J Plant Mol Breed (2017) 5(1): 19 - 30
DOI: 10.22058/JPMB.2017.68421.1141

RESEARCH ARTICLE

Expression pattern analysis of transcription factors from *Aeluropus littoralis* in response to salt stress and recovery condition

Hamidreza Ghorbani[1], Habibollah Samizadeh Lahiji[2], Ghorban-Ali Nematzadeh[3*]

[1]Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran
[2]Department of Plant Biotechnology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran
[3]Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari agricultural sciences and natural resources university, Sari, Iran

ABSTRACT: Salinity is one of the most important abiotic stresses that decrease crop production. Halophyte plant can tolerate high sodium chloride (NaCl) concentration and transcription factors (TFs) are prominent regulators in plant response to stress. In this study, the expression pattern of four salt-induced genes encoding transcription factors in *Aeluropus littoralis*, namely, *MYB*, *RF2*, *GTF,* and *ARID* was studied. Plants were exposed to salt stress (600 mM) and recovery condition and samples were collected at three timepoints (6, 12 and 24 hours). The results of quantitative real-time PCR (qPCR) showed that the effect of NaCl was statistically significant on genes expression. The expression level was increased after 6 hours in all genes and after that, it drastically decreased with promoting of stress duration in both tissues. The highest expression was observed for *MYB* gene in root (68.44) that was higher than shoot (38.57) after 6 hours. At the recovery condition, the genes expression was gradually decreased in different tissues and finally got a stable value. The result showed that the studied TFs play an important role in tolerance of *A. littoralis* to salinity and could be used as an informative resource in the breeding programs. Also, the response of *A. littoralis* to salt stress depends on the tissue type and plant exposure duration.

KEYWORDS: Abiotic stress, Halophyte, Recovery condition, Reference genes, Transcription factor.

INTRODUCTION

Salt stress is one of the most important problems in agriculture [1]. Approximately 25% of the world's total area (including 15% of Iran's area) has this problem [2]. Both grain yield and quality are downgraded by high salinity. Thus, improving level of abiotic stress tolerance has become a key objective in many crop breeding programs. Genetic variation for stress tolerance is rather limited in the domesticated gene pool, but a number of wild plant of Poaceae family have been proved to process notable high-level tolerance *Aeluropus littoralis*, a halophyte plant, belongs to Poaceae that can tolerate NaCl up to 600 mM [3] and grows in marshes, salty and drought lands. C4 photosynthesis system in *A. littoralis* is one of the reasons for being more flexible against salt and drought stresses. Therefore, *A. littoralis* may know as a great source of genes related to salt tolerance [4, 5]. Nowadays, stress responses understanding is one of the most important issues in plant research. Plants response

*Corresponding author (✉): gh.nematzadeh@sanru.ac.ir
Received: 19 July 2017/ Revised: 26 August 2017
Accepted: 11 September 2017

to abiotic stress with the change in many functional and structural mechanisms by altered metabolic pathways. Stress tolerance in plants can be enhanced via both ABA-dependent and ABA-independent pathways [6]. Various plant transcription factors (TFs) are up- or down-regulated in response to abiotic stress [7], and the response is typically quite rapid [8, 9]. TFs generally act as key regulators of gene expression and about 7% of the genes encoding the TFs in plants are specified to stress or defense responses. [10]. These TFs involve in responses to stress by encoding proteins with specific functions [11] and some of theme which plays the important roles in signaling and regulatory pathway could be used in genetic engineering [12]. Recently, the roles of some TF families in transgenic plants such as Arabidopsis, rice, maize and tobacco in abiotic stress have been reported in some literatures [13-21]. Most MYB proteins have domain repeats that able to bind DNA. In plants, most of the MYB proteins are involved in the responses to regulation mechanisms, such as abiotic and biotic stress [22], hormone signaling [23] and regulation of differentiation [24]. AtMYB60 and AtMYB96 are implicated in both biotic and abiotic stresses [25, 26]. AtMYB2 controls the ABA induction of salt and dehydration responsive genes [23]. *RF2a* is a *bZIP* transcription factor and control the promoter activity of rice in biotic stress. Studies in rice indicated that *RF2a* found in vascular tissues, leaves and stems cells, but in roots, the level of *RF2a* is very low [27]. bZIP proteins have been identified in all eukaryotes, including yeasts, vertebrates, and plants [28]. A family of transcription factors with basic region and Leu-zipper motif that defined by the conserved bZIP domain [29]. ABA activates bZIP proteins to bind to ABRE and initiate transcription of ABA-inducible genes [30] and modulates the signaling networks for a number of hormones and regulate the biosynthesis of starches, storage proteins and lipids in response to biotic and abiotic stresses [31]. The cells that respond to environmental changes typically induce the expression of different genes.

According to the crucial role of TFs in response to environmental stress and regulation of gene expression and the importance of *A. littoralis* as a salt tolerant wild member of Poaceae, this study was carried out to assess the expression pattern of four salt-induced genes encoding transcription factors. Moreover, the effect of different plant tissues (root and shoot) on gene expression was investigated.

MATERIALS AND METHODS

Plant material

A. littoralis seeds were collected from Isfahan province and the sterilized seeds plated on full strength MS medium (pH 5.8). After germination, two weeks' seedlings transferred to pots which containing Hoagland's solution [32]. The 30 day-old seedlings were exposed to 500 mM of sodium chloride at five passages (received 100 mM sodium chloride per 48 hours (h) up to 500 mM) [33]. Leaves and roots were sampled in parallel. At the end of the fifth passage, samples were collected at 6, 12 and 24 h time-course. In order to plant recovery, the remained plants were transferred to a sodium chloride-free Hoagland's solution, and then were collected after 6, 12 and 24 h. All samples and control plant were immediately frozen in liquid nitrogen and stored at –70 ℃.

