



ROLE OF *GmNAC019* TRANSCRIPTION FACTOR IN SALINITY AND DROUGHT TOLERANCE OF TRANSGENIC *ARABIDOPSIS THALIANA*

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SUMMARY

Increasingly severe drought and salinity stress due to global climate change have made these stresses bigger threats to ecosystem and agriculture. Previous studies reported that *GmNAC019*, a soybean NAC transcription factor - encoding gene, displayed induced expression upon drought treatment in wild-type cultivars. In this study, drought and salinity stresses were applied on *GmNAC019*-overexpressing *Arabidopsis* plants to verify the contribution of *GmNAC019* in regulating plant response to the stress conditions. Results from the water loss rate and survival rate assays revealed that the transgenic line conferred improved tolerance to drought stress as evidenced by lower leaf water loss and significantly higher rate of survival than seen in the wild-type plants. Similarly, the survival rate assay for testing salinity effects on plants by growing the plants on MS medium supplemented with different NaCl concentrations also indicated that the transgenic plants had a better tolerance to salt stress as they displayed lower rate of root growth inhibition and higher survival rate. Taken these results altogether, it is suggested that *GmNAC019* might play important role in aiding plant response to drought and salinity stresses. Specific functions of this gene should be elaborated in future studies to evaluate its potential application for crop improvement.

Keywords: *Arabidopsis thaliana*, drought stress, *GmNAC19*, salinity stress

INTRODUCTION

Due to the nature of being sessile, plants are exposed to various types of abiotic factors such as radiation and temperature, and biotic stresses such as pathogens and herbivores (Redondo-Gómez *et al.*, 2013). Therefore, the environmental stresses largely influence the survival, development and productivity of plants. For abiotic stresses, they are defined as non-living environmental factors that limit the growth and yield of plants (Cramer *et al.*, 2011). With human activities that cause climate changes and environmental degradation, abiotic stresses have become the larger threat to food security (Huang *et al.*, 2013). Particularly, drought, the shortage of available water in soil, is considered one of the most frequently occurring problems that agriculture has to face with. Drought hinders plant growth and development due to reduced photosynthesis, reduced stem extension and leaf expansion, and increased damage to the plant cells (Farooq *et al.*, 2008). Growth and development of the plants are also

greatly interfered by saline soils, leading to the differences in yield between crops (Rasool *et al.*, 2013). According to Qadir *et al.*, (2014), salinity stress affects 20% of irrigated land, which therefore results in significant impact to reduction of crop yields. Being exposed to this stress, the limit of plant growth is caused by osmotic stress and then ionic stress. Osmotic stress in plant cells takes places due to their reduced ability to uptake water. Prolonged salinity stress results in the accumulation of salt and ions such as Na⁺ and Cl⁻ to toxic levels which then trigger the accumulation of cellular reactive oxygen species (Wang *et al.*, 2018). As a consequence, possible changes in plants include the reduction in leaf transpiration, in stomatal conductance, and in cell division and expansion (Schachtman *et al.*, 1991; Rahnama *et al.*, 2010). In general, both drought and salinity stresses will lead to oxidative stress and therefore negatively affect the plant metabolic processes such as enzymatic reactions and protein synthesis (Flowers *et al.*, 1986; Wang *et al.*, 2018).

