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*Os*DOF18, A DOF Transcription Factor from Rice Confers Abiotic Stress Tolerance in *Escherichia coli*

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Abstract. DNA binding with one finger proteins (DOF) play vital role in many cellular including biotic and abiotic stresses. In present study, *Os*DOF18, a member of DOF gene family from *Oryza sativa* was cloned into GST expression vector (pGEX4T-1) and sequenced. The sequence was subjected to *in silico* characterisation including similarity search, multiple sequence alignment followed by phylogenetic study. The three dimensional structure was predicted by I-TASSER server followed by authentication using PROCHECK and QMEAN tools. Analysis of *Os*DOF18 by RT-qPCR confirmed the association of *Os*DOF18 with abiotic stresses including salinity and drought. DNA binding domain containing region was cloned and over-expressed in *Escherichia coli* for stress analysis. *Os*DOF18 protein improved the *E. coli* survivability against salinity and drought stresses. The results suggested *Os*DOF18 as a stress-related gene in rice that may be used in generating stress tolerant plants.

Keywords: abiotic stress, *Oryza sativa*, *Os*DOF18, *Escherichia coli*, RT-qPCR

Introduction

DNA-binding with one finger proteins (DOF) belongs to a group of plant-specific transcription factors that are characterised by the presence of conserved CX2CX21CX2C zinc finger of 50-52 amino acids containing DNA-binding DOF domain at the N-terminus. This DOF domain associates with a basic region and interacts with T/AAAAG core sequence in the promoters of target genes (Umemura *et al.,* 2004; Yanagisawa, 2002). In comparison, the C-terminal DOF region is highly variable involved in protein-protein interaction and other regulatory activities. For example, AtDOF4.2, AtDOF4.4 and ZmDOF1 contain threonine, methionine and aspartate motifs, located in the C-terminal, are responsible for activation of target gene expression. Subsequently, DOF transcription factors display a complex modular structure by forming homo and heterodimeric complexes, involve in multiple controlling processes, by acting as transcriptional activator or repressor of target genes (Yamamoto *et al.*, 2006). The regulatory action facilitated by DOF proteins is bifunctional which means it can interact with DNA as well as also with other regulatory proteins including basic leucine zippers (bZIPs) and myeloblastosis oncogenes (MYBs) (Diaz *et al.*, 2002; Washio, 2001).

DOF transcription factors have been appeared to be broadly distributed in the plant domain, for instance DOF proteins have been identified in unicellular algae, moss, and vascular plants. Since the first DOF protein identified from maize, several DOF transcription factors have been found in other plants including *Arabidopsis*, rice, poplar, barley, wheat, maize, Chinese cabbage, tomato, pepper, banana and potato (Feng *et al.,* 2016; Wu *et al.,* 2016; Gupta *et al.*, 2015; Ma *et al.*, 2015; Venkatesh and Park, 2015 Cai *et al.*, 2013; Noguero *et al.*, 2013; Yanagisawa, 2002) after the availability of partial or complete information about genome sequences. Efforts have been made to envisage the DOF genes based on *in silico* studies. In *Arabidopsis*, 36 DOF genes have been identified, 31 in wheat, 54 in maize and 30 genes have been identified in the rice genome (Shaw *et al*., 2009; Lijavetzky *et al*., 2003). Furthermore, genetic and molecular studies have advocated that DOF proteins are implicated in the regulation of biological processes exclusive to plants, for instance, light-responsiveness, photoperiodic flowering, tissue differentiation, dormancy, seed germination and maturation, metabolic regulation and nitrogen assimilation and phytochrome and phytohormone signalling (Gupta *et al.*, 2015; Noguero *et al.*, 2013; Yanagisawa *et al*., 2004). Freshly, DOF proteins were described to be involved in biotic and abiotic stress responses, perhaps via the stimulation of various stress-responsive genes (Gupta *et al.*, 2015; Sasaki *et al.*, 2015; Corrales *et al.*, 2014).

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The cultivated crops particularly rice, wheat, maize and barley inhabit significant place are grown over a large area worldwide and more than half of the world population feeds on these crops to fulfill their energy demands. Although quite a few DOF family members have been functionally characterised in rice, for instance, *Os*DOF3 was found to be involved as a pyrimidine box binding factor in the germinated aleurone (Washio, 2003), *Os*DOF12 was stated to control heading date (Li *et al*., 2009) and O*s*DOF25 is reported in carbon and nitrogen metabolism in *Arabidopsis* (Santos *et al*., 2012) and acts as a transcriptional activator of C4 photosynthesis gene, *Os*C4PPDK in rice (Zhang *et al.,* 2015). The functions of most of the rice DOF proteins remain unknown especially in abiotic and biotic stresses. The present study involves the cloning and *in silico* characterisation of *Os*DOF18 by carrying out phylogenetic and motif scan analysis followed by secondary and tertiary structure predictions. Expression pattern was investigated by RT-qPCR and effect of over-expression of *Os*DOF18 gene was studied on *E. coli* growth under different abiotic stresses.

