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CLONING OF NOVEL *BEMYB680* TRANSCRIPTIONAL FACTOR FROM *BAMBUSA EMEIENSIS*; CANVASSING TRANSACTIVATION ANALYSIS AND GENE EXPRESSION PROFILING IN THE PANORAMA OF DIFFERENT ABIOTIC STRESSES

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ABSTRACT

In this study *MYB* family related transcription factor (TF) was cloned and characterized from *Bambusa emeiensis* and tentatively named as "*BeMYB680*". We selected the TFs from bamboo because of its potentials for future improvement in terms of transgenic plants. Bamboo is fast-growing species and provides a very good podium because of its adoptable genomics to harsh climatic conditions. The *BeMYB680* was sequenced (Liuhe Gene Technology Co., Ltd, Beijing) and submitted to NCBI GenBank under accession No. (MG763924). *BeMYB680* have 1146bp open reading frame (ORF) that encodes a protein of 381 amino acids. . Transactivation analysis and subcellular localization assay revealed that *BeMYB680* is a transcriptional activator and localized in the nucleus. *BeMYB680* was up-regulated when treated with ABA, PEG, NaCl, and H_2O_2; while down-regulated with Na_2SO_4 indicating its key role in abiotic stresses particularly to the best of our knowledge it is first time to clone *BeMYB680* form *Bambusa emeiensis.*

Keywords: *BeMYB680*, Bioinformatics, Transgenic, Abiotic stress, *Bambusa emeiensis*.

INTRODUCTION

Flora is often exposed to hostile ecological conditions, such as abiotic and biotic stresses. These abiotic stresses include high salinity, extreme temperature, and drought which undesirably disturb plant metabolic activities. Plants in response to these abiotic strains acclimatize to the prevailing environmental conditions by developing various response mechanisms. The enactment of various response mechanisms be determined by innumerable transcription factors (TFs), such as NAC, WRKY, and *MYB* (Wei *et al.*, 2017). *MYB* transcriptional factors play a major role in plant morphogenesis such as specialized cells, organ, and leaf development by enhancing the metabolic activities of plants against abiotic stresses (Xu *et al.*, 2015). *MYB* superfamily is comprised of four subdivisions. i.e. *MYB1R, R2R3-MYB, MYB3R,* and *4R-MYB* (Liu *et al*., (2017). Various studies have been conducted on different plants in order to know the response of plants against abiotic stresses having different *MYB* transcriptional factors; such as *Arabidopsis thaliana*, tobacco, cotton, wheat, and rice (Chen *et al.*, 2015; Li *et al.*, 2015). However, a diminutive research has been carried out on Bamboo species especially on *Bambusa emeiensis.* The *AtMYB96* was isolated from Arabidopsis and its functional analysis has highlighted that it plays a major role in plants tolerance and boosts drought forbearance by activating the fraternize pathways of ABA and auxin (Guo *et al.*, 2013). *AtMYB44* and *AtMYB60* advances the plants tolerance against drought in *Arabidopsis thaliana* and *Hibiscus Sabdariffa L.* (Chen *et al.*, 2013; Mohamed *et al.*, 2017). Meanwhile, *TaMYBsdul, OsMYB91*, and *OsMYB511* are playing an important role in improve plants ability to encounter salt stress (Rahaie *et al.*, 2010; Zhu *et al.*, 2015). While *AtMYB14* and *OsMYB30* are involved to improve plants ability to stand still against cold environmental conditions (Lv *et al.*, 2017). The *MYB* Transcriptional factors are also playing its role in the cell wall development and also improve fibre quality in the plants (Guo *et al.*, 2017).

In the present research work we have cloned and functionally analysed a novel transcriptional factor *BeMYB680* from *Bambusa emeiensis*. Presently, limited information is available on the genetic resources of *Bambusa emeiensis* because of its incomplete genome sequence. Thus, identification and characterization of genes related to abiotic stresses from *Bambusa emeiensis* is a need of an hour. Bamboo is fast-growing species and it plays the anchor role in China's economy and provides very good raw material for pulp and paper industry. Its adaptability to harsh climatic conditions is really a hallmark. Therefore, investigation and comprehensive studies on the genomic and molecular level will be very significant; as to know the mechanisms of adaptability for these unique characteristics of bamboo. The results provide a very good platform for getting plants with high tolerance to abiotic stresses. This study increases our knowledge of the crosstalk between the abiotic stress and *BeMYB680.*

