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Research article

Pearl millet stress-responsive NAC transcription factor *PgNAC21* enhances salinity stress tolerance in *Arabidopsis*

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ABSTRACT

Pearl millet (*Pennisetum glaucum*) is the sixth-leading cereal crop and a staple food crop. It is known for its high tolerance to abiotic stress and good nutrient profile. NAC (NAM, ATAF1/2 and CUC) transcription factors (TFs) play an important role in abiotic stress tolerance. In our study, the pearl millet stress-responsive NAC TF gene *PgNAC21* was characterized. Gene expression analysis revealed that *PgNAC21* expression is induced by salinity stress and abscisic acid (ABA) treatment. *In silico* promoter analysis showed the presence of ABA response elements (ABREs) and MYB TF binding sites. A yeast one-hybrid assay indicated that a putative MYB TF in pearl millet, PgMYB1, binds to the promoter of *PgNAC21*. A transactivation assay in yeast cells revealed that *PgNAC21* functions as a transcription activator and that its activation domain is located in its C-terminus. Relative to control plants, *Arabidopsis* plants overexpressing *PgNAC21* exhibited better seed germination, heavier fresh weight and greater root length under salinity stress. Overexpression of *PgNAC21* in *Arabidopsis* plants also enhanced the expression of stress-responsive genes such as *GSTF6* (*GLUTATHIONE S-TRANSFERASE 6*), *COR47* (*COLD-REGULATED 47*) and *RD20* (*RESPONSIVE TO DEHYDRATION 20*). Our data demonstrate that *PgNAC21* functions as a stress-responsive NAC TF and can be utilized in transgenic approaches for developing salinity stress tolerance in crop plants.

1. Introduction

Soil salinity severely limits plant growth and yields worldwide (Shrivastava and Kumar, 2015). Plants have developed numerous mechanisms to cope with salinity stress at physiological, biochemical and molecular levels. At the molecular level, plants activate stress-responsive genes and pathways. Genes encoding ion transporters, transcription factors (TFs), protein kinases, and osmolytes can confer salinity tolerance (Kasuga et al., 1999). TFs activate or suppress the expression of such stress-responsive genes. Major stress-responsive TF families include the NAC (NAM, ATAF1/2 and CUC), DREB, bZIP, ERF, zinc finger, WRKY and MYB families (Joshi et al., 2016); these families can be individually or synergistically involved in regulating the expression of other stress-response genes in plants. Among these different TF families, the NAC TF family is a plant-specific family with many members (Nuruzzaman et al., 2013). NAC TFs in *Arabidopsis*, rice and wheat are relatively well characterized and are known to have versatile functions (Nuruzzaman et al., 2013; Saidi et al., 2017). NAC TF proteins have highly conserved N-terminal domains with DNA binding function and highly variable C-terminal domains (Olsen et al., 2005). NAC TFs with specific combinations of N- and C-terminal domains have been functionally characterized with respect to their roles in responding to salinity stress (Puranik et al., 2012).

The stress-responsive TF ONAC022 enhances drought and salinity stress tolerance in rice by modulating a pathway mediated by the stress-related phytohormone abscisic acid (ABA) (Hong et al., 2016). When overexpressed, the *SlNAC8* gene of the halophyte plant *Suaeda liaotungensis* improves the salt stress tolerance of *Arabidopsis* plants by regulating the expression of stress-responsive genes (Wu et al., 2018). The *Miscanthus* NAC TF gene *NAC9* enhances the salt stress tolerance of *Arabidopsis* plants by enhancing scavenging capacity for reactive oxygen species (Zhao et al., 2016). *AtNAC2*, a salt stress-responsive

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NAC TF gene in *Arabidopsis*, promotes salt stress tolerance via the ethylene and auxin signaling pathways; *AtNAC2* is also involved in lateral root development during salt stress (He et al., 2005).

Pearl millet is an abiotic stress tolerant crop that has been attracting attention since its genome was sequenced (Varshney et al., 2017). QTLs for salinity tolerance were identified earlier on Linkage group 2 in pearl millet (Sharma et al., 2011). Recently, many drought and salinity stress-related genes in pearl millet were found using transcriptome (RNA-sequencing) analyses (Dudhate et al., 2018; Shinde et al., 2018). *PgNAC21* is among the genes found to be upregulated by both drought and salinity stress in such analyses. Here, we cloned and characterized *PgNAC21* and found that *PgNAC21*-overexpressing *Arabidopsis* plants show enhanced tolerance of salinity stress. To our knowledge, this article is the first report describing the functional characterization of a pearl millet NAC TF.

2. Materials and methods

2.1. Plant material and stress treatments

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India provided pearl millet seeds. The salinity-tolerant pearl millet genotype ICMB 01222 (Shinde et al., 2018; Yadav et al., 2012) was used to isolate *PgNAC21* and to examine its expression pattern. ICMB 01222 seeds were sown in composite soil in a greenhouse maintained at 28 °C during the day and at 25 °C at night with a relative humidity of 55–75%. To induce salinity stress, 18-day-old pearl millet seedlings were transferred to a Hoagland liquid medium supplemented with 250 mM NaCl, and sampled for RNA isolation at the time points indicated in Fig. 1.

