Research Article

CMYB1 Encoding a MYB Transcriptional Activator Is Involved in Abiotic Stress and Circadian Rhythm in Rice

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Through analysis of cold-induced transcriptome, a novel gene encoding a putative MYB transcription factor was isolated and designated Cold induced MYB 1 (CMYB1). Tissue-specific gene expression analysis revealed that CMYB1 was highly expressed in rice stems and nodes. qRT-PCR assay indicated that CMYB1 was dramatically induced by cold stress (>100-folds) and induced by exogenous ABA and osmotic stress. Interestingly, CMYB1 showed rhythmic expression profile in rice leaves at different developmental stages. Subcellular localization assay suggested that CMYB1-GFP (green fluorescent protein) fusion protein was localized in the nuclei. Moreover, CMYB1 exhibited the transcriptional activation activity when transiently expressed in rice protoplast cells. Taken together, CMYB1 probably functions as a transcriptional activator in mediating stress and rhythm responsive gene expression in rice.

1. Introduction

Environmental stresses such as cold, drought, and salinity adversely affect plant growth and crop productivity. To cope with such stress, plants initiate a number of molecular, cellular, and physiological changes to respond and adapt to various stresses. The transcriptional gene regulation of stress-related genes is crucial in these responses and adaptations [1, 2]. The transcription factors play a predominant role in transcriptional regulation of stress responses in plants. Based on microarray or next-generation sequencing (NGS) technologies, many transcription factors regulated by abiotic stress were identified. However, compared with the big number of transcription factor genes present in plant genome, only a few of them have been functionally analyzed so far. The DREB/CBF transcription factors play a central role in both ABA-dependent and ABA-independent pathways via binding the C-repeat/dehydration response elements (CRT/DRE), which often exist within the promoter regions of the stress related genes [3, 4]. The DREB/CBF genes have been used in engineering tolerance against abiotic stress in crops [5–7]. Except for DREB/CBF transcription factors, some other types of transcription factors have been identified to be involved in stress signal transduction, such as bZIP transcription factors [8, 9], MYB proteins [10], NAC transcription factors [11, 12], WRKY proteins [13], and zinc finger proteins [14].

MYB transcription factors are present widely in eukaryotes [15]. MYB proteins contain one, two, or three imperfect repeats (51–53 amino acids) in their DNA-binding domain, and they are further classified into three subfamilies, type MYBR2R3, type MYBR1R2R3, and MYB-related, depending on the number of repeats in their MYB domains [16, 17]. Among them, the R2R3-type MYB proteins form a big MYB subfamily containing more than 100 members in high plants, such as Arabidopsis and rice. A number of MYB genes have been found to be induced by abiotic stress and their genetic engineering may improve stress tolerance in plants. AtMYB60, a R2R3-MYB gene of Arabidopsis, was the first identified transcription factor involved in the regulation of stomata movements [18]. AtMYB60 was specifically expressed in guard cells, and its expression was negatively modulated by drought. A null mutation in AtMYB60 resulted in the constitutive reduction of stomata opening and decreased wilting under water stress conditions. OsMYB3R-2 is a MYB transcription factor induced by cold, drought, and salt stresses in rice. The Arabidopsis and rice transgenic plants overexpressing OsMYB3R-2
showed increased cold tolerance [10, 19]. Further studies showed that OsMYB3R-2 functions as a MYB transcription factor targeting OsCycB1;1, which was involved in the G2/M phase transition at low temperature. The transcript level of OsCPT1, a putative member of DREB1/CBF pathway, was also enhanced by OsMYB3R-2 [19]. Recently, another rice MYB gene OsMYB2 was functionally studied. Expression of OsMYB2 was upregulated by salt, cold, and dehydration stresses. Overexpression of OsMYB2 increased salt, cold, and dehydration tolerance. The enhanced proline and soluble sugar accumulations and activities of antioxidant enzymes, including peroxidase, superoxide dismutase, and catalase, may underlie the enhanced stress tolerance [20]. Besides in Arabidopsis and rice, several MYB genes from other plants, such as apple, wheat, and tobacco, also showed the roles in improving abiotic stress tolerance [21–23].

In the present study, a novel MYB transcription factor gene CMYB1 was identified through transcriptome analysis in rice under cold stress. Gene expression analysis suggested that CMYB1 was largely induced upon cold stress and also induced by osmotic and drought stresses. Interestingly, CMYB1 showed the significant rhythmic expression profile, implying that CMYB1 might play an important role in cold tolerance through a circadian rhythm regulation manner.

