 doi:10.20944/preprints202208.0315.v1

MYB Transcription factor family in pearl millet: Genome-wide identification, evolutionary progression and expression analysis under abiotic stress and phytohormone treatments

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 doi:10.20944/preprints202208.0315.v1

Abstract

Transcription factors (TFs) are the regulatory proteins that act as molecular switches in controlling stress responsive gene expression. Among them MYB transcription factor family is one of the largest TF family in plants, playing a significant role in plant growth, development, phytohormone signaling and stress-responsive processes. Pearl millet (*Pennisetum glaucum L.*) is one of the most important C4 crop plant of the arid and semi-arid regions of Africa and South-east Asia for sustaining food and fodder productions. To explore the evolutionary mechanism and functional diversity of the MYB family in pearl millet, we conducted a comprehensive genome-wide survey and identified 279 MYB TFs (PgMYB) in pearl millet and distributed unevenly across seven chromosomes of pearl millet. Phylogenetic analysis of identified PgMYBs classified them into 18 subgroups and members of the same group showed a similar gene structure and conserved motif/s pattern. Further, duplication events were identified in pearl millet that indicated towards evolutionary progression and expansion of the MYB family. Transcriptome data and relative expression analysis by qRT-PCR identified differentially expressed candidate *PgMYBs* (*PgMYB2*, *PgMYB9*, *PgMYB88* and *PgMYB151*) under dehydration, salinity, heat and phytohormones (ABA, SA and MeJA) treatment. Taken together, this study provides valuable information for a prospective functional characterization of MYB family members of pearl millet and genetic improvement of crop plants.

Keywords: MYB Transcription Factors, Evolutionary progression, Pearl millet, Phytohormones, Abiotic stress

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Introduction

Environmental stresses and climate change pose a serious threat to global agricultural productivity. Plants face multiple stresses that challenge their growth and survival [1,2]. As a result, plants respond to these stress conditions, by activating various signalling pathways and by synthesis of specialized metabolites. These responses are regulated by various transcription factors encoding genes [3]. Transcription factors (TFs) bind to their cognate sites in the promoter region of the target gene and regulate the gene expression. Upon environmental stress on plants, TFs either induce or repress the expression of target genes [4,5]. Understanding the involvement of particular TF in various stress-signaling pathways will be helpful in developing stress-resistant and genetically modified (GM) crop plants with enhanced productivity. In recent times, several studies have identified and characterized various TF gene families (b-ZIP, NAC, WRKY, GRAS, THX, ERF etc.) that are known to participate in multiple stresses and involved in phytohormonal signalling pathways [6,7].

Increasing world population challenges food and nutrition security across the globe [8]. In search for the suitable staple food for overcoming such difficult scenarios, millets are one of the potential candidates. Among them, Pearl millet (*Pennisetum glaucum L.*) is a monocot plant from grass family (*Poaceae*), which is considered on 6th position in food importance after rice, wheat, maize, and sorghum all over the world. It is an annual cereal crop species, cultivated in arid and semi-arid tropical regions of Africa and South-east Asia including India [9]. It has the potential to grow in adverse climate conditions like high temperature, drought, less fertile soil and can uptake high CO_2 concentration. In addition, there are various nutritional aspects of pearl millet such as rich in protein content, carbohydrates and fibers; beside this pearl millet also contains vitamins, micronutrients such as zinc, iron, and magnesium [10]. IPMGSC (International Pearl Millet Genome Sequencing Consortium) has sequenced the whole genome of pearl millet [11] to improve crop production and abiotic stress responses. Further studies on gene family identification and characterization will be helpful in identifying stress associated genes and TFs.

MYB gene family is one of the major TF family found in both plants and animals. The discovery of first MYB gene is an oncogene found in avian myeloblastosis virus known

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as 'ʋ-MYB'. The MYB protein has a conserved signature structure i.e., DNA binding module of MYB TF with regularly interspaced three tryptophan residues to form a 3D helix-turn-helix structure which comprises 52 amino acids. Based on the adjacent repeats, MYB TFs, are classified into four groups viz. 1R-MYB, 2R-MYB (R2R3-MYB), 3R-MYB (R1R2R3-MYB), and 4R-MYB (R1R2R2R1/2). In plants, generally R2R3-MYB type proteins are seen and studies have shown that the R2R3-MYB gene evolved from R1R2R3-MYB. To date, MYB TF members have been identified in various plant species such as rice (*Oryza sativa; OsMYB*), wheat (*Triticum aestivum* L.; *TaMYB*), maize (*Zea mays*; *ZmMYB*), Arabidopsis (*Arabidopsis thaliana; AtMYB*), soybean (*Glycine max; GmMYB)* etc. [12-14]. Being a large and diverse family, MYB TF plays a vital role in biotic/abiotic stress responses, plant growth, seed and flower development, cell cycle regulation, secondary metabolites synthesis, and hormonal signaling [15]. Overexpression of *AtMYB44* and *OsMYB4* conferred tolerance to abiotic stress in Arabidopsis and rice respectively [16-18]. Similarly, overexpression of *TaMYB344* in tobacco resulted in enhanced resistance of the plants to dehydration, heat and salinity stress [19]. Tang et al. 2019, reported the role of *OsMYB6* under drought and salt stress. It was seen that *OsMYB6* transgenic plants showed enhanced tolerance compared to wild plants [20].

Phytohormones play a crucial role in various physiological processes as well as in mediating responses against biotic and abiotic stresses [21]. The levels of signal molecules such as calcium, jasmonic acid, methyl jasmonate and salicylic acid are seen to be altered in plants encountering stresses [22]. The abscisic acid (ABA) plays a vital role in plant physiological processes and is an important mediator in environmental stresses such as drought, cold, light, salinity and temperature [23]. Signal molecules jasmonates (methyl jasmonate (MeJA) and jasmonic acid (JA)) are lipid-derived molecules and play key roles in plant biotic stress responses and other biological processes. Several studies showed the upregulation of JA biosynthetic genes in roots under salt stress [24,25]. Similarly, signaling molecule salicylic acid also regulates plant metabolism and is crucial in induction of stress responses in plants [26,27].

Though MYB TF has been identified in various plant species, but there are no reports on the identification and functional analysis of MYB family in pearl millet (*P. glaucum*). Considering the relevance, this nascent study aims for genome-wide identification of

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MYB TFs in pearl millet. *In-silico* characterization and evolutionary progression of identified MYB family members were performed through analysis of motif composition, gene structure, phylogeny and synteny relationship. Moreover, Transcriptome profiling and relative expression profile of selected *PgMYBs* were evaluated under dehydration, salinity, heat-stress and phytohormone (ABA, SA and MeJA) treatments with an aim to identify stress inducible MYB TFs. Further, candidate *PgMYBs* could appear as potential tools for developing engineered plants to ensure better crop productivity.

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Materials and Methods

Identification and *sequence analysis of MYB TFs in pearl millet*

Pearl millet protein and nucleotide sequences were downloaded from genome database of pearl millet (http://cegsb.icrisat.org/ipmgsc/). The MYB family protein sequences of rice were downloaded from Oryzabase (http://rice.plantbiology.msu.edu/) [28] and GRASSIUS (Grass Regulatory Information Server) [29]. Arabidopsis and foxtail millet MYB domain protein sequences were obtained from plant genomics database Phytozome [30]. Identification of MYB in pearl millet was initiated by constructing a Hidden Markov model (HMM) profile of reference sequences (Rice, Arabidopsis and Foxtail millet) through HMMbuild program of HMMER tool v3.2 [31], followed by a HMMsearch against pearl millet proteome. The initially identified sequences were then scanned against HMM profiles obtained from Pfam site [32]. All the non-redundant hits with an expected value cut-off of (0.01) were retained and redundant sequences were removed. Further, putative MYB genes were confirmed for the presence of MYB by using HMMscan (https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan), SMART (Simple Modular Architecture Research Tool) (http://smart.embl-heidelberg.de/) and CDD (Conserved Domains Database) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Prediction of theoretical isoelectric point, protein molecular weight, instability index, GRAVY and aliphatic index for each protein was checked by using Prot-Param tool (https://web.expasy.org/protparam/). Further, TMHMM - 2.0 tool [33] was employed for the location of transmembrane helices in PgMYB sequences. WoLF PSORT [34] was used for predicting subcellular localization sites in PgMYB sequences.

Chromosomal localization, gene structure and motif analysis

The PgMYBs genes were mapped to pearl millet chromosomes according to their physical positions (bp) with help of MapInspect software v1.0 (http://mapinspect.software informer.com/). The exon/intron structures of each *MYB* gene in pearl millet were determined by GSDS (Gene Structure Display Server) tool [35]. In order to identify conserved motifs in PgMYB sequences, MEME suite [36] was used with the following parameters: (20; maximum number of motifs, 6; minimum width and 50; maximum width). Further, the results obtained were visualized by TBtools [37].

Phylogenetic analysis, gene duplication and synteny analysis

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For phylogenetic analysis, MYB sequences of pearl millet, rice, *Arabidopsis* and foxtail millet were used. Multiple sequence alignment was done using MUSCLE with default parameters [38]. The phylogenetic tree was constructed by MEGA V7.0 (Molecular Evolutionary Genetics Analysis) program [39] using neighbour-joining method (with 1000 bootstrap replicates) with following parameters (Jones-Taylor-Thronton model; Rates among sites: gamma distributed (G) and partial deletion of gaps). *MYB* gene duplication events were examined by Multiple Collinearity Scan toolkit (MCScanX) with default parameters [40]. Synteny relationship between *PgMYB* genes and *MYB* genes from *Oryza sativa, Arabidopsis thaliana and Setaria italica* were visualized by AccuSyn software [41].

Cis-element analysis

The promoter sequences (2000 bp upstream) of all identified *PgMYB* genes were extracted from pearl millet genome database. The sequences were uploaded into PlantCARE server [42] for identifying *cis*-regulatory elements present in promoter region of identified *PgMYBs*

In-silico expression analysis

For investigation of expression profiles of *PgMYB* genes, publicly available transcriptome datasets of drought and salinity stress in pearl millet were downloaded from NCBI Sequence Read Archive (SRX3556461, SRX3556459, SRX6918725, SRX6918726) [43,44]. Transcriptome datasets were mapped using Bowtie 2.0 tool and the RSEM (RNA-Seq by Expectation-Maximization) software was used to quantify RNA-seq reads [45-47]. Further, differentially expressed genes were identified and matrix file was used to generate heatmap.

