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Genome-wide identification and expression analysis of DOF transcription factor in tomato (*Solanum lycopersicum*) and its effect against developmental and stress condition

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ABSTRACT

The transcription factor known as DNA-binding with one finger (DOF) is a plant-based regulator involved in stress responses, growth, and development. Specifically, DOFs play key roles in essential biological processes, including signal transduction, cellular morphogenesis, and reactions to environmental stress. We aim to identify and characterize the DOF transcription factors in tomato (*Solanum lycopersicum*) and examine their expression under various developmental and stress conditions. In this study, we conducted a genome-wide identification of the DOF family in tomato, which involved phylogenetic analysis, conserved motif identification, predictions of sub-cellular localization, gene structure analysis, gene expression profiling, and protein-protein interaction studies. We identified, classified, and analyzed the expression of 8 DOF genes in tomato. The sequences of these genes showed similarity to those in *S. lycopersicum*, including DOF5.1, DOF3.1, DOF2.4-like, DOF2.5-like, DOF3.4-like, DOF1.4, DOF3.4-like, and DOF3.1. The zf-DOF (pfam ID: pfam02701) and the zf-DOF superfamily (pfam Cl: 03664) were identified as two common superfamily domains across all eight genes. Through phylogenetic analysis, we identified two genes associated with stress response and six genes related to developmental processes. Notably, DOF1.4 was found to be expressed in both stress and developmental contexts. The distinct expression profiles of DOF genes in response to abiotic stimuli suggest their significant involvement in the plant's defense mechanisms. These findings enhance our understanding of the mechanisms underlying plant growth, development, and stress responses, providing valuable insights that could improve crop productivity and resilience in agricultural practices.

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1 Introduction

The transcription process and the role of transcription factors are the primary regulatory mechanisms for various plant processes, including cell growth, stress responses, and environmental signaling. Transcription factors (TFs) are essential for regulating gene expression by binding to cis-regulatory elements located within the promoter region of genes (Waschburger et al. 2022). Plants have developed an extraordinary ability to respond to internal and external signals, allowing them to adapt to changing environmental conditions and support their growth and development (Tabassum et al. 2022). A crucial aspect of this regulatory mechanism is the precise control of gene expression, in which TFs play a significant role.

The DOF transcription factor family is unique to plants and is recognized for its involvement in several biological processes, including signal transduction, cellular morphogenesis, and stress responses (Li et al. 2022). TFs are proteins that bind to specific DNA sequences in the promoter regions of their target genes, modulating transcriptional activity by either enhancing or suppressing gene expression (Jiao et al. 2022). The DNA-binding domains of the DOF family are characterized by a single, consistent zinc finger motif that enhances their ability to connect with specific cis-acting regions found in the promoters of target genes (Li et al. 2022).

The roles of DOF family members in leaf vein development have attracted significant attention. Arabidopsis VDOF1 (VASCULAR-RELATED DOF1) and VDOF2 (VASCULAR-RELATED DOF2) may inhibit cotyledon vein formation and lignin deposition by regulating brassinosteroid (BR) signaling and the transcription of genes related to lignin in inflorescence stems (Ramachandran et al. 2020). Recent studies by Zhang et al. (2022) indicate that CDF4 regulates cotyledon vein development. Additionally, DOFs have been implicated in jasmonic acid (JA)-induced leaf senescence in both monocots and dicots. For instance, OsDOF24 in rice delays leaf senescence by suppressing the activity of the OsAOS gene, which is associated with JA biosynthesis (Renard et al. 2020). Conversely, Arabidopsis AtDOF2.1 has been shown to actively contribute to JA-induced leaf senescence through a MYC2-DOF2.1-MYC2 actively feed-forward transcription loop (Negi et al. 2013). These findings suggest that plant-specific DOF TFs may regulate various processes associated with developmental stages in plants or their roles in long-distance signaling.

In the context of stress conditions, several studies indicate that DOF TFs respond to biotic stresses by enhancing the ability of plants to defend against pathogens. For instance, the transient expression of the DOF genes BBF2 and BBF3 in tobacco has increased plant resistance to pathogens (Sasaki et al. 2015).

S. lycopersicum, a significant economic crop worldwide, requires a deep understanding of the regulatory mechanisms governing its growth, development, and stress responses for agricultural improvement (Bedinger et al. 2011). While research on DOF transcription factors in various plant species is growing, comprehensive studies on this family in tomatoes remain sparse (Zou and Sun 2023). The TF TMO6, a member of the DOF family that responds to auxin signals, is central to the pathway affected by MP 77. Additionally, several members of the DOF family have been demonstrated to play vital roles in promoting cell divisions in vascular tissues. Specific DOF genes, like TMO6, are regulated by cytokinin, making TMO6 responsive to both cytokinin and auxin signals (Smit et al. 2020).

Given these considerations, we aim to identify and characterize the DOF transcription factor in tomatoes and study its expression during developmental and stress conditions to understand its role fully. This information is crucial for crop improvement strategies, including genetic engineering approaches to enhance stress tolerance, fruit development, and crop productivity in tomatoes and related species.

2 Material and methods

2.1 Discovery of Potential DOF Genes Associated with Stress and Developmental Conditions in Tomato Plants

Nucleotide sequences of DOF genes, which are responsible for responses to stress and developmental conditions, were retrieved from the TAIR Database (https://www.arabidopsis.org). The TAIR (The Arabidopsis Information Resource) is a comprehensive web-based database with complete genetic and molecular biology information for the *Arabidopsis thaliana* model. A total of 39 DOF nucleotide sequences were obtained from the TAIR database. Following the retrieval, BLAST (with an e-value of 1e-10 and a minimum identity of 50%) was performed using the nucleotide sequences to identify homologous genes in *S. lycopersicum* (Table 1). This process resulted in eight blast sequences that exhibited more than 50% similarity (Li et al. 2022). The BLAST sequences (with an e-value of 1e-10 and a minimum identity of 50%) from the *S. lycopersicum* genome were employed as queries against the total expressed sequence tags (ESTs) of *S. lycopersicum* to identify the expressed genes in that genome. Additionally, NCBI's Conserved Domain search tool was utilized to calculate and analyze conserved motifs (https://www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi).

2.2 The Analysis of Phylogenetic Trees

The Blast DOF gene sequences identified from the *S. lycopersicum* genome were aligned using Clustal W2. A phylogenetic tree was constructed using the Neighbor-Joining method in MEGA 11

software (Larkin et al. 2007; Kumar et al. 2018). Bootstrap values were calculated to explore the relationships among the DOF genes of *S. lycopersicum*, which were identified through the Blastn similarity search. Additionally, using phylogeny.fr (https://www.phylogeny.fr/), a separate phylogenetic tree was created to examine the relatedness of eight genes based on stress and developmental characteristics derived from the Blastn analysis of the *S. lycopersicum* genome.

