Comparative expression profiles of four salt-inducible genes from *Aeluropus littoralis*

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Abstract

Abiotic stresses such as salinity influence agricultural production. Plants generally respond to stimulus conditions in a complex manner involving many genes and proteins. In the evolution process, halophyte plant *Aeluropus littoralis* has been proven to have abiotic stress-tolerance capacity. *A. littoralis* is a salt-resistant halophyte providing a wealthy genetic resource for developing salinity tolerance in crop plants. In the present study, the expression of four candidate ESTs from *PKL*, *5PTase*, *NUC-L2* and *GLY I* genes, in *A. littoralis* shoot and root tissues exposed to multiple time points of 600 mM NaCl stress and recovery condition, were analyzed via quantitative Real-Time PCR. *Al5PTase* gene showed the highest significant up-regulation in shoot and root tissues. However, a significant down-regulation was found for *AlGLY I* gene in root tissues. Furthermore, we found the unique up-regulations for *AlPKL* and *AlNUC-L2* genes expression magnitudes in root tissues under recovery conditions. These results may provide useful information for further understanding of the role of *A. littoralis* genes and their regulatory pathways, revealing important genetic resources for crop improvement.

Key words: *Aeluropus littoralis*, Genes expressions profiling, Real-Time PCR, Stress-responsive mechanisms.

Abbreviations

PKL: PICKLE, 5PTase: Inositol polyphosphate 5-phosphatase, NUC-L2: Nucleolin-like2, GLY I: Glyoxalase I.

INTRODUCTION

Salinity is one of the severe environmental factors limiting the plant growth and production, in arid and semi-arid regions (Flowers and Colmer, 2008). Besides the enormous financial cost of production, there are other serious impacts of salinity on infrastructure, water supplies, social structure and stability of communities. The significant areas of cultivated lands are affected by salinity in more than one hundred countries. Thus soil salinity poses a serious threat to crop yield and food production in future. A wide range of plant species, including the majority of our most important crops, grow in moderately saline environments (Flowers and Colmer, 2008). High concentrations of salt ions result in a hyperosmotic shock and ionic imbalance (Flowers and Colmer, 2008; Moller and Tester, 2007). Also, these effects can lead to the secondary stresses such as nutritional imbalance and oxidative stress (Moller and Tester, 2007).

The plant responses to high concentrations of salt are complex and comprehensive; they include many different processes, which should be correctly coordinated. Salt tolerance appears to be due to a suite of genes and proteins, which contribute in salt and osmotic regulation in different parts of the plant cells in

order to maintain photosynthesis, reproduction and seed generation under saline conditions. Notwithstanding negative influence of salinity on plants, many plant species, so called halophyte, can survive under high salinity circumstances. According to Flowers and Colmer (2008) halophytes are characterized as the plants having potential capability of desalination and restoration of saline soils, thus can survive and reproduce in environments where the salt concentration exceeds 200 mM of NaCl (~ 20 dSm^{-1}). These species form nearly 1% of the world's flora (Barhoumi *et al*., 2007; Flowers and Colmer, 2008), which are completing their life cycle under highly saline situations. These plants encompass various protective mechanisms, which allow them to cope with unfavorable environments to achieve the continued survival and growth. Cellular responses to saline environments are well-studied (Barhoumi *et al*., 2007), but the complex systems are far from being completely understood yet.

Despite the application of halophyte plant *Aeluropus littoralis* as a valuable cash crop, there is only little information regarding the salt impacts on gene expression profiles and responsive mechanisms in this genus. This plant with a small genome (2n=14) is capable to endure salt (NaCl) stress (Modarresi *et al*., 2013) and can be a rich genetic source for gene manipulation, which makes it helpful for crop improvement. Therefore, investigation of the mRNA variation profiles and characterization of the momentous stress related genes may be potentially useful for identification of significant genetic resources for genetic engineering of crop species.

Plants employ different nuclear and cytoplasmic mechanisms such as signaling pathways, stress-related protein production and compatible solutes synthesis in dealing with the multiple stimuli. In the present study four genes comprising of PICKLE, inositol polyphosphate 5-phosphatase, nucleolin and glyoxalase I, involved in stress signals transduction, stress sensing, and carbohydrate metabolism and compatible solutes synthesis, were selected to investigate their expression levels in response to salinity (600 mM NaCl) and recovery after stress removing, in *Aeluropus* root and shoot tissues. These genes have extremely been proposed to play important roles in salt tolerance of multiple plant species, but no available studies described the responses of these genes under salinity stress in *A. littoralis* yet.

