EXPRESSION ALTERATION ANALYSES IN THE TRANSGENIC ARABIDOPSIS CARRYING SOYBEAN HISTIDINE-CONTAINING PHOSPHOTRANSmitter GENE UNDER SALINITY STRESS CONDITION

Thai Chi Hung¹,², Hoang Thi Lan Xuan¹,², Nguyen Thien Quang¹,², Nguyen Phuong Thao¹,²,³

¹Applied Biotechnology for Crop Development Research Unit, School of Biotechnology, International University, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
²Vietnam National University, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
³To whom correspondence should be addressed. E-mail: npthao@hcmiu.edu.vn

Received: 21.6.2021
Accepted: 30.8.2021

SUMMARY

Productivity of many crops is highly vulnerable to extreme external conditions. Environmental stress factors such as drought and salinity have become more and more serious due to climate change and appear in many areas worldwide with higher frequency. As both drought and salinity belong to osmotic stress, they have similar negative effects on plant growth, development, and productivity as well as trigger similar stress responses by plants. In a previous study analyzing the expression profile in two soybean (Glycine max) cultivars with contrasting drought-tolerant phenotypes, a member of two-component system (TCS) in soybean, GmHP08, was proposed to associate with the plant tolerance capacity to drought. Subsequent in planta study confirmed its action as a positive regulator under drought conditions, as the transgenic Arabidopsis plants ectopically expressing GmHP08 acquired better drought tolerance. Following this, the presented research further explored the possible function of GmHP08 in mediating plant response to salinity. The obtained data from RT-qPCR analyses suggested that GmHP08 might positively enhance the salt tolerance of the Arabidopsis transgenic plants by altering the transcriptional abundance of several stress-related genes, including RD29A, RD29B, ABI5, SAG13, and CSD1. Activities of these genes are known to be associated with osmoprotection, senescence process, and antioxidation, which contribute to salt-tolerance ability of the transgenic plants. These results provided the first line of molecular evidence regarding GmHP08 function in plant response to salinity conditions. Therefore, extensive studies should be conducted in future studies to elaborate on the mechanisms by which this TCS member could improve various types of osmotic stress tolerance in plants.

Keywords: Arabidopsis, GmHP08, RT-qPCR, salt tolerance, two-component systems

INTRODUCTION

Salinity is considered as one of the major abiotic stress factors that not only reduces plant growth and productivity but also accelerates the cutting-down of land usage (Gong et al., 2020). According to a recent report, soil salinization affects almost one-fifth of the cultivated land all around the world, especially in the flooded or saltwater-intrusive areas (Morton et al., 2019). Negative impacts of salinity on plant growth and development mainly come from three types, which are (i) water deficit stress (i.e. dehydration) due to reduced water potential in soil causing difficulty of water uptake by plant root system, (ii) ion toxicity due to excessive accumulation of specific ions such as Na⁺ and Cl⁻ in plant cells, and (iii) oxidative stress due to overproduction of endogenous reactive oxygen species (ROS) under the adverse condition (Munns, 1993; Chaitanya et
al., 2003). Under prolonged salinity conditions, plants suffer decreased photosynthetic efficiency and yield loss, due to disturbed activities of carbon-reduction cycle and light reactions, growth retardation as well as promoted senescence process (Lawlor, Tezara, 2009).

