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Research Article

Isolation of Multi-Abiotic Stress Response Genes to Generate Global Warming Defense Forage Crops

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ABSTRACT

Forage crop management is severely challenged by global warming-induced climate changes representing diverse a/biotic stresses. Thus, screening of valuable genetic resources would be applied to develop stress-tolerant forage crops. We isolated two NAC (NAM, ATAF1, ATAF2, CUC2) transcription factors (ANAC032 and ANAC083) transcriptionally activated by multi-abiotic stresses (salt, drought, and cold stresses) from Arabidopsis by microarray analysis. The NAC family is one of the most prominent transcription factor families in plants and functions in various biological processes. The enhanced expressions of two *ANAC*s by multi-abiotic stresses were validated by quantitative RT-PCR analysis. We also confirmed that both ANACs were localized in the nucleus, suggesting that ANAC032 and ANAC083 act as transcription factors to regulate the expression of downstream target genes. Promoter activities of *ANAC032* and *ANAC083* through histochemical GUS staining again suggested that various abiotic stresses strongly drive both *ANAC*s expressions. Our data suggest that ANAC032 and ANAC083 would be valuable genetic candidates for breeding multi-abiotic stress-tolerant forage crops via the genetic modification of a single gene.

(Key words: ANAC032, ANAC083, Multi-abiotic stress, NAC transcription factor)

Ⅰ. Introduction

Forage crop production and grassland are essential for managing livestock industries; however, the production is widely challenged by global warming (Wheeler and Reynolds, 2013). As forage crop cultivation is based on outdoor farming, crops are primarily affected by environmental factors such as drought, high salinity, and extreme temperature such as hot and cold temperatures. Plants respond and adapt through various biochemical and physiological processes to overcome these environmental stresses by the transcriptional activation of diverse gene sets encoding heat-shock proteins, antioxidants, ion transporters, and so on (Bray et al., 2000). Extensive screening of genes responding to various abiotic stresses has been applied in the model plant Arabidopsis and in various forage crops (Lee et al., 2018; Tawab et al., 2020). Thus, the genetic modification will be helpful to develop forage crops defending to current dynamic climate changes.

The stress signaling pathways mediating these adaptations are controlled mainly by transcription factors (TFs) and *cis*-regulatory elements (Shinozaki et al., 2000). TFs are proteins that respond to environmental stimuli through a signaling cascade and bind to specific regulatory sites upstream of constituent genes in a regulatory network by direct physical interaction or in combination with other proteins. Consequently, an alteration of the expression of transcription factor genes results in dramatic differences in the expression of multiple genes in plants (Liu et al., 1999). The *cis*-regulatory elements of some genes that have a typical stress-inducible expression profile and the transcription factors that affect the expression of these genes have been identified (Shinozaki and Yamaguchi-Shinozaki, 2007). One of the most prominent families of plant-specific transcription factors is NAC proteins (Riechmann et al., 2000). NAC (NAM, ATAF1, ATAF2,

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CUC2)-domain proteins are a class of TFs known to control multiple processes, including plant development, defense, and hormone and stress responses (Riechmann et al., 2000). NAC genes have also been involved in light responses, programmed cell death, senescence, and various abiotic stresses (Shao et al., 2015). Although efficient transformation techniques have been developed in forage crops (Lee et al., 2014), identifying valuable genetic materials responding to multi-abiotic stresses will be a fast-approaching strategy to develop the forage crops.

In this study, we screened two multi-abiotic stress-responsive NAC TFs (*ANAC032* and *ANAC083*) through microarray analysis, and identified their transcriptional levels, subcellular localization, and promoter activity under various stresses.

Ⅱ. Materials and Methods

1. Plant materials and stress treatments

Arabidopsis thaliana (ecotype Columbia) were grown on germination medium (GM) agar plates under a 16 h light/8 h dark condition. For microarray analysis, mRNA treated with salt, drought, and cold stresses for 3 h were labeled with Cy5 and Cy3 (for untreated) fluorescent probes. The cDNA probes were hybridized with the cDNA microarray prepared from ~1300 full-length Arabidopsis cDNAs on the DNA chip. For treatment under different stress conditions, 7-day-old seedlings were transferred to the MS liquid medium with or without 100 μM abscisic acid (ABA), 250 mM (for Northern blot analysis) or 200 mM NaCl (for semi-quantitative RT-PCR and histochemical analyses), 20% (v/v) polyethylene glycol (PEG) 6000 or 200 mM mannitol then incubated in the culture room by shaking for indicated time. For dehydration and cold treatments, plants were incubated on 3 MM paper for 30 min and placed in a 4 ℃ chamber for 24 h, respectively. For wounding stress treatment, half of the rosette leaves were scratched with a sterile needle and maintained for 1 day.

