The responses of *L-gulonolactone oxidase* and *HKT2;1* genes in *Aeluropus littoralis'* shoots under high concentration of sodium chloride

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ABSTRACT: Salinity is one of the most important abiotic stresses that limit crop growth and production. Salt stress influences plants in two ways: by affecting ion toxicity and increasing osmotic stress. Ion homeostasis, the excretion of Na^+ and using antioxidant systems are the major strategies of salt tolerance in plants. Na^+ and K^+ transporters with enzymes that are involved in detoxification of reactive oxygen species play key roles in salt tolerance in plants. The aim of this study was to investigate the responses of high affinity K^+ transporter2;1 gene (*HKT2;1*) which is involved in regulation of ion homeostasis and *L-gulonolactone oxidase* (*GLOase*) which is involved in the ascorbic acid biosynthesis pathway, under different concentrations of NaCl over different time points in *Aeluropus littoralis* shoots. Results from Real Time PCR data showed that expressions of both genes were influenced by external and internal concentrations of Na^+ and the internal K^+ content. *AlHKT2;1* was significantly upregulated by increasing Na^+ concentration at all time points. Furthermore, its highest expression level in shoots occurred after 6 days in 300mM NaCl in shoots which was 25folds more than untreated shoots. *AlGLOase* expression levels increased 54 h after initiation of salt stress. These results indicate that *AlHKT2;1* and *AlGLOase* respond to different salinity conditions and probably are part of the mechanisms involved in tolerance to high salt concentrations in *A. littoralis.*

KEYWORDS: *Aeluropus littoralis,* Ascorbic Acid, Gene Expression, *HKT* Genes, Salt Stress

INTRODUCTION

The main aim of plant breeding is to increase crops yields and generate new cultivars that tolerate different growth conditions. World population is growing rapidly and it is needed to produce more food annually (12, 25).

Abiotic stresses have significant effects on crops and limit their growth and yield (31). Among abiotic stresses, salinity is a major stress that affects more than 20 percent of irrigated agricultural land worldwide (6). In arid areas, due to improper use of natural resources and applying unsuitable technologies, especially irrigation in crop production, considerable parts of cultivated lands have faced salinity (6, 17). Unfortunately, because of co-occurrence of several types of stresses and a variety of mechanisms that occur simultaneously, in many cases, the mechanisms that crops use tolerate abiotic stresses and maintain their yield, are poorly understood (25). Not only abiotic stresses have complicated signals, but also they have complex responses in plants (12, 21, 25). In addition, the variation in sensitivity of crops to abiotic stresses in different growth stages makes difficulties to apply a reverse genetics approach to study the tolerance of crops (8, 19). Therefore, identifying natural variation of abiotic stress tolerance in varieties, landraces and wild relatives of crops, and studying the traits that are involved in

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tolerance are highly prior approaches (25). Salinity is a soil condition characterized by a high concentration of soluble salts. Sodium chloride is highly soluble and the most abundant salt in saline soils (21) which causes plants to expose Na^+ toxicity (20). High concentration of salt also makes water uptake harder and leads to water deficiency. Osmotic stress of water shortage leads to the generation of reactive oxygen species (ROS) (23). When ROS accumulates in a large amount, cell membranes will damage and chlorophyll degradation will occur that will lead to the decline of net photosynthesis (5, 23). Plants have different strategies to reduce pernicious influences of high concentrated sodium, such as limiting the entry of sodium ion into root cells, Na^+ exclusion from leaf blades, cellular compartmentalization of excessive Na^+ into the vacuole and increasing K^+ uptake to rebalance K^+/Na^+ ratio (6, 21, 30) and improving ROS detoxification systems including enzymatic and non-enzymatic antioxidants, such as ascorbic acid (1, 6, 10, 22). Ascorbic acid (AsA) is the most abundant antioxidant that plays a main role in minimizing the damage caused by ROS in plants. It is found in all plant tissues and its highest concentration is in green matured leaves (10).