MATERIALS AND METHODS

Plant materials and stress treatments

The seeds of *A. littoralis* were collected from Isfahan province in Iran and cultured in the greenhouse. A same aged clones selected and sterilized in 0.2% (w/v) sodium hypochlorite for 1 min (Murashige and Skoog, 1962) then were transferred to 1/2 MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.7% agar and growing at controlled conditions: 25–30 °C temperature, 70–80% relative humidity and 8/16 h night-day photoperiod. After four weeks, seedlings were moved to half and then full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) for further growth. The nutrient solution was exchanged once a week. Two-month-old seedlings were subjected to 600 mM NaCl treatment. NaCl concentration in the culture medium was increased by 100 mM day–1 to avoid osmotic shock. Salt-treated plants were harvested after 6 h, 24 h and 1 week time courses, as well as 6 h, 24 h and 1 week after stress removing (Stępiński, 2012). Non-treated seedlings were also considered as the control sample. Collected root and leaf samples were frozen immediately in liquid nitrogen, and stored at -80 °C until use.

RNA isolation and cDNA synthesis

Total RNA was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The purified total RNA was qualified and quantified by agarose gel electrophoresis and Nano-Drop ND 1000 Spectrophotometer (Wilmington, USA), respectively. DNase I treatment was performed, to eliminate genomic DNA contamination, using ThermoFisher (ThermoFisher Scientific) DNase Kit, as the producer's instructions. DNase I-treated RNA was used for first strand cDNA synthesis using the QuantiTect reverse transcription kit (Qiagen, Germany) and oligo (dT) primers in a 20 µL final volume, according to the manufacturer's instructions. RT-PCR was performed using 3 µL RNA, 1 µL of specific primers, 10 µM dNTPs, 5×PCR-buffer and 1 µL RiboLock RNase Inhibitor. The no template control (NTC) was used as a control reaction that contained all essential components of the amplification reaction except the template, to detect the contamination due to contaminated reagents or foreign DNA. Finally, the PCR reaction was performed and the products were separated on 1% agarose gel to check the true amplification of the gene fragments. The first strand cDNA mixtures were utilized as the templates for Real-Time PCR analysis.

Primer designing and quantitative Real-Time PCR analysis

The EST sequences of the *A. littoralis AlPKL* (JK671232), *Al5PTase* (JK671224), *AlNUC-L2* (JZ191093) and *AlGLY I* (JZ191094) genes were

Table 1. Specific primers (Forward and Reverse) used for gene expression by the qReal-Time PCR. β-actin and *Ef1a* were used as two housekeeping genes.

EST accession number	Primer name (5' → 3')	Sequence	Length of amplicon (bp)	Tm (°C)
JK671232	*PKL*-F *PKL*-R	AGGGGTATGCTGAACTTGT CACCTTCGCCTCAATCAA	146	62
JK671224	*5PTase*-F *5PTase*-R	GGCCAGACATTTCAGACCACA AGCCCTGATGACCGTGTTTC	99	62
JZ191093	*NUC-L2*-F *NUC-L2*-R	AAGTCCAGTGTTGCGGTTGC CCGCATTTCTCTTCCCCTTC	79	63
JZ191094	*GLY I*-F *GLY I*-R	GTGGCATGGACTTGCTACGG CCGTGGCATCACAGAGGATT	72	64
AB181991	*β*-actin-F *β*-actin-R	TGCTGGCCGAGACCTTAC GGCGAGCTTTTCCTTGATG	113	59
M90077	*Ef1a*-F *Ef1a*-R	ACCTTCTCTGAATACCCTCCTCTG CTTCTCCACACTCTTGATGACTCC	90	65

selected and retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) database. Specific primers were designed using Oligo software (Rychlik, 2007) for the internal controls and the candidate genes, based on *A. littoralis* EST sequences (Table 1). Two housekeeping genes: *β*-actin and *elongation factor 1-a* (*Ef1a*) were used as the internal controls (because their expressions had not been influenced by stresses) for data normalization in leaf and root samples, respectively.