RNA extraction, cDNA synthesis, and qPCR

Shoot and root tissues were grounded in liquid nitrogen and total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol and treated with RNase-free *Dnase*I (Promega). The RNA quality and quantity were controlled using agarose gel electrophoresis and spectrophotometry, respectively. First strand cDNA synthesis and qPCR were performed according to Fermentas kit instructions. The cDNA used as a template for subsequent PCR. The *ACTIN* gene was used as internal reference gene and to calculate relative expression levels of specific genes. The studied genes were selected based on the search on NCBI databases (https://www.ncbi.nlm.nih.gov/) of *A. littoralis* and then they isolated from the extracted cDNAs through the specific primers designed by Primer3 and Oligo Analyzer 1.2 soft wares [34].

The quantitative PCR was performed in a 10-µl reaction containing SYBR Green I PCR Master Mix (Thermo Scientific, USA), 10 pmol each of forward and reverse gene-specific primers and 2 µl of diluted cDNA (1:9). PCR amplification was performed in C1000™ Thermal Cycler (Bio-Rad, USA) according to the company's suggestions. The qPCR program was as follows: 95℃ for 5 min, 15 S of denaturation, 30 S of annealing (depending on primer used) (Table 1) and 15 S of extension at 72℃ for 40 cycles and a final extension at 72℃ for 7 min(s). Melt curve analysis was performed to check the specificity of the amplified product. To minimize sample

Table 1. Sequences of primers used for real-time PCR amplification

Gene	Description	Primer Sequence	Accession Number	Amplicon Size	Tm
ACT11	Actin-11	5'- GTATGGCAACATTGTCCTCAG -3'	EE594539.1	118	59.4
		5'- TGGAGCAACGACCTTGAT -3'			58.5
ARID	AT Rich Interactive Domain	5´-TCGCCATGCTTTGATGTCTG-3	JK671186	90	58.7
		5´-ATAGCCGGAAGGATGCATGA-3´			58.4
MYB	Myeloblastosis	5'-GGAGGCGTACATGGAGGTG-3'	EF534705	166	60.1
		5'-CGCCACCTTCTTCCAGTCC-3'			59.8
RF2	Transcription Factor RF2a-like	5'-GACATCACACCCACAGAACG-3'	EE594580	184	58.85
		5'-TCACCTAGACACTGCGCTAG-3'			58.95
GTF	General Transcription Factor 3C Polypeptide	5'-TTCCAAGTGGCCATCAGGTT-3'	JZ191082.1	108	60
		5'-AAAGGGCTTCCTGCCTCTTG-3'			60.5

variation, the samples were normalized using actin expression mRNA expression. To reveal the absence of contamination or primer-dimers, a non-template control (NTC) reaction with each primer pair was run. The $2^{-\Delta\Delta CT}$ method was used for the quantitative analysis [35]. Data were analyzed by Microsoft Excel 2010. Following analysis of variance (ANOVA), Duncan's multiple range tests was used to assess the significance of differences between mean values.

RESULTS

Salinity is an abiotic stress that causes various detrimental effects on plant growth and development [36]. The expression of 4 salt-induced genes encoding transcription factors, namely, *MYB, RF2*, *GTF* and *ARID* were screened in this study using qPCR method. The results displayed different expression patterns in *A. littoralis* under salt stress in different tissues and the significant effect of salinity on genes expression.

Expression pattern of *MYB* gene under salt stress

The result of expression analysis showed that the *MYB* gene expression was induced significantly by salt treatment in 6 h after stress but drastically decreased with promoting of stress duration in both root and shoot tissues, which reached to the lowest level of expression after 24 h after salt stress treatment (Fig 1). This expression in roots was higher than shoots. The Arabidopsis *MYB* transcription factor, *AtMYB44*, behaves in a very similar manner [37]. In the case of recovery, the *MYB* genes expression increased to 2.55, 5.2 and 5.92 folds in the shoot and 2.03, 7.97 and 3.66 folds in root respectively in comparison to control plants in a non-significant manner while the expression decreased significantly in both of tissues in comparison to stress condition (table 2 and table 3).

Expression pattern of *RF2* gene under salt stress

Comparison of *RF2* expression patterns in the different times showed significant differences in gene expression rates. The increasing in *RF2* transcript levels was observed in shoots and roots of salt treated plants (Figure 2). After 6 h salt stress, the expression levels in shoots was in maximum (5.16-fold higher than control) and then decreased in the next times of exposure to stress treatment (table 2). In roots, the expression levels increased significantly until 12 h (2.04-fold higher than control plants) and then decreased in 24 h after salt stress (table 3). The result showed that the mRNA abundance of *RF2* in shoots was higher than roots in all treatments. At the recovery stage (return of plants to non-stress condition), the expression of *RF2* gene was gradually decreased compared to treated plants until reached to lowest value expression in both root and shoot samples in 24 h after recovery. This expression difference was significant statistically in shoots after 6 and 12 h recovery than control (table 2) but not significant with expression after 24 h recovery.

Expression pattern of *GTF* gene under salt stress

Sequence analysis indicated that JZ191082.1 EST refers to general transcription factor 3C polypeptide 5. As shown in Fig 3, the *GTF* expression in treated plant was induced by treatment with 500 mM NaCl. In the first 6 h,

Table 2. The means comparison of genes expression in shoot tissue

	cntr	6 h	12 h	24 h	6 h rec	12 h rec	24 h rec
MYB	1.0 ^{c}	38.57 ^{a}	9.94 ^{b}	5.73 ^{bc}	2.55 ^{c}	5.20 ^{bc}	5.92 ^{bc}
RF2	1.0 ^{c}	5.16 ^{a}	4.53 ^{a}	2.87 ^{b}	2.59 ^{b}	2.32 ^{b}	2.11 ^{bc}
GTF	1.0 ^{b}	3.85 ^{a}	2.14 ^{b}	1.76 ^{b}	1.83 ^{b}	1.23 ^{b}	1.38 ^{b}
ARID	1.0 ^{d}	5.93 ^{a}	4.91 ^{ab}	5.17 ^{a}	2.07 ^{cd}	3.04 ^{bc}	2.92 ^{c}

Control (Cntr), stress after 6 h, 12 h, 24 h, stress after 6 h recovery, 12 h recovery and 24 h recovery, respectively. Means with the same letters are not significantly different. ($P > 0.05$).

