Journal of Plant Physiology and Breeding

2021, 11(2): 161-172 ISSN: 2008-5168

Transcriptional changes of *AOS*, *AOC*, *OMT*, *NHX1*, and *L1S1* genes in roots of barley genotypes under salinity stress

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Received: June 25, 2021 Accepted: September 15, 2021
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Abstract
Salinity is a big problem for agriculture and crop productivity worldwide. Barley is considered notably salt-tolerant and there is considerable genetic variation in barley in response to salinity. In the present study, the expression pattern of *AOS*, *AOC*, *OMT*, *L1S1*, and *NHX1* genes were investigated in the roots of three barley genotypes, Sahara3771, Clipper, and an advanced breeding line (A-line) 24 hours, 3 days, and 3 weeks after 100 and 200 mM NaCl treatments as well as control (no NaCl). Analysis of variance revealed significant salinity x genotype x salt exposure time interaction for all the studied genes, except *AOC*. The highest expression level of the *AOC* gene was noted under 200 mM NaCl in Clipper and the lowest expression was recorded under 200 mM and 100 mM NaCl in A-line and Clipper, respectively. For the *AOS* gene, the highest expression level was recorded 3 weeks after 200 mM NaCl treatment. The maximum expression level of *NHX1* was measured in A-Line 24 hours after 100 mM NaCl treatment and the lowest in $Sahara_{3771}$ 3 weeks after 200 mM NaCl treatment. The *OMT* gene showed the highest expression in $Sahara_{3771}$ 3 weeks after 100 mM NaCl treatment and the lowest was observed in A-Line under 200 and 100 mM NaCl. For the *LlS1* gene, the highest level of the transcripts was measured 3 weeks after 100 mM NaCl treatment. *LlS1* gene showed the lowest expression level 24 hours after 200 mM NaCl in Sahara3771.

Keywords: Gene expression; *Hordeum vulgare*; Real-Time RT-PCR; Salt stress

How to cite: Ghaffarian S, Mohammadi SA, and Toorchi M, 2021. Transcriptional changes of AOS, AOC, OMT, NHX1, and L1S1 genes in root of barley genotypes under salinity stress. Journal of Plant Physiology and Breeding 11(2): 161-172.

Introduction

Among cereals, barley (*Hordeum vulgare* L.) ranks as the fourth most important cereal crop in production quantity and cultivation area. Barley is a highly resilient crop, adaptable to various environmental conditions and the most salt-tolerated crop along with oat. Therefore, it is cultivated in a wide land area of the world. (Shahid and Jaradat 2013). Also, it is an ideal model crop for studying the mechanism and inheritance of salinity tolerance in cereals. Investigating the physiological and molecular pathways involved in barley's response to salinity, could provide global insight into the characteristics of plants' responses to salinity and helps to improve plants' salinity tolerance by manipulating the key genes (Xu *et al*. 2012). Some barley genotypes can thrive in saline conditions using various approaches. The Plant Genetic Resource Laboratory of ICBA studies on Omani Batini barley landraces showed high levels of within and among sub-populations diversity for salt tolerance,

at different growth stages (Shahid and Jaradat 2013).

Ligaba *et al.* (2011) studied the expression of five tonoplast intrinsic protein isoforms (MIPs) coding genes in five-day-old barley seedlings. The result revealed that 24 hours of treatment with 100 mM NaCl significantly upregulated the transcripts level of *HvTIP1;2* and *HvPIP2;1* genes but suppressed the expression of *HvNIP2;1* gene by eight-folds in roots. They stated that the enhanced accumulation of *HvTIP1;2* and *HvPIP2;1* gene transcript may suggest their role in salt-stress tolerance.

Plant response to salt stress is modulated by regulation of various genes expression in different pathways. Jasmonates have diverse functions such as initiation of stress responses and growth and development regulation. Allene oxide synthase (*AOS*) and allene oxide cyclase (*AOC*) genes participate in the Jasmonate biosynthesis from polyunsaturated fatty acids. *AOS* and *AOC* convert fatty acid hydroperoxides to 12-oxophytodienoic acid (OPDA) and dinor-OPDA, respectively. Finally, jasmonates' characteristic cyclopentanone ring is established by OPDA reductase. Jasmonic acid has been known as the bioactive hormone (Schaller and Stintzi 2009).

The main part of salinity stress's destructive effects is the Na^+ toxicity. Endosomal transporters of Na^+, encoded by the NHX gene family, play important roles in vacuolar accumulation and K^+ homeostasis (Pardo *et al*. 2006). By expressing Na^+/H^+ antiporter (*NHX*), plants release sodium out of cells or store that in vacuoles (Barkla and Blumwald 1991). Methyltransferases (MTs) create S-adenosyl-L-homocysteine (AdoHcy) by transferring the methyl group from S-Adenosyl-L-methionine (AdoMet) to acceptor molecules (Ibrahim and Muzac 2000) and the acceptor molecule for O-methyltransferase (*OMT*) is an oxygen atom. Oxygen atom methylation of the secondary metabolites has an important role in plant's resistance to disease and abiotic stress tolerance (Lam *et al.* 2007). Pheophorbide *a* oxygenase (PaO), a nonheme iron monooxygenase, is a key control point in the chlorophyll degradation regulation. The role of PaO in cell death suppression has been demonstrated in some plants. The lethal leaf-spot 1 (*LlS1*) gene is a PaO homologue in wheat and studies showed that wounding treatment and infection by *Puccinia striiformis* induced the cell death suppressor protein, *Lls1*, in the wheat leaves (Tang *et al.* 2013).