In recent decades, many studies have been conducted to investigate the mechanisms and the pathways that plants utilize to respond to abiotic stresses including salinity (Gong et al., 2020). So far, various resistant mechanisms have been identified in plants, which involve anatomical, physiological, biochemical and molecular adjustments, with the engagement of diverse signaling transduction pathways (Cramer et al., 2011). Among these include the two-components system (TCS), which exists not only in plants but also in other group species such as bacteria and fungi. The action of the TCSs confers the plants capabilities to sense and respond to environmental stimuli (Thu et al., 2015). The simplest form of a TCS contains two basic components, which are a sensor histidine kinase (HK) that receives the input signal, and an effector response regulator (RR) that delivers the signal to regulate the expression of its downstream target genes (Hwang et al., 2002). There are also other complex forms of the TCS that have an extra component, known as histidine-containing phosphotransfer (HPT). This is an intermediate protein connecting the phosphor transfer from the HKs to the RRs, which is referred to as multistep histidine-aspartate phosphorelay (Schaller, 2000; Lohrmann, Harter, 2002). Additionally, several TCS members were identified to participate in abiotic stress response. For example, Arabidopsis HK1 (AHK1), Arabidopsis HP2 (AHP2), AHP3 and AHP4 were shown to act as positive and negative regulators under drought stress conditions, respectively (Tran et al., 2007; Wohlbach et al., 2008; Tran et al., 2010).

Due to its economic and nutritional importance, soybean (Glycine max L. Merrill) is one of the most essential crops worldwide (Andres et al., 2009; Le et al., 2012). It is, however, very susceptible to drought and salinity, thus suffers a significant decrease in productivity (Wang et al., 2016). Previously, nine soybean TCS-related genes including GmHK07, GmHK16, GmHP08, GmRR04, GmRR16, GmRR32, GmRR34, GmPRR39, and GmPRR44 which might potentially contribute to the drought tolerance capacity in plants have been identified (Le et al., 2011; Thu et al., 2015). Regarding GmHP08, it was found that expression of this gene was significantly induced in the soybean shoot tissue after 10 hours of dehydration treatment (Le et al., 2011) or under 15-day-drought stress conditions (Thu et al., 2015). Subsequent in planta study indicated that transgenic Arabidopsis carrying GmHP08 acquired better drought tolerance (Chuong et al., 2021), confirming the critical role of this protein in response to drought.

As drought and salinity cause similar impacts on plant growth and development, which particularly results in osmotic stress and oxidative stress (Muñns, 2002; Uddin et al., 2016), the role of GmHP08 in plant response to salinity is of interest for investigation. By utilizing transgenic Arabidopsis carrying GmHP08, the assessment of salt-tolerance related to GmHP08 was carried out in this study, based on expression analyses of several key osmotic stress-related genes. These were two well-known marker genes [Responsive to desiccation 29A (RD29A), RD29B], one regulatory gene [ABA-insensitive 5 (ABI5)], one senescence-related gene [Senescence-associated gene 13 (SAG13)], and one antioxidant enzyme-encoding gene [Superoxide dismutase (Cu-Zn) 1 (CSD1)]. According to previous studies, expression of RD29A and RD29B, which belong to Late embryonic abundance (LEA) family, was enhanced by drought, cold, abscisic acid (ABA) and high salinity conditions (Jin et al., 2013; Li et al., 2013; Zhou et al., 2015). The third selected gene for examination, ABI5, is a basic leucine zipper-typed transcription factor. It functions in the core of the ABA signaling, known as to play a crucial role in controlling seed germination, post-germination growth (Skubacz et al., 2016) and also participate in regulating plant responses

Thai Chi Hung et al.
to adverse environmental conditions such as drought and salinity (Finkelstein, Lynch, 2000; Nakamura et al., 2001). Meanwhile, relative expression of SAG13 normally increases when plant aging is accelerated such as under stress conditions (Huang et al., 2015). The last chosen gene in our study, CSD1, encodes superoxide dismutase (SOD) [Cu-Zn], which is an important antioxidant enzyme acting in eradication of excessive superoxide (a type of ROS) from plant cells (Jagadeeswaran et al., 2009).

**MATERIALS and METHODS**

**Materials**

The wild-type (WT) Arabidopsis thaliana ecotype Col-0 was utilized in this research as control and as material to generate transgenic plants carrying GmHP08 under the regulation of Cauliflower mosaic virus (CaMV) 35S promoter. The procedures for construction of recombinant vector carrying 35S::GmHP08, plant transformation as well as selection of homogenous transgenic plant progenies were described in our previously published study (Chuong et al., 2021).