Table 3. The means comparison of genes expression in root tissue

	cntr	6 h	12 h	24 h	6 h rec	12 h rec	24 h rec
MYB	1.0 ^{c}	68.44 ^{a}	18.58 ^{b}	3.79 ^{c}	2.03 ^{c}	7.97 ^{bc}	3.66 ^{c}
RF2	1.0 ^{bc}	1.45 ^{b}	2.04 ^{a}	1.16 ^{bc}	0.85 ^{c}	1.07 ^{bc}	0.87 ^{c}
GTF	1.0 ^{a}	0.98 ^{a}	0.58 ^{a}	0.82 ^{a}	0.89 ^{a}	1.18 ^{a}	1.18 ^{a}
ARID	1.0 ^{b}	0.83 ^{b}	0.26 ^{b}	0.56 ^{b}	2.49 ^{a}	1.24 ^{b}	2.66 ^{a}

Control (Cntr), stress after 6 h, 12 h, 24 h, stress after 6 h recovery, 12 h recovery and 24 h recovery, respectively. Means with the same letters are not significantly different. ($P > 0.05$).

there was a drastic increase in expression compared with the control in the shoot (3.85 folds) but the expression was significantly decreased 12 and 24 h after salt stress treatment (table 2). In root, transcript levels were decreased gradually to 0.98, .058 and 0.82 folds in response to 500 mM NaCl stress after 6, 12 and 24 h, respectively and the differences was not significant statistically (table 3). In recovery mode, changes in gene expression were different. In the shoot, *GTF* expression was decreased while in the root, expression of *GTF* increased in 6, 12 and 24 h after salt stress treatment, so that the expression in both tissues was closed to the expression level of control and in a non-significant manner. The result showed that *GTF* gene expression in shoots was higher than roots in all hours.

Expression pattern of *ARID* under salt stress

Bioinformatic analysis in NCBI database predicted that JK671186 EST refers to AT-Rich Interaction Domain protein. *ARID* proteins have an ancient DNA-binding domain that is conserved throughout higher eukaryotes and as a transcription factor, have a vital role in cell cycle and division [38]. The results indicated that *ARID* was highly expressed in shoots and the transcript levels of *ARID* were induced significantly by NaCl treatment (table 2). The expression levels of *ARID* in shoots increased 5.93, 4.91 and 5.17-fold relative to the control at 500 mM NaCl concentrations after 6, 12 and 24 h, respectively, while the level of transcripts in roots was 0.83, 0.26 and 0.56-fold lower than non-stressed controls (Fig 4) and the differences were not significant than control (table 3). Generally, higher transcript levels of *ARID* were detected in shoots compared to roots in all treatments (Fig. 4). The 5.93-fold expression of *ARID* in stress condition rather than non-stress one in the shoot, indicating a positive regulation role of *ARID* in salt stress. These results indicated that *ARID* may be involved in responding to salt stress in the plant. In recovery condition compared to stress one, *ARID* expression was increased significantly in roots and was roughly equal to gene expression in shoot tissue (Fig 4).

DISCUSSION

Plants have developed various strategies to respond to unfavorable environmental conditions [39]. To improve salt tolerant in plants, a proper understanding of molecular mechanisms and the gene expression of different factors that affect growth and developmental stage is essential. One of the most common used techniques for gene expression analysis is qPCR and has very advantages such as specificity, sensitivity, reproducibility, and accuracy. In this study, expression of genes was significantly induced by salt stress and reached

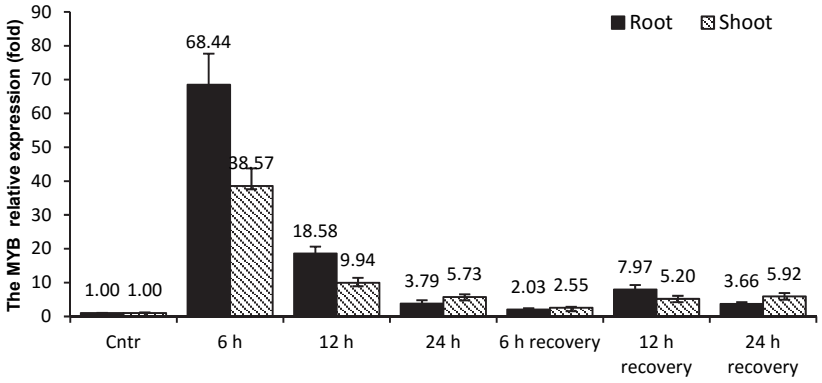

Figure 1. Expression pattern of *MYB* gene using q-PCR under salt stress and recovery condition in *A. littoralis*. Control (Cntr), stress after 6 h, 12 h, 24 h, stress after 6 h recovery, 12 h recovery and 24 h recovery, respectively. Values are Means and bars indicate Standard Error (SE).

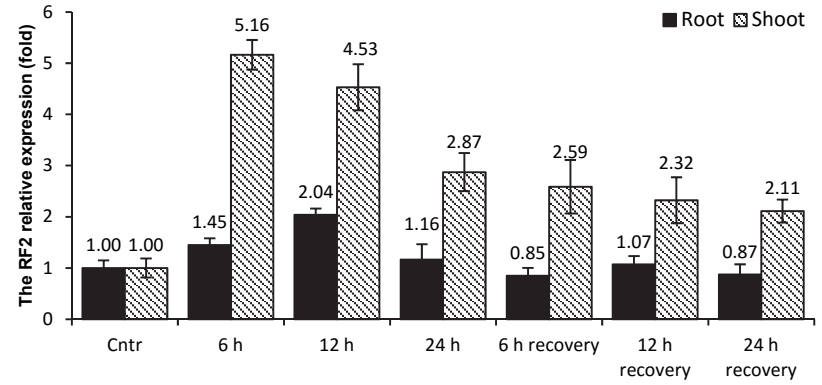

Figure 2. Expression pattern of *RF2* gene using q-PCR under salt stress and recovery condition in *A. littoralis*. Cntr, stress after 6 h, stress after 12 h, stress after 24 h, stress after 6 hours' recovery, stress after 12 h recovery and stress after 24 h recovery, respectively. Values are Means and bars indicate SE.

