

## Identifying strawberry DOF family transcription factors and their expressions in response to crown rot

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### Abstract

Crown rot is one of the most destructive diseases of cultivated strawberry. The DOF family transcription factors, which involved in biotic stress, has not been studied in responding to strawberry crown rot. In this study, the DOFs of *Fragaria × ananassa*, *F. iinumae*, *F. nilgerrensis*, *F. viridis*, and *F. vesca* were characterized. One hundred and eighteen *FaDOFs*, twenty-two *FiDOFs*, twenty-three *FnDOFs*, twenty-five *FviDOFs* and thirty-seven *FvDOFs* were identified. Gene cluster analysis showed nearly seventy segmental duplication and seventeen tandem duplications for DOF family expansion in octaploid strawberry. In addition, 59 *FaDOFs* showed syntenic relationships with 32 *AtDOFs*, which were located on all *F. × ananassa* chromosomes except *Fvb4-1* and *Fvb4-2*. Except for five DOFs of diploid strawberries had syntenic relationships to one *FaDOF*, most of them corresponded to multiple *FaDOFs*. Gene expression analysis revealed that 107 *FaDOFs* were expressed in crown, and most of them were downregulated by crown rot, while some *FaDOFs* such as *FaDOF107*, *12*, *82*, *91*, *90* and *101* were upregulated, whose regulation was not always consistent with the *cis*-elements in their promoters. Together, these results provided a basis for further functional studies of the *FaDOFs*.

**Keywords:** crown rot; gene family; gene structure; phylogenetic analysis; strawberry; transcriptional expression

### Introduction

Cultivated strawberry (*Fragaria × ananassa*), the important fruit crop species whose fruits with distinctive flavour and rich nutritious value, is widely grown all over the world, but its productivity and quality are seriously limited by crown rot (Han *et al.*, 2016). Crown rot occurs in the root neck, which is manifested as a short plant. After infection, the root neck produces red streaks, and then rapidly expands to dark, sunken spots, and finally the whole plant wilts and withers, which is a destructive disease of strawberry. Under suitable conditions, crown rot can reduce the yield of strawberries by up to 80% (Han *et al.*, 2016).

DOF proteins are a family of plant-specific transcription factors that contain a particular class of zinc-finger DNA-binding domain, which has been reported to be involved in biotic stresses. These DOF transcription factors can interact with other related transcription factors and regulate the transcription process by activating or inhibiting target genes, and then regulate their expression to participate in plant resistance to stress. For instance, in barley, two DOFs, *BPDF* and *SAD*, involved in gene regulation during seed development

Received: 20 Jan 2022. Received in revised form: 03 Mar 2022. Accepted: 07 Mar 2022. Published online: 11 Mar 2022.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

have been characterized, as part of the transcriptional regulation of the barley *Cystatin Hv-CPI* gene upon barley seed germination which has the plant defence function (Martínez *et al.*, 2005). Pepper *DOFs CaDOF10* and *11* showed relatively high expression levels after infection of PepMoV and *Phytophthora capsici*, suggesting that they could play a role in defense response against pathogen (Kang *et al.*, 2016). In Arabidopsis, motif *DOF* are proven functional in the regulation of *ACBP3* confers resistance to *Pseudomonas syringae* (Zheng *et al.*, 2012).

The *DOF* family has been characterized in several plants, including *A. thaliana* (Lijavetzky *et al.*, 2003), *Oryza sativa* (Lijavetzky *et al.*, 2003), *Hordeum vulgare* (Moreno-Risueno *et al.*, 2007), *Glycine max* (Guo and Qiu, 2013), *Populus trichocarpa* (Yang *et al.*, 2006), *Triticum aestivum* (Shaw *et al.*, 2009), *Sorghum bicolor* (Kushwaha *et al.*, 2011), *Zea mays* (Jiang *et al.*, 2012), *Solanum lycopersicum* (Cai *et al.*, 2013), and Chinese cabbage (Ma *et al.*, 2015). However, *F. ananassa* specific *DOF* studies are lacking. In the present study, the strawberry *DOF* family transcription factors members were identified via bioinformatics tools, and their expression patterns in response to biotic stress were characterized. This study provides basic information on the protein structures, subfamily divisions, chromosome localization in the strawberry genome, and expression patterns of the *DOF* proteins in response to crown rot.

## Materials and Methods

### *FaDOF* searching and characteristics

The complete genome assembly of strawberries (*F. × ananassa* ‘Camarosa’, *F. iinumae*, *F. vesca*, *F. viridis* and *F. nilgerrensis*) were downloaded from the strawberry Genome Database (<https://www.rosaceae.org>). Thirty-six *A. thaliana* *DOF* protein sequences (*AtDOFs*) were obtained from the *A. thaliana* Information Resource (TAIR) (<https://www.arabidopsis.org/>) (Kushwaha *et al.*, 2011). *F. × ananassa* *DOF* (*FaDOFs*), *F. iinumae* *DOF* (*FiDOFs*), *F. vesca* *DOF* (*FvDOFs*), *F. viridis* *DOF* (*FviDOFs*) and *F. nilgerrensis* *DOF* (*FnDOFs*) sequences were selected by comparison to the *A. thaliana* query sequences via BlastP. Redundant proteins were manually deleted based on their E-values. The molecular weight (MW) and isoelectric point (pI) of the candidate protein sequences were determined by ExPASy (<https://web.expasy.org/compute/pi/>) (Gasteiger *et al.*, 2003). Conserved *DOF* domains were verified for all potential *DOF* proteins using the NCBI Batch CD-Search program (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>).