MATERIALS AND METHODS

Cloning of a *BeMYB680* from *Bambusa emeiensis:* A novel gene *"BeMYB680"* was isolated by using the information from the genome database of moso bamboo and transcriptome database of the *Bambusa emeiensis.* Based on the obtained DNA sequence a pair of gene-specific primers (Table.1) was designed. To amplify the full length *BeMYB680*, The leaf, shoot, and root tissues were collected from *Bambusa emeiensis*. The RNA was isolated from the leaf, stem and root tissues using instructions provided by manufacturer (Omega BIO-TE, US). The prime Script^{tm} RT reagent kit (RR047A, TaKaRa Dalian, China) and oligo T (18) primer were used to prepare cDNA as per the directions given with the kit. 25µL PCR mixture was carried for the cloning of the full length sequence of *BeMYB680*. The reaction mixture containing 12.5µL of 2XGC PCR buffer (TaKaRa Dalian, China), 0.2µL of polymerase (5U/µl) (TaKaRa Dalian, China), 4µL of dNTPs (2.5mM each), 1µL of each of forward and reverse primers (10µM), 5µL of cDNA as a template and ddH_2O was added to maintain the volume of 25µL. The PCR was adjusted as; the initial denaturation achieved at 95 ℃ for 3 min, followed by 34 cycles of 95 ℃ for 30 sec, 58 ℃ for 30 sec, 72 ℃ for 1:30 min, and 72 ℃ were used for 10 min as final extension. The amplified PCR products were separated on 1 % agarose gel stained with gold-view. Positive PCR products were extracted from agarose gel using universal DNA purification kit (TaKaRa Dalian, China). Purified PCR product was cloned into the pMD19-T vector (TaKaRa Dalian, China). The positive colonies were picked and sent for sequencing to company for further confirmation.

Bioinformatics analysis: The conserved *MYB* domain was searched using MEME (http://memesuite.org/tools/meme). Alignment of relevant sequences was performed using DNAMAN (version.7) and the phylogenetic tree was constructed using software MEGA (version.7) (Kumar *et al*., 2016). The domain structure modal were constructed using phyre2.

Y1H screening of the *BeMYB680:* The Y1H screening was performed using yeast strain EGY48 and PEG202 (Zhu *et al.*, 2003). The coding sequence of the *BeMYB680* was amplified by PCR using a pair of primers i.e. (forward and reverse primers) having EcoRI and XhoI restriction sites (Table.1). The PCR products were ligated into pEG202 vector after digestion by EcoRI and XhoI. The pEG202 empty vector was used as the negative control. The derived plasmids were integrated into yeast strain EGY48. The transformed yeast was confirmed by the PCR (Lopato *et al.*, 2006). The yeast strain was streaked on synthetic dropout (SD) plates (SD /-Ura), (SD /- Ura-His) and (SD /- Ura-His- X-Gal) incubated at 30 ℃ for 3 days.

Essay of *BeMYB680* through Subcellular localization: The open reading frame (ORF) of *BeMYB680* was amplified using PCR with specific primers of *BeMYB680*-Kpnl-F and *BeMYB680*-EcoRI-R (Table.1). The PCR product was cloned into the pTEX-GFP vector to generate a *BeMYB680*: GFP in-frame fusion protein. Subsequently, the onion epidermal cells were plated on the MS medium containing mannitol (0.2mol/L) and sorbitol (0.2mol/L) and incubated into the dark for 4 hours. *BeMYB680*:GFP and green fluorescent protein (GFP) empty vector were introduced into onion (*Allium cepa*) epidermal cells using the biolistic transformation system (PDS-1000/He, Bio-Rad, and Hercules, USA). Each plate was bombarded twice at particle travel distances of 9cm. All bombardments were performed at a pressure of 1350 psi, 80µg of gold were used per each bombardment. After the bombardment, the onion tissue was incubated into the dark for 16 hours followed by the DAPI staining and fluorescence was observed under a fluorescent microscope (LeicaTCSSP8) (Duan *et al.*, 2014).

Plant material and stress treatment: The 10 inches shoots were collected from wild-type *Bambusa emeiensis.* These shoots were treated with variable concentrations of PEG, H_2O_2, NaCl, Na_2SO_4, and ABA for abiotic stress analysis. Several abiotic stress treatments were applied by immersing the shoots in 250µM solution of ABA, 20% solution of PEG, 250mM solution of NaCl, 15mM solution of H_2O_2 and 50mM solution of Na_2SO_4. At different time interval i.e. 0, 3, 6, 12 and 24hrs. Three biological replicates of samples were collected. The samples were instantly frozen in liquid nitrogen and then stored at -80 ℃ till total RNA extraction.

***BeMYB680* expression analysis through Quantitative Real-time PCR (qRT-PCR):** The stored samples were used for full RNA extraction and cDNA production. The qRT-PCR primers were designed as given in table 1. qRT-PCR was carried out for the analysis of BeYMY680 expression in response to stress treatments. The cDNA was used as the template for amplification. The qRT-PCR was performed using SuperReal PreMix Plus (FP205-02, SYBR Green, and Tiangen) on a CFX Connect ™Optics Module (Bio-Rad) Real-Time PCR System. The PCR conditions were adjusted as follows; 95 ℃ for 15min (initial denaturation), followed by 39 cycles at 95 ℃ for 10 s, 60℃ for 30 sec, the fluorescent signals were recorded at 60℃ for 30 sec. A melting curve was constructed in order to determine the specificity of each PCR primer by maintaining the reaction at 95℃ for 0.5sec, cooling to 65℃ for 0.05 sec. For internal control Tubulin gene expression level was used. The experiments

were performed in triplicate. 2−ΔΔCt method was used for the analysis of gene expression (Livak and Schmittgen, 2001).

