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Research Article

Identification and Expression Profiling of the Dof Gene Family in *Panicum virgatum* Revealing Their Role in Abiotic Stress Tolerance

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ABSTRACT

Switchgrass (*Panicum virgatum*) is a C4, perennial grass, with immense potential for biofuel production due to its adaptability, high biomass yield, and ecological benefits. Despite its agronomic importance, transcription factor regulation underlying stress responses in this crop remains unexplored. Therefore, we performed genome-wide analysis of Dof TF family in *Panicum virgatum* in which 57 non-redundant *PvDof* genes were identified and mapped across 17 chromosomes. Performing various bioinformatic analyses including phylogeny, motif, and gene structure, we classified these *PvDof*s into 7 phylogenetic groups (A-G) based on their conserved domains and sequence homology with *Arabidopsis thaliana* and *Oryza sativa*. Gene structure and conserved motif analyses demonstrated low intron counts and conserved functional motifs respectively, indicating functional conservation and potential involvement in stress signaling pathways. Promoter analysis uncovered 55 distinct cis-regulatory elements, including those associated with hormone signaling, light responsiveness and abiotic stress adaptation. Synteny analysis revealed more than 50 orthologous gene pairs between *P. virgatum* and *A. thaliana*, while 50 duplicated segments were identified in *PvDof* genes during collinearity analysis. Expression profiling under cadmium chloride exposure showed that *PvDof06, PvDof32, PvDof38,* and *PvDof57* were strongly upregulated, whereas most other members were downregulated. Under phosphorus deficiency, *PvDof32, PvDof38,* and *PvDof57* were induced in shoots, while *PvDof05, PvDof06,* and *PvDof57* were highly expressed in roots, revealing tissue-specific regulation. These findings established a foundational framework for regulatory roles of Dof transcription factors in switchgrass and offer promising targets for genetic improvements of stress resilience in switchgrass through molecular breeding.

Keywords: Dof gene family, Genome-wide analysis, *Panicum virgatum*, Expression analysis, Abiotic stresses

INTRODUCTION

Switchgrass (*Panicum virgatum*) is a warm season, C4, perennial grass of Poaceae family native to North America with pronounced adaptability and use potentials (Bai *et al*., 2022). It normally attains a height of 3 to 5 feet (Boykov *et al*., 2019). The practice of growing switchgrass started within the United States in the decades of 1930s and 1940s with the development of several cultivars (Matin *et al*., 2023). In 1991, switchgrass was selected by the Department of Energy in the United States as an exemplar crop for bioenergy feedstock (Gao *et al*., 2021). Switchgrass, based on its very high productivity potential, wide genetic diversity, and strong natural

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geographic distribution, is perceived as a promising candidate for future bioenergy needs (Hussain *et al*., 2024). It demonstrates huge potential for bioethanol production due to minimal soil erosion, low disease incidence, geographical adaptability, and bioproducts such as acetic acid, isopentanol, enzymes, and biochar (Larnaudie *et al*., 2022). Several bioactive phenolic compounds, such as vanillic acid, p-coumaric acid, ferulic acid, rutin, and quercitrin, have been identified in switchgrass extracts, exhibiting anti-inflammatory, anticancer, antibacterial, and antioxidant properties (Ho *et al*., 2022). Switchgrass can improve soil conservation and carbon sequestration, minimizing chemical runoff, leaching and on farm energy use, making it a highly sustainable biofuel feedstock (Kumar *et al*., 2019).

Transcription factors (TFs) are proteins that can selectively attach to specific regions upstream of genes, ensuring that the genes are transcribed at certain levels in response to particular stimuli at appropriate times and locations (Fu *et al*., 2024). The Dof TFs were first identified in maize in 1995 and demonstrated their essential role in regulating the expression of genes related to carbon metabolism and light regulation (Lohani *et al*., 2021). In gene expression, Dof proteins act as plant-specific transcription factors and attach to the promoter regions of target genes containing the T/AAAAG sequence.

These proteins are typically conserved at their N-terminal region, which comprises 50–52 amino acids and includes a characteristic C2-C2 zinc finger motif (Chattha *et al*., 2020). Members belonging to Dof transcription factor have been found widely distributed in several plant species, including *Arabidopsis* (*Arabidopsis thaliana*) 36 (Li *et al*., 2025), Rice (*Oryza sativa*) 30 (Wang *et al*.,2025), Maize (*Zea Mays*) 18 (Gou *et al*., 2025), Sorghum (*Sorghum bicolor*) 28 (Cao *et al*., 2020), Tomato (*Solanum lycopersicum*) 37 (Luo *et al*., 2022), Pepper (*Capsicum annuum*) 33 (Kang *et al*., 2016), Cucumber (*Cucumis sativus*) 36 (Zou & Zhang, 2019) and Apple (*Malus domestica*) has 60 Dof members (Zhou *et al*., 2020).

Transcription factors (TFs) regulate gene expression by interacting with plant-specific cis-regulatory elements in the promoter region, where they facilitate the activation or suppression of transcription of target genes (Cai *et al*., 2013). Pollen production, polarity as well as guard cell elongation and cell cycle control, are typically controlled by Dof transcription factors (Fang *et al*., 2020). Therefore, Dof transcription factors are essential for controlling the plant shapes formation, elongation of hypocotyl, root and leaf development and floral organ development (Hu *et al*., 2025). In addition, Dof proteins contribute to plant responses against both biotic and abiotic stress conditions, phytohormone and light regulation responses and controlling seed germination and maturation (Gupta *et al*., 2015). They also play roles in seed dormancy, tissue formation, control of carbon and nitrogen uptake, and carbohydrate metabolism (Zou & Sun., 2023).

In *P. virgatum,* the Dof transcription factor remains relatively unstudied. Therefore, the aim of this study was to identify and characterize the functions of *PvDof* genes in switchgrass using available genomic and transcriptional datasets. Comparative analysis with corresponding and related genes from *O. sativa* and *A. thaliana* was performed. Other analyses include conserved motifs, domain, gene structure, cis-regulatory elements, synteny and chromosome localization. Furthermore, we analyzed the expression profiles of Dof genes across different tissues and under abiotic stress conditions such as cadmium chloride exposure and phosphorus deficiency. These findings provide meaningful interpretations and build a framework for upcoming functional studies of Dof genes in *P. virgatum*.

MATRIALS AND METHODS

Database searches for sequence retrieval of *Panicum virgatum*

We retrieved the Dof protein sequences of *P. virgatum* from Plant TFDB (https://planttfdb.gao-lab.org/family.php?sp=Pvi&fam=Dof) (Jin *et al*., 2016). We also retrieved data about physiochemical properties including molecular weight (MW), Isoelectric point (pI), and the amino acid length of *PvDof* genes from plant TFDB database. We utilized the BLAST tool available on the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) to confirm the identified Dof protein sequences. We also used this resource to retrieve the corresponding coding sequences (CDS) and genomic DNA sequences. Furthermore, chromosomal locations and other genomic details related to *P. virgatum* were also extracted from the NCBI database.

Chromosomal distribution of *PvDof* genes, gene structure and conserved protein motif analyses

The chromosomal locations of Dof genes within the *P. virgatum* genome were retrieved from NCBI. A software called Mapchart (v.2.32) was utilized to perform mapping of chromosomes (Voorips, 2002). The demonstration of intron and exon patterns of *PvDof* genes was conducted by using Gene Structure Display Server (GSDS) v2.0 (https://gsds.gao-lab.org/) (Hu *et al*., 2015). The conserved protein motifs of *PvDof* genes were visualized by MEME (v5.5.8) (https://meme-suite.org/meme/tools/meme) (Bailey *et al*., 2009).