The potential of plants to tolerate salt stress is also significantly dependent on K^+ availability (20). Potassium plays an essential role in a wide range of both biophysical and biochemical processes such as enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress tolerance (4, 30). K^+ deficiency can be usually observed under salinity stress. Although most plants are adaptable to different concentrations of external K^+, but the range of physiological concentrations of K^+ is strictly limited in the presence of increasing amounts of sodium (20). The increasing potassium in saline culture solution not only takes up K^+ concentrations in plant tissue, but also decreases Na^+ content. Moreover it was shown that it led to growth increase and salt tolerance. Most reports show that it is not only pure internal concentration of Na^+ that affects on salt tolerance, but the K^+/Na^+ ratio is also more important to determine plant salt tolerance (30). The maintenance of K^+/Na^+ ratio depends on coordinated activities of ion transport systems located at plasma and vacuolar membranes (20). Transporters, belonging to the HKT (high affinity potassium transporter) family play a key role in salt tolerance by regulating transportation of Na^+ and K^+ (4, 12, 30). Two classes of HKT Transporters have been identified with different properties and cell membrane location to regulate Na^+ and K^+ homeostasis (14, 15). Studies on crops HKT transporters indicated that they are more effective in potassium related salt tolerant responses than potassium nutrition (4). This study is carried out due to the role of ascorbic acid and potassium in salt tolerance and the importance of *A. littoralis* as a salt tolerant wild member of *Poaceae*. In the present study, the responses of *L-gulonolactone oxidase* (*GLOase*) a gene in ascrobate synthesis pathway and a gene of HKT family (subclass II) are evaluated under salt stress condition. Moreover some physiological traits related to K^+ balance in shoots of *A. littoralis* are investigated.

MATERIALS AND METHODS

Plant material

The *A. littoralis* seeds were received from ICRASN (Isfahan Center for Research of Agricultural Science and Natural Resources). Seeds were grown in pots containing sand and remained in a growth chamber (12/12 day/night, Temp.18/22). Twenty-day old seedlings were moved to a hydroponic system containing Yoshida solution (11) (pH: 5-6, 16/8 day/night, Temp. 25 °C). The pH of the Yoshida solution in the hydroponic system was checked every day and the medium was refreshed every eight days. The salinity stress was induced on two-month old seedlings by adding NaCl in the solution gradually at a rate of 100 mM per 48 h until reaching 300 mM. Half of these plants were grown as control plants on a non-stress condition. The salinity stress experiment was conducted based on a completely randomized design with three biological replicates. The sampling of shoots was done at 6h/100mM, 6h/200mM, 6h/300mM, 48h/300mM, 6d/300mM and 11d/300mM. The sampling of control plants was also done simultaneously with treated ones.

Sodium and Potassium Concentrations

To determine the concentrations of Na^+ and K^+ in the shoot tissues, the sampling was done simultaneously with qRT-PCR sampling with three biological replicates. After sampling, each sample was weighed and then placed in a 72ºC oven for 48 hours. The measurement of Na^+ and K^+ concentrations was performed according to a wet ashing method. The protocol was adjusted for 15 mg of tissues (7).

RNA extraction and qRT-PCR

RNA was extracted from shoots using Denazist Column RNA Isolation Kit (#S-1020, Iran) followed by DNase digestion (Thermo Scientific DNase I #EN0525, USA). The synthesis of first strand cDNAs was performed in a 20 µL reaction containing 1.5 µg total RNA by using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (#K1622).

GLOase primers were designed according to an EST sequence related to L-gulonolactone oxidase (gb: EE594874.1) and *AlHKT* primers were designed based on the membranous *HKT2;1* of *A. lagopoides* (gb:KP081769.1). In order to normalize the qRT-PCR data, primers for two housekeeping genes: *βActin* and *Elongation factor (Elf)* were designed based on *A. littoralis* gene sequence and EST databases (Table 1).

qPCR reactions were carried out using RealQ Plus Master Mix (AMPLIQON #A314402). Moreover, 5µL of 1/16 diluted cDNA of each samples were used as a template in 20 µL final volume. qRT-PCR was performed on a Bioer thermo cycler machine (Applied Bioer, LineGeneK) with PCR condition of 94ºC/10 min, 40 cycles of 94ºC/10sec, Ta (Table 1) /20sec and 72ºC/25sec. Normalization of qRT-PCR data was done by geometric averaging of multiple internal control genes (28) and the relative gene expression was calculated according to the comparative C_T method (26).