Materials and Methods

***In Silico* analysis.** The *Os*DOF18 gene sequence was used as a query in BLAST with NCBI database for homology search. InterProScan and MOTIFSCAN tools were used to locate and analyze conserve DNA binding domain. CLUSTALW online tool was used to align the protein sequences showing similarity with *Os*DOF18 followed by construction of phylogenetic tree using Neighbor-Joining method (Kumar *et al.,* 2016) by employing MEGA7 software. Expasy's ProtParam Proteomic server was used for computing basic physiochemical properties of *Os*DOF18. Primary sequence of *Os*DOF18 was analysed by DISOPRED (http://bioinf.cs.ucl.ac.uk/psipred/?disopred=1) to measure the degree of intrinsic disorder.

Prediction of *Os*DOF18 three dimensional structure. An attempt was made to predict the structure of *Os*DOF18 using I-TASSER (Iterative Threading ASSEmbly Refinement) web server (Yang *et al.,* 2015). For energy minimization, the rough model was then subjected to GROMOS96 43B1 executed using Swiss-PDBViewer version 4.0.1. To validate the backbone conformation of the predicted structure, the Phi/Psi Ramachandran plot was obtained through PROCHECK server (Laskowski *et al.,* 2001). Moreover, the quality of DOF18 protein model was evaluated using Qualitative Model Energy Analysis (QMEAN) server (http:// swissmodel. expasy.org/qmean/cgi/index.cgi) which tells about the degree of nativeness of predicted model. The PDB sumserver (https://www.ebi.ac.uk/ thornton-srv/databases/ pdbsum/Generate.html) was used for structural motif analysis of modelled DOF protein. The final model of DOF protein was then subjected to raptorX tool (http://raptorx.uchicago.edu) for identifying the possible binding sites. Metal detector V1.0 (http:// metaldetector. dsi.unifi.it/) was used for metal detection studies.

Plant materials and stress treatments. *Oryza sativa* cv. KS282 obtained from Rice Program, Crop Science Institute, National Agricultural Research Centre (NARC) Islamabad Pakistan was selected for expression analysis of *Os*DOF18 gene. Seeds were germinated on half strength MS medium and kept at 25 °C. Ten day old seedlings were subjected to different abiotic stresses. For drought, seedlings were placed on aluminium foil till visible leaf rolling and seedlings were then transferred to 4 °C for 48 h for cold treatment. After 48 h of chilling treatment, seedlings were moved to the control environment and samples were collected after 24 h. Plant roots were immersed in 200 mM NaCl solution for 3 h for salt stress. Seedlings were subjected to 45 °C for 6 h heat stress. For wounding stress, rice leaves were cut into pieces and then left in water for 6 h at room temperature. After respective treatments, samples were collected separately, frozen in liquid nitrogen and preserved at -80 ºC till analysis. Untreated plants were used as control. Experiments were performed in replicates to confirm precision and reproducibility.

RNA isolation and RT-qPCR. Total RNA was isolated from different stressed and control plants using the RNeasy Plant Mini Kit (Qiagen). RNA purity and integrity was ensured by running 1% agarose gel. Primers were designed using PrimerBLAST tool. Quantitative real time PCR was performed using Brilliant II SYBR Green RT-qPCR master mix Kit (Agilent Technologies). Primers used are mentioned in Supplementary Table 1. Samples were assayed in a 10 µL reaction mixture containing 5 µL of 2× reaction mix, 0.5 µL (5µmol) of each forward and reverse primers, 2 µL of RNA (100 ng), 0.1 µL of reverse transcriptase and 1.8 µL of nuclease free water. No template and no primer controls were also included. The thermal profile consists of 30 min of reverse transcription at 50 °C one cycle and 10 min of polymerase activation at 95 °C, followed by 40 cycles of PCR at

95 °C for 30 sec, 53 °C for 1 min and 72 °C for 30 sec. Melting curve analysis (60 to 95 °C after 40 cycles) and agarose gel electrophoresis were performed to examine the amplification specificity. The relative change in transcript level was calculated using 2-CT method (Schmittgen and Livak, 2008) with actin as internal standard to determine relative expression levels. RT-PCR assays were repeated at least twice and each repetition had three replicates.