Comparative phylogenetic analysis of *Dof* Proteins in *P. virgatum*, *A. thaliana*, and *O. sativa* and conserved domain analyses of *PvDof* genes

Protein sequences of *P. virgatum* (*PvDof*), *A. thaliana* (*AtDof*), and *O. sativa* (*OsDof*) were downloaded from Plant TF database. Naming of *PvDof* genes was done as described by (Yu *et al*., 2020) on the basis of their position on the chromosomes. A multiple sequence alignment of *P. virgatum*, *O. sativa*, and *A. thaliana* Dof protein sequences was performed using ClustalW (Larkin *et al*., 2007) with default parameters in MEGA11 software (Tamura *et al*., 2021). Phylogenetic and molecular evolutionary analyses were conducted in MEGA11 using the neighbor-joining method (Saitou & Nei, 1987) based on pairwise distances. Moreover, 1,000 bootstrap replicates were performed to provide strong support for the evolutionary relationships among the Dof gene families. The resulting phylogenetic tree was exported in newick format and visualized using iTOL v7.0 (Interactive Tree of Life; https://itol.embl.de/) (Letunic & Bork, 2024). For the identification of conserved domains in *PvDof* proteins, the protein sequences were uploaded on CDD (Conserved Domain Database) available on NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) to generate hit-data file (Marchler-Bauer *et al*., 2015). The conserved domain structure visualization of each protein was done by using TBtools-II software (Chen *et al*., 2023).

Collinearity analysis and comparative synteny analysis of *PvDof* and *AtDof* genes

Collinearity relationships among Dof family members were investigated by integrating the resulting files into the Advanced Circos feature of TBtools-II. Duplicate gene pairs within the *PvDofs* were then identified and analyzed. The synteny analysis was performed by using dual synteny plot feature of TBtools-II software to find evolutionary similarities and relationships among *PvDof* and *AtDof* genes.

Cis-regulatory elements identification in the promoter regions of *PvDof* genes

To perform promoter analysis, 1000bp upstream regions of each *PvDof* gene were extracted from NCBI. Plant Care Database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was utilized to locate the cis-regulatory elements within the *PvDof* gene promoters (Lescot *et al*., 2002). Each gene was represented by a tab-delimited file that contained all the information on the cis-regulatory elements. The identified cis-regulatory elements were then visualized using the TBtools-II software.

Retrieval of RNA-Seq based expression profiles under different abiotic stresses

The transcriptomic data for *PvDof* genes under cadmium chloride ($CdCl_2$) treatment and phosphorus deficiency were retrieved from the NCBI SRA repository (https://www.ncbi.nlm.nih.gov/) under accession numbers SRP551653 and SRP449352, corresponding to BioProjects PRJNA1198715 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1198715) and PRJNA994525 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA994525), respectively. Each dataset comprises 24 runs, encompassing 40.61 Gb and 34.55 Gb of experimental data, respectively. The reference genome of *P. virgatum* (NCBI RefSeq assembly: GCF_016808335.1) was used on the Galaxy platform (https://usegalaxy.org/) to read mapping with Bowtie2 using built-in parameters (De Almeida *et al*., 2016). FPKM values were generated using Feature Count tool on Galaxy server, based on experimental data (Liao *et al*., 2014). A heatmap was generated with TBtools-II software after calculating FPKM values of all *PvDof* genes.

RESULTS

Identification and distribution of *PvDof* TFs

In this study, we discovered 72 Dof genes using the whole-genome data of *P. virgatum*. A total of 15 redundant genes were found and removed to eliminate duplication. The remaining 57 non-redundant *PvDof* genes were named *PvDof01-PvDof57* according to their location on chromosomes by following Dai et al. (2025). The protein length of putative 57 *PvDofs* ranged from 139 (*PvDof11*) to 583 (*PvDof14*) amino acids with an average length of 331 amino acids. The isoelectric points (Pi) varied from 4.5371 (*PvDof20*) to 12.5657 (*PvDof37*) with a mean value of 8.36. The molecular weights ranged from 12.8kDa to 58.2kDa, averaging at 32.18 kDa (Table 1).

Genomic localization of *PvDof* genes

Based on the genomic annotation data of *P. virgatum* retrieved from NCBI, Dof genes were distributed on 17 chromosomes (Figure 1). Aside from chromosome 4N, on which no *PvDof* was identified, the distribution of genes varied from 1 to 9 on different chromosomes. Chr 9K carried the highest number of *PvDof* genes (9 genes, 15.78%) followed by chr 5K, chr 5N and chr 9N, each contained 6 *PvDofs* (10.52%) and chr 3K had 5 *PvDof*s (8.77%). Chr 1K, chr 2K, chr 7K, chr 1N, chr 2N, and chr 3N, each demonstrated 3 *PvDof*s (5.26%) and chr 7N had 2 *PvDof*s (3.50%). Chr 4K, chr 6K, chr 8K, chr 6N, and chr 8N, each possessed only one gene (1.57%). Primarily, two regulatory pathways, molecular functions and biological processes are associated with Dof genes. These genes play a vital role in hormone signaling, carbon and nitrogen uptake, and respond to a range of abiotic stresses like drought, salinity and extreme temperatures (Zou & Sun, 2023).