Data analysis

All experiments were performed based on a completely randomized statistical design with three biological replicates. The gene expression analysis was done with two identical technical replicates to increase the reliability of data. Statistical tests of significance were performed using the ANOVA in MINITAB v16 following the Tukey multiple comparison procedure ($\alpha = 0.05$) to separate the means.

Table 1. Gene specific and reference primers used for qRT-PCR in *A. littoralis*

Primer	Primer sequence 5'→3'	Ta (ºC)	Product (bp)
GLOase	GCCAGGGTCTGCCGCTC TCAGTTATTGCCGCTGCTTG	61	136
AlHKT2;1	GAAACCGAGCAACCCTGAC ATCCTAAGTATCTAACGCTC	63	369
βActin	TGCTGGCCGAGACCTTAC GGCGAGCTTTTCCTTGATG	59	113
Elf	ACCTTCTCTGAATACCCTCCTCTG CTTCTCCACACTCTTGATGACTCC	65	90

RESULTS

The Na^+ concentration in treated plants increased from 4.4 to 25 mg g^{-1}DW shoot tissues and then it maintained constantly after ten days of inducing salt stress. The increasing of sodium concentration was not continuous and was constant between 6h/200mM and 6h/300mM NaCl (Figure 1). But, it appeared that the concentration of Na^+ was consistent for about 2.5 mg g^{-1}DW in the shoot of control *Aeluropus* plants at all time points. Also, as it is shown in Figure 2, the K^+ concentration in the shoot tissue of control plants did not have significant changes and maintained about 38 mg g^{-1}DW at all time points. In contrast, concentration of K^+ in treated shoots decreased gradually and reduced from 40 to 17 mg g^{-1}DW.

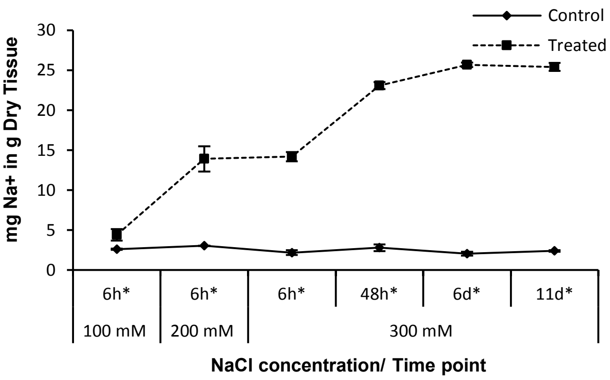

Figure 1. The fluctuation pattern of Na+ concentration in *A. littoralis* shoot in the control and salt treated samples with three biological replicates.
*Shows times from the initiation of the concentration.

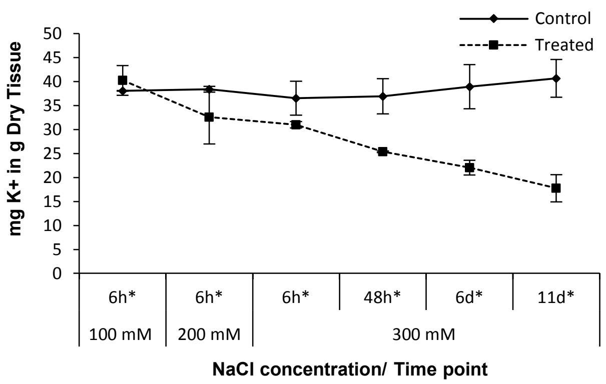

Figure 2. The fluctuation pattern of K^+ concentration in *A. littoralis'* shoot in the control and salt treated samples with three biological replicates.
*Shows time from the initiation of the concentration.

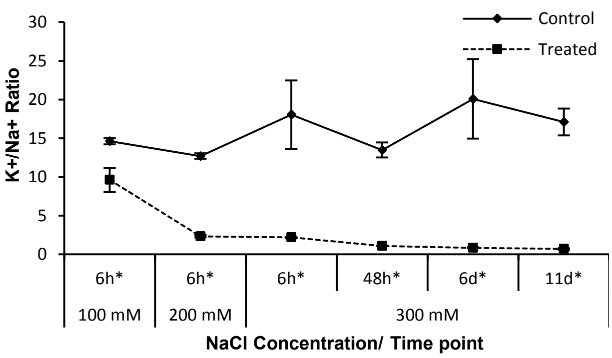

Figure 3. K^+/Na^+ ratio in shoot of *A. littoralis* in the control and salt treated samples with three biological replicates.
*Shows time from the initiation of the concentration.