Quantitative Real-Time PCR (qPCR) was performed in a 20 µL volume containing: 1 µL of cDNA (50 ng), 5 µL of 2×SYBR Green Master Mix, 0.3 µL of each 10 µM primers and 3.4 µL of RNase-free water. The amplification reactions were carried out in a two-step thermal cycler protocol (Thermo Scientific), according to the company's procedures as follows: 10 min initial activation step at 95 °C followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. After 40 cycles, the specificity of the amplifications was checked based on the melting curves by heating the amplicons from 55 to 95 °C. All amplification reactions were repeated twice under identical conditions in addition to a negative control and five standard samples. To ensure that the PCR products were generated from cDNA and not from genomic DNA, proper control reactions were carried out without reverse transcriptase. For quantitative Real-Time PCR data, relative expressions for *AlPKL*, *Al5PTase*, *AlNUC-L2* and *AlGLY I* genes were calculated based on the threshold cycle (CT) method. The CT for each sample was calculated using the CFX96 manager software (Bio-Rad) and Larionov *et al*. (2005) method. Accordingly, fold expression of the target mRNAs over the reference values were calculated by the $2^{-\Delta\Delta CT}$ equation (Livak and Schmittgen, 2001).

To increase the reliability of the gene expression analysis, Real-Time PCR experiments were performed with two identical technical replications. Data analyses were carried out using SPSS version 18 and Microsoft Excel program software.

RESULTS AND DISCUSSION

Amplification specificity for the candidate genes

The melting curve analysis demonstrated that all the curves were similar and showed a single peak, suggesting specific amplification (Figure 1). The detailed transcription rates of the candidate genes dealing with stress were as follows:

The *AlPKL* mRNA expression level

The *AlPKL* gene expression level at 600 mM salt concentration is shown in Figure 2. After salinity exposure for 6 h, the significant up-regulations of *PKL* expression was observed in *A. littoralis* shoot tissue (3-fold) compared to that of the control. Although approximately a 2-fold increment was observed in the recovered shoot samples (at 24 h after recovery), there was a highly substantial reduction in the gene expression one week after removing salinity compared with the control. Meanwhile, the *AlPKL* mRNA expression increased 2-fold compared to the control conditions after exposure to salinity for 6 h in root sample. However, one week after salinity treatment, a significant decrease was observed. Whereas, *AlPKL* showed remarkably significant increments in transcript