2. Materials and Methods

2.1. Plant Material and Stress Treatments. Rice cultivar Jiu-caicing (Oryza sativa L. subsp. japonica) was used in this experiment. The rice seeds were sterilized in 0.3% NaClO for 15 minutes, germinated, and cultured in Yoshida’s nutrition solution in the growth chamber with a light cycle of 16 h light at 28 °C and 8 h dark at 22 °C until three-leaf stage. Some seedlings were transferred to the field for further growing; then the stem, node, leaf blade, leaf sheath, immature panicle, mature panicle, and anther were harvested at proper time.

For abiotic stress treatments, the three-leaf seedlings were transferred to Yoshida’s nutrition solution containing 100 mM NaCl, 20% PEG 6000 (providing an osmotic potential of –0.54 MPa), and 0.1 mM ABA, respectively, or cultured in the nutrition solution under the condition of 4 °C, −80 °C until RNA was isolated. For dehydration treatment, the seedlings were taken off from solution and kept at a natural dehydration situation for 12 h and then put back in the nutrition solution for recovery.

2.2. RNA Isolation and First-Strand cDNA Synthesis. The total RNA was extracted using the Trizol reagent (Invitrogen, USA) according to the manufacturer’s protocol. The remaining genomic DNA in the RNA samples was removed by treating with DNase I (Promega, USA) at 37 °C for 15 min. The first-strand cDNA was synthesized with 2 μg of purified total RNA using the reverse transcription system (Promega, USA) according to the manufacturer’s protocol.

2.3. Cloning of CMYB1. The coding sequence of CMYB1 was amplified from cDNA of three-leaf rice seedling by PCR using the primers as follows: forward primer: 5'-TGGAGAGATTCGTTT-3'; reverse primer: 5'-CTTCTCCGGCCTTA-3'. The PCR product was purified and cloned into pEASY-T vector (TransGen, China) and sequenced. Plasmid was isolated from single clone, which was confirmed with correct sequencing result, and used as template for next construction of expression proteins.

2.4. Quantitative Real-Time RT-PCR. The CMYB1 primers (forward primer: 5'-TGCGAGGCACAATACATAGTG-3'; reverse primer: 5'-CCACTACGCTTTCGGAATTGCC-3') were used for quantitative real-time PCR analysis. A constitutively expressed gene 18S rRNA was used as an internal control [25]. The primers of 18S rRNA were as follows: forward primer: 5'-ATGGTGTGAAGGTGAGCC-3'; reverse primer: 5'-CAGACACTAAAGCGCCCGTGA-3'.

2.5. Subcellular Localization of CMYB1::GFP Fusion Protein. To construct CMYB1::GFP fusion protein, the CMYB1 fragment was amplified using the primers as follows: forward primer: 5'-GTCTCTAGAAATGGAATGGCCTGTGTT-3'; reverse primer: 5'-GTTCGGCGGTGTCAGACACGA-3', where the underlines indicate the restriction digestion site of Xba I and Sma I, respectively. Then this fragment was cloned into the Xba I and Sma I sites of plant transient expression vector pA7 [26] to construct CMYB1::GFP fusion protein under the control of CaMV35S promoter. 35S::GFP fusion protein was used as control. Both of the fusion proteins were then transferred into onion epidermal cells by particle bombardment and the cells were examined with confocal laser scanning microscopy (Nikon ECLIPSE 80i, Japan).

2.6. Transcriptional Activity Assay in Rice Protoplast. The transcriptional activity assay of CMYB1 was determined in rice protoplast by dual luciferase reporter assay system (Promega, USA). To construct reporter plasmids, we used the pGL3-Basic vector, in which two random cis-elements, 5×GAL4 and 3×DRE, and a minimal TATA promoter [27] were cloned in the MCS before luciferase reporter gene. The 35S::DBD-(GAL4-DNA binding domain) CMYB1 effector plasmid was constructed by inserting DNA fragments containing CMYB1 coding region and GAL4-DNA binding domain sequence of pGKT7 vector (Clontech, USA) into the Spe I and Xba I sites of pA7. Similarly, 35S::BD and 35S::BD-ZFP179 plasmids [14] were constructed as negative control and positive control, respectively. To enhance the background expression level, 35S::OsDREB1A effector plasmid that can bind to DRE cis-element was constructed [28]. The renilla luciferase was used as endogenous control. All the plasmids were transferred into rice protoplast by polyethylene glycol and cultivated overnight for the expression of luciferase genes. The luciferase activity was detected via GLOMAX 20/20 Luminometer (Promega, USA).
3. Results and Discussion