Statistical analysis: Three biological replicates of each experiment were performed, and the means were calculated as the average of three replicates, the data are shown here are the means±SD. The SPSS (Chicago, IL, US) was used for Statistical analyses. The t-test was performed to analyse the significant difference. Asterisks represent significant difference of expression level after the the treatments at different time interval ($^{*}p. < 0.05$; $^{**}p. < 0.01$; $^{***}p. < 0.001$).

RESULTS

Cloning and bioinformatics analysis: The full-length amplification of open reading frame (ORF) of *BeMYB680* revealed that the ORF is comprised of 1146bp which translates into a protein with a projected comparative molecular mass of 40.97185kDa. Multiple Sequence alignment (DNAman software version 7.0) shown that the *BeMYB680* had a characteristic *MYB* domain structure arrangement dependable with *MYB* conserved domain features. The *BeMYB680* has a typical *MYB* domain structure it contained 6 α-helix (Fig.3.1d). The phylogenetic analysis revealed that *BeMYB680* belongs to the *MYB* family from *Bambusa emeiensis* as shown in (Fig.3.1c). This novel gene *BeMYB680* has similarity with *atMYB46*-83% (ACCESSION:Z95770), *AtMYB61*-80% (ACCESSION:KJ137595), *AtMYB86*-79% (ACCESSION:Z95808), *HvMYB33*-77% (ACCESSION:EU550610) and *AtMYB26*-74% (ACCESSION:Z95749) as shown in (Fig.3.1b). *BeMYB680* demonstrated maximum homology with transcriptional factors which paly key role in abiotic stresses, such as salinity, drought and lignin biosynthesis in *Arabidopsis thaliana* (Zhong *et al*., 2007).

Yeast one-hybrid screening of the *BeMYB680:* The transformed yeast (EYG-48-pEG202-*BeMYB680*), pEG202 empty vector (EYG-48-pEG202) and yeast (EYG-48) were plated on (SD /-Ura), (SD /-Ura -His) and (SD /-Ura-His-X-gal) medium. The positive transformation was analysed by the PCR (Fig.3.2b). The transformed yeast (EYG-48-pEG202-*BeMYB680*) successfully grows on all three kinds of medium and stained blue in the presence of X-gal. The EYG-48 only grows on (SD /-Ura) medium; meanwhile on other two mediums growth were not observed. The pEG202 empty vector (EYG-48- pEG202) showed the normal growth but the blue staining was not observed (Fig.3.2a). The outcome shows that *BeMYB680* is a transcriptional activator.

Subcellular localization of *BeMYB680:* Our data revealed that *BeMYB680* is found in the nucleus of the cell (Fig.3.3).

The expression pattern analysis of *BeMYB680*: The *BeMYB680* TF expression profiling of the *BeMYB680* revealed both up and downward trends in its expression toward various kinds of stress inducers at different interval of times (Fig.3.4). In response to the ABA treatment the mRNA accumulation of the *BeMYB680* slightly changed up to the 3hrs; during 6, 12 and 24hrs the expression level goes down abruptly to very lower level (Fig.3.4a) and a slight change in the expression level was observed when exposed to the PEG and H_2O_2 during 0, 3, 6, and 12hrs; with immediateup-regulation of *BeMYB680* at 24 hours (Fig.3.4b, Fig.3.4d). While in response to NaCl treatment, *BeMYB680* was rapidly induced and reached the peak during first 3 hours. Then, the mRNA accumulation started declining with passage of time (Fig.3.4c). The mRNA accumulation in response to Na_2SO_4 was decreased very quickly during first 3rd and at 6th hours , its expression level increased followed by gradual decrease during 12th and 24th hours . (Fig.3.4e). Collectively the *BeMYB680* is up-regulated significantly when treated with ABA, PEG, NaCl, and H_2O_2 and down-regulated significantly when treated with Na_2SO_4.

Table 1. Primers used for *MYB680* TF for various experiments.

BEMYB680	**PRIMERS DIRECTION**	**VARIOUS SEQUENCES OF PRIMERS USED**	**ENZYME SITE**
Cloning primers	F	GCTCTAGAGCATGAGGAAGCCGGAGTGCCCGG	Xba-I
	R	GGGGTACCCCTCATTCAACTTGGAAATCAACC	Kpn-I
Sub-cellular localization primers	F	GGGGTACCCCGATGAGGAAGCCGGAGTGCCCGG	Kpn-I
	R	CCGGAATTCCGGTCATTCAACTTGGAAATCAACC	EcoR-I
Yeast experiment primers	F	CGGAATTCCGATGAGGAAGCCGGAGTGC	EcoR-I
	R	CCGCTCGAGCGGTCATTCAACTTGGAAA	Xho-I
Tubulin Primers	F	GCCGTGAATCTCATCCCCTT	
	R	TTGTTCTTGGCATCCCACAT	
RT-PCR primers	F	GTTCAAGCAAGTTGCAGCAT	
	R	TTGTTGGATGATGCCATTAT	