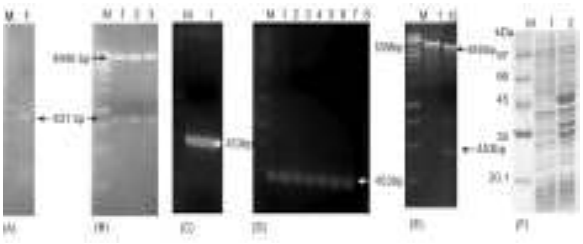
Cloning and expression in *E. coli.* *Oryza sativa* cDNA clone J065152E11 with assigned accession number AK241364.1 was acquired from NIAS Databank of Japan and primers were designed specific for full length and DNA binding domain containing region of *Os*DOF18 containing BamHI and XhoI sites to aid in the cloning (Supplementary Table 1). PCR amplification was performed using 25 ng of cDNA clone with 66 °C and 63 °C annealing temperature for full length and DNA binding domain containing region, respectively. The amplified product was analysed on agarose gel, amplicon was gel eluted using Gene JET Gel Extraction Kit (Thermo Scientific), and cloned in GST expression vector (pGEX4T-1). Putative cloned DOF18 gene was commercially sequenced. The putative recombinant plasmids were confirmed by PCR amplification, restriction digestion and commercial sequencing. The pGEX4T/*Os*DOF18 expression was induced with 1.0 mM isopropyl b-D-thiogalactoside (IPTG) for 6 h at 37 °C and analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Assays for abiotic stress tolerance in *E. coli.* Spot assay was performed to establish the function of *Os*DOF18 gene in *E. coli* cells. BL21 (DE3) cells were subjected to transformation pGEX4T1-*Os*DOF18 and control plasmid (pGEX4T-1). Cells were allowed to grow in LB broth till OD600 reached 0.6. Afterward, expression of recombinant protein was induced by 1 mM IPTG and cells were incubated for further 4 h at 37 °C. OD_{600} was measured and cultures were diluted to OD_{600} 1. Then cells were diluted to 50-fold, 100-fold and 200-fold. Then diluted samples were spotted on IPTG agar plates (100 µg/mL ampicillin and 1.0 mM IPTG) supplemented with 400, 500, and 600 mM concentration gradient of NaCl for salt stress and 500 mM, 800 mM and 1 M mannitol for drought. For heat stress, 1 mL of each sample was kept at 50 °C and 100 µL of each sample was taken at different periods of 1, 2 and 3 h, successively. Samples were diluted by 50-fold, 100-fold and 200-fold and 10 µL of each sample was spotted onto IPTG LB agar plates. For cold stress, samples were placed at -80 °C for 24 h. Then samples were allowed to thaw at 35 °C for 1 h and took 100 µL at different periods of 2, 4, 6 and 8 h, successively. Samples were diluted by 50-fold, 100-fold and 200-fold and 10 µL of each sample was spotted onto IPTG LB agar plates. All these plates were incubated overnight at 37 °C and photographed.

Results and Discussion

Plant growth and survival are greatly affected by abiotic stresses. However, plants have the ability to endure the stress condition by cascade of events at cellular and molecular level. A number of genes are involved in providing adaptation to plants against abiotic stresses for instance, genes involved in ion homeostasis, osmoprotectants, and free redical-scavenging and genes encoding transcription factors. It is important to characterise the unknown proteins to decipher their role in providing tolerance to plants. In present study, OsDOF18 cDNA fragment of entire open reading frame (ORF) and DNA binding domain containing region were cloned and characterised.

Cloning and sequence analysis of *Os*DOF18 gene. The *Os*DOF18 gene was amplified by PCR from cDNA clone using specific primer pair. The eluted PCR product was inserted into pGEX4T-1 vector in between BamHI and XhoI restriction sites. Then, cloned products were subjected to transformation in *E. coli* cells, and cloned gene was confirmed by digesting the plasmid with suitable restriction enzymes (Fig. 1) and commercial



A=PCR amplification of *Os*DOF18 full length; B=Restriction digestion of pGEX-*Os*DOF18 (Lane M: 1 Kb DNA ladder, Lane 1-3: Restricted plasmids; C=PCR amplification of *Os*DOF18 DNA binding domain containing region; D=Colony PCR (Lane M: 1 Kb DNA ladder, Lane 1-7: Colonies 1-7, Lane 8: -ve control); E=Restriction digestion of pGEX-*Os*DOF18 (Lane 1: Uncut plasmid, Lane 2: Restricted plasmid); F=Over-expression of pGEX-*Os*DOF18 in BL21 cells (Lane 1= uninduced sample; Lane 2: Induced sample).