2.2. Quantitative real-time PCR assay

Total RNA was isolated from pearl millet leaves and Arabidopsis seedlings as previously described [14]. cDNA was synthesized using PrimeScript reverse transcriptase (Takara Bio, Japan), and quantitative real-time PCR (qRT-PCR) was performed using this cDNA and TB Green Premix Ex Taq II (Takara). Relative gene expression was calculated using the ΔΔCT method (Livak and Schmittgen, 2001). The pearl millet actin gene *PgActin* was used as a reference gene, as described previously (Shivhare and Lata, 2016). Other primers are listed in Supplementary S1.

2.3. Analysis of cis-acting elements in the promoter region

The presence of cis-acting regulatory elements in the promoter region (2000 bp from the start codon) of *PgNAC21* was predicted using the online search tool at the PlantCARE website (Lescot et al., 2002; Rombauts et al., 1999).

2.4. Yeast one-hybrid analysis

For the yeast one-hybrid analysis, the 100-bp *PgNAC21* promoter sequence with two MYBHv1-binding sites (CAACGG) was amplified by genomic PCR and cloned into the yeast expression vector pHIS2 to generate the pHIS2-PgNAC21 promoter. The coding region of pearl millet MYB1 (PgMYB1) was amplified by PCR and cloned into the pGADT7-Rec2 vector to generate pGADT7-Rec2-PgMYB1. The yeast strain Y187 was co-transformed with pGADT7-Rec2-PgMYB1 and the pHIS2-PgNAC21 promoter. pGAD-Rec2-53, which has the murine p53 transcription factor gene, and p53HIS2, which has three tandem copies of p53-binding elements upstream of the *HIS3* reporter gene, were co-transformed as a positive control according to Yeast Protocols Handbook (TaKaRa Bio USA). The pGADT7-rec2 and pHIS2 empty vectors were co-transformed as a negative control. DNA-protein interactions were evaluated by growing transformed yeast cells on SD/-His/-Leu/-Trp selective medium (synthetic dextrose medium lacking histidine, leucine and tryptophan) with 100 mM 3-amino-1,2,4-triazole (3-AT) for 3 days at 28 °C.

2.5. Transactivation assay

The full-length coding sequences of *PgNAC21* and the coding sequences of the N-terminal and C-terminal domains of PgNAC21 were separately cloned into the pGBKT7 vector. The resulting vectors were then transformed into the yeast strain AH109 with the pGBKT7 vector. The transformed yeast cells were streaked on SD (-Trp) and SD (-His) media (synthetic dextrose medium lacking tryptophan and histidine, respectively). LacZ activity was assessed via a β-galactosidase assay (Yeast Protocols Handbook; Clontech, USA).

2.6. Generation of Arabidopsis overexpression lines

The coding sequence of *PgNAC21* was amplified and cloned into the pCAMBIA1300 binary vector. This vector was then transformed into the *Agrobacterium* strain EHA 105. Transgenic plants were generated using the floral dip method as previously described (Clough and Bent, 1998). Transformed plants were selected using 30 μg/ml hygromycin media plates. T3 homozygous transgenic lines harbouring a single copy of transgene were selected on the basis of the segregation ratio of antibiotic-resistant plants and susceptible plants in their progeny, and used for further analysis.

2.7. Analysis of salinity stress tolerance

Wild-type (Col-0) seeds and seeds from three T3 homozygous transgenic lines were sown on half-strength MS medium containing 125 mM NaCl to measure germination rates and perform a survival assay. In a primary root length assay, plants were grown on half-

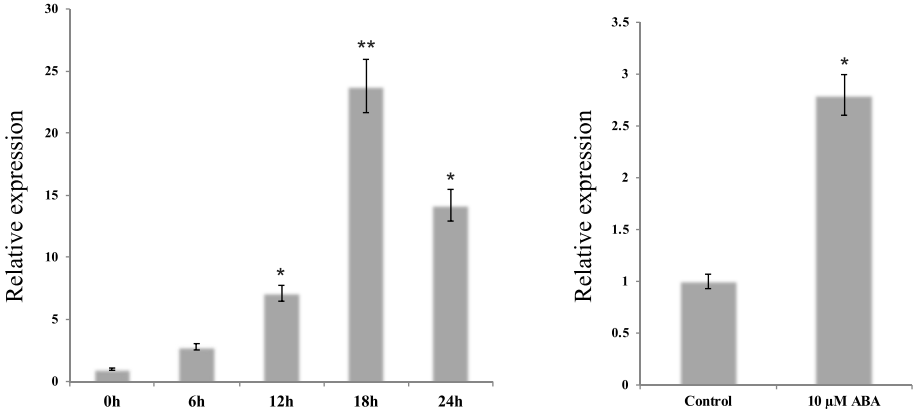


Fig. 1. Induction of P*gNAC21* by salinity stress and ABA treatment in pearl millet. For the left panel, plants were incubated for the indicated periods in 250 mM NaCl, and used for the analysis. For the right panel, plants were treated with 0 (Control) or 10 μM ABA for 24 h, and used for the analysis. The presented data represent means ± SD from three independent experiments. *: $P < 0.01$ vs. non-stressed samples in Student's t-test.

strength MS medium for 6 days and transferred to half-strength MS medium containing 100 mM NaCl. They were grown for an additional 6 days, and their root length was then measured using ImageJ software (Rasband, 2015; Schindelin et al., 2015; Schneider et al., 2012).

