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Molecular Studies on bZIP Transcription Factor from Sorghum (*Sorghum bicolor* L) for Mitigation of Stress Factors

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Abstract

Cereals are the most important sources of carbohydrate acquisition and are the most widely planted crops in global agriculture. Plants are sessile beings that are continuously exposed to various changes in the environmental conditions. Variations in the environment involving both biotic and abiotic stresses have negative effects on economically important crops. Although, transgenic plants overexpressing the bZIP proteins have been reported to exhibit a higher tolerance to abiotic stresses such as salinity, drought and extreme temperature conditions, the physiological and molecular basis of tolerance in these plants largely remains unexplored. Furthermore, the role of bZIP proteins towards maintaining the yield in crop plants under stress conditions is yet to be reported. In addition, only limited data are available on genome-wide identification and their characterizations in the sorghum genome. In the current study we aimed to study characterization of bZIP, a transcription factor from a cereal sorghum (*Sorghum bicolor* L). Study results demonstrated that The expression analysis of the bZIP gene from the sorghum local variety revealed 6-fold increases in response to salt and 9-fold increases in response to drought stress. As a result, bZIP gene can be used as a candidate gene to increase abiotic stress tolerance and to improve crop productivity of *Sorghum bicolor* L under stressful environmental conditions.

Keywords: *Sorghum*, Abiotic stress, bZIP, Transcription factor,

Introduction

Cereals are the most important sources of carbohydrate acquisition and are the most widely planted crops in global agriculture. On a worldwide basis, the main crops grown in Asia are maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), broomcorn millet (*Panicum miliaceum* L.), and Sorghum (*Sorghum bicolor* L.); rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.).[1] Abiotic stresses, for instance drought, salinity, cold, high temperatures, and mineral toxicity, are the main cause of major crop yield reductions worldwide, reducing expected average yields of the major crops by more than 50%.[2,3] Plants are sessile beings that are continuously exposed to various changes in the environmental conditions. Variations in the environment involving both biotic and abiotic stresses have negative effects on economically important crops.[4]

bZIP (basic leucine zipper) group is one of the major group of transcription factors which has expanded and evolved in genomes to play a crucial role in plant growth and stress response. bZIP proteins are a class of unique TFs with the bZIP domain and two structural features: a leucine zipper domain and a basic DNA binding domain.[5] bZIP proteins are evidently seen throughout the plant kingdom participating in various physiological processes such as seed germination, flower development and fertility, plant senescence, abiotic stress responses and ABA signal transduction.[6-8] Although, transgenic plants overexpressing the bZIP proteins have been reported to exhibit a higher tolerance to abiotic stresses such as salinity, drought and extreme temperature conditions,[9-12] the physiological and molecular basis of tolerance in these plants largely remains unexplored. Furthermore, the role of bZIP proteins towards maintaining the yield in crop plants under stress conditions is yet to be reported.

Sorghum genome has been sequenced using grain sorghum AT×623/BT×623 as a genome donor.[13,14] Its small genome (around 730 Mb) makes itself as a model for functional genomics of C4 grasses.[14] Sorghum has a remarkable ability to endure both drought conditions and waterlogging and it grows well on marginal lands.[13] Furthermore, sweet sorghum has been regarded as a biofuel crop of growing importance for ethanol production due to its high biomass yield and sugar content. However, in sorghum, to our knowledge, only one bZIP gene has been isolated, which is an Opaque2 (maize) homolog gene,[15] and no other data are available in sorghum so far, which illustrates the expression regulation of this gene family under drought, high salinity and cold stresses as well as the role in sugar mediated signaling. In addition, only limited data are available on genome-wide identification and their characterizations in the

sorghum genome. Therefore, it is important to genome widely identify and to annotate the sorghum bZIP gene family with relation to the regulation of abiotic stresses and/or sugar metabolism. Therefore, in the present study we aimed to study characterization of bZIP, a transcription factor from a cereal sorghum (*Sorghum bicolor* L)

Materials and Methods

Stress induction

Seeds of sorghum (*Sorghum bicolor* L) was purchased from local market and were grown under field conditions. After 10 days of growth saplings were transferred to quarter strength Hoaglands media in order to impose drought and salt stress. Drought stress was stimulated by withholding water and for salt stress was imposed by adding 200mM NaCl to the quarter strength Hoaglands media and grown hydroponically for 24hours. After the stress treatment, 24hours control and stress exposed tissues were harvested and stored at -80°C for further analysis.