Table 1. Different attributes of identified *PvDof* genes and proteins

TF ID	Given names	AA length	Mol. Wt. (Da)	pI	Gene symbol	Chr no.	Gene start	Gene end	Exon count
Pavir.1KG444600.1.p	PvDof01	374	37586.7	8.66	LOC120712396	1K	45854584	45857430	3
Pavir.1KG470100.1.p	PvDof02	291	30470.7	8.277	LOC120649675	1K	48375047	48378123	3
Pavir.1KG489100.1.p	PvDof03	292	29333.5	8.747	LOC120640406	1K	49574424	49575753	1
Pavir.2KG402600.1.p	PvDof04	269	27512.5	8.119	LOC120682154	2K	49371746	49373128	1
Pavir.2KG433300.1.p	PvDof05	351	34735.7	7.997	LOC120689137	2K	57706435	57708743	1
Pavir.2KG583500.1.p	PvDof06	482	51023.9	7.354	LOC120694887	2K	67918527	67922134	2
Pavir.3KG359300.1.p	PvDof07	220	22754.8	10.99	LOC120701295	3K	19226204	19227474	1
Pavir.2KG194000.1.p	PvDof08	362	38050.6	6.69	LOC120698550	3K	24404595	24406107	1
Pavir.3KG494800.1.p	PvDof09	338	34257.3	8.875	LOC120699846	3K	57340116	57342573	2
Pavir.J557300.1.p	PvDof10	196	20150.3	9.832	LOC120699962	3K	59595668	59597002	1
Pavir.3KG487300.1.p	PvDof11	139	15416.5	9.233	LOC120701588	3K	59616553	59617924	1
Pavir.4KG127600.1.p	PvDof12	284	28576.7	7.31	LOC120705108	4K	18495976	18497190	1
Pavir.5KG052600.1.p	PvDof13	242	24245.8	9.083	LOC120710429	5K	3615794	3617305	1
Pavir.5KG172400.1.p	PvDof14	583	63574	4.818	LOC120706439	5K	13003016	13006499	2
Pavir.5KG210600.1.p	PvDof15	419	44524.8	7.191	LOC120709722	5K	14738576	14743514	2
Pavir.5KG434900.1.p	PvDof16	174	17377.6	10.43	LOC120707581	5K	43515253	43516729	1
Pavir.5KG551000.1.p	PvDof17	218	23554.4	10.24	LOC120708104	5K	49026523	49027798	1
Pavir.5KG648900.1.p	PvDof18	327	34768.6	4.603	LOC120708780	5K	55904631	55905965	1
Pavir.6KG333000.1.p	PvDof19	241	25160	7.303	LOC120712722	6K	41428986	41430224	1
Pavir.7KG049200.1.p	PvDof20	256	26479.4	4.537	LOC120639910	7K	10254922	10256003	3
Pavir.7KG301100.1.p	PvDof21	375	37348.4	8.667	LOC120641472	7K	43392193	43395354	2
Pavir.7KG374000.1.p	PvDof22	211	22609	7.218	LOC120642124	7K	51762891	51764026	1
Pavir.3KG000100.1.p	PvDof23	260	27642.9	9.016	LOC120646435	8K	204327	207450	2
Pavir.6NG206000.1.p	PvDof24	406	41262.8	9.28	LOC120649274	9K	2415219	2417596	2
Pavir.9KG224200.1.p	PvDof25	372	37837.9	9.464	LOC120649658	9K	6113291	6114961	1
Pavir.9KG094400.1.p	PvDof26	194	19267.1	11.32	LOC120650353	9K	15490488	15491722	1
Pavir.9KG468600.1.p	PvDof27	365	36050.2	8.403	LOC120648552	9K	16935473	16937143	1
Pavir.9KG148500.1.p	PvDof28	253	25078.2	9.327	LOC120650645	9K	19960127	19961828	2
Pavir.9KG169000.1.p	PvDof29	438	45248.7	8.006	LOC120651098	9K	27927279	27929754	2
Pavir.9KG387700.1.p	PvDof30	445	45339.7	8.546	LOC120648095	9K	50931985	50933142	2
Pavir.9KG491100.1.p	PvDof31	383	38924.3	9.995	LOC120652831	9K	59291087	59293178	2
Pavir.9KG607600.1.p	PvDof32	434	46201.8	8.161	LOC120647170	9K	66150872	66153586	2
Pavir.1NG303700.1.p	PvDof33	372	37525.5	8.478	LOC120656784	1N	55356189	55358919	3
Pavir.1NG451300.1.p	PvDof34	277	29074.1	8.334	LOC120656968	1N	57733556	57736317	2
Pavir.8KG323200.1.p	PvDof35	181	19153.7	10.44	LOC120657098	1N	59284128	59285457	1
Pavir.2NG397500.1.p	PvDof36	257	26454.5	8.582	LOC120660992	2N	50282809	50284277	1
Pavir.2NG506100.1.p	PvDof37	307	31671.9	12.57	LOC120661456	2N	59527430	59529756	1
Pavir.2NG632700.1.p	PvDof38	347	37560.2	7.99	LOC120662207	2N	69396893	69401055	2
Pavir.3KG103600.1.p	PvDof39	345	35243.5	8.993	LOC120664028	3N	7235600	7237646	2
Pavir.J349300.1.p	PvDof40	126	12873.5	9.841	LOC120664933	3N	19866986	19868121	1
Pavir.5NG325800.1.p	PvDof41	357	37641.3	6.952	LOC120665346	3N	25660741	25662703	1
Pavir.5NG035700.1.p	PvDof42	245	24522	9.637	LOC120672100	5N	4155325	4156702	1
Pavir.5NG175800.1.p	PvDof43	582	63571	4.912	LOC120672726	5N	14164184	14167686	2
Pavir.5NG191200.1.p	PvDof44	541	58273.8	6.378	LOC120673509	5N	15576987	15579258	1
Pavir.5NG428200.1.p	PvDof45	167	16812	10.43	LOC120675222	5N	51056466	51057892	1
Pavir.5NG505100.1.p	PvDof46	219	23735.6	9.886	LOC120672059	5N	57633247	57634848	1
Pavir.8KG185900.1.p	PvDof47	329	34819.6	4.544	LOC120672144	5N	64860712	64862067	1
Pavir.J117600.1.p	PvDof48	214	22159.7	5.828	LOC120679600	6N	45456444	45457732	1
Pavir.7NG291500.1.p	PvDof49	372	36916.9	8.842	LOC120682393	7N	41643680	41647542	3
Pavir.7NG441000.1.p	PvDof50	220	23590	6.932	LOC120682093	7N	49886752	49887926	2
Pavir.8NG007300.1.p	PvDof51	310	32658.2	8.563	LOC120685470	8N	283085	284254	1
Pavir.9NG035500.1.p	PvDof52	408	41114.5	9.248	LOC120692689	9N	2455755	2458272	2
Pavir.9NG042700.1.p	PvDof53	367	37299.3	9.286	LOC120689992	9N	6756582	6758281	1
Pavir.J404100.1.p	PvDof54	435	45311.9	7.959	LOC120690901	9N	30624788	30627285	2

Pavir.9NG532000.1.p	PvDof55	404	41126.1	9.982	LOC120689175	9N	59120742	59121808	2
Pavir.9NG697900.1.p	PvDof56	375	38194.5	9.995	LOC120692536	9N	69487195	69489306	2
Pavir.9NG730400.1.p	PvDof57	435	46237.8	8.163	LOC120688493	9N	77649464	77652252	2

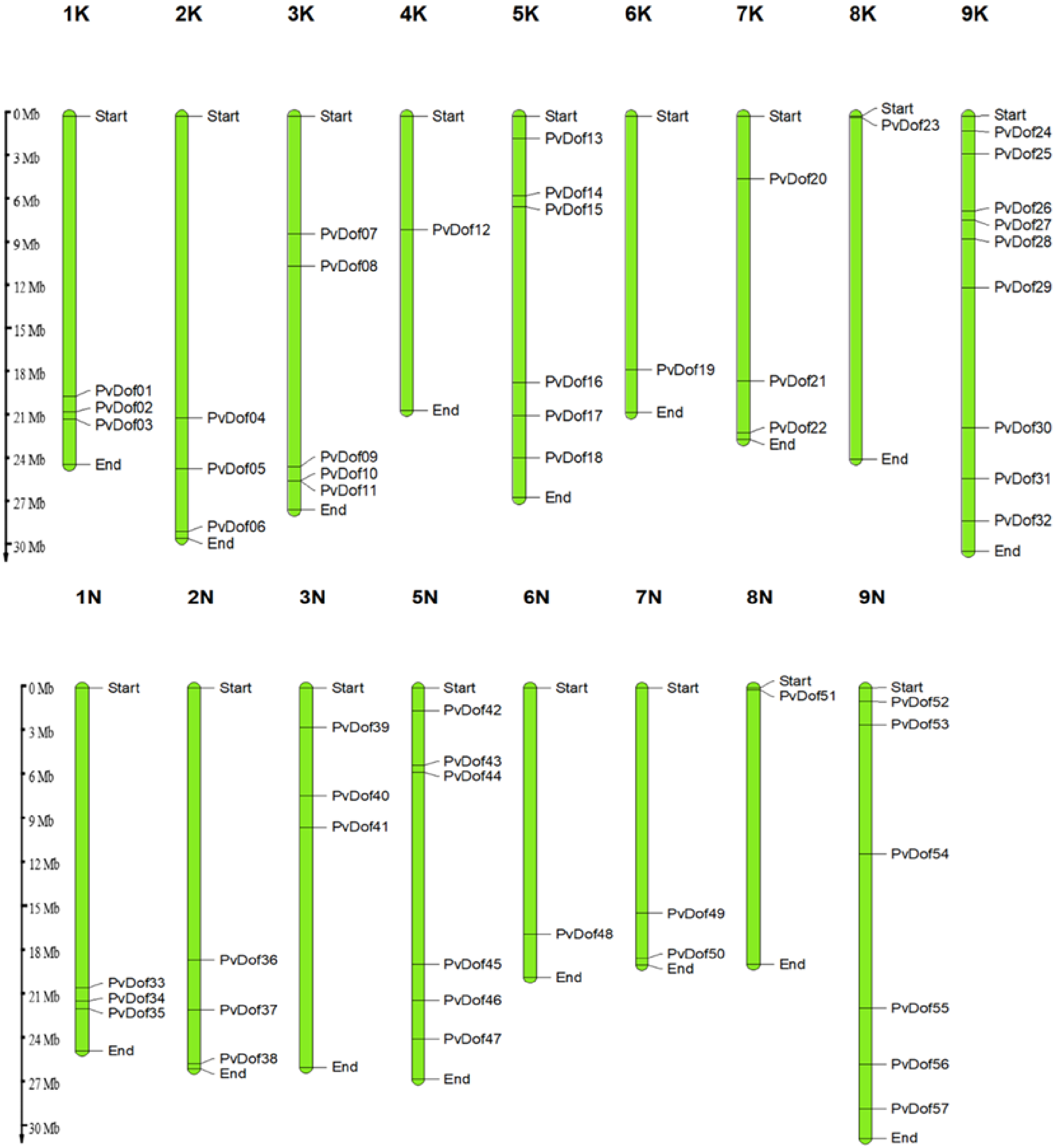

Figure 1. Distribution of *PvDof* genes. The dark lines on the chromosomes indicate the specific position of Dof genes). The scale on left side represents the chromosomes length in mega-bases ranging from 0 to 30 Mb.