This study aimed to assess the expression pattern of *AOC*, *AOS*, *NHX1*, *OMT*, and *LlS1* genes in three barley genotypes with differential responses to salt stress under different exposure times to NaCl treatments.

Materials and Methods

Plant material, cultivation, and experimental condition

The plant materials included three barley genotypes, Clipper, Sahara3771, and Advanced breeding line (A-line), with differential responses to salinity. Clipper is a salt-susceptible commercial Australian cultivar, Sahara3771 is a salt-tolerant landrace from North Africa, and A-line is a salt-tolerant line obtained from a cross between Sahra and Kavir. The seeds of Clipper and Sahara3771 were kindly provided by the

University of Western Australia and A-line by the Seed and Plant Improvement Institute, Karaj, Iran.

Uniformly sized seeds from each genotype were sterilized with sodium hypochlorite and then germinated in Petri dishes containing one layer of wet filter paper. After seven days, uniformly germinated seedlings were transferred to a hydroponic medium containing a modified Hoagland nutrient solution in the greenhouse under controlled conditions [16/8 h photoperiod, 25/23±1°Cday\night,35 μmolm^{-2}s^{-1} photosynthetic photon flux density (PPFD), and 60% relative humidity]. After establishment, the seedlings were exposed to 100 and 200 mM NaCl and root samples were harvested 24 hours, 3 days, and 3 weeks after the salt treatments. The control treatment had no NaCl. The experimental design was split plot-factorial based on the completely randomized. RNA was extracted from root samples using RNX-Plus kit (CINNAGEN, Iran) according to the manufacturer's instruction. The extracted RNA samples were treated with DNase (Fermentas, USA). The quantity and quality of RNA were checked using a spectrophotometer (PicoDrop, UK) and 1.2% agarose gel electrophoresis. The cDNA was synthesized using Revert Aid first-strand cDNA synthesis kit (Thermo Scientific, America).

The expression pattern of allene oxide synthase *(AOS)*, allene oxide cyclase *(AOC)*, O-methyltransferase *(OMT)*, Na^+/H^+ antiporter *(NHX)*, and lethal leaf-spot 1 *(LlS1)* genes under slat treatments and control conditions was analyzed using gene-specific primer pairs (Table 1) with real-time PCR. The α-tubulin gene was used as a housekeeping gene to normalize the transcript of the studied genes. Then, the data were quantified by the comparative Ct method ($2^{-\Delta\Delta Ct}$ method) based on the Ct values (Livak and Schmittgen 2001). The PCR reaction was performed in the volume of 10 μl consisting of 3.5 μl ddH2O, 1 μl cDNA, 0.7+0.7 μl forward and reverse primers (5ng/μl), and 4 μl SYBR Green premix Ex *Taq*TMII PCR master mixture (TAKARA, Japan). The amplification reactions were performed on the C1000™ Thermal Cycler system (Bio-Rad) with an initial denaturation at 94 °C for 5 minutes, 40 cycles of 94 °C for 45 seconds, primer specific annealing temperature for 45 seconds (Table 1), and 72 °C for 45 seconds, and final extension at 72 °C for 5 minutes.

Data analysis

The PROC GLM procedure of the SAS 9.2 software (SAS Institute Inc., NC, USA) was used to do the analysis of variance (ANOVA) for the morphological and gene expression data, and then Duncan's multiple range test was performed to compare the means. The normality of residuals was tested before ANOVA by the UNIVARIATE procedure of the SAS software.

Results

Based on ANOVA, genotype, salt stress, and salt exposure time significantly affected the expression level of all studied genes. The two-way and three-way interactions of salt, salt exposure time, and genotype were also significant for all genes except the three-way interaction for the *AOC* gene (Table 2).

Table 1. The sequences and annealing temperature of the gene-specific primers used for amplification of *PEAMT*, *OMT*, *BFRUCT3*, *NHX1*, and *α-tubulin2* genes

Gene	Forward primers (5´-3´)	Reverse primers (5´-3´)	AT (˚C)
AOC	GCAACACACGGAGATTCATTCA	ACAGCATGTACTTCGGCGACTA	68
AOS	TCATACATAGCCGGTGCAGGTTT	CGACATGAACATCGAGAGCCA	65
OMT	TCCCTCGTCCCACTATCATACC	AACCTTTCCCCCCATTTCG	60
NHX	TACGGTTTTCTGCCTCTGTCACA	ACAA ATCTGGTCATACTGCCG	68
LlS1	GCCAGAAGCATTTCGTGTTT	TGGTTTTCAACCCGACTTTT	55

Table 2. Analysis of variance for the genes expression in Sahara$_{3771}$, Clipper, and A-Line barley genotypes, 24 hours, 3 days, and 3 weeks after exposure to 100 and 200 mM NaCl treatment as well as the control (no NaCl)

