Genome wide identification and characterization of Dof transcription factor and its expression analysis under salinity stress in Avena sativa L.

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Abstract

The Dof transcription factor family is essential to several biological processes that occur in different plants, including regulating the growth and developmental stages of many plant tissues, such as root growth, hypocotyl elongation, plant morphogenesis, leaf development, and floral organ development. and environmental stress. But as of yet, *Avena sativa's* Dof family has not undergone a comprehensive analysis. In this study, 32 Dof genes (along with transcripts) in *Avena sativa* were identified from GrainGenes database and given named based on the chromosomal location. The conserved motifs, gene structures, gene duplication events, physiochemical properties, cis-acting elements, and expression patterns were further examined. These 32 *AsDof* members are classified into 8 subfamilies according to phylogenetic analysis. All proteins include Dof domains. All genes randomly dispersed throughout the 21 chromosomes with one tandem duplication and 10 pairs of segmental duplications; this may be the primary cause of the Dof gene family's expansion. Gene structure exposed intron number varying from zero to one and exons from one to two. Expression profiling identified 7 Avena Dof genes that respond to salt stress based on RNA-Sequence data available on the NCBI SRA database and validated through qRT-PCR. This study advances our knowledge of the genetic foundations of salinity tolerance and provides prospective towards genetic engineering to increase the resilience of oats to salt stress, supporting sustainable farming techniques.

INTRODUCTION

Numerous cellular functions, such as morphogenesis, cell signalling, and resistance to biotic and abiotic stressors, are impacted by the transcriptional and post-transcriptional regulation of gene expression in crop plants (Bokolia et al. 2023). Certain transcription factors characterize these alterations. The TFs are the proteins that attach to specific nucleotide sequences found in the promoter region to start the upregulation or downregulation of a gene's expression. Therefore, developing models of transcriptional control requires the discovery and functional characterisation of transcription factors. Thus far, about one hundred different DNA-binding domains have been discovered (Kummerfeld and Teichmann 2006). Based on their DNA-binding domains, these TFs are divided into numerous families, including MYB, bHLH, DREB, WRKY, bZIP and NAC, ERF, and ARF (Bokolia et al. 2025a; Bokolia et al. 2025b). Dof (DNA-binding with a single finger) is one of these transcription factor families. The Dof proteins are encoded by the zinc finger multigene family, which is unique to plants. The N-terminal of these Dof proteins is made up of 50–52 amino acids with a single C2–C2 type zinc finger motif that attaches to the T/AAAAG elements found in the promoter region. Several different TF families may interact physically with the Dof transcription factors. These Dof transcription factors have been linked to a number of physiological processes, including vascular development and the creation of the interfascicular cambium, flower abscission, photoperiodic flowering, guard cell differentiation, circadian clock, hormonal signaling in plants, and plant tolerance to biotic and abiotic stresses. ZmDof1, the first Dof protein, was discovered and described in maize. As a result, several Dof genes have been discovered in many plant genomes, including 36 in *Arabidopsis thaliana*, 30 in Rice, 26 in Barley, 28 in Sorghum, 27 in *Brachypodium distachyon*, 34 in Tomato, 46 in Maize, 36 in Cucumber, 33 in Pepper, 29 in Eggplants, and 31 in Bread Wheat (Liu et al., 2020, Wei et al., 2018, Wen et al., 2016, Wu & Cheng et al., 2016, Chen et al., 2015, Cai et al., 2013, Hernando et al., 2012, Kushwaha et al., 2011, Shaw et al., 2009, Moreno-Risueno et al., 2007, Lijavetzky et al., 2003,). Dof TFs are necessary for plants' growth and development as well as their capacity to react to various environmental stressors. Many investigations have demonstrated the function of Dof genes in *Arabidopsis*

thaliana. In a similar vein, MdDof15 was elevated in the roots of apple plants under drought or salt stress and in the leaves of plants under heat stress. Similarly, overexpression of the GhDof1 gene demonstrated extremely high resistance in *G. hirsutum* L. in the direction of salt and cold stressors (Huaizhu L et al., 2020). Nowadays, salt stress poses the most harm to the environment among all the abiotic stresses, including drought, high temperatures, cold, and heavy metals. The second biggest worldwide cause of harm to many physiological, biochemical, and molecular processes that impact plant agriculture productivity is salinity, often known as salt stress. Improving salt resistance is essential for maintaining global agricultural output because the majority of crop plants are vulnerable to salinity (Nerago et al., 2017). The function of Dof under salinity stress has been studied in a variety of plants, but *Avena sativa* L. has not been the subject of a thorough investigation. In light of this research gap, a thorough investigation was carried out in this specific crop.

One of the most understudied plants in genome and transcriptome research is *Avena sativa*, an allohexaploid (2n = 6 = 42) plant with a huge and complex genome. China is lucky to have high-quality, high-yielding oats, which are crucial to the country's cattle output. Additionally, because oats can tolerate harsher conditions than other food crops like rice and wheat, they are a pioneer crop for enhancing soil fertility. Through identification, phylogenetic analysis, domain and motif recognition, gene structure analysis, chromosomal mapping, subcellular localization, multiple sequence alignment, cis-regulatory element analysis, gene duplication, expression analysis by real-time PCR, and other methods, the genome-wide study of the Dof transcription factor in *Avena sativa* was analyzed.

MATERIALS AND METHODS

1. To study the genome wide identification and functional analysis of Dof TF gene family.

The complete genome data and GFF (general feature format) of the hexaploid-cultivated *Avena sativa* (OT3098) were downloaded from the Grain Genes database (https://wheat.pw.usda.gov/GG3/graingenes-downloads/pepsico-oat-ot3098-v2-files-2021). To search all the possible Dof TFs in *Avena sativa*, the Dof gene family members evolutionary study of Arabidopsis, wheat, and rice were obtained from the plant transcription factor database v5.0 (PlantTFDB - Plant Transcription Factor Database @ CBI, PKU (gao-lab.org). The Dof protein sequences of *Avena sativa* from Arabidopsis, wheat, and rice were then searched by using all required queries from desired Dof protein sequences using the default settings of the BLASTP program. 32 potential Dof sequences were reclaimed from the BlastP findings. Additionally, by using three databases such as Batch CD-Search(https://www.ncbi.nlm.nih.gov/cdd/Structure/cdd/wrpsb.cgi), Interproscan (https://www.ebi.ac.uk/interpro/search/sequence) and genome browser (PepsiCo OT3098 v2 Hexaploid Oat (2021) - GrainGenes (usda.gov) were used to confirm all potential Dof domain. As a result, 32 gene sequences were identified as Dof genes and utilized for further investigation. The *AsDof* gene and protein sequences are listed in Table S1 (Supplementary Table1).

2. Gene structure, Domain, motif analysis of *AsDof* genes transcription factor

By using different bioinformatics tools, we examine the presence of conserved motifs in the 32 *AsDof* proteins using the online application MEME-suite version 5.5.1 (https://meme-suite.org/meme/tools/meme): Motif widths varied within the range of 6 to 50, with a maximum count of 10 motifs in each gene (Bailey et al., 2009). By Using the NCBI-CDD database or the Interproscan, we predicted the peptide sequences of the 32 *AsDof*

proteins were retrieved. The Gene Structure Display Server 2.0 tool (http://gsds.gao-lab.org) was used to predicted the exon-intron structure of 32 *AsDof* genes by using the CDS as input (Hu et al., 2015). Following confirmation from the Batch CD-search and TBtools finished the domain analysis of the potential 32 *AsDof* protein.

3. Chromosomal mapping and gene nomenclature

Avena sativa's Dof gene chromosomal locations were predicted using the MapInspect1.0 tool (https://mapinspect.software.informer.com/). We used *As* as prefix for naming the Dof genes, where *As* refers to *Avena sativa*, and gene numbers were assigned according to the chromosomal locations from top-to-bottom location.

4. Physiochemical properties, subcellular localization, and 3D modeling of *AsDof* genes

The Expasy ProtParam (https://web.expasy.org/protparam) tool was used to examine the physicochemical properties and calculated the aliphatic index, number of amino acid residues, instability index, GRAVY (Grand average of hydropathicity), molecular weight (MW), isoelectric point (pI) etc. Furthermore, ProtComp 9.0 tool (ProtComp - Predict the sub-cellular localization for Plant proteins, softberry.com), WoLF PSORT (https://www.genscript.com/wolf-psort.html), and CELLO (http://cello.life.nctu.edu.tw/) were used to predict the subcellular localization of 32 potential *AsDof* genes. The proteins' secondary structure was predicted and manipulated using the Endscript (https://endscript.ibcp.fr/ESPript/cgi-bin/ENDscript.cgi) and the tertiary structure was predicted by using I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Yang et al., 2015). The PyMOL tool was used to manipulate the 3D model of the desired Dof proteins.

5. Gene Ontology and identification of promoter region cis-regulatory elements

The Omics box tool (https://www.biobam.com/omicsbox) provided the Gene Ontology results for the Dof genes found in oats. In order to obtain the gene annotation files (cellular component, molecular function, and biological process), the complete CDS sequences were submitted to the Omics box database. Furthermore, to find out the cis-regulatory elements in the 32 *AsDof* promoter regions, the sequences which are present 1.5 kb upstream from the transcription start site (ATG) were retrieved by using the PlantCARE tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

6. Phylogenetic analysis and multiple sequence alignment

Multiple sequence alignments of the amino acid sequences of the Dof protein in *Avena sativa* and *Arabidopsis* were taken into consideration for the phylogenetic analysis of the Dof gene family. Based on the provided information about the two species a phylogenetic tree was constructed using the MEGA-XI software with 1000 bootstraps, by Neighbour-Joining (NJ) method. The ClustalW was used to align several Dof's, 32 sequences of *AsDof* were aligned as a result. Later the aligned sequences were subjected to endscript.

7. Gene duplication event of *AsDof*

The desired sequences were aligned using ClustalW, which was used to assess the chromosomal gene duplication events. In order to identify the paralogous gene pairs having at least 80% sequence similarity. The CDS sequences of gene pairs were taken into consideration by using KaKs calculator (Computational Biology

Unit (CBU) uib.no) the substitution rates were determined for each duplicated gene pair, where Ka represents for (non-synonymous) and Ks (synonymous). The type of duplication was depended upon on the gene which was found in that particular area on the chromosome. Genes that were located within the 5 Mb zone of the same chromosome were regarded as tandem duplications. Similarly, if the blocks of DNA that typically share more than 90% sequence identity and occur at more than one site within the genome are categorised as segmental duplication, these duplications were taken into consideration when the genes were positioned outside of the 5Mb region of either the same or different chromosome. (Shumayla et al., 2016). The formula T = Ks/2λ × 10–6 was used to determine the estimated timing of the duplication event million years ago (Mya).