2.8. qRT-PCR analyses of stress-responsive genes in Arabidopsis

Total RNA was isolated from transgenic *Arabidopsis* plants under salinity-stressed and control conditions. qRT-PCR was performed using TB Green Premix Ex Taq II (Takara). The primers used in this study were used in prior research (Wu et al., 2018) and are listed in Supplementary S1.

3. Results

3.1. Gene expression analysis of PgNAC21 under salinity stress

PgNAC21 expression in pearl millet leaves under salinity stress (250 mM NaCl) was characterized using qRT-PCR. Under salinity stress, there was higher *PgNAC21* expression at 6 h, 12 h, 18 h and 28 h than at 0 h. The *PgNAC21* expression level was highest at 18 h (23.8 times higher than the corresponding expression level at 0 h). *PgNAC21* expression was also upregulated (2.8-fold) by exogenous ABA application (10 μM) (Fig. 1). These results suggest that *PgNAC21* is a salinity stress- and ABA-responsive gene.

3.2. PgNAC21 is a transcriptional activator gene

The transcriptional activation activity of the PgNAC21 was assessed in yeast. Yeast cells containing the pGBKT7 empty vector or the pGBKT7-PgNAC21-N vector (which included the coding sequences of the N-terminal region of PgNAC21) could not grow on SD (-His) medium. However, cells containing the pGBKT7-PgNAC21-C vector (which included the coding sequences of the C-terminal region of PgNAC21) or pGBKT7-PgNAC21 (which included full-length PgNAC21) could grow on SD (-His) medium and turned blue in that context, indicating enhanced β-galactosidase activity (Fig. 2). These results suggest that PgNAC21 functions as a transcription activator and that its activation domain is located in its C-terminus.

3.3. In silico promoter analysis

In silico analysis of the *PgNAC21* promoter showed the presence plant hormone-responsive element, such as an ABA-responsive element (ABRE). In addition, four MYB1 binding sites (*Hordeum vulgare* MYB protein (MYBHv1)-binding elements) were detected in the vicinity of the putative transcription start site of *PgNAC21*. Drought responsive elements and MYC transcription factor binding sites were also detected (Supplementary S2).

3.4. Putative PgMYB1 protein binds to the promoter of PgNAC21

To confirm that the *PgNAC21* promoter is bound by PgMYB1, which is highly similar to MYBHv1, we performed yeast one-hybrid analysis. In this analysis, yeast cells grew on the selection medium when co-transformed with pGADT7-Rec2-PgMYB1 and the pHIS2-PgNAC21 promoter (Fig. 3), suggesting that PgMYB1 binds to the *PgNAC21* promoter.

3.5. Analysis of PgNAC21-overexpressing Arabidopsis plants under salinity stress

Germination rates of Col-0 and the *PgNAC21*-overexpressing Arabidopsis plants were similar under control conditions (72–85%). Under salinity stress (125 mM NaCl), the germination rate of Col-0 was 21%, whereas the germination rates of the transgenic lines were 42–80% (Fig. 4A and B). The average fresh weight of 6-day-old Col-0 plants under salinity stress was 1.1 mg, whereas significantly heavier fresh weights (3.11–4.8 mg) were observed for the transgenic plants (Fig. 4C). When salinity stress was imposed at a post-germination stage, the *PgNAC21*-overexpressing plants showed less severe reduction in primary root length than the wild type (Fig. 5). The *PgNAC21*-overexpressing plants did not show any abnormal phenotypes under normal growth conditions at later developmental stages (Shinde et al., unpublished data).