SOV	**df**	**Mean Squares**				
		AOS	*AOC*	*OMT*	*NHX1*	*L1S1*
Replication	1	0.047	0.240	0.007*	0.005	0.004
Salinity	2	1.131**	1.907*	0.748**	2.963**	4.209**
Error 1	2	0.004	0.102	0.000	0.010	0.003
Genotype	2	7.549**	6.780**	0.380**	0.581**	1.759**
Salinity x Genotype	4	3.908**	4.800**	5.301**	2.741**	1.132**
Salinity exposure time	2	1.228**	20.180**	4.184**	1.280**	0.586**
Salinity x Salinity exposure time	4	4.505**	8.770**	1.483**	1.619**	1.008**
Genotype x Salinity exposure time	4	1.076**	7.630**	3.839**	0.717**	0.547**
Salinity x Genotype x Salinity exposure time	8	1.098**	1.060	1.689**	0.518**	0.245**
Error 2	24	0.050	1.009	0.006	0.012	0.069
CV (%)		2.30	10.10	0.73	1.09	2.64

*,**: significant at the 0.05 and 0.01 probability levels, respectively

AOC gene

The expression of the *AOC* gene was significantly affected by salinity treatments, genotypes, time courses, and their two-way interaction. In the roots of A-Line, salt stress decreased the transcription of *AOC* as compared with the control. Under 200 mM NaCl, the expression of this gene significantly decreased in the salt-tolerant A-Line as compared with 100 mM NaCl but increased in Sahara$_{3771}$ as compared to the salt-susceptible cultivar Clipper (Figure 1a). Twenty-four hours after salt treatment, the *AOC* gene expression pattern was similar in Clipper and A-Line. Prolonging salt stress from 24 hours to 3 weeks resulted in the higher expression of the *AOC* gene in Sahara$_{3771}$ as compared to the other two genotypes (Figure 1b). On average of the genotypes, 24 hours and 3 weeks after 100 and 200 mM NaCl treatment, the *AOC* gene was highly down-regulated in all of the studied genotypes compared with the 3-days treatment. Under 200 mM NaCl treatment, the highest level of *AOC* transcript was recorded 3 days after salt treatment as compared to the control. A significant reduction was observed in the gene expression 24 hours after the 100 mM NaCl treatment compared to the control. Assessing the effect of 200 mM NaCl on the *AOC* gene expression as compared to 100 mM NaCl revealed significant down-regulation of the gene with prolonging of salt treatment from 24 hours and 3 days to 3 weeks (Figure 1c).

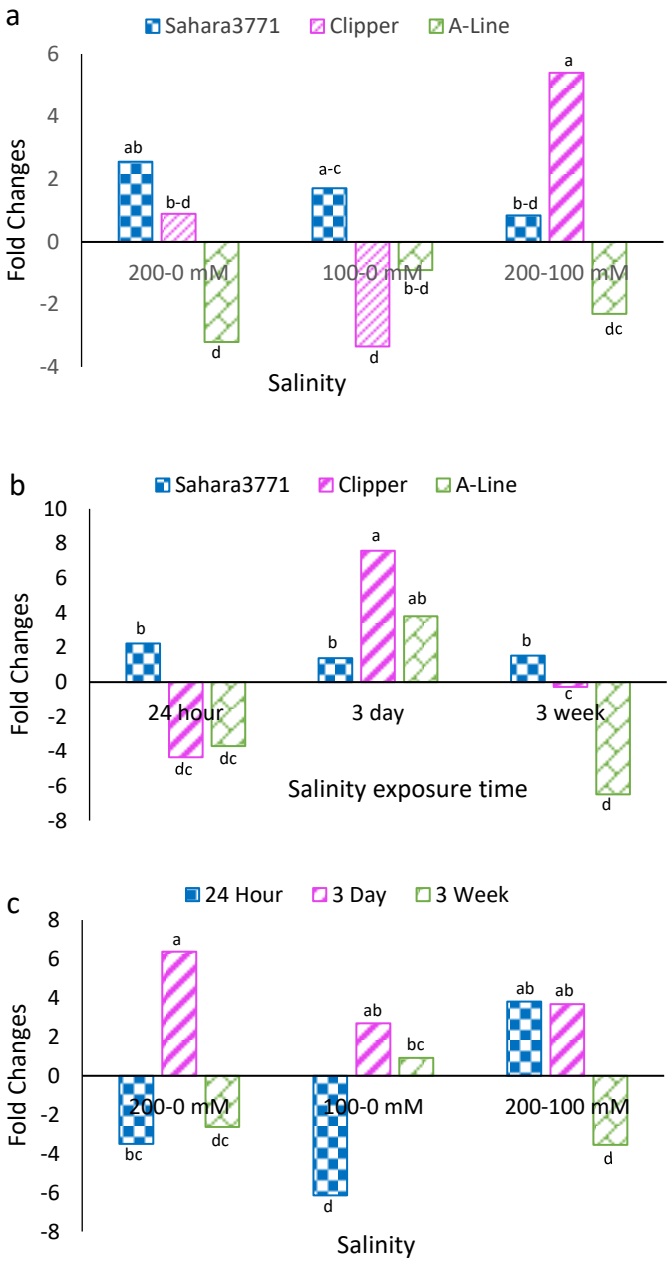

Figure 1. Changes in the *AOC* gene expression under 100-0, 200-0, and 200-100 mM NaCl treatment comparison; A) genotype x salinity interaction, B) genotype x salinity exposure time interaction, and C) salinity x salinity exposure time interaction; means with different letters are significantly different at the 0.05 level of probability.