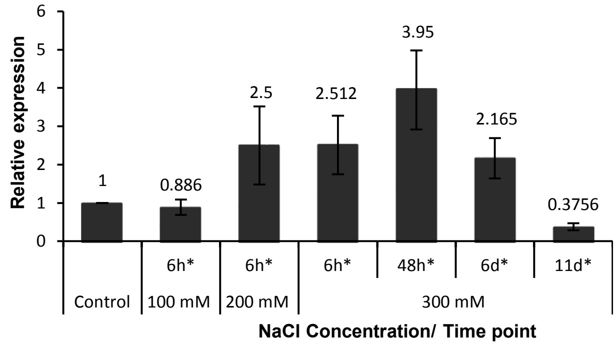

Figure 5. Relative expression pattern of *L-gulonolactone oxidase* in *A. littoralis'* shoot under salinity stress.
*Shows time from the initiation of the concentration.

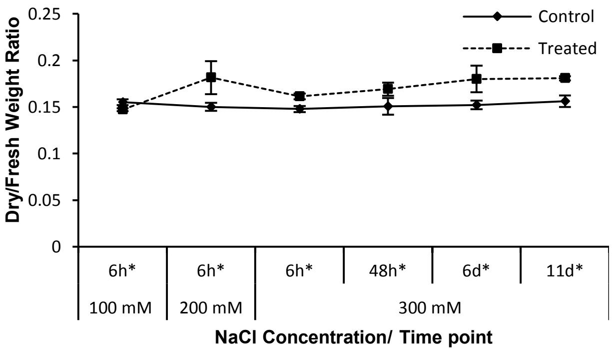

Figure 4. Dry/Fresh weight Ratio in *A. littoralis'* shoot in the control and salt treated samples with three biological replicates.
*Shows time from the initiation of the concentration.

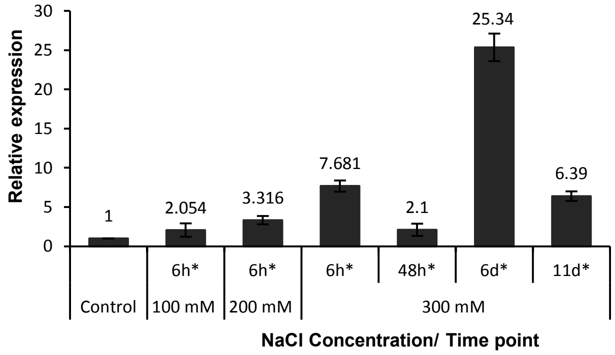

Figure 6. Relative expression pattern of *ALHKT2.1* in *A. littoralis'* shoot under salinity stress.
*Shows time from the initiation of the concentration.

The increasing sodium and decreasing K^+ concentration in the shoot of treated plants led to reduction in K^+/Na^+ ratio significantly (Figure 3).

As it is illustrated, this ratio decreased rapidly for 6h/200mM NaCl, and after that, it decreased slowly. The shoot dry/fresh weight (Dw/Fw) ratio was calculated for both control and salt treated plants. As it is shown in Figure 4, Dw/Fw ratio had been constantly maintained for approximately 0.15 in control plants, however in treated plants it increased from 0.15 to 0.18 for 6h/200mM NaCl and then remained constant.

The expression level of L-gulonolactone oxidase gene (*GLOase*) in shoots was affected by NaCl treatment. The results showed *GLOase* relative expression was constant for 6 h in 100mM NaCl, but then it increased for 48h/300mM NaCl, and it was about 4folds higher in treated plant shoots than untreated ones (Figure 5).

Subsequently, the level of *GLOase* transcript decreased and it reached a third of the level in the controls. Also transcript levels of *AlHKT2* showed a significant increase at all time points in shoots of salt treated plants compared to the controls (Figure 6).

The increase of *AlHKT2* expression in treated shoots was about 2 times more than the controls for 6h/100mM and 48h/300mM NaCl. 6 days after reaching NaCl concentration to 300mM, the mRNA abundance of *AlHKT2* in the shoots of salt treated plants showed the highest level and it was 25.34 times more than in shoots of the controls.

