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A N I N T E R N A T I O N A L J O U R N A L

Molecular Characterization of a MYB Protein from *Oryza sativa* for its Role in Abiotic Stress Tolerance

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ABSTRACT

The MYB family represents one of the most abundant classes of transcriptional regulators that perform pivotal role under different developmental processes and abiotic stresses. In present study, a MYB gene from Oryza sativa *was selected for functional characterization. Bioinformatics analysis revealed that OsMYB1 cDNA encodes R2-R3 type DNA binding domain consisting of 413 amino acids having size of 44 kDa and pI of 6.24. DNA binding domain containing region was cloned and over-expressed in E. coli. Then, the survival of pGEX-OsMYB1 transformed E. coli cells was compared with control plasmid under different concentrations of NaCl, mannitol, high and low temperature. pGEX-OsMYB1 enhanced the survival of cells at high temperature and salinity. Electrophoretic mobility shift assays (EMSAs) have shown that recombinant OsMYB1 protein was able to bind with DIG labeled probe containing MYB binding site. RT-qPCR analysis revealed high MYB1 expression under wounding, salt, drought and heat stresses in rice. Expression was 23 fold higher in response to wounding demonstrating the worth of OsMYB1 up-regulation in wounding. Intrinsic disorder profile predicted that OsMYB1 exhibits 60% degree of intrinsic disorder proposing that these regions might be involved in DNA binding specificity and protein-protein interaction. The positive response of OsMYB1 suggests that its over-expression in crop plants may help in providing protection to plants to grow under abiotic stresses.*

Keywords: abiotic stress, *E. coli*, *Oryza sativa*, *Os*MYB1, pGEX, Real time qPCR

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INTRODUCTION

Various environmental factors have negative impact on plant growth, development and productivity including salinity, high and low temperature, water logging, wounding and mineral deficiencies. Plants survive these abiotic stresses by unleashing sophisticated survival approaches involving initiation of stress specific adaptive responses to overcome the adverse effects of these stresses[1]. Expression of several genes is initiated by different stresses which may be divided into two classes, i.e. functional and regulatory genes. The regulatory genes are mostly comprised of transcription factors, grouped into different families such as bZIP, DREB, DOF, MYC/MYB, NAC and WRKY. These transcription factors manage the expression of numerous downstream genes involved in providing stress tolerance to plants. High throughput technologies have provided considerable insight in deciphering the molecular approaches of abiotic stress tolerance linking a number of functional and regulatory genes. In addition to regulatory *cis*-elements in plant promoters and transcription factors, information about several stress responsive genes have been gathered[2].

The MYB transcription factors encompass one of the major class in the plant domain. MYB transcription factors are composed of 2 distinct regions, a conserve MYB DNA binding domain at the N-terminus and a varied modulator region at the C-terminus which is involved in controlling regulatory activities of proteins. Based on number of domains, MYB proteins can be distributed into different groups, out of which R2-R3 MYB proteins are specific and most copious type in plants having more than 100 members in higher plants including Arabidopsis and rice[3]. It is named as MYB because the first gene of MYB domain protein was isolated from avian myeloblastosis virus[4]. Afterwards, numerous MYB genes were documented in animals, plants and fungi[5]. The MYB proteins in plants are conserved both in structure and function in comparison to animals and yeasts.

The plant MYB transcription factors have been documented to contribute in several phases of plant growth and development, for instance, the regulation of primary and secondary metabolism, the control of cell cycle and response to abiotic and biotic stresses[6,7]. The expression profiles of numerous MYB genes were investigated for their role in responding to phytohormones and stress conditions in higher plants. For instance, *At*MYB13, *At*MYB15, *At*MYB33, *At*MYB102 and *At*MYB96 are implicated in responding to wounding, salinity, ABA signaling and osmotic stresses[8,9]. In rice, *Os*MYB55 is a key regulatory component in response to high temperature[10] and *Os*MYB2 is involved in responding to salt, cold and drought stresses[11].These annotations proposed the participation of MYB proteins under abiotic stresses and hormonal changes. Nevertheless, little is known about the role of rice MYB proteins in abiotic stresses.