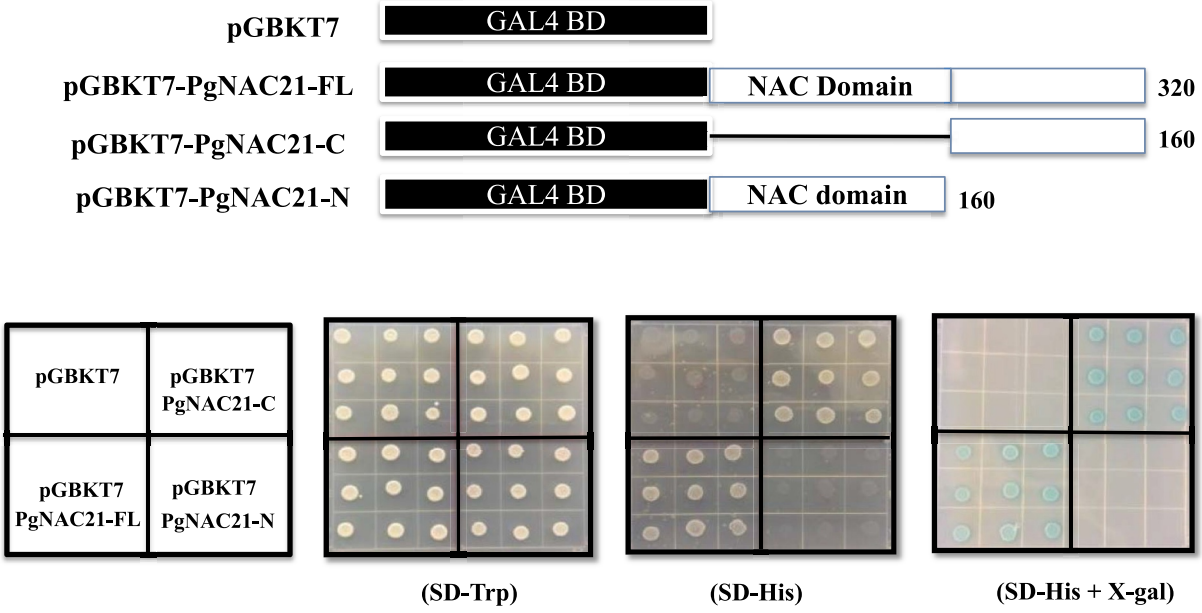
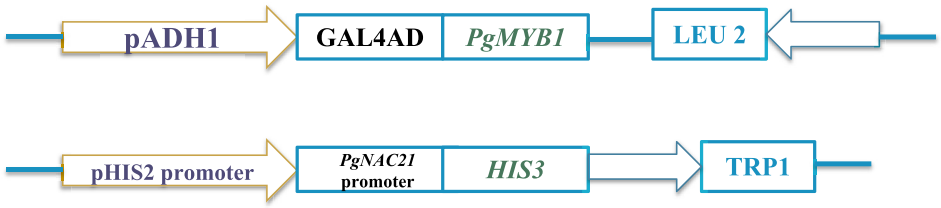


Fig. 2. PgNAC21 exhibits transactivation activity. Yeast cells containing the pGBKT7 empty vector ("pBD"), pGBKT7-PgNAC21-FL ("pBD-PgNAC21-FL", which is to express full-length PgNAC21 as GAL4 DNA-binding domain-fused protein), pGBKT7-PgNAC21-N ("pBD-PgNAC21-N", to express an N-terminal region of PgNAC21) or pGBKT7-PgNAC21-C ("pBD-PgNAC21-C", to express a C-terminal region of PgNAC21) were streaked on either the SD-His medium or the SD-His + X-gal medium to examine reporter gene activation. The SD-Trp medium was used for a control. The media with yeast cells were incubated for 3 days at 28 °C.



PgNAC21 promoter (100bp) cloned into pHIS2 vector (CAACCA is putative MYB binding site)

AGAACACCGCAGAACCCACAGGACA**CAACCA**GAGGGCCGGCCGGCCGCCGGAGCAACAGC**CAACCA**ACAAGAGCACTGTTGC
GGAGTGGGAGCGAGCGAG

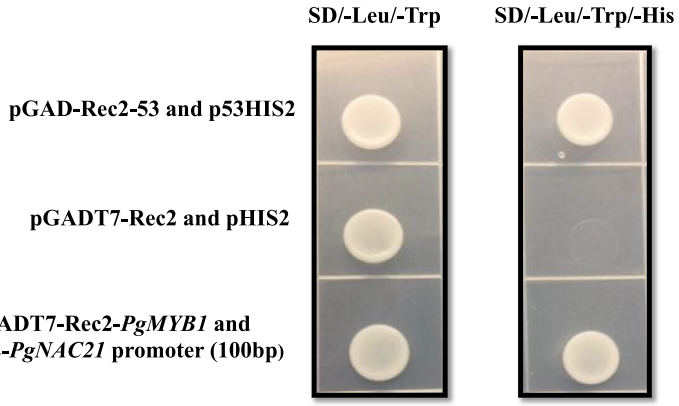


Fig. 3. Yeast one-hybrid analyses using the PgMYB1 and PgNAC21 promoters. The upper panel shows the schematic structure of the effector construct pGADT7-Rec2-PgMYB1 and the reporter construct pHIS2-PgNAC21 promoter (100 bp region) utilized for the yeast one-hybrid analysis. These constructs were transformed into the yeast strain Y187, and the growth of transformed cells on SD/-Leu/-Trp and SD/-Leu/-Trp/-His medium was observed to examine reporter gene activation in yeast. pGAD-Rec2-53, which can express the murine p53 transcription factor, and p53HIS2, which has p53-binding elements upstream of the reporter gene, were used as a positive control. pGADT7-Rec2 and pHIS2 were used as a negative control (lower panel).

3.6. Analysis of the expression of stress-responsive genes in transgenic Arabidopsis

We analyzed the expression of stress-responsive genes in PgNAC21-overexpressing *Arabidopsis* plants and Col-0 plants. The expression levels of the three stress-responsive genes *GSTF6* (*GLUTATHIONE S-TRANSFERASE 6*), *COR47* (*COLD-REGULATED 47*) and *RD20* (*RESPONSIVE TO DEHYDRATION 20*) were higher in *PgNAC21*-overexpressing plants than in Col-0 plants (Fig. 6).