Plant growth and stress treatments

Pearl millet seeds PRLT 2/89-33 were obtained from International Crops Research Institute for Semi-Arid Tropics (ICRISAT) through a material transfer agreement (MTA). The seeds were sown in nutrient soil (1:1 mix of black and red soil) and allowed to grow in greenhouse with 16:8 h light: dark cycle at 28 °C (± 2).

Four-week-old pearl millet seedlings were subjected to drought condition by withholding water for 8 days whereas the control plants were watered on alternate days. On 9th day, both control and treated plants were rewatered for their recovery. Samples of both

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control and treated plants were collected on 0, 5, 7, 9 and 11 days respectively. For salt stress, four-week-old seedlings were transferred to Hoagland solution containing 250 mM NaCl and for control ½ strength Hoagland solution was used. Samples were collected from both control and treated plants at time points of 0 hr, 3 hr (early) and 24 hr (late). For heat stress, plants were transferred to a 45ºC chamber for 12h and samples were collected at 0, 3, 12 and 24 hr [48,49].

For hormonal stress experiments, four-week-old seedlings were treated with 100 µM Abscisic acid (ABA), 100 µM Salicylic acid (SA) and 100 µM methyl jasmonate (MeJA) [50]. Both control and treated samples were collected at different time points of 0 hr, 3 hr (early) and 24 hr (late). Tissue specific expression of *PgMYB* genes was also studied by harvesting leaf, stem and root, from 4-week-old plants under normal conditions. The harvested samples were snap-frozen in liquid nitrogen and stored at -80 °C until further analysis. All the samples were collected in biological triplicates.

Quantitative qRT-PCR analysis

Total RNA was isolated from the samples by using Spectrum Plant Total RNA Kit (Sigma-Aldrich, MO, USA) according to manufacturer's instructions. RNA quality was checked on a 1.2% agarose gel with 18% formaldehyde. The purity and yield were estimated by NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Waltham, USA) and RNA samples with 260/280 nm ratio between 2.0 to 2.1 were used for further analysis. RNA purification was done by treating with DNAse I (Sigma-Aldrich, MO, USA) as per manufacturer's protocol.

cDNA synthesis was carried out by first-strand cDNA synthesis kit (Thermo Scientific, MA, USA). Expression analysis of selected *MYB* genes were performed using the QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All the primers used in this study were designed by Prime quest tool of IDT. The qRT-PCR reaction mixture included a total volume of 20 µl containing 2 µl (20 ng) of cDNA, 10 µl of SYBR Premix buffer (Mesa green qPCR master mix-Eurogentec), 1.0 µl each of forward and reverse primer (5 µM) and 6 µl of nuclease free water. The qRT-PCR run profile was as follows: 95 °C for 10min, followed by 40 cycles of 95ºC for 15s, 60ºC for 1min. EF1α and *GAPDH [51]* genes were taken for data normalization.

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Results and Discussion

Identification and physicochemical characteristics of MYB TF family in pearl millet

The HMM tool was used for screening of MYB TF family members in pearl millet. A total of 306 probable MYB members were identified using HMM-search. All redundant sequences were removed using Expasy tool and MYB DNA binding domain (PF00249) was confirmed in non-redundant putative MYB sequences using HMMScan, SMART and CDD tools. Finally, a total of 279 MYB TFs were identified in pearl millet and designated as PgMYB1 to PgMYB279 based on their chromosomal location & coordinates. Interestingly, identified MYB members in pearl millet were higher in number compared to that of *Arabidopsis* (155), *O. Sativa* (197) *S. bicolor* (134), *Soyabean* (244), *S. italica* (209) and *Z. maize* (157) [52-56].

The physical and chemical properties such as protein length, molecular weights (MWs), isoelectric point (PI), and subcellular localization of identified PgMYBs were analyzed (Additional Table S1). The amino acid length of PgMYBs varies from 73 (PgMYB190) to 2299 (PgMYB250) amino acids and relative molecular weight ranges from 7.46 kDa (PgMYB190) to 255.88 kDa (PgMYB250). Moreover, the theoretical isoelectric point (pI) of identified PgMYB proteins ranges from 4.37 to 11.34 and the grand average of hydropathy (GRAVY) value of all PgMYB proteins was found to be relatively low (<0), which suggests their hydrophilic nature. The prediction of subcellular localization showed that most of PgMYB proteins (234 PgMYBs) were located in the nucleus which suggests their role as a TF. However, some PgMYB were localized in chloroplast (23), cytoplasm (11), mitochondria (7), peroxisome (1) and plasma membrane (1). Further, PgMYB66 and PgMYB152 were predicted to contain transmembrane (TM) helices by TMHMM server. (Additional Figure S1). Membrane-bound TFs (MTFs) are known to play a significant role in biotic and abiotic stress responses. The MTFs get activated by proteolytic cleavage during environmental stresses [57,58].

Chromosomal localization, gene structure and motif analysis of PgMYBs

Pearl millet genome comprises of seven chromosomes with varying lengths; chromosome 3 being the longest (300.9 Mb) and chromosome 5 being the shortest (158.6 Mb). The 279 PgMYBs were mapped on all chromosomes based on their positions and chromosomal coordinates in pearl millet genome using MapInspect software v1.0. The physical map positions demonstrated that PgMYBs were unevenly distributed across all

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7 chromosomes (Figure 1). The maximum number of *PgMYB* genes were located on chromosome 3 (49 *PgMYBs*), followed by chromosome 1, 2 and 6 with 45 *PgMYBs* individually. Comparatively, fewer *PgMYBs* were located on chromosome 5 (31) and 7 (21). The larger number of *PgMYBs* on chromosome 3, 1, 2 and 6 indicate a possible hot spot region for MYB family distribution during the course of pearl millet evolution.

Diversification of gene structure is an important component of gene family evolution and also contributes to phylogenetic groupings. The exon/intron organization was analyzed to gain insights into the structural variation of *PgMYB* genes by GSDS server. The number of introns in *PgMYB* genes varied from 0 to 23, however, 21 *PgMYBs* didn't contain an intron while 56 *PgMYBs* had only one intron (Additional Figure S2). Maximum number of introns were observed in *PgMYB264* (23) followed by *PgMYB223* with 21 introns. The results indicate a high structural diversity of *PgMYB* genes in pearl millet.

A total of 15 distinct conserved motifs were identified in PgMYBs (Figure 2). Motif 1, Motif 2, Motif 4 and Motif 5 were present in most of the PgMYBs with conserved residues that are identical among all R2R3-MYB domains; while, Motif 3 was found to be a core conserved motif in identified PgMYBs. Along with typically conserved residue Trp (W), other residues such as Gly (G), Glu (E), Asp (D), Cys (C), Arg (R), Leu (L), Thr (T), Asn (N) and Lys (K) were also conserved. However, few motifs such as Motif 6, Motif 9, Motif 10, Motif 13 and Motif 15 were present in less than 10% of PgMYB sequences. We also observed Motif 1, Motif 2, Motif 3, Motif 5 and Motif 7 were located towards N-terminal and while Motif 4 and Motif 6 were found towards C-terminal. Most of PgMYB members from the same clade of the phylogenetic tree displayed similar motif compositions, which indicates their functional similarities within the same subgroup.

Phylogenetic relationship and gene duplication events of PgMYBs

To explore the evolutionary relationships among 279 PgMYBs, 126 OsMYBs, and 141 AtMYBs, a phylogenetic tree was constructed by using MEGA7 software. We perceived from the phylogenetic tree map (Figure 3) that, most of the *PgMYB*s were aligned in 18 (Group I-XVIII) subgroups with *OsMYB* and *AtMYB*. Among eighteen groups, Group XII found to be largest with 47 PgMYBs, followed by Group XIII (34 PgMYBs), Group XVII (22 PgMYBs), Group II (21 PgMYBs), Group XVIII (20 PgMYBs), Group XVI (19 PgMYBs), Group XIV (17 PgMYBs), Group XV (15 PgMYBs), Group V (14

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PgMYBs), Group IV (13 PgMYBs), Group VIII (12 PgMYBs), Group III (11 PgMYBs), Group VI (8 PgMYBs), Group I (8 PgMYBs), Group VII (6 PgMYBs), Group IX (5 PgMYBs), Group XI (4 PgMYBs) and Group X was found to be smallest with 4 PgMYBs.

Further, we performed synteny analysis to identify duplication events among MYB family members of pearl millet, rice and foxtail millet using MCScanX tool. We observed both orthologous and paralogous events across all seven chromosomes of pearl millet. Moreover, PgMYBs from chromosome 1, chromosome 3 and chromosome 6 of pearl millet were predominantly involved in orthologous relationship. A total of 198 paralogous pairs including 22 tandem and 176 segmental events were identified in the MYB family members of pearl millet (Figure 4).

A total of 204 *PgMYBs* were showing orthologous relationship with MYB family members of Arabidopsis, rice and foxtail millet. Foxtail millet showed the highest collinear relationship (70%) with 343 collinear genes followed by Arabidopsis (48%) with 205 collinear genes and rice (28%) with 111 collinear genes (Additional Table S2). Interestingly, we found 9 collinear *PgMYB* pairs with rice and foxtail millet but not with Arabidopsis; indicating their probable formation after monocot and dicot divergence. Whereas, 14 collinear pairs of *PgMYB* occurred with Arabidopsis but not with rice and foxtail millet which suggest their probable disappearance during divergence of monocot and dicot. Similarly, 9 collinear *PgMYB* pairs were found only with foxtail millet but not with Arabidopsis and rice which suggest these pairs may be formed during millets evolution; while 6 collinear *PgMYB* pairs were found with Arabidopsis and rice but not with foxtail millet indicates towards their loss during millet evolution. The formation and loss of collinear pairs shows the evolutionary progression of the MYB family members in pearl millet [59,60].