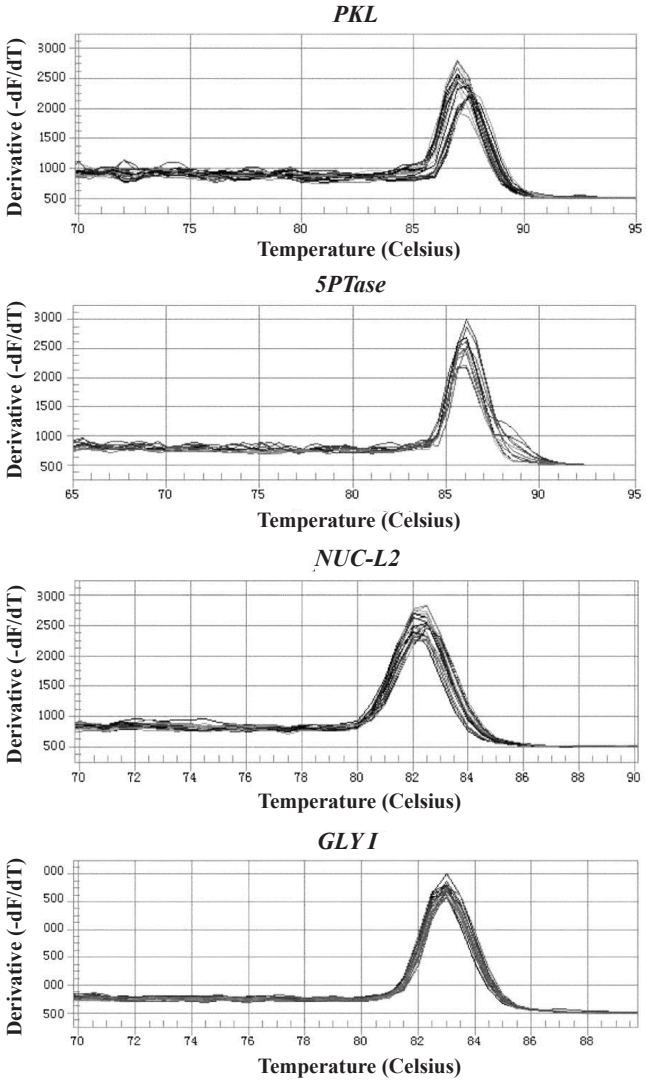

Figure 1. The melting curve analysis results obtained from the *AlPKL*, *Al5PTase*, *AlNUC-L2* and *AlGLY I* genes amplifications under 600 Mm NaCl stress by the Real-Time PCR technique. All four meltingcurves revealed almost the same and single temperature peak, showing the absence of the non-specific amplifications.

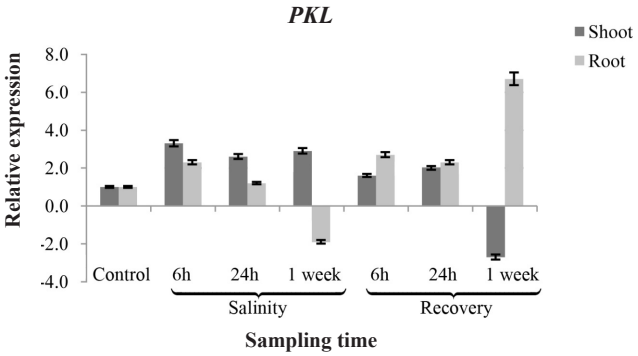

Figure 2. Relative expression levels of *AlPKL* gene after exposure to 600 mM NaCl stress and recovery conditions in *A. littoralis* shoot and root tissues. X axis shows the elapsed time from salt treatments and Y axis displays relative expression calculated via $2^{-\Delta\Delta ct}$ formula according to the *β*-actin and *Ef1a* genes as the internal reaction control in shoot and root tissues, respectively.

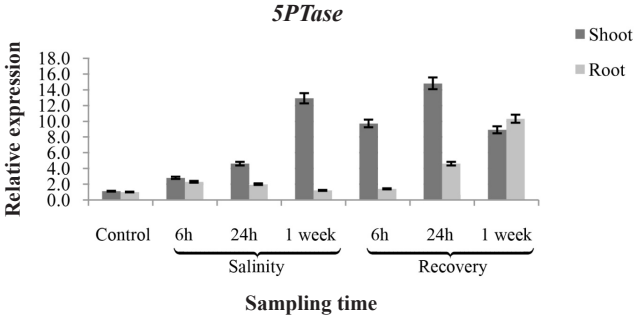

Figure 3. Relative expression levels of *Al5PTase* gene after exposure to 600 mM NaCl stress and recovery conditions in *A. littoralis* shoot and root tissues. X axis shows the elapsed time from salt treatments and Y axis displays relative expression calculated via $2^{-\Delta\Delta ct}$ formula according to the *β*-actin and *Ef1a* genes as the internal reaction control in shoot and root tissues, respectively.

expression 6 h and one week after recovery with approximately 3- and 7-fold, respectively, compared to that of the control.

Aichinger *et al*. (2009) reported that *PKL* mediated activation of *PcG* genes seem to be restricted to primary roots and has not been observed in aerial parts of the seedlings. *PICKLE* genes are the evolutionary conserved chromatin factors, which define cell identity by regulating the expression of key developmental genes (Schuettengruber *et al*., 2007). The *PKL* genes in *Arabidopsis* and rice genomes were identified to play critical roles in the regulation of a series of genes involved in developmental or stress response processes (Hu *et al*., 2014). It was reported that the *AtPKL* in *Arabidopsis* is a necessary factor for the regulation of *ABSCISIC ACID–INSENSITIVE3* (*ABI3*) and *ABI5* genes in response to ABA (Perruc *et al*., 2007). This may be implicating the possible involvement of the PKL protein in osmotic stress response. Also, the microarray data sets from the rice genome revealed that the expression of *CHR729* (a rice *PKL* gene) was resulted in the up-regulations of many stress-responsive genes (Hu *et al*., 2014), suggesting the possible role of .this protein in stress responses

Transcription level of the *Al5PTase* gene

The variation profile of the *Al5PTase* mRNA expression in 600 mM NaCl treatment is shown in Figure 3. The significant up-regulations (an up-ward

trend) were observed in the *Al5PTase* gene expression in response to both salinity and recovery situations in both shoot and root samples. The maximum expression level of the gene was found one week after exposure to salt stress in shoots with approximately 13-fold compared with the control sample. The recovered shoot tissues exhibited significant increases regarding time duration, so that an increment of about 15-fold was detected 24 h after recovery, compared with that of the control. Transcription level of this gene also revealed approximately a 2-fold increase at the beginning of salinity stress in root samples, while an increment of 10-fold was observed at the last sampling time (one week after recovery), comparing to the control condition.