4. Discussion

Soil salinity is one of the major abiotic stresses limiting agricultural productivity worldwide. Climate change is expected to increase soil salinity, posing serious threat to food security (Su and Hock, 2016). Developing the salinity tolerant crop varieties will help to improve the agricultural production in such a situation. Conventional plant breeding approaches take some time in achieving this goal (Ashkani et al., 2015). Use of biotechnological approches such as genetic engineering can reduce the time required for this process. Salinity stress causes both dehydration stress and ion toxicity-derived stress in plants. Salinity stress tolerance of plants should therefore be a complex trait, acquired by functions of multiple genes. Utilization of stress-responsive TFs that regulate wide array of downstream genes is one of the most powerful approaches to enhance plant stress tolerance (Wang et al., 2016). Among the different TF families, the NAC TF family has gained lots of attention because of their functional diversity and importance (Nakashima et al., 2007; Tran et al., 2010). Our results also show that one of the NAC TFs, PgNAC21, is important in regulating salinity stress tolerance in plants.

Pearl millet is the sixth most important staple crop. It is grown in arid and semi-arid tropical regions of Asia and Africa (Vadez et al., 2012). This is because pearl millet has strong tolerance to abiotic stresses, and several genes such as *PgDREB2A* (dehydration responsive element-binding protein), *PgNHX1* (Na+/H + antiporter), *PgDHN* (dehydrin), *PgVDAC* (voltage-dependent anion channel), and *PgLEA* (late embryogenesis abundant protein) are linked to the strong tolerance of pearl millet (Agarwal et al., 2010; Desai et al., 2006; Reddy et al., 2012; Singh et al., 2015; Verma et al., 2007). It should be interesting to determine whether *PgNAC21* contributes to the expression of these genes.

NAC TFs represent a major plant-specific TF family, and pearl millet has 155 members of the NAC TF family. This article is the first report of functional characterization of a pearl millet NAC TF. In this study, *PgNAC21* was induced by salinity stress, exogenous ABA treatment (Fig. 1), PEG-induced dehydration stress and cold (4 °C) stress (Supplementary S3). Because salinity stress, dehydration stress and cold stress all can activate ABA biosynthesis (Tuteja, 2007), and because the promoter of *PgNAC21* contains an ABRE, the *PgNAC21* induction by these stresses may partially be dependent on ABA. However, because the salinity stress induced *PgNAC21* more greatly than the ABA treatment (Fig. 1), an ABA-independent yet salinity stress-dependent inducer of *PgNAC21* may be present. This idea is consistent with a previous finding that some genes such as *NCED3*, *AAO3* and *ABA1* in *Arabidopsis* are induced by both ABA-dependent and ABA-independent pathways under a salinity-stressed condition (Barrero et al., 2006). The promoter of *PgNAC21* contains not only the ABRE but also four MYB TF-binding elements (Supplementary S2), and the *PgNAC21* promoter fragment containing two of these elements were bound by PgMYB1 (Fig. 3). These results raise the possibility that PgMYB1 regulates the

