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TRANSGENIC SOYBEAN OVEREXPRESSING *GmNAC085* ENHANCED EXPRESSION OF IMPORTANT GENES UNDER SALINITY STRESS CONDITION

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SUMMARY

Climate change has made abiotic stresses such as drought and salinity bigger threats to ecosystem and global food security. In response to unfavorable conditions, physiological, biochemical and molecular activities in plants are altered. Particularly, various members of NAC (NAM, ATAF1/2, CUC2) transcriptional factor family have been reported to be the key regulators in modulating multiple biological processes of plant responses to osmotic stress conditions caused by drought and salinity. Previously, transgenic studies have shown the positive regulatory role of GmNAC085, a NAC transcription factor from soybean (*Glycine max*), in plant resistance against drought stress. Therefore, in this study, we extended the investigation on its contribution to salinity stress. According to our RT-qPCR analyses, expression of several important stress-related genes was significantly induced in the transgenic soybean plants in comparison with the wild-type plants, including antioxidant enzyme-encoding genes (*GmSOD*, *GmAPX* and *GmCAT*), sodium-proton antiporter encoding gene (*GmNHX1*) and proline metabolic gene (*GmP5CS*). Furthermore, biochemical results were also in agreement with the molecular data, with enhanced antioxidant enzyme activities of peroxidase and catalase, and in couple with lower cellular content of the reactive oxygen species hydrogen peroxide in the transgenic plants. Taken these altogether, the transgenic plants might acquire advantages in dealing with oxidative stress using enzymes and proline, as well as cellular Na^+ removal under salinity stress conditions. Therefore, underlying mechanisms of GmNAC085 associated with salinity stress should be fully elaborated to find out its potential utility in crop improvement.

Keywords: *antioxidant enzyme activities, Glycine max, GmNAC085, salinity, stress resistance*

INTRODUCTION

During their life cycle, plants continuously experience a constantly changing environment that often leads to various adverse biotic and abiotic conditions, due to their sessile nature (Shao *et al.*, 2015). These environmental stresses may prevent the plants from fulfilling their maximum potential performance, even threaten their survival. Environmental disturbance caused by human activities has increased the frequency of abiotic stresses, thus alerting the difficulties in maintenance of adequate food supply (Hussain *et al.*, 2015). Among the stressors, drought and salinity are considered as the most serious factors limiting crop production (Shabala *et al.*, 2013). Salinity is estimated to affect approximately 20% of the cultivated area, and the regions getting

salinized are projected to rise in the forthcoming years (Gupta, Huang, 2014). Salinity increases concentrations of certain ions in soil such as Na^+ and Cl^-, thus causing plants to suffer not only osmotic and ionic stresses but also oxidative stress, which influence their normal growth, development and reproduction. Few examples for the negative effects caused by salinity stress on plants are stunted shoot growth, reduced transpiration and photosynthesis, tissue necrosis and yield loss (Rushton *et al.*, 2008). Therefore, improvement of salinity stress tolerance in plants has become an important mission for ensuring agricultural productivity and sustainability (Zhu, 2016).

Upon being exposed to environmental stress, a complicated, responsive network is initiated by plant perception to the external trigger. The signal is then relayed to the nucleus for regulating gene expression (Shinozaki, Yamaguchi-Shinozaki, 2007; Nakashima *et al*., 2014). Changes in transcription and translation often include activation of various adaptive and defensive processes, such as promotion of stomatal closure to minimize water loss, and enhanced production of compatible osmolytes to protect plant cells from osmotic and oxidative damages (Shinozaki, Yamaguchi-Shinozaki, 2007; Hu, Xiong, 2014).