### Sequence alignment and phylogenetic analysis

The full-length *DOF* protein sequences from *A. thaliana* and strawberries were aligned via muscle in MEGA version 7.0, with default parameters (Edgar, 2004; Kumar *et al.*, 2016). A neighbour-joining (NJ) tree was also generated with bootstrapping (1000 replicates). The phylogenetic relationships among the five kinds of strawberries *DOFs* and *A. thaliana* *DOFs* were estimated.

### Conserved motifs and gene structure analysis

Motif analysis was conducted on the MEME website (<http://meme-suite.org/tools/meme>) to identify conserved motifs with the following optimized parameters: zero or one occurrence per sequence, a maximum of 10 motifs and an optimum motif width between 6 and 50 residues. The default settings were used for all other parameters. The structure of *FaDOFs* was determined with TBtools by comparing the coding sequences and the corresponding genomic sequences (Chen *et al.*, 2020).

The Strawberries Generic Feature Format (GFF) files were downloaded from the strawberry Genome Database and used to elucidate the structure information of the *DOF* gene. An illustration of the *FaDOFs* protein motifs, conserved domain, gene structures and a phylogenetic tree was also constructed in TBtools (Chen *et al.*, 2020).

### *Chromosomal distribution, gene duplication and collinearity*

The chromosome locations of the candidate strawberry *DOF* genes were analyzed from the GFF information and visualized by TBtools (Chen *et al.*, 2020). Gene duplication events of the *FaDOFs* and collinearity between the *A. thaliana* *DOF* protein sequences and five kinds of strawberries *DOF* protein sequences were investigated by MCScanX (Wang *et al.*, 2012). The results were visualized in TBtools (Chen *et al.*, 2020).

### *FaDOFs expression in response to biotic stress*

A single factor experiment was performed using different treatments causing inoculation with *Colletotrichum siamense* *SCR-7*, which caused crown rot in Hubei province, China. The healthy and consistent one year old strawberry (*F. × ananassa* Duch 'Benihoppe') seedlings were divided into *SCR-7* inoculation treatment group and control group with 12 pots each. The *SCR-7* inoculation treatment group was inoculated with *C. siamense* *SCR-7*, while the control group was inoculated with non-toxic medium using the same method. The seedlings were grown with or without *C. siamense* *SCR-7* and 0 or 6 days after vaccination, resulting in four treatment groups: 0-day post inoculation with medium (0DPM), 6-day post inoculation with medium (6DPM), 0-day post inoculation with *C. siamense* *SCR-7* (0DPI) and 6 days post inoculation with *C. siamense* *SCR-7* (6DPI). All the seedlings were grown in light incubator, with 16h photoperiod, 900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 25/15 °C day/night temperatures, and a 68% relative humidity. The crowns of seedlings were sampled for transcriptome and qRT-PCR analysis, each treatment had three biological replicates.

Transcriptomic data of seedling crowns from the four treatments were analyzed as described by Shu *et al.* (2016). Twelve libraries of seedling crowns were sequenced using the Illumina HiSeq 2000 system. Reads that contained adapters, more than 10% unknown nucleotides, and more than 50% bases with a quality value  $\leq 5$  were removed to obtain uncontaminated sequences based on the raw data. Uncontaminated sequences were mapped to the genome of *F. × ananassa* 'Camarosa' (v1.0.a2) for annotation (Liu *et al.*, 2020). The transcriptomic data were uploaded to the NCBI Sequence Read Archive as PRJNA715088. Gene expression was analyzed based on the transcriptomic data, where the transcriptional abundance of *FaDOF* was calculated as fragments per kilobase of exon model per million mapped reads (FPKM) using the Cufflinks package cuffdiff version 2.2.1. The FPKM value of 0DCK was considered the relevant control. Heat maps were created using TBtools software based on the transformed data of  $\log_2(\text{FPKM}+1)$  values (Chen *et al.*, 2020).

qRT-PCR was performed as in Luo *et al.* (2020) on three independent biological samples having three technical replications each. Eight genes were selected for RNA-seq verification and the primers used for qRT-PCR were shown in Table S1. The relative gene expression was calculated using the  $2^{-\Delta\Delta C_t}$  method, where house-keeping gene (gene11892) was taken as the reference gene. The measured transcripts were normalized to the relative expression value in 0-day post inoculation with medium. Significant differences between treatments were determined by Duncan's Multiple Range Tests at  $p = 0.05$  with SAS 8.1 (SAS Institute, Inc., Cary, NC, USA). Different letters indicate statistically significant differences.

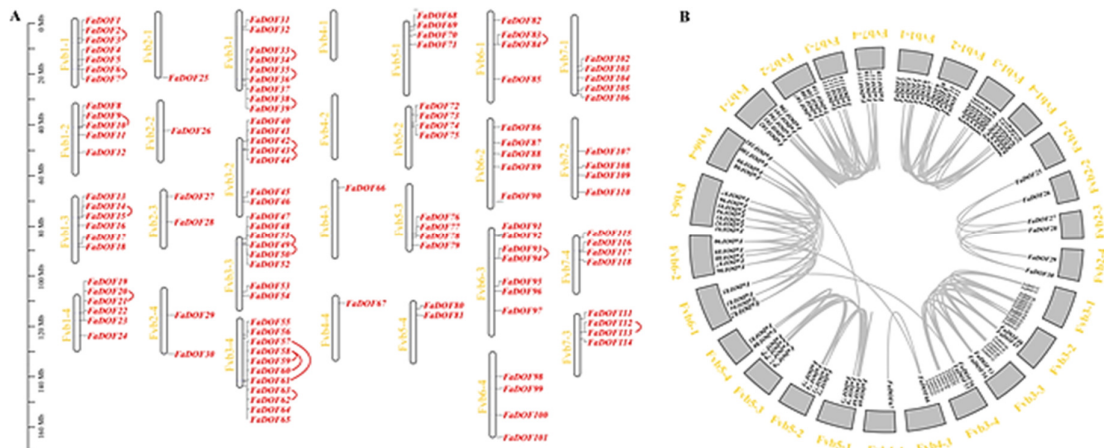
### *Cis-acting elements of the FaDOFs*

The 2000 bp sequences upstream of the transcription initiation site of the candidate genes were extracted from the strawberry genome sequences. The PlantCARE software (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html>) was used to search for *cis*-acting elements (Rombauts *et al.*, 1999), and the results were visualized in TBtools (Chen *et al.*, 2020).