AOS gene

The results revealed that the *AOS* gene was involved in the late response to salt stress such that 24 hours after salt treatment, there were no significant differences among the studied genotypes. The highest level of the *AOS* gene expression was observed in A-Line after 3 weeks of exposure to 200 mM NaCl as compared to 100 mM NaCl. Under 100 mM NaCl treatment, the salt-susceptible cultivar Clipper showed the

maximum gene expression 3 days after salt treatment compared with the control. Under all salt treatments and exposure times to salinity, the level of *AOS* gene transcript was low in the salt-tolerant cultivar Sahara$_{3771}$ (Figure 2).

NHX1 gene

The maximum level of *NHX1* gene transcript was recorded in the salt-tolerant A-Line, 24 hours after 100 mM NaCl treatment followed by Sahara$_{3771}$ under 100 mM NaCl and Clipper under 200 mM NaCl as compared with the control. Prolonging salt stress to 3 days resulted in a significant down-regulation of *NHX1* gene in all three genotypes under 200 mM NaCl and Sahara$_{3771}$ and Clipper under 100 mM NaCl as compared to the control. Three days after salt treatment with 200 mM, compared with 100 mM NaCl, the expression level of this gene in the salt-tolerant genotypes was significantly less than the salt-susceptible genotype Clipper and among the genotypes, A-Line showed the highest level of the *NHX1* gene expression which was achieved under 100 mM NaCl as compared to the control. Long time exposure (3 weeks) to salt stress resulted in the down-regulation of the gene in Clipper under all salt treatments. In total, 3 weeks of exposure to 200 mM NaCl resulted in the down-regulation of the *NHX1* gene in all genotypes as compared to 100 mM NaCl. However, the expression of the *NHX1* gene in the salt-tolerant A-Line was up-regulated under 100 mM NaCl at all three salt exposure times as compared to the control (Figure 3).

OMT gene

The highest level of *OMT* gene transcript was observed in Sahara$_{3771}$, 3 days and 3 weeks after 100 mM NaCl treatment compared to the control and the lowest level was measured in A-Line, 24 hours after 100 and 200 mM NaCl treatments compared to the control (Figure 4). Short-time exposure (24 hours) to 200 mM NaCl treatment significantly reduced the expression level of the *OMT* gene in the salt-tolerant genotypes Sahara3771 and A-Line as compared with the salt-susceptible cultivar Clipper. However, 3 days of exposure to 200 mM NaCl resulted in a significant increase of the *OMT* transcript in the salt-tolerant genotypes over the salt-susceptible Clipper. Three days of exposure to 100 mM NaCl treatment also significantly elevated the level of *OMT* gene expression in Sahara3771 and A-line compared with Clipper. But, under 200 mM NaCl treatment at all three exposure times the level of the *OMT* gene transcript was significantly higher than that in the two salt-tolerant genotypes (Figure 4).

L1s1 gene

The salt-tolerant A-Line showed the highest level of *L1S1* gene expression 24 hours and 3 weeks after treatment with 100 mM NaCl compared to the control followed by Sahara$_{3771}$. Under both conditions, the expression level of the *L1S1* gene was significantly reduced in the salt-susceptible Clipper compared with the two salt-tolerant genotypes. The increase in NaCl concentration from 100 to 200 mM resulted in a significant down-regulation of the L1S1 gene in all genotypes at three salt exposure times, although the level of reduction was not the same in the