Availability of comprehensive knowledge about MYB proteins will allow modifying them in beneficial ways in the current situation of increasing global warming. Present study was carried out to decipher the role of MYB1 protein from rice in abiotic stresses. Different properties of *Os*MYB1 were analyzed using various bioinformatics tools. DNA binding domain containing region was cloned and functionally characterized by analyzing its response in *E. coli* under different abiotic stresses. *OsMYB1* gene expression level was also monitored by real time qPCR in rice in response to cold, salt, drought, heat and wounding/mechanical stress.

MATERIAL AND METHODS

Bioinformatics Analysis

The NCBI database was used for searching *Os*MYB1 nucleotide and protein sequences and CLUSTALW for sequence alignment. SMART (http://smart.embl-heidelberg.de/) online tool was used to predict conserve DNA binding domain. Secondary structure was predicted by PSIPRED Protein Structure Prediction server (http://bioinf.cs.ucl.ac.uk/psipred/). Nuclear localization signals (NLS) prediction was carried out using online tool WoLF pSORT (http://www.genscript.com/wolf-psort.html). Primary sequence was analyzed by DISOPRED (http://bioinf.cs.ucl.ac.uk/psipred/?disopred=1) to measure the degree of intrinsic disorder. Protein-protein interaction network was constructed by STRING (http://string-db.org/newstring_cgi/show_input_page.pl?UserId=TwZ3I284aTRf&sessionId=AzWVpTDVECpt). The interactions comprise of physical and functional combinations.

Plant Material and Growth Conditions

Seeds of *Oryza sativa* cv. KS282 obtained from Rice Program, Crop Science Institute, National Agricultural Research Centre (NARC) Islamabad were germinated on half strength MS medium and kept at 25^{o}C in growth room for ten days. Total RNA was isolated using RNA extraction kit (Qiagen) and subjected to cDNA synthesis.

Stress Treatments and RT-qPCR

Gene expression database, Genevestigator (Plant Biology) (https://genevestigator.com/gv/plant.jsp), was searched out to find the microarray data for *Os*MYB1 (Os01g0850400) and up-regulation of *OsMYB1* gene was observed in various stresses. The data acquired was for abiotic stresses including cold, heat, drought and salt stresses.

Oryza sativa cv. KS282 seeds were grown on MS medium at 25°C for 10 days. Ten day old rice seedlings were subjected to different abiotic stresses. For drought treatment, plants were placed on aluminium foil till visible leaf rolling appeared in the plants. Cold treatments were performed by transferring 10 day old seedlings to 4°C with a 12 h light/12 h dark photoperiod. After 48 h of cold treatment, seedlings were moved to 25°C and samples were collected after 24 h following recovery from cold stress. High salt stress was carried out by immersing the plant roots in a beaker containing 200 mM NaCl solution for 3 h at 28 ± 1°C. Seedlings were subjected to 45°C for 6 h for heat stress. For wounding, seedlings were cut into pieces 7 mm in length and then left in water at room temperature under continuous light for 6 h. Samples were collected separately and kept at -80^{o}C until analysis. RNA was isolated from different stressed and control plants using the RNeasy Plant Mini Kit (Qiagen). Primers were designed for actin and *Os*MYB1 (Supplementary table 1) and Quantitative real time PCR was performed using Brilliant II SYBR Green QRT-PCR Master Mix Kit (Agilent Technologies). Samples were assessed in a 10 µL reaction mixture containing 5 µL of 2× reaction mix, 100 ng of each forward and reverse primers, 100 ng of RNA, 0.1 µL of reverse transcriptase and 1.8 µL of nuclease free water. Control reactions having no primer and no template were also included. The RT-qPCR profile consists of one cycle for reverse transcription at 50°C for 30 min and polymerase activation at 95°C for 10 min, followed by 40 cycles of PCR at 95°C for 30 s, 53°C for 1 min and 72°C for 30 s. The $2^{-\Delta\Delta CT}$ method was employed to compute the relative change in transcript levels with actin as

internal standard to determine relative expression levels. RT-qPCR assays were repeated at least twice and each repetition had three replicates.