## Results

### Identification and characteristics of the *FaDOFs* in the strawberry genome

One hundred and eighteen *FaDOF* genes were identified in the *F. × ananassa* genome after searching for DOF domain sequences. All of the *FaDOFs* were predicted to contain one DOF. One hundred and eighteen *FaDOFs*, twenty-two *FiDOFs*, twenty-three *FnDOFs*, twenty-five *FviDOFs* and thirty-seven *FvDOFs* were identified for *F. × ananassa* (Figure 1), *F. iinumae*, *F. nilgerrensis*, *F. viridis*, and *F. vesca* (Figure S1, Table S2), respectively. The *FaDOFs* were named according to their positions on each chromosome. The *FaDOF* protein lengths ranged from 135 aa (*FaDOF30*) to 525 aa (*FaDOF66*), and the pI was ranged from 4.78 (*FaDOF29*) to 10.51 (*FaDOF30*). The molecular weight ranged from 15.29 kDa (*FaDOF30*) to 56.22kDa (*FaDOF66*) (Table S2). The *FaDOFs* were distributed across twenty-six chromosomes, while no *FaDOF* were found on chromosomes Fvb4-1 and 4-2 (Figure 1). Chromosome Fvb3-4 contained the largest number of *FaDOFs* (eleven genes), chromosome Fvb2-1 and Fvb2-2 contained one *FaDOF*. There are a total of 17 tandem repeats were obtained from gene cluster analysis, among which *FaDOF2* and 3, *FaDOF6* and 7, *FaDOF9* and 10, *FaDOF14* and 15, *FaDOF20* and 21, *FaDOF33* and 34, *FaDOF35* and 36, *FaDOF38* and 39, *FaDOF42*, 43 and 44, *FaDOF49*, 50 and 51, *FaDOF57* and 61, *FaDOF58* and 59, *FaDOF58* and 60, *FaDOF62* and 63, *FaDOF83* and 84, *FaDOF93* and 94, *FaDOF112* and 113 exhibited tandem duplication. While the Chr 7 of the four chromosome sets showed the highest 34 segmental duplications, followed 33 numbers of Chr 3, 26 numbers of Chr 5, 24 numbers of Chr 6, 21 numbers of Chr 1, 6 numbers of Chr 2 and 2 numbers of Chr 2 (Figure 1).



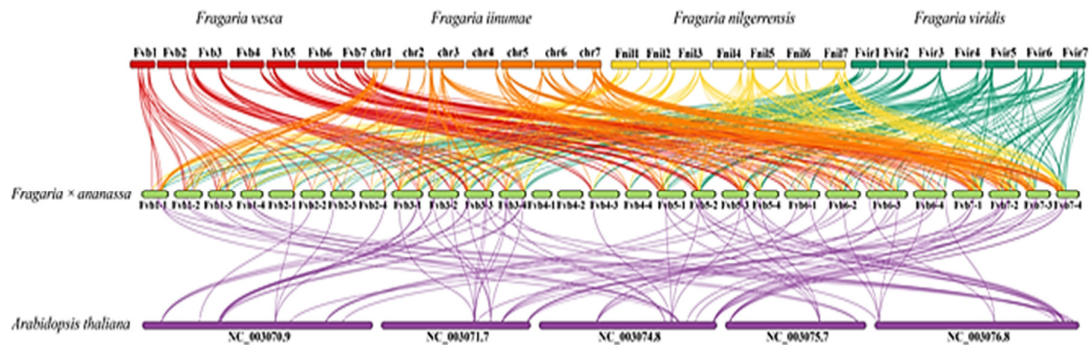
**Figure 1.** Chromosomal distribution of the *FaDOF* genes

Chromosome numbers are provided at the top of each chromosome together with the approximate size. The *FaDOFs* were named *FaDOF1* to *FaDOF118* based on their order on the chromosomes. A and B represent tandem duplication and segmental duplication by gene cluster analysis, respectively.

### Syntenic analysis of the *DOFs* in *A. thaliana* and five strawberries

To further investigate the phylogenetic patterns of the *FaDOFs*, a comparative syntenic map of five strawberries and *A. thaliana* was constructed. Among them, different numbers of *FaDOFs* showed syntenic relationships with the same *AtDOF* and the number ranges from 1-6, where at least one *FaDOF* corresponds to one *AtDOF* (*FaDOF26* to *AtDOF2*, *FaDOF90* to *AtDOF9*, *FaDOF96* to *AtDOF19* and *FaDOF113* to *AtDOF20*), and at most six *FaDOFs* correspond to one *AtDOF* (*FaDOF102*, 103, 107, 108, 114 and 118 to *AtDOF21*). At the same time, we found that 22 *FaDOFs* (*FaDOF2*, 6, 10, 14, 21, 33, 36, 38, 42, 43, 49, 50, 57, 58, 60, 61, 62, 83, 97, 97, 93 and 112) did not have syntenic relationships with the other four kinds of strawberries and *A. thaliana* (Figure 2). DoF of *F. × ananassa* showed different collinearity with DoF of *F. iinumae*, *F. vesca*, *F. viridis* and *F. nilgerrensis*. On the one hand, 38 *FaDOFs* (*FaDOF4*, 7, 9, 11, 15, 16, 18, 19,