Among different groups of regulators, NAC (NAM, ATAF1/2, CUC2) proteins form one of the largest transcription factor (TF) families in plants (Nuruzzaman *et al*., 2013). A typical NAC will have two distinct domains, one locating at the N-terminus responsible for nuclear localization and DNA binding, whereas the other locating at the C-terminus responsible for gene expression regulation (Nuruzzaman *et al.*, 2013). The NAC TFs mediate various developmental processes including flowering, cell division, leaf senescence, formation of secondary walls, as well as plant responses to biotic and abiotic stress conditions (Tran *et al.*, 2010). A great number of studies have reported the improvement of plant stress tolerance by manipulating *NAC* gene expression. For example, overexpression of tomato (*Solanum lycopersicum*) *SlNAC3* gene could enhance both drought and salt tolerance in the transgenic tomato (Al-Abdallat *et al*., 2015). Similarly, transgenic ramie (*Boehmeria nivea*) overexpressing the rice (*Oryza sativa*) *ONAC022* also acquired better adaptation to both these stress factors (An *et al.*, 2015). Meanwhile, *Arabidopsis* harboring the pumpkin (*Cucurbita moschata*) *CmNAC1* could be tolerant to not only drought and salinity but also low temperature conditions (Cao *et al*., 2017). In soybean (*Glycine max*), several abiotic stress-related *NAC* genes have also been identified. *GmNAC2*, *3* and *4* were found to be responsive to osmotic stress (Pinheiro *et al.*, 2009; Morishita *et al.*, 2009). Another gene, *GmNAC019*, was shown to be up-regulated in root (Thao *et al.*, 2013) and shoot tissues (Thu *et al.*, 2014) of soybean plants under drought condition. Investigation on the effects of soybean *GmNAC15* revealed that overexpression of this gene in hairy roots of transgenic soybean plants conferred positive effects on the plant adaptation to salinity (Ming *et al.*, 2018). In addition, transgenic *Arabidopsis* ectopically expressing *GmNAC085* (Nguyen *et al.*, 2018) or *GmNAC109* (Nguyen *et al*., 2019) could acquire better drought tolerance capacity.

As drought and salinity result in similar negative effects on plants (i.e. osmotic and oxidative stresses) and plants also using overlapping mechanisms to respond to these stress factors (An *et al.*, 2015; Al-Abdallat *et al*., 2015; Nxele *et al*., 2017), the main objective in this study was to find out if the drought-related GmNAC085 also plays beneficial role in plant response to salinity stress. For preliminary information, we studied expression of several important stress-related genes and activities of hydrogen peroxide-scavenging enzymes in the transgenic soybean plants overexpressing *GmNAC085*.

MATERIALS AND METHODS

Plant materials and growing conditions

The wild-type (WT) soybean (Williams 82) was supplied by Vietnam Legumes Research and Development Center. The transgenic soybean seeds were generated by University of Missouri (USA) using *Agrobacterium*-mediated

transformation method. The transgenic line used in this study has been previously confirmed to carry a single copy of transgene in the form of homozygosity (Hoa *et al*., 2018).

The seedlings were grown in plastic pots (10 cm in diameter, 40 cm in height, one plant per pot) in net-house condition (natural photoperiod, temperature range of 27-33 ^{o}C, and relative humidity 60-70 %). For salinity stress treatment, NaCl solution 100 mM was applied to 12-day-old seedlings (100 ml/pot) every 2 days until the samples were collected for analysis.

Total RNA isolation and cDNA synthesis

Root tissues from plants that were salt-treated for 0 and 10 days were frozen in liquid nitrogen for molecular analyses. The procedures of RNA isolation and cDNA synthesis were described in Thao *et al*. (2013). In brief, the collected tissue (0.1 g/sample) was ground into powder in liquid nitrogen. Total RNA was then extracted using the GeneJET Plant RNA Purification Kit (Thermo Scientific), followed by DNA removal using RapidOut DNA Removal Kit (Thermo Scientific). Synthesis of cDNA was conducted using 700 ng total RNA and RevertAid First strand cDNA synthesis kit (Thermo Scientific).

Real-time quantitative PCR assay

Each real-time PCR reaction was prepared in volume of 25 µL, including Maxima SYBR Green qPCR Master Mix (Thermo Scientific), 0.4 µM each for forward and reverse primers (Table 1), and 1 µL cDNA. The thermal cycle for RT-qPCR reactions and melting curve analysis was performed following Thao *et al*. (2013). The relative gene expression level was determined using $2^{-\Delta Ct}$ method, with *Fbox* as the reference gene for normalization (Table 1).

Table 1. List of primers using in RT-qPCR.