Regarding the *5PTase* gene expression pattern under salt stress, a deficiency of 5PTase protein can lead to a disruption in phosphatidylinositol pathway (Xue *et al*., 2009), which may result in many metabolic disorders in stressed plants. According to DeWald *et al*. (2001) the *Arabidopsis* plants revealed the substantial increases in 5PTase protein synthesis in response to treatments with NaCl, KCl and sorbitol, suggesting an important role of phosphatidylinositol pathway in plant salt tolerance. Functional analysis of the four *5PTases* from *Saccharomyces cerevisiae* showed that although they are not essential for viability under normal conditions, they have an important role in osmotic stress tolerance (Ooms *et al*., 2000). In fact, *5PTase* gene regulates the plant NADPH oxidase activity (Munnik and Vermeer, 2010), leading to an increase in ROS (reactive oxygen species) generation and finally overexpression of the other stress responsive genes. Therefore, it can be mentioned that the 5PTase acts as the signaling molecule under stress conditions. Kaye *et al*. (2011) reported that, the *At5ptase* mutants in *A. thaliana* failed to induce the *RbohJ* gene, which is responsible for ROS production during salt stress; this resulted in the reduced induction of the other salt-responsive genes such as *RD29* and *RD22*. Recent studies revealed that mutation in *5PTase* genes led to an increased salt sensitivity in *A. thaliana* and *O. sativa* (Kaye *et al*., 2011), revealing the importance of *5PTases* for stress coping in *A. littoralis* and the other important plants.

The *AlNUC-L2* stress response pattern

The *AlNUC-L2* expression pattern in response to NaCl and recovery conditions is shown in Figure 4. A significant increase of about 4-fold was detected in transcription level of *AlNUC-L2* gene in *A. littoralis* shoot tissue, after 6 h of exposure to NaCl, compared with that of the control. This level dropped to 3.5-fold with recovery after stress removing in comparison

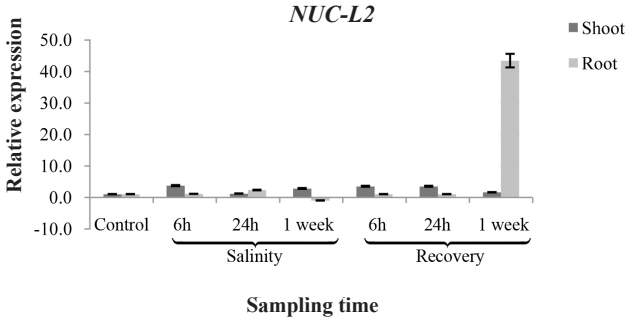

Figure 4. Relative expression levels of *AlNUC-L2* gene after exposure to 600 mM NaCl stress and recovery conditions in *A. littoralis* shoot and root tissues. X axis shows the elapsed time from salt treatments and Y axis displays relative expression calculated via $2^{-\Delta\Delta ct}$ formula according to the *β*-actin and *Ef1a* genes as the internal reaction control in shoot and root tissues, respectively.

to the control. Transcription level of *AlNUC-L2* in root samples followed the same pattern as *AlPKL*; it means that the expression level of this gene reached almost to the control level with the onset of salinity. 24 hours after stress a 2-fold increase was observed in root compared to the control, but a substantial down-regulation of *AlNUC-L2* was detected one week after NaCl exposure. An interesting enhancement of the *AlNUC-L2* gene expression occurred one week after stress removing, which transcription level of this gene increased to approximately 43-fold compared to the control condition.