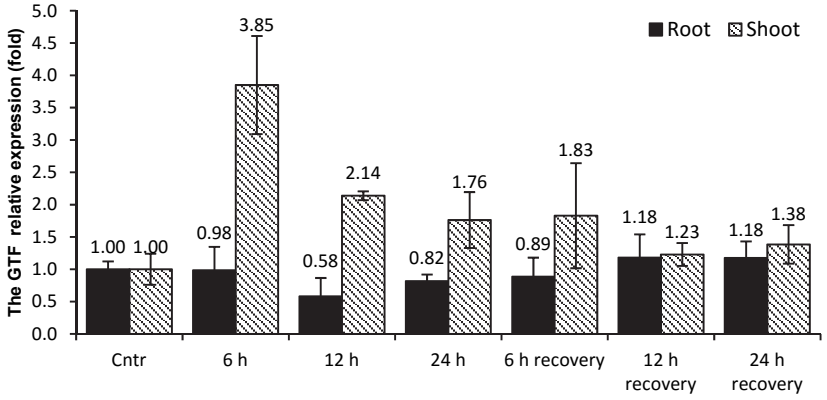

Figure 3. Expression pattern of *GTF* gene using q-PCR under salt stress and recovery condition in *A. littoralis*. Cntr, stress after 6 h, 12 h, 24 h, stress after 6 h recovery, 12 h recovery and 24 h recovery, respectively. Values are Means and bars indicate SE.

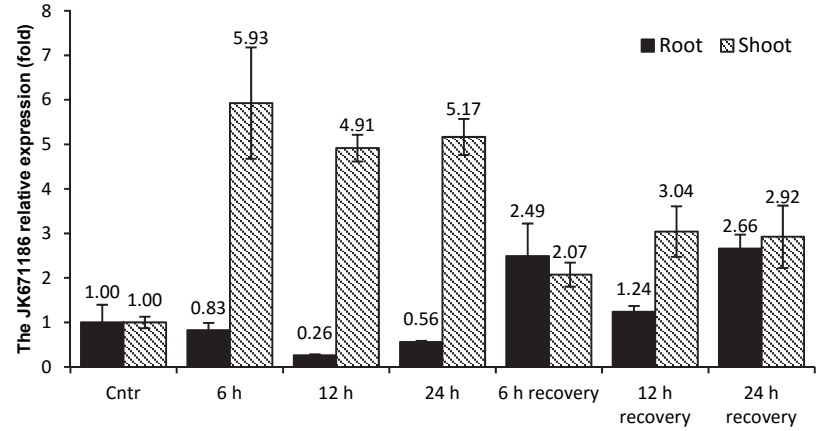

Figure 4. Expression pattern of *ARID* gene using q-PCR under salt stress and recovery condition in *A. littoralis*. Cntr, stress after 6 h, 12 h, 24 h, stress after 6 h recovery, 12 h recovery and 24 h recovery, respectively. Values are Means and bars indicate SE.

the highest peaks at 6 h after salt stress. *MYB* transcription factors play important roles in plant growth and stress responses. The highest transcript level of MYB in *A. littoralis* was nearly 68-fold over that in the non-treated control, indicating a positive regulation role of MYB in salt stress. Most MYB genes were positive and negative regulators of transcription [40] and play important roles in the regulation of many secondary metabolites at the transcriptional level [41]. MYB genes are involved in the signal transduction pathways of salicylic acid [42], abscisic acid (ABA) [23], gibberellic acid [43] and jasmonic acid [44] as well. The phytohormone ABA, produced under water deficit conditions, caused stomatal closure and played an important role in the adaptation of vegetative tissues to abiotic environmental stresses, such as drought and high salinity [45, 46]. Recently, the role of *AtMYB44* and *AtMYB15* Over-expression in stress tolerance [37, 47], *TaMYB2A* role in making Arabidopsis tolerance to multiple abiotic stresses [48], AtMYB2 role in dehydration and salt stress tolerance at the transcriptional level [49] and TaMYBsdu1 role in salt tolerance in wheat cultivars [50] were reported. In this study, transcriptional levels of *MYB* in the shoot and root of *A. littoralis* plants significantly increased in response to salinity after 6 h of salt stress which was consistent with the report that Tamyb2 was rapidly induced under osmotic stress for 1 h [51]. The result indicated the prominent roles of *MYB* family in quick response to stresses in plants as well as other TFs which regulated rapidly in response to biotic stress [7]. Because the roots are the first area which sense salt stress so the first response should occur in the root rather than the leaf. Furthermore, some MYB-encoding genes showed preferential accumulation of transcripts in tissues or under a specific condition. For example, some MYB genes in *Gossypium raimondii* were mainly expressed in leaves and roots under drought and saline conditions. A number of *GrMYB* genes such as *GrMYB050* and *GrMYB074* strongly and preferentially expressed in leaves under drought stress, whereas some of them were highly expressed in roots under drought and salt stress [52]. By contrast of *MYB*, in *RF2* gene expression pattern, whereas gene expression was increased in both tissue but it was in shoot higher than root and the peak level in the shoot was at 6 h and in root in 12 h after stress. *RF2a* was found in different tissues such as leave and stem in high level and low level in the root. *RF2* plays a critical role in leaf tissue differentiation and reducing the amount of it in transgenic rice plants severely disrupted shoot development. *RF2a* is a *bZIP* transcription factor that affects shoot development in *Oryza sativa*. With reducing of *RF2a* levels in transgenic rice line, some characteristics, such as severe stunting of shoots and leaf twisting and aberrant tissue organization were presented [53]. The RF2a protein has a bZIP domain which is similar to the domain from Arabidopsis, tobacco, tomato, and other plants [54]. Different studies indicated that the plants *bZIP* factors are vital regulators of plant specific processes including pathogen defense, light and stress signaling [55]. This group of *bZIP* proteins is involved in regulating gene expression in vascular tissues and shoot tissue organization in rice [27] and involved in ABA signaling pathway and Acts as positive regulator of the expression of abiotic stress-responsive genes through an ABA-dependent signaling pathway [56]. It Plays an important role in ABA and auxin responses by directly binding to the ABA-responsive element (ABRE)-containing genes, especially WRKY family genes. Suppresses auxin signaling by targeting ABRE-containing genes related to auxin metabolism or signaling [57]. Consistent with these studies, the expression of bZIP in Arabidopsis [58] and Rice [31] validated the role of bZIP in response to abiotic stresses. Some bZIP TFs such OsbZIP50 and OsbZIP39 are transcription factors that are involved in the activation of numerous chaperone genes [59, 60] and someone such an OsbZIP46 may be activated by post translational phosphorylation modification and Improves abiotic stress tolerance [61]. Although, the gene expression of *GTF* and *ARID* was increased in shoot tissue under 500 mM NaCl, but the expression level of these genes was decreased in root compared to the control. Recently reported that in *A. littoralis*, *GTF* expression level in leaf was higher and more stable than root tissue [62]. *GTFs* are involved in transcription initiation step and have the main role in RNA polymerase II regulation. Recent studies indicated that early event in the activation of genes is the binding of GTFs and Pol II to enhancer elements [63] and form the TATA-box complex [64] that involves in assembling of general transcription factors. Tfg3 (a small subunit of TFIIF general transcription factor) of *Schizo-saccharomyces pombe* was characterized and proposed that Tfg3 had an important role in high temperatures stress as a gene expression regulator [65]. Some ARID protein members are expressed ubiquitously and others in a highly tissue-specific pattern [66] and exhibited some cellular functions, including transcriptional regulation, participation in cell differentiation and development,