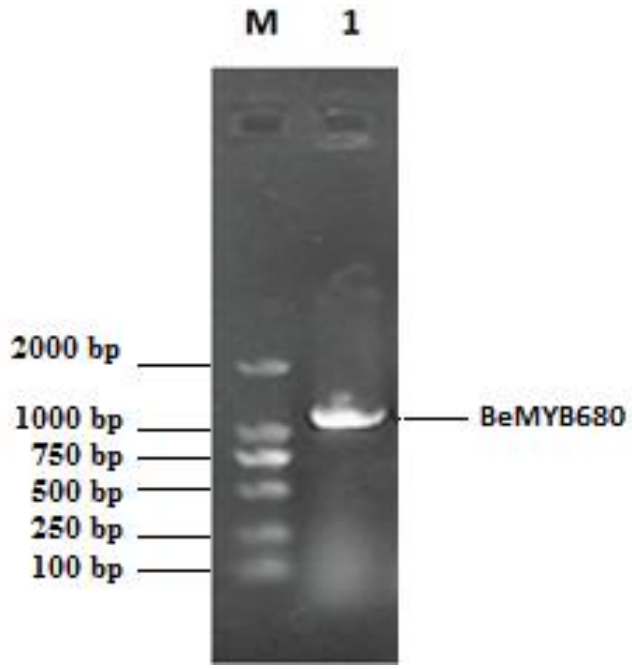

Figure.3.1a. The PCR amplification of *BeMYB680*, the PCR product was analyzed by the Gel Electrophoresis.

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MYB680     MRKPECPAMKGSGGGAAAGPCGGNGINAAAAVKLRKGLWSPEEDKKLVAYMLRSGQGSWSDVARNAG.........LQRCGKSCRLRWINYLRPDLKRGA     91
AtMYB46    MRKPEVAIAAST.................HQVRKMKKGLWSPEEDSKLMQYMLSNGQGCWSDVAKNAG.........LQRCGKSCRLRWINYLRPDLKRGA     75
AtMYB61    MGRHS..CCYKQ.....................KLRKGLWSPEEDKKLLTHITNHGHGCWSSVPKLAG.........LQRCGKSCRLRWINYLRPDLKRGA     69
AtMYB86    MGRHS..CCFKQ.....................KLRKGLWSPEEDKKLLNYITRHGHGCWSSVPKLAG.........LQRCGKSCRLRWINYLRPDLKRGA     69
HvMyb33    MGRPSSGAVGQP.....................KVRKGLWSPEEDKKLYNHIIRHGVGCWSSVPRLAA.........LNRCGKSCRLRWINYLRPDLKRGC     71
AtMYB26    MGHHS..CCNKQ.....................KVKKGLWSPEEDKKLINYINSYGHGCWSSVPKHAGTYTHIHGFCLQRCGKSCRLRWINYLRPDLKRGS     78
Consensus  m                                 k   glwspeed kl       g g ws v   a          l rcgkscrlrwinylrpdlkrg

MYB680     FSPQPEELIVNLHAILGNRWSQIAARLPGRTDNEIKNFWNSTIKKKLKNSASSSPATTQCASLPEPKLAVDGGSSCLDLAGLEDGAHHAIKSMWVDSSSS    191
AtMYB46    FSPQPEDLIIRFHSILGNRWSQIAARLPGRTDNEIKNFWNSTIKKKLKKMSDTSNLINNSSSSPNTAS.........................DSSSN    148
AtMYB61    FSPEPENLIVELHAVLGNRWSQIASRLPGRTDNEIKNLWNSSIKKKLKQRGIDPNTHKPISEVESFSDKDKPTTSNNKRSGNDHKSPSSSSATNQDFFLE    169
AtMYB86    FSQDPESLIIELHAALGNRWSQIATRLPGRTDNEIKNFWNSCLKKKLRRKGIDPTTHKPLITNELQS....LNVIDQKLTSSEVVKSTGSINNLHDQSMV    165
HvMyb33    FSQQPEDHIVALHQILGNRWSQIASHLPGRTDNEIKNFWNSCIKKKLRQQGIDPATHKPMASADTAT.....................AAAALPDAEEE    149
AtMYB26    FSPQPAALIIELHSILGNRWAQIAKHLPGRTDNEVKNFWNSSIKKKLMSHHHHGHHHHHLSSMASLLTN.LPYHNGFNPTTVDDESSRFMSNIITNTNPN    177
Consensus  fs  e   i   h  lgnrw qia  lpgrtdne kn wns  kk l