2. Northern blot analysis

Total RNA from untreated and stress-treated Arabidopsis seedlings was extracted using the phenol/LiCl method (Ausubel et al., 2003). Total RNA (20 μg) was fractionated on 1.2% formaldehyde agarose gel and blotted on Hybond-N nylon membrane (Amersham) with 10x SSC. Blots were hybridized overnight at 65 ℃ with a ^{32}P-labeled full-length DNA using the Megaprime DNA labeling system (Amersham). After hybridization, the blots were washed twice with 2x SSC containing 1% (w/v) SDS for 15 min at room temperature and twice with 0.1x SSC containing 0.5% SDS for 10 min at 65 ℃. The blots were exposed to X-ray film (Fuji Photo Film).

3. Semi-quantitative RT-PCR analysis

RNA was extracted from wild-type Arabidopsis plants using the Trizol reagent (Invitrogen). Five micrograms of RNA of each genotype with 1 μg oligo (dT) primer were heated at 70 ℃ for 5 min and then chilled on ice immediately. RNA was then subjected to RT with RevertAid™ M-MuLV reverse-

Tabel 1. List of oligonucleotide primers used in this study

Gene Name	Application	Primer Name	Sequences
ANAC032 (At1g77450)	GFP-Localization	F032GFP R032GFP	5'-GCTCTAGAATGATGAAATCTGGGGCTGA-3' 5'-GCGGATCCGAAAGTTCCCTGCCTAACC-3'
	GUS-Promoter	F032GUS R032GUS	5'-AAAAAGCAGGCTAGACGTTAACATGTCAA-3' 5'-AGAAAGCTGGGTCATAATTAACCTATTTT-3'
ANAC083 (At5g13180)	GFP-Localization	F083GFP R083GFP	5'-GCGGATCCATGGATAATGTCAAACTTGTT-3' 5'-GCGAATTCTCTGAAACTATTGCAACTA-3'
	GUS-Promoter	F083GUS R083GUS	5'-AAAAAGCAGGCTAACATATATTTGGACGAAAT-3' 5'-AGAAAGCTGGGTCATGGTGGTTCCAAAC-3'
RD29A	Gene expression	FRD29A RRD29A	5'-GCCGGATCCTTTTCTGATATGGTTGCC-3' 5'-GCCCTCGAGCCGAACAATTTATTAACC-3'
Cor15A	Gene expression	FCor15A RCor15A	5'-CTCTCTCATGGCGATGTC-3' 5'-CTGATTAGGTAAGACCCTA-3'
Tubulin	Gene expression	FTUB2 RTUB2	5'-GTTCTCGATGTTGTTCGTAAG-3' 5'-TGTAAGGCTCAACCACAGTAT-3'

transcriptase (Fermentas) at 42 ℃ for 1 h according to the manufacturer's protocol. Synthesized cDNA was used as a template for PCR. The PCR amplify of expected genes was conducted with the following primers in Table 1, and the tubulin gene was used as a loading control.

4. ANAC032- and ANAC083-GUS promoter constructs

The promoter regions (1500 bp upstream from the start codon) of the *ANAC032* and *ANAC083* genes were PCR-amplified with the following primer pair for GUS-expression in Table 1. The PCR fragment was inserted into entry vector *pDONR221* and then recombined into the destination vector *pBGWFS7* by Gateway methods (Invitrogen). These *ANAC032* and *ANAC083* promoter-GUS constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 and transformed into wild-type Arabidopsis. T1 transgenic lines resistant to Basta herbicide (glufosinate) were used for generations. T2 transgenic lines were selected on MS medium containing phosphinothricin (4 mg ml^{-1}).