20, 22, 26, 28, 29, 34, 39, 40, 44, 47, 48, 51, 59, 65, 68, 69, 72, 74, 77, 79, 80, 84, 86, 88, 94, 95, 96, 99, 100 and 101) only had syntenic relationships to four genes, while *F. iinumae*, *F. nilgerrensis*, *F. vesca* and *F. viridis* each corresponded to a gene. On the other hand, 46 *FaDOFs* (*FaDOF5*, 8, 13, 25, 27, 30, 31, 32, 45, 46, 53, 54, 55, 56, 66, 67, 70, 71, 72, 73, 75, 76, 78, 81, 82, 85, 89, 90, 97, 98, 102, 03, 104, 105, 106, 107, 108, 109, 110, 111, 113, 114, 115, 116, 117 and 118) were corresponded to more than 4 genes, and *F. iinumae*, *F. nilgerrensis*, *F. vesca* or *F. viridis* had multiple genes of the same species corresponding to the same *FaDOF*. In addition, we found that among them, there were 33 *FaDOFs* showing syntenic relationships to multiple *F. iinumae* *DOFs*, 38 *FaDOFs* showing syntenic relationships to multiple *F. nilgerrensis* *DOFs*, 43 *FaDOFs* showing syntenic relationships to multiple *F. viridis* *DOFs*, but only 13 *FaDOFs* had syntenic relationships to multiple *F. vesca* *DOFs*. From the perspective of diploid strawberries, there were 12 *FvDOFs* (*FvDOF4*, 6, 12, 14, 1, 17, 18, 9, 31, 32, 33, 35), 2 *FviDOFs* (*FviDOF10* and 22) and 1 *FidDOF* (*FidDOF10*) not having syntenic relationships to *FaDOFs*, but all *FnDOFs* were related. Except for a few *DOFs* of diploid strawberries (*FnDOF10*, *FviDOF4*, *FvDOF7*, *FidDOF4* and *FidDOF5*) exhibited syntenic relationships to one *FaDOF*, most of them corresponded to multiple *FaDOFs*, with the most *DOFs* (*FnDOF12*, 18 and 21, *FviDOF30*, *FidDOF11*, 15, 17 and 20) having syntenic relationships to 14 different *FaDOFs*. This showed that in the process of strawberry evolution, genes had been duplicated, and the *DOFs* were highly conserved (Table S3).



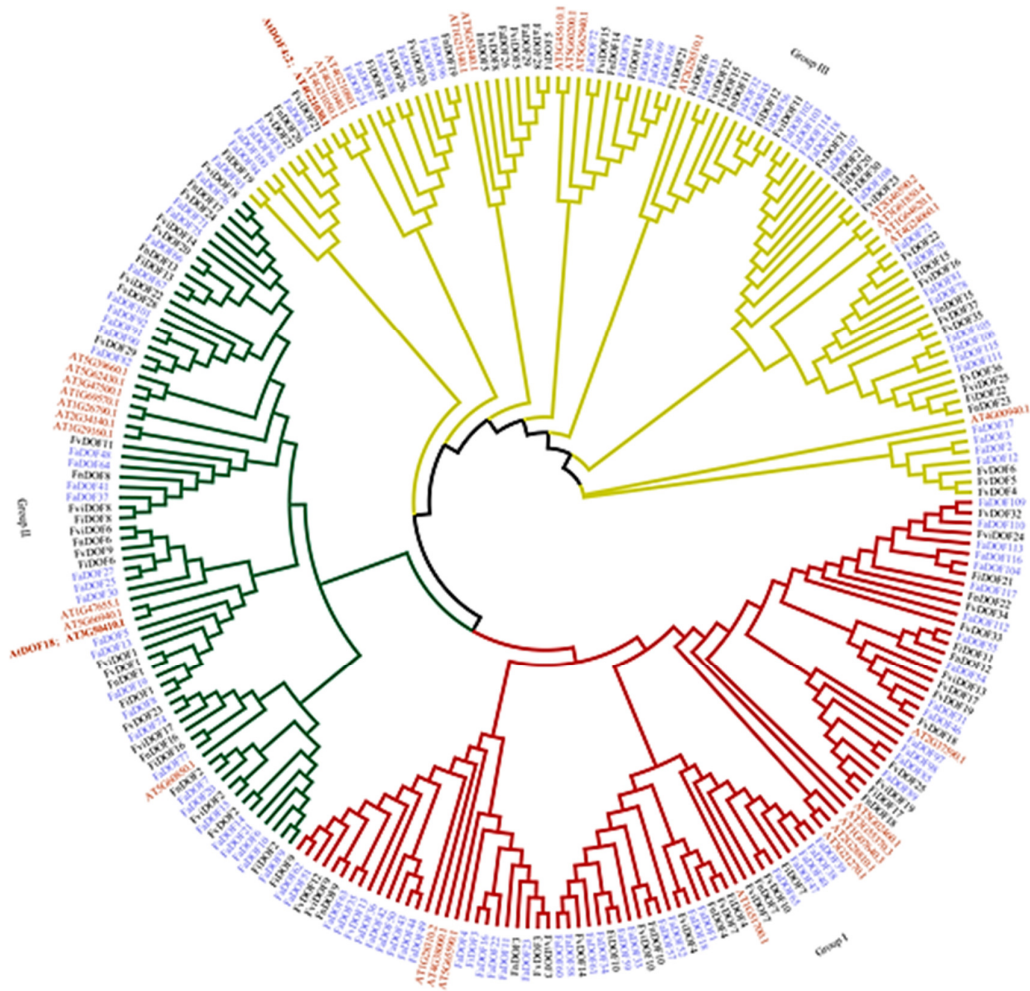
**Figure 2.** Synteny analysis of *Fragaria × ananassa* *DOFs* and *Arabidopsis thaliana* *DOFs* or other four diploid strawberries

Purple lines in the background indicate the collinear blocks within *F. × ananassa* and *A. thaliana* genomes. And lines indicate the collinear blocks within four diploid strawberries and *F. × ananassa* genomes.