On the basis of the obtained results, it can be supposed that nucleolin level was correlated with transcriptional activities of the nucleoli of active cells in *A. littoralis* tissues. The same results also reported by Stępiński (2012) regarding the root tissue of soybean plants subjected to chilling stress (10 °C) and recovery. This study showed that the transcriptional activity of the soybean nucleolin-like 2 gene under chilling stress was considerably reduced in root samples, while it was significantly higher during recovery after chilling, in comparison to the control. Furthermore, there are numerous reports for increased expression level of *NUC-L2* under abiotic stress conditions in various plant species. Sripinyowanich *et al*. (2013) reported that *OsNUC-L1* and *OsNUC-L2* play important roles in salt resistance during salinity stress in rice plants. Accordingly, *OsNUC-L* genes were shown to be highly expressed in rice leaf blades during salinity (Sripinyowanich *et al*., 2013), indicating a potential involvement of nucleolin protein in salt stress responses. Pontvianne *et al*. (2007) suggested that *NUC-L2* is required for coping with multiple stresses

in *A. thaliana*. Many studies showed that simultaneous disruption of *NUC-L1* and *NUC-L2* is lethal to plants, while *NUC-L2* can fulfill some essential functions of *NUC-L1* under stress situations (Pontvianne *et al*., 2007; Durut *et al*., 2014).

The variation profile of *AlGLY I* gene

The changing profile of *AlGLY I* gene under 600 mM NaCl concentration is shown in Figure 5. The higher expression level of *AlGLY I* gene (6-fold) was observed in *A. littoralis* shoot tissues exposed to 6 h salinity, compared with that of the control. But no substantial increases occurred in the expression level of *AlGLY I* transcripts among the shoots recovered groups at the fifth to seventh sampling-times. Significant lower expression profiles of *AlGLY I* gene were detected in *A. littoralis* root tissues after exposure to both salinity and recovery circumstances, which were not considerable compared with the control.

This likely represents a minimal potential of *AlGLY I* gene in *Aeluropus* root tissues to deal with salt stress. These mean that glyoxalase I activity in *A. littoralis* root tissue, unlike the *A. thaliana* and *O. sativa* orthologous genes (Mustafiz *et al*., 2014), probably cannot be the main factor in salinity tolerance. Since the responsiveness of this gene was very substantial in leaf samples, glyoxalase pathway in *A. littoralis* aerial parts may play a key role for stress tolerance (Saad *et al*., 2010). Mustafiz *et al*. (2014) revealed that *OsGLY I* gene in salt resistant Pokkali cultivar is considered as one of the most responsible genes for salinity and heavy metal stresses. Also, the transgenic tobacco plants over expressing *GLY I* gene in leaves exhibited high resistance to salinity and metal stresses (Veena *et al*., 1999). Moreover, it was proved that the enzyme activity and glyoxalase I transcription can be enhanced by some abiotic and biotic stresses such as NaCl, mannitol, osmotic, Zinc, Methylglyoxal (MG), *Aspergillus flavus*, etc (Lin *et al*., 2010; Mustafiz *et al*., 2014).

Therefore, plants reveal various responses to the biotic and abiotic stresses including salinity. Changes in gene expression play a central role in the plant stress responses (Kawasaki *et al*., 2001), so understanding the potential salinity tolerance of cereal related families may help the breeders to develop new salt tolerant varieties. The Real-Time PCR results suggested that *AlPKL*, *Al5PTase*, *AlNUC-L2* and *AlGLY I* genes might play defense functions during NaCl exposure, particularly in the aerial parts of *A. littoralis*. Among the studied genes, *Al5PTase* revealed the best expression pattern in response to both salt and recovery conditions in shoot and root samples. It revealed that the *Al5PTase* coordinates plant responses to multiple stresses by modulating the interactions in the cellular protein-lipid network (Xue *et al*., 2009). In addition, *5PTase* gene can be potentially suitable as a biomarker for indication of different stresses. Recovery condition can also improve salt tolerance of *A. littoralis* probably by restoration of the other responsive genes (Stępiński, 2012). These findings indicated that the candidate genes have distinct expression patterns, which are related to their varied roles in response to stimuli.