8. Recovery of transcriptomic data and *AsDof* gene expression profiling under salinity stress

We used NCBI SRA (Sequence Read Archive) (https://www.ncbi.nlm.nih.gov/sra) database RNA sequencing data to examine the expression profile of Dof genes in *Avena sativa* under salt stress. *Avena sativa's* salt stress expression profiles were investigated using the RNA-Seq data associated with project numbers SRP094911 (Wu et al., 2017). First, the seeds were sterilized and germinated at room temperature on moist filter paper till the shoot and roots are appeared. Later they were moved to Hoagland solution media under ideal conditions (16 hours of light and 8 hours of darkness, 20–25 degrees Celsius, and 70% relative humidity). Then these plants were allowed to grow till the second leaflet stage and the salt treatment was provided to the plants (100 mM NaCl). The samples of the entire plant were taken at 0-, 2-, 4-, 8-, 12-, and 24-hours following intervals after the treatment under the salt stress. The plant sample without NaCl treatment was collected which was used as the control. Then they were preserved in -80 degrees freezer and used for further gene expression analysis. Using the Trinity-V 2.03 tool, the expression profiling of Dof genes was carried out (Haas et al., 2013). To validate the expression of various genes, RSEM (RNA-Seq by Expectation-Maximization) software computed FPKM (fragments per kilobase of transcript per million fragments mapped) values were taken into consideration. To determine which genes are differentially expressed, EdgeR and RSEM are used in tandem. Base on the one-fold change value of 1.0 and significant score value using 0.05 and the Log2 of various gene-expressed data, heatmaps were generated by using TBtools.

9.Treatment of salt stress and plant material in *Avena sativa*

The Punjab Agricultural University provided the oat (OL-14) seeds sample which we used in this investigation. The seeds were cultivated on the moist filter paper till the root and shoot was developed. The ideal temperature ranges were 16–18 °C at night and 22–24 °C during the day, with a relative humidity of 70–80% and a photoperiod of 14/10 hours. Later these plants were grown up to second leaflet stage and the plants were treated with 100 mM NaCl solution when the second leaf appeared, which occurred after two weeks. The plant that received no treatment was regarded as the control. At 0-, 2-, 4-, 8-, 12- and 24-hours following treatment, the control and treated seedlings were harvested. All treatments and sampling were performed in three biological replicates for each time point from different individual plants grown under identical conditions to ensure reproducibility and statistical reliability and after that, the samples were kept at -80 °C for as long as possible before the RNA was extracted.

10. RNA extraction and expression analysis by qRT-PCR

The stress subjected *Avena sativa's* plant RNA was extracted by using RNeasy® Plant Mini Kit (Hilden, Germany) Kit. Then, cDNA was synthesized by using theApplied biosystems by thermofisher high-capacity

cDNA reverse transcription kit, then the synthesized cDNA was extracted. 5 *AsDof* genes primers were designed using primer 3 or primer quest tool (Table S2). By using the Biorad Taq universal green SYBR supermix (Vilnius, Lithuania) and the JENWAY Genova nano instrument, we performed qRT-PCR for the expression analysis study. The following was the reaction system: Ten minutes at 95 °C, forty cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. (Jia et al. 2020) employed the 2-DDCt technique to analyze gene expression, with β-actin serving as the reference gene.

RESULTS

1. Identification of Dof genes across the genome and their chromosomal distribution.

Using the InterProScan and NCBI CD-search databases, we identified 32 probable candidates. Genes were found when DOF domain confirmation was completed. The positions of the various Dof genes on the various *Avena sativa* chromosomes are depicted in Fig. 1. Based on the information gathered, we called the Dof genes *AsDof1* to *AsDof29*. These even include transcriptomes identified by the genome database data as *AsDof4.2, AsDof9* and *AsDof14.4*. We created a map file for each chromosome in order to identify the locations of the 32 *AsDof* genes. The *AsDof* genes were discovered to be randomly distributed throughout the 21 chromosomes (Fig. 1). The greatest number of genes is found on chromosome 5D. On the other hand, there are the fewest genes discovered on chromosome 1C.

2. Gene structure, domain, motif analysis of *AsDof* transcription factor

Genes have evolved in a variety of ways from prehistoric times. They evolved vividity in their functions and the gene structure of *AsDof* genes for their survival as a result of their circumstances, environmental changes, or habitat changes. The gene structure display server (GSDS) was used to retrieve the gene's exons and introns, which are displayed in Fig. 2. The findings demonstrated that every Dof gene has both exons and introns. *AsDof1, AsDof3, AsDof4.2, AsDof5, AsDof9.2, AsDof10, AsDof13, AsDof14.4, AsDof17, AsDof19, AsDof22, AsDof25, and AsDof27* have no introns. Of the 32 genes, the maximum introns ranged from 0-2. Additionally, the NCBI CDD and Batch CD search were used to examine the domain analysis of Dof genes in *Avena sativa*. The Dof domain is encoded by all 32 *AsDof* genes (Fig 3). In addition to the Dof domain, *AsDof7* contains the DUF5585 superfamily, whose family function is still unknown. Similarly, *AsDof9.2* contains the MND1 superfamily, which is crucial in controlling the development of inflorescences and shoot branching (Agatha Walla et al., 2020). Similar to this, *AsDof19* contains the PRK13108 superfamily, a member of the heat stress-related protein family, which is essential for plant development, growth, and abiotic stress response (Hua, Y et al., 2023). Similarly, the function of the PTZ00121 superfamily in *AsDof 9.1* is yet unknown (Q Xiao et al., 2022).

To identify the conserved motifs of the 32 *AsDof* genes in *Avena sativa*, we used the MEME suit analysis. Ten motis in all, numbered 1 through 10, were discovered. Based on the data retrieved, we were able to obtain the protein sequences of the Dof genes. We discovered that the amino acid sequence, which ranges from a maximum of 50 to a minimum of 15, has a vividity Fig. 4(B) and Table S3. The findings demonstrated that while motif 1 is found in every gene, certain genes also include additional motifs. While the genes *AsDof8, AsDof9.1*, and *AsDof9.2* include two motifs (motif1, motif2), *AsDof17, AsDof18, AsDof19, AsDof22*, and *AsDof23* only contain one motif (motif 1). Similar to this, *AsDof24* and *AsDof25* also have two motifs (motif 1, motif 8), *AsDof10* and *AsDof11* have four motifs (motif 1, motif 4, motif 7, motif 8), *AsDof16* has four motifs (motif 4,

motif 7, motif 8, AsDof19), and the genes *AsDof 4.1* and *AsDof 4.2, AsDof 6, AsDof7* contains 7 motifs (motif 1, motif 3, motif 5, motif 8, motif 9, motif 10, AsDof6) and so on. The same motif composition of the proteins in the same group suggests that they may also play similar biological roles. Even while these expectations might apply to the same group, their amino acid composition may differ, and as a result, they may serve a specific purpose Fig. 4(A) and Table S3 displays these genes' sequences.

3. Physiochemical properties, subcellular localization, and 3D modeling of *AsDof* genes

The general physicochemical characteristics of Dof genes, include molecular weight (MW), isoelectric point (pI), amino acid length (AA), instability index, and aliphatic index etc. of the 32 *AsDof* genes and the trancriptomes listed in Table 1. These 32 *AsDof* genes have molecular weights between 2000 and 7000, pI values larger than 9, and aliphatic indices with minimum values of 42 and maximum values of 76.

Table 1. The physiochemical properties of 32 *AsDof* genes such as molecular weight (MW), no. of amino acid residues (AA), instability index, GRAVY and also shows the subcellular localization of the genes.

ID	AMINO ACID	MOL WT	PI	INSTABILITY INDEX	ALIPHATIC INDEX	GRAVY	SUBCELLULAR LOCALISATION
AsDof 1	272	27672.76	9.34	57.78	53.68	-0.288	Extra/Chlo/nucl
AsDof2	428	46389.67	7.51	49.97	52.73	-0.727	Peri/Chlo/nucl
AsDof3	329	34929.13	5.11	52.21	64.5	-0.347	Peri/nucl
AsDof4.1	382	39067.17	8.65	52.38	46.2	-0.53	Extra/nucl
AsDof4.2	367	37436.29	8.66	54.54	45.42	-0.526	Extra/nucl
AsDof5	354	35354.83	7.22	45.26	58.42	-0.274	Extra/nucl
AsDof6	374	38436.58	8.65	55.46	44.3	-0.552	Extra/nucl/mito
AsDof7	376	38423.36	8.65	51.88	51.88	-0.551	Extra/nucl/mito
AsDof8	476	51419.24	8.79	62.33	64.75	-0.559	Peri/nucl
AsDof9.1	559	60963.87	5.06	59.93	51.52	-0.807	Peri/nucl
AsDof9.2	486	53150.25	5.29	58.19	51.21	-0.791	Peri/nucl
AsDof10	354	35641.4	9.59	47.89	44.8	-0.451	Extra/Chlo
AsDof11	373	38144.63	8.86	59.28	36.62	-0.66	Extra/nucl
AsDof12	432	46990.38	7.9	51.68	53.36	-0.725	Peri/nucl
AsDof13	374	38744.98	9.33	69.54	59.57	-0.333	Extra/nucl
AsDof14.2	438	45202.68	9.22	51.14	46.23	-0.63	Extra/nucl
AsDof14.4	354	35475.18	9.48	47.04	44.27	-0.447	Extra/nucl/Chlo
AsDof15	369	38068.53	8.86	57.96	36.72	-0.687	Extra/nucl
AsDof16	387	40265.68	9.58	69.28	43.28	-0.673	Extra/nucl
AsDof17	361	36484.85	4.67	42.05	58.31	-0.373	Extra/nucl/Chlo
AsDof18	325	34031.68	8.4	44.24	42.65	-0.579	Extra/nucl
AsDof19	304	30909.32	4.53	49.65	47.99	-0.609	Extra/nucl
AsDof20	384	40050.45	9.36	69.97	45.13	-0.661	Extra/nucl
AsDof21	348	35975.81	8.91	57.47	55.95	-0.473	Extra/nucl
AsDof22	303	30989.71	4.51	54.54	51.39	-0.527	Extra/nucl
AsDof23	480	51162.13	6.21	59.73	51.48	-0.671	Peri/nucl
AsDof24	214	22530.09	8.56	76.73	50	-0.507	Peri/nucl/Chlo
AsDof25	286	29582.96	8.8	68.13	68.13	-0.319	Extra/nucl/Chlo/mito
AsDof26	386	39440.58	8.82	46.5	47.23	-0.571	Extra/nucl/mito/cyto

AsDof27	285	29669.09	8.42	72.73	55.65	-0.353	Extra/nucl/Chlo/mito
AsDof28	367	38040.42	8.62	54.18	49.67	-0.428	Extra/nucl
AsDof29	242	25647.55	6.17	67.79	52.56	-0.557	Peri/nucl

Protcomp6 or CELLO were utilized for subcellular localization. The findings demonstrated that the precise locations of the genes in the cells where they are found are displayed in Table 1. We discovered that the majority of these 32 *AsDof* genes are distributed in several cell locations, including the extracellular space, periplasm, nucleus, chloroplast, and mitochondria.