5. Histochemical GUS assay

For GUS assay, T3 seedlings grown on MS agar plates were was incubated in a solution containing 50 mM phosphate buffer, 10 mM EDTA (pH 8), 0.1% (v/v) Triton X-100, 1.25 mM potassium ferricyanide, 1.25 mM potassium ferrocyanide, and 0.1% (w/v) 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc) for 12 h at 37 ℃ and then washed with ethanol to remove chlorophylls.

6. *In vivo* targeting of GFP fusion constructs

Subcellular localization analysis of *ANAC032* and *ANAC083* was prepared using leaf tissues of 10-day-old Arabidopsis plants grown on MS in the growth chamber. Leaves were cut with a razor blade and incubated with 30 ml of enzyme solution containing 1% (w/v) macerozyme R-10 (Yakult Honsha Co), 4% (w/v) cellulase onozuka R-10 (Yakult Honsha Co), 0.1% (w/v) pectolyase Y23 (Yakult Honsha Co), 400 mM mannitol, 1 mM $CaCl_2$, and 0.5% (w/v) BSA (pH 5.6) at 22 ℃ for 24 h with gentle agitation (50 to 75 rpm). To construct *ANAC032::GFP* and *ANAC083::GFP*, cDNA was amplified with the primer pairs F032GFP and R032GFP for *ANAC032*, and primer pairs F083GFP and R083GFP for *ANAC083* (Table 1) by deletion of the stop codon. The PCR fragments were then fused into *pUC19* modified vector (smGFP) driven by *35S promoter*. The construct was transformed to protoplast using polyethylene glycol-mediated transformation. The images were monitored and captured by IX71 microscope camera (Olympus). The filters sets used are U-MGFPHQ mirror units (exciter, 460~480HQ: dichroic, 485: and emitter, 495~540HQ).

Ⅲ. RESULTS AND DISCUSSION

1. Isolation of multi-abiotic stresses responsible genes by microarray analysis.

Microarray analysis was applied to isolate multiple abiotic stress responsive genes against significant environmental stresses such as salt, drought, and cold. Over 500 genes among 1300 TFs prepared on DNA chip were commonly induced by these treatments. We regarded genes with an expression ratio greater than three-folds (3 h salt vs. untreated, 3 h drought vs. untreated, or 3 h cold vs. untreated) as multi-stress-inducible genes (Fig. 1A) (Ooka et al., 2003). For further functional analysis in abiotic stress responses, two TFs (*ANAC032* and *ANAC083*) genes were selected. NAC TFs including ORESARA1 (ORE1)/ANAC092 are well characterized in leaf senescence as positive regulators (Kim et al., 2009; Yang et al., 2011; Mahmood et al., 2016). However, our two NAC TFs are not well characterized yet under diverse abiotic stresses in detail.

Expression patterns of *ANAC032* and *ANAC083* were validated using Northern blot analysis. NaCl (250 mM) treatment as salt stress, PEG 6000 (20%) treatment as alternative drought stress, and ABA treatment (100 μM) as a stress-inducible phytohormone, and cold stress were applied to Arabidopsis seedlings and examined transcriptional levels with their representative marker genes such as *RD29A* for salt, drought and ABA treatments and *Cor15A* for cold treatment. Both *ANAC* genes were gradually increased by NaCl, ABA, or cold treatment and peaked at 1 h of PEG 6000 treatment (Fig. 1B). Thus, *ANAC032* and *ANAC083* could be positive regulators and candidates for developing multi-abiotic stress-responsive forage crop plants.

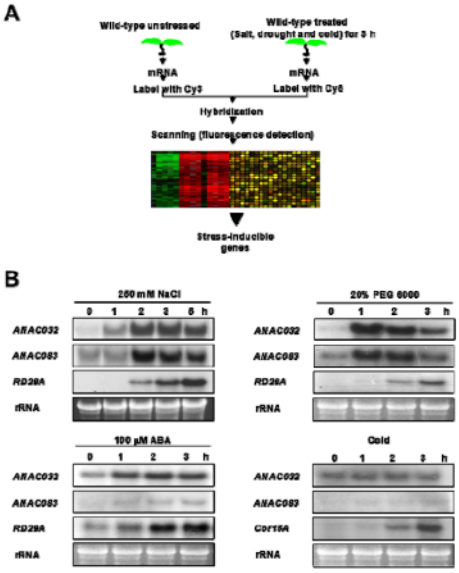

Fig. 1. Microarray preparation and Northern blot analysis. (A) A schematic performing of microarray analysis for multi abiotic stress inducible genes. (B) Conformation of isolated *ANAC* genes under abiotic stresses by Northern blot analysis. Seven-day-old Arabidopsis wild-type seedlings were exposed to NaCl (250 mM), PEG (20%), ABA (100 μM), or cold (4 ˚C) for indicated time period.

