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Insilco gene characterisation and Promoter analysis of drought inducible *MYB* gene from *Eleusine coracana*

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Abstract

Drought is a major abiotic factor that limits agricultural crop production and productivity. Plants experience drought stress either due to limited water supply to roots or when the transpiration rate becomes very high. These two conditions often coincide under arid and semi-arid climates. Plants such as millets, sorghum and maize which are native to such climate have acclimatized themselves in such a way that they can survive better. Finger millet is most important among them as it grows widely and is staple food of South Africa and many parts of Asia. It is nutritionally rich, contain high amount of calcium and good for diabetic patient due to low glycaemic index. Therefore, finger millet can be taken as a model plant for investigating the pathway underlying drought tolerance in plants. Present study was focussed on isolation of full length gene corresponding to the online publically available EcMYB gene partial transcript from the UGENE transcriptome data of *Eleusine coracana* and open reading frame was deduced from it. The protein BLAST results and the presence of DNA binding domain and helix turn helix domain ensured that the isolated full transcript was MYB gene from *Eleusine coracana*. In order to predict their ABA responses the ABRE (ABA Responsive Elements) was predicted in the promoter of MYB gene from *Oryza sativa* that was homologous to MYB gene in Finger millet with more than 80% identity. Thus EcMYB follows ABA dependent signalling pathways and accumulation of ABA in response to several abiotic stress is responsible for up and down regulation of several gene especially transcription factors which provide drought tolerance to plants.

Keywords: enzymes, pigments in mango, card board carton, salicylic acid

Introduction

In India, the year 2018 was declared as national year of millets and the year 2023 is going to be international year of millets as declared by FAO. The world's millet production was estimated at 28.45 million tons (www.fao.org). India is the largest producer of millets with a 41.04 per cent global market share. Finger millet (*Eleusine coracana*) is hardy crop and can be grown in arid regions where rainfall is less. It is extensively cultivated in the tropical and sub-tropical regions of Africa and Asia. It is known to save the lives of poor farmers from starvation at times of extreme drought (Kotschi, 2006) [7]. Out of the total production of minor millets in India, *ragi* accounts for about 85% of production (Divya, 2011) [6]. In India it covers an area of 1.19 million hectares with an average productivity of 1661 kg per ha leading to the production of 1.98 million tonne. Finger millet can be grown under rain fed as well as irrigated condition. It is grown extensively as a kharif (rainfed) crop and sown during May-June while in irrigated area it can be grown in more than one season. It is a popular food among diabetic patients in the countries like India and Sri Lanka due to low glycemic index (Pradhan *et al.*, 2010) [12]. It contains more fibre, minerals and vitamins, which are normally deficient in Indian diet, and has eight times more calcium than other cereals. The characteristic attributes of this crop is its adaptability to adverse agro-ecological conditions with minimal inputs and good nutritional properties.

Finger millet has internal cellular and molecular mechanism to tolerate adverse environment to a large extent. Genes which impart tolerance to the plants against adverse environmental conditions can act as molecular tag and can be used to track genetic loci controlling stress tolerance, thus reducing the need for extensive field testing over time and space.

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In response to adaptation against stresses, some of the genes are up regulated and some are down regulated. Study of the mechanisms of the regulation of the expression of these genes during tolerance to adverse environment conditions is necessary. In general, abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that affect plant growth, development and productivity. Abiotic stress such as drought leads to accumulation of ABA endogenously. Accumulation of ABA triggers ABA dependent signalling pathway that causes up regulation and down regulation of ABA responsive gene. This up regulation and down regulation occurs due to a number of factors; among these transcription factors (TFs) and RNA binding proteins are especially notable.

Transcription factors are proteins that act together with other transcriptional regulators, including chromatin remodelling/modifying proteins to employ or obstruct RNA polymerases to the DNA template (Udvardi *et al.*, 2007) [15]. TFs play a critical role in the drought stress response (Chaves and Oliveira, 2004) [5]. Plant genomes assign approximately 7% of their coding sequence to TFs which proves the complexity of transcriptional regulation (Udvardi *et al.*, 2007) [15]. The TFs interact with *cis*-elements in the promoter regions of several stress-related genes and thus up-regulate the expression of many downstream genes resulting in imparting drought stress tolerance (Agarwal and Jha, 2010) [2]. Drought responsive MYB proteins are TFs which work under the influence of ABA accumulation. MYB TFs are composed of one, two or three imperfect helix-turn-helix repeats that recognize the major groove of DNA and are thought to play an important role in imparting drought tolerance to plants (Yanhui *et al.*, 2006) [17]. A MYB recognise the *cis* acting elements in the drought-induced expression of the *rd22* gene, which brings about stomatal closure and thus save the plants from water loss (Abe *et al.*, 1997) [1].