2.3 Gene Ontology Study

2.3.1 Sub-cellular localization

We used Busca (Bioinformatic Utility for Species Context Analysis) (https://busca.biocomp.unibo.it/) to predict specific subcellular localization that starts from the protein sequence, which was translated using the ExPaSy translation tool from the gene obtained through BLASTn.

2.3.2 Protein-protein interaction

We investigated complex pathways by analyzing protein-protein interaction (PPI) networks to enhance our understanding of the relationships between the identified DOF genes and uncover unknown proteins' roles. STRING (https://string-db.org/) performed the PPI interactions, focusing on the stress-related functions associated with the DOF genes. Additionally, we annotated and integrated functional files detailing various functions, from which we categorized the genes according to their respective developmental and stress-related conditions.

2.4 Plant material, pathogen inoculation, and biotic stress treatments

Two tomato cultivars, *ArkaSamrat* (resistance to BW) and *Pusa Ruby* (susceptible to BW) were selected for the present study. These cultivars were chosen based on their known resistance and susceptibility to *Ralstonia solanacearum* bacteria. *R. solanacearum* was grown in separate growth conditions to ensure its availability for plant inoculation. The growth conditions involved a suitable culture medium, such as nutrient agar or broth, and appropriate temperature and humidity for bacterial growth. A temperature-controlled growth chamber was set up to maintain specific temperature conditions necessary for the growth of tomato plants and *R. solanacearum*. The chamber had temperature and humidity sensors and control systems to maintain the desired conditions. Tomato plants of both AS and PR cultivars were selected for inoculation. The bacterial suspension was prepared by diluting the culture to an appropriate concentration. The tomato plants were inoculated with *R. solanacearum* root immersion. Care was taken to avoid cross-contamination between cultivars during inoculation. The inoculated tomato plants were placed inside the temperature-controlled growth chamber. The chamber was set to maintain the required temperature, typically around 28-30°C, which is favorable for *R. solanacearum* infection and disease development. The chamber's humidity level was also adjusted to create an optimal environment for the plants and the bacterial pathogen. The tomato plants were regularly monitored for disease progression and symptoms caused by *R. solanacearum* infection. Disease severity and symptoms, such as wilting, leaf yellowing, necrosis, or stunting, were recorded for each cultivar separately.

2.5 Inoculation of R. solanacearum

The bacteria were cultivated in TTC (Triphenylterazolium chloride) medium, which contains 3 g of sucrose, 5–10 g of beef extract, 7 g of tryptone, and 7 g of agar per liter. The medium was treated with 1% TTC for two days and maintained at 30 °C. *R. solanacearum* was injected into plants at the six-leaf stage through root wounds. The plants underwent a mock inoculation using sterile water and were allowed to incubate for 30 minutes in a bacterial solution containing 10^8 colony-forming units (cfu)/ml. All inoculated plants were then placed in plastic containers. The Arka Samrat plants inoculated with pathogens and mock-inoculated were labeled R-5dpi, R-10dpi, R-15dpi, and R-mock (control). Similarly, the Pusa Ruby plants subjected to pathogen injection and mock inoculation were designated S-5dpi, S-10dpi, S-15dpi, and S-mock (control). We maintained both susceptible and resistant plant varieties at three distinct developmental stages: the vegetative state (40 days after germination), the blooming stage (90 days after germination), and the seedling stage (20 days after germination). Tissue samples from plants at different developmental stages were then quickly frozen in liquid nitrogen and stored at –80 °C for further examination. Each experiment was conducted three times to ensure reliability.

2.6 Isolation of RNA and Analysis of Gene Expression

Five stem tissue samples were collected at each designated time point and developmental stage to obtain RNA. Total RNA was isolated from the ice-frozen control and treatment samples using Trizol reagent (Invitrogen, Darmstadt, Germany). DNAse I (supplied by Promega, Madison, USA) was then utilized to purify the resulting RNA according to the manufacturer's instructions. The concentration of the RNA was evaluated using the NanoDrop spectrophotometer (Thermo Scientific, Waltham, USA). RNA samples with a 260/280 nm ratio between 2.0 and 2.1 were selected for further examination. Following the instructions, 2 µg of RNA were transcribed using the high-capacity cDNA synthesis kit (Life Technologies, Burlington, CA) to synthesize the first strand of cDNA. After diluting the cDNA tenfold, it served as the template for quantitative real-time PCR (qRT-PCR). A set of gene-based primers developed from the conserved regions of eight DOF genes was used for the qRT-PCR. Before qPCR, RT-PCR was performed to verify the specificity of the primers, and agarose gel

electrophoresis was used to separate the amplified cDNA products. Each RT-PCR reaction was conducted in a total volume of 10 mL, which included 5 µL of FASTSYBR Green mix from Kappa Biosystems (D Mark, Toronto, CA), 1 µL of each forward (F) and reverse (R) primer (at a concentration of 5 µM), 1 µL of reverse-transcribed cDNA (with a concentration of 5 ng), and 2 µL of nuclease-free (NF) water.

Using the Step One Plus real-time PCR equipment (Life Technologies, Burlington, Canada), the reactions underwent rapid qPCR with the following thermal cycling conditions: an initial denaturation step for one minute at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing at 60 °C for 30 seconds. A melting curve analysis was performed by first holding at 65 °C for 15 seconds, then gradually increasing the temperature to 95 °C at a rate of 0.2 °C per second to confirm the accuracy of the PCR product. Nine reactions, consisting of three biological and three technical replicates, were used to calculate the Ct values for each sample. Glyceraldehyde-3-phosphate dehydrogenase (SlGADPH) and Solanum lycopersicum Ubiquitin-containing enzyme 3 (SlUBC3) were endogenous controls.

Using the comparative 2−ΔΔCt method, the threshold cycle values were converted into relative expression values for the genes, with the control transcript set at 1 (Bedinger et al. 2011). The results of the qRT-PCR were statistically significant, as evidenced by a two-way analysis of variance (ANOVA) and numerous evaluations using the uncorrected Fisher's LSD test. A p-value of less than 0.05 was used to determine the significance of the differences in mean values.

