of genes in each category; (C) Pie charts show the proportion of different cis-acting elements in each category.


(A)
Plant growth and development
Phytohormone responsiveness
Stress defense responsiveness
A-box
AT-rich element
CAT-box
CCAAT-box
GCN4_motif
HD-Zip 1
MBSI
O2-site
RY-element
as-1
ABRE
AuxRE
AuxRR-core
CGTCA-motif
ERE
GARE-motif
P-box
TATC-box
TCA-element
TGACG-motif
TGA-element
ARE
box S
DRE core
DRE1
GC-motif
LTR
MBS
STRE
TC-rich repeats
W box
WRE3
WUN-motif
VcDof1
VcDof2
VcDof3
VcDof4
VcDof5
VcDof6
VcDof7
VcDof8
VcDof9
VcDof10
VcDof11
VcDof12
VcDof13
VcDof14
VcDof15
VcDof16
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VcDof40
VcDof41
VcDof42
VcDof43
VcDof44
VcDof45
VcDof46
VcDof47
VcDof48
VcDof49
VcDof50
VcDof51
(B)
Plant growth and development
Phytohormone responsiveness
Stress defense responsiveness
(C)
A-box, 12%
AT-rich element, 3%
CAT-box, 16%
CCAAT-box, 8%
GCN4_motif, 1%
HD-Zip 1, 2%
MBSI, 5%
O2-site, 18%
RY-element, 3%
as-1, 32%
Plant growth and development
TGA-element, 7%
ABRE, 30%
AuxRE, 1%
AuxRR-core, 2%
CGTCA-motif, 18%
ERE, 9%
GARE-motif, 1%
P-box, 5%
TATC-box, 2%
TCA-element, 7%
TGACG-motif, 18%
Phytohormone responsiveness
WUN-motiof, 7%
ARE, 24%
boxS, 2%
DRE core, 2%
DRE1, 1%
GC-motif, 3%
LTR, 9%
MBS, 3%
STRE, 28%
TC-rich repeats, 6%
W box, 7%
WRE3, 8%
Stress defense responsiveness

Figure 5

Figure 5: Expression profiles of Dof TFs in different tissues and fruit development stages of blueberry.

The blue or red indicated lower or higher expression levels of each transcript in each sample, respectively.