Plants do possess intrinsic mechanisms to

minimize the impact of environmental stress (Bohnert, Jensen, 1996). It is thus believed that more understanding about plant defense strategies would be beneficial for the scientists in terms of deploying interventional approach such as genetic engineering. Through genetic studies, numerous pathways involving in the growth of plants under drought or salinity condition have been identified for facilitating the understanding of how plants thrive to cope with adverse conditions (Bartels, Sunkar, 2005; Shinozaki, Yamaguchi-Shinozaki, 2007; Deinlein *et al.*, 2014). The findings indicate that many pathways are conserved among plant species and the determined adaptation to these stressors are changes at transcription levels. Many genes have been identified to respond to drought at the transcriptional level, and their products are thought to involve in drought tolerance (Shinozaki, Yamaguchi-Shinozaki, 2000; Shinozaki, Yamaguchi-Shinozaki, 2007). Among different groups of transcription regulators, NAC transcription factors are known to form one of the largest families and associate with transcriptional regulation in plants (Nuruzzaman *et al.*, 2013). Basic structures of a NAC transcription factor are a DNA binding-NAC domain at the N-terminus and a transcriptional activation domain at the C-terminus (Puranik *et al.*, 2012). NAC is the abbreviation of the three different genes initially found to contain NAC domain, including NAM (no apical meristem of petunia), ATAF (*Arabidopsis* transcription activating factors) and CUC (cup-shaped cotyledon in *Arabidopsis*). Members of this group act on various biological pathways relating to plant morphogenesis and development, and stress signal transduction and regulation (Olsen *et al.*, 2005). For example, NAC1 involves in the formation of root hair and auxin signaling pathway in *Arabidopsis* (Xie *et al.*, 2000). Within the NAC family, a number of members have been reported to be the transcription activators while others function as repressors. Important progresses on analyzing NAC family members in different species have been made. For instance, the findings revealed that there are approximately 117 NAC genes in *Arabidopsis*, 151 in rice (Nuruzzaman *et al.*, 2010), 152 each in soybean (Le *et al.*, 2011) and tobacco (Rushton *et al.*, 2008, Nuruzzaman *et al.*, 2012).

Many NAC genes have been shown to involve in plant response to abiotic stresses. In study of Tran *et al.*, (2004), drought, salt and ABA treatments up-regulated the expression of

Arabidopsis ANAC019, *ANAC055*, and *ANAC072* and consequently, overexpression of these genes stimulated the drought resistance in transgenic *Arabidopsis* plants. Taking the rice *OsNAC10* as another example, its overexpression in root of rice induced the drought tolerance (Jeong *et al.*, 2010). Overexpression of rice *OsNAC6* developed the higher plant resistance to drought and salt stress conditions in transgenic rice lines (Rachmat *et al.*, 2014). In another example, expression of *OsNAC2* was induced by osmotic stress and ABA (Shen *et al.*, 2017). In soybean, several NAC genes have also been found to involve in abiotic stress response of plants under salinity, cold or drought conditions such as two *GmNAC* genes (IDs EU40353 and EU40354), which participates in regulating plant response to salinity stress (Hao *et al.*, 2011). Meanwhile, the expression of *GmNAC019*, *022*, *027*, *043*, *085*, *092*, *095*, *099*, *101*, *102* and *109* was induced under drought treatment (Thao *et al.*, 2013). Among NAC group genes, *GmNAC019* was found to be heavily induced by dehydration and suggested as a potential gene for improving drought resistance in soybean (Tran *et al.*, 2009). The study of Tran *et al.* (2009) also showed the increase in expression of this gene in the root tissue upon drought condition. Under both normal and drought conditions, expression of the gene was proved to be higher in shoot of drought-tolerant cultivar (Thao *et al.*, 2013). Particularly based on the analyzed RT-qPCR data for subset of soybean NAC genes, *GmNAC019* (ID Glyma04g38990.1) was also shown to express at high level under drought stress in soybean roots (Thu *et al.*, 2014). This suggested that *GmNAC019* might play an important role in supporting plant response to osmotic stress conditions such as water deficit and salinity. Therefore, the main objective in this study was to analyze the salinity and drought tolerance of *Arabidopsis thaliana* overexpressing *GmNAC019* using several physiological parameters. The obtained results are expected to provide more information on the role of *GmNAC019* and its potential for improving transgenic crops' performance under drought and salinity conditions.

MATERIALS AND METHODS

Plant materials

Arabidopsis thaliana seeds from the wild-type

(WT) and its transgenic lines were kindly provided by Dr. Lam-Son Phan Tran from Signaling Pathway Research Unit, RIKEN Center for Sustainable Resource Science, Yokohama, Japan. The transgenic seeds carrying *GmNAC019* under the regulation of CaMV35S promoter.