MYB680     SNSSSSMQ........SRPSTMAAARSYGGLLPSPSKSAAWRSTLRRRFS...TTTHRSSKLQHCIGGGYYGSTRHGPMAMEGGGSFMGGESSVLYGVPP    280
AtMYB46    SASSLDIK........DIIGSFMSLQEQG..FVNPSLTHIQTNNPFPTGN...MISHPCNDDFTPYVDGIYGVN..............AGVQGELY.FP.    219
AtMYB61    RPSDLSDYFGFQKLNFNSNLGLSVTTDSSLCSMIPPQFSPGNMVGSVLQTPVCVKPSISLPPDNNSSSPISGGDHVKLAAPNWEFQTNNNNTSNFFDNGG    269
AtMYB86    VSSQQGPWW......FPANT.TTTNQNSAFCFSSSNTTTVSDQIVSLISS...MSTSSSPTPMTSNFSPAPN.NWEQLNYCNTVPSQSNSIYSAFFGN.Q    253
HvMyb33    ...DRKP..............LCPAVDGSLVPKQPAVFDPFPLCVDYGAG.......FAEELGAANAAALYG....QLCGGKEVADDDAG.....FGAAD    216
AtMYB26    FITPSHLSLP..SPHVMTPLMFPTSREGDFKFLTTNNPNQSHHHDNNHYNN.LDILSPTPTINNHHQPSLSSCPHDNNLQWPALPDFPASTIS...GFQE    271
Consensus

MYB680     LSEPIAVEDQITIMASSNNPKNNTAETTTLSSNNGSNITDNSSNSNSNKNINISLVSNSVVYWVGHQQQQQHMSRNVMGEWDLEELMKDVSSLPFLDFQV    380
AtMYB46    ...PLECEEGDWYNANINN......HLDELNTNGSGNAPEG..............................MRPVEEFWDLDQLMNTEVPSFYFNFKQ    278
AtMYB61    FSWSIPNSSTSSSQVKPNHNFE...EIK.WSEYLNTPFFIGSTVQSQTSQPIYIKSETDYLANVSNMTDPWSQNENLGTTETSDVFSKDLQRMA.VSFGQ    364
AtMYB86    YTEASQTMNNNNPLVDQHHHHQ...DMKSWASEILHYTEHNQSSETVIEAEVKPDIANYYWRSASSSSSPNQEAATLLHDANVEVYGKNLQKLNNMVFDQ    350
HvMyb33    YSCVLD........VSENLGYG...ESSSNSSNWNYGGEVGSVLDGEVPHWAKAEPAFAEMERQQQHS.PAEQKLSLPCQEQSLLASFDFNLELEPYF..    302
AtMYB26    TLQDYDDANKLNVFVTPFNDN....AKKLLCGEVLEGKVLSSSSPISQDHGLFLPTTYNFQMTSTSDHQHHHRVDSYINHMIIPSSSSSSPISCGQYVIT    367
Consensus

MYB680     E                                                                                                      381
AtMYB46    S                                                                                                      279
AtMYB61    S                                                                                                      365
AtMYB86    S                                                                                                      351
HvMyb33    .                                                                                                      302
AtMYB26    .                                                                                                      367
Consensus
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Figure.3.1b. Multiple sequence alignment analysis of *BeMYB680* with the homologs proteins from *Arabidopsis thaliana* (*AtMYB46, AtMYB26, AtMYB61, AtMYB86*) and barley (*HvMYB26*).