3 Results

3.1 Computational discovery of genes associated with developmental and stress conditions

We downloaded 39 nucleotide sequences of *A. thaliana* from the TAIR database to identify the DOF genes associated with developmental and stress conditions. A nucleotide BLAST was performed against the whole genome of *S. lycopersicum* using NCBI BLASTn. Out of the 39 sequences from *A. thaliana*, only 8 exhibited similarity with *S. lycopersicum*: DOF5.1, DOF3.1, DOF2.4-like, DOF2.5-like, DOF3.4-like, DOF1.4, DOF3.1, and DOF3.4-like. Their identity and query coverage are detailed in Table 1. The stress and developmental conditions for these sequences were assigned based on a literature review, as indicated in Table 1.

Out of 8 DOF sequences, one was categorized under stress conditions, while the remaining were associated with developmental conditions. To assess the expression of the derived DOF sequences of *S. lycopersicum* from BLASTn, we conducted an analysis using Expression Sequence Tags (EST). It was found that DOF2.4-like, DOF3.4-like, and DOF1.4 did not show any expression in the EST database, as shown in Table 2.

3.2 Motif and conserved domain analysis

The MEME analysis of DOF genes identified three significant motifs that reflect potential regulatory elements crucial for their function. The genes analyzed include DOF5_1, DOF3_1, DOF2_4_like, DOF2_5_like, DOF3_4_like, DOF1_4, DOF3_1_1, and DOF3_4. These motifs were found at various positions across the genes, with highly significant p-values ranging from 1.49e-4 for DOF5_1 to 8.16e-49 for DOF3_4, indicating strong statistical support for their presence. Motif 1, which has a consensus sequence of "AACA CAAAG TTYGT TACTA CAACA AYTYA RYYKT CTCAG CCAGC CACGCCA," is prominently found in all the analyzed genes, suggesting a conserved functional role. In addition, Motif 2, with the sequence "AGGT AYTGGC ATYRA GGNGG AACTY TAMG BAAY RTHC CWGT ITGG IGWGG," and Motif 3, with the sequence "YTTY TGCA AAGACT YG," were also identified. Each of these motifs contributes to the regulatory landscape of the DOF genes. The motif logos illustrate

Table 1 Identity/similarity between *A. thaliana* and *S. lycopersicum* Dof sequences.

Est– results			
Gene name	Identity	Similarity	e-value
DOF5.1	100%	100%	5e-06
DOF3.1	100%	100%	4e-89
DOF2.4-like	-	-	-
DOF2.5-like	100%	100%	3e-91
DOF3.4-like	-	-	-
DOF1.4	-	-	-
DOF3.1	98.73%	98%	2e-33
DOF3.4-like	100%	100%	5e-62

Table 2 Represents similarity/identity and e value. Where Accession no. (AT) represents the *A. thaliana*, and Accession no. (sol) represents the *S. lycopersicum.*

Gene name	Accession no. (AT)	Accession no. (sol)	Identity	E value	Condition
DOF5.1	AT1G29160.1	XM_004250915.4	100%	4e-04	Seed coat Development (Renard et al. 2020)
DOF3.1	AT2G28510.1	XM_004235567.4	77.53%	1e-19	Tissue regeneration development (Zhang et al. 2022)
DOF2.4-like	AT2G37590.1	XM_004253279.1	96.19%	0.0	Radial growth development (Miyashima et al., 2019)
DOF2.5-like	AT2G46590.2	XM_004229950.4	82.87%	3e-37	Stress (Zou and Sun 2023)
DOF3.4-like	AT3G52440.2	XM_004233894.4	81.61%	1e-09	Positive regulator of light-mediated seed germination l (Santopolo et al. 2015)
DOF1.4	AT4G24060.1	XM_004232850.4	78.99%	1e-16	Regulate vascular cell differentiation and lignin biosynthesis (Ramachandran et al. 2020)
DOF3.1	AT5G60850.1	XM_004241893.4	79.84%	4e-16	Control Vascular Development in Arabidopsis (Smit et al. 2020)
DOF3.4-like	AT5G65590.1	XM_004233894.4	80.77%	5e-19	Stomatal guard cell maturation and differentiation (Negi et al. 2013)

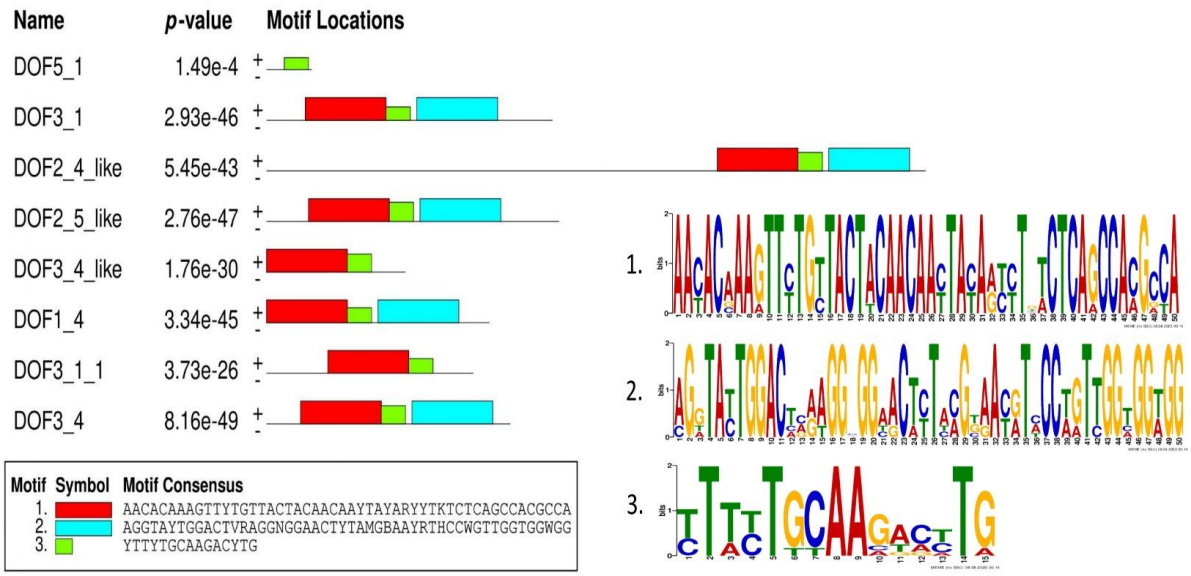

Figure 1 Conserved motif analyses of DOF protein sequences using the MEME 4 program. The length of each distinct protein sequence is shown by a solid line in the graphical representation, and the various discovered motifs are shown as colour boxes.

the conservation of specific nucleotide positions within each motif, highlighting their potential importance in gene regulation (Figure 1). DOF3_1 exhibited Motifs 1, 2, and 3 at multiple locations, with a highly significant p-value of 2.93e-46, underscoring its complex regulatory functions. Similarly, DOF2_5_like, which also contains Motifs 1, 2, and 3, had a p-value of 2.76e-47, suggesting robust regulatory roles as well. The presence of these motifs across different DOF genes indicates their involvement in essential regulatory mechanisms that could influence gene expression and functional specificity. The high conservation of these motifs among various DOF genes and their significant p-values emphasize their critical role in the regulatory networks governing gene expression.