Fig. 1(A-F). Cloning and over-expression of *Os*DOF18 full length and DNA binding domain.

sequencing. Sequence analysis revealed that the complete open reading frame of OsDOF18 is 831 bp and predicted protein consists of 276 amino acids having molecular weight of 28.9 kDa and the isoelectric point (pI) of 6.75. It was observed that it is intronless gene which is a key feature of prokaryotic genes. Although there are many intronless genes in eukaryotes and to study those genes might help in understanding the evolutionary array of related genes and genomes. Research also revealed the conservation of many intronless genes in archaea, bacteria, fungi, plants and other eukaryotes during the process of evolution. The translated protein sequence of *Os*DOF18 gene was then subjected to BLAST search for finding homology with already available sequences in NCBI databases. Search showed similarity with DOF protein like sequences belonging to different species including maximum similarity with ZmDOF protein (57%), *Si*MNBIA-like (57%) and SbDOF22 (48%). The *Os*DOF18 ortholog sequences were retrieved and multiple sequence alignment was carried out using CLUSTALW tool followed by phylogenetic analysis which showed the clustering of *Os*DOF18 with *Zea mays* and *Sorghum bicolor* whereas other DOF proteins were clustered into different groups (Fig. 2).

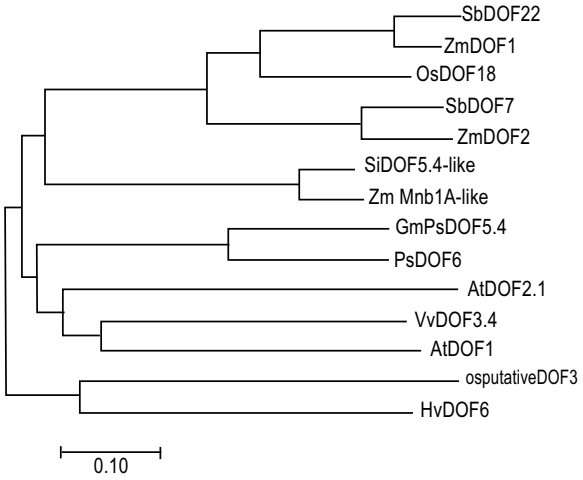


Fig. 2. The phylogenetic analysis of DOF proteins from rice and other plant species by MEGA 7 from CLUSTALW alignments. The neighbor-joining method was used to construct the tree with p-distance.

Tertiary structure prediction. Although protein sequences of a number of DOF proteins is available in protein sequence database, but the information about the 3D structure of DOF proteins is missing in protein database. The absence of 3D structure has encouraged us to build the 3D structure of *Os*DOF18 protein. Information about molecular function and active site residues can be gathered from 3D structure. 3D structure was acquired by multiple threading from online accessible I-TASSER server utilizing diverse threading templates as given in Table 1. C-score of the I-TASSER models was used to deduce the final result from the consensus of top structural matches. C-score is calculated on the basis of threading template alignment and the conjunction parameters of the structure assembly imitations and describe about the quality of predicted models. Template modeling score (TM-score) was used to evaluate the structural similarity between templates and models and the sequence identity was determined in the structurally aligned region.

I-TASSER server predicted five structural models and on the basis of maximum C-score and maximum number of decoys for evaluation and verifications, most appropriate predicted structure was chosen. The selected model has predicted TM score of 0.37 ± 0.13 and RMSD (root mean square deviation) score of 13.3±4.1Å and found in correct topology C-score, TM-score, and RMSD value. Swiss-pdbViewer was then used for stabilizing their stereochemical properties by energy minimization. Kushwaha *et al.* (2013) also predicted the structure of four DOF proteins from Sorghum in the same way.

Table 1. Primers used in present study

Primers name	Primers sequences	Product size (bp)	Accession numbers
*Os*DOF18(1-277)-For	5′-CGCGGATCCATGCAGGAGCAGCAGCCG-3′(BamHI)	831	NM_001068645.2
*Os*DOF18(1-277)-Rev	5′-CCGCTCGAGTCATGGGAGGTTGAGGAACAC-3′(XhoI)		
*Os*DOF18(1-148)-For	5′-CGCGGATCCATGCAGGAGCAGCAGC-3′(BamHI)	444	NM_001068645.2
*Os*DOF18(1-148)-Rev	5′-CCGCTCGAGTCACTCGGGAGTCGTGACG-3′(Xho1)		
RTActin-F	5′-GAAGATCACTGCCTTGCTCC-3′	226	X16280.1
RTActin-R	5′-CGATAACAGCTCCTCTTGGC-3′		
RTDOF18-F	5′-AAGACGACGACTTCCACAAC-3′	182	NM_001068645
RTDOF18-R	5′-AGACTCTTGGTGATGGACGG-3′		