Cloning and Expression in *E. coli*

DNA binding domain containing region of *OsMYB1* was PCR amplified using Forward 5'-CGCGGATCCATGGATGACGTCGTCATC-3' and Reverse 5'-CCGCTCGAGTCAACCACCTTGTGTGCA-3' using cDNA as template. Underlined regions are restriction sites for BamHI and XhoI respectively. PCR was carried out in 20 µL reaction mix containing 4 µL of 5x HF Phusion buffer, 0.4 µL 10 mM dNTPs,10.8 µL water (nano pure), 1 µL of each primer (10 µM), 0.2 µL of Phusion DNA polymerase, 0.6 µL DMSO and 100 ng of cDNA. PCR cycle conditions included heating the PCR reaction for pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 20 sec, annealing at 65°C for 20 sec and extension at 72°C for 20 sec. A final extension was given at 72°C for 10 min. The amplicon with BamH1 and XhoI sites was subcloned into pGEX4T-1 vector and transformed in the *E. coli* BL21 strain to generate the putative pGEX-*Os*MYB1 (Supplementary Figure 1). The desired recombinant plasmids were identified by PCR amplification, restriction digestion and commercial sequencing. The prokaryotic expression product was induced with 1.0 mM isopropyl b-D-thiogalactoside (IPTG) for 6 h at 37°C and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Electrophoretic mobility shift assay (EMSA)

Oligonucleotides (30 bp) containing MYB binding site (WAACCA) were annealed by heating at 95ºC for 10 min and then slowly cooled to room temperature in TEN buffer (Tris (10 mM, pH 8), 1 mM EDTA 100 mM NaCl). A DIG Gel Shift kit (Roche) was used to detect DNA-protein interaction. Digoxigenin (DIG) was labeled at the 3′ end of the double-stranded oligonucleotides. The labelled probes were then purified by ethanol precipitation. The pellet was air dried and resuspended in ddH_2O to a final concentration of 2.5 pmol/µL. Labelling efficiency was tested by spotting serial dilutions on nylon membrane along with control labelled oligonucleotides provided with the kit. DIG labeled (50 fmol) and unlabeled (10 pmol) oligonucleotides were mixed with GST-*Os*MYB1 (1 µg) in binding buffer and incubated at room temperature for 30 min. The reaction products were fractionated through a pre-run 6% non-denaturing polyacrylamide gel for 90 min in 0.5X TBE buffer at 4°C, then electrophoretically transferred to a positively charged nylon membrane (Roche) by applying a constant current of 300 mA for 30 min. The DNA was cross-linked to the membrane using a UV stratalinker (Stratagene). The nylon membrane was blocked in 1X blocking reagent for 1 h at room temperature and then incubated with a 1:20,000 dilution of anti-DIG antibody coupled to alkaline phosphatase for 30 min. The membrane was washed twice in 0.1 M maleic acid (pH 7.5), 0.15 M NaCl, 0.3% (v/v) Tween 20 for 20 min each. Then after a 5 min equilibration in detection buffer (100 mM Tris-HCl pH 9.5, 100 mM NaCl), membrane was kept carefully on a plastic sheet and 1:100 dilution of the CSPD substrate was added on membrane dropwise around membrane edges and then covered the whole membrane by tilting it. Membrane was incubated at room temperature for 5 min and then placed in a plastic folder and incubated at 37°C for more 15 min. Then membrane was exposed to X-ray film for 3 h to capture the chemiluminescent signal.

Assays for Abiotic Stress Tolerance in *E. coli*

Spot assay was performed to establish the function of *Os*MYB1 in *E. coli* cells. BL21 (DE3) cells were subjected to transformation with recombinant plasmid

(pGEX4T1-MYB1) and control plasmid (pGEX4T-1). Cells were grown in LB broth till OD_{600} 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.

To determine salt tolerance of transformed *E. coli* cells, the diluted samples were spotted (10 µL) on LB agar plates (100 µg/mL ampicillin and 1.0 mM IPTG) containing 400, 500, and 600 mM concentration gradient of NaCl. For drought stress, LB agar plates were supplemented with 500 mM, 800 mM and 1 M mannitol. For heat stress, 1 mL of each sample was kept at 50°C and 100 µL aliquots were taken at different periods of 1, 2 and 3 h successively. Samples were subjected to dilution by 50-fold, 100-fold and 200-fold and each sample was spotted (10 µL) onto LB agar plates. For cold stress, liquid cultures were placed at -80°C for 24 h. Then these cultures were allowed to thaw at 35°C for 1 h and 100µL sample was collected at time intervals 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