Cis-element analysis in promoter regions of PgMYBs

The cis-regulatory elements analysis of promoters helps in understanding gene regulation at transcriptional level. Therefore, putative cis-acting elements present in 2000bp upstream region of identified PgMYBs were identified using PlantCare database. The results demonstrated the presence of versatile cis-regulatory elements (Figure 5) linked to plant growth and development (CAT-box, dOCT, E2Fb, HD-Zip, OCT etc.), hormonal signalling (AuxRR, ERE, JERE, GA-motif, GARE-motif, P-box etc.), circadian cycle,

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metabolism (O_2-site), cell cycle regulation (MSA-like), seed-specific regulation (RY element), XYLEM specific expression (AC-I & II) and abiotic/biotic stress (ARE, ABRE, DRE, LTRMYB, W-box, WUN-motif, MBS etc.,) responses. The presence of versatile cis-regulatory elements in presumptive promoter regions of *PgMYBs* shows their functional diversity and their probable involvement in multiple biological plant processes [61,62].

In-silico expression analysis of PgMYBs under dehydration and salinity stress

RNA-seq data was analyzed to assess the expression level of identified 279 *PgMYB* genes under dehydration and salinity stress conditions in pearl millet. For that, the publicly available transcriptome data and the Sequence Read Archive (SRA-NCBI) files were accessed and explored for differential expression profiling of *PgMYBs*. As shown in Figure S3a and S3b, most of the *PgMYBs* showed a differential transcripts level under both dehydration and salinity stress. Specifically, upon dehydration stress *PgMYB2, PgMYB9, PgMYB61, PgMYB8, PgMYB106, PgMYB110, PgMYB114, PgMYB169, PgMYB198, PgMYB250, PgMYB269 and PgMYB275* showed a higher expression level, whereas expression level of *PgMYB138, PgMYB141, PgMYB228, PgMYB229, PgMYB245* and *PgMYB246* was decreased. Similarly, under salinity stress, expression level of *PgMYB2, PgMYB9, PgMYB35, PgMYB49, PgMYB101, PgMYB102, PgMYB134, PgMYB146, PgMYB151, PgMYB241, PgMYB249, PgMYB267* and *PgMYB271* was induced, while the transcripts accumulation for *PgMYB126, PgMYB199, PgMYB201, PgMYB235* and *PgMYB240* was reduced compared to control samples.

We tried to correlate the expression pattern of *PgMYBs* in dehydration and salinity stress. We found the similar upregulated expression profile of *PgMYB2* and *PgMYB9* under both dehydration and salinity stresses. Which suggest their probable involvement in abiotic stress responses. We also observed few *PgMYBs* of same phylogenetic clade or group showed a similar expression pattern under both dehydration and salinity stress which suggest the similar functional profiling of subfamily members [15].

Relative expression analysis of PgMYBs

Previous studies have shown the role of *MYB* family members in regulating environmental stress responses [15]. We have selected 15 *PgMYB* genes (Table S3) based on *in-silico* expression profiling, phylogenetic analysis, sequence homology and synteny

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analysis to explore their expression profiling in different tissues and under abiotic stress conditions.

Tissue-specific expression profiling of PgMYBs

Tissue-specific expression analysis helps in understanding the role of a particular TF/gene in growth and development of the plant. Therefore, we evaluated the spatial expression pattern of selected 15 *PgMYBs* in leaf, stem and root tissues of pearl millet. As shown in Figure 6, transcript level of *PgMYB9*, *PgMYB44*, *PgMYB61*, *PgMYB88*, *PgMYB132*, *PgMYB151* and *PgMYB198* was predominant in leaf tissues. Whereas expression level of *PgMYB49*, *PgMYB187*, and *PgMYB263* was higher in root tissues. We also observed higher expression of four *PgMYBs* namely *PgMYB2*, *PgMYB134*, *PgMYB176*, and *PgMYB240* in both leaf and root tissues. *PgMYB229* was only a member expressed in stem tissues predominantly. Taken together, the tissue-specific expression profiling of *PgMYBs* provides a basis for better understanding of pearl millet growth and development [63].

Relative expression analysis of PgMYBs under abiotic stress

To investigate the role of *PgMYB* genes under different abiotic stresses in pearl millet, expression profile of 15 selected *PgMYB* genes was generated at different time points under dehydration, salinity and heat stress.

PgMYBs showed a differential expression pattern under dehydration stress condition (Figure 7). Among the 15 selected *PgMYBs*, 13 *PgMYB* genes showed an upregulation pattern upon dehydration stress whereas only 2 *PgMYBs* showed downregulated expression pattern. Moreover, *PgMYB2*, *PgMYB49*, *PgMYB88* were significantly induced and while the expression level of *PgMYB151* was prominently downregulated. Interestingly, we observed recovery in transcripts level of *PgMYB44*, *PgMYB134*, *PgMYB151*, *PgMYB187*, and *PgMYB198* on 11th day (after rewatering). This suggests their possible involvement in dehydration stress responses of pearl millet. We also noticed that qRT-PCR analysis data of most of the *PgMYBs* corroborated with transcriptome profile under dehydration stress.

In course of salinity stress, upregulation of 7 *PgMYBs* and downregulation of 7 *PgMYB* was evidenced at early or late time points (Figure 8). *PgMYBs* showed a differential expression pattern at different time points such as *PgMYB2* and *PgMYB151* showed

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changes in expression level at early time point (3 hr). Similarly, the expression level of *PgMYB61*, *PgMYB132*, *PgMYB240*, *PgMYB263* and *PgMYB198* was affected at late time points (24 hr). Interestingly, we observed significant downregulation of *PgMYB9*, *PgMYB49*, *PgMYB187* was *PgMYB229* at both early (3 hr) and late (24 hr) time points. Most of the *PgMYBs* showed similar expression pattern in both transcriptome data and relative expression profiling.

In response to heat stress, 9 *PgMYBs* showed upregulation and 4 *PgMYBs* displayed downregulation (Figure 9). A significant increase in transcripts accumulation of *PgMYB49*, *PgMYB132* was observed, while the expression level of *PgMYB61*, *PgMYB151* and *PgMYB240* was significantly reduced upon heat stress. Interestingly, the transcript level of few *PgMYB* were comparable to control samples after recovery (at 24 hr), suggesting their possible involvement in heat stress response of pearl millet.

Notably, *PgMYB2*, *PgMYB88* and *PgMYB263* were upregulated under dehydration, salinity and heat stress. Similarly, the transcript accumulation of *PgMYB9* and *PgMYB151* was reduced under abiotic stress treatments. Differential expression profile under multiple stress conditions indicates their crucial role in abiotic stress responses in pearl millet. Several studies have demonstrated the role of MYB TFS in multiple abiotic stresses [19,52,53].

Relative expression analysis of PgMYBs upon phytohormone treatments

Phytohormones are well known for activating the specific signal cascades in response to various environmental stress [21]. MYB TFs are reported to be involved in phytohormonal signalling pathways under various stress conditions [64]. Thus, in the present study, the expression pattern of *PgMYB* genes was evaluated in response to ABA, MeJA and SA treatment (Figure 10).

The ABA treatment led to the upregulation of *PgMYB2*, *PgMYB134* and downregulation of *PgMYB9*, *PgMYB61*, *PgMYB240* at 3 hr and 24 hr time points. We have also observed upregulated expression of 8 PgMYBs at early time point (3 hr). While *PgMYB151* showed downregulation at early time point (3 hr) upon ABA treatment. Furthermore, it was showed that *MYB* family members involved in the ABA-dependent signal pathway and activates antioxidant enzymes to improve plant stress tolerance [65]. Similarly, under dehydration stress and ABA treatment, *PgMYB2*, *PgMYB44*, *PgMYB49*, *PgMYB88*, *PgMYB134*, *PgMYB187*, *PgMYB198*, and *PgMYB229* were induced; meanwhile

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PgMYB9 and *PgMYB151* were downregulated. Therefore, these *PgMYBs* might play an important role in providing drought tolerance through ABA signalling pathway in pearl millet, though further validation is necessary to confirm these findings.

The significant role of jasmonates and salicylic acid in stress responses has been very well documented [66]. Upon treatment with MeJA, transcript accumulation of most of *PgMYBs* was reduced, however, the expression level *PgMYB134*, *PgMYB198*, *PgMYB229* and *PgMYB240* was increased. Meanwhile, SA treatment caused downregulation of majority of *PgMYB* genes in pearl millet. The transcript level of *PgMYB229* was upregulated at both 3 hr and 24 hr time points. Early upregulated response (at 3 hr) of 9 *PgMYBs* was also observed in response to exogenous SA application. However, at 24 hr time point the expression level of these 9 *PgMYBs* were reduced. Differential expression profile of *PgMYBs* upon exogenous phytohormone treatments indicating a possible involvement of *PgMYBs* in phytohormone stress signaling for implying plants tolerance [67].

Taken together, differential expression analysis of *PgMYB* genes under abiotic stress and phytohormone treatment indicate their probable involvement in different signalling pathways linked to stress responses in pearl millet.

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Conclusion:

In the present study, A total of 279 putative PgMYBs were identified and distributed unevenly across seven chromosome of pearl millet genome. The phylogenetic analysis, motif conservation and gene structure analysis provided insights into the structural diversity of *PgMYBs*. Tandem and segmental duplication events indicate towards the expansion of MYB family in pearl millet. In addition, transcriptome data and relative expression analysis of selected *PgMYBs* in different tissues and upon various stress treatments enabled us to understand their role in pearl millet development and stress responses. The majority of *PgMYB* genes also showed differential expression profile under abiotic stress as well upon phytohormone treatments, which suggest their probable participation in various phytohormone signalling pathways for mediating stress responses in pearl millet. Therefore, our work provides a comprehensive understanding of the MYB family members and their functional role in pearl millet. Information obtained will be useful for detailed evolutionary study and characterization of candidate PgMYB TFs. Further, candidate *PgMYBs* could contribute significantly towards the development of engineered multiple stress-tolerant crop plants to ensure better crop productivity.