embryonic development and cell lineage [67]. The expression of *GTF* and *ARID* genes in recovery stage reached nearly or higher than to the control. Furthermore, their expression level in the shoot was higher than root. Similar results have been reported about the role of transcription factor by other researchers in plants under salt stress [68-71, 47]. This expression pattern in *A. littoralis* was indicated that timing and tissue type is an important factor in stress-related gene response in salt stress condition. The results showed that when plants were transferred to a NaCl-free Hoagland's solution after salt stress duration as a recovery, the expression of up-regulated genes under salt stress was decreased gradually in different tissues and finally depending on the function of genes such as plant growth, cell cycle and division, developmental processes and et cetera was placed in a constant value. Quantitative differences in the expression of genes involved in tolerance induction during abiotic stress could also be the result of the timing and intensity of transcriptional activation of involved genes rather than their presence/absence or other mechanisms [72]. Therefore, to make a plant with multiple resistances to abiotic stresses, it's important to increase the capability of genetical control of one or several key regulatory factor(s) [73]. Furthermore, considering transcription factors as candidate genes in breeding programs will give us a clear understanding the net of abiotic stress events and will finally lead us to improve crop varieties with higher stress tolerance. In the present study, the results showed that in *A. littoralis* the expression patterns of genes encoding transcription factors changes in response to salt stress and it is depending on the tissue type and treatment exposure duration. So it could be mentioned in designing breeding and biotechnology programs of salt tolerance plants.

ACKNOWLEDGMENTS

This study was conducted using funds provided by Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT). The authors also wish to thank the laboratory staff of GABIT for their technical assistance.

REFERENCES

[1] Modarresi, M., Nematzadeh, G.A. and Zarein, M. 2013. Glyceraldehyde-3-phosphate Dehydrogenase Gene from Halophyte *Aeluropus lagopoides*: Identification and Characterization. J Crop Imp, 27: 281-290.

[2] Kamkar, B., Kafi, M. and Nassiri-Mahallati, M. 2004. Determination of the most sensitive developmental period of wheat (Triticumaestivum) to salt stress to optimize saline water utilization. Australian Agronomy Conference, 12th AAC, 4th ICSC.

[3] Gulzar, S., Khan, M.A., and Ungar, I.A. 2003. Salt tolerance of a coastal salt marsh grass. Commun Soil Sci Plant Anal, 34: 2595-2605.

[4] Malekzadeh, Kh., Niazi, A., Shahriari-Ahmadi, F., Amin Mirshamsi-Kakhaki, A. and Zare-Mehrjerdi, M. 2015. The responses of L-gulonolactone oxidase and HKT2;1 genes in *Aeluropus littoralis'* shoots under high concentration of sodium chloride. J Plant Mol Breed, 3(2):28-35.

[5] Mohseni, A., Nematzadeh, G.A., Dehestani, A., Shahin, B. and Soleimani, E. 2015. Isolation, molecular cloning and expression analysis of *Aeluropus littoralis* Monodehydroascorbate reductase (MDHAR) gene under salt stress. J Plant Mol Breed, 3(1):72-80.

[6] Barrero, J.M., Rodriguez, P.L., Quesada, V., Piqueras, P., Ponce, M.R. and Micol, J.L. 2006. Both abscisic acid (ABA)-dependent and ABA-independent pathways govern the induction of NCED3, AAO3 and ABA1 in response to salt stress. Plant Cell and Envir, 29: 2000-2008.

[7] Yamaguchi-Shinozaki, K. and Shinozaki, K. 2006. Cross-talk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol, 9:436-442.

[8] Tran, L.S., Nakashima, K., Sakuma, Y., Simpson, S.D., Fujita, Y., Maruyama, K., Fujita, M., Seki, M., Shinozaki, K. and Yamaguchi- Shinozaki, K. 2004. Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a droughtresponsive cis-element in the early responsive to dehydration stress promoter. The Plant Cell, 16: 2481-2498.

[9] Liu, K., Wang, L., Xu, Y., Chen, N., Ma, Q., Li, F. and Chong, K. 2007. Overexpression of OsCOIN, a putative cold inducible zinc finger protein, increased tolerance to chilling, salt and drought, and enhanced proline level in rice. Planta, 226: 1007-1016.

[10] Udvardi, M.K., Kakar, K., Wandrey, M., Montanari, O., Murray, J., Andriankaja, A., Zhang, J.Y., Benedito, V., Hofer, J.M., Chueng, F. and Christopher, D.T. 2007. Legume transcription factors: Global regulators of plant development and response to the environment. Plant Physiol, 144:538–549.