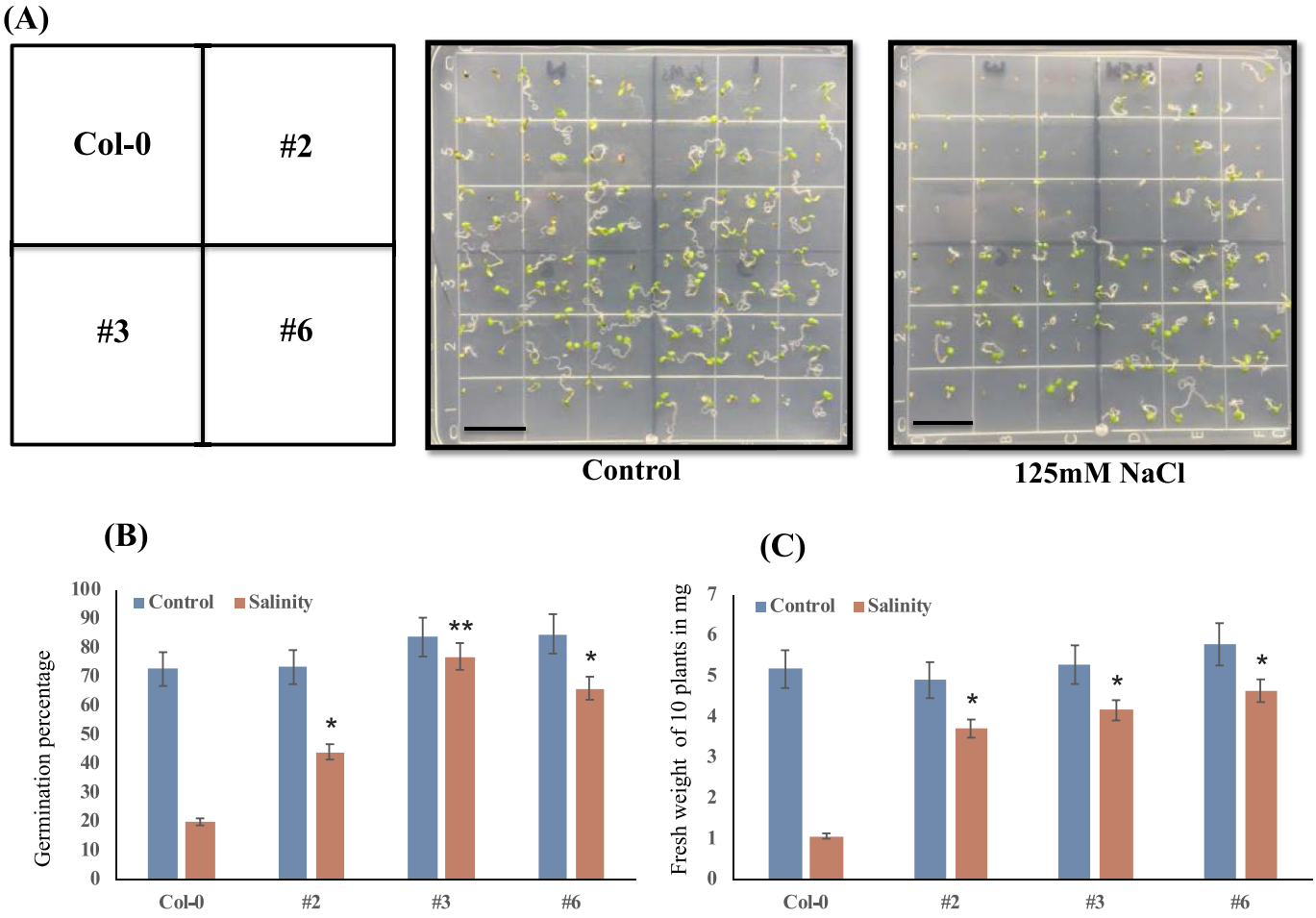


Fig. 4. Characterization of PgNAC21-overexpressing *Arabidopsis* T3 lines (#2, #3 and #6) with respect to salinity stress tolerance. (A) Germination on MS media with 0 ("Control") and 125 mM NaCl. Scale bars = 1 cm. (B) Germination rates of seeds sown on MS media with 0 ("Control") and 125 mM NaCl ("Salinity"). Data are means ± SD of three replicates. Thirty plants were assessed in each replicate. *: $P < 0.05$ vs. "Control" in the Tukey-Kramer test. (C) Fresh weights of plants grown on MS media with 0 ("Control") and 125 mM NaCl ("Salinity"). Data are means ± SD of three biological replicates. Ten plants were weighed for each replicate. *: $P < 0.05$ vs. "Control" in the Tukey-Kramer test.

expression of *PgNAC21*. This idea is consistent with a recent finding that *Arabidopsis* MYB108 binds the promoter of ANAC003 (*Arabidopsis* NAC 003), although neither MYB108 nor ANAC003 has been shown to be involved in regulating salinity stress tolerance. The promoter of *PgNAC21* also contains abiotic stress responsive element (such as DRE) and MYC transcription factor binding site (Supplementary S2). Thus, it should also be interesting to examine whether these elements mediate the salinity-dependent induction of *PgNAC21*. (Chou et al., 2018).

In this study, *PgNAC21*-overexpressing *Arabidopsis* plants showed enhanced salinity stress tolerance. *PgNAC21* should therefore be a positive regulator of salinity stress tolerance. This idea is consistent with previous findings that certain NAC TFs act as positive regulators of salinity stress tolerance (Chou et al., 2018; Hong et al., 2016; Huang et al., 2015). *GSTF6, COR47* and *RD20* showed significant upregulation in *PgNAC21*-overexpressing plants. Promoters of these genes have multiple copies of the NAC TF-binding site CACG. *PgNAC21* may therefore directly bind to these promoters to regulate the expression of these genes. It is known that NAC TFs bind to promoters of their target genes, preferentially to the NAC core binding site CACG (Tran et al., 2004). Levels of GSTF6 protein are significantly increased in roots by NaCl-induced salinity stress, and this protein plays a role in scavenging reactive oxygen species generated by such stress (Jiang et al., 2007). COR47 belongs to the dehydrin protein family and responds to osmotic stress, ABA and cold stress (Puhakainen et al., 2004). RD20 is a salinity and drought stress-inducible caleosin protein that participates in regulating stomatal apertures, transpiration and abiotic stress tolerance in *Arabidopsis* (Aubert et al., 2010). The salinity stress tolerance of *PgNAC21*-overexpressing *Arabidopsis* plants may be attributed to strong expression of these genes (Fig. 7).

N-terminal regions (i.e., NAC domains) of NAC TF members are highly conserved between each other, and cause these proteins to localize in the nucleus, to homo- and heterodimerize, and to bind DNA (Nuruzzaman et al., 2013). Their C-terminal regions are less conserved, and can fine-tune their DNA-binding specificity. Interestingly, the C-terminal region of *PgNAC21* is highly homologous to that of rice SNAC1, overexpression of which enhances tolerance to drought and salinity stresses in rice (Liu et al., 2014). Functions of *PgNAC21* examined in our study are shared with *SNAC1*: The C-terminal region of SNAC1 exhibits transcriptional activation potential in a yeast one-hybrid system (Hu et al., 2006); *SNAC1* is induced by drought stress, salinity stress, cold stress and ABA treatment. *SNAC1* overexpression increases ABA sensitivity in rice, not consistent with our finding that *PgNAC21* overexpression does not affect ABA sensitivity in Arabidopsis (Supplementary S4). Nevertheless, these results together support the idea that *SNAC1* and *PgNAC21* are both useful to enhance salinity stress tolerance of cereal crops.