The conserved region analysis of the DOF genes revealed significant insights into their structural domains, particularly the zinc finger-Dof (zf-Dof) domain, which is crucial for DNA binding and gene regulation. The gene DOF5.1 (XM_004250915.4) spans residues 69 to 127, with an E-value of 3.6e-35, indicating a highly conserved domain (pfam02701). DOF3.1 (XM_004235567.4) spans residues 1 to 144, with an E-value of 1.61e-28, also showing a conserved zf-Dof domain. DOF2.4-like (XM_004253279.1), covering residues 250 to 408, presents an E-value of 4.78e-36 and belongs to the zf-Dof superfamily (cl03664), indicating a broader conserved region. DOF2.5-like (XM_004229950.4) spans residues 3 to 164 with an E-value of 2.56e-32, confirming its conserved zf-Dof domain. DOF3.4-like (XM_004233894.4) spans residues 1 to

Table 3 Representation of interval conserved part in the sequence of the DOF gene their 'e' value with pfam id.

Conserved region of genes	Gene Name	Accession no.	Interval	E-value	Gene name (cd)	pfam id
	Dof 5.1	XM_004250915.4	69-127	3.6e-35	zf-Dof	pfam02701
	Dof 3.1	XM_004235567.4	1-144	1.61e-28	zf-Dof	pfam02701
	Dof 2.4-like	XM_004253279.1	250-408	4.78e-36	zf-Dof superfamily	Cl03664
	DOF2.5-like	XM_004229950.4	3-164	2.56e-32	zf-Dof	pfam02701
	DOF3.4-like	XM_004233894.4	1-84	7.28e-15	zf- Dof superfamily	cl03664
	DOF1.4	XM_004232850.4	1-138	2.41e-27	zf-Dof	pfam02701
	DOF3.1-like	XM_004241893.4	9-128	1.71e-23	zf-Dof superfamily	cl03664
	DOF3.4-like	XM_004233894.4	1-150	4.44e-32	zf-Dof	pfam02701

84 with an E-value of 7.28e-15 and belongs to the zf-Dof superfamily (cl03664), indicating a shorter conserved region. DOF1.4 (XM_004232850.4) spans residues 1 to 138 with an E-value of 2.41e-27, highlighting a conserved zf-Dof domain (pfam02701). DOF3.1-like (XM_004241893.4) spans residues 9 to 128 with an E-value of 1.71e-23, belonging to the zf-Dof superfamily (cl03664). Finally, DOF3.4 (XM_004233894.4) spans residues 1 to 150 with an E-value of 4.44e-32, indicating a highly conserved zf-Dof domain (pfam02701) (Table 3). These conserved regions across the DOF genes underscore their evolutionary conservation and functional importance in DNA binding and gene regulation.

3.3 Phylogenetic analysis

The phylogenetic analysis revealed that the DOF genes can be categorized into two main groups: development-related genes and stress-related genes. In the development-related cluster, the genes XM004250915.4 (DOF5.1) and XM004253279.1 (DOF2.4-like) had branch lengths of 0.00 and 0.14, respectively. XM004233894.4 (DOF3.4-like) and its variant, DOF3.4-like (2), exhibited a branch length of 0.00, indicating a close relationship between them. The genes XM004235567.4 (DOF3.1) and XM004241893.4 (DOF3.1) showed branch lengths of 0.12 and 0.04, respectively. In the stress-related gene cluster, XM004229950.4 (DOF2.5-like) and XM004232850.4 (DOF1.4) exhibited branch lengths of 0.07 and 0.14, respectively. The tree's root connected these two clusters, with branch lengths of 0.05 for the development-related genes and 0.02 for the stress-related genes. This analysis emphasizes these genes' evolutionary relationships and genetic distances, highlighting their functional divergence in response to developmental processes and stress conditions (Figure 2).

3.4 Functional Characterization of the DOF Genes

3.4.1 Subcellular localization

The physiological positions of the genes were determined using subcellular localization data (Table 3) with the assistance of BUSCA. Most genes, represented as 5 DOF, are located in the chloroplast thylakoid lumen, except DOF 2.4-like, which is found in the extracellular space and scored 0.67 (Table 4).