3.1. Sequence Analysis of CMYB1. Based on the cold-responsive transcriptome analysis in rice (our unpublished data), an EST probe (probe ID: Os.8149.1.S1_at) encoding a putative MYB family transcription factor (Gene locus name: LOC_Os02g46030.1) was identified. The predicted protein product comprised 491 amino acids with a convered MYB domain at the N-terminal and was designated CMYB1.

CMYB1 belongs to the RI-type subfamily of MYB proteins and was homologous to many other MYB transcription factors containing single MYB domain (Figure 1(a)). Furthermore, a phylogenetic tree was constructed using neighbor-joining method to investigate the evolutionary relationship among several MYB proteins (Figure 1(b)). The result revealed that CMYB1 was clustered with AtRVE1 [29], AtRVE2 [30], and GmMYB177 [31], whereas other MYB proteins containing two or three MYB repeats were categorized into another big branch. GmMYB177 has been reported to confer stress tolerance in transgenic plants [31]. The phylogenetic clustering suggests that CMYB1 might functionally relate to its orthologues in Arabidopsis and soybean.

3.2. The Tissue-Specific Expression Pattern of CMYB1. To explore the tissue-specific gene expression pattern, a total of nine rice tissues at different stages were employed to analyze CMYB1 expression. Through qRT-PCR assay, it was observed that CMYB1 was highly expressed in rice stem and node under normal growth conditions (Figure 2). The stems and nodes of rice plants contain more active cells being proliferating and growing. The MYB proteins have been suggested with the roles in the regulation of cell cycle genes by directly binding their promoter regions. Stress regulated OsMYB3R-2 could directly target OsCycB1;1, which was involved in the G2/M phase transition and further regulated cell cycle [19]. Therefore, CMYB1 probably also plays a role in modulating cell cycle in rice stem and node and involves the internodes elongation.

3.3. CMYB1 Is Induced by Multiple Abiotic Stresses and ABA Treatment. To verify the transcriptome data, the expression pattern of CMYB1 in rice seedlings under different stress conditions was investigated. All seedlings were kept under continuous light for 48 h before treatment and the light was continuous during the whole treatments. Consistent with the transcriptome data, the transcription level of CMYB1 was induced dramatically under 4°C in a very short time and gradually accumulated up to 12 hour with nearly 140-fold as compared to control (Figure 3(a)). For 20% PEG6000 stress, CMYB1 was upregulated in 20 minutes and the expression reached to the top in 1 h with about 8-fold as compared to control (Figure 3(b)). However, CMYB1 was negatively regulated by salt treatment at the later stage of the treatment (Figure 3(c)). For dehydration, the CMYB1 was upregulated after 6 h treatment and decreased thereafter. After recovery in nutrition solution, it was found that CMYB1 expression level decreased to the baseline (Figure 3(d)). The expression of CMYB1 was also slightly induced by exogenous ABA treatment (Figure 3(e)).

3.4. Expression of CMYB1 Is Involved in Circadian Rhythm. Through investigation of CMYB1 expression in RiceXPro microarray datasets (http://ricexpro.dna.affrc.go.jp/), it was found that expression of CMYB1 was significantly subjected to circadian rhythm regulation. The similar results were obtained in rice leaves during different developmental stages, including vegetative, reproductive, and ripening stages (Figure 4). It seems that CMYB1 gene was highly expressed in middle night and lowly expressed in middle day in every stage, suggesting that CMYB1 might function more specifically in the night. As CMYB1 was largely induced upon cold stress, it was hypothesized that CMYB1 probably functions in regulating expression of genes involved in cold tolerance to maintain plants normal growth, and its expression is downregulated in the day with the increased temperature. Interestingly, two other orthologues of CMYB1, REVEILLE1 (RVE1) and RVE2, were also involved in circadian rhythm. Although inactivation of RVE1 does not affect circadian rhythmicity, it was speculated that RVE1 was a clock output affecting plant development. It was also suggested that RVE2 was possibly part of a regulatory feedback loop that controls a subset of the circadian outputs. As CMYB1 expression was also strictly circadian rhythm regulated, this branch of MYB family might be involved in circadian rhythmicity, especially functioning in the regulation of circadian outputs regulating plant development and adaptation to environment changes.