[11] Kilian, J., Peschke, F., Berendzen, K.W., Harter, K. and Wanke, D. 2012. Prerequisites, performance and profits of transcriptional profiling the abiotic stress response. Biochim Biophys Acta, 1819:166-175.

[12] Golldack, D., Lüking, I. and Yang, O. 2011. Plant tolerance to drought and salinity: Stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep, 30:1389-1391.

[13] Zhang, H.X. and Blumwald, E. 2001. Transgenic salt tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotechnol, 19:765–768

[14] Mohanty, A., Kathuria, H., Ferjani, A., Sakamoto, A., Mohanty, P., Murata, N. and Tyagi, A.K. 2002. Transgenics of an elite indica rice variety Pusa Basmati 1 harbouring the codA gene are highly tolerant to salt stress. Theor Appl Genet, 106:51-57.

[15] Puranik, S., Bahadur, R.P., Srivastava, P.S. and Prasad, M. 2011. Molecular cloning and characterization of a membrane associated NAC family gene, SiNAC from foxtail millet [*Setaria italica* (L.) P. Beauv.]. Mol Biotech, 49:138-150.

[16] Zheng, X., Chen, B., Lu, G. and Han, B. 2009. Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem. Biophys. Res. Commun, 379: 985-989.

[17] Wang, Q., Guan, Y., Wu, Y., Chen, H., Chen, F. and Chu, C. 2008. Overexpression of a rice OsDREB1F gene increases salt, drought, and low temperature tolerance in both Arabidopsis and rice. Plant Mol Biol, 67: 589-602.

[18] Chen, H., Chen, W., Zhou, J., He, H., Chen, L., Chen, H. and Deng, X.W. 2012. Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. Plant Sci, 193:8-17.

[19] Fujita, Y., Fujita, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. 2011. ABA-mediated transcriptional regulation in response to osmotic stress in plants. J. Plant Res., 124:509-525.

[20] Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K. and Yamaguchi-Shinozaki, K. 2012. NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta, 1819:97-103.

[21] Rushton, D.L., Tripathi, P., Rabara, R.C., Lin, J., Ringler, P., Boken, A.K., Langum, T.J., Smidt, L., Boomsma, D.D. and Emme, N.J., Chen, X., Finer, J.J., Shen, Q.J. and Rushton, P.J. 2012. WRKY transcription factors: Key components in abscisic acid signalling. Plant Biotechnol J, 10(1):2–11.

[22] Lippold, F., Sanchez, D.H., Musialak, M., Schlereth, A., Scheible, W.R., Hincha, D.K. and Udvardi, M.K. 2009. AtMyb41 regulates transcriptional and metabolic responses to osmotic stress in Arabidopsis. Plant Physiol, 149:1761-1772.

[23] Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K. and Yamaguchi- Shinozaki, K. 2003. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell, 15:63-78.

[24] Xie, Z., Lee, E.K., Lucas, J.R., Morohashi, K., Li, D., Murray, J.A.H., Sack, F.D. and Grotewold, E. 2010. Role of the stomatal development regulators FLP/ MYB88 in abiotic stress responses. The Plant Cell, 22:2306-2321.

[25] Seo, P.J., Xiang, F.N., Qiao, M., Park, J.Y., Lee, Y.N., Kim, S.G., Lee, Y.H., Park, W.J. and Park. C.M. 2009. The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in Arabidopsis. Plant Physiol, 151: 275-289.

[26] Seo, P.J., and Park, C.M. 2010. Myb96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in Arabidopsis. New Phytol, 186: 471-483.

[27] Dai, Sh., Petruccelli, S., Ordiz, M.I., Zhang, Zh., Chen, Sh. and Beachy, R.N. 2003. Functional Analysis of RF2a, a Rice Transcription Factor. J Biol Chem, 278(38): 36396-36402.

[28] Deppmann, C.D., Alvania, R.S. and Taparowsky, E.J. 2006. Cross-species annotation of basic leucine zipper factor interactions: Insight into the evolution of closed interaction networks. Mol Biol Evol, 23: 1480-1492.

[29] Nijhawan, A., Jain, M., Tyagi, A.K. and Khurana, J.P. 2008. Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice. Plant Physiol, 146: 333-350.

[30] Shinozaki, K., and Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. J exp Bot, 58: 221-227.

[31] E, Z.G. Zhang, Y.P., Zhou, J.H. and Wang, L. 2014. Roles of the bZIP gene family in rice. Gene and Mol Res. 13(2): 3025-3036.

[32] Hoagland, D.R. and Arnon, D. I. 1950. The Water-Culture Method For Growing Plants Without Soil. Circular. California Agricultural Experiment Station, 347.

[33] Hashemipetroudi, S.H., Nematzadeh, Gh., Ahmadian, Gh., Yamchi, A. and Kuhlmann, M. 2016. Expression analysis of salt stress related expressed sequence tags (ESTs) from *Aeluropus littoralis* by quantitative real-time PCR. Biosci. Biotech. Res. Comm. 9(3): 445-456.

[34] Moradi, N., Rahimian, H., Dehestani, A. and Babaeizad, V. 2016. Cucumber Response to Sphaerotheca fuliginea: Differences in Antioxidant Enzymes Activity and Pathogenesis-Related Gene Expression in Susceptible and Resistant Genotypes. J of Plant Mol Breeding, 4(2): 33- 40.

[35] 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.

[36] Ghanem, M.E., Albacete, A., Martínez-Andújar, C., Acosta, M., Romero-Aranda R., Dodd, I.C., Lutts, S. and Pérez-Alfocea, F. 2008. Hormonal changes during salinity induced leaf senescence in tomato (*Solanum lycopersicum* L.). J Exp Bot, 59(11):3039-3050.

[37] Jung, C., Seo, J.S., Han, S.W., Koo, Y.J., Kim, C.H., Song, S.I., Nahm, B.H., Choi, Y.D. and Cheong, J.J. 2008. Over-expression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol, 146:623-635.

[38] Zhu, H., Chen, T., Zhu, M., Fang, Q. and Kang, H. 2008. A novel ARID DNA binding protein interacts with SymRK and is expressed during early nodule development in Lotus japonicus. Plant Physiol, 148: 337–347.