Growth of plants before stress treatments

Arabidopsis seeds were cleaned with distilled water (DW) for 5 minutes, sterilized using 70% ethanol for 1 minute followed by 10% Javel detergent for 13 minutes. Subsequently, the seeds were washed repeatedly (3-5 times) with DW to completely remove all the bleach residues. These sterilized seeds were placed on plates containing Murashige Skoog (MS) medium, stratified at 4°C for 2 days and transferred to the growth room at 22°C and 16 h of light for germinating and growing of plants.

Survival rate assay for evaluating drought tolerance

The procedure was adopted from Zhang *et al.* (2016). At first, seeds were germinated on MS plates for 2 weeks and then transferred to trays with sterilized soil for further 2 weeks under normal daily watering. Following, irrigation was ceased until the soil moisture level dropped below 10% to record the change in plant phenotype. After that, the plants were re-watered for 7 days and recovered plants were recorded as the survival rates of wild-type and transgenic plants.

Measurement of water loss rate in dehydrated leaf samples

Leaves from the 5-week old plants (2 weeks on MS plates and 3 weeks on soil) were cut and weighed immediately to record their fresh weights (FWs). These leaves were then placed on a laboratory bench for slowly drying and were weighed at regular intervals. Water loss rate was estimated based on the percentage of recorded weight at the time point of measurement relative to the initial tissue FW (Zhang *et al.*, 2016).

Survival rate assay for evaluating salinity tolerance

The assay followed the protocol of Zhang *et al.* (2016). Seedlings germinated and grown on normal MS medium for 14 days were transferred to half-strength MS medium containing various sodium chloride concentrations (0, 50, 100 and 150 mM). Survival rates of wild-type and transgenic line (n=32 per genotype) were recorded 7 days since NaCl treatment.

Examine effects of salt to root elongation

Root elongation was examined according to Orsini *et al.*, (2010). After 7 days of growth on MS

medium, the seedlings exhibiting similar root lengths were transferred onto MS medium with 0 (control), 50, 100 and 150 mM NaCl. Under standard long day conditions (16 h light/ 8 h dark), the plants were grown in a vertical position for 5 days. Relative root elongation of these seedlings was then evaluated.

Statistical analysis

The data were analyzed using Student's t-test or ANOVA for identification of statistical significance with *p*-value below 0.05 (* *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001).

RESULTS AND DISCUSSION

Overexpression of *GmNAC019* enhanced tolerance to drought in *Arabidopsis thaliana*

In response to drought, plants can implicate a variety of drought resistance mechanisms such as drought avoidance, drought tolerance or/and drought escape (Price *et al.*, 2002; Levitt *et al.*, 1980). For example, minimizing water loss can aid the avoidance of low water potentials under water deficit condition, and as result, help plants survive under drought stress condition (Villar-Salvador *et al.*, 2004). In order to generally examine the drought tolerance of WT and transgenic plants, the survival rate assay was conducted. In this study, these two genotypes were grown in alternate order in the same tray with appropriate space between two adjacent plants to avoid shielding effect to water evaporation. The WT and transgenic plants displayed similar phenotypes under normal growing condition (Fig. 1A). At the end of 13-day drought stress period when the soil moisture content lowered to less than 10%, the plants of both lines were heavily affected whereby the leaves showed symptoms of being wilted, senescence and chlorophyll degradation (Figs. 1A and 1B). Interestingly, when re-irrigation was applied, only 54.2% of WT plants could recover while the survived proportion of the transgenic line was 24% higher (78.5%, *p*-value < 0.05) (Fig. 1C). This assay demonstrated the significantly better drought tolerance of the transgenic plants compared to the WT.

Next, to assess the ability of water maintenance by the plants, water loss assay for the leaf tissue was performed. The leaves at the same growth stage and similar sizes were cut from 5-week-old plants of WT and *GmNAC019* transgenic line for measuring the decrease in leaf weight over a time course of dehydration treatment. The analyses indicated that a distinct difference in water loss rate between WT and transgenic leaves could be seen after 30-minute dehydration and become more apparent after 1 and 4 hours. After 5 hours since leaf excision, the control plant leaves

lost 66% water while the transgenic one lost about 50% (Fig. 2). These results revealed the significantly higher ability to minimize water loss

upon dehydration in the transgenic *Arabidopsis* compared to the WT.