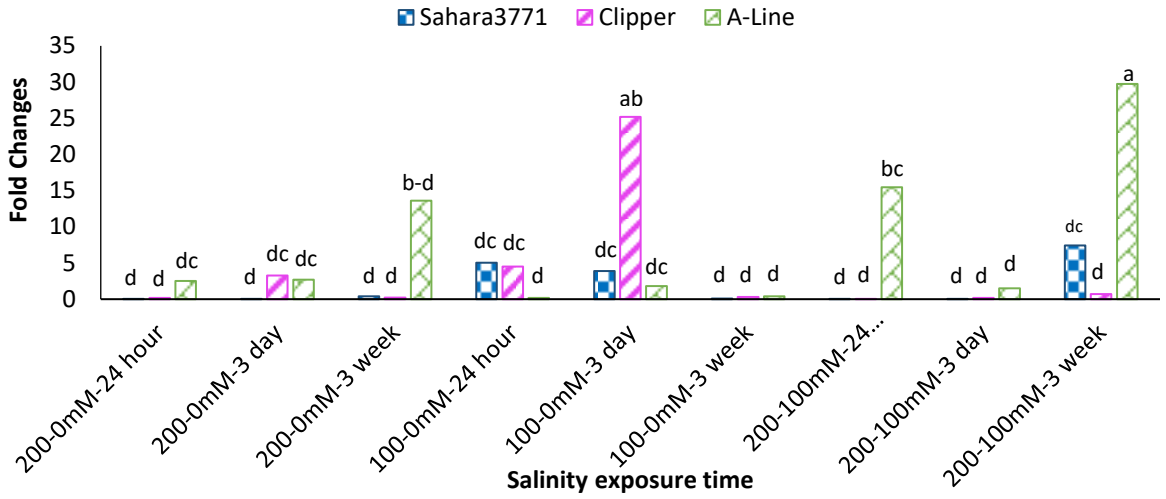

Figure 2. Genotype x salinity x salinity exposure time interaction for the changes in *AOS* gene expression under 100-0, 200-0, and 200-100 mM NaCl; means with different letters are significantly different at the 0.05 level of probability.

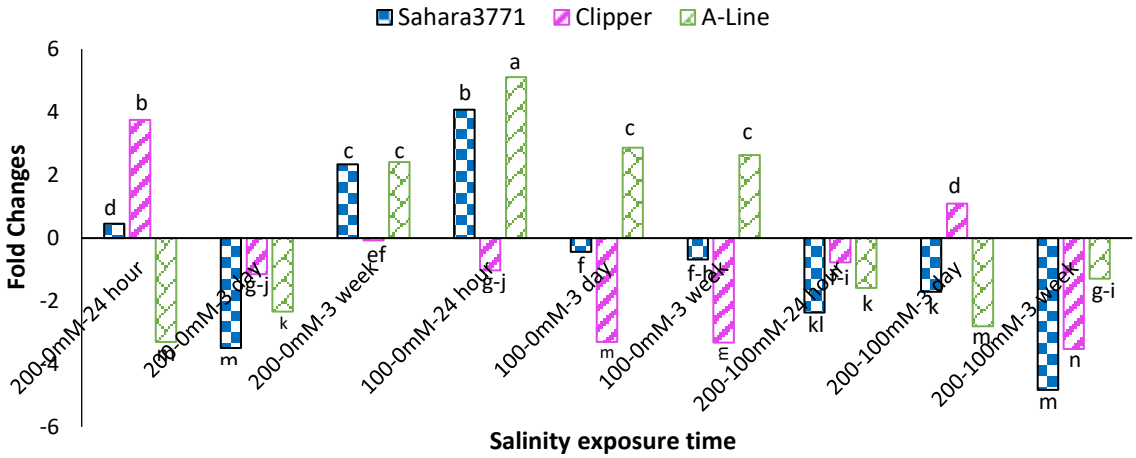

Figure 3. Genotype x salinity x salinity exposure time interaction for the changes *in NHX1* gene expression under 100-0, 200-0, and 200-100 mM NaCl; means with different letters are significantly different at the 0.05 level of probability.

studied genotypes. Similar results were also observed under the 200 mM NaCl treatment as compared with the control (Figure 5).

Discussion

Barley is one of the salt-tolerant crop plants and can maintain its growth under high water and soil salinity, however, there is a large variation in salt tolerance among genotypes and growth stages (Munns *et al*. 1988). It is well-known that barley is highly tolerant to salinity at the germination and sensitive at the seedling stage. Salinity tolerance at the seedling stage can determine the stability of the plant to maintain its productivity under saline conditions (Greenway 1965). In this study, the expression profile of some salt-induced genes were assessed in two salt-tolerant (Sahara3771 and A-Line) and one salt-susceptible (Clipper)

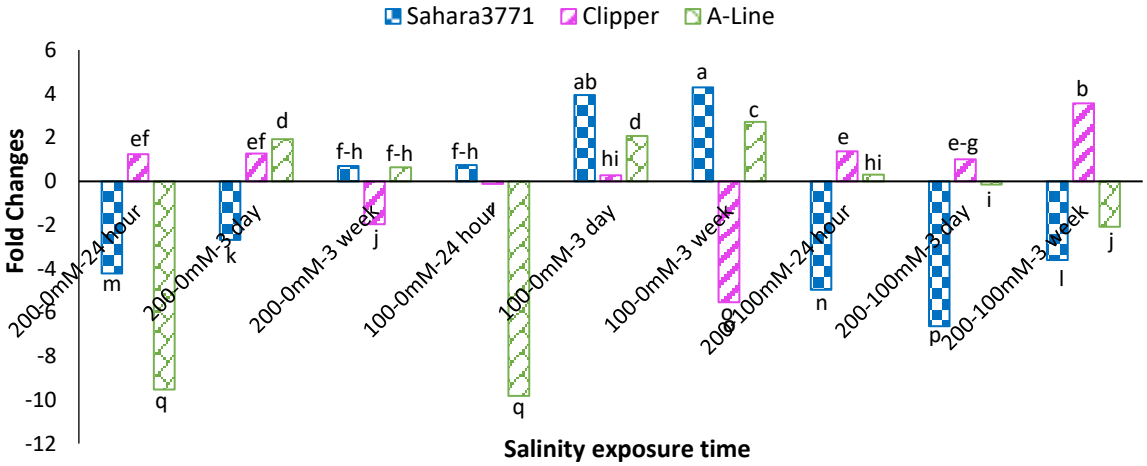

Figure 4. Genotype x salinity x salinity exposure time interaction for the changes in OMT gene expression under 100-0, 200-0, and 200-100 mM NaCl; means with different letters are significantly different at the 0.05 level of probability.