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Figure Legends:

Figure 1: Chromosomal mapping of identified 279 PgMYB members on pearl millet genome

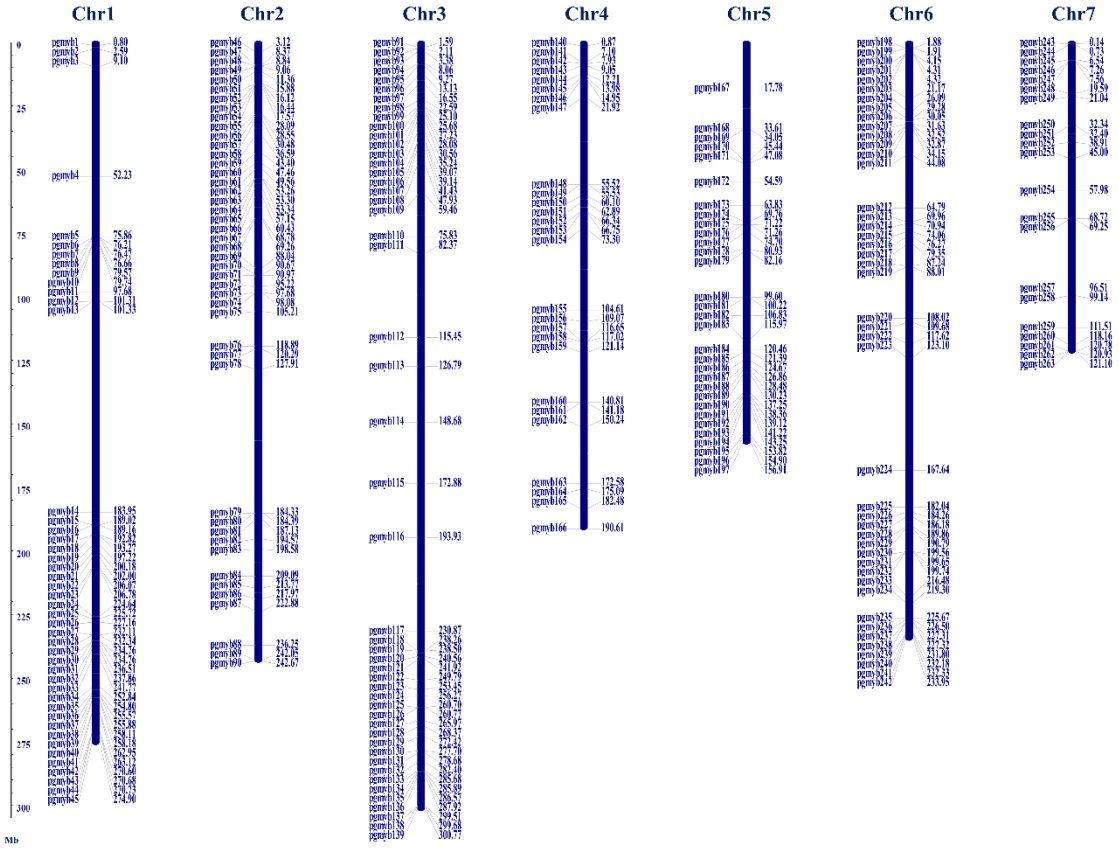

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Figure 2: Conserved motif distribution in identified 279 PgMYB proteins. All identified 15 motifs are indicated by different colours and their respective sequence logo.

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Figure 3: Phylogenetic tree of MYB family members of *P. glaucum*, *A. thaliana* and *O. sativa*. A total of 534 MYB proteins were aligned by Clustal, and tree was constructed by MEGA v7.0 using maximum likelihood method with 1000 bootstrap replication. The different groups are numbered from (I-XVIII).

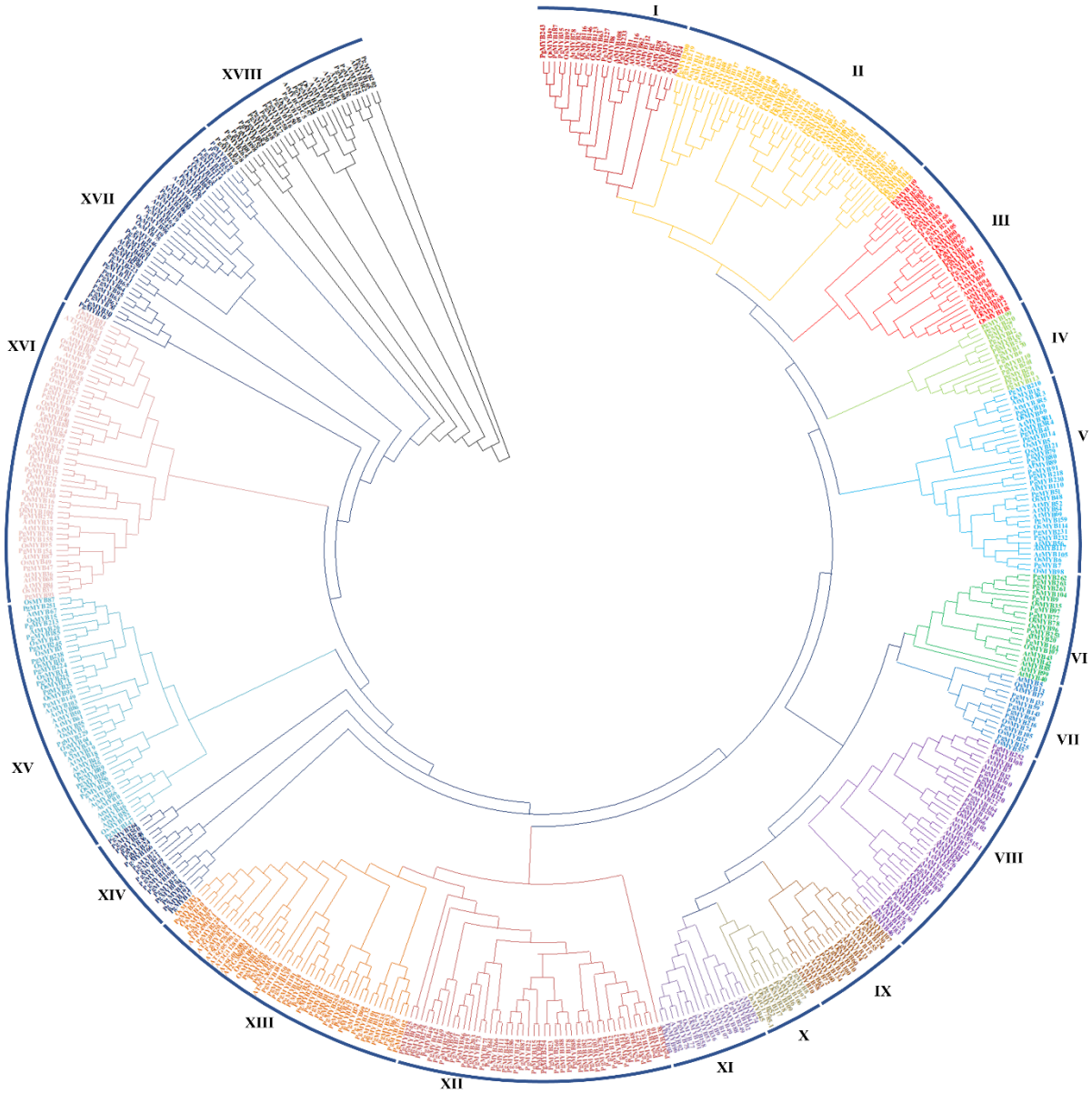

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Figure 4: Synteny relationship of MYB family members between *P. glaucum*, *A. thaliana*, *O. sativa* and *S. italica*. Each connected coloured line shows orthologous and paralogous pairs among the species. **A)** Paralogous relationship in *P. glaucum* **B)** Orthologous relationship between *P. glaucum* and *A. thaliana* **C)** Synteny relationship between *P. glaucum* and *O. sativa* **D)** Synteny relationship in *P. glaucum* and *S. italica.*

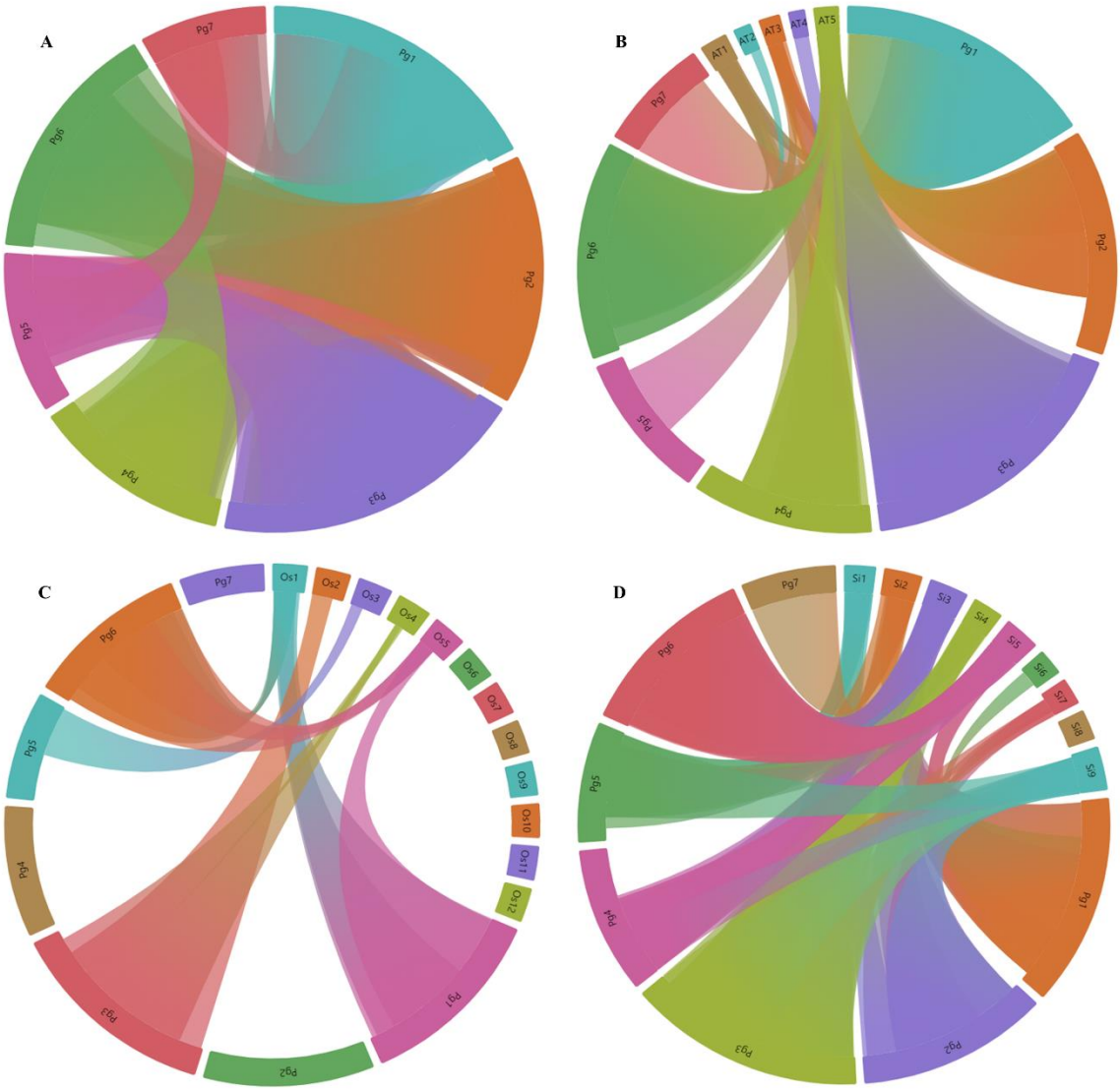

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Figure 5: Presence of Cis regulatory elements in 2000bp upstream region of identified PgMYBs. Color of bars indicates the group (shown in legend) it belongs.