Therefore investigating the pathway underlying drought tolerance in plants is important and finger millet can be taken as a model plant for this purpose. Present study was performed to characterize MYB gene from *Eleusine coracana* and their promoter was analysed for the presence of ABA responsive elements in the promoter of MYB gene of finger millet.

Materials and Methods

Retrieval of Gene Sequence of MYB for *Eleusine coracana*

Drought responsive transcription factor gene, EcMYB of *Eleusine coracana* was downloaded from NCBI with accession number JN107890.1 (<https://www.ncbi.nlm.nih.gov/>).

Full length gene isolation of MYB from *Eleusine coracana*

The downloaded EcMYB gene sequence was UGENE blasted with transcriptome data of *Eleusine coracana*, available at the Department of MBGE, G.B.P.U.A. & T., PANTNAGAR.

Prediction of open reading frame (ORF) and peptide sequences of isolated MYB gene

ORF finder was used for finding of translating region among the isolated gene sequence of MYB from *Eleusine coracana*. The ORF nucleotide sequence of MYB gene was translated to amino acid sequence by using online ExPASy translation tool.

Confirmation of isolated gene as MYB

The predicted and extracted amino acid sequence of MYB

gene from *Eleusine coracana* were blasted at NCBI and look for their similarity to MYB genes.

Prediction of functional domain in the MYB gene

Isolated ORF was used for prediction of functional domain in the MYB gene using SMART (Simple Modular Architecture Research Tool) and PROSITE.

Isolation of homologous promoter for MYB gene sequence from *Oryza sativa*

MYB gene sequence was used as a query to protein blast to NCBI. The top hit was downloaded along with their corresponding chromosome in which it was resides. Gene mapped over the chromosome and around 1.5 Kbp genomic sequences upstream to the gene were taken for further analyses.

Promoter analysis of MYB gene

Upstream 1.5 Kbp gene sequences were used for promoter analysis using Eukaryotic transporter prediction tools and PlantCARE online tools.

Results and Discussion

Abiotic stress cause major crop loss worldwide. Drought and salinity particularly are becoming widespread in many regions and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Wang *et al.*, 2003). Plant generates the responses to combat the stress phenomena through a set of gene expression that is regulating through the transcription factor. Drought responsive genes are activated through both the ABA dependent and independent pathway that activate the four major family of transcription factors; MYB/MYC-regulon, bZIP regulon, DREB regulon and NAC regulon respectively (Shinozaki and Yamaguchi-Shinozaki, 1996) [13]. ABA is a key regulator for most of the biotic and abiotic responses as it convey signal throughout the plant. The accumulation of ABA is highly correlated with the response generated under the abiotic stress tolerance mechanism. The details of ABA homeostasis under stress condition have been discussed in details by Kumari *et al.* (2018) [8]. Upstream regulatory element such as; transcription factor is quickly activated through ABA signalling; however, it is not necessary that the entire transcription factor and their all member will be activated through ABA. Similarly, members of MYB transcription factor are not necessarily will induced by ABA. In the present studies a drought responsive EcMYB gene regulated through the ABA dependent pathway has been further Insilco analysed for the functional characterisation of the gene and presence of ABA responsive element in their promoter to identify their ABA dependent pathway (Kumari *et al.*, 2017) [9].

A drought responsive EcMYB transcription factor with accession number JN107890.1 was downloaded from NCBI. Publically available EcMYB gene was basically a transcript with the length of 636 nucleotides, isolated from *Eleusine coracana*. In order to fully characterize the gene, full length of MYB gene was required. The full-length gene was isolated from the transcriptome data of *Eleusine coracana* by UGENE BLAST. The length of the assembled UGENE transcript that aligned with the EcMYB gene was 2413 bp (Fig.1). Isolated transcript was screened for the presence of ORF (open reading frame) that was translated further to peptide sequence in order to identify that isolated transcript as a true MYB gene and not a faulty alignment. the isolated transcript code for the ORF of the length 1287 bp (Fig.2) that was translated in to peptide

