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Insilco gene characterisation and Promoter analysis of drought inducible *MYB* gene from *Eleucine 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 is 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.

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 coracan*, 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

using online ExPASy tools in to six possible reading frames (Artimo *et al.*, 2012) [4]. The longest reading frame was 428 amino acids (Fig. 3) that had no internal stop codon, used as a true peptide corresponding to the ORF.

The isolated gene was true MYB transcription was ensured by looking their homology to the MYB gene from heterologous species. The protein blast was performed to predict their homology and it was observed that all the significant alignment to the homologous gene was MYB gene (Fig. 4) (Altschul *et al.*, 1990) [3]. The result was confirmed with the presence of DNA binding domain and helix turn helix (HTH) domain in the isolated MYB gene by PROSITE and SMART online tools (Sigrist *et al.*, 2012; Letunic *et al.*, 2015) [14, 11] (Fig. 5).

As in the previous studies it was observed that MYB gene expressed under the influence of ABA. The expression of any gene is regulated by the promoter. So, the responses of any gene are highly depending upon the type of response element present in the promoter apart from the core promoter. The promoter analysis of MYB gene of *Eleusine coracana* was performed through the analysis of homologous MYB gene in *Oryza sativa*. The ORF of MYB gene was submitted to online tool UNIPROT BLAST, the top hit was MYB 16 belongs to *Oryza sativa* with 85 % identity. The sequence of MYB 16 and chromosome of *Oryza sativa* both were downloaded from NCBI and located the sequence of MYB 16 on complete chromosome and then 1.5 kb sequence upstream to the located sequence was selected and submitted to online tool Plant Care for promoter analysis (Lescot *et al.*, 2002) [10]. The ABREs (ABA responsive elements) were observed over promoter apart from the core promoter motifs (Fig. 6). The presence of ABRE on the promoter region confirmed their activities under ABA. However, the upregulation of reporter gene associated with the isolated promoter in the heterologous system will further validate the results.