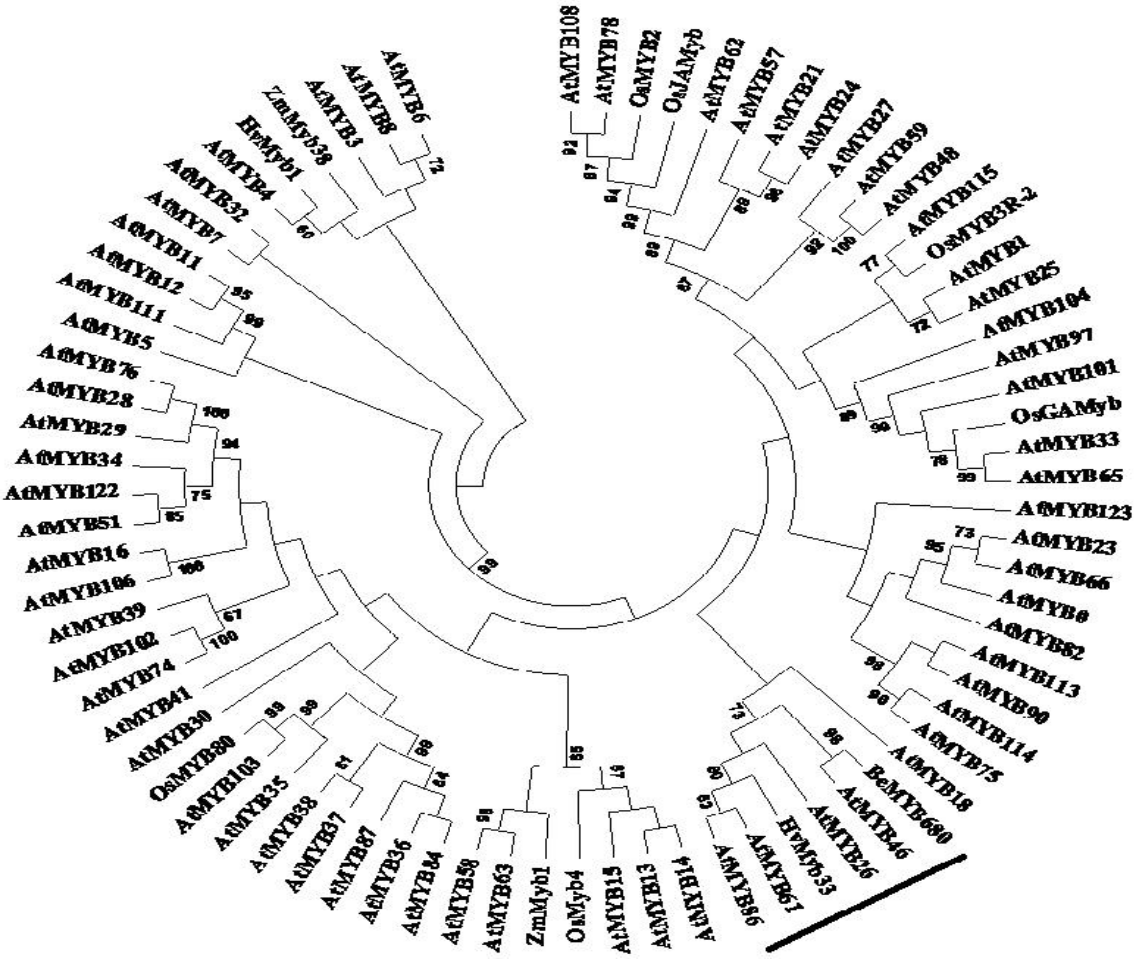

Figure.3.1c. The MEGA7 software was used for phylogenetic tree analysis of designated *MYB* (TFs) protein sequences from altered plant species. *AtMYB46* is the most similar sequence of *BeMYB680* as shown in the figure.

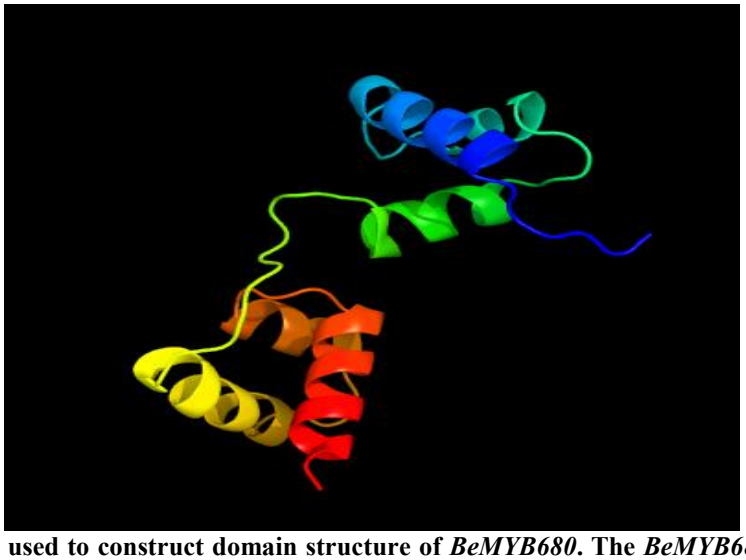

Figure.3.1d. phyre2 was used to construct domain structure of *BeMYB680*. The *BeMYB680* domain contains 6 α-helix without β-sheets.

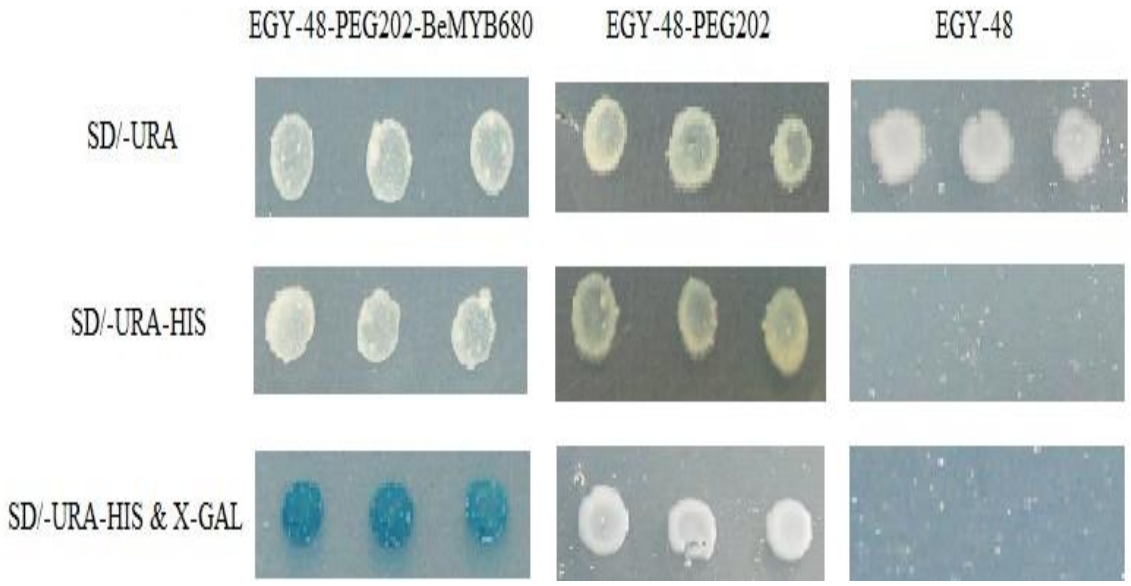

Figure.3.2a. Transactivation assay of *BeMYB680*. The blue staining indicates the transactivation of EYG-48-PEG202- *BeMYB680* in comparison with EYG-48-PEG202.