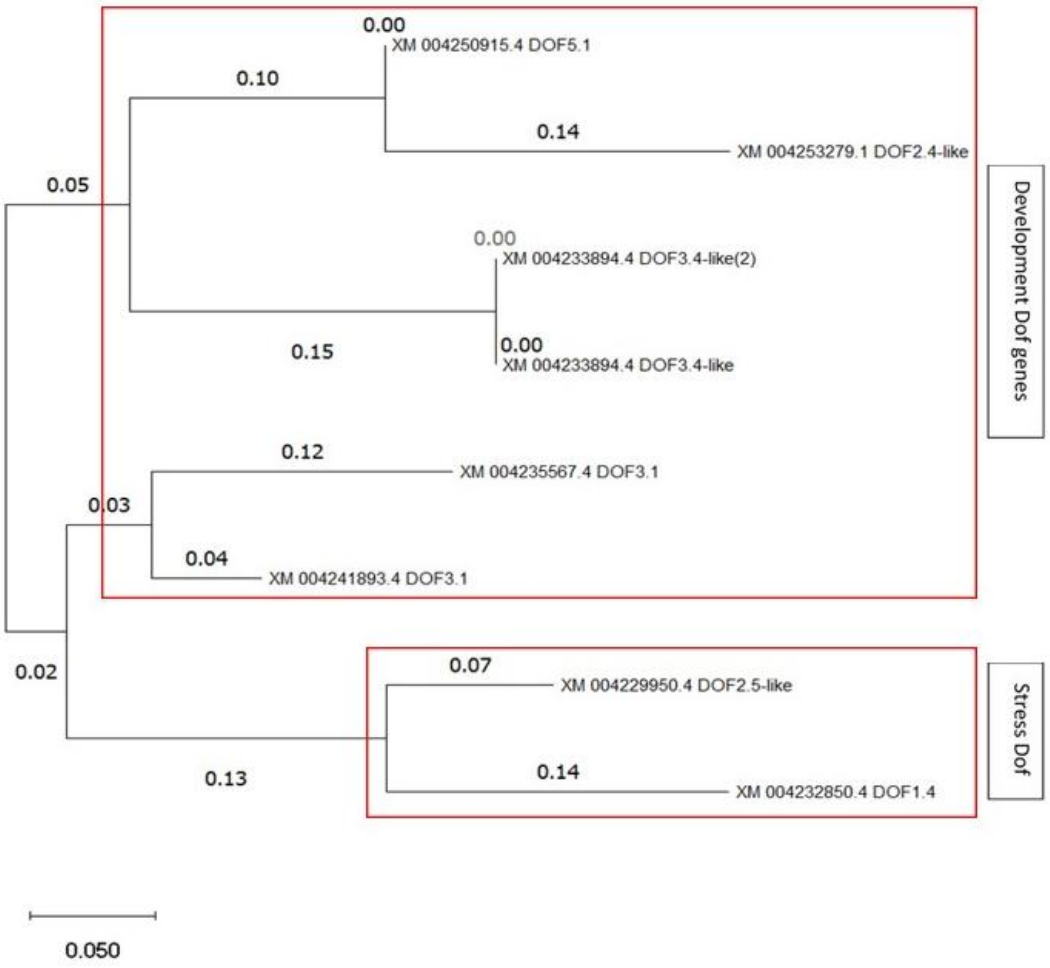

Figure 2 Phylogenetic analysis of *S. lycopersicum* DOF genes differentiate with development and stress condition.

Table 4 Dof genes with accession and location, with the score mentioned for identifying the location.

Gene Name	Accession	GOids	GOterms	Score
Dof 3.1	XM_004235567.4 479-655	GO:0009543	C:chloroplast thylakoid lumen	0.75
Dof 2.4-like	XM_004253279.1	GO:0005615	C:extracellular space	0.67
DOF2.5-like	XM_004229950.4 303-483	GO:0009543	C:chloroplast thylakoid lumen	0.8
DOF3.4-like	XM_004233894.4 449-534	GO:0009543	C:chloroplast thylakoid lumen	0.77
DOF1.4	XM_004232850.4 309-446	GO:0009543	C:chloroplast thylakoid lumen	0.78
DOF3.1-like	XM_004241893.4 176-303	GO:0009543	C:chloroplast thylakoid lumen	0.74

3.4.2 Protein-protein interaction

To better understand the functional relationships among the derived DOF genes, a protein-protein interaction (PPI) network analysis was conducted. We calculated and identified the different functions of the stress-related genes, DOF2.5-like and DOF1.4 (Figure 3).

Based on the STRING protein-protein interaction network, the analysis revealed significant interactions among several proteins. The central protein, Solyc00g024680.1.1, interacts with multiple other proteins, each supported by various types of evidence. The interaction with Solyc01g005300.2.1 is robustly supported by text mining data (light green line), curated database information (ocean blue line), and experimental data (magenta line), indicating a well-

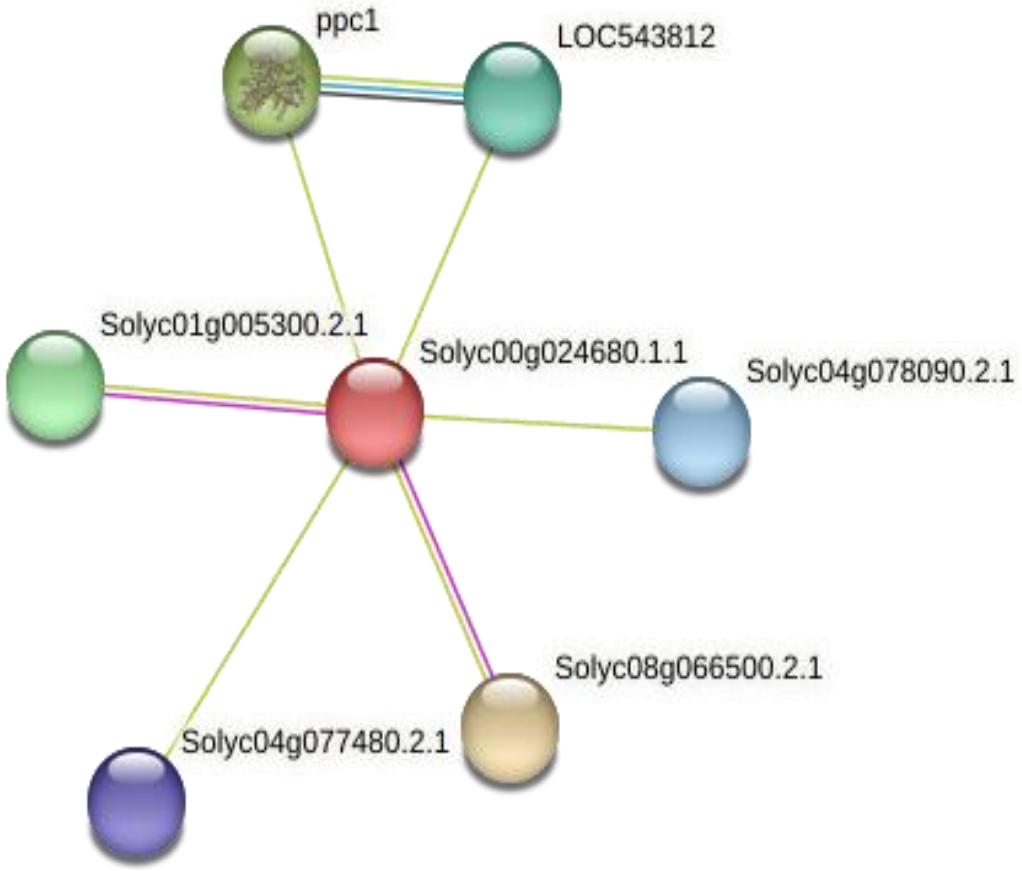

Figure 3 Protein-protein interaction analysis between the genes and their interaction pattern is represented.

documented relationship. Other proteins, such as ppc1, LOC543812, Solyc04g078090.2.1, and Solyc08g066500.2.1, also show potential interactions with Solyc00g024680.1.1 based solely on text mining data. Additionally, Solyc04g077480.2.1 is linked to Solyc00g024680.1.1 through text mining data and co-expression evidence (black line), suggesting these proteins are co-expressed in the same or different species. This comprehensive network illustrates the intricate web of protein interactions, underpinned by diverse evidence sources, providing a deeper understanding of the functional connections between these proteins.