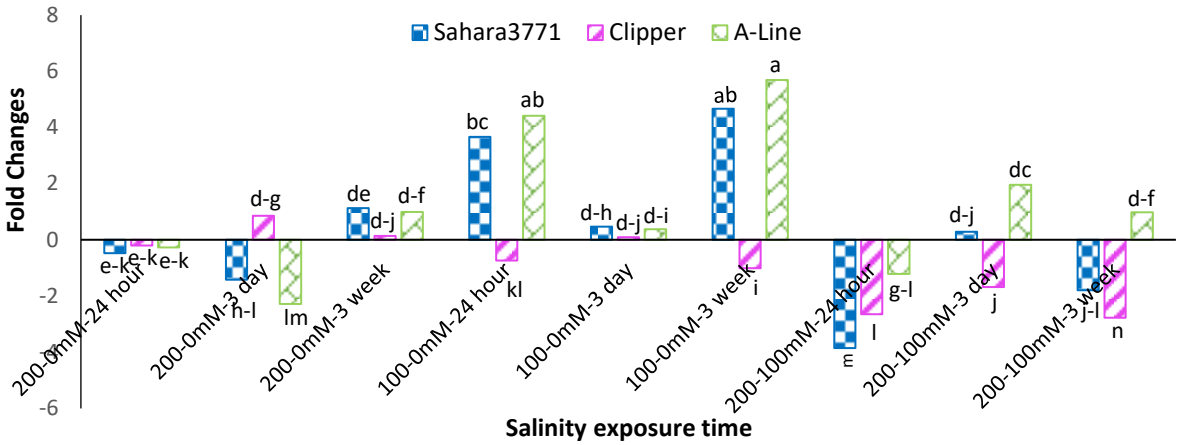

Figure 5. Genotype x salinity x salinity exposure time interaction for the changes in *L1S1* gene expression under 100-0, 200-0, and 200-100 mM NaCl; means with different letters are significantly different at the 0.05 level of probability.

genotypes under different salt treatments and time of exposure to salinity. The up-regulation and down-regulation of the *AOC* gene in the salt-tolerant Sahara$_{3771}$ and salt-susceptible Clipper genotypes, respectively, showed the *AOC* involvement in the salt-stress tolerance in barley. In our study, the expression of the *AOC* gene increased with the increase of salt concentration and exposure time in Sahara$_{3771}$. At all salt exposure times, the *AOC* gene expression increased compared to the control. Zhao *et al.* (2014) reported a significant increase in the expression of the *AOC* gene in the salt-tolerant wheat genotypes under salt stress and concluded that the continuous expression of the *AOC* gene in wheat and Arabidopsis improves salinity tolerance by increasing jasmonic acid levels[9]. Pedranzani *et al.* (2003) reported stable levels of the expression of lipoxygenase (*LOX*) and *AOS* genes coding two key enzymes in the jasmonic acid biosynthesis in

the salt-tolerant tomato genotypes.

Ionic stress is the result of excess Na^+ accumulation in the plant. Na^+ interferes with the K^+ homeostasis, and since it participates in many metabolic processes, maintaining a balanced cytosolic Na+/K+ ratio is essential in the salinity tolerance (Assaha *et al.* 2017). Plants' NHX antiporters catalyze the exchange of Na^+ and/or K^+ for H^+, have an important role in K1 homeostasis, and play a critical role in response to salt stress (Pardo *et al*. 2006). Several studies have demonstrated the role of the Na^+/H^+ antiporter genes overexpression in the salt- tolerance improvement. According to Fukuda *et al.* (2004), the *HvNHX1* gene expression increased up to two folds 24 hours after the 200 mM NaCl treatment. Ligaba and Katsuhara (2010) compared the expression of Na^+/H^+ antiporters including *HvNHX1*, *HvNHX3*, and *HvNHX4* in roots of salt-tolerant and salt-susceptible genotypes of barley and reported their higher transcripts in the tolerant genotypes. In our study, under 100 mM NaCl treatment, the transcript level of the *NHX1* gene in the salt-susceptible Clipper decreased with prolonging the salt-stress treatment but increased in the salt-tolerant A-Line compared to the control. But there were no significant changes in the expression pattern of the gene under the 200 mM NaCl treatment between salt-tolerant and salt-susceptible genotypes.

Methylation of the oxygen atom in the secondary metabolites by OMT plays an important role in stress tolerance (Lam *et al.* 2007). In this study, the expression level of the *OMT* gene decreased in the salt-tolerant A-Line 24 hours after the salt treatment but increased with prolonging the salt treatment duration. In the salt-tolerant $Sahara_{3771}$ cultivar, the expression of the *OMT* gene was also increased under the prolonged exposure to the 100 mM NaCl treatment but decreased under the 200 mM NaCl treatment. This indicates the role of the *OMT* gene in late response to salt stress. Walia *et al.* (2006) and Sugimoto *et al.* (2003) also reported an increase in the level of the *OMT* gene transcripts under salinity stress in barley.