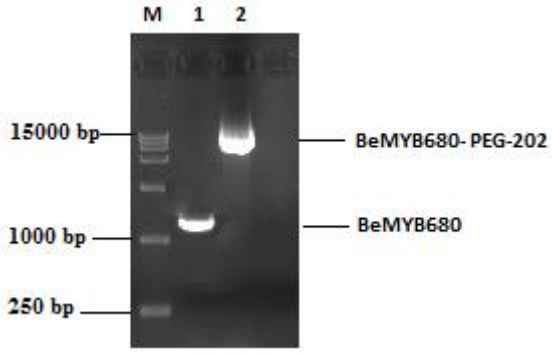

Figure.3.2b. PCR confirmation of ligated *BeMYB680* with Vector pEG-202. Afterward PCR verification of *BeMYB680* from yeast EYG-48.

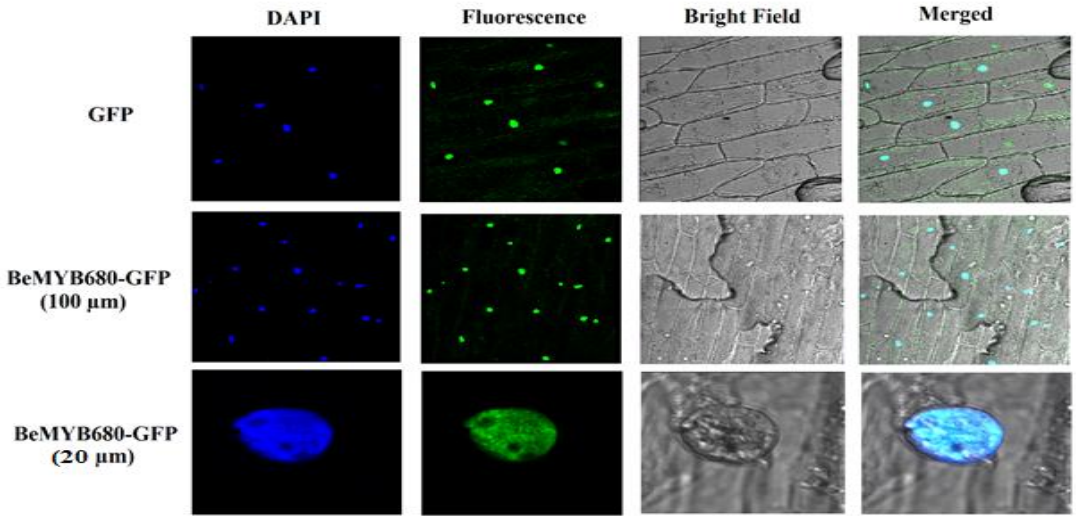

Figure.3.3. Onion epidermal cells were used for the pTEX-*BeMYB680*-GFP through bombardment for purpose of Subcellular localization. The pTEX-*BeMYB680*-GFP and empty vector pTEX-GFP were applied to Onion epidermal cells; which were afterward analyzed through a fluorescent microscope.