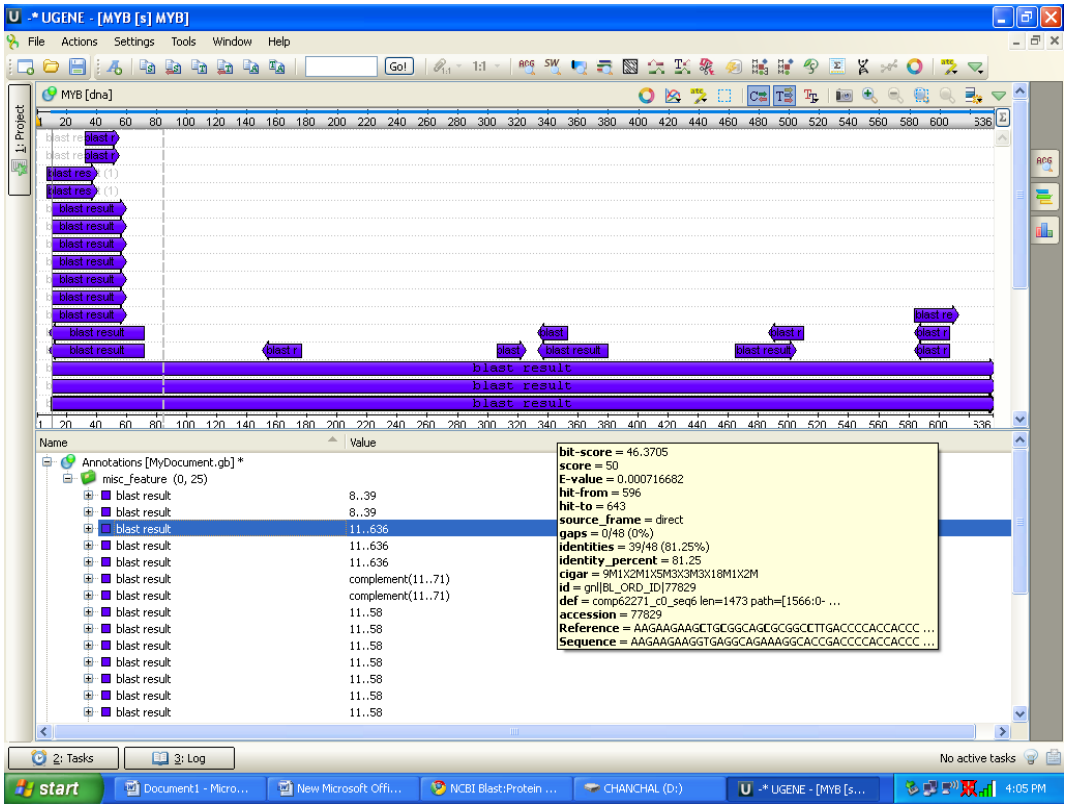

Fig. 1: UGENE BLAST result of partial EcMyb transcript with transcriptome data of *Eleusine coracana*

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ATGGGGAGGCATTCTTGCTGTTACAAGCAGAAGTTGAGAAAGGGGCTCTGGTCTCCTGAGGAAGATGAGAAACTTATGAATCACATAACAAAAC
ATGGCCATGGCTGCTGGAGCTCTGTCCCTAAACTAGCAGGGCTCCAAAGGTGCGGAAAGAGCTGCAGGCTCAGGTGGATAAACTACCTGAGGCC
GGATCTCAAGAGAGGGGCATTTTCTCAGGAGGAAGAGGACCTCATCATTGAGCTGCATGCTGTCCTTGGGAACAGGTGGTCCCAAATCGCAGCA
CAGCTGCCTGGTAGGACTGATAATGAGATCAAGAATCTGTGGAACTCCTGCATCAAGAAGAAGCTGAGGCAGAAAGGAATTGACCCCAACACCC
ACAAGCCCCTCGCCAAGGCTGATAGAAGTGGAGCAGCTCCCACAATCAGCACCGAGAGAACCTCAGGATCCAGTGATGCCAACCCGTCAAGCAC
TGGTGCTCTGGGGAACTTAAGCCACCTCCTCAGCGAGACGGCCCAATCATCAATGCTCCTGCCAGTGTATGACAAGAATTGTGCTGAAACTCCA
AACTTGGCACGCCCTAAGGTGCCACCAAAGGAGTTATTTCTGGACCAGCTTGCTGCTGGCCATGAGAGCCCATCAACCTGCCGCTCATCAGGAC
CGACTCTGTATTTCCCTTTCCATCAGCCATTGGGTTATAGCAGTGAAAGTGGCAGTGGCGATGGTGCAAATATGAATTCACTATGGTTCAACCA
GAGTGACTTCAACTGCAGCACAATTTCTACCATTATGCCACCAGTTTCACCATCAGCCCTCTCAACGTCAATGGGGCTGAATCTCCCACCAGAT
AATCCTCGACATGTGGGCATTGGCAATGCCCCGGTAGACTCATTCTACTGGGATGGTACTAATCCAAGCAGCAGTAGCAGTACTGGGAGCAGGG
GAAGCAACAGTATGGGATTCGAGCCACAGAGCACAAGCTCGATTTTGGAGAACAGTGTATTTCCATGGACAGACATTGGTCAAGAAAAAGATAC
TAGAAGTCAATTAGTTGAGGAACTTAAGTGGCCTGACTTGCTCCATGGAACCTTTGCTGAGACGACCACAACCATGCAGAACCAAAGCCAATCT
CTCTACGATGACGTGATTAAGGCAGAGTGCCAATTCAACATGGAAGGGATATGTGCTTCTTGGTTTCAGAATCAACAACCGCAGCAACAGCTGC
AGGCAGCACCAGACATGTATGACAAGGACTTGCAAAAATGCAACTGTCTTTTGAGAATATCTAG
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Fig. 2: The ORF of Myb gene from *Eleusine coracana*