3.5. cis-Acting Elements Present in CMYB1 Promoter Region. The promoter sequence of CMYB1 was analyzed through the MatInspector program (http://www.genomatix.de/). The result showed that the promoter sequence of CMYB1 contained many putative plant hormone-related cis-acting elements, such as ABA-responsive element (ABRE), auxin response element (AuxRE), brassinosteroid (BR) response element (BRRE), and ethylene response element factors (EREF), indicating that CMYB1 might be a central cross-talk of various plant hormones regulating plant growth and development. The presence of ABRE in the promoter region and the fact that CMYB1 was induced by exogenous ABA treatment suggested that CMYB1 probably functions in the ABA-dependent pathway of stress response in rice. Interestingly, we also found a circadian-related cis-element existing in the promoter region (Table 1), indicating that regulation of CMYB1 by circadian rhythm might be mediated by this element.

3.6. CMYB1 Is Localized in the Nuclei. To investigate the subcellular location of CMYB1, the coding regions of the whole CMYB1 cDNA were fused with GFP gene, and the resulting construct was introduced into onion epidermal cells by particle bombardment. Localization of fusion protein was determined by visualization with fluorescence microscopy. The 35S::CMYB1::GFP fusion protein was localized to the nuclei of onion epidermal cell (Figure 5). By contrast, the
Figure 1: Multiple sequences alignment and phylogenetic tree analysis of amino acid sequences of CMYB1 with other MYB proteins. (a) Multiple sequences alignment of CMYB1 with the other R1-type MYB proteins. The upper line indicates the R1 repeat. GenBank accession numbers for the sequences: ArvRE1 (AT5G17300), ArvRE2 (AT5G37260), ArvCCA1 (ATU28422), ArvLHY1 (ATIG01060), and GmMYB177 (DQ822925). (b) Phylogenetic tree analysis of CMYB1 with other MYB proteins. The neighbor-joining tree was constructed with MEGA 4.0. Branch numbers represent a percentage of the bootstrap values in 1,000 sampling replicates and the scale bar indicates branch length. GenBank accession numbers for the sequences: AtMYB15 (AY42407), OsMYB4 (D886620), GmMYB92 (DQ822903), AtMYB90 (ATIG06390), AtMYB51 (ATIG08570), AtMYB60 (ATIG08880), GmMYB76 (DQ822885), OsGAMYB (AK19607), AtMYB52 (ATIG17950), OsMYB3R-2 (BAD8765), AtMYB3R4 (ATSGH510), AtMYB3R1 (AF176005), NmMYB1A1 (AB056122), and NmMYB2A2 (AB056123).
Table 1: Predicted cis-elements in the promoter region of CMYB1.

| Element name                        | Element sequence               | Position^b |
|-------------------------------------|--------------------------------|------------|
| ABA response elements (ABRE)        | tctgatgaCGTGgaccg             | −1441–1425 |
|                                     | tggcctgaCGTGTcaaa             | −988–972  |
|                                     | cacataaGCTGgcct              | −688–652  |
|                                     | gactcAGTggcgcg               | −264–248  |
| Auxin response element (AuxRE)      | ttaTGTCcccgtc                | −1422–1410 |
| Brassinosteroid response element (BRRE) | cctgaCGTGtcaaaa         | −985–969  |
|                                     | gcgctCGTGtggacggg            | −914–898  |
| Ethylene response element factors (ERE) | aGCGAaagtccaaagtcc          | −964–946  |
| GCC box family (GCC)                | gactccGCGCaag               | −1270–1258 |
| Heat shock element (HSE)            | gcgtTTCgtaatt                | −1205–1189 |
|                                     | gaaaaaaaataAGAAAaa            | −368–352  |
| NAC transcription factor recognition site | ttttaacctccgcCACGtcatccc | −1368–1342 |
| Salt/drought responsive element     | ttgcgGCGGgtgatt              | −1039–1025 |
| Circadian LEtLHC                    | catCAAcaacATCgag             | −123–108  |

^aThe capital letters represented the core sequence in the predicted cis-element.