The expression level of *Lls1* gene coding cell death suppressor protein in wheat and tobacco (Tang *et al*., 2013) significantly increased in the salt-tolerant genotypes $Sahara_{3771}$ and A-Line as compared with the salt-susceptible Clipper under the 100 mM NaCl treatment. But 24 hours after the 200 mM NaCl treatment, compared with 100 mM NaCl, *LlS1* expression was higher in A-Line as compared to two other genotypes. Temel and Gozukirmizi (2015) and Ozturk *et al.* (2002) also reported a significant increase in the *LlS1* transcripts in response to salinity.

Conclusion

In this study, the expression pattern of *AOC*, *AOS*, *NHX1*, *OMT*, and *LlS1*genes was analyzed in roots of three barley genotypes with differential responses to salinity after 24 hours, 3 days, and 3 weeks of the exposure to the 100 and 200 mM NaCl treatments. The expression pattern of all genes except *AOC* was significantly affected by salinity x genotype x exposure time to salinity interaction. The *AOC* gene expression in the salt-tolerant $Sahara_{3771}$ cultivar increased by increasing the NaCl concentration. The expression level of the *AOS* gene was significantly increased under

salinity stress at different salt exposure time points indicating the important role of this gene in salt tolerance. Under the 100 mM NaCl treatment, the level of the *NHX1* transcript increased in the salt-tolerant Sahara$_{3771}$ and A-Line genotypes and decreased in the salt-susceptible Clipper indicating the possible role of the *AOS* gene in differentiating the salt-tolerant and salt-susceptible genotypes. It is reported that the ability of salt-tolerant genotypes to sequester Na^+ into sub-cellular compartments and/or maintain K^+ homeostasis may be the cause of their better performance compared to salt-sensitive genotypes. In our study, the expression of the *NHX1* gene decreased under the prolonged exposure to salt stress revealing the role of this gene in the early response to salt stress. The significant increase in the expression of *OMT* and *LlS1* genes in the salt-tolerant Sahara$_{3771}$ cultivar under 100 mM NaCl, compared to 200 mM, showed their involvement in the tolerance to low-salt levels.

Acknowledgments

The authors are grateful for the fund and laboratory facilities provided by the Center of Excellence in Cereal Molecular Breeding, University of Tabriz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

References

Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, and Galili G, 2003. Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. The Plant Cell 15: 439-447.

Assaha DVM, Ueda A, Saneoka H, Al-Yahyai R, and Yaish MW, 2017. The role of Na^+ and K^+ transporters in salt stress adaptation in glycophytes. Frontiers in Physiology 8: 509.

Barkla BJ and Blumwald E, 1991. Identification of a 170-kDa protein associated with the vacuolar Na+/H+ antiport of Beta vulgaris. Proceedings of the National Academy of Sciences of the United States of America 88: 11177-11181.

Brash AR, Baertschi SW, Ingram CD, and Harris TM, 1988. Isolation and characterization of natural allene oxides: unstable intermediates in the metabolism of lipid hydroperoxides. Proceedings of the National Academy of Sciences of the United States of America 85: 3382-3386.

Fukuda A, Chiba K, Maeda M, Nakamura A, Maeshima M, and Tanaka Y, 2004. Effect of salt and osmotic stresses on the expression of genes for the vacuolar H+-pyrophosphatase, H+-ATPase subunit A and Na+/H+ antiporter from barley. Journal of Experimental Botany 55: 585-94.

Greenway H, 1965. Plant response to saline substrates. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare*. Australian Journal of Biological Sciences 18: 763-779.

Hamberg M and Fahlstadius P, 1990. Allene oxide cyclase: a new enzyme in plant lipid metabolism. Archives of Biochemistry and Biophysics 276: 518-526.

Ibrahim RK and Muzac I, 2000. The methyltransferase gene superfamily: a tree with multiple branches. In: Romeo JT, Ibrahim R, Luc V, and Luca VD (Eds). Evolution of metabolic pathways. Pp. 349–384. Pergamon Press, New York, USA.

Isayenkov SV, 2012. Physiological and molecular aspects of salt stress in plants. Cytology and Genetics 46: 302-318.

Lam KC, Ibrahim RK, Behdad B, and Dayanandan S, 2007. Structure, function, and evolution of plant O-