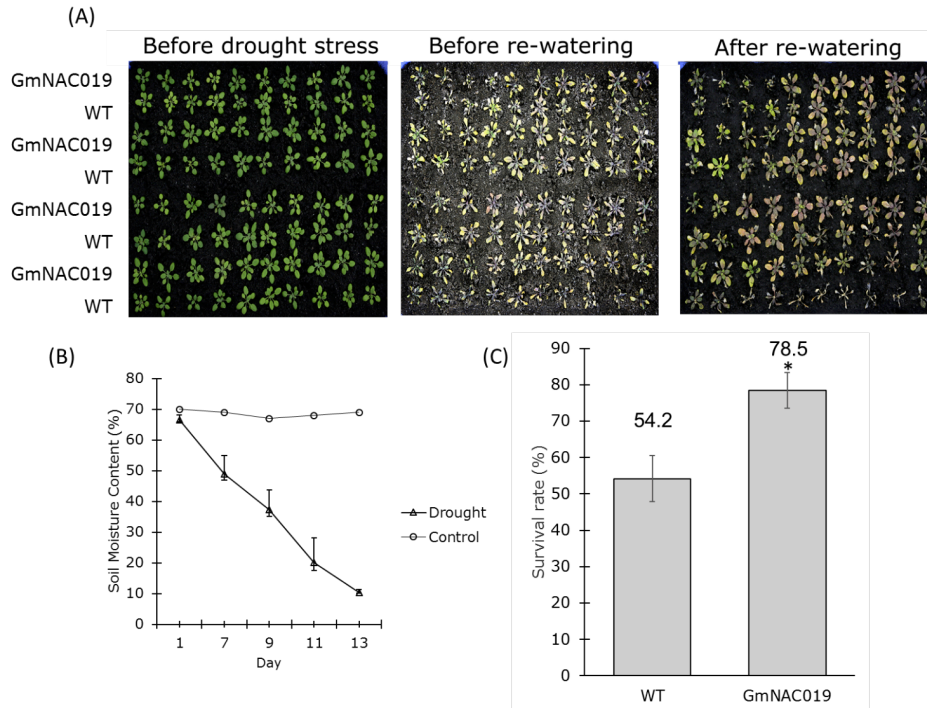


Figure 1. Survival rate experiment of *Arabidopsis* transgenic plants overexpressing *GmNAC019*. (A) Phenotypes of wild-type (WT) and transgenic plants grown under normal, drought and post-drought (with re-irrigation) conditions; (B) Soil moisture content measured over 13-day-period of drought treatment; (C) Survival rates of transgenic and wild-type plants recorded after 13-day drought stress and 7-day re-watering application. * indicates statistically significant difference between survival rate of WT and transgenic plants. The experiment was performed with 40 plants for each genotype.

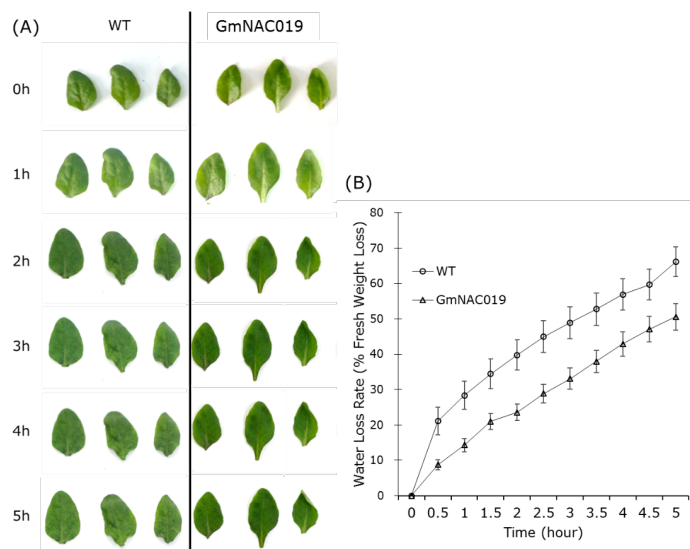


Figure 2. Effects of drought stress on water loss rate of *Arabidopsis* transgenic plants overexpressing *GmNAC019*. (A) Phenotypes of wild-type (WT) and transgenic rosette leaves after cutting; (B) Water loss rate shown in fresh weight loss (%) recorded at 30 min interval during the indicated time (n=18 per genotype).