Genes	Forward (5'-3')	Reverse (5'-3')	References
Fbox	AGATAGGGAAATTGTGCAGGT	CTAATGGCAATTGCAGCTCTC	Thao *et al*., 2013
GmSOD	GCACCACCAGACTTACATCAC	AACGACGGCGGAGGAATC	Jiao *et al*., 2016
GmAPX	AGTTGGCTGGCGTTGTTG	TGGTGGCTCAGGCTTGTC	Jiao *et al*., 2016
GmCAT	CCACAGCCATGCCACTCAAG	CAGGACCAAGCGACCAACAG	Jiao *et al*., 2016
GmNHX1	CTTTCCACTCCAACACACAC	GGTGAGCCAGGTTCTATAGG	Li *et al*., 2017
GmP5CS	TGTCTCTCAGATCAAGAGTTCCAC	CAGCCTGCTGGATAGTCTATTTTT	Stolf-Moreira *et al*., 2010

Extraction and determination of cellular hydrogen peroxide levels

The hydrogen peroxide (H_2O_2) contents were determined based on the method described by Patterson *et al.* (1984) with modification. In brief, 0.2 g soybean leaf tissue was used for cellular H_2O_2 extraction in 2 ml PBS solution (0.1 M, pH 7.4). The reaction solution containing 1 ml of extracted cellular H_2O_2 solution mixed with 1 ml of 0.1% titanium sulphate in 20% H_2SO_4 (v/v) was centrifuged at 12,000 rpm at room temperature for 10 min. After that, absorbance of the reaction solution was measured at 410 nm.

The standard curve was constructed using known H_2O_2 concentrations (0; 0.2; 0.4; 0.6; 0.8 and 1 mM), with PBS 0.1 M as diluent. The samples were analyzed at day 0, 6th and 12th since salt solution application, using three biological replicates.

Extraction and quantification of total protein content

The antioxidants enzymatic activities were determined from a crude enzyme solution by

homogenizing 0.2 g of soybean leaves, which had been treated by salinity stress for 0, 6 and 12 days, in 2 ml cold extraction buffer (pH 7.0, containing 1 mM EDTA and 2% polyvinylpyrrolidone (PVP-8000) in 50 mM potassium phosphate buffer). The supernatant collected from the homogenate after centrifugation at 15,000 rpm, 4°C for 15 min was used for measuring total protein contents, catalase and peroxidase activities.

To measure total soluble protein contents, the extracted protein solution was diluted 2 times followed by the protein quantification by Bradford method (reading at 595 nm) using commercial kit (Thermo Scientific). The standard curve was constructed for bovine serum albumin solutions (BSA; Sigma) with concentrations of 0; 0.25; 0.5; 1; 1.4 and 2 mg/mL.

Measurement of peroxidase activity

Peroxidase activity was determined based on the method described by Shannon *et al.* (1966) and calculated following the formula of Rodríguez *et al.* (2001). The 3 ml-reaction mixture (pH 5.4) consisted of 2.8 ml POD substrate solution (1.375 ml acetate 0.1 M, 0.05 mL *O*-dianisidine 0.5% (pH = 6.0), 100 µl H_2O_2 0.1 M) and 100 µl of the supernatant solution was incubated at 37°C for 30 min. The enzyme activity was measured based on the change in absorbance at 460 nm over 3-minute duration since the reaction took place. For each group, three biological replicates were used.

Measurement of catalase activity

Catalase activity was determined based on the method described by Wang *et al.* (2012). Briefly, each reaction consisted of 2.79-ml-catalase substrate solution (containing 0.3 ml potassium phosphate buffer 1 M, 3 µL EDTA 0.1 mM (pH = 7.0), 10 µL H_2O_2 0.1M) and 200 µL of the extracted protein solution. The enzyme activity was measured at wavelength 240 nm based on the decreased absorbance of H_2O_2 over one min. Three biological replicates were used for each group of samples.

Statistical analysis

The data were analyzed using Student's *t*-test for identification of statistically significant difference between genotypes or treatments.