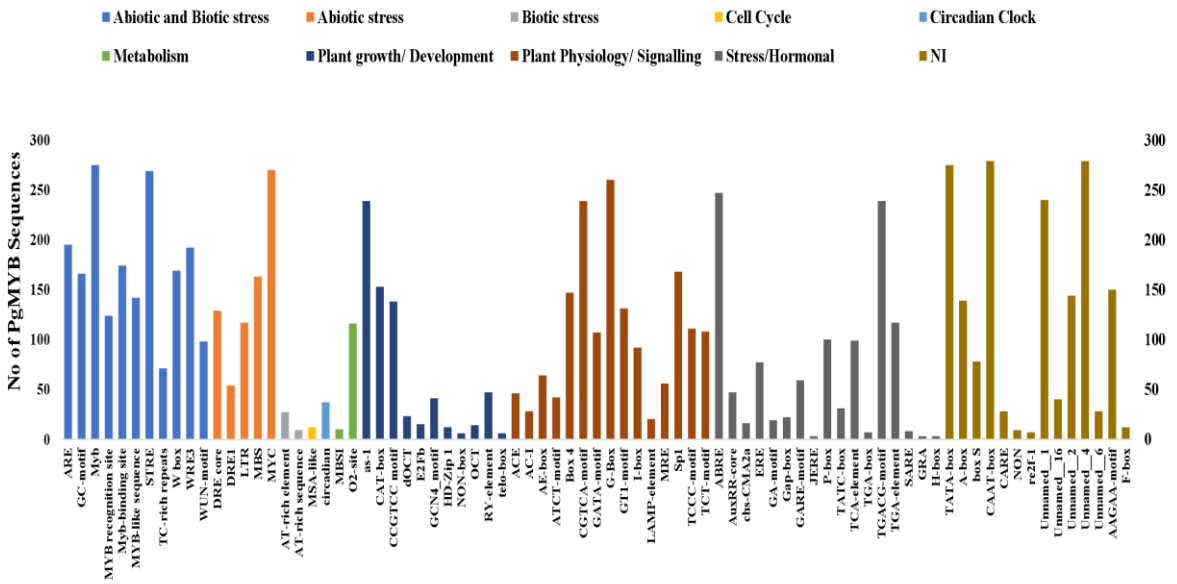

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Figure 6: Tissue specific expression analysis of *PgMYB* genes in Leaf, Stem and Root tissues. Significant difference in mean indicated by *P < 0.05, **P < 0.01, as obtained by Student's t-test.

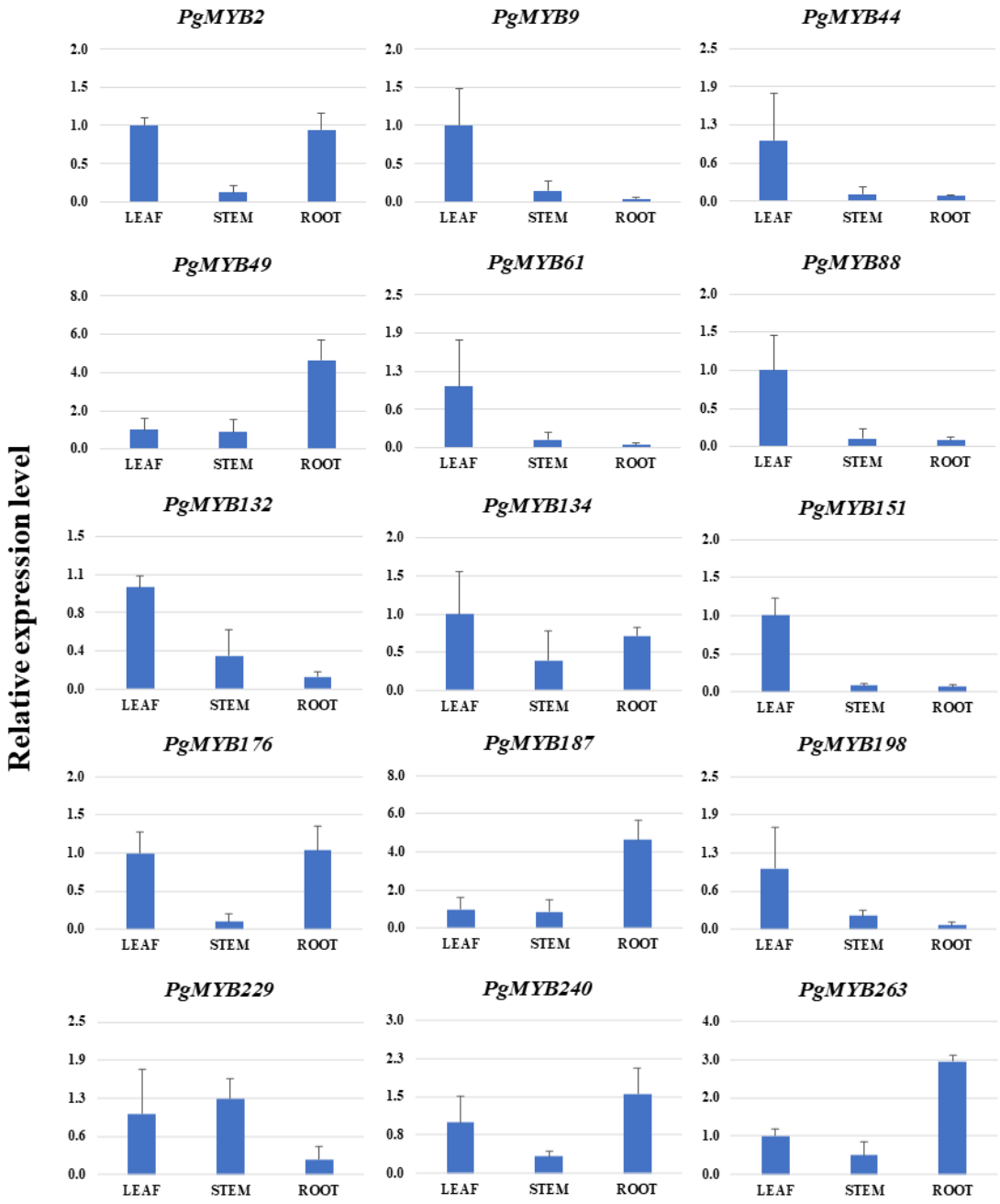

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Figure 7: Relative expression analysis of *PgMYB* genes under dehydration stress at 0th day, 5th day, 7th day, 9th day and 11th day time points. The X-axis represents different time points and the Y-axis indicates relative expression level. Significant difference in mean indicated by *P < 0.05, **P < 0.01, as obtained by Student's t-test.

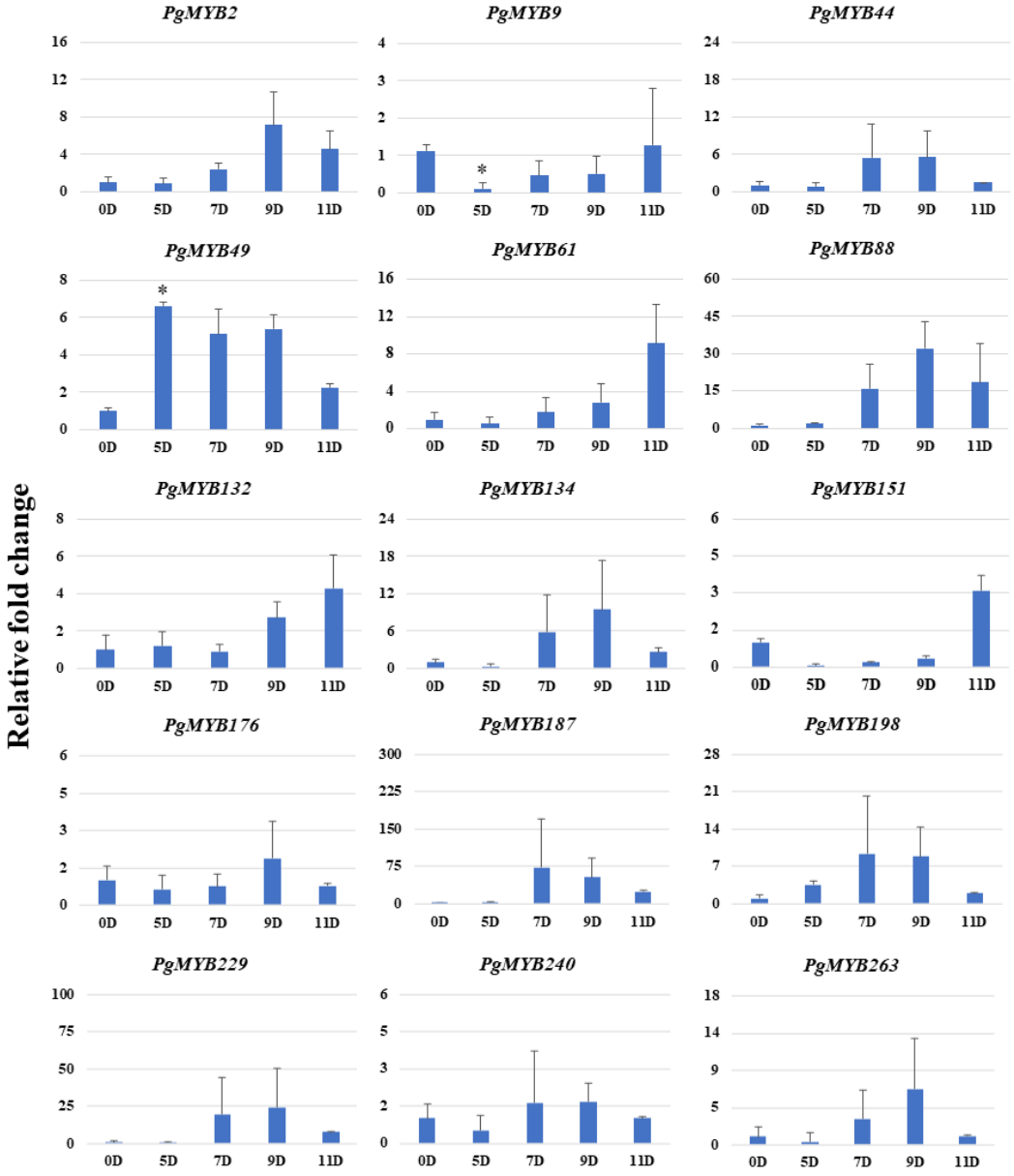

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Figure 8: Relative expression analysis of *PgMYB* genes under Salinity stress at 0-hour, 3-hour, and 24-hour time points. The X-axis represents different time points and the Y-axis indicates relative expression level. Significant difference in mean indicated by *P < 0.05, **P < 0.01, as obtained by Student's t-test.