During root development, *PvDof37* acts as an essential transcriptional regulator, modulating gene expression by interacting with transcription regulatory regions of DNA in a template manner. *PvDof27* plays a crucial role in stomatal dynamics, regulating transcription through sequence-specific DNA binding and directing guard cell differentiation and pectin metabolism to optimize physiological responses. Moreover, *PvDof39* serves as a light-responsive transcription factor facilitating photomorphogenesis via sequence-specific binding to regulatory DNA regions and positively regulating gene expression in a DNA templated mechanism. *PvDof02* promotes transcription in a similar manner and is essential for procambium, phloem, and xylem histogenesis, making it a crucial regulatory element in vascular tissue development.

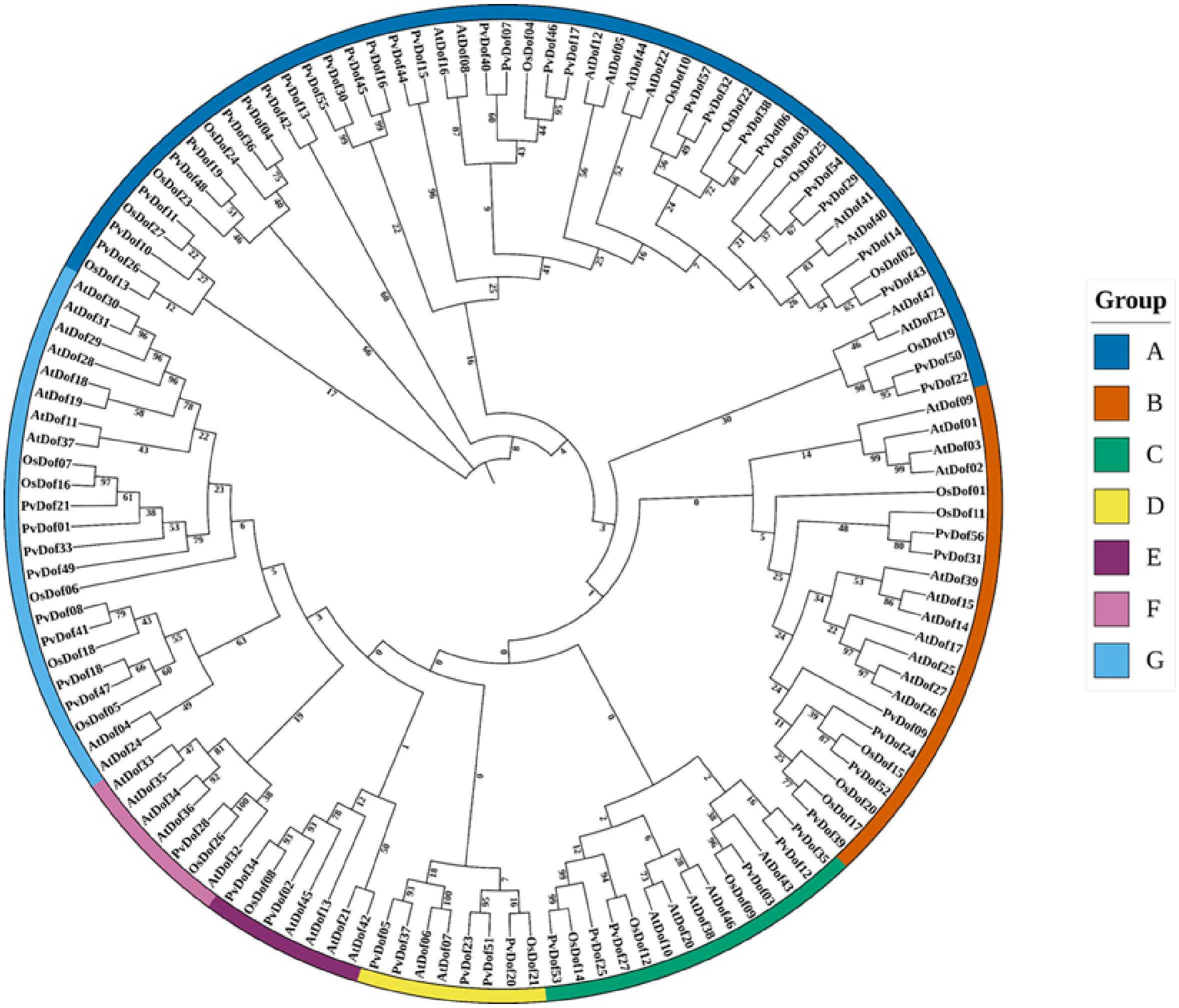

Figure 2. Comparative phylogenetic tree of 131 Dof proteins from *P. virgatum*, *A. thaliana*, and *O. sativa*. The tree was constructed in MEGA 11 using the neighbor-joining method based on the poisson model and visualized using iTOL v7.0. The evolutionary relationships were resolved into seven distinct groups, each represented by unique colors.

Comparative phylogeny among *PvDof, AtDof* and *OsDof* genes and conserved domain analysis of *PvDof* genes

The evolutionary relationship between the Dof family members of *P. virgatum, A. thaliana*, and *O. sativa* was studied by construction of an unrooted neighbor-joining phylogenetic tree, based on 131 peptide sequence from these species (Figure 2). The Dof proteins were classified into seven groups (A, B, C, D, E, F and G) of orthologous genes. The largest group was group A with 50 Dof members from all three species while the smallest groups were group E and F with 7 Dof members each. Group B, C, D and G contained 22, 14, 8 and 23 members, respectively. In the constructed phylogenetic tree, most of the Dof proteins of *A. thaliana* was grouped together while Dof proteins of *O. sativa* and *P. virgatum* were intermixed to form several distinct orthologous and paralogous groups.

In all *PvDof* genes, six conserved domains were identified which include zf-Dof, PTZ00121 superfamily, PABP-1234 superfamily, Herpes_BLLF1 superfamily, PRK12323 superfamily, and zf-Dof superfamily. zf-Dof domain was detected in all *PvDof*s except *PvDof41*, *25*, *09* and *20* and these proteins contained zf-Dof superfamily domain. PTZ00121 superfamily domain was conserved in *PvDof14* and *43* along with zf-Dof domain. Moreover, PRK12323 superfamily domain was observed in *PvDof04* and *36*. Herpes_BLLF1 superfamily was only detected in *PvDof44*.

Gene structure and motif analyses

Further details about the evolution of the Dof family in *P. virgatum genes* were investigated via gene structure analysis, focusing on intron/exon distribution. Within a genomic region, intron and exon distribution serves as supporting evidence for the expansion of gene families and their evolutionary relationships with ancestral genes. *PvDof20* and *PvDof29* showed the highest intron count among the analyzed genes, with a maximum of two introns each. A total of twenty-four *PvDof* genes contained a single intron, while the remaining genes completely lacked introns (Figure 4).

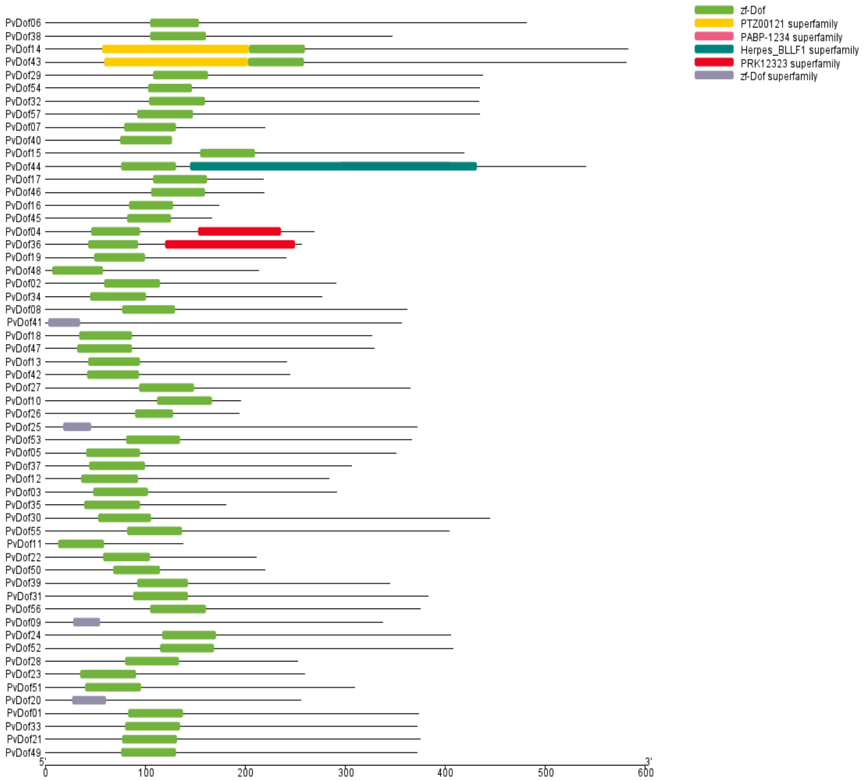

Figure 3. Conserved domain regions of *PvDof* genes are illustrated in green, yellow, zinc, red, and grey, representing the zf-Dof, PTZ00121, Herpes_BLLF1, PRK12323, and zf-Dof superfamilies domains, respectively.