We also found that under a salinity stress condition *PgNAC21*-overexpressing *Arabidopsis* plants had longer primary roots and more lateral roots than wild type plants (Fig. 5). This indicates a possible role of *PgNAC21* in regulating the plant root system under salinity stress and

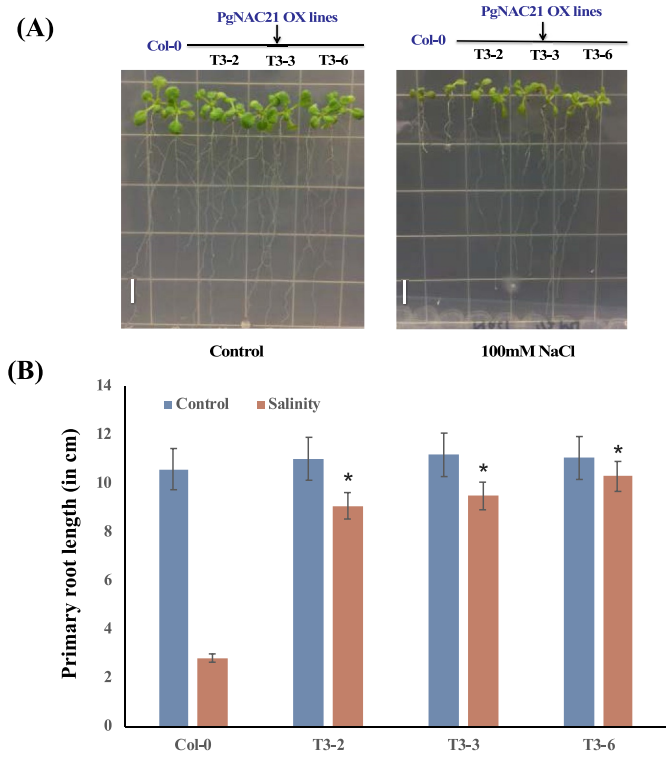


Fig. 5. Analysis of root length for *PgNAC21*-overexpressing lines (T3-2, T3-3 and T3-6). Plants were grown for six days on an MS medium with 0 mM NaCl, transferred to an MS medium with either 0 ("Control") or 100 mM NaCl ("Salinity"), and further grown for six days. (A) Plant images. Scale bars = 1 cm. (B) Primary root lengths. Data are means ± SD of three representative plants for each genotype for each treatment. To choose the representative plants, at least 20 plants were observed. *: $P < 0.05$ vs. "Control" in the Tukey-Kramer test.

thereby improving the salinity stress tolerance. This result is consistent with several previous findings that root system has a role in abiotic stress tolerance (Jeong et al., 2010; Redillas et al., 2012). Our analysis also showed that under salinity stress *PgNAC21*-overexpressing Arabidopsis plants had better seed germination than wild type plants. This result is consistent with previous finding that *ThNAC13* from *Tamarix hispida* confers salinity stress tolerance in Arabidopsis by improving its germination rate under salinity stress (Wang et al., 2017).

In summary, our study demonstrates that *PgNAC21* is a stress-responsive gene and encodes a transcription activator that regulates salinity stress tolerance. *PgNAC21* can improve salinity stress tolerance in not only *Arabidopsis* but also pearl millet and other crops. Further investigation of *PgNAC21* target genes in pearl millet will be helpful for elucidating mechanisms underlying salinity stress tolerance in this plant species.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Nucleotide sequence of the reported gene *PgNAC21* from pearl millet is available in the NCBI-GenBank database (Accession # MK084913).

CRediT authorship contribution statement

Harshraj Shinde: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. **Ambika Dudhate:** Data curation, Formal analysis, Methodology, Software, Validation. **Daisuke Tsugama:** Supervision, Validation, Writing – review & editing. **Shashi K. Gupta:** Resources, Supervision. **Shenkui Liu:** Funding acquisition, Investigation, Resources, Supervision. **Tetsuo Takano:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Visualization, Writing – review & editing.