In Silico Analysis

The *Os*MYB1 cDNA (GenBank accession number XM_015775202.1) was 1560 bp long, contained a 36 bp 5' UTR, a 1242 bp open reading frame and a 282 bp 3' UTR region. The cDNA encoded a polypeptide of 413 amino acid residues with a predicted molecular mass of 44.3 kDa and an isoelectric point of 6.24. *OsMYB1* gene was kept with *AtMYB1, AtMYB25* and *AtMYB109* in NCBI homologene database. Multiple sequence alignment with Arabidopsis proteins showed sequence conservation especially in DNA binding domain region (Figure 1). The secondary structure of *Os*MYB1 analyzed by PSIPRED protein structure prediction software showed that it contains 6 alpha helices in DNA binding domain region forming two helix-turn-helix motifs, and one alpha helix at the C terminus (Figure 2) indicating that it belongs to R2R3 type MYB family. Subcellular localization was predicted by WoLF PSORT and found to be localized in nuclei (Supplementary Figure 2)

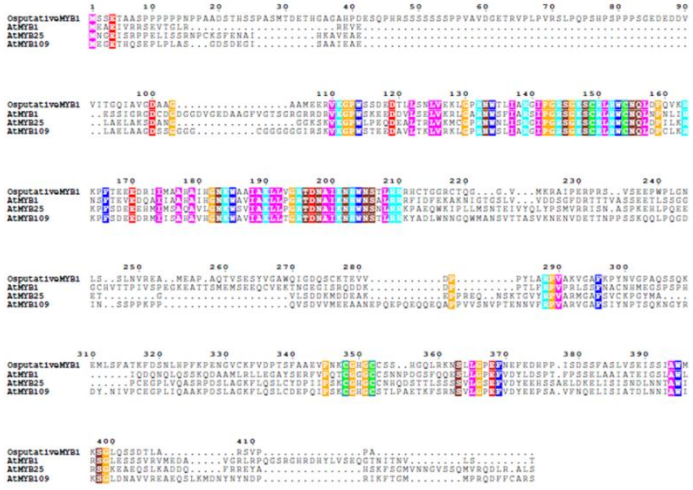


Figure 1: Protein sequence alignment of *Os*MYB1 with putative homologs from *Arabidopsis thaliana*. Conserved residues are shaded in different colors. Genbank accession numbers for *At*MYB1, *At*MYB25 *and At*MYB109 are NP_187534.1, NP_181517.1and NP_191132.1 respectively.

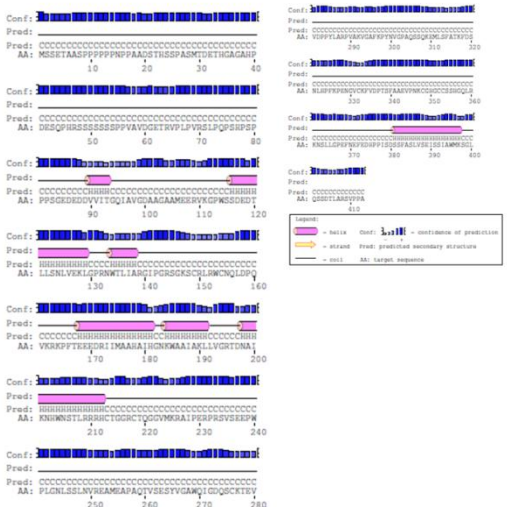


Figure 2: PSIPRED results showing secondary structures features of *Os*MYB1.

Expression Analysis of *Os*MYB1 by RT-qPCR

To elucidate the role of *Os*MYB1 in plant responses to environmental stimuli, we analyzed gene expression profile in rice under various abiotic stresses using Genevestigator tools. The results indicated that the *Os*MYB1 expression is up-regulated by some abiotic stresses, demonstrating that *Os*MYB1 could play significant roles in plants in response to different abiotic stresses. We then conducted RT-qPCR tests to validate the results from the Genevestigator analysis. Gene expression was examined in rice seedlings using high and low temperatures, salinity, drought and wounding. For *Os*MYB1, the transcript level was increased upto 23 fold upon wounding compared to control. Expression was increased (~3 fold) by salt, drought and heat stresses (Figure 3). These results strongly suggest that *Os*MYB1 gene play important roles in providing tolerance to various environmental stresses.