RESULTS AND DISCUSSION

Studies in soybean showed that *GmNAC085* was one of the most highly induced genes among the *NAC* members by dehydration and drought conditions (Le *et al*., 2011; Thao *et al*., 2013; Thu *et al*., 2014). Additionally, *GmNAC085* is considered as an attractive candidate gene, which encodes a protein sequence sharing 39% amino acid identity to the most extensively characterized rice SNAC1, whose overexpression resulted in the enhanced drought tolerance in the transgenic rice plants (Hu *et al*., 2006; Thao *et al*., 2013). In addition, transgenic *Arabidopsis* overexpressing *GmNAC085* also acquired enhanced drought tolerance, with better cell membrane integrity, lower transpiration rate and higher antioxidant enzyme activities (Nguyen *et al*., 2018). Therefore, in this study, we were interested in exploring GmNAC085 function in relation to salinity, which also results in osmotic and oxidative stresses (Gupta, Huang, 2014).

Several important stress-related genes had enhanced expression in the transgenic plants

Under prolonged salinity stress conditions, superoxide and H_2O_2 are two kinds of reactive oxygen species (ROS) that are accumulated in plant cells (Gupta, Huang, 2014). In plant defense, superoxide dismutase enzymes can be employed to scavenge superoxide while catalases and peroxidases are responsible for removing H_2O_2. Therefore, for RT-qPCR, we examined the expression of superoxide dismutase-encoding gene (*GmSOD*), ascorbate peroxidase-encoding gene (*GmAPX*) and catalase-encoding gene (*GmCAT*). From our results, expression levels of *GmSOD* were similar between the WT and transgenic plants under non-stressed conditions (Fig. 1a). However, significantly higher transcript abundance of *GmAPX* and *GmCAT* was observed

in the transgenic plants, suggesting due to the transgene expression activity under the normal condition, which was driven by the constitutive promoter *CaMV 35S* (Fig. 1b, c). When comparing the expression of these antioxidant genes between the two genotypes under salinity condition, the transgenic plants displayed higher expression levels by 2.4-fold for *GmSOD*, by 2.5-fold for *GmAPX1* and by 2.2-fold *GmCAT* (Fig. 1a-c).