**Plant growth**

The seeds of transgenic and WT plants were sterilized using 70% ethanol and 10% Javel before being sown on germination medium (GM) (Murashige and Skoog medium supplemented with 1% glucose and 0.8% agar, pH 5.8). They were then incubated for 2 days in dark and cold (4 °C) environment for breaking seed dormancy. After that they were cultivated in normal growth conditions (22 °C, 16-h-day/8-h-night period).

**Salt-stress assay**

The WT and transgenic plants were treated with NaCl following methods in previous studies (Li et al., 2014; Jiang et al., 2015) with some modifications. In brief, fourteen-day-old WT and transgenic plants were transferred from the GM to water-saturated soil and grown under normal conditions for the next 16 days. After that, the salt-stress assay was applied by irrigating 120 mL of 200 mM NaCl to each tray every 2 days. The aerial parts of plants were harvested at day 0, day 3rd and day 7th since salt application by freezing in liquid nitrogen. For each time point of sample collection, three biological replicates were used for each genotype.

**Total RNA extraction, cDNA synthesis and reverse-transcription quantitative PCR**

Pure total RNA of all collected samples were obtained by using commercial kits (Thermo Fisher Scientific, USA) for RNA extraction and DNA removal (Thao et al., 2013). After this, cDNA synthesis was performed using 1,000 ng of RNA from each sample and following the instruction of the kit manufacturer (RevertAid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific, USA).

RT-qPCR reactions were prepared in 25 µL of total volume, which contained SYBR Green PCR Master mix (Thermo Scientific), primers (0.4 µM each) and 1 µL cDNA. The PCR thermal profile was established in accordance with our previous study (Thao et al., 2013).

Actin 2 (ACT2) (Yang et al., 2016) was used as the reference gene for gene expression analysis. The sequences of primers for five target genes were obtained from previous studies including RD29A (Rasheed et al., 2016), RD29B (Liu et al., 2018), AB15 (Huang et al., 2015); CSD1 (Chen et al., 2013) and SAG13 (Huang et al., 2015). The relative transcript abundance target genes between WT and transgenic plants under different conditions was calculated using the 2^\(^{-\Delta\Delta CT}\) method (Livak, Schmittgen, 2001).

**Statistical data analysis**

The obtained results were analyzed by Student’s t-test. The significant difference was confirmed if the p-value was below 0.05 (Thu et al., 2015).

**RESULTS AND DISCUSSION**

**Expression of RD29A and RD29B**

Upon being irrigated by salt-water, a significant
increase in transcript abundance of $RD29A$ and $RD29B$ was observed in both transgenic and WT plants (Figure 1), especially after 7 days. Particularly, $GmHP08$-transgenic plants displayed a much higher induction level in expression of these genes, compared with that in the WT plants at the same time point of analysis under the stressed conditions. To be specific, the expression levels of $RD29A$ were 3.39-fold higher after 3 days and 1.95-fold higher after 7 days in the transgenic plants. Meanwhile under normal conditions, although expression levels were comparable between the two genotypes (Figure 1A), that of $RD29B$ was higher in the WT plants than in the transgenic plants by 1.47-fold (Figure 1B).

According to literature, $RD29$ genes have been highlighted as markers for plant response to dehydration and high salinity conditions, with an increase in gene expression (Yamaguchi-Shinozaki, Shinozaki, 1994; Msanne et al., 2011). Although the function of hydrophilic proteins encoded by these genes remained elusive, they share similarity to LEA proteins. Therefore they are called LEA-like proteins and suggested to have similar function with LEA proteins (Yamaguchi-Shinozaki, Shinozaki, 1993a; Yamaguchi-Shinozaki, Shinozaki 1993b; Msanne et al., 2011). LEA proteins are responsible for osmoprotection, along with other elements such as osmotin, chaperones, sugars and proline (Shinozaki, Yamaguchi-Shinozaki, 2007; Msanne et al., 2011). Therefore, the higher expression of $RD29A$ and $RD29B$ in $GmHP08$-transgenic plants might confer them better osmoprotection under salinity conditions (Figure 1).