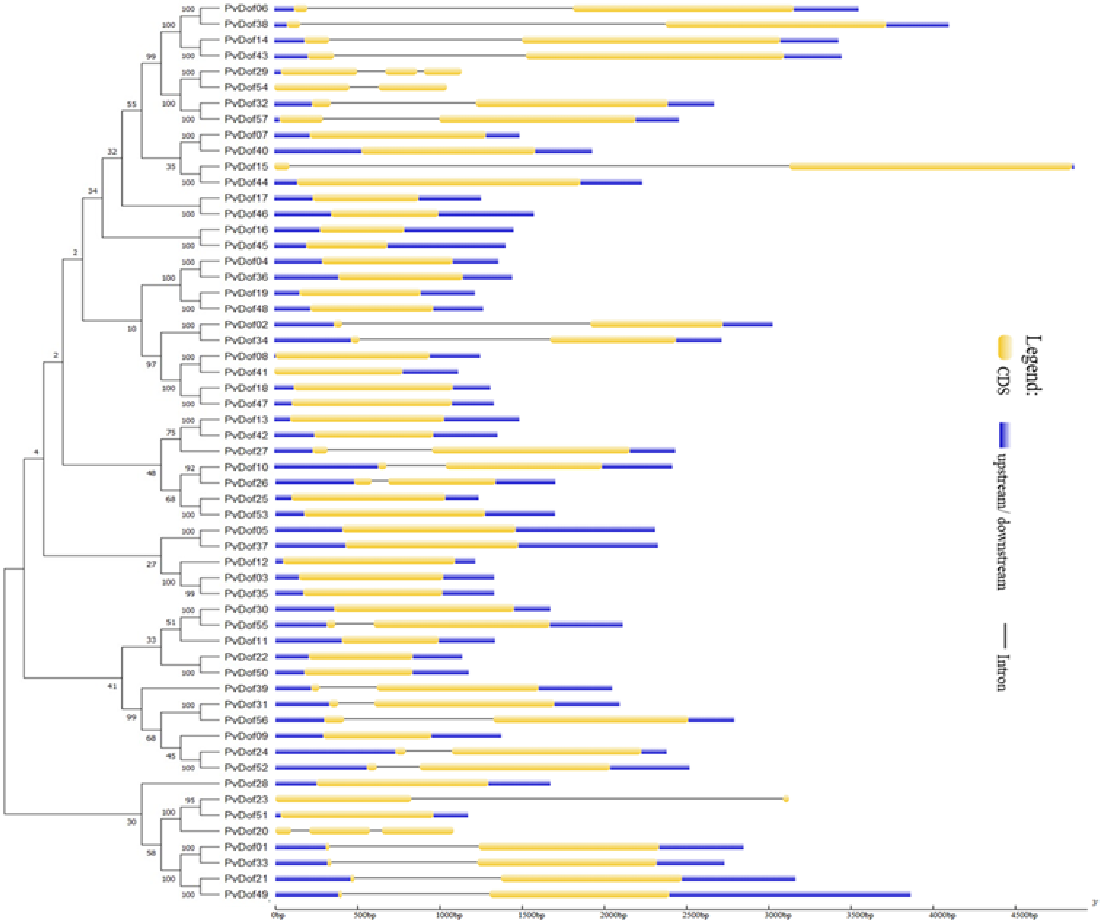

Figure 4. An unrooted, neighbor-joining tree (1000 bootstraps) was constructed from *PvDof* protein sequences. CDS, regulatory regions, and introns are visualized by yellow, blue, and gray colors respectively.

To predict the conserved motifs in 57 *PvDof* proteins, MEME online tool was utilized through which 10 conserved motifs (1-10) were obtained (Figure 5A). Motif1 and 2 were repeatedly observed in most of the *PvDof* proteins. Both were aligned together, suggesting their potential role in similar biological functions. Motif 3 was identified in 8 proteins, motif 4 in 11 proteins, motif 5 in 7 proteins, motif 6 in 8 proteins, motif 7 in 10 proteins, motif 8 in 4 proteins, motif 9 in 8 proteins, and motif 10 in 6 proteins, indicating a variable distribution pattern of these conserved motifs across the *PvDof* proteins. The names and sequence logos of the conserved motifs are presented in Figure 5B. The longest motif was motif 7, comprising 50 amino acids with the sequence (SSGBESVGAESSSHKEKLDGVNSTEKKMASDPKNEAVTKDEGSGGZEK), whereas motif 2 was the shortest, consisting of only 15 amino acids (GGALRNVPVCGGCRK). Various features of all 10 motifs are detailed in Table 2.