The amino acid sequence and the locations of the alpha helix and beta pleated sheets are illustrated in Fig. 5(A) of the protein's secondary structure, which was predicted using the Endscript software. For instance, 10 beta pleated sheets and 5 alpha helices make up the protein *AsDof4*. Similar to this, the I-TASSER tool is used to determine the 3D structure of proteins. It displays the protein's clear structure as well as the locations of the alpha helix and beta pleated sheets, which allow us to interpret and alter the structures, as illustrated in Fig. 5(B). We were able to determine the protein's tertiary structure using this study and subsequently by utilizing PyMOL 3D structure for visualization.

4. Gene ontology and identification of promoter region cis regulatory elements

The three categories—cellular component, biological process, and molecular function—were used to analyze the gene ontology of the 32 Dof genes listed above. DNA binding transcription factor (F: GO:0003700), DNA binding (F: GO:0003677), metal ion binding (F: GO:0046872), regulation of transcription factor, DNA template (F: GO:0003700), nucleus (C: GO:0005634), and binding (F: GO:0005488) were among the components whose expression varied according to GO analysis. The examination of all the GO elements and their controlled functions through DNA is depicted in Figure 6.

We examined the cis regulatory elements of the 32 *AsDof* genes using the plantTFDB. Modules found in non-coding DNA areas that control the transcription of nearby genes are known as cis regulatory elements. For this investigation of cis regulatory elements, the *AsDof* genes subjected to salt stress were utilized. Sequences that are found more than 1.5 kb upstream from the gene's start point are often taken into account. This site contains a variety of cis regulatory elements that are classified according to their functions, such as light-mediated response, wound and defense related, anaerobic induction.

There are numerous cis regulatory elements under these functions, such as zein metabolisms. For instance, light-mediated response elements include CCGTCC-box, CCGTCC motif, AE-box, GATA-motif, TCT-motif, Sp1, AE-box, TCCC-motif, CCGTCC-box, etc. Similarly, hormonal-responsive elements include MYC, MYB, STRE, DRE-core, LTR, DRE1, MBS, and TC-rich repeats elements. Similar to how the as-1 functions as a growth and development element, the wound and defense-related elements are W box, WUNmotif, and WRE3.

Similarly, O2 is the zein motif element, ARE functions as the anaerobic induction activity, and the promoter region contains numerous other parts. All of the cis regulatory elements found in the promoter region are displayed in Fig. 7 and Table S4.

5. Phylogenetic analysis

Using MEGA XI, we constructed a phylogenetic tree using 36 Arabidopsis genes as the reference genome and 32 *AsDof* genes to investigate evolutionary links. The naive version of the created tree was utilized. The iTOL tool was used to do alterations, including adding color. The neighbor joining method (NJ) with a bootstrap value of 1000 was used to build the tree. The results show that the 32 *AsDof* genes were separated into eight subgroups, including A+B2, B1, C1, C2.1, C2.2, C3, D1, and D2. *AsDof5, AsDof13, AsDof17, AsDof22, AsDof19, AsDof29*, and *AsDof27* make up the family A+B2. Their motifs range from 1-2, and they have 0 introns and a maximum of 2 exons. *AsDof14.2, AsDof14.4, AsDof10, AsDof21, AsDof16, AsDof20, AsDof11, AsDof15,* and *AsDof28* genes make up the B1 family. These motifs range from 3 to 4 and have a minimum of 0 introns and a maximum of 2 exons. It is the only family with the greatest number of *AsDof* genes. There are no *AsDof* genes in family C1, which is made up solely of Arabidopsis genes. *AsDof 26, AsDof7, AsDof6, AsDo4.2*, and *AsDof4.1* make up the family C2.1. They have motifs ranging from 3 to 7 and have a minimum of 0 introns and a maximum of 2 exons. With just three genes—one of which is from *Avena sativa* (*AsDof3*) and the other two are from Arabidopsis—the C2.2 family is the smallest in the tree. The single Dof gene has a single motif, 0 introns, and 1 exon. The lone gene in the family C3 is *AsDof1*, which is made up of two motifs with one exon and zero introns. *AsDof9.1, AsDof9.2, AsDof23, AsDof8, AsDof2, AsDof12,* and *AsDof18* make up the family D1. These motifs range from 1 to 3 and have an exon maximum of 2 and a minimum of 1. This evolutionary tree's final family, D2, has a single Dof gene, *AsDof24*, which has two motifs, two exons, and one intron. The phylogenetic tree is explained in detail in Fig. 8.

6. Gene duplication event of 32 *AsDof* genes

Gene duplication, which includes both segmental and tandem duplication, is essential to gene evolution. We must identify the genes' positions on the chromosome before we can analyze the gene duplication. The locations of the 32 *AsDof* genes across the 21-oat reference genome are revealed by the chromosomal mapping results. *AsDof1* is found in chr 1C, *AsDof2* and *AsDof3* are found in chr 1D, *AsDof4.1* and *AsDof4.2* are found in chr 2A, *AsDof5* and *AsDof6* are found in chr 2C, and *AsDof7* is found in chr 2D, *AsDof8*, *AsDof9.1*, and *AsDof9.2* make up chr 3C. Similarly, the chr 4A contain *AsDof10, AsDof11. AsDof12* makes up the chr 4C. *AsDof13, AsDof14.2, AsDof14.4,* and *AsDof15* make up the chr 4D. Similarly, *AsDof16* and *AsDof17* are found in chr 5A, and *AsDof18* and *AsDof19* are found in chr 5C. *AsDof20, AsDof21, AsDof22, AsDof23,* and *AsDof24* make up the chr 5D, while *AsDof25* and *AsDof26* are found in the chr 6A. Similarly, *AsDof27* makes up the chr 6C. *AsDof28* is found in chr 7A, and *AsDof29* is found in chr 7D. The highest number of chromosomes discovered is found in chr 5D. *AsDof2, AsDof6, AsDof7, AsDof19, AsDof17, AsDof16, AsDof10*, and *AsDof25* exhibited 80% sequence similarity across the 32 *AsDof* genes, according to the ClustalW aligned sequences. We determined the substitution ratios of Ka (nonsynonymous) to Ks (synonymous substitution rate) for each duplicated paralog pair in order to understand the evolutionary influences of the *AsDof* gene family (Tomar et al., 2022). The ka/ks was computed to calculate the selection pressure that propels a gene's evolution. Positive selection is indicated if the ka/s is larger than 1, neutral selection is indicated if the ka/ks is equal to 0 and the purifying selection is displayed if the ka/ks ratio is less than 1. We examined the duplication time using the parameters and found that the duplication time ranged from 3.78573 mya (Ks = 0.045656) to 8.34173 mya (Ks = 0.100601). The findings of the gene duplication are displayed in Table 2. According to the data, all 32 genes had duplications that were segmental and had ka/ks values greater than 1, indicating purifying selection.

Table 2. The above table represents the gene duplication events of the *AsDof* genes duplication which was retrived by ka/ks formula.

Seq_1	Seq_2	Ka	Ks	Ka_Ks	MYA	Purifying selection	Duplication
AsDof12	AsDof2	0.00929	0.084389	0.110081	6.99746	yes	segmental
AsDof7	AsDof6	0.014584	0.100601	0.14497	8.34173	yes	segmental
AsDof4.1	AsDof6	0.014418	0.099636	0.144704	8.26169	yes	segmental
AsDof4.1	AsDof7	0.004751	0.05871	0.080917	4.86813	yes	segmental
AsDof17	AsDof19	0.079454	0.228294	0.348035	18.9298	yes	segmental
AsDof22	AsDof19	0.072219	0.294397	0.245313	24.411	yes	segmental
AsDof22	AsDof17	0.044966	0.230941	0.194708	19.1493	yes	segmental
AsDof20	AsDof16	0.011702	0.046596	0.251134	3.86368	yes	segmental
AsDof14.2	AsDof10	0.007652	0.045656	0.167608	3.78573	yes	segmental
AsDof27	AsDof25	0.030974	0.087996	0.351996	7.29651	yes	segmental

7. RNA-Seq expression profile of the Dof gene family during salt stress

For the expression analysis of these genes, we used the TBtools to create a heatmap that illustrates the relative expression differences in the entire plant RNA-sequence data that is subjected to salinity stress. The FPKM values were obtained by using the Trinity software. Seven of the 32 *AsDof* genes—*AsDof22, AsDof14.4, AsDof23, AsDof9.2, AsDof4.2, AsDof24*, and *AsDof9.1*—had differential expression. Nevertheless, these genes were first downregulated and subsequently increased. Figure 9 shows the distinct differential expression.

8. Expression analysis of *AsDof* genes with response to salt stress treatment

AsDof gene expression was shown to be induced based on the transcriptomic data analysis. We used seven *AsDof* genes—*AsDof22, AsDof14.4, AsDof23, AsDof9.2, AsDof4.2, AsDof24,* and *AsDof9.1*—that were approved by qRT-PCR analysis to confirm the RNA-seq of Dof genes under salt stress. All five graphs, with the exception of 14.4, had consistent results. However, the 4.2 graph shows that while the RNA values are consistent, the RT-PCR values are not. This could be because of environmental factors that affect the expression levels of both RNA and RT-PCR data. The results of qRT-PCR are shown in Fig.10 (A), (B), (C), (D), and (E).