2. The transcriptional pattern of *ANAC032* and *ANAC083*.

To confirm whether expression of *ANAC032* and *ANAC083* genes is affected by abiotic stresses, seven-day-old seedlings of Arabidopsis wild-type were subjected to various abiotic stresses. Quantitative PCR analyses showed that transcript levels of *ANAC032* and *ANAC083* genes in wild-type plants were highly increased by ABA, high salt, or drought treatments (Fig. 2A, C). The increasing expressions were 2-fold over than non-treated plants. Cold, osmotic stress or wounding treatments also highly induced the transcript levels of *ANAC032* and *ANAC083* (Fig. 2A–C).

To obtain further clues about their functions, we analyzed the expression pattern of *ANAC032* and *ANAC083* in different tissues. As shown in Fig. 2B, the *ANAC032* gene was expressed in silique, leaves, and roots, mature plant tissues. Moreover, *ANAC083* was highly induced in both cauline and rosette leaves (Fig. 2D). These accelerated expression patterns in leaves may be correlated with leaf longevity as positive regulators for delaying leaf senescence (Yang et al., 2011; Mahmood et al., 2016).

3. Subcellular localization of *ANAC032* and *ANAC083*.

TFs control transcriptional regulation in massive biological processes in plant development and environmental stresses (Ooka et al., 2003; Chapman and Estelle, 2009). TFs are localized in the nucleus and regulate their expression of downstream target genes via their DNA-binding motifs. ANAC032 protein contains the NAC-domain, divided into 5 subdomains and has high similarity with other members of the NAC family. However, the C-terminal region shows no significant similarity with other members of NAC family. A putative nuclear localization signal (NLS) sequence (PRDRKYPNGS) was found in its N-terminal region. The closest relative of ANAC032 is ATAF1, with which it shares around 65% amino acid similarity (data not shown). ATAF1 is involved in dehydration stress and ABA-dependent (Lu et al., 2007). In addition, the N-terminal region of ANAC083 shares a high identity to other NAC domain-containing proteins and a variable C-terminal domain. The five motifs (A to E) comprising the NAC domain presented in the N-terminal region. A putative NLS sequence (AKYPNGN) of ANAC083 was predicted in the third motif of NAC domain.

Thus, we constructed GFP-fused ANAC032 and ANAC083

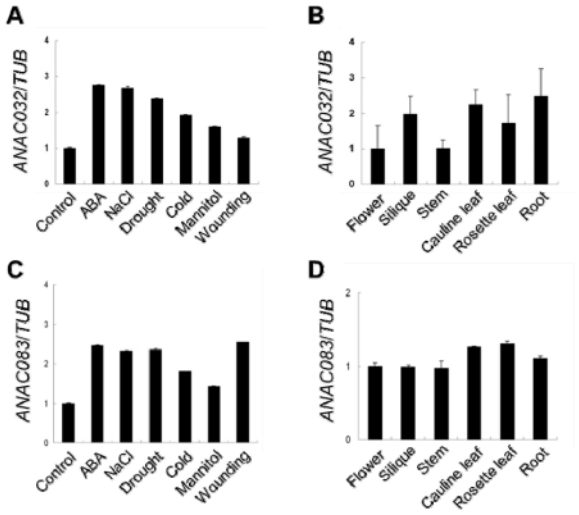

Fig. 2. The expression of *ANAC* genes in wild-type plants. Semi-quantitative RT-PCR analyses of *ANAC032* (A and B) and *ANAC083* (C and D) transcripts. Seven-day-old Arabidopsis Col-0 seedlings were treated as follows, control (no treatment), ABA (100 μM) for 12 h, NaCl (200 mM) for 12 h, drought as dehydration on 3MM paper for 1 h, cold (4 ℃) for 24 h, mannitol (200 mM) for 12 h, and wounding. Data represent means±SD of three independent experiments.