Expression of $ABI5$, $SAG13$ and $CSD1$

When exposed to the salt stress condition, $ABI5$ expression significantly increased in both transgenic and WT plants (Figure 2A), especially after 7 days with higher level in the transgenic than in the WT plants. Meanwhile, $SAG13$ expression levels were only induced in both studied genotypes after 3 days of the stress application. At the stage of one-week treatment, further enhancement in $SAG13$ expression was only observed in the $GmHP08$-transgenic plants but not the in WT plants, thus leading to substantially higher expression level of $SAG13$ in the former group over its WT counterparts at this time point (Figure 2B). With the expression pattern of $CSD1$, this gene was always expressed more highly in the ectopic expression line than in
the WT plants at the same time point of analysis, in both growing conditions. It is noticed that compared to the non-stressed WT plants, the expression of this gene after 3-day stress treatment only slightly increased and followed by a significant decrease after 7 days of treatment. Meanwhile, the expression levels of CSD1 in the transgenic plants increased significantly after 3-day exposure to salinity before dropping to almost similar level at day 0 (Figure 2C).

Among ABI5, SAG13 and CSD1, under non-stressed condition, transcriptional levels of ABI5 between the two genotypes were similar (Figure 2A), whereas those of the other two genes showed a significant difference between the transgenic and WT plants (Figures 2B, 2C). Under salinity conditions, expression of ABI5 was 1.6-fold higher after 3 days and 1.9-fold higher after 7 days than the corresponding levels in the WT counterparts (Figure 2A). In contrast, the expression of SAG13 was higher in the WT plants than in transgenic plants by 2.9-fold after 3 days, although after 7 days, the reverse trend was observed (i.e. SAG13 expression was significantly more upregulated in the transgenic plants than in WT plants by 1.54-fold) (Figure 2B). For CSD1, its expression showed an increase in transcript abundance, which was higher in the transgenic plants in both time points of analyses (3rd and 7th day) under salinity, with the higher levels approximately about 1.6 and 2.4-fold, respectively (Figure 2C).

**ABI5** is normally known as an inhibitory regulator of plants during seed germination but positive regulator in processes of protection, water retention and toxicity isolation in the chloroplasts as well as in preventing cellular damage when being exposed to adverse conditions (Lopez-Molina et al., 2001; Brocard et al., 2002; Skubacz et al., 2016). Thus, an increase in transcriptional level of this gene might assist the GmHP08-transgenic plants to have a better cellular protection and higher possibility to survive under prolonged high salinity conditions in comparison with the WT plants. With SAG13, this protein acts as a marker of senescence in Arabidopsis and other plant species (Brodersen et al., 2002; Espinoza et al., 2007). It was also reported that SAG13 plays a role in germination process, seedling development under oxidative stress and has a crucial role in mediating plant response to ROS attack in combination with light stress. Furthermore, the presence of SAG13 might assist accumulation of anthocyanin – an important compound not only in reproduction but also in protection during oxidative stress (Liu et al., 2018; Dhar et al., 2020). Therefore, based on the

**Figure 2.** Relative expression of genes ABI5 (A), SAG13 (B) and CSD1 (C) in wild-type (WT) and transgenic plants under normal (0 day) and salinity conditions. Significant difference in expression between conditions for the same genotype was shown by the plus symbol above the drawing lines (+: p-value < 0.05, ++: p-value < 0.01, +++: p-value < 0.001) and between the two genotypes under the same condition by the star symbol displayed above the transgenic bar (*: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001).
obtained data, it is hypothesized that GmHP08-transgenic plants might have delayed senescence under short stress duration conditions for maintaining photosynthesis but promoted leaf senescence under prolonged stress conditions to prioritize their survival (Figure 2B).