DISCUSSION

Dof (DNA binding with One Finger) transcription factors constitute a plant specific family of transcription regulators known to play pivotal roles in various physiological and developmental processes, including seed development, germination, carbon metabolism and responses to environmental stresses (Gupta et al., 2015). In *Avena sativa* (oat), an important cereal crop with increasing agricultural and nutritional significance, understanding the function and regulation of Dof transcription factors is essential for advancing molecular breeding and crop improvement. In the present study, we identified 32 Dof gene family members in *Avena sativa* randomly distributed on 21 chromosomes. This identification and characterization of Dof genes in *A. sativa* reveal a conserved domain architecture typical of Dof family with the characteristic zinc finger motif enabling specific binding to target gene promoters. This conservation suggests functional parallels to Dof proteins in other cereals such as wheat, barley and rice where Dof factors have been implicated in regulating

genes related to starch biosynthesis and seed storage protein accumulation (Khan et al., 2021, Liu et al., 2020). Till now, 36 Dof gene family members have been identified in *Arabidopsis thaliana*, 96 in Wheat (Liu et al., 2020), 26 in Barley (Moreno-Risueno et al., 2007), 28 in Sorghum (Kushwaha et al., 2011), 30 in Rice (Lijavetzky et al., 2003), 46 in Maize (Chen et al., 2015) and 25 in Sugarcane (Gupta et al., 2014) which suggests their significant divergence among different plants.

The phylogenetic analysis explains the evolutionary relationships between Dof transcription factors from *Avena sativa* (*AsDof*) and *Arabidospsis thaliana.* The Dof proteins are grouped into eight major clades- A + B2, B1, C1, C2.1, C2.2, C3, D1 and D2 revealed evolutionary relationships consistent with those reported in other cereals such as wheat, barley and rice (Moreno-Risueno et al., 2007). The widespread distribution of *AsDof* genes across all clades suggests high degree of evolutionary diversification and functional specialization in oat. Notably, clades such as A + B2, B1 and C2.1 contain larger number of *AsDof* members indicating potential expansion and lineage specific adaptation. This diversification may reflect the involvement oat Dof transcription factors in a range of biological functions including light signaling, hormonal regulation, seed development and responses to abiotic stress. The close clustering of certain *AsDof* proteins with their Arabidopsis counterparts also points to conserved functions suggesting orthologous relationships. Gene structure analysis provided additional evidence of this evolutionary conservation. Members within each phylogenetic subgroup displayed highly similar exon-intron arrangements with the A + B2 clade characterised predominantly by intronless genes, a structural pattern also observed in rice and wheat Dof families (Liu et al., 2020, Moreno-Risueno et al., 2007). Such conservation in exon-intron architecture suggests that the structural integrity of Dof genes is under purifying selection to preserve their functional capacity. Conversely, the more complex structures observed in subgroups such as C2.1 and D1 may reflect functional diversification or subgroup specific regulation of gene expression. Conserved motif analysis revealed that all *AsDof* proteins possess motif 1 representing the C2-C2 zinc finger DNA-binding domain essential for recognising the AAAG core motif in target promoters (Yanagisawa et al., 1999). This universal presence underscores its central role in Dof function across plant species. Especially, subgroup specific motifs were also identified- motif 2, 3 and 4 were enriched in C2.1 members, while motif 7 and 10 were unique to certain D clade proteins suggesting possible functional specialisation. In cereals, such motif divergence has been associated with regulation of starch biosynthesis, seed storage protein accumulation and abiotic stress responses (Liu et al., 2020, Gupta et al., 2015, Moreno-Risueno et al., 2007). The integration of phylogenetic, structural and motif data supports a model in which *AsDof* transcription factors have evolved under dual constraints maintaining a highly conserved DNA-binding function while diversifying regulatory regions to acquire new roles. This balance between conservation and innovation likely contributes to the adaptation of oats to specific ecological niches and agricultural requirements. Given that *A. sativa* seeds are valued for their high beta-glucan content, balanced protein composition and health promoting properties (Kim et al., 2021), the subgroup specific motif diversity observed here may point to candidate Dof genes involved in metabolic pathways influencing these quality trades. This combined analysis reveals that the *Avena sativa* Dof gene family retains the hallmark features of cereal Dof proteins while exhibiting structural and functional diversity that could underlie species-specific traits. Future research employing transcriptomic profiling under developmental and environmental conditions, coupled with functional validation through gene editing, will be crucial to explicate the precise contribution of these transcription factors to oat growth, stress tolerance and grain quality, thereby enabling their integration into molecular breeding programmes.

Promoter analysis of *Avena sativa* Dof genes identified a diverse collection of CREs, importance of their potential roles in complex transcriptional networks. Across the *AsDof* genes analysed, numerous functional classes of CREs were detected, including those involved in light responsiveness, hormone signaling, stress responses and developmental regulation. Light-responsive elements such as G-box, I-box, GATA-motif and TCT-motif were abundant across almost all *AsDof* promoters. The prevalence of these elements suggests that light-regulated transcriptional control may be a universal feature of oat Dof genes, consistent with earlier reporters that Dof factors participate in photoperiod-dependent processes including seed germination and flowering time regulation in cereals (Gupta et al., 2015, Yanagisawa et al., 2002). Hormone responsive CREs were also predominantly represented. ABRE motifs (abscisic acid responsiveness) were widely distributed, indicating possible roles in ABA-mediated pathways, particularly under drought and seed maturation conditions (Nakashima & Yamaguchi-Shinozaki et al., 2013). AuxRR-core and TGA-element (auxin responsiveness), GARE-motif and P-box (gibberellin responsiveness) and ERE (ethylene responsiveness) suggests that *AsDof* genes may integrate multiple hormonal signals, enabling fine-tuned regulation of seed development and stress adaptation, as has been documented in rice and wheat Dof orthologs (Zou & Sun et al.,2023). Stress-related elements such as MBS (MYB binding site involved in drought response), W-box (WRKY binding site associated with pathogen and defence responses), LTR (low-temperature responsiveness) and WUN-motif (wound responsive) were detected in several *AsDof* promoters. The presence of multiple stress-responsive motifs in certain genes, such as *AsDof19, AsDof23* and *AsDof29*, suggests that these members could play prominent roles in abiotic and biotic stress tolerance. This is in line with studies in barley and maize, where Dof TFs modulate stress-inducible gene expression via interactions with MYB and WRKY factors (Kushwaha et al., 2011, Chen et al., 2015). In addition to stress and hormone signaling, elements such as ARE (anaerobic induction), O2-site (zein metabolism regulation) and as-1 (pathogen and salicylic acid responsiveness) point toward potential roles in nutrient metabolism and pathogen defense. Notably, the detection of O2-site motifs in several oat Dof genes mirrors finding in maize, where the Dof protein PBF regulates prolamin storage protein genes during endosperm development (Liu et al., 2020, Vicente-Carbajosa et al.,1997). The variation in CRE abundance among *AsDof* genes with some members such as *AsDof19* and *AsDof29* containing more than 50 elements, indicates potential differences in regulatory complexity and responsiveness to multiple signals. Genes with a higher density of CREs are likely to act as a transcriptional hub, integrating diverse environmental and developmental signals, thereby contributing to the fine-tuning of metabolic and physiological pathways. Collectively, these findings suggest that *Avena sativa* Dof transcription factors are embedded within an intricate regulatory network governed by multiple environmental and hormonal cues. This complexity underscores their potential roles not only in growth and developmental processes but also in adaptive responses to changing environmental conditions.

The heatmap analysis further revealed functional divergence with certain genes (*AsDof14.4, AsDof22*) being strongly upregulated while others (*AsDof4.2, AsDof9.1*) were stable or downregulated. Such variation supports subfunctionalisation within the Dof family, consistent with reports of lineage-specific diversification in plants (Zou et al., 2023, Gupta et al., 2015). The expression validation of *Avena sativa* Dof transcription factors under salinity stress demonstrated consistent patterns between RNA-Seq and qRT-PCR data sets like *AsDof22* showed strong early induction while *AsDof23* displayed a delayed expression peak suggesting temporally distinct regulatory roles in stress adaptation. These results align with previous findings in cereals where Dof genes function in stress-responsive transcriptional reprogramming (Liu et al., 2020, Yanagisawa et al., 2002). Functional studies, including gene silencing or overexpression in model systems, could further elucidate the

specific regulatory circuits governed by oat Dof transcription factors. For example, manipulating Dof gene expression could improve seed nutritional quality or enhance tolerance to abiotic stresses, thereby stabilizing yield under variable climatic conditions. Integration of Dof gene function studies with genomics and breeding efforts will accelerate the development of superior oat varieties tailored to future agricultural challenges. Overall, these findings indicate that oat Dof TFs act in coordinated, stage-specific responses to salinity stress and continued investigation into their molecular functions and interactions will not only deepen our understanding of oat biology but also provide valuable tools for improving this important cereal crop.

Declarations

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The authors declare that no specific funding was received for this study.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Ethical Approval

This study did not involve human participants or animal experiments.

Consent for Publication

All authors have given their consent for publication of the manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Authors' Contributions

Muskan Bokolia: Conceptualization, Methodology, Investigation, Writing – Original Draft.

Tanu Singh: Methodology, Investigation, Writing – Original Draft.

Baljinder Singh: Supervision, Project Administration, Validation, Data Curation, Visualization, Writing Review & Editing.

All authors read and approved the final manuscript.

References

1. Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., ... & Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching. Nucleic acids research, 37(suppl_2), W202-W208.