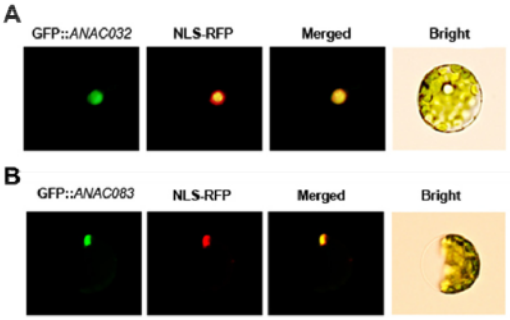

Fig. 3. In vitro targeting of the GFP-fused ANAC032 or ANAC083 proteins. The GFP::ANAC032 (A) or GFP::ANAC083 (B) fusion protein was introduced into Arabidopsis protoplast. NLS-RFP was used as a nuclear marker.

constructs driven by 35S promoter and confirmed their subcellular localization in Arabidopsis protoplasts. Both ANAC032 and ANAC083 were presumably targeted to nuclei (Fig. 3). The merged images of ANACs::GFP with a positive marker, NLS-RFP (a control for nuclear localization), clearly showed nuclear localization (Fig. 3). These implied that the general characteristics of NAC proteins are nuclear proteins, and function as TFs for regulating transcription of downstream genes.

4. Promoter activity of *ANAC032* and *ANAC083*.

To identify more detail, the temporal and spatial expression patterns of *ANAC032* and *ANAC083* were determined by histochemical β- glucuronidase (GUS) staining of transgenic plants that harbored an *ANAC032* or *ANAC083* promoter-GUS construct. With the *ANAC032* or *ANAC083* promoter (1.5 Kb from start codon) construct, GUS activity was monitored at various growth stages of seedlings. First, GUS activity of *ANAC032* was constitutively expressed in 2-, 5- and 7-day-old

seedlings (data not shown). However, its activity was not detected in 2-week-old seedlings. Interestingly, treatment with exogenous ABA, NaCl or mannitol in 2-week-old seedlings for 12 h enhanced the *ANAC032* promoter activity, mainly in the roots (Fig. 4A). Wounding treatment was not induced the expression of *ANAC032* promoter (data not shown), even though in the promoter region of *ANAC032* consists of six recognition sites for WUN boxes and two sites for W-boxes (Fig. 4B), which are known as recognition sites in response to wounding and pathogen attack, respectively (Pastuglia et al., 1997; Maleek et al., 2000). Other *cis*-elements found in the promoter sequence of *ANAC032* using Plant CARE software http://bioinformatics. psb.ugent.be/webtools/plantcare/html/) are six ABA-responsive elements (ABRE) and one dehydration responsive element (DRE), known as elements response in drought and salt stresses (Shinozaki et al., 2003). An as-1 motif is also present in the promoter, known to be oxidative stress-responsive and hypersensitive responsive elements (Lam et al., 1989; Selth et al., 2005). Second, the spatial and temporal expression pattern of *ANAC083* was also determined by GUS staining of transgenic plants harboring an *ANAC083* promoter-GUS reporter construct. In germination stages, GUS activity of *ANAC083* promoter was constitutively expressed in 2-, 5- and 7-day-old seedlings (data not shown). In 2-week-old