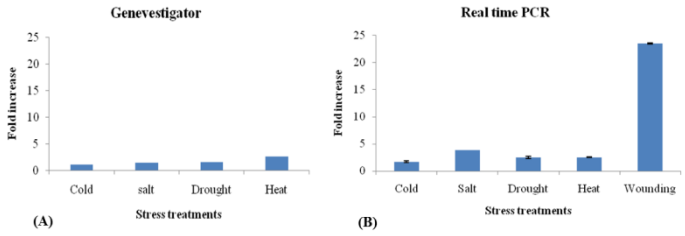


Figure 3: Expression analysis of *OsMYB1* gene. **A:** Microarray data of *OsMYB1* expression in abiotic stresses from Genevestigator . **B:** Relative expression of *OsMYB1* in abiotic stresses by Real-time qPCR. Bars represent standard errors of the mean based on three independent experiments.

Recombinant *Os*MYB1 protein binds to WAACCA *cis*-element

To analyze the possible physical interaction of *Os*MYB1 with WAACCA containing probe, *Os*MYB1 was produced as recombinant protein (GST-*Os*MYB1). DNA binding domain with flanking regions were cloned, fused to the GST tag in the expression vector pGEX-4T-1, expressed in BL21 and purified as GST-*Os*MYB1 (Supplementary Figure 3). Using the 30 bp probe containing WAACCA binding region, we used EMSA to test whether the GST/*Os*MYB1 protein can directly bind to the MYB-binding motif *in vitro*. There was no shift in both negative controls i.e labeled probe without protein and labeled probe with GST. GST-*Os*MYB1 bound to core sequence and a shift was observed (Figure 4). The DIG-labeled probe competed

with unlabeled cold competitor probes. Protein-DNA complex was reduced by the addition of 100 and 200 fold molar excess of unlabeled competitor oligos respectively, suggesting that the signal from the probe represents the specific binding ability of the *Os*MYB1 with the probe. These results demonstrated that *Os*MYB1 was able to directly and specifically bind to the MYB-binding site in *in vitro* conditions.

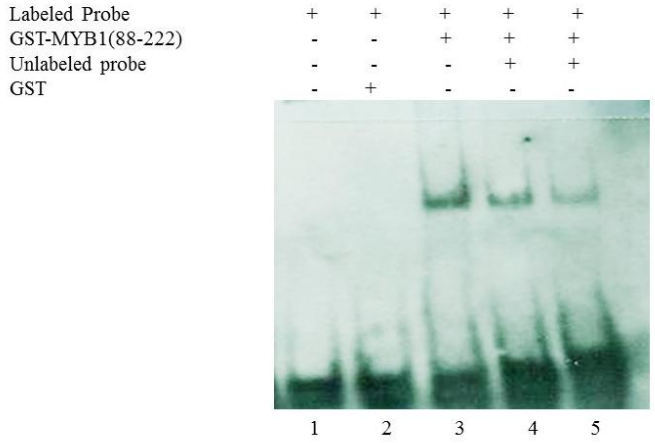


Figure 4: EMSA of recombinant *Os*MYB1 with a DIG-labelled probe containing WAACCA motif. **Lane 1:** *Os*MYB1 oligos negative control, **Lane 2:** GST negative control, **Lane 3:** 1 µg GST-*Os*MYB1 with *Os*MYB1 oligos, **Lane 4:** with 100 molar excess of unlabeled *Os*MYB1 oligos, **Lane 5:** with 100 molar excess of unlabeled *Os*MYB1 oligos.

Expression Patterns of GST-*Os*MYB1 in *E. coli* under Different Abiotic Stresses

To study the consequences of the over-expression of the *OsMYB1* gene on the growth of recombinant *E. coli* cells in response to various environmental stresses, cultures of BL/pGEX4T-1 and BL/*Os*MYB1 were diluted and spread on supplemented plates. Figure 5 showed that BL/pGEX4T-1, BL/*Os*MYB1 containing cells have same growth pattern on standard LB plates. However, the recombinant *E. coli* cells showed enhanced growth in comparison to control cells on plates supplemented with high salt and samples exposed to high temperature. On plates supplemented with 400 to 600 mM NaCl, the survivability rate of BL/*Os*MYB1 cells and BL/pGEX4T-1 cells was different i.e better survival was observed for BL/*Os*MYB1. There were small and few BL/*Os*MYB1 cells on plates supplemented with 600 mM NaCl in comparison to BL/pGEX4T-1. Furthermore, similar results were obtained with different mannitol concentrations; the colony number of BL/*Os*MYB1 was more as compared to BL/pGEX4T-1 at high mannitol concentrations. These findings suggest that the expression of the *OsMYB1* gene augmented the salt and drought tolerance in *E. coli* cells. With the aim of finding the effect of over-expression of *OsMYB1* gene on the growth of *E. coli* recombinants under low and high-temperature stresses, IPTG containing bacterial cultures were transferred to -80°C and 50°C respectively. The number of cells were compared in BL/*Os*MYB1 and BL/pGEX4T-1 plates after the temperature treatment for different time periods. Number of control cells was less as compared to BL/*Os*MYB1 but growth rate was stagnant for both control and BL/*Os*MYB1 cells after 2, 4, 6 and 8 h of cold treatment. Even though both cultures have less number of cells after heat shock, BL/*Os*MYB1 cells were more as compared to control cells. Most of the BL/pGEX4T-1 cells died after 3 h at 50°C, while, less BL/*Os*MYB1 cells died at the same temperature. These outcomes indicated that the *OsMYB1* gene has significantly induced tolerance to high-temperature.