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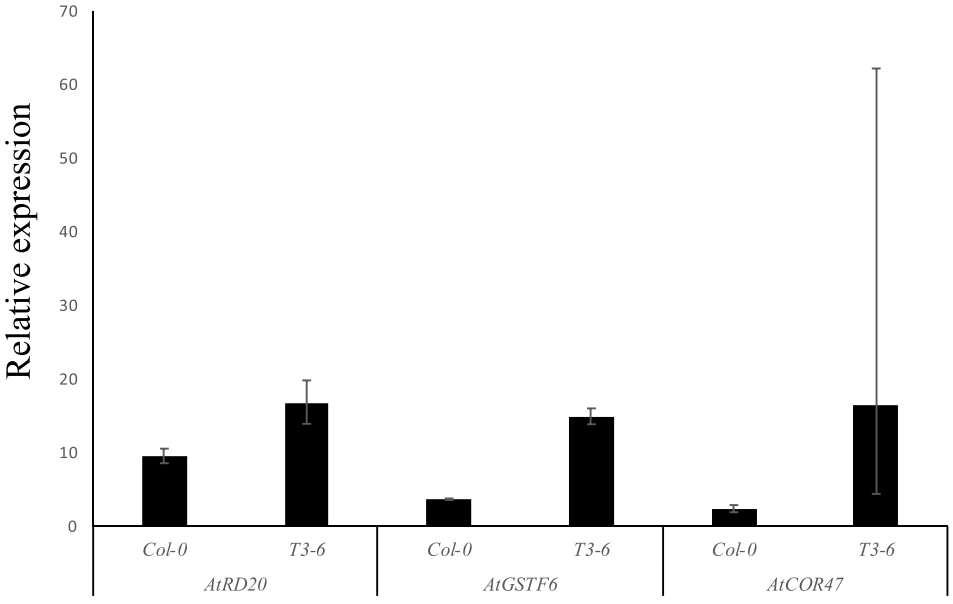

Fig. 6. Analysis of the expression of stress-responsive genes (*RD20, GSTF6* and *COR47*) in *PgNAC21*-overexpressing *Arabidopsis* plants ("OX plants", the line T3-6) and Col-0 plants. Seven-day-old seedlings were exposed to salinity stress (100 mM NaCl) for 7 days. Levels of gene transcripts were assessed using qRT-PCR. Each value represents the mean ± SE of three biological replicates. *: $P < 0.01$ vs. Col-0 in Student's t-test.

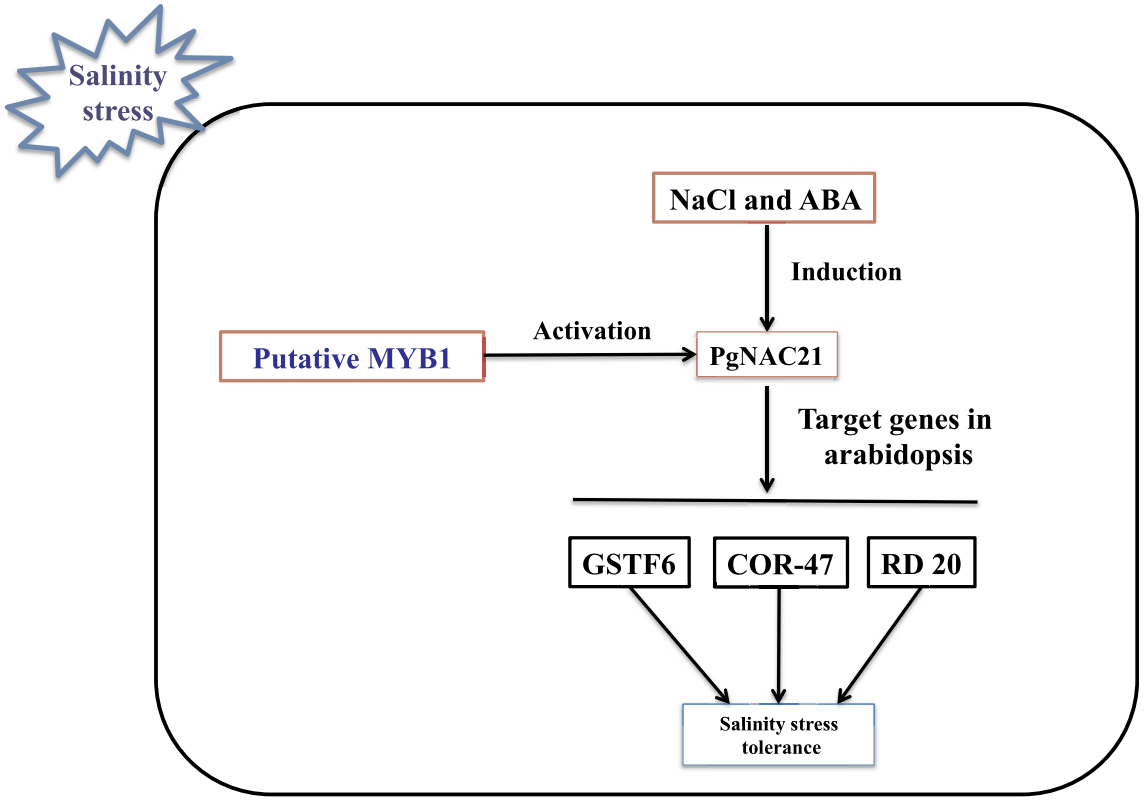

Fig. 7. A schematic model for *PgNAC21*-mediated salinity stress tolerance. NaCl and ABA induce *PgNAC21* expression; PgMYB1 also activates PgNAC21 expression by binding to the activator region of the promoter. PgNAC21 then activates stress-responsive genes, such as *GSTF6*, *COR47* and *RD20*, to increase salinity stress tolerance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.11.004.

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