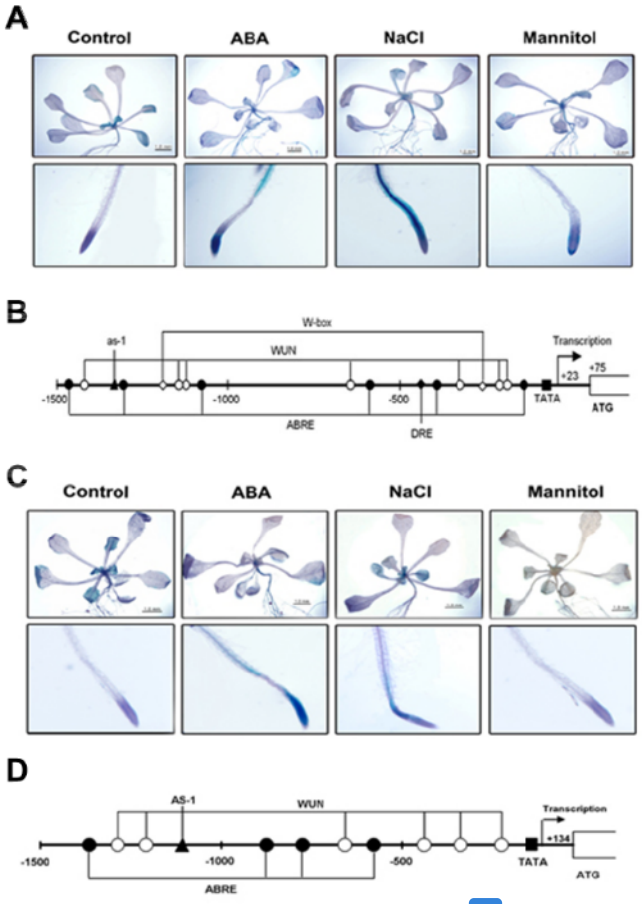

Fig. 4. Histochemical analysis of *ANAC032* and *ANAC083* promoter-GUS. GUS staining of transgenic plants harboring ANAC032 (A) or ANAC083 (B) promoter-GUS. Two-week-old plants were subjected to distilled water as a control, ABA (100 μM), NaCl (200 mM) or mannitol (200 mM) for 12 h. Distribution of *cis*-acting element in the promoter region of *ANAC032* (B) *and ANAC083* (D).

seedlings, the GUS expression in leaves and roots was very low. However, the GUS expression in seedlings treated by ABA, NaCl, or mannitol increased mainly in the roots (Fig. 4C). These results indicated that the upstream sequence present in *ANAC083* contains sufficient cis-regulatory elements to respond to ABA, NaCl, or osmotic treatment. In silico analysis showed that the upstream sequence (1.5 Kb) of *ANAC083* contains ABRE, WUN, and As1- elements, which are involved in ABA, wounding, and oxidative responses (Fig. 4D) (Yamaguchi-Shinozaki and Shinozaki, 1994).

Ⅳ. CONCLUSION

Although massive genetic resources responding to various environmental stresses have been identified, most of them are restricted in a single abiotic stress response. However, current global warming-induced climate changes are complex due to the regions and nations. Thus, multi-abiotic stresses responsive genetic materials are urgently required for developing stress-tolerant crops. TFs regulate the transcriptional expression of diverse downstream target genes via direct association with the chromosome. This study found two *ANAC* genes induced by multi-abiotic stresses including salt, drought and cold stresses by microarray analysis. Both *ANAC* genes were localized in the nucleus. Histochemical analysis revealed that various abiotic stresses activated their promoters of *ANAC032* and *ANAC083*. Taken together, the heterologous expression of *ANAC032* and *ANAC083* in forage crops would enhance stress tolerance against multi-abiotic stresses.

Ⅴ. ACKNOWLEDGEMENTS

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Ⅵ. REFERENCES

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. 2003. Current protocols in molecular biology. John Wiley & Sons, Inc., New York. pp. 4.3.1-4.3.4.

Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. 2000. Responses to abiotic stresses. Biochemistry and molecular biology of plants. In: B. Buchannan, W. Gruissem and R. Jones (Eds.), American society of plant biologist. Roclville, MD. pp. 1158-1203.

Chapman, E.J. and Estelle, M. 2009. Mechanism of auxin-regulated gene expression in plants. Annual Review of Genetics. 43:265-285.

Kim, J.H., Woo, H.R., Kim, J., Lim, P.O., Lee, I.C., Choi, S.H., Hwang, D. and Nam, H.G. 2009. Trifurcate feed-forward regulation of age-dependent cell death involving miR164 in Arabidopsis. Science. 323(5917):1053-1057.

Lam, E., Benfey, P.N., Gilmartin, P.M., Fang, R.X. and Chua, N.H. 1989. Site-specific mutations alter in vivo factor binding and change promoter expression pattern in transgenic plants. Proceeding of National Academy of Science USA. 86(20):7890-7894.