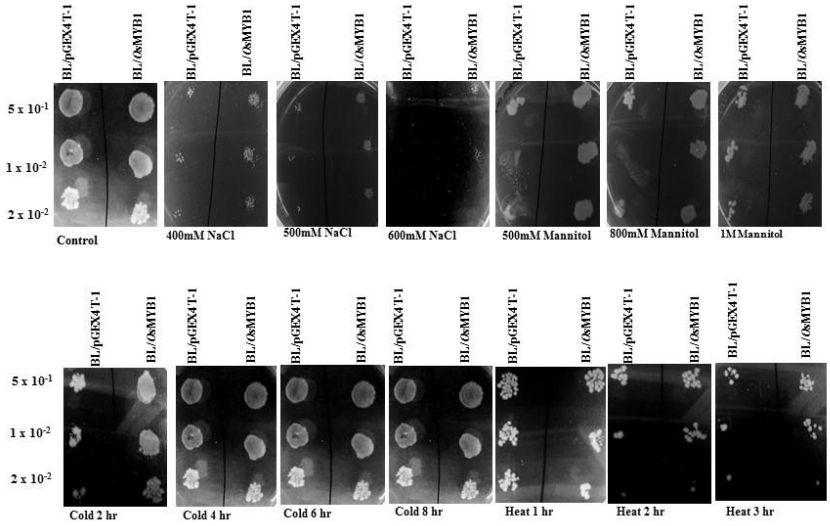


Figure 5: Spot assay of BL/pGEX4T-1 and BL/*Os*MYB1 recombinants. Transformed *E. coli* cells were subjected to different abiotic stresses. Induced cultures OD was adjusted to OD_{600}=1. Then 10 µL of 50-, 100- and 200-fold diluted bacterial suspension was spotted on LB plates containing 400, 500 and 600 mM NaCl for salt stress; 500, 800 mM and 1M mannitol for desiccation. Samples were spotted after 2, 4, 6 and 8 h of cold stress and after 1, 2 and 3 h of heat stress.

Intrinsic Disorder in *Os*MYB1

Plant transcription factors belonging to different transcription factors families, such as MYB, NAC, WRKY and bZIP, have significant degrees of Intrinsic disorder regions (IDRs) which play vital role in interaction with DNA and other regulatory proteins[12]. DISPORED analysis predicted 60% residues to be disordered in *Os*MYB1. Only the DNA binding domain region is structured whereas amino acids residues surrounding the DNA binding domain are intrinsically disordered (IDs). Structure of DNA binding domain predicted by SMART contains two MYB DNA binding domains that interact with promoter region of target genes. The flanking disordered regions are supposed to be involved in DNA binding affinity and specificity. But how these disordered regions affect binding is still to be determined. DISPORED has also predicted four disordered regions involved in protein-protein interaction (Figure 6).