^bPosition displayed the nucleotide position relative to the up-stream of ATG of CMYB1.

Figure 2: The qRT-PCR analysis of expression of CMYB1 in various rice tissues.

In this paper, we reported a novel MYB transcription factor gene CMYB1 which was dramatically induced by cold stress. CMYB1 is a nuclear protein exhibiting transcriptional activation activity, implying that CMYB1 functions as a transcription activator in rice cells. Interestingly, we found that expression of CMYB1 was significantly regulated by circadian rhythm. As CMYB1 was largely induced upon cold stress, it is hypothesized that CMYB1 probably promotes cold-responsive gene expression to respond to low temperature in the night. With temperature increases in the day, expression of CMYB1 gradually decreases. However, this hypothesis needs further experimental validation by use of CMYB1 null mutant or transgenic approach.

4. Conclusion

In this paper, we reported a novel MYB transcription factor gene CMYB1 which was dramatically induced by cold stress. CMYB1 is a nuclear protein exhibiting transcriptional activation activity, implying that CMYB1 functions as a transcription activator in rice cells. Interestingly, we found that expression of CMYB1 was significantly regulated by circadian rhythm. As CMYB1 was largely induced upon cold stress, it is hypothesized that CMYB1 probably promotes cold-responsive gene expression to respond to low temperature in the night. With temperature increases in the day, expression of CMYB1 gradually decreases. However, this hypothesis needs further experimental validation by use of CMYB1 null mutant or transgenic approach.
Figure 3: The abiotic-stress-induced expression pattern of CMYB1. Jiucaiqing three-leaf seedlings were treated with 4°C (a), 20% PEG6000 (b), 100 mM NaCl (c), dehydration (d), and 0.1 mM ABA (e), respectively.
Figure 4: Expression of CMYB1 in rice response to circadian rhythm in leaves under different developmental stages. Veg.: vegetative; Rep.: reproductive; Rip.: ripening. The gene expression analysis is based on RiceXPro microarray data [24].

Figure 5: Subcellular localization of CMYB1. The 35S::CMYB1::GFP which was constructed in pA7 vector and the empty vector harboring 35S::GFP were transformed into onion epidermis cells, respectively.
Figure 6: Transcriptional activity analysis of CMYB1 in rice protoplast via dual luciferase reporter assay system. (a) Construction of reporter, effector, and internal control vectors. (b) The luciferase activities. Vector control contained reporter and internal control plasmids; DREB1A contained reporter, internal control, and 35S::DREB1A protein plasmids; DREB1A+GAL4DBD contained reporter, internal control, 35S::DREB1A protein, and 35S::GAL4DBD protein plasmids; DREB1A+G-CMYB1 contained reporter, internal control, 35S::DREB1A protein, and 35S::GAL4DBD-CMYB1 fusion protein plasmids; DREB1A+G-ZFP179 contained reporter, internal control, 35S::DREB1A protein, and 35S::GAL4DBD-ZFP179 fusion protein plasmids.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution
Min Duan and Peng Huang contributed equally to this work.

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References
[1] K. Yamaguchi-Shinozaki and K. Shinozaki, “Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses,” Annual Review of Plant Biology, vol. 57, pp. 781–803, 2006.
[2] D. Golldack, I. Lüking, and O. Yang, “Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network,” Plant Cell Reports, vol. 30, no. 8, pp. 1383–1391, 2011.
[3] M. Q. Zhou, C. Shen, L. H. Wu, K. X. Tang, and J. Lin, “CBF-dependent signaling pathway: a key responder to low temperature stress in plants,” Critical Reviews in Biotechnology, vol. 31, no. 2, pp. 186–192, 2011.
[4] J. Mizoi, K. Shinozaki, and K. Yamaguchi-Shinozaki, “AP2/ERF family transcription factors in plant abiotic stress responses,” Biochimica et Biophysica Acta: Gene Regulatory Mechanisms, vol. 1819, no. 2, pp. 86–96, 2012.
[5] Q. Wang, Y. Guan, Y. Wu, H. Chen, F. Chen, and C. Chu, “Over-expression of a rice OsDREB1F gene increases salt, drought, and low temperature tolerance in both Arabidopsis and rice,” Plant Molecular Biology, vol. 67, no. 6, pp. 589–602, 2008.
[6] M. Cui, W. Zhang, Q. Zhang et al., “Induced over-expression of the transcription factor OsDREB2A improves drought tolerance in rice,” Plant Physiology and Biochemistry, vol. 49, no. 12, pp. 1384–1391, 2011.
[7] Z.-S. Xu, M. Chen, L.-C. Li, and Y.-Z. Ma, “Functions and application of the AP2/ERF transcription factor family in crop improvement,” Journal of Integrative Plant Biology, vol. 53, no. 7, pp. 570–585, 2011.
[8] G. Lu, C. Gao, X. Zheng, and B. Han, “Identification of OsbZIP72 as a positive regulator of ABA response and drought tolerance in rice,” Planta, vol. 229, no. 3, pp. 605–615, 2009.
A. Yang, X. J. Dai, and W. H. Zhang, “A R2R3-type MYB gene,” 2011.