2. Bokolia, M., Nibha, T. V., Priya, J. C., & Singh, B. (2025a). GRAS transcription factor-Genome-wide identification, characterization and expression analysis in Avena sativa L. under salinity stress. *Plant Stress*, *15*, 100739.
3. Bokolia, M., Singh, B., Kumar, A., Goyal, N., Singh, K., & Chhabra, R. (2023). Genome-wide identification of NAC transcription factors in Avena sativa under salinity stress. *Plant Stress*, *10*, 100276.
4. Bokolia, M., Singh, T., Goyal, N., Kumar, A., Singh, K., & Singh, B. (2025b). Genome-wide identification and characterization of GATA transcription factors in Avena sativa L. and expression profiling under salinity stress. *Journal of Applied Genetics*, 1-20.
5. Cai, X., Zhang, Y., Zhang, C., Zhang, T., Hu, T., Ye, J., ... & Ye, Z. (2013). Genome-wide analysis of plant-specific Dof transcription factor family in tomato. *Journal of Integrative Plant Biology*, *55*(6), 552-566.
6. Chattha, W. S., Atif, R. M., Iqbal, M., Shafqat, W., Farooq, M. A., & Shakeel, A. (2020). Genome-wide identification and evolution of Dof transcription factor family in cultivated and ancestral cotton species. Genomics, 112(6), 4155-4170.
7. Chen, P., Yan, M., Li, L., He, J., Zhou, S., Li, Z., ... & Guan, Q. (2020). The apple DNA-binding one zinc-finger protein MdDof54 promotes drought resistance. Horticulture Research, 7.
8. Chen, Y., & Cao, J. (2015). Comparative analysis of Dof transcription factor family in maize. Plant Molecular Biology Reporter, 33, 1245-1258.
9. Chen, Y., & Cao, J. (2015). Comparative analysis of Dof transcription factor family in maize. *Plant Molecular Biology Reporter*, *33*(5), 1245-1258.
10. Gupta, S., Kushwaha, H., Singh, V. K., Bisht, N. C., Sarangi, B. K., & Yadav, D. (2014). Genome wide in silico characterization of Dof transcription factor gene family of sugarcane and its comparative phylogenetic analysis with Arabidopsis, rice and sorghum. *Sugar Tech*, *16*(4), 372-384.
11. Gupta, S., Malviya, N., Kushwaha, H., Nasim, J., Bisht, N. C., Singh, V. K., & Yadav, D. (2015). Insights into structural and functional diversity of Dof (DNA binding with one finger) transcription factor. *Planta*, *241*, 549-562.
12. Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., ... & Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature protocols, 8(8), 1494-1512.
13. Hernando-Amado, S., González-Calle, V., Carbonero, P., & Barrero-Sicilia, C. (2012). The family of Dof transcription factors in Brachypodium distachyon: phylogenetic comparison with rice and barley Dofs and expression profiling. *BMC Plant Biology*, *12*(1), 202.
14. Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics, 31(8), 1296-1297.
15. Hua, Y., Liu, Q., Zhai, Y., Zhao, L., Zhu, J., Zhang, X., ... & Wang, D. (2023). Genome-wide analysis of the HSP20 gene family and its response to heat and drought stress in Coix (Coix lacryma-jobi L.). BMC genomics, 24(1), 478.
16. Jia, S., Yobi, A., Naldrett, M. J., Alvarez, S., Angelovici, R., Zhang, C., & Holding, D. R. (2020). Deletion of maize RDM4 suggests a role in endosperm maturation as well as vegetative and stress-responsive growth. Journal of experimental botany, 71(19), 5880-5895.

17. Khan, I., Khan, S., Zhang, Y., & Zhou, J. (2021). Genome-wide analysis and functional characterization of the Dof transcription factor family in rice (Oryza sativa L.). *Planta*, *253*, 1-14.
18. Kim, I. S., Hwang, C. W., Yang, W. S., & Kim, C. H. (2021). Multiple antioxidative and bioactive molecules of oats (*Avena sativa* L.) in human health. *Antioxidants*, *10*(9), 1454.
19. Kummerfeld, S. K., & Teichmann, S. A. (2006). DBD: a transcription factor prediction database. Nucleic acids research, 34(suppl_1), D74-D81.
20. Kummerfeld, S. K., & Teichmann, S. A. (2006). DBD: a transcription factor prediction database. Nucleic acids research, 34(suppl_1), D74-D81.
21. Kushwaha, H., Gupta, S., Singh, V. K., Rastogi, S., & Yadav, D. (2011). Genome wide identification of Dof transcription factor gene family in sorghum and its comparative phylogenetic analysis with rice and Arabidopsis. *Molecular Biology Reports*, *38*(8), 5037-5053.
22. Li, H., Dou, L., Li, W., Wang, P., Zhao, Q., Xi, R., ... & Ren, Z. (2018). Genome-wide identification and expression analysis of the Dof transcription factor gene family in Gossypium hirsutum L. Agronomy, 8(9), 186.
23. Lijavetzky, D., Carbonero, P., & Vicente-Carbajosa, J. (2003). Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis Dof gene families. *BMC evolutionary biology*, *3*(1), 17.
24. Liu, Y., Liu, N., Deng, X., Liu, D., Li, M., Cui, D., ... & Yan, Y. (2020). Genome-wide analysis of wheat DNA-binding with one finger (Dof) transcription factor genes: evolutionary characteristics and diverse abiotic stress responses. BMC genomics, 21, 1-18.
25. Liu, Y., Liu, N., Deng, X., Liu, D., Li, M., Cui, D., ... & Yan, Y. (2020). Genome-wide analysis of wheat DNA-binding with one finger (Dof) transcription factor genes: evolutionary characteristics and diverse abiotic stress responses. *BMC genomics*, *21*, 1-18.
26. Lohani, N., Babaei, S., Singh, M. B., & Bhalla, P. L. (2021). Genome-wide in silico identification and comparative analysis of Dof gene family in Brassica napus. Plants, 10(4), 709.
27. Moreno-Risueno, M. Á., Martínez, M., Vicente-Carbajosa, J., & Carbonero, P. (2007). The family of Dof transcription factors: from green unicellular algae to vascular plants. *Molecular Genetics and Genomics*, *277*(4), 379-390.
28. Nakashima, K., & Yamaguchi-Shinozaki, K. (2013). ABA signaling in stress-response and seed development. *Plant cell reports*, *32*(7), 959-970
29. Negrão, S., Schmöckel, S. M., & Tester, M. J. A. O. B. (2017). Evaluating physiological responses of plants to salinity stress. Annals of botany, 119(1), 1-11.
30. Shaw, L. M., McIntyre, C. L., Gresshoff, P. M., & Xue, G. P. (2009). Members of the Dof transcription factor family in Triticum aestivum are associated with light-mediated gene regulation. *Functional & integrative genomics*, *9*(4), 485-498.
31. Shumayla, Sharma, S., Pandey, A. K., Singh, K., & Upadhyay, S. K. (2016). Molecular characterization and global expression analysis of lectin receptor kinases in bread wheat (Triticum aestivum). PLoS One, 11(4), e0153925.
32. Tomar, S., Subba, A., Bala, M., Singh, A. K., Pareek, A., & Singla-Pareek, S. L. (2022). Genetic conservation of CBS domain containing protein family in Oryza species and their association with abiotic stress responses. International Journal of Molecular Sciences, 23(3), 1687.

33. Vicente-Carbajosa, J., Moose, S. P., Parsons, R. L., & Schmidt, R. J. (1997). A maize zinc-finger protein binds the prolamin box in zein gene promoters and interacts with the basic leucine zipper transcriptional activator Opaque2. *Proceedings of the National Academy of Sciences*, *94*(14), 7685-7690.
34. Walla, A., Wilma van Esse, G., Kirschner, G. K., Guo, G., Brünje, A., Finkemeier, I., ... & von Korff, M. (2020). An acyl-CoA N-acyltransferase regulates meristem phase change and plant architecture in barley. Plant Physiology, 183(3), 1088-1109.
35. Wei, Q., Wang, W., Hu, T., Hu, H., Mao, W., Zhu, Q., & Bao, C. (2018). Genome-wide identification and characterization of Dof transcription factors in eggplant (Solanum melongena L.). *PeerJ*, *6*, e4481.
36. Wen, C. L., Cheng, Q., Zhao, L., Mao, A., Yang, J., Yu, S., ... & Xu, Y. (2016). Identification and characterisation of Dof transcription factors in the cucumber genome. *Scientific reports*, *6*(1), 23072.
37. Wu, B., Hu, Y., Huo, P., Zhang, Q., Chen, X., & Zhang, Z. (2017). Transcriptome analysis of hexaploid hulless oat in response to salinity stress. PloS one, 12(2), e0171451.
38. Wu, Z., Cheng, J., Cui, J., Xu, X., Liang, G., Luo, X., ... & Qin, C. (2016). Genome-wide identification and expression profile of Dof transcription factor gene family in pepper (Capsicum annuum L.). *Frontiers in Plant Science*, *7*, 574.
39. Xiao, Q., Liu, T., Ling, M., Ma, Q., Cao, W., Xing, F., ... & Liu, Z. (2022). Genome-wide identification of Dof gene family and the mechanism dissection of SbDof21 regulating starch biosynthesis in sorghum. International Journal of Molecular Sciences, 23(20), 12152.
40. Yanagisawa, S. (2002). The Dof family of plant transcription factors. *Trends in plant science*, *7*(12), 555-560.
41. Yanagisawa, S., & Schmidt, R. J. (1999). Diversity and similarity among recognition sequences of Dof transcription factors. *The Plant Journal*, *17*(2), 209-214.
42. Yang, J., & Zhang, Y. (2015). Protein structure and function prediction using I-TASSER. Current protocols in bioinformatics, 52(1), 5-8.
43. Zhang, Z., Yuan, L., Liu, X., Chen, X., & Wang, X. (2018). Evolution analysis of Dof transcription factor family and their expression in response to multiple abiotic stresses in Malus domestica. Gene, 639, 137-148.
44. Zou, X., & Sun, H. (2023). Dof transcription factors: Specific regulators of plant biological processes. *Frontiers in Plant Science*, *14*, 1044918.