3.5 Expression of DOF-related gene under biotic stress in resistance and susceptible Plant

In this study, we analyzed the 8DOFs gene for gene expression. Under biotic stress conditions, the genes Solyc04g077480.2.1 (DOF 1.4) and Solyc01g005300.2.1 (DOF2.5-like) were differentiated according to gene ontology in Table 4.

After exposure to stress, changes in the transcriptome of the plants were monitored. The comparison was conducted in two steps. In the first step, to characterize the role of DOF genes in disease management, a transcriptome comparison was made between three stages of the resistant Arka Samrat line (Figure 4) and the susceptible Pusa Ruby line (Figure 5). The RNA expression was studied at 5-day intervals to monitor the gradual changes in DOF expression in response to the induction of disease (Figure 6). The results showed that the genes resembling DOF 2.5 and DOF 1.4 exhibited significant downregulation, while genes like DOF 3.1, DOF 3.4, and DOF 2.4 showed moderate downregulation. In contrast, the genes resembling DOF 5.1, DOF 3.4.2, and DOF 3.1.2 displayed minimal changes compared to the control in the resistant Arka Samrat line. The changes in RNA expression in response to abiotic stress were different in the susceptible Pusa Ruby line. The DOF 2.5 and DOF 1.4 genes exhibited only minor changes, while the other genes showed substantial to moderate downregulation compared to the control.

In the second step, similar trends were observed in the gradual changes in DOF expression. The DOF 2.5 and DOF 1.4 genes showed significant changes throughout the infection period, whereas the other six DOF genes displayed little to moderate changes when compared to the control data taken at 0 hours post-infection.

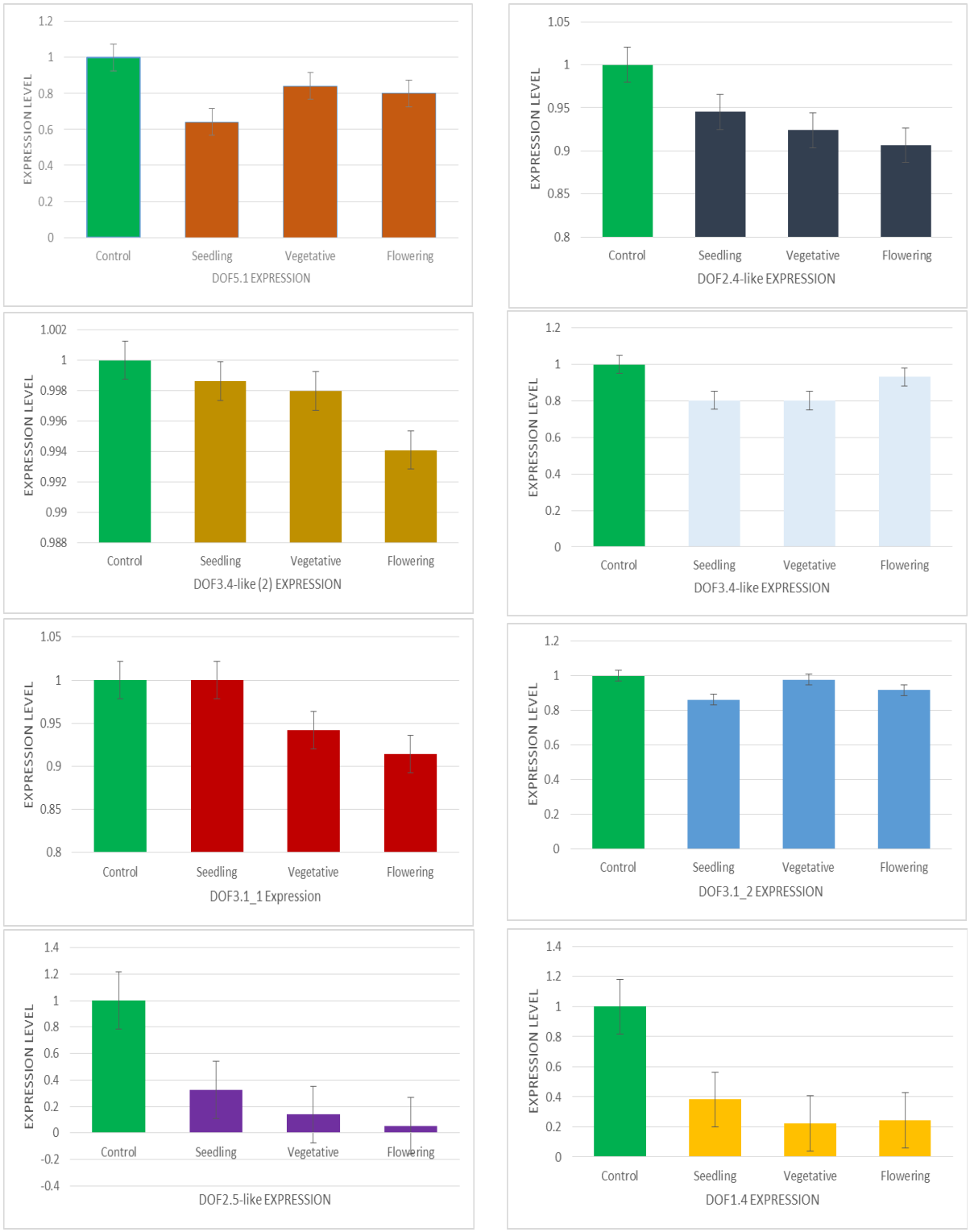

Figure 4 shows the expression analysis of eight selected DOF genes in the resistant tomato variety Arkasamrat under abiotic stress. After the stress treatment, RNA was extracted from plants collected at three developmental stages. GAPDH was used as the housekeeping gene to normalize the mRNA levels compared to the untreated sensitive line, ArkaSamrat. The error bars represent the standard deviations from three separate real-time PCR tests.