MGRHSCCYKQKLRKGLWSPEEDEKLMNHITKHGHGCWSSVPKLAGLQRCGKSCRLRWINYLRPDLKRGAFSQEEEDLIIEIHLAVLGNRWSQIAA
 QLPGRTDNEIKNLWNSCIKKLKRQKIDPNTHKPLAKADRSGAAPTISTERTSGSSDANPSSTGALGNLSHLLSETAQSSMLLPVYDKNCAETP
 NLARPKVPPEKELFDQLAGHESPTCRSSGPTLYFFPHQPLGYSSSESGSGDGMNMSLWFNQSDFNCSSTISTIMPPVSPALSSTSMGLNLPPD
 NPRHVIGIGNAPVDSFYWDGTPNPSSSSSTGSRGNSMGEFEPQSTSSILENSVFPWTDIGQEKDTRSOLVEELKWPDLHGTFAETTTTMONQSQS
 LYDDVIKAECCQFNMEGICASWFQNPQQPQQQLQAAPDMYDKDLQRMQLSFENI

Fig. 3: The Amino acid sequences of Myb gene from *Eleusine coracana*

Description	Max score	Total score	Query cover	E value	Ident	Accession
MYB transcription factor [Zea mays]	773	773	100%	0.0	93%	NP_001132070.1
PREDICTED: transcription factor MYB28-like isoform X2 [Setaria italica]	772	772	100%	0.0	93%	XP_004960435.1
PREDICTED: transcription factor MYB46-like [Oryza brachyantha]	754	754	100%	0.0	92%	XP_006654025.1
MYB18 [Saccharum hybrid cultivar Co 740]	751	751	100%	0.0	91%	ACT98139.1
putative MYB DNA-binding domain superfamily protein [Zea mays]	750	750	100%	0.0	91%	AFW82675.1
Os05g0140100 [Oryza sativa Japonica Group]	747	747	100%	0.0	90%	NP_001054597.1
uncharacterized protein LOC100272313 [Zea mays]	744	744	100%	0.0	90%	NP_001140269.1
PREDICTED: uncharacterized protein LOC100837565 [Brachypodium distachyon]	735	735	100%	0.0	88%	XP_003568972.1
Transcription factor MYB86 [Aegilops tauschii]	734	734	100%	0.0	88%	EMT11047.1
MYB75 [Triticum aestivum]	733	733	100%	0.0	88%	AFH08282.1
PREDICTED: transcription factor MYB28-like isoform X1 [Setaria italica]	675	781	100%	0.0	92%	XP_004960434.1
hypothetical protein SORBIDRAFT_09g003100 [Sorghum bicolor]	665	665	84%	0.0	93%	XP_002440559.1
MYB protein [Phyllostachys edulis]	633	633	100%	0.0	76%	ADQ53510.1
hypothetical protein SORBIDRAFT_03g011640 [Sorghum bicolor]	631	631	100%	0.0	75%	XP_002457686.1

Fig. 4: Protein BLAST result

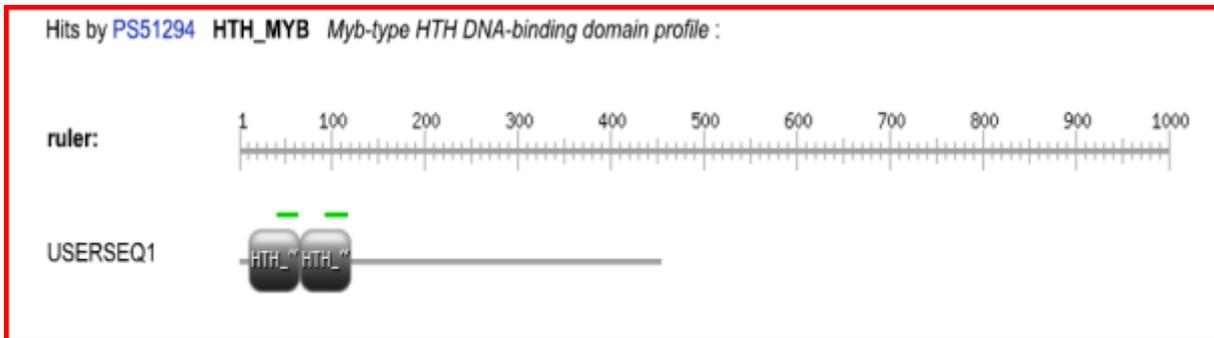


Fig. 5: PROSITE result for domain

Menu

Query CARE

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Classification

Gene...