Isolation and amplification of bZIP gene and cloning

The total RNA was isolated by modified Chomczynski method.[16] The total RNA template was converted to complementary DNA (cDNA) by reverse transcription with total RNA using cDNA Synthesis Kit for Reverse Transcriptase PCR according to manufacturer's instructions. cDNA concentration was checked using Nanodrop2000. EcbZIP coding DNA sequence and protein sequence were retrieved from NCBI Nucleotide Database and Genpept of Accession number KP033192.1 and AJP67539.1 and validated by using SMART (simple modular architecture tool) Domain Tool. For EcbZIP sequence primers were designed using Primer3 plus Software (http://bioinfo.ut.ee/primer3-0.4.0/) with the following parameters – Forward and Reverse primers of 26bp, 50-55% GC content, Tm of 60.13 and 59.92 respectively. The restriction sites were inserted in both forward and reverse primers at 5´end for BamHI (GGATCC) and XhoI (CTCGAG) restriction enzymes along with 4bp sitting sequence. The molecular cloning of bZIP was carried out using standardized mole technique for cloning.

Results and Discussion

RNA isolation: Total RNA was isolated from 14 days old saplings. The concentration of the total RNA was determined using Nanodrop and was found to be 265 µg/µl. Total RNA was taken as template for the synthesis of cDNA by reverse transcriptase. The concentration of the cDNA was determined using Nanodrop 2000 and was found to be 100 µg/µl. The coding DNA sequence and protein sequence of bZIP, obtained from NCBI Nucleotide data base and Genepept were as below

Coding DNA sequence

AATATCCCAATCGAGCTACATGGACGCGGACTTCTTCGCTGACCTCGACTTTGTCGCCCTCCTCGCATCC
TTCTCCGGCGAGCCCGCCGCCGTCTCCGATATTATCATCCCCTCCCCTCCTCCGCCGTCGCCGCCGGCGC
GCGATGCGGAGGCGGGGTCGCCGGAGTCAGTGACTTCCCGGTCGAGCCCTCCGGCGGAGGCCCTATCGGA
GATCGAGAGGTTCCTGATGGAGGAGGGGGAGGCGGAGGGCGACGGGGCGGTGGAAGGGATCAGCGTGGAG
GAATTCTTCGACGCGCTGTACGACGGCGGGGAGGAGAAAGAGAAGGAGAGCGAGGCGGGCGGGAGCACGG
ATTCGGGCTCCGGCAGGGAGGAGGAGGTTGCGACGCCGGAGGCGGAGAAGTTGGAGGTGGATGGCGATGA
CCCCGTCAGCAAGAAGAAGAGGAGGCAAATGAGGAACAGGGATTCTGCCATGAAATCGAGGGAGAGGAAG
AAGTTATATGTAAAGGAATTGGAGACGAAGAGCAAGTATTTGGAGGCAGAGTGCCGCCGTCTCAGCTACG
CACTCCAGTGCTACGCAGCTGAGAACATGACACTGCGCCAGAGCTTGCTGAAGGATAGGCCTGTTGGTGC
TCCCACAGCCATGCAGGAGTCTGCCGTACTCACGGAAACCCTGCCGATGGTTTCCCTGCTTTGGCTGGTG
AGCATCGTGTGCCTATTCCTAATGCCCGGTCTACCCAACCCAAGCTCAGCTGCTCCAAGAAGCAGCGGGA
GAGATCTCGTGACGGTAGCCGGAAAGCCAAGCAGTGAAAACCCAGAGACACTGGACCTCATCCTCCATGG
AAGGCGTTGCAAGGGCACAAGGGCGAAGATCAAGCTATCTACCTTACCGTTTCATGCTGCAGCAGCTTGT
TAGGCTAGGCTTATCATGATCTGTAGTAGTCGCCTTTCCAAGGCTTAGCATGCCTATGGCATGTGCATCC
ATGGAGTATGTACCTATTTTTTTTGTTCTTTTTTCCTCCTGATTTATGATCCCATCGTACCCAGAGTAGT
TTTCAAATCTGGTTTGTGTTATGTCTTTTCGTCTCGCTGCTCCAGGATCGAAGAATAGTCGGGACCAAAA
CAGTGAAGTATGAAGCTTGGACCTATAGTCATCTATAAAATCTTCGATTATATGCAAAAAAAA

Protein sequence

bZIP transcription factor

MDADFFADLDFVALLASFSGEPAAVSDIIIPSPPPPSPPARDAEAGSPESVTSRSSPPAEALSEIERFLM
EEGEAEGDGAVEGISVEEFFDALYDGGEEKEKESEAGGSTDSGSGREEEVATPEAEKLEVDGDDPVSKKK
RRQMRNRDSAMKSRERKKLYVKELETKSKYLEAECRRLSYALQCYAAENMTLRQSLLKDRPVGAPTAMQE
SAVLTETLPMVSLLWLVSIVCLFLMPGLPNPSSAAPRSSGRDLVTVAGKPSSENPETLDLILHGRRCKGT
RAKIKLSTLPFHAAAAC

Using primer3 plus software specific primers for bZIP were design:

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Table 2: Primer for EcbZIP

Forward PRIMER having BamH1 restriction site	5'atatGGATCCCCGTCAGCAAGAAGAAGAGG 3'
Reverse PRIMER having Xho1 restriction site	5'atatCTCGAGGGGCATTAGGAATAGGCACA 3'

In order to amplify bZIP gene, cDNA was used as template and PCR reaction was set up with gene specific forward and reverse primers along with an NTC. The amplified mixture was run on 1.5% agarose gel along with 100bp and 1kb ladder (Figure 1). A single band was obtained, and the amplified mixture was eluted from gel by using gene extraction kit.