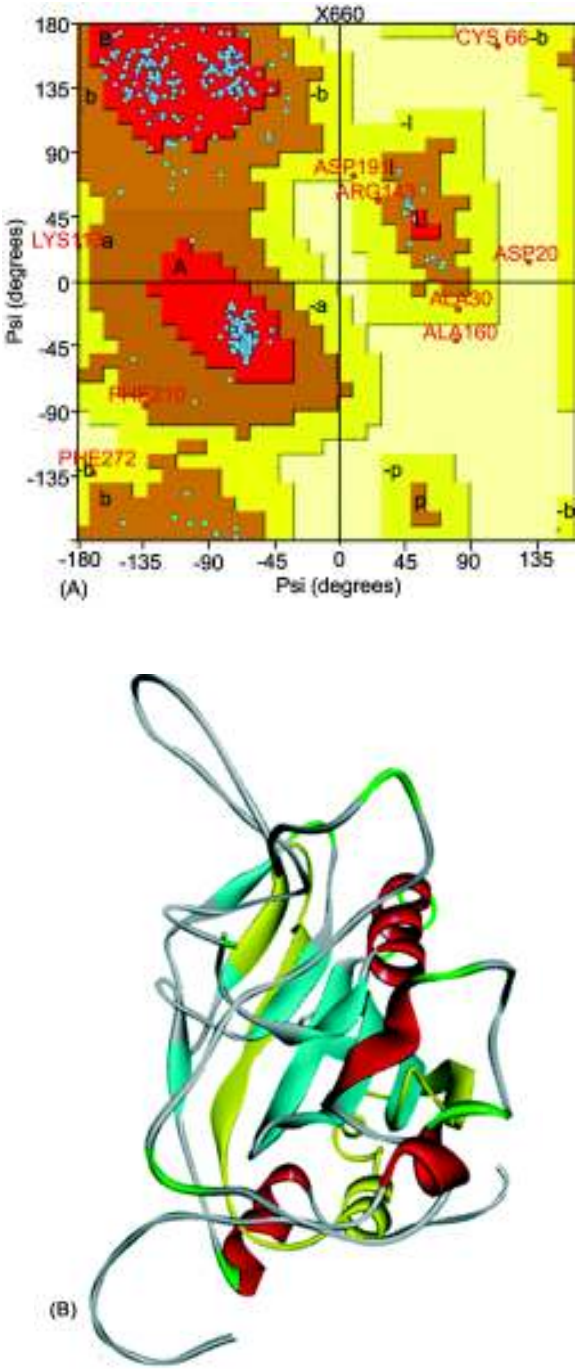
Validation of the predicted 3D structure. PDB files were subjected to PDBsum and PROCHECK server for predicted model authentication. Ramachandran plot and % of the residues in the core region, allowed regions and in the disallowed region are shown in Fig. 3A. Maximum likelihood of finding residues of protein (>90%) in the core regions proposes better stereochemical quality. PROCHECK study revealed the low percentage of residues having phi/psi angles in the disallowed region suggesting the acceptability of Ramachandran plots. The percentage of residues in the allowed/core region were found to be 98.7% while residues in disallowed regions were found to be 1.3% as shown in Table 2. Additionally, the quality of 3D structure was estimated using QMEAN server. The QMEAN Z score were found to 0.262 (Z-score: -5.76). The presence of significant QMEAN Z score suggested the predicted model quality to be acceptable. The final modeled structure is shown in Fig. 3B.

Structural motif, active site and metal binding site analysis. The PDB file of putative *Os*DOF18 protein was subjected to PDBsum server for structural motif analysis. *Os*DOF18 protein contains more frequency of α-helix, β-sheets, turns and coil. The DNA binding domain region primarily contains turns but sheets, helix and coil are also present. The predicted structure is shown in Fig. 4. Motif scan analysis showed the presence of amino acid-rich profiles along with zinc finger DOF type profile. Alanine-rich (28-57), serine-rich (114-125) and threonine-rich (145-198) profiles were found in motif scan output. The alanine-rich and serine rich profiles mainly consist of β-turns and sheets while threonine-rich profile consists of helix, β-turns and sheets.

The binding site of targeted protein was predicted by raptor X tool and shown in 3D structure of DOF

Table 2. Ramachandran plot statistics

	No. of residues	Percentage
Most favoured regions [A, B, L]	178	76.7%
Additional allowed regions [a, b, l, p]	45	19.4%
Generously allowed regions [~a, ~b, ~l, ~p]	6	2.6%
Disallowed regions [XX]	3	1.3%
Non-glycine and non-proline residues	232	100 %
Glycine residues	14	
Proline residues	29	
Total number of residues	276	



A) Ramachandran plot of *Os*DOF18 protein. The plot calculations on the 3D models were computed with the PROCHECK server. Most favored regions are colored red, additional allowed, generously allowed, and disallowed regions are indicated as yellow, light yellow, and white fields, respectively. B) The final 3D structure of *Os*DOF18 protein generated by Discovery studio ver3.0. The α-helix is represented by red cylinders, β-sheet by cyan arrows, coils by green, and loops by gray lines. DOF domain is marked with yellow colour (Colour figure online)

Fig. 3(A-B). Prediction and validation of 3D structure of *Os*DOF18 protein.