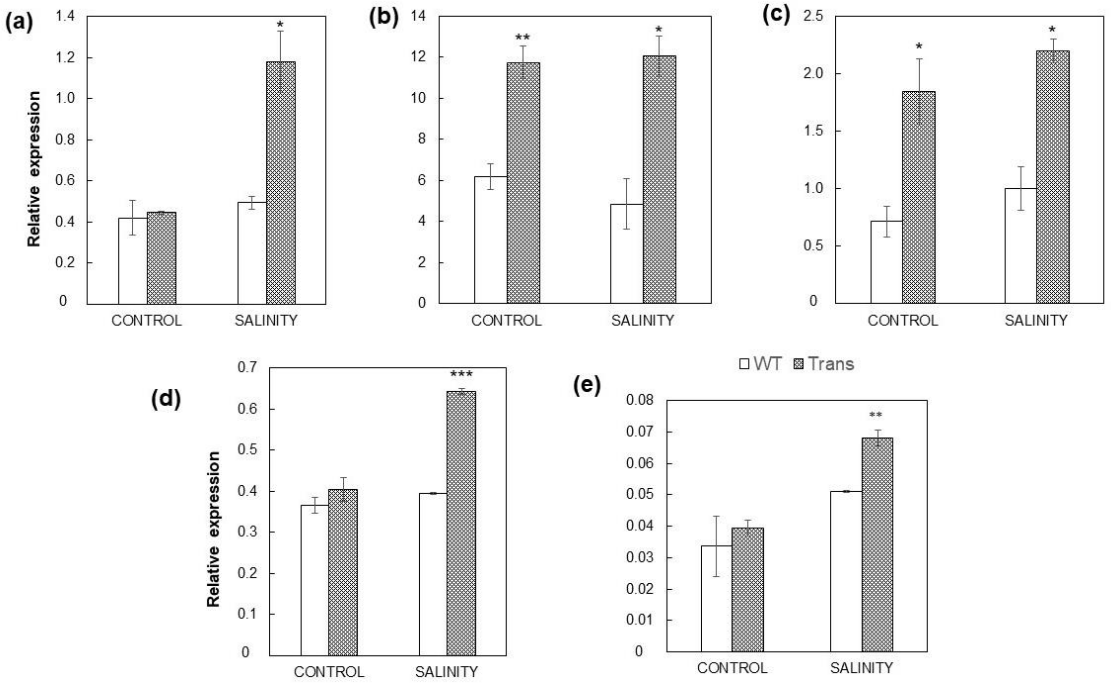

Figure 1. Expression of five stress-responsive genes in wild-type soybean plants (WT) and transgenic soybean plants overexpressing *GmNAC085* (Trans). (a) Expression of superoxide dismutase encoding gene *GmSOD*; (b) Expression of ascorbate peroxidase encoding gene *GmAPX*; (c) Expression of catalase encoding gene *GmCAT*; (d) Expression of Na^+/H^+ antiporter-encoding gene *NHX1*; (e) Expression of proline metabolic gene *P5CS*. The root tissues from 10-day-salt-treated plants were used for relative expression analyses of target genes by real-time PCR, using *Fbox* as the house-keeping gene for normalization. Significant difference between genotypes under the same treatment was illustrated by an asterisk, with * *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001.

We also analyzed other important stress-responsive genes, including ion transporter protein-encoding gene (*GmNHX1*) and proline metabolic gene (*GmP5CS*). Similar to the expression profile of antioxidant genes, a higher expression levels of these genes were also found in the salt-stressed transgenic plants in comparison with those in the salt-stressed WT plants (Fig. 1d, e). *GmNHX1* encodes Na^+/H^+ antiporter which facilitates the removal of sodium ions out of the cells. Under salinity stress conditions, accumulation of Na^+ will disrupt cellular ionic balance and normal metabolic activities (Apse *et al*., 2003; Shi *et al*., 2000). Therefore, enhancement of Na^+/H^+ antiporter activities in the transgenic plants would reduce the negative effects caused by salinity stress. Regarding *GmP5CS*, this gene encodes delta-1-pyrroline-5-carboxylase synthase, which is the key enzyme in biosynthesis of proline (Stolf-Moreira *et al*., 2010). This amino acid can function as an osmoprotectant molecule, with the capacity to adjust cellular water potential, stabilize macromolecules and remove ROS (Bayoumi *et al*., 2008; Stolf-Moreira *et al*., 2010). Increased expression of *GmP5CS*

indicates the likelihood of increased proline content in the soybean overexpressing *GmNAC085*, thus bringing certain advantages for the transgenic plants to tolerate better than the WT under salinity conditions.

Transgenic plants had better hydrogen peroxide removal capacity

In getting evidence to support expression data of antioxidant genes, we analyzed activities of enzymes that can scavenge H_2O_2, catalase and peroxidase. This is also one of important biochemical parameters in evaluation of plant tolerance to salinity. According to Fig. 2, the enzyme activities were comparable between the two genotypes under non-stressed condition. However, over the course of 12-day-salinity treatment, peroxidase enzyme activities were enhanced strongly in the transgenic plants but not in the WT (Fig. 2a), whereas an increase in catalase activities was observed in both WT and transgenic plants, but it was more pronounced in the latter group (Fig 2b). These results imply different regulation of peroxidase and catalase enzymes in dealing with salinity stress in WT and transgenic plants. In addition, we also investigated the cellular contents of H_2O_2. As shown in Fig. 2c, a lower level of H_2O_2 was accumulated in the soybean plants overexpressing *GmNAC085* under the same time-point of analysis (i.e. at both day sixth or day twelfth since stress application), which could be explained by the more efficient H_2O_2 detoxification carried out by catalase and peroxidase enzymes.

Previously, study on GmNAC085 function in relation to drought also revealed the enhanced activities of antioxidant enzymes and lower intracellular H_2O_2 contents in the transgenic *Arabidopsis* ectopically expressing *GmNAC085* (Nguyen *et al*., 2018). Reports on improved salinity tolerance by manipulating other NAC transcription factors have been also documented. For example, *Arabidopsis* ectopically expressing *Suaeda liaotungensis SlNAC8* acquired better salinity resistance, partially due to the enhanced antioxidant enzyme activities and higher proline contents (Wu *et al*., 2018). In another example, transgenic rice (*Oryza sativa*) overexpressing the rice gene *ONAC022* became more tolerant to salinity, with lower Na^+ accumulation in the root tissue (Hong *et al*., 2016). Taken altogether, our data indicate the positive regulatory role of GmNAC085 in mediating plant response to salinity conditions.