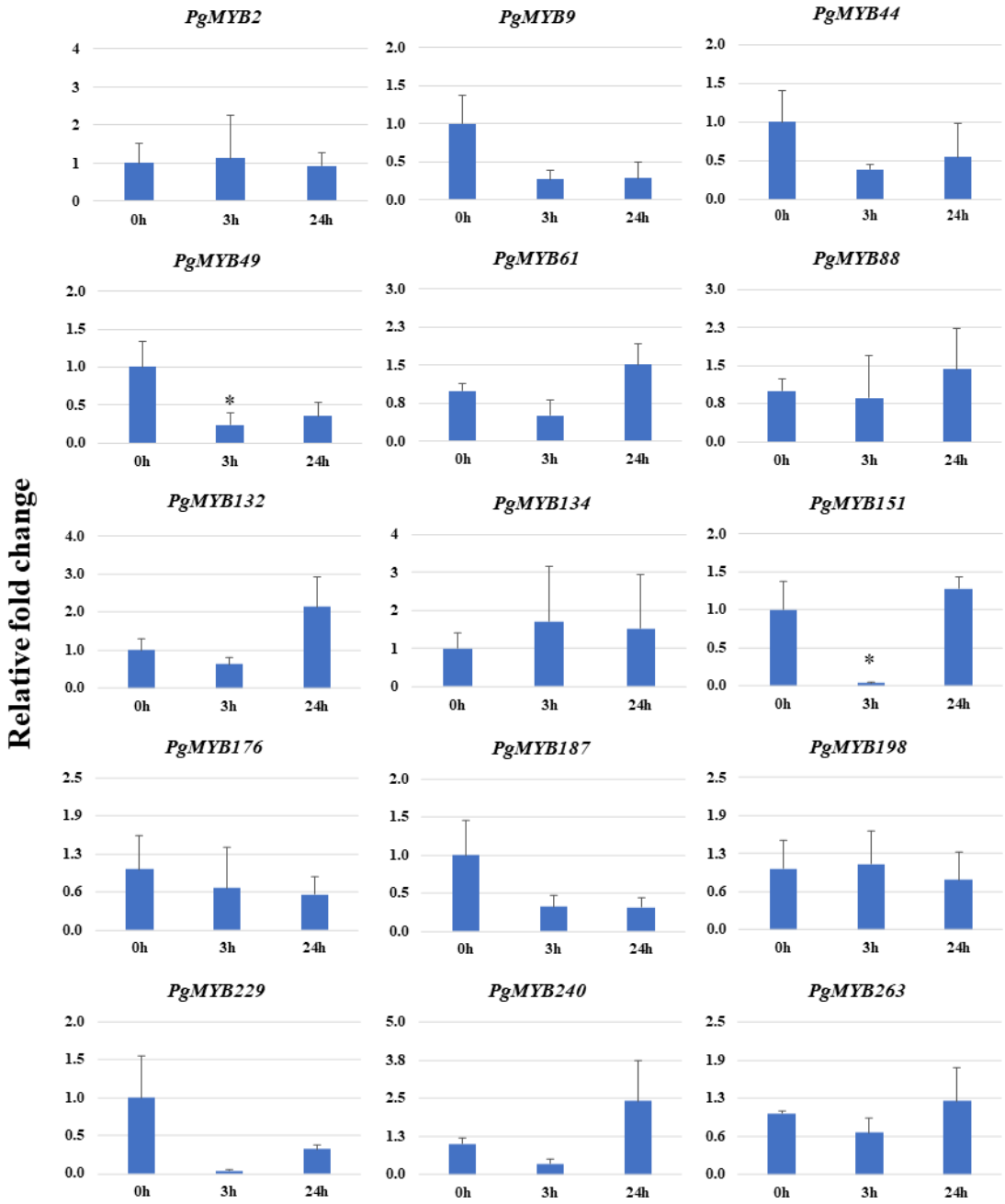

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Figure 9: Relative expression analysis of *PgMYB* genes under Heat stress at 0-hour, 3-hour, 12-hour and 24-hour time points. The X-axis represents different time points and the Y-axis indicates relative expression level. Significant difference in mean indicated by *P < 0.05, **P < 0.01, as obtained by Student's t-test.

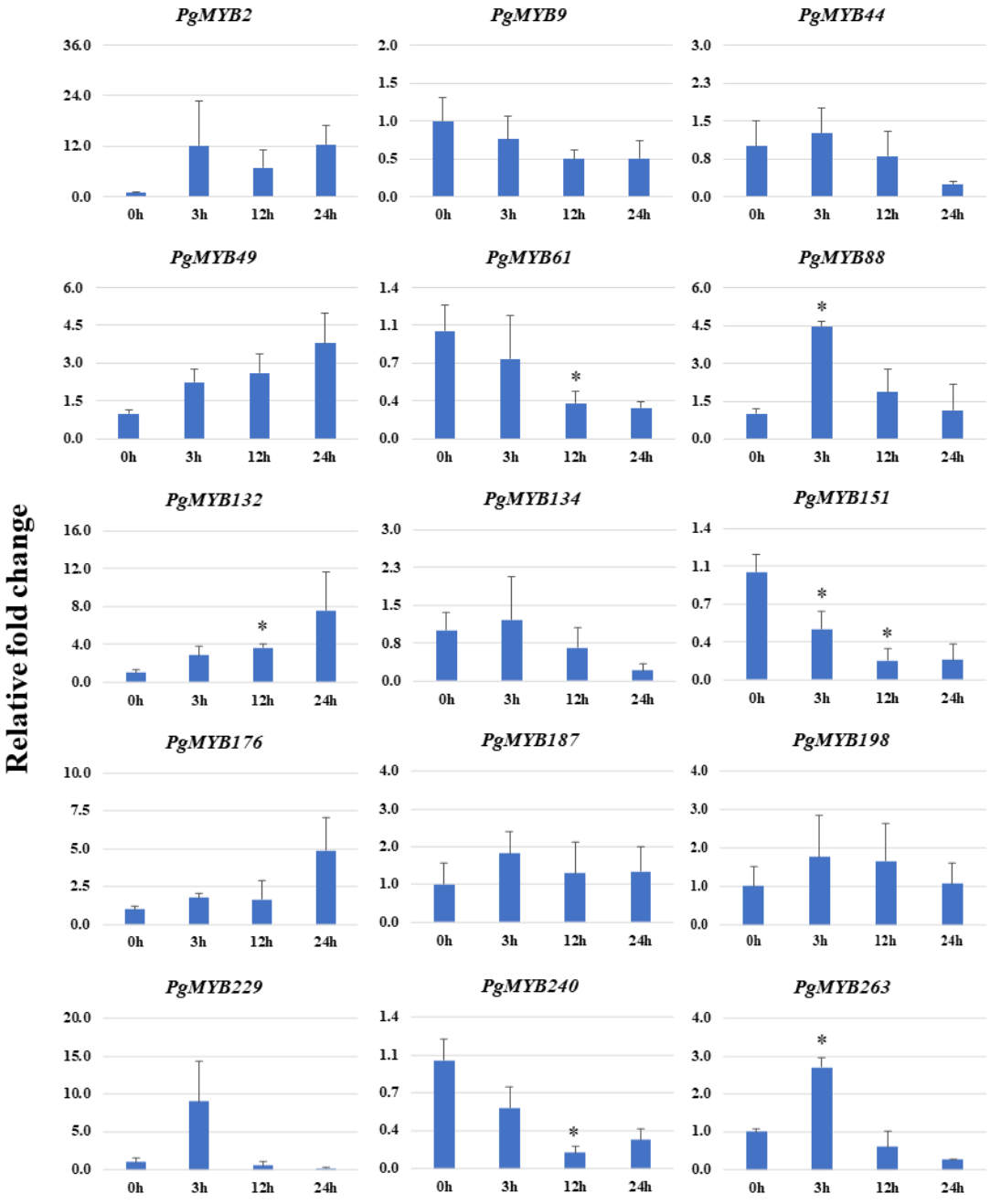

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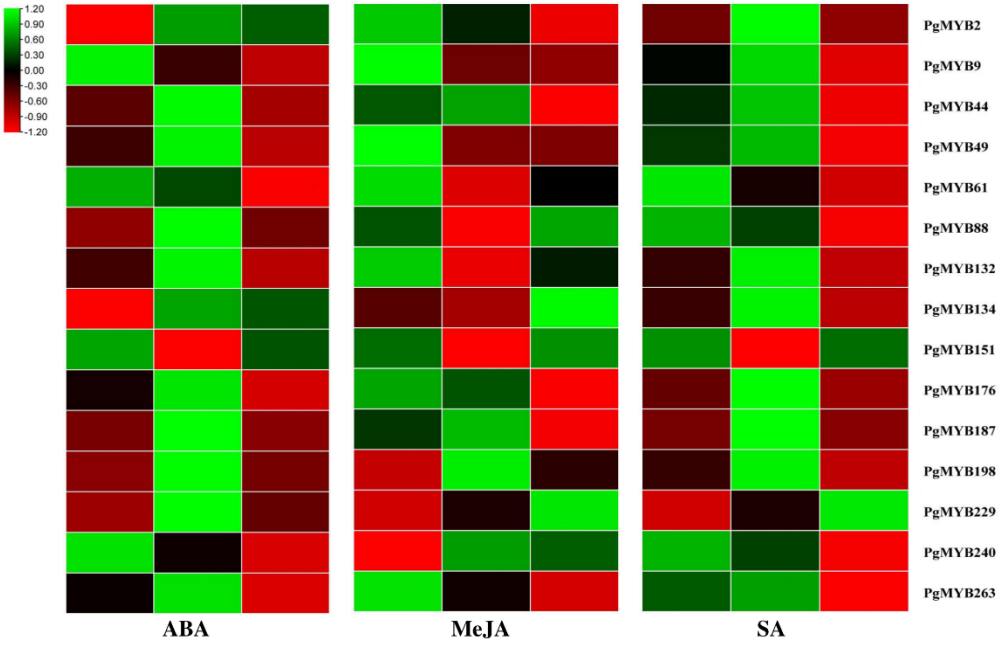
Figure 10: Heat map showing expression profile of *PgMYB* genes upon exogenous phytohormones (ABA, MeJA and SA) treatments at 0-hour, 3-hour, and 24-hour time points. The transcript abundance level has been normalized and hierarchically clustered. The heat map was generated using TBtools v0.66831