[39] Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C. and Lepiniec, L. 2010. MYB transcription factors in Arabidopsis. Trends in Plant Sci, 15: 573-581.

[40] Jin, H., Cominelli, E., Bailey, P., Parr, A., Mehrtens, F., Jones, J., Tonelli, C., Weisshaar, B. and Martin, C. 2000. Transcriptional repression by AtMYB4 controls production of UV- protecting sunscreens in Arabidopsis. EMBO J, 19: 6150-6161.

[41] Gonzalez, A., Zhao, M., Leavitt, J.M. and Lloyd, A.M. 2008. Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/ Myb transcriptional complex in Arabidopsis seedlings. Plant J, 53:814-827.

[42] Raffaele, S., Rivas, S. and Roby, D. 2006. An essential role for salicylic acid in AtMYB30-mediated control of the hypersensitive cell death program in Arabidopsis. FEBS Lett, 580: 3498–3504.

[43] Murray, F., Kalla, R., Jacobsen, J. and Gubler, F. 2003. A role for HvGMYB in anther development. Plant J, 33:481-491.

[44] Lee, M.W., Qi, M. and Yang, Y. 2001. A novel jasmonic acid-inducible rice MYB gene associates with fungal infection and host cell death. Mol Plant Microbe Interact, 14: 527-535.

[45] Vannini, C., Locatelli, F., Bracale, M., Magnani, E., Marsoni, M., Osnato, M., Mattana, M., Baldoni, E. and Coraggio, I. 2004. Overexpression of the rice OsMYB4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J, 37: 115-127.

[46] Maeda, K., Kimura, S., Demura, T., Takeda, J. and Ozeki, Y. 2005. DcMYB1 acts as a transcriptional activator of the carrot phenylalanine ammonia-lyase gene (DcPAL1) in response to elicitor treatment, UV-B irradiation and the dilution effect. Plant Mol Biol, 59: 739-752.

[47] Ding, Z., Li, S., An, X., Liu, X., Qin, H. and Wang, D. 2009. Transgenic expression of MYB15 confers enhanced sensitivity to abscisic acid and improved drought tolerance in Arabidopsis thaliana. J Genet Genomics, 36:17-29.

[48] Mao, X., Jia, D., Li, A., Zhang, H., Tian, S., Zhang, X., Jia, J. and Jing, R. 2011. Transgenic expression of TaMYB2A confers enhanced tolerance to multiple abiotic stresses in Arabidopsis. Funct Integr Genomics, 11:445-465.

[49] Ambawat, S., Sharma, P., & Yadav, N.R. and Yadav, R.C. 2013. MYB transcription factor genes as regulators for plant responses: an overview. Physiol Mol Biol Plants, 19(3): 307-321.

[50] Rahaie, M., Xue, G.P., Naghavi, M.R., Alizadeh, H. and Schenk, P.M. 2010. A MYB gene from wheat (*Triticum aestivum* L.) is up-regulated during salt and drought stresses and differentially regulated between salt tolerant and sensitive genotypes. Plant Cell Rep, 29: 835–844.

[51] Chen, R.M., Ni, Z.F., Nie, X.L., Qin, Y.X., Dong, G.Q. and Sun, Q.X. 2005. Isolation and characterization of genes encoding Myb transcription factor in wheat (*Triticum aestivum* L.). Plant Sci, 169: 1146-1154.

[52] He, Q., Don, C., Li, J.W., Xie, F., Ma, J., Sun, R., Wang, Q., Zhu, S. and Zhang, B. 2016. Genome wide identification of R2R3-MYB genes and expression analyses during abiotic stress in *Gossypium raimondii*. Sci Rep, 6, 22980. doi: 10.1038/srep22980.

[53] Yin, Y., Zhu, Q., Dai, S., Lamb, C. and Beachy, R.N. 1997. RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. EMBO J, 16: 5247-5259.

[54] Jakoby, M., Weisshaar, B., Droge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T. and Parcy, F. 2002. bZIP transcription factors in Arabidopsis. Trends Plant Sci, 7(3): 106–111.

[55] Vincentz, M., Schlögl, P.S., Corrêa, L.G.G., Kühne, F. and Leite, A. 2001. Phylogenetic relationships between Arabidopsis and sugarcane bZIP transcriptional regulatory factors. Genet Mol Biol., 24(1-4): 55-60.

[56] Hossain, M.A., Cho, J.I., Han, M., Ahn, C.H., Jeon, J.S., An, G. and Park, P.B. 2010. The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. J Plant Physiol, 167: 1512-1520.

[57] Yang, X., Yang, Y.N., Xue, L.J., Zou, M.J., Liu, J.Y., Chen, F. and Xue, H.W. 2011. Rice ABI5-Like1 regulates abscisic acid and auxin responses by affecting the expression of ABRE-containing genes. Plant Physiol, 156: 1397-1409.

[58] Jin, X.F., Xiong, A.S., Peng, R.H., Liu, J.G., Gao, F., Chen, J.M. and Yao, Q.H. 2010. OsAREB1, an ABRE-binding protein responding to ABA and glucose, has multiple functions in Arabidopsis. BMB Rep, 43: 34-39.

[59] Lu, S.J., Yang, Z.T., Sun, L., Sun, L., Song, Z.T. and Liu, J.X. 2012. Conservation of IRE1-regulated bZIP74 mRNA unconventional splicing in rice (*Oryza sativa* L.) involved in ER stress responses. Mol. Plant 5: 504-514.

[60] Takahashi, H., Kawakatsu, T., Wakasa, Y., Hayashi, S., and Takaiwa, F. 2012. A rice transmembrane bZIP transcription factor, OsbZIP39, regulates the endoplasmic reticulum stress response. Plant Cell Physiol. 53: 144-153.

[61] Tang, N. Zhang, H. Li, X. Xiao, J. and Xiong, L. 2012. Constitutive Activation of Transcription Factor OsbZIP46 Improves Drought Tolerance in Rice. Plant Physiol, 158: 1755-1768.