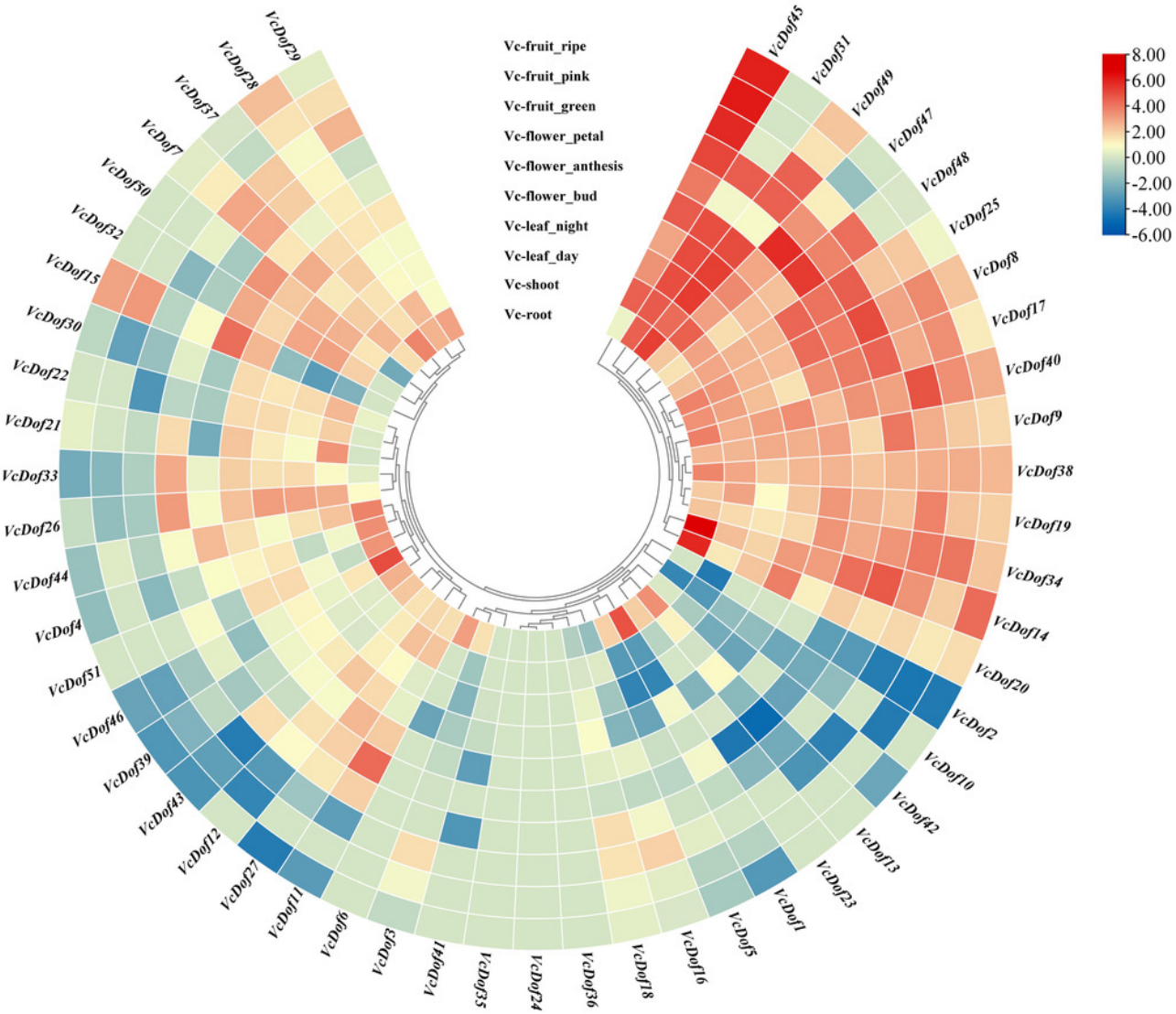
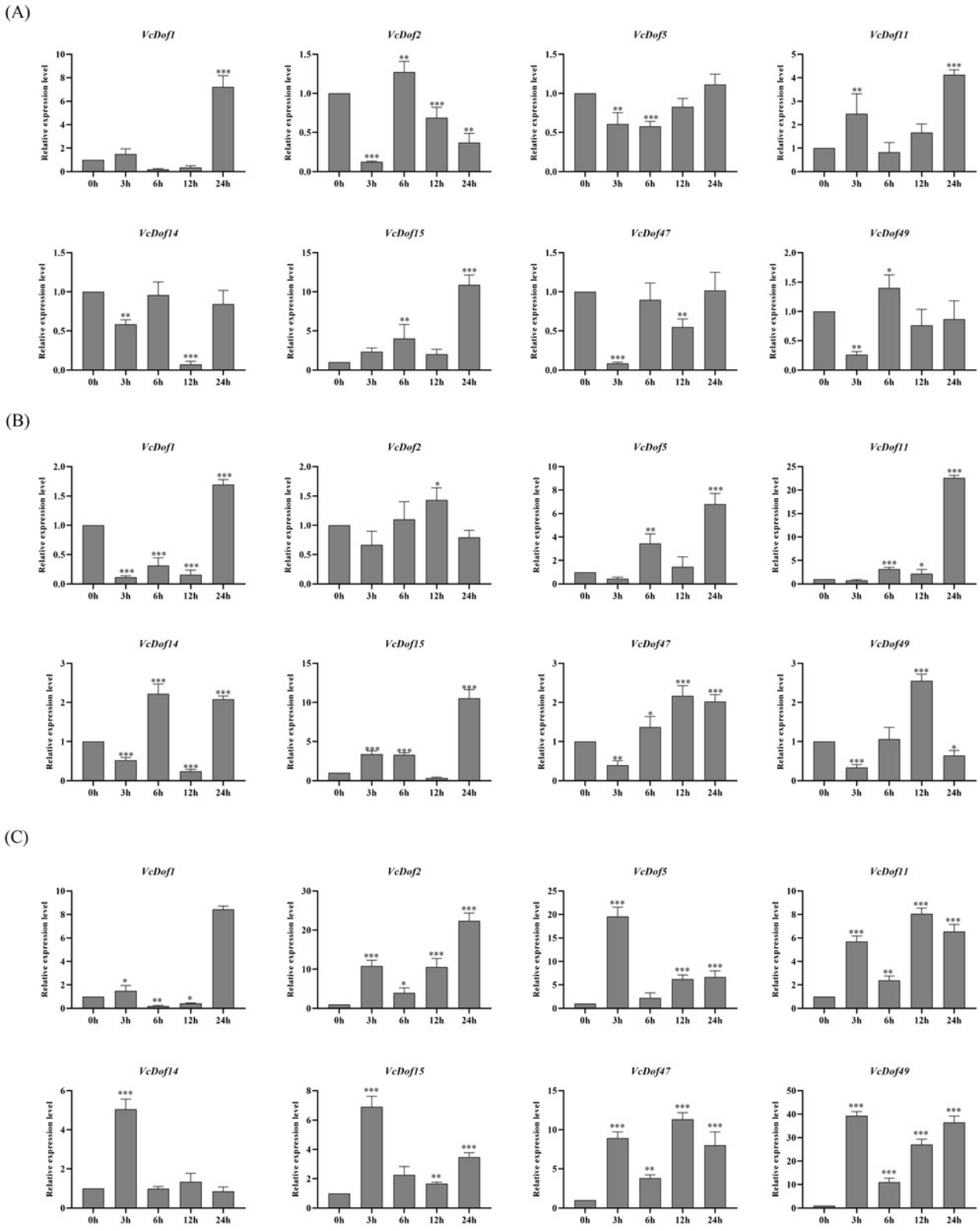


Figure 6

Figure 6: Expression profiles of blueberry Dof TFs in response to salt, drought and abscisic acid.

(A) Expression profiles of *VcDof* genes under salt stress. (B) Expression profiles of *VcDof* genes under drought stress. (C) Expression profiles of *VcDof* genes under abscisic acid. Error bars indicate standard deviation, and asterisks indicate significant differences between the control and treatments, * $p < 0.05$, ** $p < 0.01$, *** $p<0.001$.


(A)
VcDof1
VcDof2
VcDof5
VcDof11
VcDof14
VcDof15
VcDof47
VcDof49
(B)
VcDof1
VcDof2
VcDof5
VcDof11
VcDof14
VcDof15
VcDof47
VcDof49
(C)
VcDof1
VcDof2
VcDof5
VcDof11
VcDof14
VcDof15
VcDof47
VcDof49
Relative expression level
0h
3h
6h
12h
24h