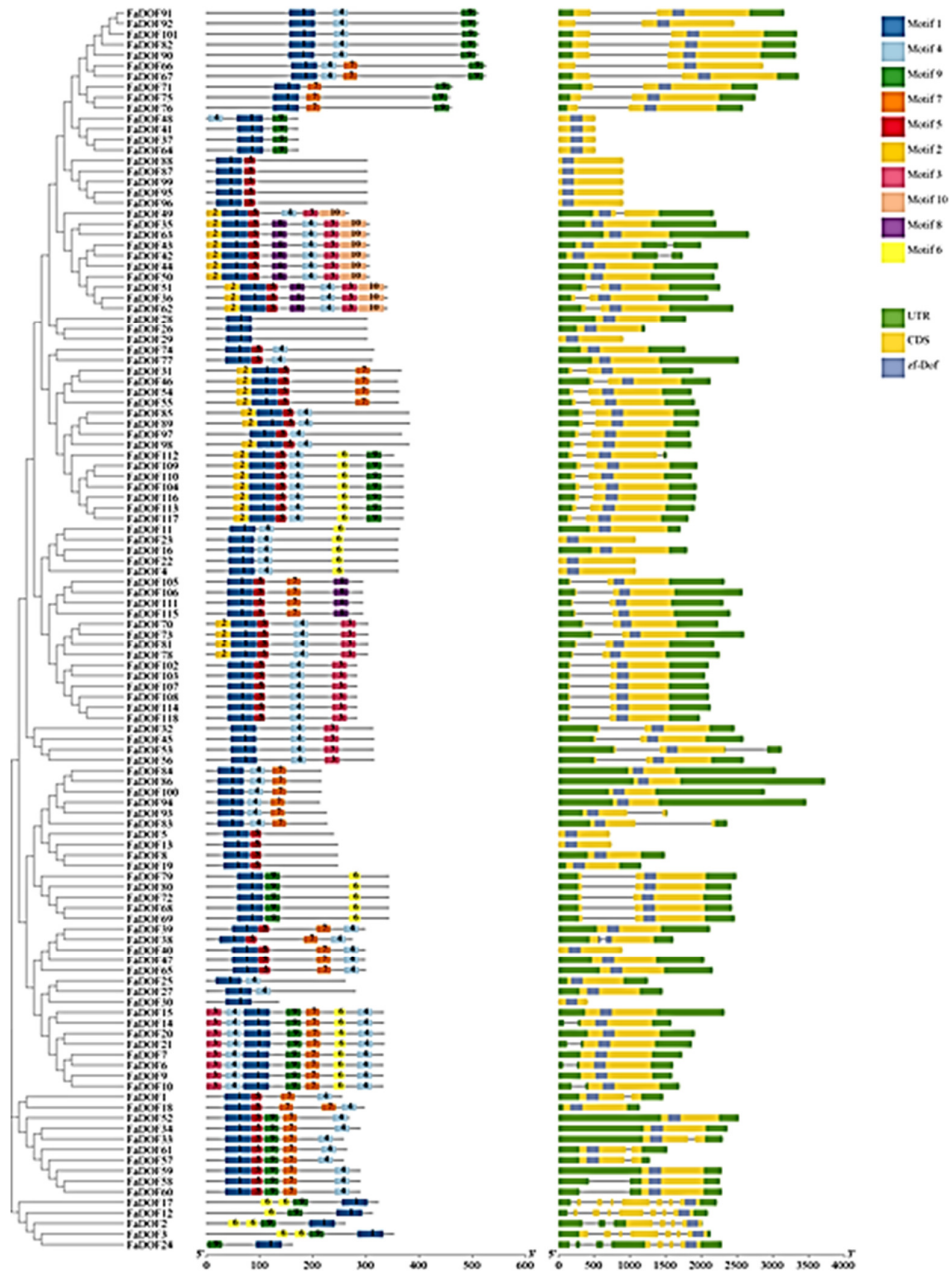
#### *Phylogenetic, exon-intron structure, conservative domains and motifs analysis of the FaDOFs*

The phylogenetic relationships of five different kinds of strawberries and *A. thaliana* *DOFs* were analyzed by a phylogenetic tree of the protein sequence alignment. As shown in Figure 3, the strawberry and *A. thaliana* *DOFs* clustered into three major groups. Groups I to III possessed 93, 72 and 100 members, where the number of members in group III was larger than the other two groups. The number of *AtDOFs* in groups I and II were nearly equal (10-11) (Figure 3).

The *FaDOFs* in different groups were characterized according to their *DOF* domain numbers and exon–intron structures. Motifs 1 composed the *DOF* domain, and all 118 *FaDOFs* had *DOF* characteristic domain. The number of introns varied from 0 (42 *FaDOFs*) to 7 (*FaDOF12* and *FaDOF17*). Most *FaDOFs* contained motif 1, 5, 7 and 4 or motif 3, 4, 1, 9, 7, 6 and 4 in the group I. Most of the group II had motif 2, 1, 5 and 4 or motif 10 as the end of 3'UTR side. Most of the group III contained one or more codons, and it was also the only group with motif 3 and 10 as the end of 3'UTR side. Most members of the fourth group IV only contained two or three motifs (Figure 4).