[62] Hashemi, S.H., Nematzadeh, Gh., Ahmadian, Gh., Yamchi, A. and Kuhlmann, M. 2016. Identification and validation of *Aeluropus littoralis* reference genes for Quantitative Real-Time PCR Normalization. J of Biol Res-Thessaloniki, 23: 18.

[63] Szutorisz, H., Dillon, N. and Tora, L.S. 2005. The role of enhancers as centres for general transcription factor recruitment. Trends Biochem Sci, 30(11): 593-599.

[64] Kuhlman, T.C., Cho, H., Reinberg, D. AND Hernandez. N. 1999. The General Transcription Factors IIA, IIB, IIF, and IIE Are Required for RNA Polymerase II Transcription from the Human U1 Small Nuclear RNA Promoter. Mol Cell Biol, 19(3): 2130-2141.

[65] Kimura, M. and Ishihama, A. 2004. Tfg3, a subunit of the general transcription factor TFIIF in *Schizosaccharomyces pombe*, functions under stress conditions. Nucleic Acids Res, 32: 6706-6715.

[66] Zheng, B., He, H., Zheng, Y., Wu, W., and McCormick, S. (2014). An ARID domain-containing protein within nuclear bodies is required

for sperm cell formation in Arabidopsis thaliana. PLoS genetics, 10(7), e1004421.

[67] Wilsker, D., Probst, L., Wain, H.M., Maltais, L., Tucker, P.W. and Moran, E. 2005. Nomenclature of the ARID family of DNA-binding proteins. Genomics, 86: 242–251.

[68] Zou, M., Guan, Y., Ren, H., Zhang, F. and Chen, F. 2008. A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. Plant Mol Biol, 66: 675–683.

[69] Jia, Z., Lian, Y., Zhu, Y., He, J., Cao, Z. and Wang, G. 2009. Cloning and characterization of a putative transcription factor induced by abiotic stress in Zea mays. Afr J Biotech, 8: 6764-6771.

[70] Dai, X., Xu, Y., Ma, Q., Xu, W., Wang, T., Xue, Y. and Chong, K. 2007. Overexpression of an R1R2R3 MYB gene, OsMYB3R-2, increases tolerance to freezing, drought, and salt stress in transgenic Arabidopsis. Plant Physiol, 143: 1739-1751.

[71] Xu, Z.S., Ni, Z.Y., Li, Z.Y., Li, L.C., Chen, M., Gao, D.Y., Yu, X.D., Liu, P. and Ma, Y.Z. 2009. Isolation and functional characterization of HvDREB1-a gene encoding a dehydration-responsive element binding protein in *Hordeum vulgare*. J Plant Res, 122: 121-130.

[72] Qiang, L., Nanming, Z., Yamaguchi-Shinozaki, K. and Shinozaki, K. 2000. Regulatory role of DREB transcription factors in plant drought, salt and cold tolerance. Chinese Sci Bull, 45(11): 970-975.

[73] Lata, C., Bhutty, S., Bahadur, R.P., Majee, M. and Prasad, M. 2011. Association of a SNP in a novel DREB2-like gene SiDREB2 with stress tolerance in foxtail millet [*Setaria italica* (L.)]. J Exp Bot, 62(10): 3387-3401.

بررسی الگوی بیان فاکتورهای رونویسی در شرایط تنش شوری و محیط بازیابی در گیاه هالوفیت *Aeluropus littoralis*

حمیدرضا قربانی[۱]، حبیب اله سمیع‌زاده لاهیجی[۲]، قربانعلی نعمت‌زاده[۳]*

[۱] گروه زراعت و اصلاح نباتات، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت، ایران

[۲] گروه بیوتکنولوژی کشاورزی، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت، ایران

[۳] پژوهشکده ژنتیک و زیست‌فناوری کشاورزی طبرستان، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

* نویسنده مسئول: gh.nematzadeh@sanru.ac.ir

چکیده

تنش شوری یکی از مهمترین تنش های غیر زیستی است که رشد و تولید محصولات زراعی را محدود می کند. گیاهان هالوفیت غلظت های بالای کلرید سدیم را تحمل کرده و فاکتورهای رونویسی، عوامل تنظیم کننده مهمی در پاسخ گیاه به تنش‌ها می‌باشند. در این تحقیق، الگوی بیانی ۴ ژن کد کننده فاکتورهای رونویسی در گیاه آلوروپوس لیتورالیس با نام‌های، *MYB*, *RF2*, *GTF* و *ARID* در پاسخ به تیمار تنش شوری بررسی شد. بدین منظور، گیاهان در معرض تنش شوری ۶۰۰ میلی مولار و سپس شرایط ریکاوری قرار گرفته و نمونه برداری از گیاهان در سه زمان (۶، ۱۲ و ۲۴ ساعت) انجام گرفت. نتایج داده های حاصل از q- PCR نشان داد که کلرید سدیم تأثیر معنی‌داری از لحاظ آماری بر میزان بیان ژن‌ها داشت. این بیان در ۶ ساعت ابتدایی تنش افزایش ولی با افزایش زمان تنش، میزان آن در بافت‌های ریشه و اندام هوایی کاهش یافت. بالاترین میزان بیان برای ژن *MYB* در ۶ ساعت ابتدایی تنش در ریشه گیاه (۶۸/۴۴) مشاهده شد که بیشتر از بیان ژن در اندام هوایی گیاه (۳۸/۵۷) بود. در شرایط ریکاوری، بیان ژن‌ها در بافت‌های مختلف به تدریج کاهش و در نهایت به مقدار ثابتی رسید. نتایج این تحقیق نشان داد که فاکتورهای رونویسی مورد مطالعه نقش مهمی در تحمل گیاه آلوروپوس به تنش شوری داشته و می توانند به عنوان منبع اطلاعاتی مهمی در برنامه های اصلاحی معرفی شوند. همچنین، پاسخ گیاه آلوروپوس لیتورالیس به تنش شوری به نوع بافت و مدت زمان در معرض تنش بودن گیاه نیز بستگی دارد.

کلمات کلیدی: آلوروپوس لیتورالیس، تنش زیستی، ژن مرجع، فاکتور رونویسی، محیط ریکاوری