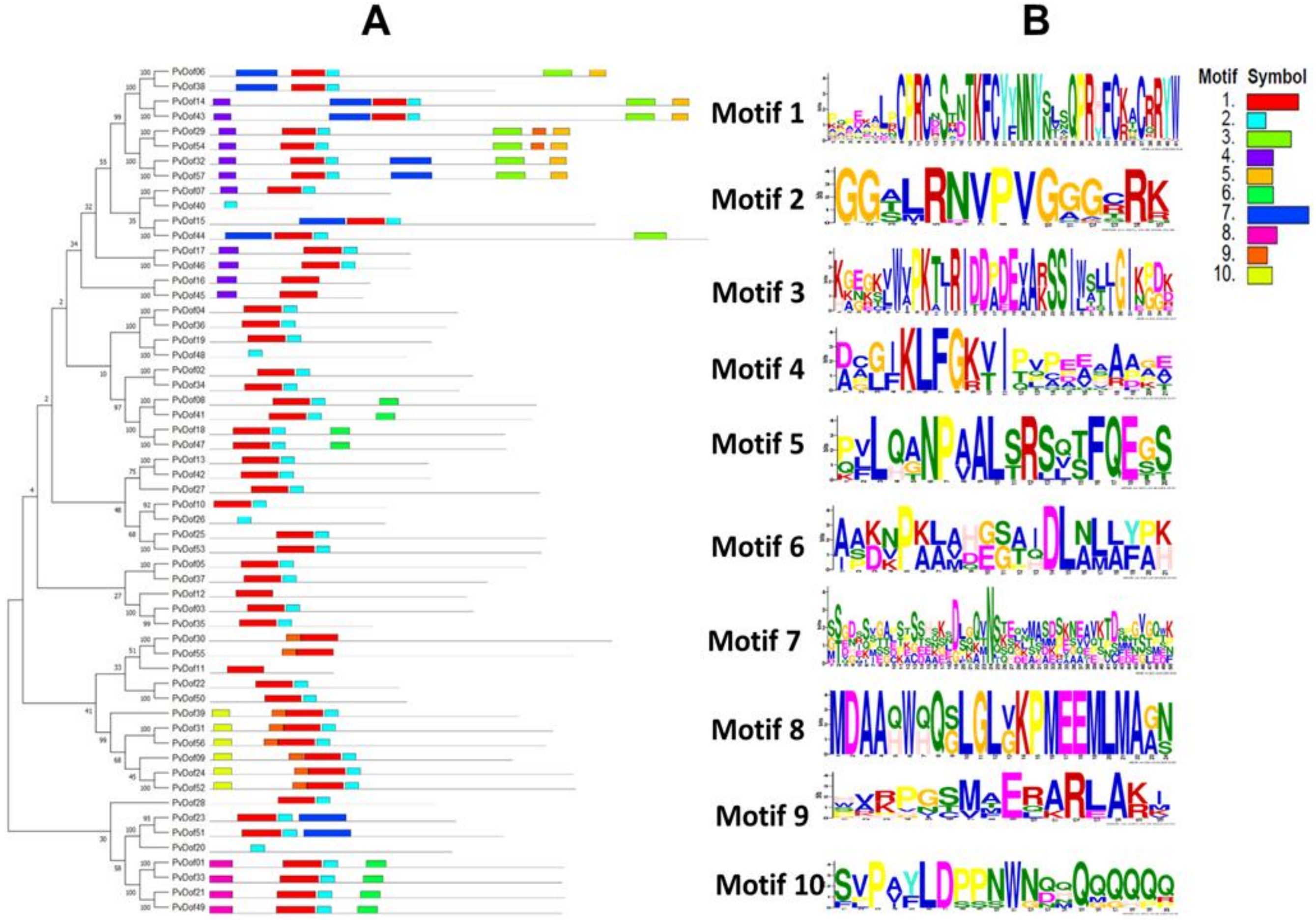

Figure 5. The conserved protein motifs of *PvDof* genes were visualized using MEME (v5.5.8). (A) Distribution pattern of conserved motifs to their particular regions. (B) Conserved sequence motifs represented by logos, where the height of each letter indicates the relative frequency of that amino acid at a given position.

Table 2. Different attributes of motifs identified by using MEME suite.

Motif No.	Motif sequences	E-value	Sites	Width
1	PQPEKALPCPCDSTBTKFCYYNNYSLSQPHRFKCACRRYW	5.0e-1864	53	41
2	GGALRNVPVCGGCRK	9.1e-501	51	15
3	KGEGKVWPKTJRIDDDPEVAKSSIWSLJGIKPDK	3.10E-134	8	35
4	DCGIKLFGKVIVPPEEAAAAE	1.30E-62	11	21
5	PVLQANPAALSRQTFQEGS	7.10E-36	7	20
6	AANDPKLAHEGIDIALIYPK	3.20E-35	8	21
7	SSGBESVGAESSSHKEKLDGVNSTEKKMASDPKNEAVTKDEGSGGZEK	1.70E-51	10	50
8	MDAPAQWQGLGKPMEEMLMAGN	3.30E-34	4	24
9	HVKPGSMAERALRAKI	4.60E-33	10	16
10	SVPAYLDPNWNHQQQQQQQ	8.00E-34	6	20

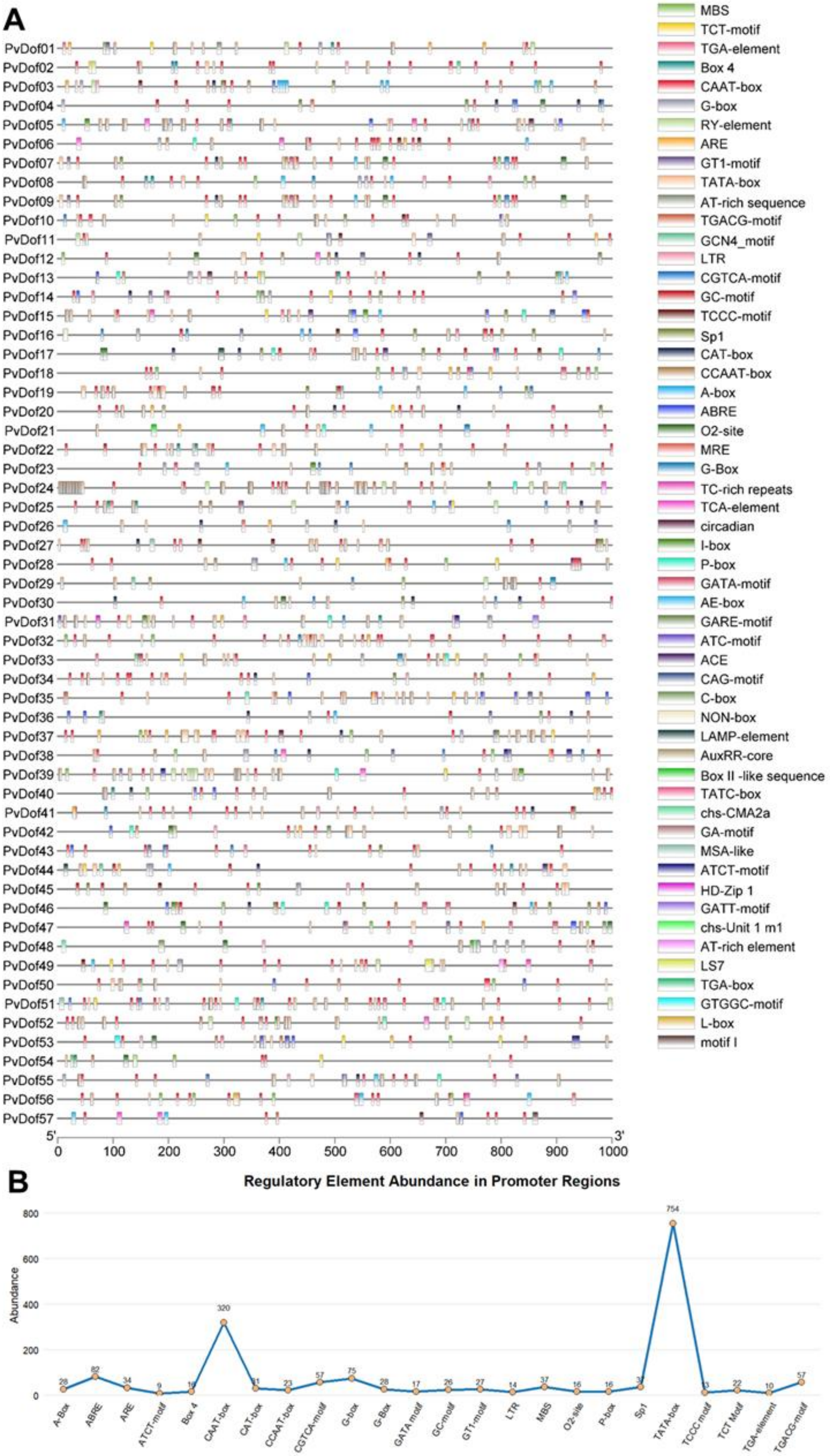

Figure 6. Prediction of cis-regulatory elements in *PvDof* genes. (A) Distribution and visualization of cis-acting regulatory elements across *PvDof* genes. Promoter regions are shown with color-coded ligand mapping for each element type. (B) The regulatory elements abundance graph illustrates TATA-box is abundant, followed by CAAT-box.

Promoter analysis of cis-acting regulatory elements of *Dof* genes

Cis-acting regulatory elements are non-coding DNA sequences in promoter regions that regulate gene expression by interacting with transcription factors during environmental and developmental responses (Dsilva & Galande., 2024; Ali et al., 2024). Promoter analysis of *PvDof*s detected the presence of 55 distinct cis-regulatory elements (Figure 6). Various conserved cis-regulatory elements like MBS involved in drought inducibility, TC rich repeats in defense and stress responsiveness and RY-element are responsible for seed specific regulation. G box, GT-1 motif, TCT motif, AE box, and TCCC motifs are light responsive elements. ABRE and TCA-elements are essential for abscisic acid and salicylic acid responsiveness, respectively. Moreover, TGA-element and AuxRR-core are important auxin responsive elements while P-box is gibberellin responsive element. TATA box, a core promoter element around -30 of transcription start, found frequently in *PvDof*s. Among all Cis regulatory elements, TATA box accounted for the largest number (754, 40.42%). These findings strongly support the integral role of *PvDof* genes in mediating various stress responses.