**Figure 3.** Phylogenetic tree of the DOFs based on an alignment of strawberries and *Arabidopsis thaliana* proteins  
The phylogenetic tree was constructed using the neighbour-joining method implemented in MEGA 7.0. Reliability of the predicted tree was tested using bootstrapping with 1,000 replicates.



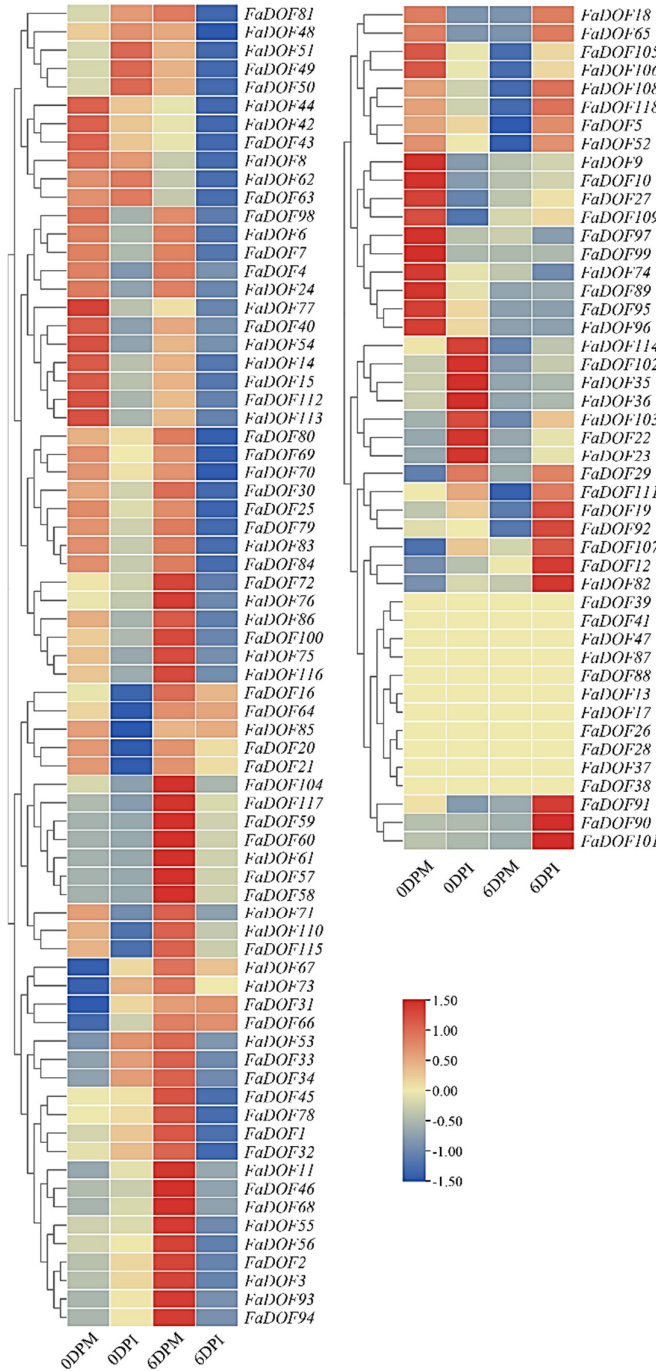
**Figure 4** Phylogenetic analysis of deduced *FaDOF* proteins associated with the motif composition and exon-intron composition of *FaDOF* genes

The phylogenetic tree was constructed using the neighbour-joining method (left-hand side of the figure). Reliability of the predicted tree was tested using bootstrapping with 1,000 replicates. The motif composition related to each *FaDOF* protein is displayed in the middle of the figure. The motifs were numbered 1-10. The information for each motif is provided in Figure S2.

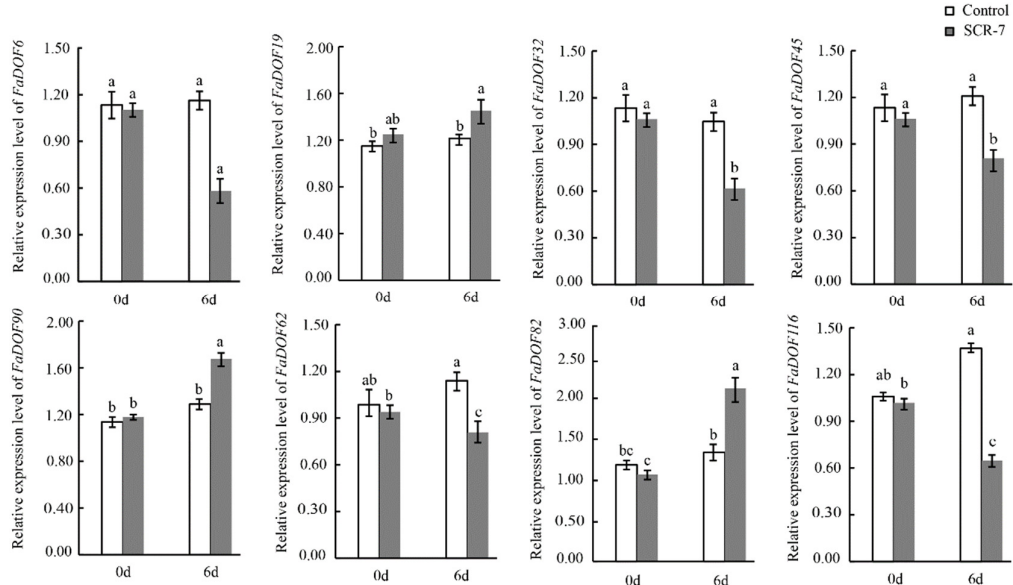
*Analysis of FaDOF expression and the cis-elements in FaDOF promoters*