Figures

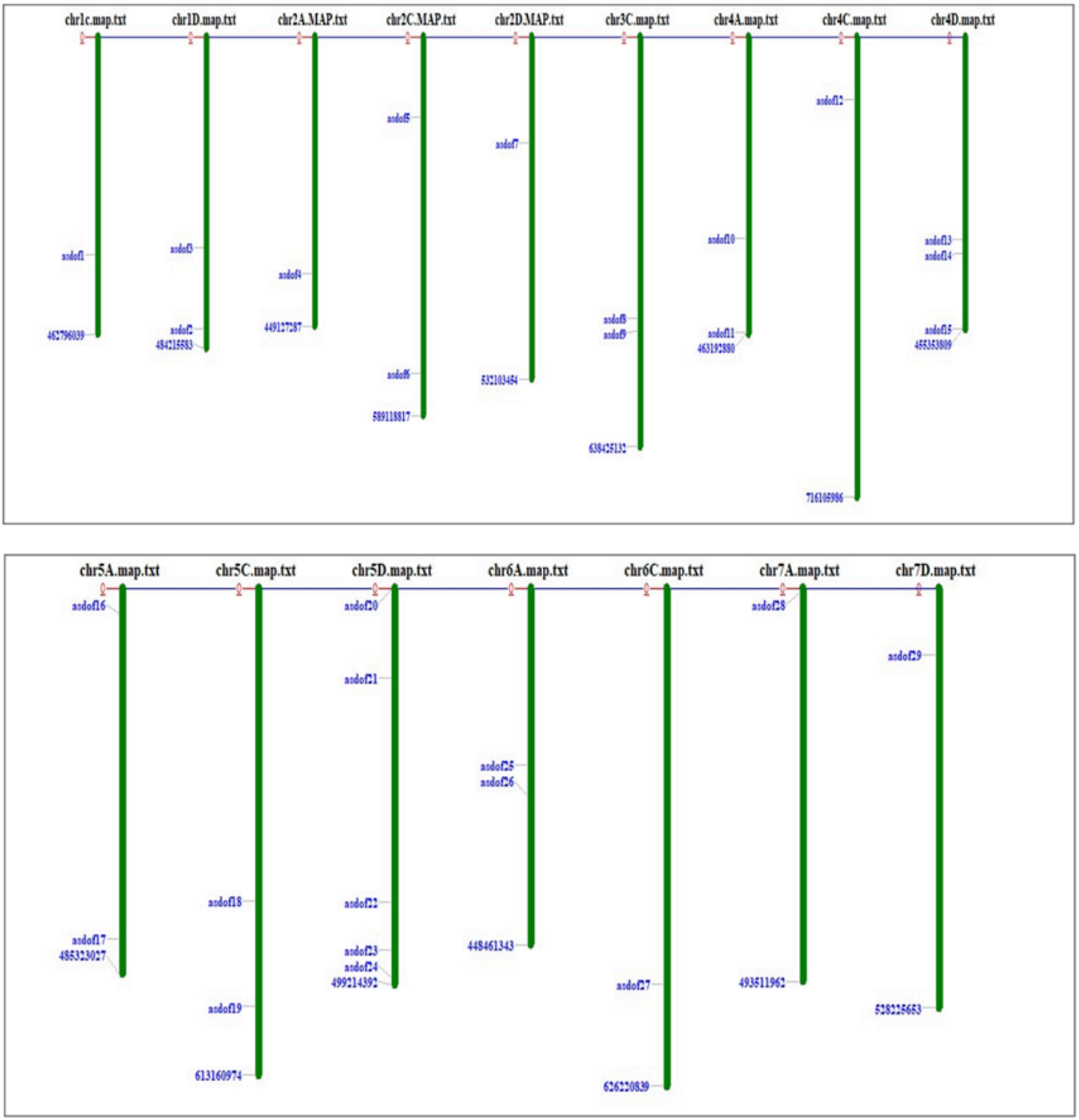


Figure 1

Random distribution of *AsDof* genes on the *Avena sativa* chromosomes. The total length of chromosome is given at the end of chromosome.

Figure 2

The sketchmap of the 32 Dof genes in *Avena sativa* which shows the no. of exons and introns present in the Dof genes. The exons are represented by purple bars and the introns are by black curved lines.

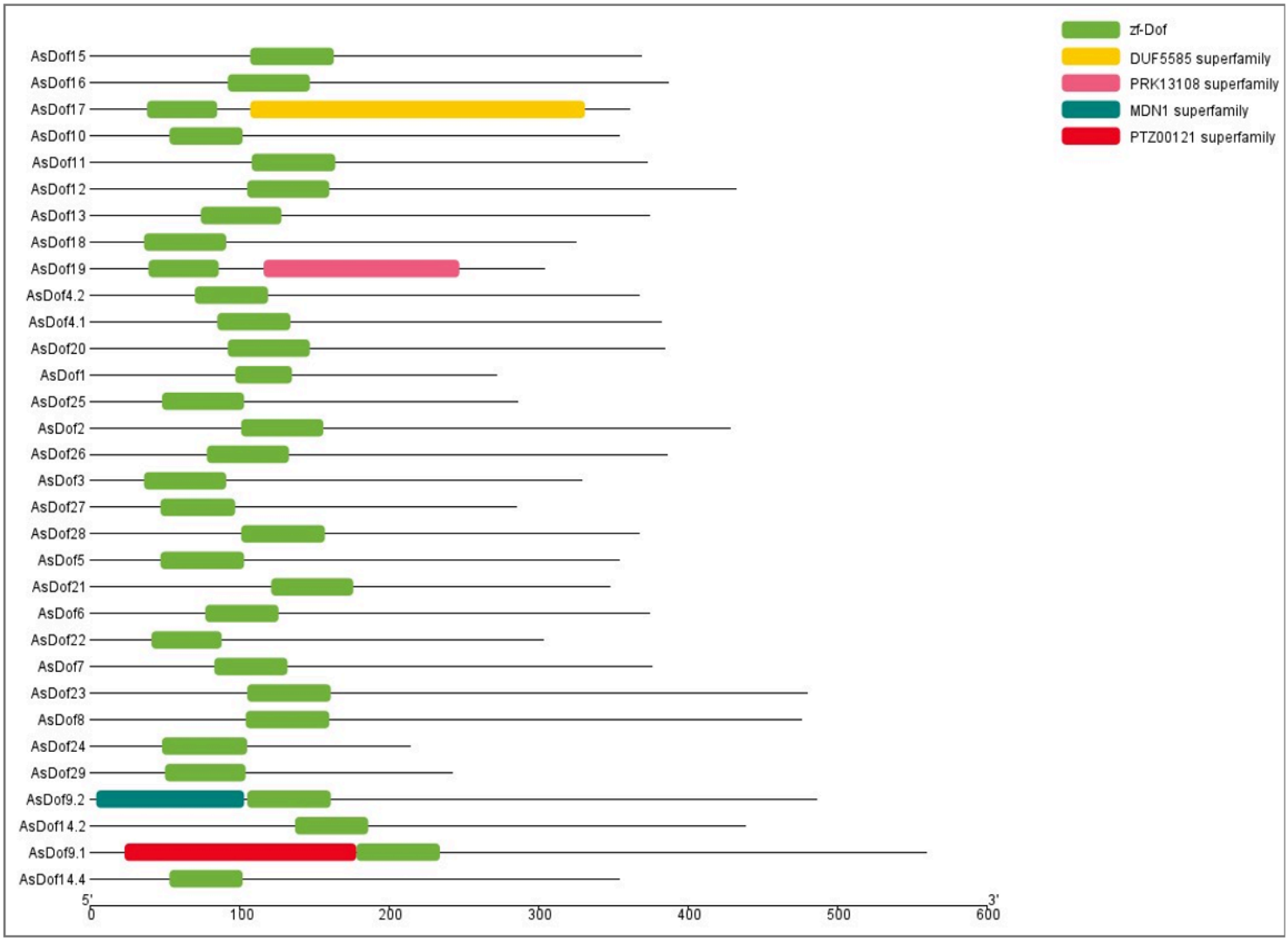
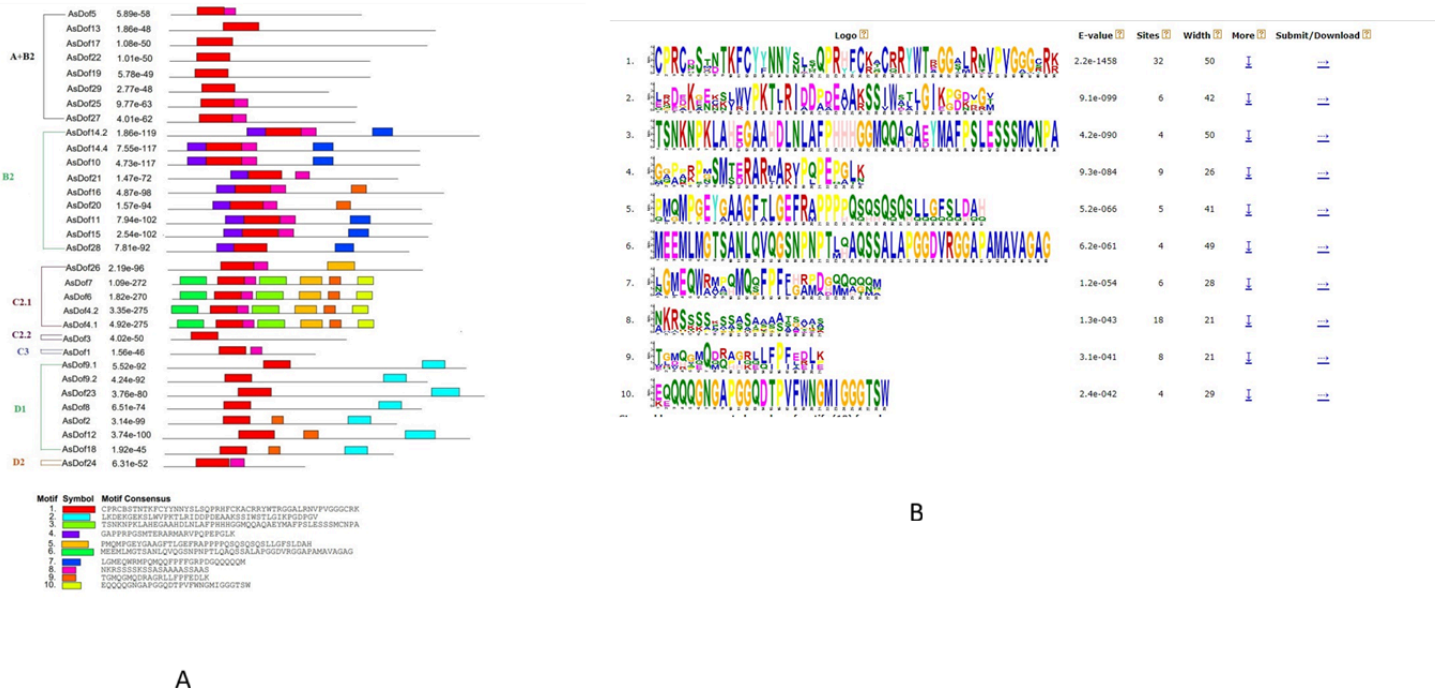


Figure 3

Batch CD search results showing the Dof domain along with four other superfamily genes which are present on *AsDof 9.1, AsDof9.2, AsDof 17, AsDof19*.