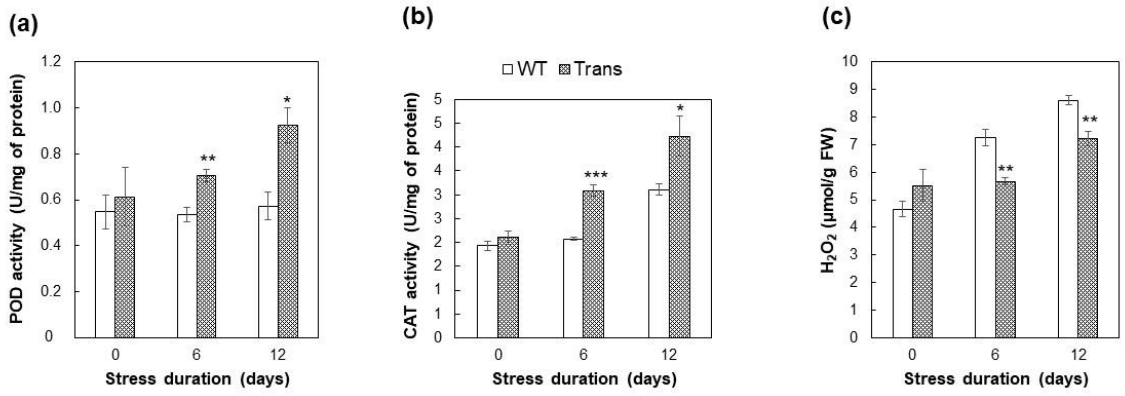

Figure 2. Biochemical analyses for hydrogen peroxide (H_2O_2)-scavenging enzyme activities and endogenous H_2O_2 contents in wild-type (WT) and transgenic soybean plants overexpressing *GmNAC085* (Trans) under salinity treatment. (a) Peroxidase (POD) enzyme activities; (b) Catalase (CAT) enzyme activities; (c) Cellular H_2O_2 contents. Difference degree between genotypes under the same treatment was illustrated by an asterisk, with * *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001.

CONCLUSION

In summary, this study provided primary molecular and biochemical evidence for importance of GmNAC085 in modulating plant response to salinity conditions, as a positive regulator. Compared with the WT plants, soybean plants overexpressing *GmNAC085* might suffer less damages caused the stress effects, probably due to stronger antioxidant enzyme activities, higher content of proline and better capacity in preventing accumulation of cellular sodium ions using transporter proteins. Therefore, underlying mechanisms and importance of GmNAC085 should be further elaborated to fully evaluate its potential utility in genetic engineering for development of crop varieties with better salinity tolerance and productivity.

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REFERENCES

Al-Abdallat AM, Ali-Sheikh-Omar MA, Alnemer LM (2015) Overexpression of two ATNAC3-related genes improves drought and salt tolerance in tomato (*Solanum lycopersicum* L.). *Plant Cell Tiss Organ Cult* 120: 989–1001.

An X, Liao Y, Zhang J, Dai L, Zhang N, Wang B, Liu L, Peng D (2015) Overexpression of rice NAC gene *SNAC1* in ramie improves drought and salt tolerance. *Plant Growth Regul* 76: 211–223.

Apse MP, Sottosanto JB, Blumwald E (2003) Vacuolar cation/H^+ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of *AtNHX1*, the *Arabidopsis* vacuolar Na^+/H^+ antiporter. *Plant J* 36: 229–239.

Bayoumi TY, Eid MH, Metwali EM (2008) Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *Afric J Biotechnol* 7: 2341–2352.

Cao H, Wang L, Nawaz MA, Niu M, Sun J, Xie J, Kong Q, Huang Y, Cheng F, Bie Z (2017) Ectopic expression of pumpkin NAC transcription factor CmNAC1 improves multiple abiotic stress tolerance in *Arabidopsis*. *Front Plant Sci* 8: 2052.

Gupta B, Huang B (2014) Mechanisms of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int J Genomics* 2014: 701596.

Hoa TTK, Tuyet HN, Thao NP, Xuan HTL (2018) Drought stress-related functional characterization of transcription factor GmNAC085 in soybean. *J Biotech* 16: 1–8.

Hong Y, Zhang H, Huang L, Li D, Song F (2016) Overexpression of a stress-responsive NAC transcription factor gene *ONAC022* improves drought and salt tolerance in rice. *Front Plant Sci* 7: 4.