Name of Factor...

Name of Site...

Referenc...

Motif Sampler

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+ TCGGCAACCA TAGAGTTCCT CAATCTCTGT CTGAGCCAAA CCCCCAATCA TCCATGGTGA TATAGTCCAC
AGGCGTTTGT ATGTCAACGA GTTAAAGACCA GAGTGGGAAA CCGGGTTAGT AAGTAGCAAT ATATCCAGTG
+ TATAGCTTCC CTTCGATITC CCACGACCCG CCGTTRAAAG CATRAATCCG TBAAGAAATC GTCCTCCCTA
ATATGSSAGG GAACGTAAAG GGTGGTGGTG GGGAACTTCG GTACTTAGCG ACTTCTTCAG CAGACCCGAT
+ CTGCTACCAC CCGGGAAAGG TTACTGCG
GACGATGGTG GCCCTTCTCT AATCGACCG
    
```

Motifs Found

- SUTR Py-rich stretch
- A-box
- AAGAA-motif
- ABRE
- AC-II
- ARE
- CAAT-box
- CCGTCC-box
- CGTCA-motif
- G-Box
- G-box
- GA-motif
- GAG-motif
- GARE-motif
- GATA-motif
- GCN4_motif
- GT1-motif

Fig. 6: ABRE motif in promoter of *Myb 16* of *Oryza sativa*

References

1. Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K. Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *Plant Cell*. 1997; 9:1859-1868.
2. Agarwal PK, Jha B. Transcription Factors in Plants and ABA Dependent and Independent Abiotic Stress Signalling. *Biol. Plant*. 2010; 54:201-212.
3. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. "Basic local alignment search tool." *J. Mol. Biol.* 1990; 215:403-410.
4. Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, de Castro E et al. ExPASy: SIB bioinformatics resource portal, *Nucleic Acids Res.* 2012; 40(W1):W597-W603.
5. Chaves MM, Oliveira MM. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany*. 2004; 55:2365-2384.
6. Divya GM. Growth and instability analysis of finger millet crop in Karnataka (Master's thesis, University of Agricultural Sciences, Bengaluru, India), 2011.
7. Kotschi J. Coping with Climate Change, and the Role of Agrobiodiversity. Conference on International Agricultural Research for Development. Tropentag 2006 University of Bonn, 2006.
8. Kumari S, Singh SK. Regulation of ABA Homeostasis in Plants during Stress. *Indian Research Journal of Genetics and Biotech.* 2018; 10(2):208-221.
9. Kumari S, Sharma S, Kumar A, Lohani P. Role of abscisic acid in regulating the expression of *EcMyb* gene for drought stress tolerance in *Eleusine coracana*. *Journal of Environment & Biotechnology Res.* 2017; 6(1):137-145
10. Lescot M, Déhais P, Thijs G et al. Plant care, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 2002; 30(1):325-327. doi:10.1093/nar/30.1.325
11. Letunic I, Doerks T, Bork P. SMART: recent updates, new developments and status in *Nucleic Acids Res.* 2015; 43 (Database issue):D257-D260. doi:10.1093/nar/gku949
12. Pradhan A, Nag SK, Patil SK. Dietary management of finger millet (*Eleusine coracana* L. Gaerth) controls diabetes. *Current Science*. 2010; 98:6.
13. Shinozaki K, Yamaguchi-Shinozaki K. Molecular responses to drought and cold stress. *Curr. Opin Biotechnol.* 1996; 7:161-167.
14. Sigrist CJA, de Castro E, Cerutti L, Cuče BA, Hulo N, Bridge A et al. New and continuing developments at PROSITE *Nucleic Acids Res.* 2012. doi: 10.1093/nar/gks1067 PubMed:23161676
15. Udvardi MK, Kakar K, Wandrey M, Montanri O, Murray J, Andraiankaja A et al. Legume transcription factors: global regulators of plant development and response to the environment. *Plant Physiology*. 2007; 144:538-549.
16. Wang XQ. G-protein regulation of ion channels and abscisic acid signalling in Arabidopsis guard cells. *Science*. 2001; 292:2070-2072.
17. Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G et al. The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. *Plant Molecular Biology*. 2006; 60:107-124.