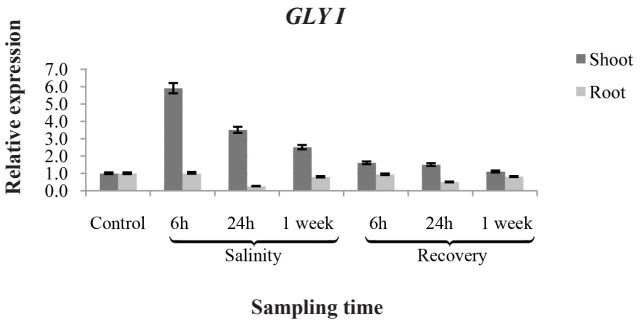

Figure 5. Relative expression levels of *AlGLY I* gene after exposure to 600 mM NaCl stress and recovery conditions in *A. littoralis* shoot and root tissues. X axis shows the elapsed time from salt treatments and Y axis displays relative expression calculated via $2^{-\Delta\Delta ct}$ formula according to the *β*-actin and *Ef1a* genes as the internal reaction control in shoot and root tissues, respectively.

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REFERENCES

Aichinger E., Villar C. B., Farrona S., Reyes J. C., Hennig L., and Köhler C. (2009). CHD3 proteins and polycomb group proteins antagonistically determine cell identity in *Arabidopsis*. *PLoS Genetics*, 5: e1000605.

Barhoumi Z., Djebali W., Chaïbi W., Abdelly C., and Smaoui A. (2007). Salt impact on photosynthesis and leaf ultrastructure of *Aeluropus littoralis*. *Journal of Plant Research*, 120: 529-537.

DeWald D. B., Torabinejad J., Jones C. A., Shope J. C., Cangelosi A. R., Thompson J. E., Prestwich G. D., and Hama H. (2001). Rapid accumulation of phosphatidylinositol 4,5 bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt stressed *Arabidopsis*. *Plant Physiology*, 126: 759-769.

Dlakić M. (2000). Functionally unrelated signalling proteins contain a fold similar to Mg^{2+}-dependent endonucleases. *Trends in Biochemical Sciences*, 25: 272-273.

Dreyfuss G., Swanson M. S., and Piñol-Roma S. (1988). Heterogeneous nuclear ribonucleoprotein particles and the pathway of mRNA formation. *Trends in Biochemical Sciences*, 13: 86-91.

Durut N., Abou-Ellail M., Pontvianne F., Das S., Kojima H., Ukai S., deBures A., Comella P., Nidelet S., Rialle S., Merret R., Echeverria M., Bouvet P., Nakamura K., and Sáez-Vásquez J. A. (2014). Duplicated nucleolin gene with antagonistic activity is required for chromatin organization of silent 45S rDNA in *Arabidopsis*. *Plant Cell*, 26: 1330-1344.

Eisen J. A., Sweder K. S., and Hanawalt P. C. (1995). Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Research*, 25: 2715-2723.

Flowers T. J., and Colmer T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 179: 945-963.

Furuta K., Kubo M., Sano K., Demura T., Fukuda H., Liu Y. G., Shibata D., and Kakimoto T. (2011). The CKH2/PKL chromatin remodeling factor negatively regulates cytokinin responses in *Arabidopsis* Calli. *Plant and Cell Physiology*, 52: 618-628.

Hoagland D. R., and Arnon D. I. (1950). The water-culture method for growing plants without soil. *Circular, California Agricultural Experiment Station*, California, 1-32.

Hu Y., Lai Y., and Zhu D. (2014). Transcription regulation by CHD proteins to control plant development. *Frontiers in Plant Science*, 5: 223.

Jacobs S. A., and Khorasanizadeh S. (2002). Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science*, 295: 2080-2083.

Jefferson A. B., Auethavekiat V., Pot D. A., Williams L. T., and Majerus P. W. (1997). Signaling inositol polyphosphate-5-phosphatase: characterization of activity and effect of GRB2 association. *Journal of Biological Chemistry*, 272: 5983-5988.

Kawasaki S., Borchert C., and Deyholos M. (2001). Gene expression profiles during the initial phase of salt stress in rice. *The Plant Cell*, 13: 889-905.

Kaye Y., Golani Y., Singer Y., Leshem Y., Cohen G., Ercetin M., Gillaspy G., and Levine A. (2011). Inositol polyphosphate 5-phosphatase7 regulates the production of reactive oxygen species and salt tolerance in *Arabidopsis*. *Plant Physiology*, 157: 229-241.

Larionov A., Krause A., and Miller W. (2005). A standard curve based method for relative Real-Time PCR data processing. *BMC Bioinformatics*, 6: 62.