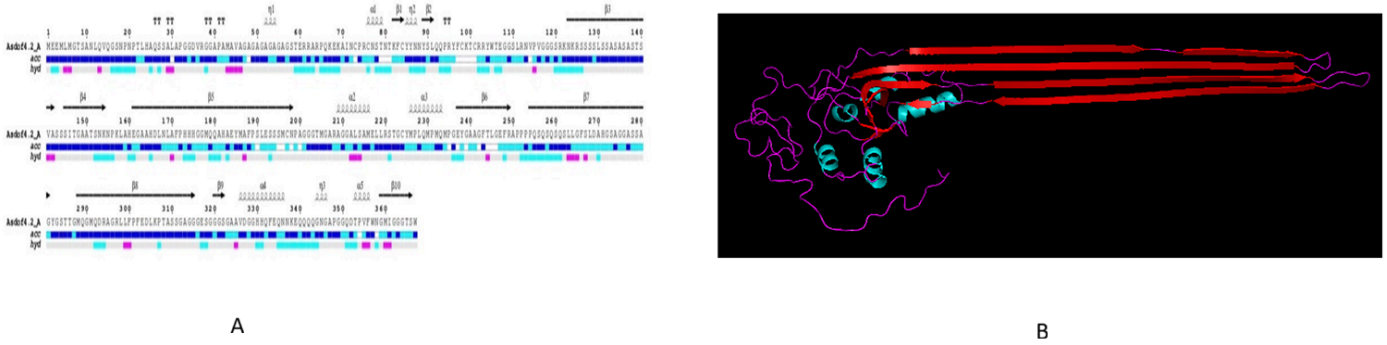


A B

Figure 4

(A). Pictorial representation of the *AsDof* genes highly conserved motifs. The position and different motifs were indicated by different coloured boxes at their particular locations.

(B). The sequences of the 10 motifs in *AsDof* genes.



A B

Figure 5

(A). The Endscript result showing the amino acid sequence and the positions of the alpha helix and beta pleated sheets and their number.

(B). *AsDof 4* protein 3D structure which describes about the tertiary structure which shows the alpha helix and the beta pleated sheets of the particular protein.

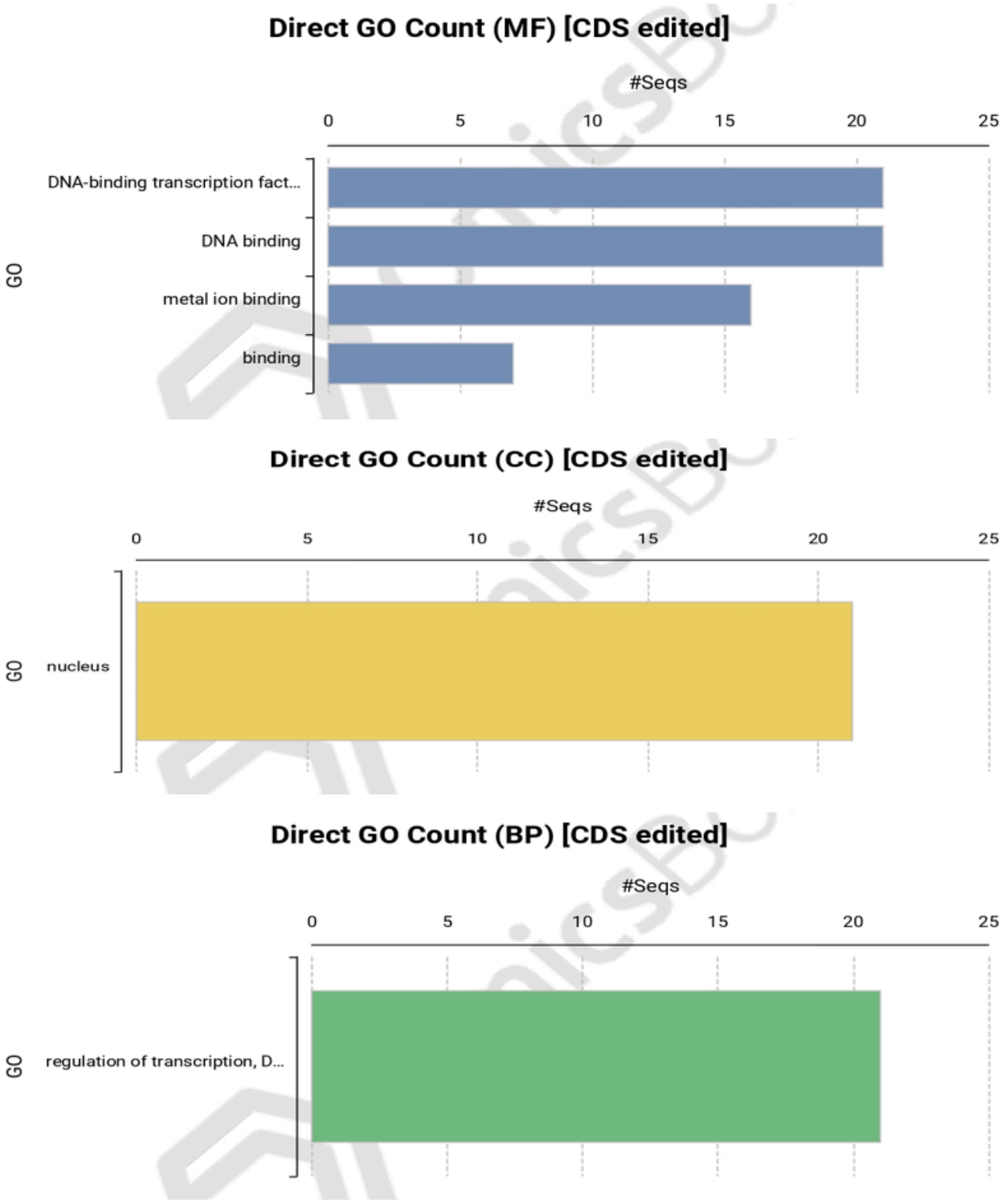


Figure 6

The gene ontology analysis of *AsDof* genes in *Avena sativa* represents the 32 Dof genes in the plant. The violet colour bars show the analysis of the molecular function (MF), the yellow bar represents the cellular component (CC), and similarly green bar represents the biological process (BP).

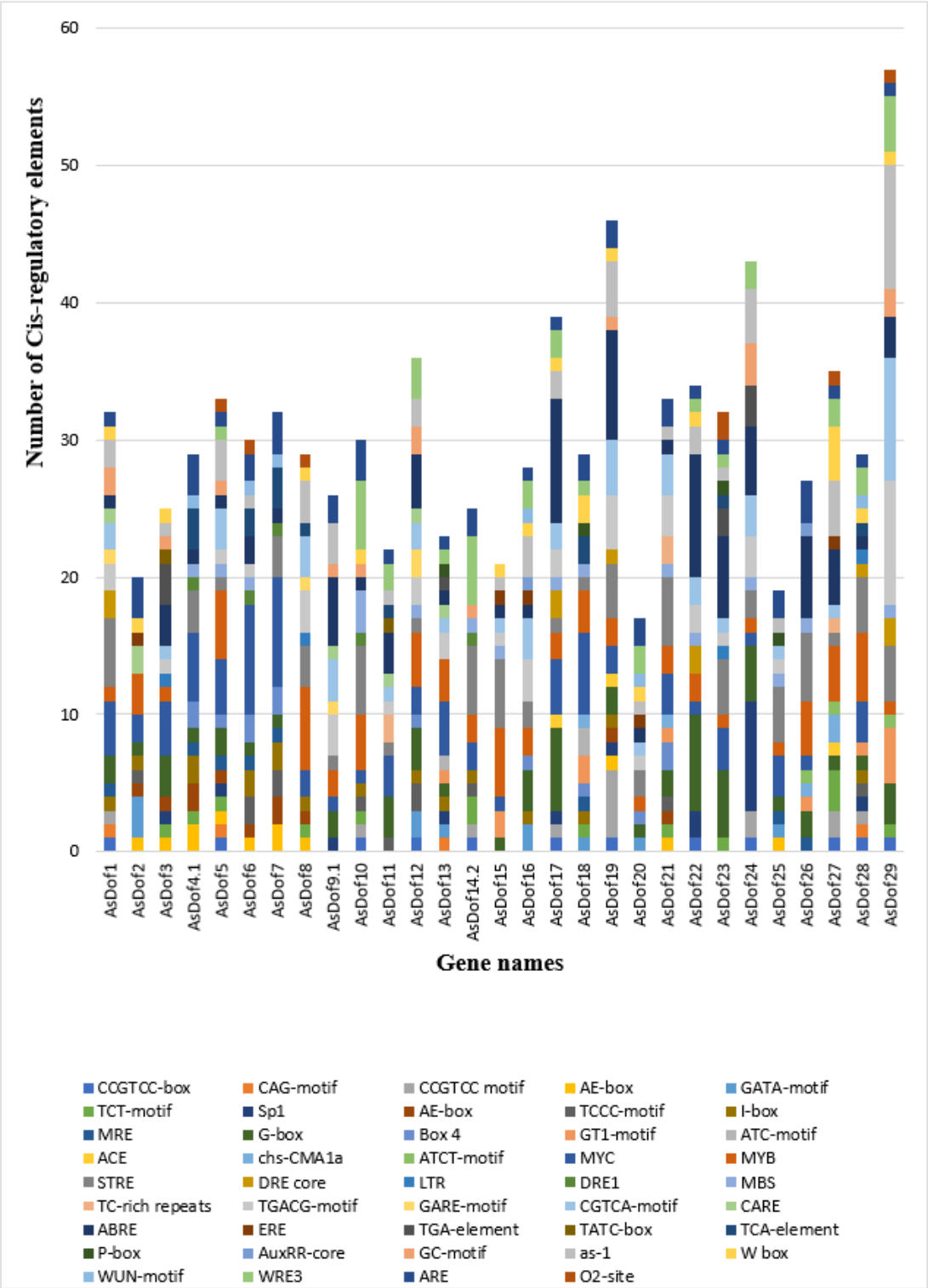


Figure 7

The graphical chart representation of the cis regulatory elements presents in the *AsDof*genes in *Avena sativa* which are extracted from the plantTFDB software.