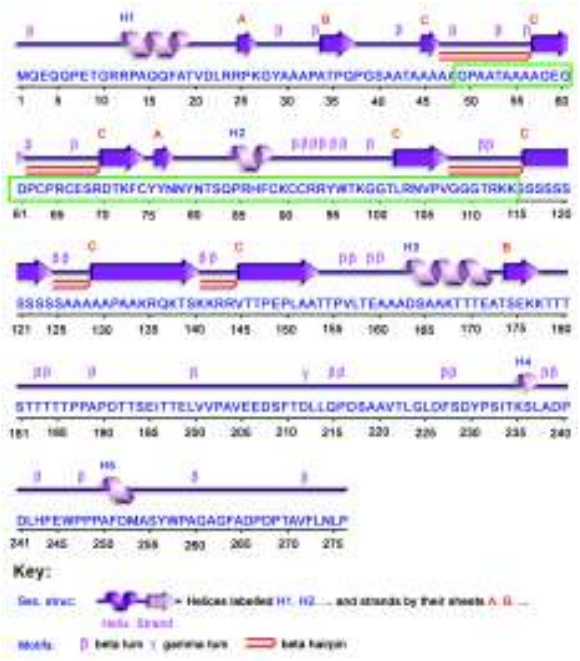


Fig 4. Secondary structure of *Os*DOF18 protein predicted by PDBsum server. The DOF domain region is highlighted in green box.

domain 1 in Fig. 5. Use of metal detector tool showed that cysteine residues were actively involved in coordination with metal on the basis of greater metal score and lesser free score value with presence of disulphide bridges. The DOF domain was truly functioning as Cys2/Cys2-type zinc finger proteins.

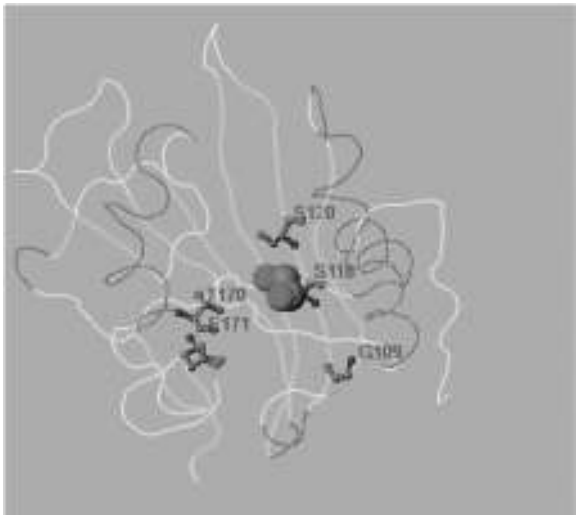


Fig. 5. Identified binding sites of DOF domain 3D structure by raptorX tool.

Intrinsic disorder in *Os*DOF18. A large degree of intrinsic disorder (ID) is observed in eukaryotes as bioinformatic analysis revealed the presence of 30 or more disordered residues in 30-60% of proteins. Plant transcription factors have significant degrees of intrinsic disorder regions (IDRs) which play vital role in interaction with DNA and other regulatory proteins (Kragelund *et al.,* 2012). The ID prediction profile showed that *Os*DOF18 has high degree of IDR in both N and C termini. A region at the N-terminus represents putative protein-protein interaction region. Degree of IDs is 65% in *Os*DOF18 including a region of high ID of 122 amino acids at C terminus adjacent to DNA binding domain. Only the region of DNA binding domain containing zinc finger is structured (Fig. 6A&B).