Lin F., Xu J., Shi J., Li H., and Li B. (2010). Molecular cloning and characterization of a novel glyoxalase I gene *TaGLY I* in wheat (*Triticumaestivum L.*). *Molecular Biology Reports*, 37: 729-735.

Livak K. J., and Schmittgen T. D. (2001). Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402-408.

Modarresi M., Nematzadeh G. A., and Moradian F. (2013). Salinity response pattern and isolation of catalase gene from halophyte plant *Aeluropus littoralis*. *Photosynthetica*, 51: 621-629.

Moller I. S., and Tester M. (2007). Salinity tolerance of *Arabidopsis*: a good model for cereals. *Trends in Plant Science*, 12: 534-540.

Munnik T., and Vermeer J. E. (2010). Osmotic stress-induced phosphoinositide and inositol phosphate signalling in plants. Plant, Cell and Environment, 33: 655-669.

Murashige T., and Skoog F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.

Mustafiz A., Ghosh A., Tripathi A. K., Kaur C., Ganguly A. K., Bhavesh N. S., Tripathi J. K., Pareek A., Sopory S. K., and Singla-Pareek S. L. (2014). A unique Ni^{2+}-dependent and methylglyoxal-inducible rice glyoxalase I possesses a single active site and functions in abiotic stress response. *Plant Journal*, 78: 951-963.

Ooms L. M., McColl B. K., Wiradjaja F., Wijayaratnam A. P., Gleeson P., Gething M. J., Sambrook J., and Mitchell C. A. (2000). The yeast inositol polyphosphate 5-phosphatases inp52p and inp53p translocate to actin patches following hyperosmotic stress: mechanism for regulating phosphatidylinositol 4,5-bisphosphate at plasma membrane invaginations. *Molecular and Cellular Biology,* 20: 9376-9390.

Perruc E., Kinoshita N., and Lopez-Molina L. (2007). The role of chromatin remodeling factor PKL inbalancing osmotic stress responses during *Arabidopsis* seed germination. *Plant Journal*, 52: 927-936.

Pontvianne F., Matía I., Douet J., Tourmente S., Medina F. J., Echeverria M., and Sáez-Vásquez J. (2007). Characterization of *AtNUC-L1* reveals a central role of nucleolin in nucleolus organization and silencing of *AtNUC-L2* gene in *Arabidopsis*. *Molecular Biology of the Cell*, 18: 369-379.

Rychlik W. (2007). OLIGO 7 primer analysis software, In: Yuryev A. (eds) PCR Primer Design. *Methods in Molecular Biology*, 402: 35-59.

Saad R. B., Zouari N., Ramdhan W. B., Azaza J., Meynard D., Guiderdoni E., and Hassairi A. (2010). Improved drought and salt stress tolerance in transgenic tobacco overexpressing a novel A20/AN1 zinc-finger "*AlSAP*" gene isolated from the halophyte grass *Aeluropus littoralis*. *Plant Molecular Biology*, 72: 171-190.

Schuettengruber B., Chourrout D., Vervoort M., Leblanc B. and Cavalli G. (2007). Genome regulation by polycomb and trithorax proteins. *Cell*, 128: 735-745.

Sripinyowanicha S., Chamnanmanoontham N., Udomchalothorna T., Maneeprasopsuk S., Santawee P., Buaboocha T., Que L. J., Gue H., and Chadchawan S. (2013). Overexpression of a partial fragment of the salt-responsive gene *OsNUC1* enhances salt adaptation in transgenic *Arabidopsis thaliana* and rice (*Oryza sativa L.*) during salt stress. *Plant Science*, 213: 67-78.

Stępiński D. (2012). Nucleolin level in plant root meristematic cells under chilling stress and recovery. *Micron*, 43: 870-875.

Veena V. S., Reddy S. K., and Sopory S. K. (1999). Glyoxalase I from *Brassica juncea*: molecular cloning, regulation and its over-expression confer tolerance in transgenic tobacco under stress. *Plant Journal*, 17: 385-395.

Verdel A., Jia S., Gerber S., Sugiyama T., GygiS., Grewal S. I., and Moazed D. (2004). RNAi-mediated targeting of heterochromatin by the RITS complex. *Science*, 303: 672-676.

Xue H. W., Chen X., and Mei Y. (2009). Function and regulation of phospholipid signaling in plants. *Biochemical Journal*, 421: 145-156.