Under stress conditions, plants rely on antioxidant molecules and enzymes to scavenge the excessive ROS contents in plant cells, as accumulation of these species can result in the disruption of cellular structure and activities (Foyer et al., 1994; Mittler, 2002). Regarding the ROS-type superoxide, its detoxification is achieved by the activities of SOD enzymes (Mittler, 2002). Many studies have shown that the transgenic plants acquired better tolerance to salinity with increased expression of SOD-related genes and SOD enzyme activities (Hu et al., 2012; Chen et al., 2017). Thus, the increase in CSD1, which is an SOD-encoding gene, in the GmHP08-transgenic plants suggests enhanced SOD activity and better superoxide removal in these plants, thus conferring a better protection from salinity-induced oxidative stress.

CONCLUSION

Taken together, the obtained results from this study demonstrate that the transgenic plants ectopically expressing GmHP08 have higher salt-tolerance capacity by upregulating the transcriptional level of several important stress-responsive genes. Hereby, GmHP08 was found to act as a positive regulator of RD29A, RD29B, ABI5, SAG13 and CSD1. This conferred the transgenic plants certain advantages in osmoprotection and endogenous ROS removal, and thus potentially better tolerance to salinity stress. Nevertheless, comprehensive understanding of GmHP08 function is required to serve for the thorough evaluation of its application potential in stress tolerance improvement. The future research should be focused on how the transgenic plants react with different concentrations of salt as well as on expression of other stress-responsive genes in connection with physiological and biochemical data analyses.

Acknowledgments: Hoang Thi Lan Xuan was funded by Vingroup Joint Stock Company and supported by the Domestic Master/ PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), Vingroup Big Data Institute (VINBIGDATA), code VINIF.2020.TS.09.

REFERENCES

Andres A, Donovan SM, Kuhlenschmidt MS (2009) Soy isoflavones and virus infections. J Nutr Biochem 20: 563-569.

Anwar HM, Golam MM, Fujita M (2014) Modulation of reactive oxygen species and methylglyoxal detoxification systems by exogenous glycinebetaine and proline improves drought tolerance in mustard (Brassica juncea L.). Int J Plant Biol Res 2: 1014.

Brocard IM, Lynch TJ, Finkelstein RR (2002) Regulation and role of the Arabidopsis abscisic acid-insensitive 5 gene in abscisic acid, sugar, and stress response. Plant Physiol 129: 1533-1543.

Brodersen P, Petersen M, Pike HM, Olszak B, Skov S, Odum N, Jørgensen LB, Brown RE, Mundy J (2002) Knockout of Arabidopsis accelerated-cell-death11 encoding a sphingosine transfer protein causes activation of programmed cell death and defense. Genes Dev 16: 490-502.

Chaitanya KV, Jutur PP, Sundar D, Reddy AR (2003) Water stress effects on photosynthesis in different mulberry cultivars. Plant Growth Regul 40: 75-80.

Chen Y, Han Y, Kong X, Kang H, Ren Y, Wang W (2017) Ectopic expression of wheat expansin gene TaEXPA2 improved the salt tolerance of transgenic tobacco by regulating Na+/K+ and antioxidant competence. Physiol Plant 159: 161-177.

Chen Y, Jiang J, Song A, Chen S, Shan H, Luo H, Gu C, Sun J, Zhu L, Fang W (2013) Ambient temperature enhanced freezing tolerance of Chrysanthemum dichotum CdICE1 Arabidopsis via miR398. BMC Biol 11: 121.

Chuong NN, Hoang XLT, Nghia DHT, Nguyen NC, Thao DTT, Tran TB, Ngoc TTM, Thu NBA, Nguyen QT, Thao NP (2021) Ectopic expression of GmHP08 enhances resistance of transgenic Arabidopsis toward drought stress. Plant Cell Rep 40: 819-834.