MGRHSCCYKQKLRKGLWSPEEDEKLMNHITKHGHGCWSSVPKLAGLQRCGKSCRLRWINYLRPDLKRGAFSQEEEDLIIELHAVLGNRWSQIAA
QLPGRTDNEIKNLWNSCIKKKLRQKGIDPNTHKPLAKADRSGAAPTISTERTSGSSDANPSSTGALGNLSHLLSETAQSSMLLPVYDKNCAETP
NLARPKVPPKELFLDQLAAGHESPSTCRSSGPTLYFPFHQPLGYSSESGSGDGANMNSLWFNQSDFNCSTISTIMPPVSPSALSTSMGLNLPPD
NPRHVGIGNAPVDSFYWDGTNPSSSSSTGSRGSNSMGFEPQSTSSILENSVFPWTDIGQEKDTRSQLVEELKWPDLLHGTFAETTTTMQNQSQS
LYDDVIKAECQFNMEGICASWFQNQQPQQQLQAAPDMYDKDLQRMQLSFENI

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

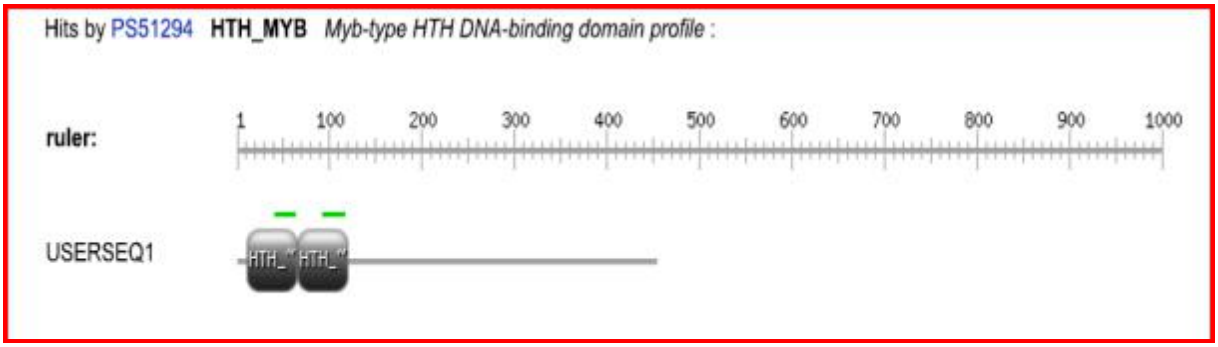

Fig. 5: PROSITE result for domain

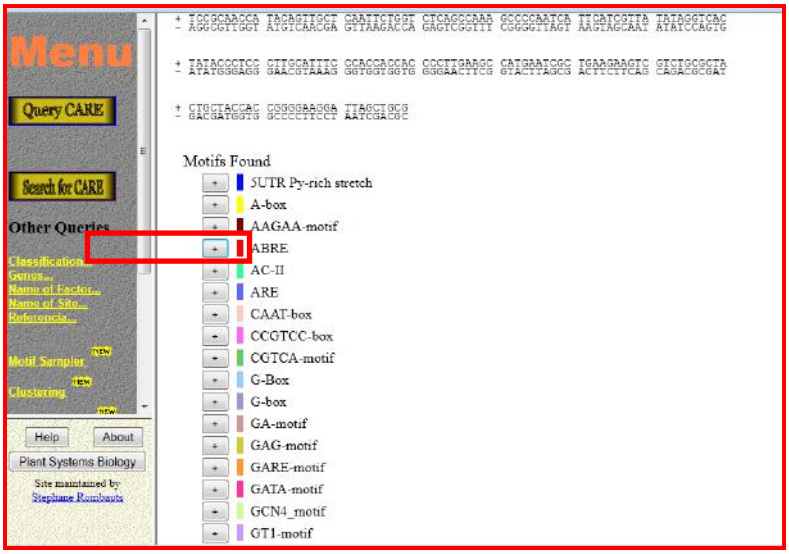

Fig. 6: ABRE motif in promotor of *Myb 16* of *Oryza sativa*

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