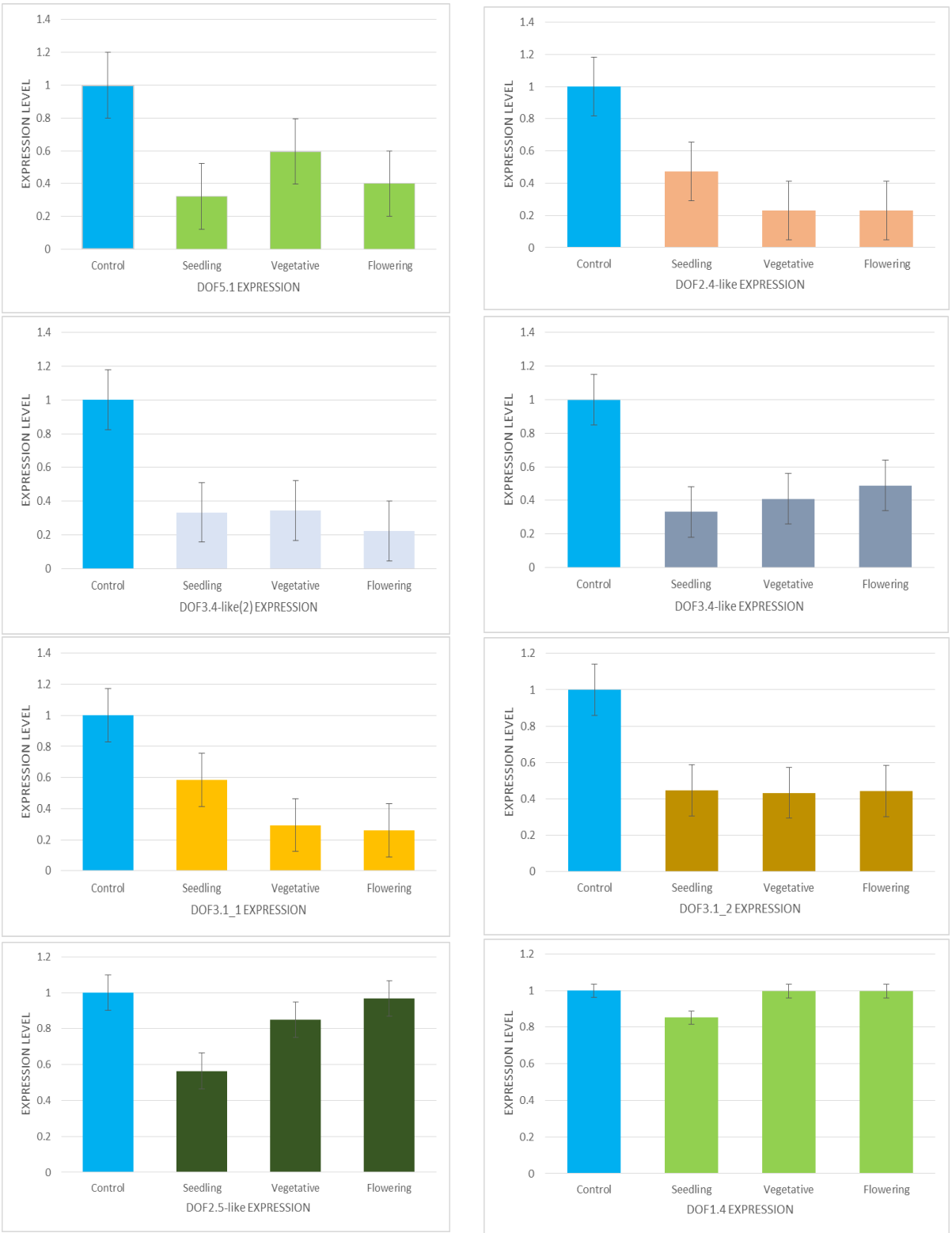

Figure 5 shows the expression analysis of eight selected tomato DOF genes in the susceptible variety Pusa Ruby under abiotic stress. After the stress treatment, RNA was extracted from plants collected at three developmental stages. The mRNA levels were normalized against those in the untreated sensitive line, Pusa Ruby. The housekeeping gene used for normalization was GAPDH.

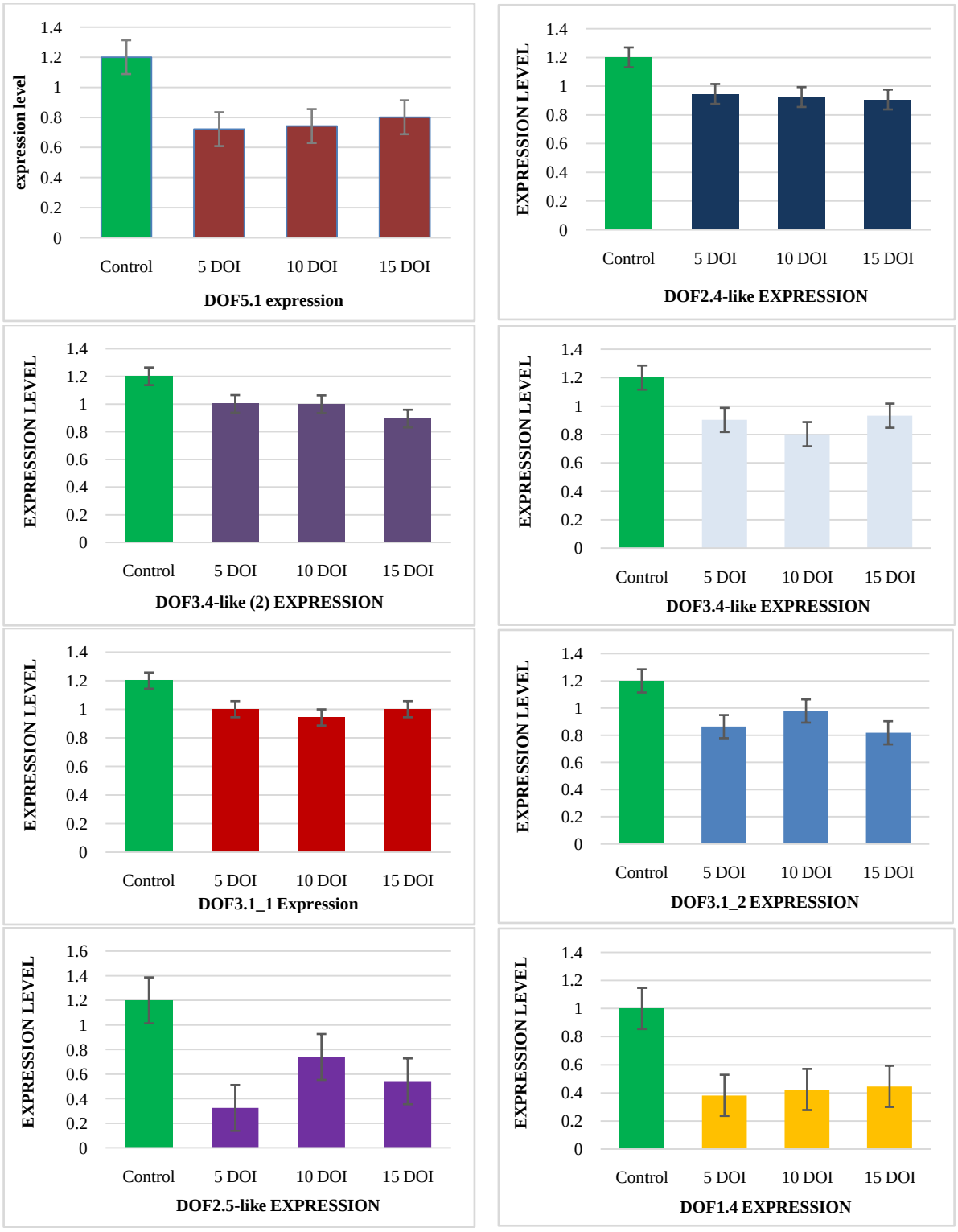

Figure 6 shows the expression analysis of eight selected DOF genes in the susceptible tomato variety Pusa Ruby under abiotic stress. RNA was extracted from the plants collected at 5, 10, and 15 days after inoculation (DAI). The mRNA levels were normalized to the untreated sensitive line Pusa Ruby (0 hours), setting the relative expression at 1 for this time point. The housekeeping genes used for normalization were GAPDH and UBI3.