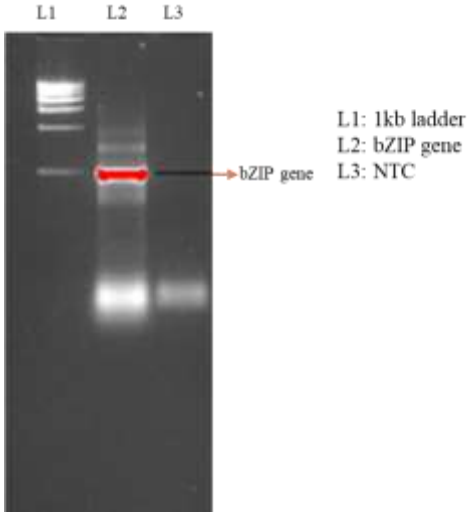

Figure 1: Showing Amplified bZIP gene.

To clone bZIP gene, pET28a plasmid was isolated by alkali lysis method and purified by phenol chloroform isoamyl alcohol. After isolation, the dissolved plasmid was given RNase treatment to remove RNA. The purified plasmid and bZIP gene were digested with Xho1 and BamH1 to create cohesive ends. The digested plasmid mixture was run on 1% agarose gel to check for complete digestion. The plasmid was linearized and showed single band of 5.3kbp (Figure 4).

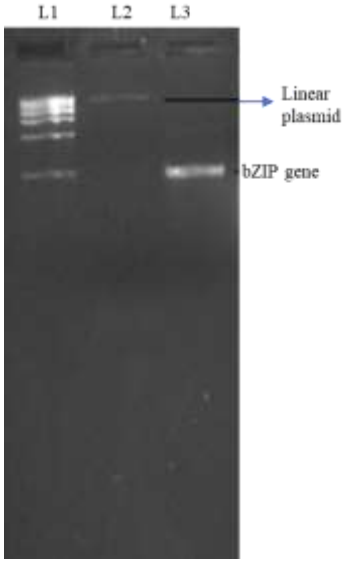

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Figure 2: Showing Restriction digestion of pET28a and bZIP

The linearized plasmid and the digested bZIP gene were ligated using T4 DNA ligase and transformed into *E. coli* host DH5α. The transformed culture was plated on kanamycin resistant LB agar and incubated at 37˚C overnight and figure 5 shows the positive control. Colony PCR is a convenient high through put method for determining the presence and absence of insert DNA in Plasmid grown up on antibiotic containing media following transformation by using gene specific primers. Colony PCR was carried out by picking three colonies from transformed culture plate and run on agarose gel to check for the bZIP amplicon. Gel was visualized under gel documentation unit. Out of three colonies picked in this experiment two were confirmed to contain insert (Figure 3).

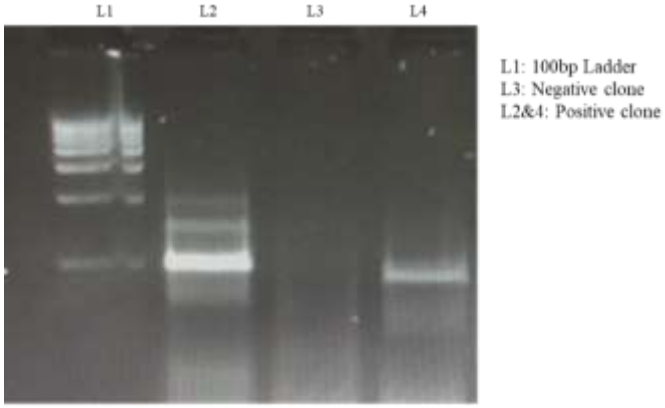

Figure 3: Showing colony PCR gel

In all eukaryotes, transcription factors from the bZIP family regulate crucial processes. In plants, bZIPs regulate a wide range of essential physiological and developmental processes, such as abiotic and biotic stress responses.[17] bZIP transcription factor genes have been cloned from Arabidopsis, rice, soybean, maize, and other plant species and have been discovered to be involved in plant biotic and abiotic stress-response.[18] These genes have been reported to be excellent candidate genes for the improvement of stress tolerance in crop plants.[19-21] According to these findings, we performed bZIP expression profiling under abiotic stress and discovered that the transcription factor significantly upregulated under both salt and drought stress conditions, indicating that it is crucial for stress tolerance.

Conclusions

The expression analysis of the bZIP gene from the sorghum local variety revealed 6-fold increases in response to salt and 9-fold increases in response to drought stress. As a result, bZIP gene can be used as a candidate gene to increase abiotic stress tolerance and to improve crop productivity of *Sorghum bicolor* L under stressful environmental conditions.

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