DISCUSSION

In most plants, the redelivery of Na^+ from shoots to roots is done in a small proportion of the Na^+ that is sent from the roots to the shoots, so a large proportion of delivered Na^+ to shoot remains in it, and the concentration of Na^+ will reach the toxic level and cause cell death (21). *A. littoralis* uses the salt excretion strategy to avoid increase of Na^+ concentration in shoot tissues. There are lots of trichomes and salt glands in the surface of *Aeluropus'* leaves that specialized in excreting NaCl (2, 3).

In our study, the initiation of salt excretion was observed five days after the addition of salt in the medium. As the investigated results implicated the concentration of Na^+ was constant during 300mM NaCl treatment which was 25 mg g^{-1}DW. The accumulation of Na^+ in shoots increased by adding NaCl to culture media which is in agreement with the results of Barhoumi *et al.* 2007. As it is illustrated in Figure 1, the constancy of Na^+ content in shoots between 6h/200mM and 6h/300mM NaCl is probably due to beginning salt excretion form leaves and the increasing of Na^+ content after that time points may be due to adding more salt to culture media.

In contrast, it is reported that the concentration of potassium in salt stressed *Aeluropus* shoots decreased by increasing NaCl concentration and this decline was more severe in shoots than roots (3). Similar results were shown in our study. Several studies indicated K^+ starvation affects regulating *HKT* genes (9, 14).

All members of HKT2 are expected to be K^+ transporters (29). In rice, two members of the subclass 2, OsHKT2;1 and OsHKT2;2 act as Na^+-K^+ symporters (16, 29). As previously mentioned, by increasing Na^+ concentration and reducing inner K^+ content, the expression of *AlHKT2;1* showed a significant increase in salt treated shoots. Jabnoune *et al.* used in situ hybridization and indicated HKT2;1 was expressed in rice in vascular tissues, phloem, xylem and mesophyll cells and expression patterns of HKT2*;1* in the leaves were not modified by different growth conditions (16). Our study showed that increasing expression levels of *AlHKT2;1* were variable in different salt concentrations and time points. The role of HKT2;1 is not well understood in shoot tissues, so, enhancing in transcription levels of *HKT2;1* by this plant probably serves to get rid of Na^+ in cells and move to outside of shoot tissues or catalyze K^+ influx to shoots. In some monocot plants like rice and barley, the expression of *HKT2;1* in roots is increased based on low external K^+ availability. *HKT2;1* is also expressed in leaves, but at a lower level than in roots (29). It seems *HKT2;1* has a role in osmoregulatory processes in leaf tissues and bulliform cells (29).

Another reaction to salt stress is osmotic stress which causes disruption in water absorption of plants (6, 21) so, plants adjust their water potential to more negative levels such as salinity increase (24). It was found that the water content was about 13% less in treated plants compared with controls. This decline in water content probably resulted in the decrease of growth rate in vegetative growth of salt treated plants. Water deficiency caused by salt stress, as well as ion toxicity and K^+ starvation strongly affects photosynthesis and causes oxidative stress (6). Most of salt tolerant genotypes apply antioxidative defense systems for the detoxification of ROS (6, 12, 21). Among plant antioxidants, the ascorbic acid acts as an agent in reducing harmful effects of ROS (1, 10, 13). Moreover, AsA plays a vital role in multiple physiological processes including photosynthesis, photoprotection, the control of cell cycles and cell elongation, gene regulation, and senescence (18). L-gulonolactone oxidase (GLOase) is one of the key enzymes as well as the final enzyme that catalyzes the transformation of L-Gulonolactone to ascorbic acid (13, 27). It is reported that in the salt stressed wheat, AsA leaf contents decreased however, adding AsA in culture media enhanced AsA leaf contents and improved growth (1). As it is illustrated in Figure 5, *GLOase* relative expression showed a significant increase at 6h/200mM NaCl and continued until 6d/300mM NaCl in treated shoots. This enhancing may be correlated with reduction in K^+ and water content, and increase in Na^+ concentration. These parameters that were previously mentioned induce oxidative stress that results in increasing ROS. It seems *A. littoralis* plants under these conditions, upregulated *GLOase* to produce more AsA and detoxified ROS and helped plants to tolerate salt stress. As it is reported over expression of *GLOase* in transgenic potato which was done by increasing AsA content, improved salt tolerance (13). Also, engineered A*rabidopsis* for over expression of *GLOase* showed the same results (18). But, we observed a significant reduction in *GLOase* expression level for 11d/300mM NaCl, compared with controls and other time points in salt treated shoots. It is probable because of the recovery pathway of ascorbic acid that produces AsA from Monodehydroascorbate (Monodehydroascorbate is generated by reaction of ASA with ROS) that provides AsA in suitable levels that are needed and it probably has a negative effect on *GLOase* expression (27).