Overexpression of *GmNAC019* enhanced tolerance to salinity in *Arabidopsis thaliana*

Plants also have numerous of physiological responses to salinity, which are often complex and multi-faceted. According to Munns, Tester (2008), there are three main salinity tolerance mechanisms, including ion exclusion, tissue and shoot ion-independent tolerance. Other physiological components such as leaf area reduction, accumulation of salt ions in cellular vacuole to prevent the ion-build up in cytoplasm and cell walls, early seedling growth and leaf transpiration maintenance are also likely to contribute to water deficit tolerance (Kingsbury, Epstein, 1984; Munns *et al.*, 2002; Harris *et al.*, 2010). In addition to upper plant parts, salinity also affects root growth and their nutrient uptake (Hasanuzzaman *et al.*, 2013).

For salinity tolerance assessment, 14-day-old seedlings were transferred onto fresh half-strength

MS medium supplemented with 0 (control), 50, 100, 150 mM NaCl and survival rates of both WT and transgenic plants were calculated after 7 days. No visible death was observed on the normal medium, yet of few died plants were found in both WT and *GmNAC019* lines grown on ½ MS culture medium with a concentration of 50 mM NaCl (Fig. 3A). However, there was no significant difference in survival rate between them. On ½ MS medium supplemented with 100 mM NaCl, the survival rate of the transgenic line was slightly higher than that of WT. The growth of most WT plants was inhibited, with yellow or white leaves at the concentration of 150 mM NaCl after 7 days of the treatment (Fig. 3A) with survival rate of 3.13% (Fig. 3B). In comparison, more transgenic plants could survive and grow under high-salinity condition (Figs. 3A, 3B). At NaCl concentration of 150 mM, the survival rate of *GmNAC019* – overexpressing transgenic line was 37.5%, which was significantly higher than that of WT plants (Fig. 3B).