X. Dai, Y. Xu, Q. Ma et al., “Overexpression of an R1R2R3 MYB gene, OsMYB3R-2, increases tolerance to freezing, drought, and salt stress in transgenic Arabidopsis,” Plant Physiology, vol. 143, no. 4, pp. 1739–1751, 2007.

H. Hu, M. Dai, J. Yao et al., “Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 35, pp. 12987–12992, 2006.

H. Sun, X. Huang, X. Xu, H. Lan, J. Huang, and H.-S. Zhang, “ENAC1, a NAC transcription factor, is an early and transient response regulator induced by abiotic stress in rice (Oryza sativa L.),” Molecular Biotechnology, vol. 52, no. 2, pp. 101–110, 2012.

C.-F. Niu, W. Wei, Q.-Y. Zhou et al., “Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants,” Plant, Cell and Environment, vol. 35, no. 6, pp. 1156–1170, 2012.

S.-J. Sun, S.-Q. Guo, X. Yang et al., “Functional analysis of a novel Cys2/His2-type zinc finger protein involved in salt tolerance in rice,” Journal of Experimental Botany, vol. 61, no. 10, pp. 2807–2818, 2010.

F. Lippold, D. H. Sanchez, M. Musialak et al., “AtMYb41 regulates transcriptional and metabolic responses to osmotic stress in Arabidopsis,” Plant Physiology, vol. 149, no. 4, pp. 1761–1762, 2009.

R. Stracke, M. Werber, and B. Weisshaar, “The R2R3-MYB gene family in Arabidopsis thaliana,” Current Opinion in Plant Biology, vol. 4, no. 5, pp. 447–456, 2001.

Y. Chen, X. Zhang, W. Wu, Z. Chen, H. Gu, and L.-J. Qu, “Overexpression of the wound-responsive gene AtMYB15 activates the Shikimate pathway in Arabidopsis,” Journal of Integrative Plant Biology, vol. 48, no. 9, pp. 1084–1095, 2006.

E. Cominelli, M. Galbiati, A. Vavasseur et al., “A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance,” Current Biology, vol. 15, no. 13, pp. 1196–1200, 2005.

Q. Ma, X. Dai, Y. Xu et al., “Enhanced tolerance to chilling stress in OsMYB3R-2 transgenic rice is mediated by alteration in cell cycle and ectopic expression of stress genes,” Plant Physiology, vol. 150, no. 1, pp. 244–256, 2009.

A. Yang, X. J. Dai, and W. H. Zhang, “A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice,” Journal of Experimental Botany, vol. 3, no. 7, pp. 2541–2556, 2012.

L. C. Zhang, G. Y. Zhao, C. Xia, J. Z. Jia, X. Liu, and X. Y. Kong, “A wheat R2R3-MYB gene, TaMYB30-B, improves drought stress tolerance in transgenic Arabidopsis,” Journal of Experimental Botany, vol. 63, no. 16, pp. 5873–5885, 2012.

R. K. Wang, Z. H. Cao, S. Z. Zhang, R. F. Zhang, and Y. J. Hao, “Genome wide analysis of the apple myb transcription factor family allows the identification of mdmyb121 gene conferring abiotic stress tolerance in plants,” PLoS ONE, vol. 8, no. 7, pp. 1–13, 2013.

C. J. Huang, G. J. Hu, F. F. Li, Y. Q. Li, J. X. Wu, and X. P. Zhou, “NtPHAN, a MYB transcriptional factor, regulates leaf development and affects drought tolerance in Nicotiana benthamiana,” Physiologia Plantarum, vol. 149, pp. 297–309, 2013.