4 Discussion

Transcription factors are crucial in plant growth and development, significantly influencing crop yield under cultivation. Their importance has increased in the context of changing climatic conditions. This research aims to identify the transcription factor gene DOF in *S. lycopersicum* (tomato). The identified DOF genes from *S. lycopersicum* include DOF5.1, DOF3.1, DOF2.4-like, DOF2.5-like, DOF3.4-like, DOF1.4, and DOF3.4-like, all derived from the selected 39 DOF gene in the TAIR database of *A. thaliana*. The selection of these candidate genes in the present study was based on information provided by Li et al. (2022). A literature survey of the eight identified genes indicated that, except for DOF2.5-like, all are involved in plants' biotic and abiotic stress conditions. This work focuses on identifying the DOF genes in the tomato genome and differentiating their roles in developmental and stress conditions. Analysis of the conserved domains indicates that, except for DOF5.1, all other genes fall under the conserved region of the zinc-finger family and superfamily group (Rao et al. 2013).

In this phylogenetic analysis, our primary objective was to elucidate the relationship between DOF genes identified in Solanum lycopersicum (commonly known as tomato) and their association with developmental and stress-related genes. Our investigation revealed intriguing findings regarding the evolutionary divergence and functional categorization of these DOFs. Specifically, we identified DOF1.4, a known transcription factor associated with developmental processes (Ramachandran et al. 2020), which is situated on the same branch as DOF2.5-like, a transcription factor linked to stress responses (Santopolo et al. 2015). This proximity in the phylogenetic tree suggests a potential relationship or shared ancestry between these two distinct DOFs despite their involvement in different biological contexts. These results provide insights into the intricate interplay between developmental pathways and stress responses in *S. lycopersicum*. The shared evolutionary lineage between DOF1.4 and DOF2.5-like indicates a possible functional overlap or co-regulation, suggesting a potential cross-talk between developmental programs and stress-related signaling pathways (Santopolo et al. 2015). Further exploring these findings may offer valuable insights into the molecular mechanisms governing plant growth, development, and adaptation to environmental stresses. Understanding the functional implications and regulatory networks associated with these DOFs could pave the way for developing novel strategies for crop improvement, such as enhancing stress tolerance while maintaining optimal developmental processes in tomato and other related plant species (Mohanty et al. 2019). Our investigation also uncovered potential interactions among proteins encoded by DOF genes through protein-protein network analysis. This analysis provided insightful information about the regulatory networks involving DOF transcription factors and their potential roles in mediating plant responses to environmental stimuli, as indicated by the gene ontology function in the PPI network analysis. By analyzing protein-protein interactions, we better understood how DOF genes may collaborate with other proteins to modulate plant responses to various environmental cues. The interactions revealed valuable insights into the molecular mechanisms underlying the regulatory networks governing plant stress responses. Our research focused on analyzing the expression patterns of DOF genes in the plant variety under biotic stress conditions, as these stress-related genes respond to such stimuli. We aimed to identify genes associated with resistance or susceptibility. We employed the qRT-PCR technique for gene expression validation (Mohanty et al. 2017). Our analysis identified distinct expression profiles of DOF genes responding to biotic stimuli, indicating their potential involvement in the plant's defense mechanisms. These findings suggest that specific DOF genes may significantly confer resistance or susceptibility traits in the examined plant variety.

However, it is important to note that additional experimental investigations are necessary to validate and expand upon these initial findings. Nevertheless, this phylogenetic analysis serves as a solid foundation for future studies, paving the way for deeper exploration of the intricate relationships between DOFs and their roles in developmental and stress conditions in *S. lycopersicum* and other plant systems. These DOF genes have been shown to play significant roles in several plants' development and responses to biotic and abiotic stresses. All identified genes exhibited essential features characteristic of the selected genes in question. Furthermore, there are variations in tomato transcription factor genes compared to those in the model plant Arabidopsis, illustrating phylogenetic divergence and distinct characteristics. With the availability of genomic sequences for several crop plants and advances in bioinformatics, new avenues are opening to decipher the genetic elements involved in controlling complex traits such as flowering.

Conclusion

The current research aimed to identify and analyze DOF and DOF-like genes in the tomato genome (*S. lycopersicum*) using sequence homology searches and various bioinformatics methods. We successfully identified eight putative DOF transcription factors. Our comprehensive genome-wide analysis investigated conserved domain architectures, evolutionary relationships, and expression patterns across tissues and stress conditions. The results of our study significantly enhance our understanding of the functional roles that DOF transcription factors play in tomatoes. By clarifying their involvement in various biological processes, we have gained insights into how these regulatory proteins contribute to tomato growth, development, and stress responses. This knowledge is crucial for

understanding the signaling pathways involved in DOF transcription factor-mediated regulation, particularly concerning resistance to *C. truncatum* in chili pepper. Moreover, our findings open new avenues for crop improvement strategies, including genetic engineering approaches to enhance stress tolerance, fruit development, and overall crop productivity in tomatoes and related species. The fundamental information provided by this study will serve as a valuable resource for future research and breeding programs focused on improving tomato resilience and yield.

Ethics approval and consent to participate

Not applicable

Declaration of competing interest

The authors declare that there is no conflict of interest and has approved for publication.

Availability of data and material

All the data generated or analyzed during this study are included in this article.

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Author's contributions

Conceptualization,J.N.M., S.J.S. and A.P.; Validation, S.J.S., B.L.P., A.J.,M.B., R.M, A.P and J.N.M.; Original draft preparation, J.N.M.,S.J.S., R.M. and A.P.; Review and editing, AP,MB, J.N.M.; Supervision, J.N.M., and A.P.

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