Expression analysis of *Os*DOF18 by RT-qPCR. RT-qPCR assay was used to investigate *Os*DOF18 expression in rice under drought, cold, heat, salinity and wounding/mechanical stress. Analyses of available microarray data demonstrated that expression of *Os*DOF18 gene is regulated by various abiotic stress

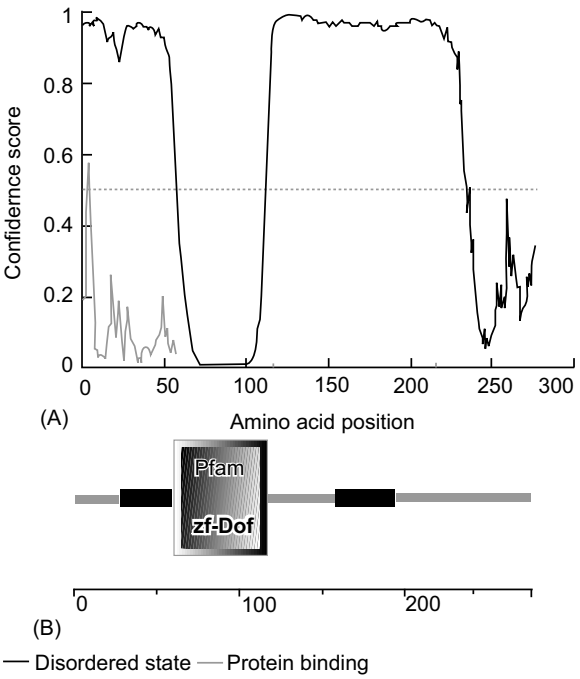
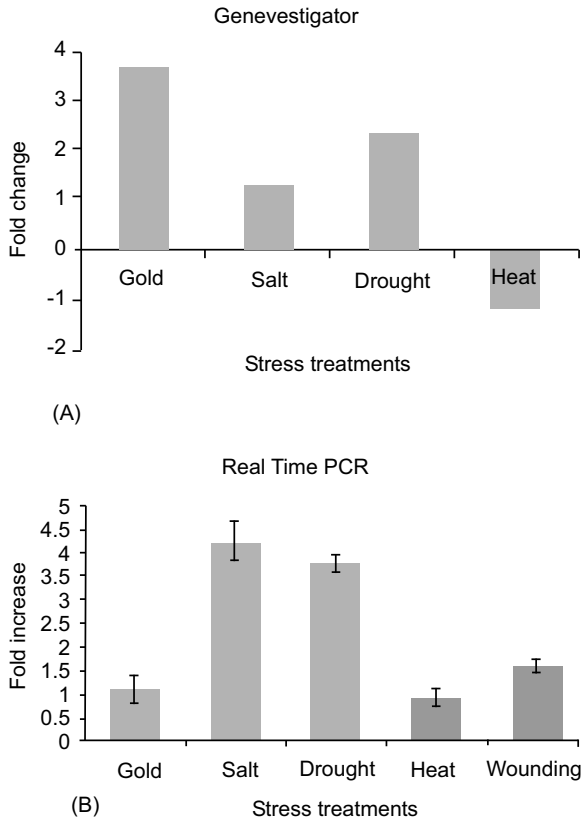


Fig. 6. Intrinsic disorder (ID) prediction for *Os*DOF18 (A) Intrinsic disorder analysis by DISPORED. A threshold was applied with disorder assigned to values greater than or equal to 0.05 (black bar). (B) Diagrammatic representation of *Os*DOF18 structure. It comprises of zinc finger DNA binding domain.

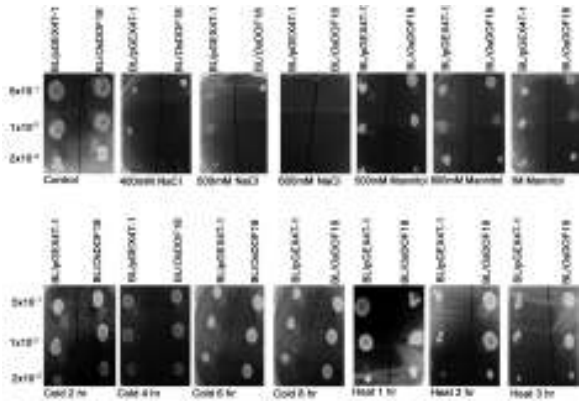
conditions. RT-qPCR analysis showed up-regulation of *Os*DOF18 transcript level in response to salt and drought stresses (Fig. 7A&B) whereas, GENEVESTIGATOR data showed the significant up-regulation under cold stress. In salinity and drought, transcript level was peaked up to 4 fold in comparison to control plants. There was no significant increase in heat, cold and wounding stresses. Corrales *et al.* (2014) reported that tomato cycling DOF factor transcript was up-regulated in response to salt, drought and threshold temperatures suggesting its role in multiple stresses. Ma *et al.* (2015) also observed the up-regulation of DOF genes in Chinese cabbage against cold, salt, heat and drought stresses.