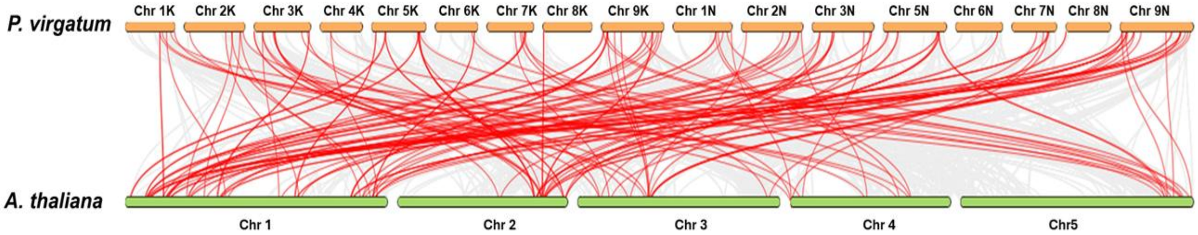

Figure 7. Synteny analysis between *PvDof* and *AtDof* genes, performed using the dual synteny plot feature of TBtools-II software. Red lines connecting the chromosomal bars represent conserved syntenic blocks, indicating homologous genomic regions between the two species.

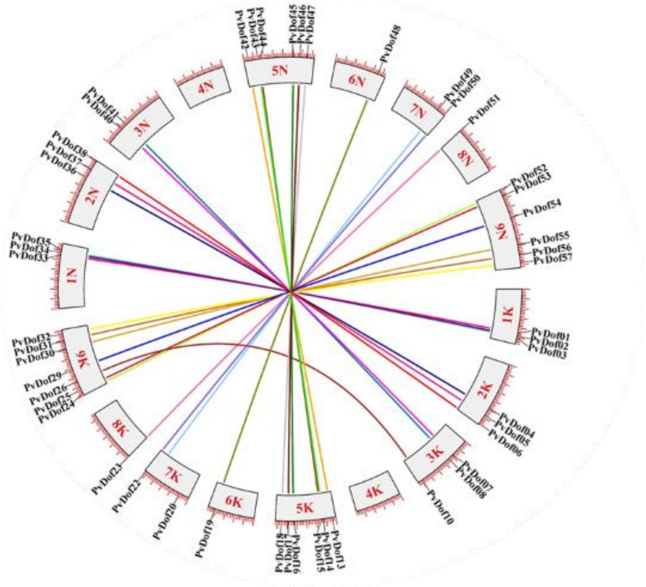

Figure 8. Circos plot shows segmental duplication of *PvDof* genes. Colored lines represent duplicated gene pairs across different chromosomal regions.

Synteny analysis and collinearity analysis

Gene synteny is widely utilized in transcriptomics and comparative genomic studies to identify orthologous genes across species and map out conserved homologous genomic regions. (Wu *et al*., 2024). A comparative synteny analysis was carried out between Dof genes in *P. virgatum* and *A. thaliana* to reveal the relationship between both studied plants. Cumulatively, more than 50 orthologous gene pairs were discovered, indicating a close evolutionary relationship within the Dof gene family between *P. virgatum* and *A. thaliana* (Figure 7). To investigate the intraspecific gene duplications of the *PvDof*s, a comprehensive analysis of 57 *PvDof* genes in *P. virgatum* was conducted to explore their syntenic relationships within the Dof gene family. Among 57 genes of *P. virgatum*, 50 gene duplication segments were identified. These gene duplication segments were widely distributed with highest count (7) on chromosome 9K followed by 6 regions on chromosomes 5N, 9N and 5K each. It is worth noting that no gene duplication segments were detected on chromosomes 4N and 4K. Collectively, the identified *PvDof* gene pairs constitute strong pattern for whole genome segmental duplication events in *P. virgatum* (Figure 8).

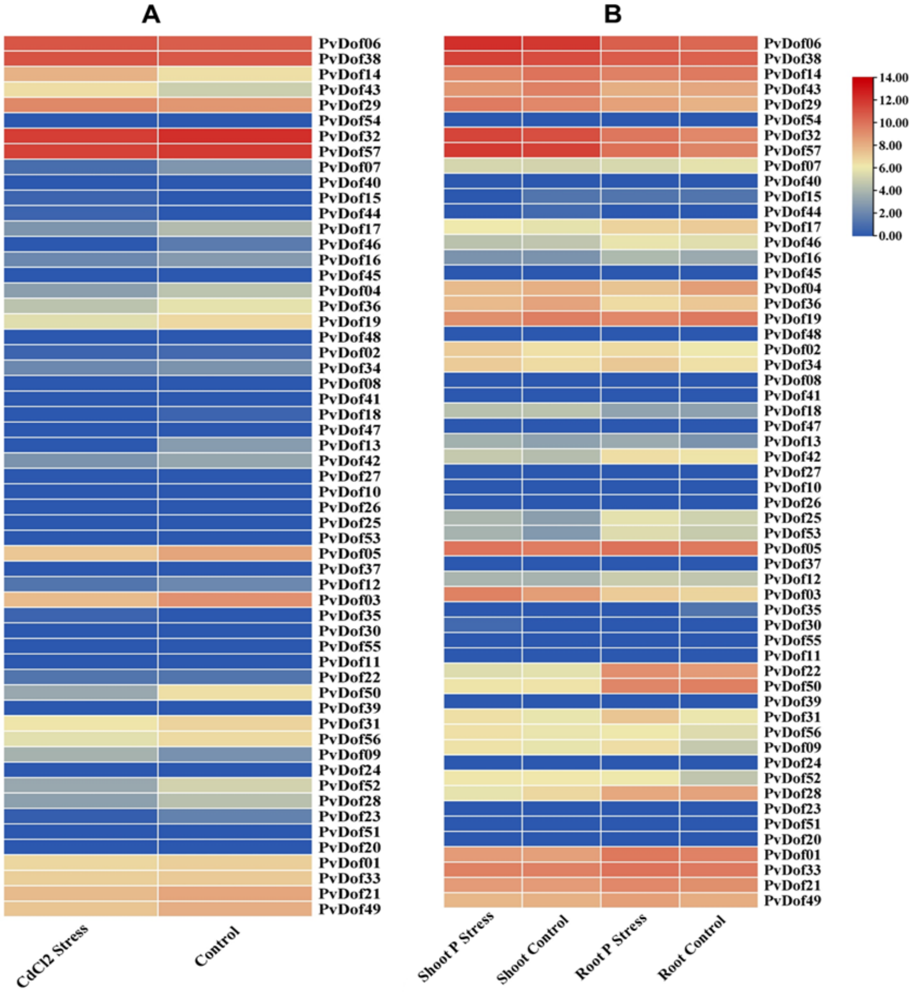

Figure 9. Transcriptomic profiling of *PvDof* genes across various tissues. (A) Expression patterns of *PvDof* genes under cadmium chloride ($CdCl_2$) exposure. (B) *PvDof* gene expression under phosphorus deficiency in root and shoot tissues. Rows represent genes and columns correspond to control and phosphorus deficient conditions. Colors reflect changes in expression levels.

Expression profiling of *PvDof* genes under cadmium chloride treatment and phosphorus deficiency

This study investigates the transcriptional response of *PvDof* genes to abiotic stressors, specifically cadmium chloride ($CdCl_2$) exposure and phosphorus deficiency. Cadmium chloride is a highly toxic compound which disrupts switchgrass growth, when high in concentration, by disrupting vital functions and inhibiting root and shoot development, reducing its bioenergy potential (Wang *et al*., 2015). However, phosphorus deficiency weakens root systems, reduces photosynthesis and energy metabolism, and lowers stress tolerance, ultimately impairing switchgrass growth and productivity (Sawyer *et al*., 2019).

P. virgatum plants were exposed to cadmium chloride ($CdCl_2$) treatment at a concentration of 250μmolL^{-1}. About 16 identified *PvDof* genes exhibited expression under stress conditions among which *PvDof06*, *PvDof32*, *PvDof38*, and *PvDof57* demonstrated the highest expression levels. Notably, these genes are classified within Group A, suggesting a potential group-specific regulatory role in abiotic stress response pathways. This indicates their potential involvement in cadmium stress response mechanisms. Some genes like *PvDof19*, *PvDof31*, *PvDof43* and *PvDof56* exhibited a neutral expression pattern. Interestingly, most *PvDof* genes were transcriptionally downregulated in response to $CdCl_2$ exposure (Figure 9).