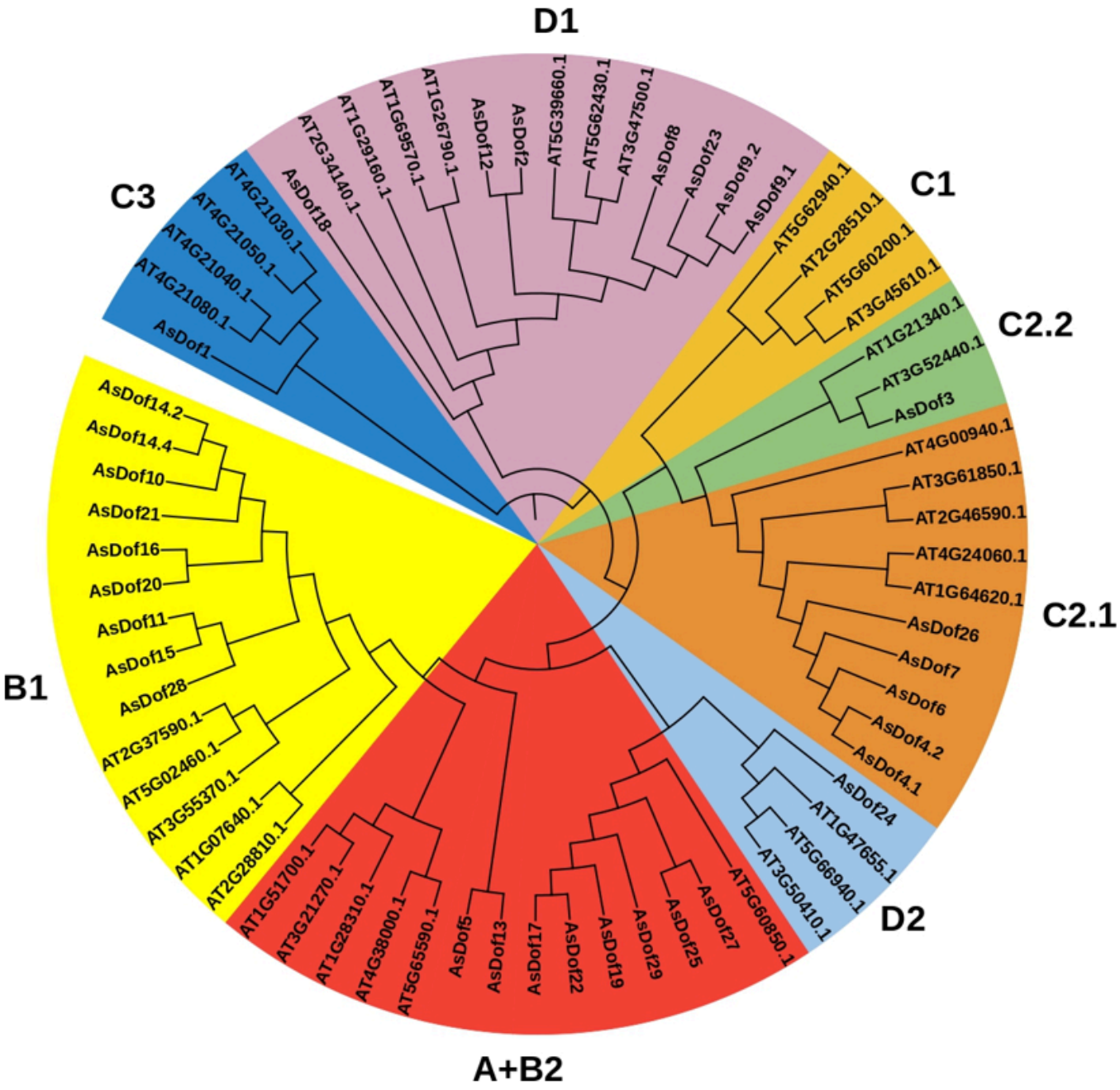


Figure 8

The MEGA XI constructed and iTOL edited phylogenetic tree (by NJ method with 1000 bootstrap value) which shows the clear clads and nodes of each gene family of the 32 *AsDof*genes along with the *Arabidopsis*genes and their 8-family classification.

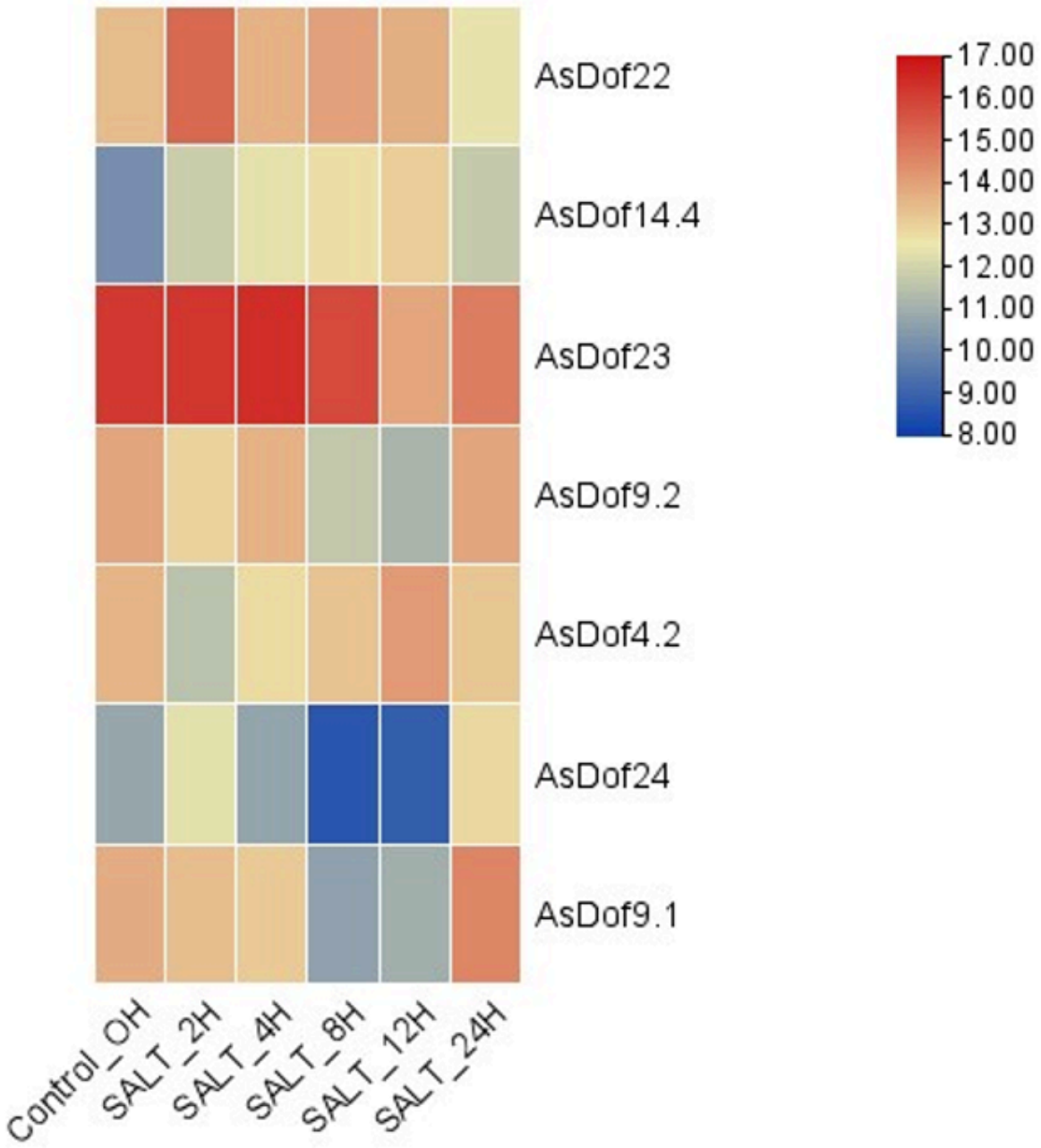


Figure 9

The heatmap which depicts the 7 differentially expressed *AsDof* genes. Different colour bars represent the different relative expression of the gene. The colour blue represents the downregulating expression of the gene and the red colour represents the upregulation of the genes.

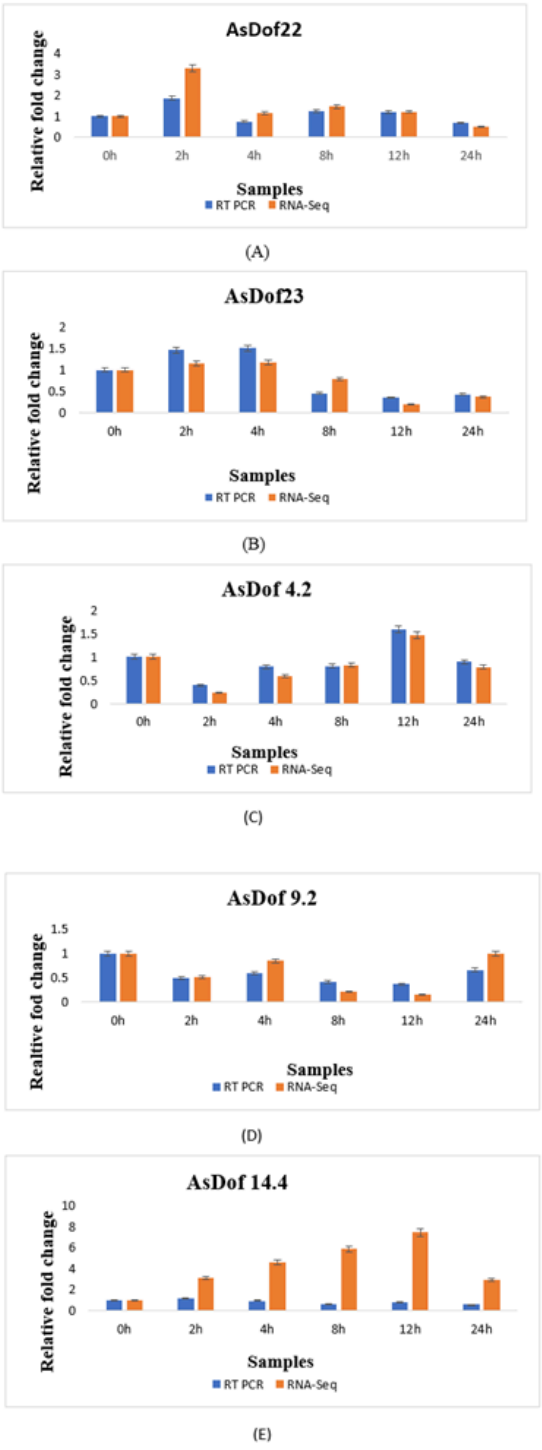


Figure 10

The expression analysis of the *AsDof22* (A), *AsDof23* (B), *AsDof4.2*(C), *AsDof9.2* (D), *AsDof14.4* (E) genes in *Avena sativa* which are extracted under salinity stress and analysed by qRT-PCR. The relative gene expression measured using 2-DDCt methodology. The seed samples were treated at different time intervals such as 0h, 2h ,4h, 8h, 12h, 24h respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS11.xlsx
- TableS21.xlsx
- TableS31.xlsx
- TableS41.xlsx