Gene expression analysis revealed that 107 *FaDOFs* were expressed in crown (Figure 5), and most of them such as *FaDOF81*, 48, 51, 49, 50, 44, 42, 43, 8, 62, 63, 98, 6, 7, 4, 24, 77, 40, 54, 14, 15, 112, 113, 80, 69, 70, 30, 25, 79, 83, 84, 72, 76, 86, 75, 116 and other *FaDOFs* were downregulated by crown rot, while some *FaDOFs* such as *FaDOF107*, 12, 82, 91, 90 and 101 were upregulated (Figures 5, 6). The *cis*-element of ‘defense

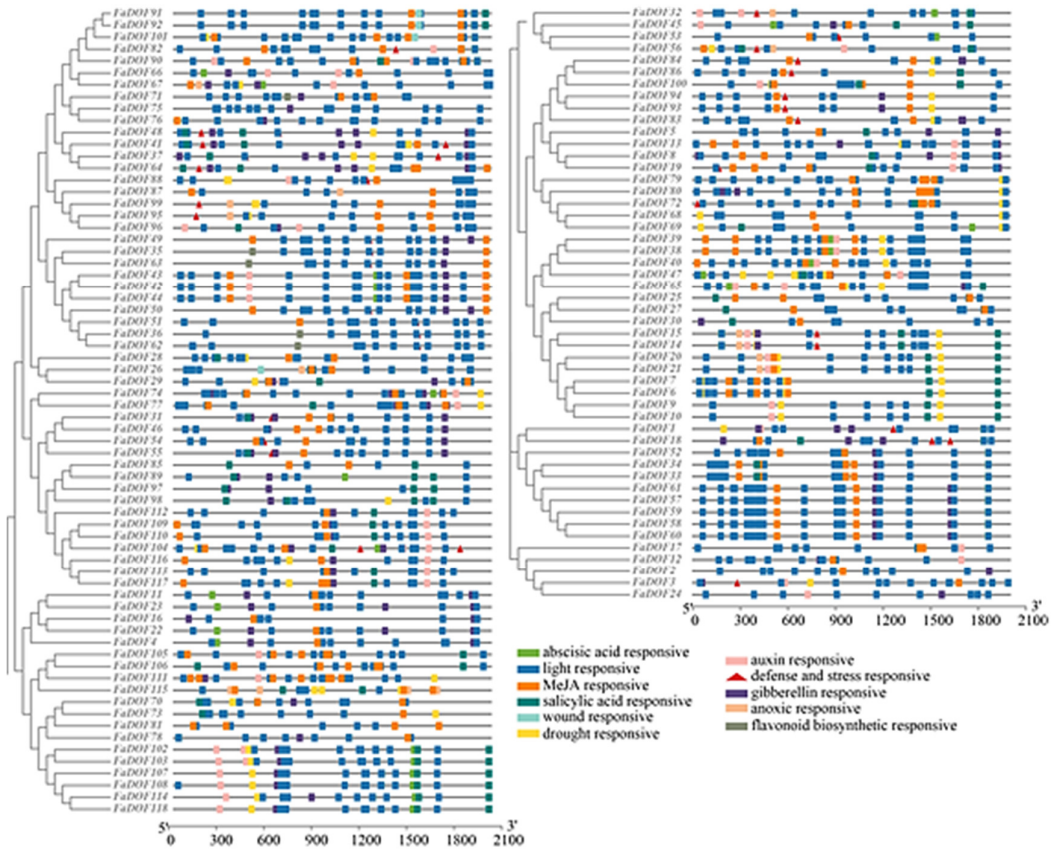
and stress responsive' was responsible for the plant response to pathogen infection. 'Defense and stress responsive' *cis*-elements were identified in the promoters of 43 *FaDOFs* (*FaDOF1*, 3, 4, 14, 15, 18, 19, 31, 32, 35, 36, 41, 48, 49, 50, 51, 53, 54, 55, 56, 62, 63, 64, 76, 77, 82, 83, 84, 86, 88, 93, 94, 95, 99, 104, 109, 110, 112, 113, 116 and 117). Among them, two 'defense and stress responsive' *cis*-elements were found in 4 genes (*FaDOF18*, 37, 48, and 56) and three were found in one gene (*FaDOF104*) (Figure 7).



**Figure 5.** Expression profiles of the *FaDOF* genes responding to crown rot. Red and blue indicate up- and downregulated genes compared to the relevant control.



**Figure 6.** Verification *FaDOF* genes expression responding to crown rot by qRT-PCR. The relative gene expression was calculated using the  $2^{-\Delta\Delta Ct}$  method, where  $\beta$ -actin was taken as the reference gene. Significant differences between treatments were determined by Duncan's Multiple Range Tests at  $p = 0.05$ . The value with different letter showed significant differences.