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Supplementary Tables/Figures

Figure S1: Membrane-bound MYB transcription factors with a transmembrane motif (TM, blue square) at the C-terminal region

Figure S2: Gene **s**tructure analysis of the 279 PgMYB genes of pearl millet. S2a;1-100 PgMYBs, S2b;101-200 PgMYBs, S2c;201-279 PgMYBs. Yellow boxes indicate exons, black lines indicate introns, blue boxes indicate upstream/downstream

Figure S3: *In-silico* expression profiling of *PgMYB* genes under drought (S3a) and salt (S3b) stress using publicly available RNA-seq data

Table S1: Sequence characteristics and chromosomal coordinates of identified 279 PgMYB members in pearl millet.

Table S2: List orthologous and paralogous pairs between PgMYB, OsMYB, SiMYB and AtMYB

Table S3: List of primers used in qRT-PCR analysis for selected PgMYB*s*

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Conflicts of Interest

The authors declare no conflict of interests.

Acknowledgements

This work was funded by the core grant of Institute of Life Sciences, Bhubaneswar, Govt. of India. We are thankful to ICRISAT, Hyderabad for providing the seed material.

Authors' contributions

JC performed all the experiments, bioinformatics data analysis, RtPCR data analysis and manuscript writing. **BK** bioinformatics data analysis and manuscript writing **DKJ** performed RtPCR experiments. **ISS** manuscript revision **ND** conceptualized the project, data analysis and interpretation, and manuscript revision. All authors have read and approved the manuscript.

References;

1. Zandalinas, S.I.; Mittler, R. Plant responses to multifactorial stress combination. **2022**, *234*, 1161-1167, doi:https://doi.org/10.1111/nph.18087.
2. Rivero, R.M.; Mittler, R.; Blumwald, E.; Zandalinas, S.I. Developing climate-resilient crops: improving plant tolerance to stress combination. **2022**, *109*, 373-389, doi:https://doi.org/10.1111/tpj.15483.
3. Baillo, E.H.; Kimotho, R.N.; Zhang, Z.; Xu, P. Transcription Factors Associated with Abiotic and Biotic Stress Tolerance and Their Potential for Crops Improvement. **2019**, *10*, 771.
4. Manna, M.; Thakur, T.; Chirom, O.; Mandlik, R.; Deshmukh, R.; Salvi, P. Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. **2021**, *172*, 847-868, doi:https://doi.org/10.1111/ppl.13268.
5. Wray, G.A.; Hahn, M.W.; Abouheif, E.; Balhoff, J.P.; Pizer, M.; Rockman, M.V.; Romano, L.A. The Evolution of Transcriptional Regulation in Eukaryotes. *Molecular Biology and Evolution* **2003**, *20*, 1377-1419, doi:10.1093/molbev/msg140 %J Molecular Biology and Evolution.
6. Khan, S.-A.; Li, M.-Z.; Wang, S.-M.; Yin, H.-J. Revisiting the Role of Plant Transcription Factors in the Battle against Abiotic Stress. **2018**, *19*, 1634.
7. Wang, H.; Wang, H.; Shao, H.; Tang, X. Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. **2016**, *7*, doi:10.3389/fpls.2016.00067.
8. Saxena, R.; Vanga, S.K.; Wang, J.; Orsat, V.; Raghavan, V. Millets for Food Security in the Context of Climate Change: A Review. **2018**, *10*, 2228.
9. Satyavathi, C.T.; Ambawat, S.; Khandelwal, V.; Srivastava, R.K. Pearl Millet: A Climate-Resilient Nutricereal for Mitigating Hidden Hunger and Provide Nutritional Security. **2021**, *12*, doi:10.3389/fpls.2021.659938.
10. Srivastava, R.K.; Yadav, O.P.; Kaliamoorthy, S.; Gupta, S.K.; Serba, D.D.; Choudhary, S.; Govindaraj, M.; Kholová, J.; Murugesan, T.; Satyavathi, C.T.; et al. Breeding Drought-Tolerant Pearl Millet Using Conventional and Genomic Approaches: Achievements and Prospects. *Frontiers in plant science* **2022**, *13*, 781524, doi:10.3389/fpls.2022.781524.
11. Varshney, R.K.; Shi, C.; Thudi, M.; Mariac, C.; Wallace, J.; Qi, P.; Zhang, H.; Zhao, Y.; Wang, X.; Rathore, A. Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. *Nature biotechnology* **2017**, *35*, 969-976.
12. Ambawat, S.; Sharma, P.; Yadav, N.R.; Yadav, R.C. MYB transcription factor genes as regulators for plant responses: an overview. *Physiology and Molecular Biology of Plants* **2013**, *19*, 307-321, doi:10.1007/s12298-013-0179-1.
13. Jiang, C.-K.; Rao, G.-Y. Insights into the Diversification and Evolution of R2R3-MYB Transcription Factors in Plants1. *Plant physiology* **2020**, *183*, 637-655, doi:10.1104/pp.19.01082 %J Plant Physiology.
14. Li, Z.; Peng, R.; Tian, Y.; Han, H.; Xu, J.; Yao, Q. Genome-Wide Identification and Analysis of the MYB Transcription Factor Superfamily in Solanum lycopersicum. *Plant and Cell Physiology* **2016**, *57*, 1657-1677, doi:10.1093/pcp/pcw091 %J Plant and Cell Physiology.
15. Wang, X.; Niu, Y.; Zheng, Y. Multiple Functions of MYB Transcription Factors in Abiotic Stress Responses. **2021**, *22*, 6125.
16. Jaradat, M.R.; Feurtado, J.A.; Huang, D.; Lu, Y.; Cutler, A.J. Multiple roles of the transcription factor AtMYBR1/AtMYB44 in ABA signaling, stress responses, and leaf senescence. *BMC Plant Biology* **2013**, *13*, 192, doi:10.1186/1471-2229-13-192.
17. Jung, C.; Seo, J.S.; Han, S.W.; Koo, Y.J.; Kim, C.H.; Song, S.I.; Nahm, B.H.; Choi, Y.D.; Cheong, J.J. Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. *Plant physiology* **2008**, *146*, 623-635, doi:10.1104/pp.107.110981.

18. Vannini, C.; Locatelli, F.; Bracale, M.; Magnani, E.; Marsoni, M.; Osnato, M.; Mattana, M.; Baldoni, E.; Coraggio, I. Overexpression of the rice Osmyb4 gene increases chilling and freezing tolerance of Arabidopsis thaliana plants. **2004**, *37*, 115-127, doi:https://doi.org/10.1046/j.1365-313X.2003.01938.x.
19. Wei, Q.; Chen, R.; Wei, X.; Liu, Y.; Zhao, S.; Yin, X.; Xie, T. Genome-wide identification of R2R3-MYB family in wheat and functional characteristics of the abiotic stress responsive gene TaMYB344. *BMC Genomics* **2020**, *21*, 792, doi:10.1186/s12864-020-07175-9.
20. Tang, Y.; Bao, X.; Zhi, Y.; Wu, Q.; Guo, Y.; Yin, X.; Zeng, L.; Li, J.; Zhang, J.; He, W.; et al. Overexpression of a MYB Family Gene, OsMYB6, Increases Drought and Salinity Stress Tolerance in Transgenic Rice. **2019**, *10*, doi:10.3389/fpls.2019.00168.
21. Verma, V.; Ravindran, P.; Kumar, P.P. Plant hormone-mediated regulation of stress responses. *BMC plant biology* **2016**, *16*, 86.
22. Ku, Y.S.; Sintaha, M.; Cheung, M.Y.; Lam, H.M. Plant Hormone Signaling Crosstalks between Biotic and Abiotic Stress Responses. *International journal of molecular sciences* **2018**, *19*, doi:10.3390/ijms19103206.
23. Vishwakarma, K.; Upadhyay, N.; Kumar, N.; Yadav, G.; Singh, J.; Mishra, R.K.; Kumar, V.; Verma, R.; Upadhyay, R.; Pandey, M. Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Frontiers in plant science* **2017**, *8*, 161.
24. Qiu, Z.; Guo, J.; Zhu, A.; Zhang, L.; Zhang, M. Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. *Ecotoxicology and environmental safety* **2014**, *104*, 202-208.
25. Wasternack, C.; Song, S. Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany* **2017**, *68*, 1303-1321.
26. Hayat, Q.; Hayat, S.; Irfan, M.; Ahmad, A. Effect of exogenous salicylic acid under changing environment: a review. *Environmental and experimental botany* **2010**, *68*, 14-25.
27. Khan, M.I.R.; Fatma, M.; Per, T.S.; Anjum, N.A.; Khan, N.A. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Frontiers in plant science* **2015**, *6*, 462.
28. Kurata, N.; Yamazaki, Y. Oryzabase. An integrated biological and genome information database for rice. *Plant physiology* **2006**, *140*, 12-17, doi:10.1104/pp.105.063008.
29. Yilmaz, A.; Nishiyama, M.Y.; Fuentes, B.G.; Souza, G.M.; Janies, D.; Gray, J.; Grotewold, E. GRASSIUS: A Platform for Comparative Regulatory Genomics across the Grasses. **2009**, *149*, 171-180, doi:10.1104/pp.108.128579 %J Plant Physiology.
30. Goodstein, D.M.; Shu, S.; Howson, R.; Neupane, R.; Hayes, R.D.; Fazo, J.; Mitros, T.; Dirks, W.; Hellsten, U.; Putnam, N.; et al. Phytozome: a comparative platform for green plant genomics. *Nucleic acids research* **2012**, *40*, D1178-D1186, doi:10.1093/nar/gkr944.
31. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER web server: interactive sequence similarity searching. *Nucleic acids research* **2011**, *39*, W29-W37, doi:10.1093/nar/gkr367.
32. Sonnhammer, E.L.L.; Eddy, S.R.; Birney, E.; Bateman, A.; Durbin, R. Pfam: Multiple sequence alignments and HMM-profiles of protein domains. *Nucleic Acids Research* **1998**, *26*, 320-322, doi:10.1093/nar/26.1.320 %J Nucleic Acids Research.
33. Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E.L. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of molecular biology* **2001**, *305*, 567-580, doi:10.1006/jmbi.2000.4315.
34. Horton, P.; Park, K.-J.; Obayashi, T.; Fujita, N.; Harada, H.; Adams-Collier, C.J.; Nakai, K. WoLF PSORT: protein localization predictor. *Nucleic acids research* **2007**, *35*, W585-W587, doi:10.1093/nar/gkm259.
35. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics (Oxford, England)* **2015**, *31*, 1296-1297, doi:10.1093/bioinformatics/btu817.

36. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* **2009**, *37*, W202-208, doi:10.1093/nar/gkp335.
37. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Molecular Plant* **2020**, *13*, 1194-1202, doi:https://doi.org/10.1016/j.molp.2020.06.009.
38. Edgar, R.C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* **2004**, *32*, 1792-1797, doi:10.1093/nar/gkh340.
39. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* **2016**, *33*, 1870-1874, doi:10.1093/molbev/msw054.
40. Wang, Y.; Tang, H.; Debarry, J.D.; Tan, X.; Li, J.; Wang, X.; Lee, T.-h.; Jin, H.; Marler, B.; Guo, H.; et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic acids research* **2012**, *40*, e49-e49, doi:10.1093/nar/gkr1293.
41. Siri, J.N.; Neufeld, E.; Parkin, I.A.P.; Sharpe, A.G. Using Simulated Annealing to Declutter Genome Visualizations. In Proceedings of the FLAIRS Conference, 2020.
42. Rombauts, S.; Déhais, P.; Van Montagu, M.; Rouzé, P. PlantCARE, a plant cis-acting regulatory element database. *Nucleic Acids Research* **1999**, *27*, 295-296, doi:10.1093/nar/27.1.295 %J Nucleic Acids Research.
43. Shivhare, R.; Asif, M.H.; Lata, C. Comparative transcriptome analysis reveals the genes and pathways involved in terminal drought tolerance in pearl millet. *Plant molecular biology* **2020**, *103*, 639-652, doi:10.1007/s11103-020-01015-w.
44. Shinde, H.; Tanaka, K.; Dudhate, A.; Tsugama, D.; Mine, Y.; Kamiya, T.; K. Gupta, S.; Liu, S.; Takano, T. Comparative de novo transcriptomic profiling of the salinity stress responsiveness in contrasting pearl millet lines. *Environmental and Experimental Botany* **2018**, *155*, 619-627, doi:https://doi.org/10.1016/j.envexpbot.2018.07.008.
45. Langmead, B.; Salzberg, S.L. Fast gapped-read alignment with Bowtie 2. *Nature methods* **2012**, *9*, 357-359, doi:10.1038/nmeth.1923.
46. Grabherr, M.G.; Haas, B.J.; Yassour, M.; Levin, J.Z.; Thompson, D.A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R.; Zeng, Q.; et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* **2011**, *29*, 644-652, doi:10.1038/nbt.1883.
47. Li, B.; Dewey, C.N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **2011**, *12*, 323, doi:10.1186/1471-2105-12-323.
48. Chanwala, J.; Satpati, S.; Dixit, A.; Parida, A.; Giri, M.K.; Dey, N. Genome-wide identification and expression analysis of WRKY transcription factors in pearl millet (Pennisetum glaucum) under dehydration and salinity stress. *BMC genomics* **2020**, *21*, 1-16.
49. Sun, M.; Huang, D.; Zhang, A.; Khan, I.; Yan, H.; Wang, X.; Zhang, X.; Zhang, J.; Huang, L. Transcriptome analysis of heat stress and drought stress in pearl millet based on Pacbio full-length transcriptome sequencing. *BMC Plant Biology* **2020**, *20*, 1-15.
50. Jha, D.K.; Chanwala, J.; Sandeep, I.S.; Dey, N. Comprehensive identification and expression analysis of GRAS gene family under abiotic stress and phytohormone treatments in Pearl millet. *Functional plant biology : FPB* **2021**, *48*, 1039-1052, doi:10.1071/fp21051.
51. Reddy, P.S.; Reddy, D.S.; Sharma, K.K.; Bhatnagar-Mathur, P.; Vadez, V. Cloning and validation of reference genes for normalization of gene expression studies in pearl millet [Pennisetum glaucum (L.) R. Br.] by quantitative real-time PCR. *Plant Gene* **2015**, *1*, 35-42, doi:https://doi.org/10.1016/j.plgene.2015.02.001.
52. Katiyar, A.; Smita, S.; Lenka, S.K.; Rajwanshi, R.; Chinnusamy, V.; Bansal, K.C. Genome-wide classification and expression analysis of MYB transcription factor families in rice and Arabidopsis. *BMC Genomics* **2012**, *13*, 544, doi:10.1186/1471-2164-13-544.
53. Muthamilarasan, M.; Khandelwal, R.; Yadav, C.B.; Bonthala, V.S.; Khan, Y.; Prasad, M. Identification and molecular characterization of MYB Transcription Factor Superfamily in C4

model plant foxtail millet (Setaria italica L.). *PLoS One* **2014**, *9*, e109920, doi:10.1371/journal.pone.0109920.

54. Singh, V.; Kumar, N.; Dwivedi, A.K.; Sharma, R.; Sharma, M.K. Phylogenomic Analysis of R2R3 MYB Transcription Factors in Sorghum and their Role in Conditioning Biofuel Syndrome. *Current genomics* **2020**, *21*, 138-154, doi:10.2174/1389202921666200326152119.
55. Du, H.; Yang, S.S.; Liang, Z.; Feng, B.R.; Liu, L.; Huang, Y.B.; Tang, Y.X. Genome-wide analysis of the MYB transcription factor superfamily in soybean. *BMC Plant Biol* **2012**, *12*, 106, doi:10.1186/1471-2229-12-106.
56. Du, H.; Feng, B.R.; Yang, S.S.; Huang, Y.B.; Tang, Y.X. The R2R3-MYB transcription factor gene family in maize. *PLoS One* **2012**, *7*, e37463, doi:10.1371/journal.pone.0037463.
57. Wang, D.; Yu, Y.; Liu, Z.; Li, S.; Wang, Z.; Xiang, F. Membrane-bound NAC transcription factors in maize and their contribution to the oxidative stress response. *Plant Science* **2016**, *250*, 30-39.
58. De Backer, J.; Van Breusegem, F.; De Clercq, I. Proteolytic Activation of Plant Membrane-Bound Transcription Factors. **2022**, *13*, doi:10.3389/fpls.2022.927746.
59. Cannon, S.B.; Mitra, A.; Baumgarten, A.; Young, N.D.; May, G. The roles of segmental and tandem gene duplication in the evolution of large gene families in Arabidopsis thaliana. *BMC Plant Biol* **2004**, *4*, 10, doi:10.1186/1471-2229-4-10.
60. Feng, G.; Burleigh, J.G.; Braun, E.L.; Mei, W.; Barbazuk, W.B. Evolution of the 3R-MYB Gene Family in Plants. *Genome biology and evolution* **2017**, *9*, 1013-1029, doi:10.1093/gbe/evx056.
61. Sheshadri, S.A.; Nishanth, M.J.; Simon, B. Stress-Mediated cis-Element Transcription Factor Interactions Interconnecting Primary and Specialized Metabolism in planta. **2016**, *7*, doi:10.3389/fpls.2016.01725.
62. Yamaguchi-Shinozaki, K.; Shinozaki, K. Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends in Plant Science* **2005**, *10*, 88-94, doi:https://doi.org/10.1016/j.tplants.2004.12.012.
63. Ma, R.; Liu, B.; Geng, X.; Ding, X.; Yan, N.; Sun, X.; Wang, W.; Sun, X.; Zheng, C. Biological Function and Stress Response Mechanism of MYB Transcription Factor Family Genes. *Journal of Plant Growth Regulation* **2022**, doi:10.1007/s00344-021-10557-2.
64. Li, C.; Ng, C.K.Y.; Fan, L.-M. MYB transcription factors, active players in abiotic stress signaling. *Environmental and Experimental Botany* **2015**, *114*, 80-91, doi:https://doi.org/10.1016/j.envexpbot.2014.06.014.
65. Jaradat, M.R.; Feurtado, J.A.; Huang, D.; Lu, Y.; Cutler, A.J. Multiple roles of the transcription factor AtMYBR1/AtMYB44 in ABA signaling, stress responses, and leaf senescence. *BMC Plant Biol* **2013**, *13*, 192, doi:10.1186/1471-2229-13-192.
66. Per, T.S.; Khan, M.I.R.; Anjum, N.A.; Masood, A.; Hussain, S.J.; Khan, N.A. Jasmonates in plants under abiotic stresses: Crosstalk with other phytohormones matters. *Environmental and experimental botany* **2018**, *145*, 104-120.
67. Kapoor, B.; Kumar, P.; Sharma, R.; Kumar, A. Regulatory interactions in phytohormone stress signaling implying plants resistance and resilience mechanisms. *Journal of Plant Biochemistry and Biotechnology* **2021**, *30*, 813-828, doi:10.1007/s13562-021-00739-0.