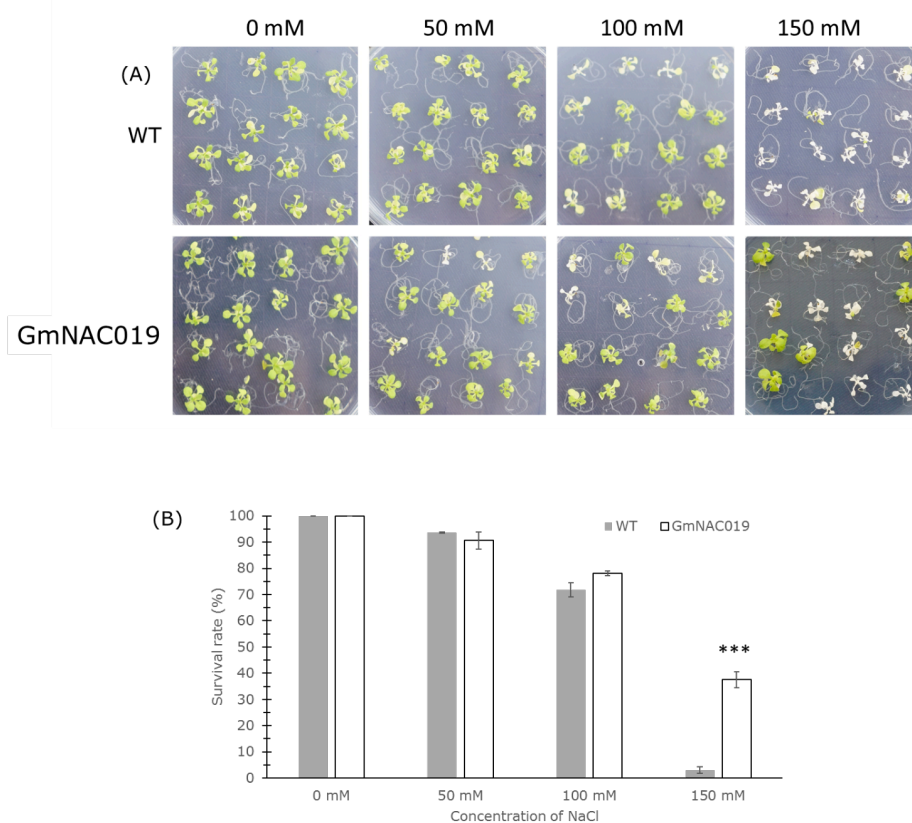


Figure 3. Effects of salinity stress on survival rates of *Arabidopsis* transgenic plants overexpressing *GmNAC019* (A) Phenotype of seedlings sown from medium supplemented with various NaCl concentrations; (B) Survival rate under high-salinity conditions in the presence or absence of NaCl. The survival rates of transgenic and wild-type (WT) plants were calculated 7 days after treatment. Student's *t*-test was used for determination of significance (***)*p*-value < 0.001).

Additional test was then performed to determine the effect of salt on growth by comparing the root elongation of WT and transgenic seedlings grown under different concentrations of NaCl (0, 50, 100, 150 mM NaCl). On medium supplemented with 50 mM NaCl, root growth of the transgenic plants was markedly greater than that of the WT (Fig. 4B). The root elongation of WT was also significantly less than of *GmNAC019* line at the concentrations of 100 mM and 150 mM NaCl (Fig.

4B). The data here suggested that overexpression of *GmNAC019* increased the high-salinity tolerance in *Arabidopsis*.

Collectively, these results indicate the possible involvement of *GmNAC019* in drought stress and high-salinity stress resistance. However, more detailed studies in molecular mechanism of *GmNAC019* product on regulating plant stress tolerance are further required.