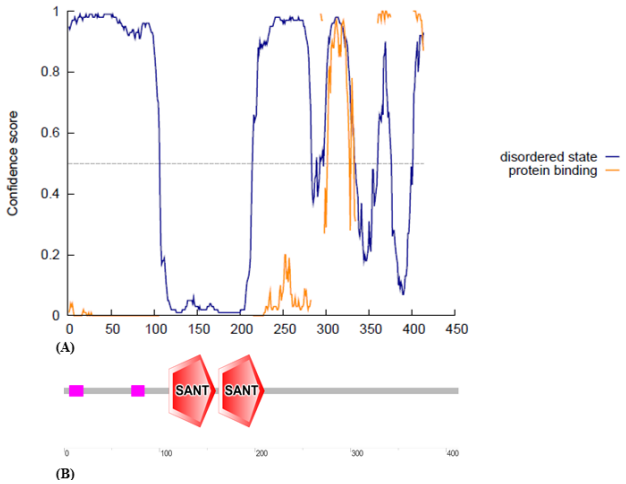
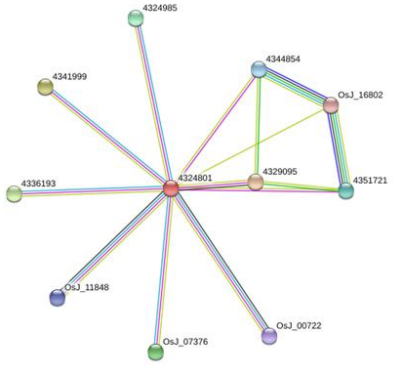


Figure 6: Intrinsic disorder (ID) prediction for *Os*MYB1 **(A)** Intrinsic disorder analysis by DISPORED. A threshold was applied with disorder assigned to values greater than or equal to 0.05 (black bar). **(B)** Diagrammatic representation of *Os*MYB1 structure. It comprises of 2 SANT DNA binding domains.

Protein-protein Interaction Analysis

To find out how *Os*MYB1 interacts with other rice proteins and alter cell function, the protein was analyzed by searching the STRING database and the protein-protein interaction network was obtained. *Os*MYB1 was predicted to bind ATP-dependent RNA helicase, TAZ zinc finger proteins, DIRP family proteins and phosphoribosylamine glycine ligase with a score of 0.8-0.9. (Figure 7).



Your Input:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
4324801	MYB family transcription factor, putative, expressed (413 aa) *(Oryza sativa Japonica)*									

Predicted Functional Partners:

4329095	pre-mRNA-splicing factor ATP-dependent RNA helicase, putative, expressed (1240 aa)						•	•	•	0.921
4341999	TAZ zinc finger family protein, expressed; Acetyltransferase enzyme. Acetylates histones, givin [...] (1428 aa)						•	•	•	0.885
4336193	BTBZ4 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-bi [...] (355 aa)						•	•	•	0.885
OsJ_07376	BTBZ3 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-bi [...] (361 aa)						•	•	•	0.885
4324985	BTBZ2 - Bric-a-Brac, Tramtrack, and Broad Complex BTB domain with TAZ zinc finger and Calmoduli [...] (347 aa)						•	•	•	0.885
4351721	phosphoribosylamine--glycine ligase, putative, expressed (518 aa)								•	0.873
4344854	phosphoribosylamine--glycine ligase, putative, expressed (527 aa)								•	0.873
OsJ_11848	DIRP family protein, putative, expressed (1255 aa)						•	•	•	0.838
OsJ_00722	DIRP family protein, putative, expressed (872 aa)						•	•	•	0.838
OsJ_16802	phosphoribosylformylglycinamidine synthase, putative, expressed (1415 aa)								•	0.819

Figure 7: Protein-protein interaction network of *Os*MYB1 with differentially expressed proteins. In the resulting proteins network, proteins are represented by nodes which are linked by lines whose thickness tells the confidence level. Various line colors denote different kinds of associations: red line represents fusion association evidence; green line represents neighborhood association; light blue line indicates database evidence; black line represents co-expression evidence; purple line denotes experimental evidence of proteins association; yellow line represents text-mining evidence and blue line indicates co-occurrence.

DISCUSSION

Transcription factors play significant roles in plant growth and development, and in responses to environmental stresses. To examine the expression of *OsMYB1* gene in response to multiple abiotic stresses in rice, we took advantage of the available microarray data from GENEVESTIGATOR. To validate this expression analysis, RT-qPCR analysis was carried out. Wounding stress was also included as MYB proteins are reported to show response in wounding. The transcript level of *OsMYB1* gene increased under different environmental stresses, however, their relative expression varies depending on the kind of the stress. Upon wounding stress, the expression of *OsMYB1* was increased up to 23 fold compared to control, while the expression behavior for salt, drought, cold and heat stresses was same for GENEVESTIGATOR and RT-qPCR results i.e ~2 fold increase. The highest expression level of *Os*MYB1 suggests that it may have an important transcriptional regulatory role in response to wounding stress, and that the *Os*MYB1-mediated pathway is likely essential for responding to signals by reactive oxygen intermediates from wounding in rice. It is documented that MYB proteins interact with jasmonate signaling pathway and mediate responses to reactive oxygen species generated during wounding and biotic stresses[13]. For instance, *At*MYB102 expression was dependent on and integrated signals from both wounding and water stress and the expression of Arabidopsis R2R3 AtMYB102 transcription factor gene