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems
biology perspective. *BMC Plant Biol* 11: 163.

Dhar N, Caruana J, Erdem I, Subbarao KV, Klosterman SJ, Raina R (2020) The *Arabidopsis* Senescence-associated gene 13 regulates dark-induced senescence and plays contrasting roles in defense against bacterial and fungal pathogens. *Mol Plant Microbe Interact* 33: 754-766.

Espinoza C, Medina C, Somerville S, Arce-Johnson P (2007) Senescence-associated genes induced during compatible viral interactions with grapevine and *Arabidopsis*. *J Exp Bot* 58: 3197-3212.

Finkelstein RR, Lynch TJ (2000) The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* 12(4): 599-609.

Foyer CH, Descourvieres P, Kunert KJ (1994) Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. *Plant Cell Environ* 17: 507-523.

Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, Chao DY, Li J, Wang PY, Qin F (2020) Plant abiotic stress response and nutrient use efficiency. *Sci China Life Sci* 63: 635-674.

Hu L, Huang Z, Liu S, Fu J (2012) Growth response and gene expression in antioxidant-related enzymes in two bermudagrass genotypes differing in salt tolerance. *J Am Soc Hortic Sci* 137: 134-143.

Huang Q, Wang Y, Li B, Chang J, Chen M, Li K, Yang G, He G (2015) TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. *BMC Plant Biol* 15: 268.

Hwang I, Chen HC, Sheen J (2002) Two-component signal transduction pathways in *Arabidopsis*. *Plant Physiol* 129: 500-515.

Jagadeeswaran G, Saini A, Sunkar R (2009) Biotic and abiotic stress down-regulate miR398 expression in *Arabidopsis*. *Planta* 229: 1009-1014.

Jiang SC, Mei C, Liang S, Yu YT, Lu K, Wu Z, Wang XF, Zhang DP (2015) Crucial roles of the pentatricopeptide repeat protein SOAR1 in *Arabidopsis* response to drought, salt and cold stresses. *Plant Mol Biol* 88: 369-385.

Jin X, Xue Y, Wang R, Xu R, Bian L, Zhu B, Han H, Peng R, Yao Q (2013) Transcription factor OsAP21 gene increases salt/drought tolerance in transgenic *Arabidopsis italiana*. *Mol Biol Rep* 40: 1743-1752.

Lawlor DW, Tezara W (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann Bot* 103: 561-579.

Le DT, Nishiyama RIE, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K., Shinozaki K, Tran LS P (2011) Genome-wide expression profiling of soybean two-component system genes in soybean root and shoot tissues under dehydration stress. *DNA Res* 18: 17-29.

Le DT, Nishiyama R, Watanabe Y, Vankova R, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2012) Identification and expression analysis of cytokinin metabolic genes in soybean under normal and drought conditions in relation to cytokinin levels. *PLoS One* 7: e42411.

Li H, Gao Y, Xu H, Dai Y, Deng D, Chen J (2013) ZmWRKY33, a WRKY maize transcription factor conferring enhanced salt stress tolerances in *Arabidopsis*. *Plant Growth Reg* 70: 207-216.

Li X, Yang X, Hu Y, Yu X, Li Q (2014) A novel NAC transcription factor from *Suaeda liaotungensis* K. enhanced transgenic *Arabidopsis* drought, salt, and cold stress tolerance. *Plant Cell Rep* 33: 767-778.

Liu Y, Li L, Zhang L, Lv Q, Zhao Y, Li X (2018) Isolation and identification of wheat gene *TaDIS1* encoding a RING finger domain protein, which negatively regulates drought stress tolerance in transgenic *Arabidopsis*. *Plant Sci* 275: 49-59.