Lee, S.H., Kim, K.Y., Park, H.S., Cha, J.Y. and Lee, K.W. 2014. Comparison of *Agrobacterium*-mediated of five Alfalfa (*Medicago sativa* L.) cultivars using the GUS reporter genes. Journal of the Korean Society of Grassland and Forage Science. 34(3):187-192.

Lee, S.H., Rahman, M.A., Kim, K.W., Lee, J.W., Ji, H.C., Choi, G.J., Song, Y. and Lee, K.W. 2018. Screening of multiple abiotic stress-induced genes in Italian ryegrass leaves. Journal of the Korean Society of Grassland and Forage Science. 8(3): 90-195.

Liu, L., White, M.J. and MacRae, T.H. 1999. Transcription factors and their genes in higher plants. Functional domains, evolution and regulation. European Journal of Biochemistry. 262(2):247-257.

Lu P.L., Chen, N.Z., An, R., Su, Z., Qi, B.S., Ren, F., Chen, J. and Wang, X.C. 2007. A novel drought-inducible gene, *ATAF1*, encodes a NAC family protein that negatively regulates the expression of stress-responsive genes in Arabidopsis. Plant Molecular Biology. 63:289-305.

Mahmood, K., El-Kereamy, A., Kim, S.H., Nambara, E. and Rothstein, S.J. 2016. ANAC032 positively regulates age-dependent and stress-induced senescence in Arabidopsis thaliana. Plant and Cell Physiology. 57(10):2029-2046.

Maleek, K., Levine, A., Eulgem, T., Morgan, A., Schmidl, J., Lawton, K., Dangl, J. and Dietrich, R. 2000 An Arabidopsis promoter element shared among genes co-regulated during systemic acquired disease resistance. Nature Genetics. 26:403-410.

Ooka H., Satoh, K., Doi, K., Nagata, T., Otomo, Y., Murakami, K., Matsubara, K., Osato, N., Kawai, J., Carninci, P., Hayashizaki, Y., Suzuki, K., Kojima, K., Takahara, Y., Yamamoto, K. and Kikuchi, S. 2003. Comprehensive analysis of NAC family genes in Oryza sativa and Arabidopsis thaliana. DNA Research. 10(6):239-247.

Pastuglia, M., Roby, D., Dumas, C. and Cock, J.M. 1997. Rapid

induction by wounding and bacterial infection of an *S* gene family receptor-like kinase gene in *Brassica oleracea*. Plant Cell. 9(1):49-60.

Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K. and Yu, G. 2000. Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science. 290(5499):2105-2110.

Selth, L.A., Dogra, S.C., Rasheed, M.S., Healy, H., Randles, J.W. and Rezaian, M.A. 2005. A NAC domain protein interacts with *Tomato leaf curl virus* replication accessory protein and enhances viral replication. Plant Cell. 17(1):311-325.

Shao, H., Wang, H. and Tang, X. 2015. NAC transcription factors in plant multiple abiotic stress response: progress and prospects. Frontiers in Plant Science. 6:902.

Shinozaki, K. and Yamaguchi-Shinozaki, K. 2000. Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. Current Opinion in Biotechnology. 3(3):217-223.

Shinozaki, K. and Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. Journal of Experimental Botany. 58(2):221-227.

Shinozaki, K., Yamaguch-Shinozaki, K. and Seki, M. 2003. Regulatory network of gene expression in the drought and cold stress responses. Current Opinion of Plant Biology. 6(5):410-417.

Tawab, F., Munir, I., Nasim, Z., Khan, M.S., Tawab, S., Nasim, A., Iqbal, I., Ahmad, M.A., Ali, W., Munir, R., Munir, M. and Asim, N. 2020. Identification and characterization of a novel multi-stress responsive gene in Arabidopsis. PLoS One. 15(12):e0244030.

Wheeler, T. and Reynolds, C. 2013. Predicting the risks from climate change to forage and crop production for animal feed. Animal Frontiers. 3(1):36-41.

Yamaguchi-Shinozaki, K. and Shinozaki, K. 1994. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell. 6(2):251-264.

Yang, S.D., Seo, P.J., Yoon, H.K. and Park, C.M. 2011. The Arabidopsis NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the COR/RD genes. Plant Cell. 23(6):2155-2168.

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