Over-expression of *Os*DOF18 in *E. coli* improves growth during abiotic stresses. An attempt was made express the pGEX vector containing the full coding sequence of *Os*DOF18. It ended in failure, perhaps because the expression of the whole coding region of DOF protein is leaky and toxic for *E. coli* cells (Yanagisawa and Schmidt, 1999) or it may have produced degraded forms of DOF18 proteins. However, a plasmid that allowed the expression of DOF18 DNA binding domain only with some flanking region was constructed. The recombinant plasmid pGEX-*Os*DOF18 and empty vector pGEX4T-1, used as a control, were transformed into *E. coli* cells. The recombinant protein was induced by IPTG treatment as confirmed by SDS-PAGE (Fig. 8). Cultures of BL/pGEX4T-1 and BL-*Os*DOF18 were spread on different plates to investigate the consequences of *Os*DOF18 over-expression on *E. coli* cells against different stresses. Figure 8 showes that recombinant and control cells have similar growth on LB medium in overnight grown culture. When *Os*DOF18 was over-expressed in *E. coli* cells, BL/*Os*DOF18 cells showed better tolerance in high salt and desiccation treatment as compared to vector alone. At low and high temperature, bacterial growth was similar in BL/*Os*DOF18 and BL/pGEX4T-1 cells. These results revealed that *Os*DOF18 gene significantly induced tolerance under salt and dehydration stresses. The improved tolerance to different abiotic stresses may be a consequence to the binding of *Os*DOF18 DNA in binding domain to the stress inducible promoters of different functional genes in *E. coli*. *Os*DOF18 proteins belong to C_2C_2 type zinc finger proteins initially considered restrained to the eukaryotes, the first



(A) Microarray data of OsDOF18 expression in abiotic stresses from Genevestigator, (B) Relative expression of OsDOF18 in abiotic stresses by Real-time PCR. Bars represent standard errors of the mean based on three independent experiments.

Fig. 7(A-B). Expression analysis of *Os*DOF18.



Transformed *E. coli* cells were subjected to different abiotic stresses. Induced cultures OD was adjusted to OD_{600}=1. Then 10 µL of 50-, 100- and 200-fold diluted bacterial suspension was spotted on LB plates containing 400, 500 and 600 mM NaCl for salt stress; 500, 800 mM and 1M mannitol for dessication. Samples were spotted after 2, 4, 6 and 8 h of cold stress and after 1, 2 and 3 h of heat stress.

Fig. 8. Growth performance of BL/pGEX4T-1 and BL/*Os*DOF18 recombinants

prokaryotic C_2H_2 zinc finger protein was identified in 1998 in the transcriptional regulator protein (Ros) in *Agrobacterium tumefaciens* (Chou *et al.,* 1998). This protein contains the sequence $CX_2CX_3FX_2$ LX_2HX_2HH located at the residues 79-97 and considerably bears a resemblance to the consensus sequence of a eukaryotic C_2H_2 zinc finger domain (Malgieri *et al.,* 2007; Kado, 2002). Structural and evolutionary studies suggest that eukaryotic zinc finger domains were evolved from Ros homologues (Moreira and Rodriguez-Valera, 2000). This imitates that there might be some similarity in regulatory systems of both eukaryotes and prokaryotes at some point of communication, and might also share similar components between them. Therefore, it is credible to presume that *Os*DOF18 is making interaction with transcriptional network in the bacterial cells and aid in stress tolerance. Like our study, few other researchers also reported that survival of *E. coli* cells gets better by over-expressing plant stress-related genes. Over-expression of a LEA protein from soybean results in salt tolerance in *E. coli* (Liu and Zheng, 2005). Over-expression of phytochelatin synthase conferred tolerance to *E. coli* against various stresses including pesticide, UV exposure, heat and salt (Chaurasia *et al.,* 2008). SbDREB2A transcription factor over-expression resulted in better *E. coli* growth under different stress conditions (Gupta *et al.,* 2010). Similarly, SbSI-1 gene over-expression confers salt and drought tolerance in *E. coli* (Yadav *et al.,* 2012). Recently, Jin-long *et al.* (2012) demonstrated that the expressed novel dirigent protein ScDir from sugarcane had enhanced the *E. coli* tolerance to PEG and NaCl.

Conclusion

There is no 3D structure available for Dof proteins. A Dof transcription factor was cloned and characterised from rice. this paper reports the *in silico* prediction of three dimensional structure of *Os*DOF18 validated by PROCHECK server and Ramachandran plot analysis, which suggested predicted model to be satisfactory. For functional characterisation, RT-qPCR analysis revealed the upregulation of *Os*DOF18 by salt and drought stresses. *Os*DOF18 DNA binding domain was transformed in *E. coli* and recombinant *E. coli* cells showed higher tolerance to desiccation and salinity compared to vector alone. The present study demonstrates that *Os*DOF18 gene might play an important positive modulation role in abiotic stress tolerance and suggest that it could be a potential bioresource for engineering abiotic stress tolerance in crop plants.

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