**Figure 7.** Predicted *cis*-elements in the promoters of the *FaDOFs*. The 'defense and stress responsive' *cis*-elements are indicated by triangle

## Discussion

DOF proteins are plant-specific transcription factors, and genes containing the DOF domain were first discovered from maize in 1993 (Yanagisawa and Izui, 1993), with the deepening of research, it is found in higher plants or in unicellular algae. The number of *DOF* transcription factor families varies greatly among different species. For example, there is only 1 in *Chlamydomonas reinhardtii* (Algae), while there are 78 in *G. max* (Dicotyledon) (Moreno-Risueno *et al.*, 2007; Guo and Qiu, 2013). It is also distributed in different species such as *Brachypodium distachyum* (Monocot), *Physcomitrella patens* (Musci) and *Pinus taeda* (Coniferopsida) (Hernando-Amado *et al.*, 2012; Wu *et al.*, 2016). One hundred and eighteen *FaDOFs* were identified on twenty-six chromosomes in the *F. ×ananassa* genome (Figure 1). Gene duplication and divergence are important in gene family expansion and in the evolution of novel functions. Nearly seventy segmental duplication and seventeen tandem duplication were obtained from gene cluster analysis, suggesting that segmental and tandem duplication may have played crucial roles in the expansion of the *F. ×ananassa DOF* gene family (Figure 1). Among them, twenty-two *FaDOFs* showed no syntenic relationships with the *AtDOFs*, which can be speculated they may be new genes produced during plant evolution. The syntenic relationships of *DOFs* between other four kinds of strawberries and the *F. ×ananassa* showed that the *F. ×ananassa* had gene duplication during the evolution process (Figure 2). Recently research showed *F. iinumae* and *F. vesca* are the diploid progenitors to the octoploid *F. ×ananassa*, whereas the other three diploids that we analyzed in this study are not parental species (Edger *et al.*, 2019; Liston *et al.*, 2020; Edger *et al.*, 2020; Feng *et al.*, 2021). There were 33 *FaDOFs*, 38 *FaDOFs* and 43 *FaDOFs* respectively exhibiting syntenic relationships to multiple *F. iinumae*, *F. nilgerrensis* and *F. viridis DOFs*, but there were only 13 *FaDOFs* having syntenic relationships to multiple *F. vesca DOFs* (Table S3). The large variation numbers of syntenic relationships between *F. iinumae* and *F. ×ananassa*, and *F. vesca* and *F. ×ananassa* need further study.

All of the *FaDOFs* clustered into three major groups, with distinct protein domains, motifs and sequences (Figure 3). DOF domain was the most conserved region in DOF protein. Motifs 1 composed the DOF domain, and all 118 *FaDOFs* had one characteristic domain (Figure S2). The phylogenetic tree which was generated based on the protein sequence alignment of strawberries and *A. thaliana* segregated the 118 *FaDOFs* into four large groups. Group members shared similar protein sequence lengths, motif compositions and exon–intron structures, suggesting a close function. Thus, *FaDOF5* with their homolog *AtDOF18* (*OBP1*) in the same branch may play similar roles in biotic stress responses (Figure 3). *AtDOF18* clustering with *FaDOF5* which was induced by crown rot (Figure 5) speculated that they may interaction with *cystatin* gene and *octopine synthase* gene respectively in biotic stress tolerance to enhance its anti-adversity (Zhang *et al.*, 1995; Martínez *et al.*, 2005). From the phylogenetic tree it was predicted that the *FaDOFs* were involved in pathogen infection interactions, but this hypothesis required verification in future studies. While *AtDOF4;2* affects *A. thaliana* resistance by negative influence on flavonoid biosynthesis and positively influences the production of hydroxycinnamic acids in phenylpropanoid metabolism (Skirycz *et al.*, 2007), the most similar genes *FaDOF24* which was repressed by crown rot (Figure 5) might regulated phenylpropanoid metabolism in octoploid strawberry. In addition, ‘defence and stress responsive’ *cis*-elements were identified in the promoters of 43 *FaDOFs* but some of them such as *FaDOF 4, 14* were down-regulated by crown rot. The results suggested *FaDOFs* expression regulations was not consistent with the *cis*-elements present in their promoters (Figure 5, 7), which might be due to the integration of other gene regulating elements, such as *trans*-acting factors (Chow *et al.*, 2018; Xie *et al.*, 2018).

## Conclusions

*DOF* family transcription factors are one class of resistance genes involved in biological stress, and the family members in strawberry and its expression pattern in responding to crown rot still unclear. In our current

study, we identified 118 *FaDOFs*, 22 *FiDOFs*, 23 *FnDOFs*, 25 *FviDOFs* and 37 *FvDOFs* in the *F. × ananassa*, *F. iinumae*, *F. nilgerrensis*, *F. viridis*, and *F. vesca* genome, respectively. In the syntenic relationship analysis with *A. thaliana*, it was found that *F. × ananassa* produced 22 new genes during the evolution process. Except for a few *DOFs* of diploid strawberries (*FnDOF10*, *FviDOF4*, *FvDOF7*, *FiDOF4* and *FiDOF5*) showing syntenic relationships to one *FaDOF*, most of them corresponded to multiple *FaDOFs*, with the most *DOFs* (*FnDOF12*, 18 and 21, *FviDOF30*, *FiDOF11*, 15, 17 and 20) had syntenic relationships to 14 different *FaDOFs*. It showed that *F. × ananassa* may have chromosomal variation during the evolution process, which also proved it is highly conserved during *DOF* evolution. In addition, 107 *FaDOFs* were expressed in crown, most of them downregulated by crown rot, while some *FaDOFs* such as *FaDOF107*, 12, 82, 91, 90 and 101 were upregulated. The analysis of phylogenetic tree and *cis*-elements in promoters indicated that the genes may have the ability to regulate the pressure of pathogen infection. However, the study of *DOFs* mechanism of action is still not thorough, so it is necessary to further study the signal pathways involved in order to further study the specific mechanism of action. Collectively, the results of this study provided a basis for future functional studies of the strawberry *DOF* and their responses to crown rot.

### Authors' Contributions

BS conceived and designed the experiments, supervised and revised the manuscript. CL conducted part of the experiments and wrote the original manuscript. YH conducted part of the experiments.

All authors read and approved the final manuscript

### Ethical approval (for researches involving animals or humans)

Not applicable.

### Acknowledgements

This work was supported by the Scientific Research Foundation for Doctor of Yangtze University, grant number 802100270303.

### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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