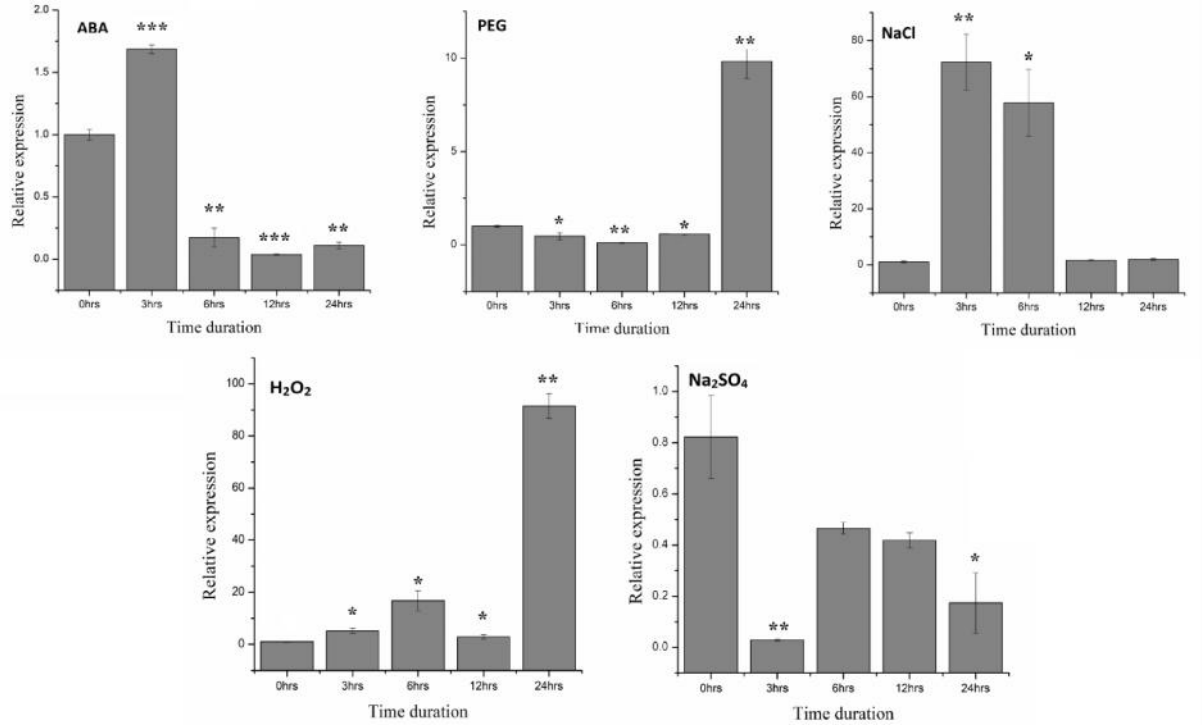

Figure.3.4. Expression of *BeMYB680* under the treatment of ABA, PEG, NaCl, H_2O_2, and Na_2SO_4. The expression level was analyzed by qRT-PCR. Data are means ± SD calculated from three replicates. The bar indicates standard deviation. Asterisks indicate the significance level after treatments were applied at different time interval. ($^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$).

DISCUSSION

MYB TFs have been extensively studied in terms of their biotic and abiotic stress tolerance (Mohamed *et al.*, 2017) such as salinity, drought, cold (Zhang *et al.*, 2012), flower development (Mohamed *et al.*, 2017), and embryogenesis (Wang *et al.*, 2009) in different plants especially in *Arabidopsis thaliana*, wheat, rice and poplar etc.. The comparative analysis resulted six very similar TFs i.e., *atMYB18, atMYB86, atMYB61, HvMYB33*, and *atMYB26*. (Figure.1b). which not only play their role in abiotic stresses but in the cellulose and fiber quality enhancement (Prabu and Prasad, 2012; Schmidt *et al.*, 2013). Therefore we hypothesized that *BeMYB680* will might have important roles in abiotic stresses as demonstrated in the current study . It has been observed that *BeMYB680* is significantly expressed when treated with different abiotic stresses i.e. ABA, PEG, NaCl, H_2O_2, and Na_2So_4 our this resulted is in the line with previous studies of TFs (Huang *et al.*, 2015; Chen *et al.*, 2015). The Furthermore, the sub-cellular localization of *BeMYB680*-GFP showed that it location in the nucleus of the onion epidermal cells. (Fig.4) our results are consistent with previous studies of subcellular localization poplar, rice and citrus(Yang *et al.*, 2014, 2017; Liu *et al.*, 2016). .Our results from Y1H very much in line with the results previously achieved by (Liu *et al*., 2015; Ramalingam *et al.*, 2015) Therefore, collectively our data revealed that *BeMYB680* TF is one of the key gene to play an important role in abiotic stresses. The results proposed that the *BeMYB680* TF is involved in retorts to salinity, which is in covenant to the networks of transcription factors regarding abiotic stress responses in Arabidopsis, *Medicago truncatula* and *Leymus chinensis* (Cheng *et al.*, 2013; Zhao *et al.*, 2016). *AtMYB44* and *AtMYB77* are two TFs which have diverse involvement in response to ABA stress (Oh *et al.*, 2011). *BeMYB680* was down regulated when treated with ABA during time interval which is of high significance. *PAP1-AtMYB75* and *PAP2-AtMYB90* TFS are positively regulated when treated with H_2O_2 (Borevitz *et al.*, 2000), as observed in *BeMYB680*. *TaMyb4*, and *TaMyb5* are TFs which are greatly involved against PEG (Yanhui *et al.*, 2006); and in-line with our studies of *BeMYB680 of Bambusa emeiensis* when treated with PEG at different interval of time. Form discussion it is evident that *BeMYB680* is a key TF through which we can get high quality of plants; which have the potential to counter abiotic stresses. Our study for the first explores and provides the evidence that *BeMYB680* of *Bambusa emeiensis* is key TF responsive for abiotic stress tolerance gene furthermore provide an insight of *BeMYB680* TFs molecular structure and its response to abiotic stresses.

Conclusion: TF *BeMYB680* was cloned from *Bambusa emeiensis and found responsible for* major defence against abiotic stresses, salinity, drought, cold and lignin biosynthesis. Furthermore our data revealed that *BeMYB680* is trans-activator and localized in the nucleus of the cell and its expression profiling against abiotic stresses resulted that it was up regulated when treated with ABA, PEG, NaCl and H_2O_2, while down regulated when treated with Na_2SO_4 . The current research offers an innovative understanding of the *BeMYB680* TFs in response to abiotic stress.

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Author contributions: Muhammad Imran and Naseem Samo have contributed equally in experiment workout and research paper writing. All authors read and approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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