was dependent on signals derived from both wounding and osmotic stress[14]. Different MYB proteins have been reported to participate in different stress response processes[15]. *Jc*MYB1 transcript level was reported to be up-regulated by PEG, NaCl and cold stresses as well as by hormones[16]. Katiyar *et al.*[17] observed the up-regulation of various *MYB* genes in rice under drought, salt and cold stresses.

It has been accounted that MYB transcription factors can recognize a specific binding motif, WAACCA and YAACKG[18]. An attempt was made to investigate the binding of *Os*MYB1 with a probe containing WAACCA core sequence. It was found that *Os*MYB1 was able to bind WAACCA *cis*-element. Binding was further confirmed by competitor EMSA and EMSA with mutant oligonucleotides. Specificity of interaction was confirmed by adding excess of unlabeled probes. These results suggest the involvement of *Os*MYB1 protein in regulating the expression of stress related genes as promoters of many stress regulated genes contain WAACCA binding motif for instance, *OsRGLP2* gene promoter has 4 copies of *Os*MYB1 binding motif[19].

In recent times, a number of plant genes and transcription factors have been evaluated for their stress analysis using heterologous expression system of *E. coli*. *SbsI-1*, a unique salt responsive gene, from *S. brachiata* displayed drought and salinity tolerance in *E. coli* cells as investigated by Yadav *et al.*[20]. Guo *et al.*[21] observed the drought tolerance in *E.coli* cells transformed with the *ThPOD3* from *Tamarix hispida*. LEA proteins from soybean improved tolerance to *E. coli* cells against salt stress[22]. In order to examine the function of proteins against diverse abiotic stresses, recombinant plasmid was expressed in *E. coli*. The differential response of *E. coli* harboring pGEX-*Os*MYB1 was inspected for their diverse level of tolerance to various abiotic stresses in comparison to empty pGEX-4T1 vector including salt, drought and heat stresses. Tolerance of the recombinant *E. coli* indicated that expression of *Os*MYB1 in host cells could confer protective function against damaging of proteins, cellular membrane, and cells and this may be a sequel to the binding of *Os*MYB1 protein to the stress-inducible promoters of the different functional genes in *E. coli*. There might be some common protective mechanisms in both prokaryotes and eukaryotes under stress conditions[23]. The MYB transcription factors are considered to be eukaryote specific and composed of helix-turn-helix domains. It is reported that eukaryotic helix-turn-helix domains are originated from prokaryotic helix-turn-helix domains[24]. This imitates that the regulatory system of eukaryotes and prokaryotes might have some resemblance at certain point of interaction, and related mechanisms do exist between them. Hence, it is credible to assume that *Os*MYB1 DNA binding domain is interacting with transcriptional network in the bacterial cells and aid in stress tolerance.

In the past decade, research revealed that eukaryotic genomes contain 25 to 30% of ID proteins out of which 90% proteins are transcription factors[25]. Various computational methods have been employed to predict IDRs based on primary protein sequence. These IDs appear to be involved in molecular recognition, for instance, protein binding to DNA to assist transcription or replication. IDP and IDR can recognize and bind to a multiple partners at different binding sites via short regions, called Molecular Recognition Features (MoRFs) that switch from disorder-to-order confirmation[26]. The large interaction potential permits IDRs to interact as hubs with a number of partners in interactomes[27]. The significance of ID for protein-protein interactions is now being valued in plant science[12]. In plants, NAC transcription factors are the only class of transcription factors in which ID was studied in detail [28]. Our results revealed that *Os*MYB1 have 60% IDRs which means that it interacts with other proteins. The protein partners were predicted by STRING. Predicted proteins partners play vital roles in photosynthesis, signal transduction, metabolism and various stress conditions.

CONCLUSION

Taken together, it is judicious to speculate that the DNA-protein interaction mechanism for *Os*MYB1 might be alike in prokaryotes and eukaryotes in response to several environmental stresses, and it is predictable that heat, salt, drought, and wounding tolerant crops can be produced through overexpression of *OsMYB1* gene.

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