In a similar experiment, *P. virgatum* plants were exposed to phosphorus deficiency stress, and tissue-specific gene expression was analyzed. Under phosphorus deficiency in shoots, *PvDof38*, *PvDof32*, and *PvDof57* genes were upregulated, suggesting a prominent role in modulating shoot responses under phosphorus-limited conditions. Conversely, in roots, *PvDof05*, *PvDof06*, and *PvDof57* genes were highly expressed, revealing a peak regulatory function in root-specific phosphorus deficiency responses (Figure 9). Interestingly, *PvDof09*, *PvDof52* and *PvDof56* genes didn't express any specific regulation in both root and shoot case of phosphorus deficiency.

DISCUSSION

Switchgrass (*Panicum virgatum* L.) is a vital bioenergy crop with high adaptability to marginal land (Hayford *et al*., 2022). Dof transcription factors are plant-specific proteins that regulate growth, development, and responses to hormones and environmental stress (Zou & Sun., 2023). Although the Dof family has been extensively characterized across different plant species*, P. virgatum* has received less attention, which led us to conduct a detailed examination. The effects of abiotic stresses like cadmium chloride treatment and phosphorus have been studied in different plant species (Gou *et al*., 2025; An *et al*.,2024). There was a significant gap in the previous understanding of Dof transcription factors in switchgrass, particularly about their regulatory roles in abiotic stresses like cadmium chloride treatment and phosphorus deficiency. The Dof transcription factors not only play vital role in developmental processes of plants, but also regulate various abiotic stresses (Ma *et al*., 2015).

We identified 57 non-redundant Dof genes in *P. virgatum*, which was notably higher than those identified several other plant species, including cucumber 36 (Zhou *et al*., 2020), barley 26 (Moreno-Risueno *et al*., 2007), sorghum 28 (Kushwaha *et al*., 2011), pigeon pea 38 (Malviya *et al*., 2015), and potato 35 (Venkatesh & Park, 2015). In contrast, certain plant species exhibit a greater number of Dof genes, such as soybean 78 (Guo & Qiu, 2013), Chinese cabbage 76 (Ma *et al*., 2015), cotton 89 (Luo *et al*., 2022), and wheat 96 (Li *et al*., 2022).

In this study, we identified 57 *PvDof* genes which were mapped across 17 chromosomes of *P. virgatum* (Figure.1). The distribution of these genes was found to be uneven, reflecting possible gene duplication events or evolutionary divergence. Among all the chromosomes, chromosome 9K carried the highest number of *PvDof* genes (9 genes). According to comparative phylogenetic analysis and similarity of protein sequences with *A. thaliana* and *O. sativa*, *PvDofs* were distributed into seven groups (A, B, C, D, E, F and G), based on the classification previously established in *Arabidopsis* by Hamdi *et al*. (2021). The largest group was group A with 29 *PvDof* members while group F was smallest with 1 *PvDof* member. Group B, C, D, E, and G contained 06, 06, 05, 02 and 08 *PvDof* members respectively. Among the 57 *PvDof* genes, 50 gene duplication segments were discovered by collinearity analysis. Segmental duplication has significantly contributed to the evolutionary expansion of Dof gene family as evident from previous studies in *A. thaliana* and rice (cannon *et al*., 2004; Wang *et al*., 2014). A comparative synteny analysis was conducted to discover more than 50 orthologous gene pairs between *A. thaliana* and *P. virgatum* that indicated a close evolutionary relationship between *PvDofs* and *AtDofs*.

Gene structure analysis helped to understand exon-intron organization and evolutionary relationships, while motif analysis revealed conserved functional domains critical for gene regulation and classification. In this study, all *PvDof* genes exhibited a low intron count, with the majority (mostly containing no or only one intron; 96%, 55/57) similar with findings of previous studies (Zhou *et al*., 2020). Fewer introns enhance transcriptional control and adaptability, potentially increasing sensitivity to environmental stresses (Shu *et al*., 2015). Phylogenetic profiling revealed that *PvDof* genes clustered within same group possess similar motif, suggesting their group specific functional roles. Group A comprised motifs 1, 2, 3, 4, 5, 7 and 9. Groups B and C share motifs 1, 2, 9 and 10. Group D contained motifs 1, 2, and 7. Groups E and F contain motifs 1 and 2. Group G was characterized by the presence of motifs 1, 2, 6, and 8. The widespread presence of motifs 1 and 2 across all groups suggested their conserved functional importance as evident from previous studies (Khan *et al*., 2021).

Cis-regulatory elements in the promoter regions of Dof genes were analyzed to investigate their functions and contributions to stress-responsive gene expression. A total of 55 cis-regulatory elements were identified in the promoter regions of *PvDof* genes, including elements responsive to environmental stimuli (e.g., low temperature, light, drought, anaerobic conditions, defense, and stress), phytohormones (abscisic acid, salicylic acid, auxin, MeJA, and gibberellin) as studied by (Li *et al*., 2021). Moreover, these elements are also involved in developmental processes (cell cycle regulation, meristem activity, seed-specific expression, and zein metabolism) as discussed by (Zhou *et al*., 2020). The most frequently observed elements included -30-core element, followed by common promoter-enhancer element and light responsive element, indicating their potential roles in transcriptional regulation. No distinct distribution pattern of cis-regulatory elements was observed among gene groups. The prevalence of these elements further suggests that the Dof gene family broadly participates in plant regulatory networks and plays a crucial role in crop growth and development.

Dof genes play a crucial role in plant abiotic stress responses, as demonstrated in previous studies (Wang *et al*., 2018; Khan *et al*., 2021). In this study, we analyzed gene expression patterns of 57 *PvDof*s under two abiotic stress conditions including $CdCl_2$ treatment and Phosphorus deficiency stress. In the $CdCl_2$ treatment experiment conducted under a 16 h light / 8 h dark photoperiod, the upregulation of *PvDof06*, *PvDof32*, *PvDof38*, and *PvDof57* in leaf tissues suggested their potential involvement in cadmium stress tolerance, likely through the regulation of stress-responsive signaling and detoxification pathways. On the other hand, under phosphorus deficiency, both roots and shoots exhibited distinct gene expression patterns. Phosphorus deficiency induced tissue-specific expression of *PvDof* genes. In shoots, the upregulation of *PvDof38*, *PvDof32*, and *PvDof57* suggested their involvement in regulating shoot-associated adaptive responses under low-phosphorus conditions. In contrast, the upregulated expression of *PvDof05*, *PvDof06*, and *PvDof57* in roots implies a critical role in modulating root-specific phosphorus deficiency signaling pathways.

CONCLUSION

Collectively, 57 non-redundant Dof genes were identified from *P. virgatum* and subsequently categorized into seven groups based on their phylogenetic relationships. The conserved motifs, gene structures, and transcriptomic profiles of these *PvDof* genes were analyzed. The expansion of the Dof gene family in *P. virgatum* was found to be chiefly attributable to segmental duplication, as evidenced by genome comparison. Expression profiling revealed that several *PvDof* genes (notably *PvDof32*, *PvDof38*, *PvDof57* and *PvDof58*) were highly expressed under cadmium chloride treatment and phosphorus deficiency, suggesting their involvement in abiotic stresses. These findings provide valuable insights into the functional role of *PvDof*s in stress signaling pathways. Overall, the identified *PvDof* candidates represent valuable genetic resources for developing stress-resilient switchgrass cultivars through advanced breeding approaches.

AUTHOR CONTRIBUTIONS

Mubashir Sharif: Writing – original draft, Writing – review & editing, Formal analysis. Muhammad Annas Shahid: Writing – original draft, Writing – review & editing, Formal analysis (†Mubashir Sharif and Muhammad Annas Shahid contributed equally to this research). Mahad-Ur-Rehman: Conceptualization, Validation, Supervision, Resources, Validation. Ikhlas Shafique: Conceptualization, Validation, Supervision, Resources, Validation. Muzammal Majeed: Writing – review & editing. Syeda Sahar Fatima: Writing – review & data analysis. Saad Kamran: Writing – review & editing. Rida Naseem: Validation, Writing – review & editing.

CONFLICT OF INTEREST,

The authors confirm that there are no conflicts of interest related to this research.

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