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci* 103: 12987–12992.

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. *Ann Rev Plant Biol* 65: 715–741.

Hussain S, Peng S, Fahad S, Khaliq A, Huang J, Cui K, Nie L (2015) Rice management interventions to mitigate greenhouse gas emissions: a review. *Environ Sci Pollut Res* 22(5): 3342–3360.

Jiao C, Yang R, Zhou Y, Gu Z (2016) Nitric oxide mediates isoflavone accumulation and the antioxidant system enhancement in soybean sprouts. *Food Chem* 204: 373–380.

Le DT, Nishiyama RIE, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2011) Genome-wide survey and expression analysis of the plant-specific NAC transcription factor family in soybean during development and dehydration stress. *DNA Res* 18(4): 263–276.

Li Y, Chen Q, Nan H, Li X, Lu S, Zhao X, Liu B, Guo C, Kong F, Cao D (2017) Overexpression of *GmFDL19* enhances tolerance to drought and salt stresses in soybean. *PLoS One*, 12: e0179554.

Ming LI, Zheng HU, Jiang QY, Sun XJ, Yuan GUO, Qi JC, Zhang H (2018) *GmNAC15* overexpression in hairy roots enhances salt tolerance in soybean. *J Integrat Agricult* 17(3): 530–538.

Morishita T, Kojima Y, Maruta T, Nishizawa-Yokoi A, Yabuta Y, Shigeoka S (2009) *Arabidopsis* NAC transcription factor, ANAC078, regulates flavonoid biosynthesis under high-light. *Plant Cell Physiol*

50(12): 2210–2222.

Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (2014) The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front Plant Sci* 5: 170.

Nguyen KH, Mostofa MG, Li W, Van HC, Watanabe Y, Le DT, Thao NP, Tran LSP (2018) The soybean transcription factor GmNAC085 enhances drought tolerance in *Arabidopsis*. *Environ Exp Bot* 151: 12–20.

Nguyen NC, Hoang XLT, Nguyen QT, Binh NX, Watanabe Y, Thao NP, Tran L-SP (2019) Ectopic expression of *Glycine max GmNAC109* enhances drought tolerance and ABA sensitivity in *Arabidopsis*. *Biomolecules* 9: 714.

Nuruzzaman M, Sharoni AM, Kikuchi S (2013) Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Front Microbiol* 4: 428.

Nxele X, Klein A, Ndimba BK (2017) Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *South Afr J Bot* 108: 261–266.

Patterson BD, MacRae EA, Ferguson IB (1984) Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Anal Biochem* 139: 487–492.

Pinheiro GL, Marques, CS, Costa MD, Reis PA, Alves MS, Carvalho CM, Fietto LG, Fontes EP (2009) Complete inventory of soybean NAC transcription factors: sequence conservation and expression analysis uncover their distinct roles in stress response. *Gene*, 444(1-2): 10–23.

Rodríguez Y, Pérez E, Solórzano E, Meneses AR, Fernández (2001) Peroxidase and polyphenoloxidase activities in tomato roots inoculated with *Glomus clarum* or *Glomus fasciculatum. Cul Tropic* 22: 11–16.

Rushton PJ, Bokowiec MT, Han S, Zhang H, Brannock JF, Chen X, Laudeman TW, Timko MP (2008) Tobacco transcription factors: novel insights into transcriptional regulation in the *Solanaceae*. *Plant Physiol* 147(1): 280–295.

Shabala S (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann Bot* 112(7): 1209–1221.

Shannon LM, Kay E, Lew JY (1966) Peroxidase isozymes from horseradish roots. I. Isolation and physical properties. *J Biol Chem* 241: 2166–2172.

Shao H, Wang H, Tang X (2015) NAC transcription factors in plant multiple abiotic stress responses: progress and prospects. *Front Plant Sci* 6: 902.

Shi HZ, Ishitani M, Kim CS, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter. *Proc Natl Acad Sci USA* 97:6896–6901.

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. *J Exper Bot* 58(2): 221–227.

Stolf-Moreira R, Medri ME, Neumaier N, Lemos NG, Pimenta JA, Tobita S, Brogin RL, Marcelino-Guimarães FC, Oliveira MCN, Farias JRB, Abdelnoor RV, Nepomuceno AL (2010) Soybean physiology and gene expression during drought. *Genet Mol Res* 9: 1946–1956.