Liu Y, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A (2018) Anthocyanin biosynthesis and degradation mechanisms in *Solanaceous* vegetables: a review. *Front Chem* 6: 52.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. *Methods* 25: 402-408.

Lohmann J, Harter K (2002) Plant two-component signaling systems and the role of response regulators. *Plant Physiol* 128: 363-369.

Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the AB15 transcription factor in *Arabidopsis*. *Proc Natl Acad Sci* 98: 4782-4787.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7: 405-410.
Morton MJL, Awlia M, Al-Tamimi N, Saade S, Pailles Y, Negrão S, Tester M (2019) Salt stress under the scalpel—dissecting the genetics of salt tolerance. *Plant J* 97: 148-163.

Msanne J, Lin J, Stone JM, Awada T (2011) Characterization of abiotic stress-responsive *Arabidopsis thaliana* RD29A and RD29B genes and evaluation of transgenes. *Planta* 234: 97-107.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell Environ* 16: 15-24.

Nakamura S, Lynch TJ, Finkelstein RR (2001) Physical interactions between ABA response loci of *Arabidopsis*. *Plant J* 26: 627-635.

Ra’sheed S, Bashir K, Matsui A, Tanaka M, Seki M (2016) Transcriptomic analysis of soil-grown *Arabidopsis thaliana* roots and shoots in response to a drought stress. *Front Plant Sci* 7: 180.

Schaller GE (2000) Histidine kinases and the role of two-component systems in plants. *Adv Bot Res* 32: 109-148.

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

Skubacz A, Daszkowska-Golec A, Szarejko I (2016) The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Front Plant Sci* 7: 1884.

Thai Chi Hung et al.

Tran LSP, Shinozaki K, Yamaguchi-Shinozaki K (2010) Role of cytokinin responsive two-component system in ABA and osmotic stress signalings. *Plant Signal Behav* 5: 148-150.

Tran LSP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. *Proc Natl Acad Sci* 104: 20623-20628.

Wang N, Zhao S, Lv M, Xiang F, Li S (2016) Research progress on identification of QTLs and functional genes involved in salt tolerance in soybean. *Yi Chuan* 38: 992-1003.

Wohlbach DJ, Quirino BF, Sussman MR (2008) Analysis of the *Arabidopsis* histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. *Plant Cell* 20: 1101-1117.

Yamaguchi-Shinozaki K, Shinozaki K (1993a) *Arabidopsis* DNA encoding two desiccation-responsive rd29 genes. *Plant Physiol* 101: 1119.

Yamaguchi-Shinozaki K, Shinozaki K (1993b) Characterization of the expression of a desiccation-responsive rd29 gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. *Mol Genet Genet* 236: 331-340.

Yamaguchi-Shinozaki K, 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: 251-264.

Yang L, Liu Q, Liu Z, Yang H, Wang J, Li X, Yang Y (2016) *Arabidopsis* C3HC4-RING finger E3 ubiquitin ligase AtAIRP4 positively regulates stress-responsive abscisic acid signaling. *J Integr Plant Biol* 58: 67-80.

Zhou L, Wang NN, Gong SY, Lu R, Li Y, Li XB (2015) Overexpression of a cotton (*Gossypium hirsutum*) WRKY gene, GhWRKY34, in *Arabidopsis* enhances salt-tolerance of the transgenic plants. *Plant Physiol Biochem* 96: 311-320.

Thao NP, Thu NBA, Hoang XLT, Ha CV, Tran LSP (2013) Differential expression analysis of a subset of drought-responsive GmNAC genes in two soybean cultivars differing in drought tolerance. *Int J Mol Sci* 14: 23828-23841.

Thu NBA, Hoang XLT, Nguyen TDH, Thao NP, Tran LSP (2015) Differential expression of two-component system–related drought-responsive genes in two contrasting drought-tolerant soybean cultivars DTS1 and MTD720 under well-watered and drought conditions. *Plant Mol Biol Rep* 33: 1599-1610.