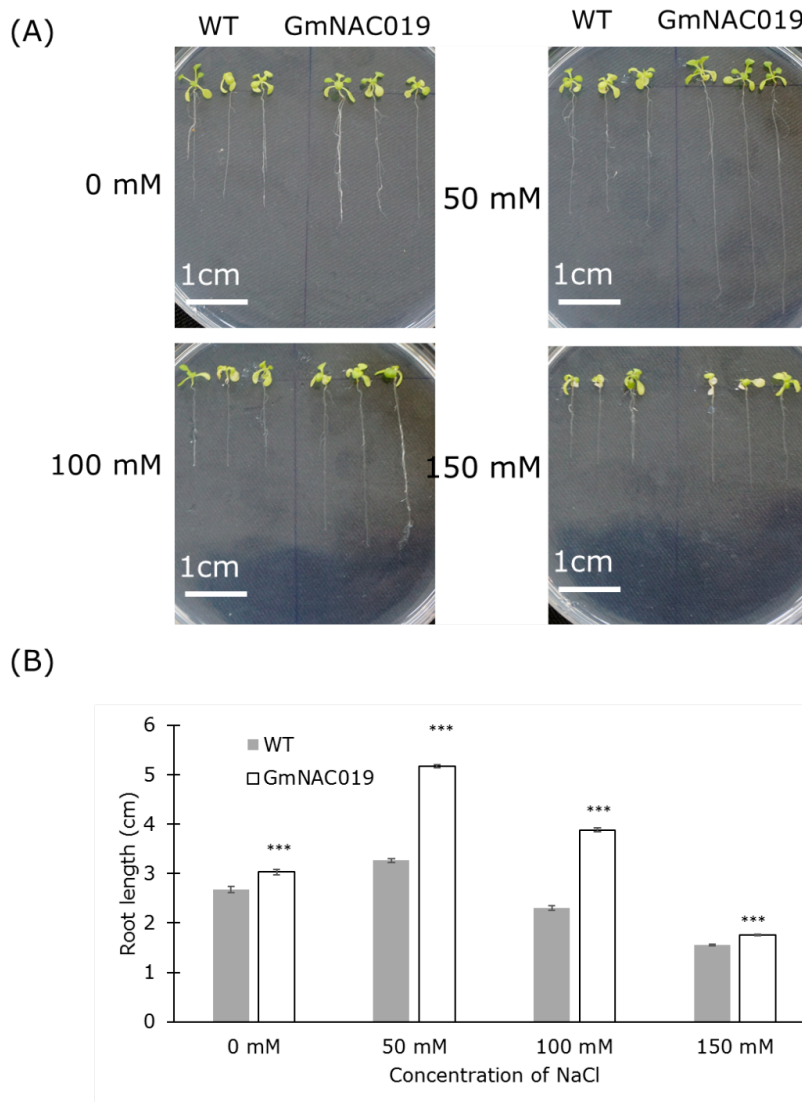


Figure 4. Root phenotypes of *GmNAC019* - overexpressing *Arabidopsis* plants compared to the wild-type (WT) plants under salinity treatment. (A) Phenotype of 12-day-old seedlings grown on $\frac{1}{2}$ MS medium containing 0, 50, 100, 150 mM NaCl; (B) Comparison of relative root length of *GmNAC019* and WT lines under the same treatment condition (n=60 per genotype). The colored bars refer to the final length of root recorded at day 5th of treatment. The experiment was repeated three times. *** indicates a statistical significance of p -value < 0.001.

CONCLUSION

In summary, the physiological analyses obtained in this study indicated that overexpression of *GmNAC019* could confer enhanced drought and salinity stress resistance in *Arabidopsis*. The tolerance was seen with lower cellular water loss rate under dehydration, lower inhibition of root growth under salt stress and better survival rates under both stress conditions. These results suggest an important role of *GmNAC019* transcription factor in supporting plant tolerance to abiotic stress factors, at least to water deficit and salinity.

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VAI TRÒ CỦA NHÂN TỐ ĐIỀU HÒA *GmNAC019* TRONG ĐÁP ỨNG KHÁNG HẠN VÀ MẶN CỦA CÂY CHUYỂN GEN *ARABIDOPSIS THALIANA*

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TÓM TẮT

Sự gia tăng về mức độ nghiêm trọng của stress hạn và mặn do hiện tượng biến đổi khí hậu toàn cầu đã khiến các tác nhân này trở thành các mối đe dọa lớn hơn đối với hệ sinh thái và sản xuất nông nghiệp. Các nghiên cứu trước đây đã cho thấy *GmNAC019*, gen mã hóa một nhân tố điều hòa thuộc họ NAC ở cây đậu

tương, có hoạt động biểu hiện mạnh hơn khi cây bị xử lý stress hạn. Ở nghiên cứu này, các yếu tố stress hạn và mặn được xử lý trên cây *Arabidopsis* chuyển gen có biểu hiện vượt mức *GmNAC019* nhằm xác nhận vai trò của *GmNAC019* protein trong việc điều hòa các phản ứng của cây dưới điều kiện stress. Các kết quả từ thí nghiệm về tốc độ thoát nước và tỉ lệ sống sót đã cho thấy được dòng cây chuyển gen có khả năng chống chịu hạn tốt hơn, thể hiện qua tốc độ thoát nước thấp hơn và tỉ lệ sống sót cao hơn một cách rõ rệt so với cây không chuyển gen. Đồng thời, thí nghiệm đánh giá tỉ lệ sống sót khi bị stress mặn bằng cách trồng cây trên môi trường MS có chứa nồng độ dung dịch muối (NaCl) khác nhau cũng cho thấy cây chuyển gen có khả năng chịu mặn tốt hơn vì sinh trưởng của rễ ít bị ức chế hơn và có tỉ lệ sống sót cao hơn. Tất cả các kết quả ghi nhận cho thấy *GmNAC019* có thể đóng vai trò quan trọng trong việc hỗ trợ cây đáp ứng với các điều kiện bất lợi về hạn và mặn. Các chức năng cụ thể của *GmNAC019* cần được phân tích sâu hơn trong tương lai để có thể đánh giá ứng dụng tiềm năng của gen dùng cho mục đích cải thiện chất lượng cây trồng.

Từ khóa: *Arabidopsis thaliana*, *GmNAC019*, stress hạn, stress mặn