Thao N, Thu N, Hoang X, Ha C, Tran LS (2013) Differential expression analysis of a subset of drought-responsive *GmNAC* genes in two soybean cultivars differing in drought tolerance. *Inter J Mol Sci* 14(12): 23828–23841.

Thu NBA, Hoang XLT, Doan H, Nguyen TH, Bui D, Thao NP, Tran LSP (2014) Differential expression analysis of a subset of *GmNAC* genes in shoots of two contrasting drought-responsive soybean cultivars DT51 and MTD720 under normal and drought conditions. *Mol Biol Rep* 41(9): 5563–5569.

Tran LSP, Nishiyama R, Yamaguchi-Shinozaki K, Shinozaki K (2010) Potential utilization of NAC transcription factors to enhance abiotic stress tolerance in plants by biotechnological approach. *GM Crops* 1(1): 32–39.

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo, JH (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth–promoting *Rhizobacterium* strains. *PLoS One* 7: e52565.

Wu D, Sun Y, Wang H, Shi H, Su M, Shan H, Li T, Li Q (2018) The *SlNAC8* gene of the halophyte *Suaeda liaotungensis* enhances drought and salt stress tolerance in transgenic *Arabidopsis thaliana*. *Gene* 662: 10–20.

Zhu JK (2016) Abiotic stress signaling and responses in plants. *Cell* 167(2): 313–324.

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CÂY CHUYỂN GEN ĐẬU TƯƠNG *GmNAC085* TĂNG CƯỜNG BIỂU HIỆN CỦA MỘT SỐ GEN QUAN TRỌNG DƯỚI ĐIỀU KIỆN STRESS MẶN

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TÓM TẮT

Biến đổi khí hậu đã khiến các stress phi sinh học như hạn hay mặn trở thành các mối đe dọa lớn hơn đối với hệ sinh thái và an ninh lương thực thế giới. Để đáp ứng và bảo vệ trước những điều kiện bất lợi, thực vật thay đổi các hoạt động sinh lý, sinh hóa và phân tử. Đặc biệt, các thành viên NAC (NAM, ATAF1/2, CUC2) đã được biết đến là nhân tố quan trọng trong điều hòa nhiều quá trình sinh học của cây trong đáp ứng các stress thẩm thấu gây ra từ hạn và mặn. Theo các nghiên cứu trước đây, GmNAC085, một nhân tố điều hòa phiên mã của đậu tương (*Glycine max*) có vai trò điều hòa dương tính đối với stress hạn. Vì vậy, ở nghiên cứu này, chúng tôi mở rộng tìm hiểu vai trò của GmNAC085 trong đáp ứng stress mặn. Theo kết quả phân tích RT-qPCR, một số gen quan trọng liên quan đến đáp ứng stress được tăng cường biểu hiện cao hơn đáng kể ở cây đậu tương chuyển gen so với cây không chuyển gen, bao gồm các gen mã hóa các enzyme chống ôxi hóa (*GmSOD*, *GmAPX* and *GmCAT*), gen mã hóa kênh vận chuyển natri-hydrogen ion (*GmNHX1*) và gen mã hóa enzyme tổng hợp proline (*GmP5CS*). Ngoài ra, các kết quả sinh hóa cũng cho thấy hoạt động của các enzyme chống ôxi hóa peroxidase và catalase tăng mạnh ở cây chuyển gen, cùng với nồng độ hydrogen peroxide nội bào thấp hơn. Tất cả những kết quả này cho thấy cây chuyển gen có thể có những thuận lợi trong việc đối phó stress ôxi hóa thông qua hoạt động enzyme và sử dụng nồng độ proline, cũng như thông qua hoạt động bài tiết Na^+ nội bào ở điều kiện stress mặn. Vì vậy, cơ chế hoạt động đầy đủ của GmNAC085 liên quan đến stress mặn cần được thực hiện nhằm đánh giá tiềm năng sử dụng gen này trong công tác cải thiện chất lượng giống cây trồng.

Từ khóa: *Glycine max, GmNAC085, hoạt động enzyme chống ôxi hóa, khả năng chịu stress, stress mặn*