methyltransferases. Genome 50: 1001-1013.
Ligaba A and Katsuhara M, 2010. Insights into the salt tolerance mechanism in barley (*Hordeum vulgare* L.) from comparisons of cultivars that differ in salt sensitivity. Journal of Plant Research 123: 105-118.
Ligabaa A, Katsuharab M, Shibasakab M, and Djirac G, 2011. Abiotic stresses modulate expression of major intrinsic proteins in barley (*Hordeum vulgare* L.). Comptes Rendus Biologies 334: 127-139.
Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25: 402-408.
Munns R, Gardner PA, Tonnet ML, and Rawson HM, 1988. Growth and development in NaCl-treated plants. II. Do Na+ or Cl− concentrations in dividing or expanding tissues determine growth in barley? Australian Journal of Plant Physiology 15: 529-540.
Pardo JM, Cubero B, and Leidi EO, 2006. Alcalication exchangers: roles in cellular homeostasis and stress tolerance. Journal of Experimental Botany 57: 1181-1199.
Pedranzani H, Racagni G, Alemano S, Miersch O, Ramírez I Peña-Cortés H, Taleisnik E Machado-Domenech E, and Abdala G, 2003. Salt tolerant tomato plants show increased levels of jasmonic acid. Plant Growth Regulation 41: 149-158.
Schaller A and Stintzi A, 2009. Enzymes in jasmonate biosynthesis: structure, function, regulation. Phytochemistry 70: 1532-1538.
Shahid M and Jaradat AA, 2013. Barley: a salt tolerant cereal crop. Biosalinity News 14: 1-2.
Sugimoto M, Okada Y, Sato K, Ito K, and Takeda K, 2003. A root-specific O-methyltransferase gene expressed in salt-tolerant barley. Bioscience, Biotechnology and Biochemistry 67: 966-72.
Tang C, Wang X, Duan X, Wang X, Huang L, and Kang Z, 2013. Functions of the lethal leaf-spot1 gene in wheat cell death and disease tolerance to *Puccinia striiformis*. Journal of Experimental Botany 64: 2955-2969.
Temel A and Gozukirmizi N, 2005. Physiological and molecular changes in barley and wheat under salinity. Applied Biochemistry and Biotechnology 175: 2950-2960.
Teaster M and Davenport R, 2003. Na+ tolerance and Na+ transport in higher plants. Annals of Botany 91: 503-527.
Ueda A, Kathiresan A, Inada M, Narita Y, Nakamura T, Shi W, Takabe T, and Bennett J, 2004. Osmotic stress in barley regulates expression of a different set of genes than salt stress does. Journal of Experimental Botany 55: 2213-2218.
Walia H, Wilson C, Wahid A, Condamine P, Cui X, and Close TJ, 2006. Expression analysis of barley (*Hordeum vulgare* L.) during salinity stress. Functional and Integrative Genomics 6: 143-156.
Xu R, Wang J, Li C, Johnson P, Lu C, and Zhou M, 2012. A single locus is responsible for salinity tolerance in a Chinese landrace barley (*Hordeum vulgare* L.). PLoS ONE e43079.
Zhao Y, Dong W, Zhang N, Ai X, Wang M, Huang Z, Xiao L, and Xia G, 2014. A wheat allene oxide cyclase gene enhances salinity tolerance via jasmonate signaling. Plant Physiology 164: 1068-1076.

تغییرات رونویسی ژن‌های *AOS*، *AOC*، *OMT*، *NHX1* و *L1S1* در ریشه ژنوتیپ‌های جو تحت تنش شوری

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چکیده

شوری یک مشکل عمده برای کشاورزی و تولید محصول در سطح جهان است. جو به عنوان گیاهی با تحمل بالا به شوری، تنوع ژنتیکی قابل توجهی در پاسخ به شوری دارد. در مطالعه حاضر، الگوی بیان ژن‌های *AOS*، *AOC*، *OMT*، *NHX1* و *L1S1* در ریشه سه ژنوتیپ جو، Sahara3771، Clipper و لاین اصلاحی پیشرفته، ۲۴ ساعت، سه روز و سه هفته پس از اعمال شوری ۱۰۰ و ۲۰۰ میلی‌مولار NaCl در مقایسه با کنترل مورد بررسی قرار گرفت. تجزیه واریانس نشان دهنده اثر متقابل سه جانبه شوری × ژنوتیپ × مدت زمان معنی‌دار برای کلیه ژن‌های مورد مطالعه غیر از *AOC* بود. بیشترین بیان ژن *AOC*، تحت شوری ۲۰۰ میلی مولار NaCl در NaCl در ژنوتیپ Clipper و کمترین میزان بیان آن، تحت تیمار ۲۰۰ و۱۰۰ میلی مولار NaCl به ترتیب در لاین اصلاحی پیشرفته و Clipper مشاهده شد. بیشترین میزان بیان ژن *AOS*، سه هفته پس از اعمال تیمار ۲۰۰ میلی‌مولار NaCl به دست آمد. حداکثر و حداقل بیان ژن *NHX1* به ترتیب در لاین اصلاحی پیشرفته ۲۴ ساعت پس از تیمار ۱۰۰ میلی مولار NaCl و در Sahara3771 سه هفته پس از اعمال شوری ۲۰۰ میلی مولار مشاهده شد. حداکثر بیان ژن *OMT* در Sahara3771، سه هفته پس از اعمال شوری ۱۰۰ میلی مولار NaCl و کمترین میزان بیان آن در لاین اصلاحی پیشرفته تحت تیمارهای ۱۰۰ و ۲۰۰ میلی مولار NaCl اندازه گیری شد. برای ژن *L1S1*، بیشترین میزان بیان سه هفته پس از اعمال شوری ۱۰۰ میلی‌مولار NaCl و کمترین بیان آن، ۲۴ ساعت پس از اعمال شوری ۲۰۰ میلی مولار در Sahara3771 حاصل شد.

واژه‌های کلیدی: بیان ژن؛ تنش شوری؛ *Hordeum vulgae*؛ Real-Time RT-PCR