In conclusion, according to the fact that tolerance to salinity is a polygenic trait, and our investigation shows that the expression of *AlHKT2;1* and *AlGLOase* genes are a body of complex reactions of *A. littoralis* to salinity, So, introducing of these genes and regulatory elements of them can be assumed as reliable element to improve other relative crops toward salinity stress.

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REFERENCES

[1] Athar, H. R., Khan, A. and Ashraf, M. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ Exp Bot, 63: 224-231.

[2] Barhoumi, Z., Djebali, W., Abdelly, C., Chaïbi, W. and Smaoui, A. 2008. Ultrastructure of *Aeluropus littoralis* leaf salt glands under NaCl stress. Protoplasma, 233:195-202.

[3] Barhoumi, Z., Djebali, W., Smaoui, A., Chaïbi, W. and Abdelly, C. 2007. Contribution of NaCl excretion to salt resistance of *Aeluropus littoralis* (Willd) Parl. J Plant Physiol, 164: 842-850.

[4] Benito, B., Haro, R., Amtmann, A., Cuin, T. A. and Dreyer, I. 2014. The twins K^+ and Na^+ in plants. J Plant Physiol, 171: 723-731.

[5] Cakmak, I. 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci, 168: 521-530.

[6] Chinnusamy, V., Jagendorf, A. and Zhu, J. K. 2005. Understanding and improving salt tolerance in plants. Crop Sci, 45: 437-448.

[7] Enders, A. and Lehmann, J. 2012. Comparison of wet-digestion and dry-ashing methods for total elemental analysis of biochar. Commun Soil Sci Plant Anal, 43: 1042-1052.

[8] Flowers, T. J. and Yeo, A. R. 1981. Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa L.*) varieties. New Phytol, 88:363-373.

[9] Garciadeblás, B., Senn M. E., Bañuelos, M. A., and Rodríguez-Navarro, A. 2003. Sodium transport and HKT transporters: the rice model. Plant J, 34:788-801.

[10] Gill, S. S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48: 909-930.

[11] Gregorio, G. B., Senadhira, D. and Mendoza, R. D. 1997. Screening rice for salinity tolerance. International Rice Research Institute discussion paper series (22).

[12] Gupta, B. and Huang, B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics, 1-18.

[13] Hemavathi, Upadhyaya, C. P., Akula, N., Young, K. E., Chun, S. C., Kim, D. H. and Park, S. W. 2010. Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. Biotechnol Lett, 32:321-330.

[14] Horie, T., Hauser, F. and Schroeder, J. I. 2009. HKT transporter-mediated salinity resistance mechanisms in Arabidopsis and monocot crop plants. Trends Plant Sci, 14: 660-668.

[15] Horie, T., Yoshida, K., Nakayama, H., Yamada, K., Oiki, S. and Shinmyo, A. 2001. Two types of HKT transporters with different properties of Na^+ and K^+ transport in *Oryza sativa*. The Plant J, 27: 129-138.

[16] Jabnoune, M., Espeout, S., Mieulet, D., Fizames, C., Verdeil, J. L., Conéjéro, G., Rodríguez-Navarro, A., Sentenac, H., Guiderdoni, E., Abdelly, C. and Véry, A. A. 2009. Diversity in expression patterns and functional properties in the rice HKT transporter family. Plant Physiol, 150:1955-1971.

[17] Koocheki, A. and M. N. Mohalati. 1994. Feed value of some halophytic range plants of arid regions of Iran. In Squires, V. R. and Ayoub, A. T. (eds.). Halophytes as a resource for livestock and for rehabilitation of degraded lands. Kluwer Academic Publishers, Printed in the Netherlands, pp 249-253.

[18] Lisko, K. A., Torres, R., Harris, R. S., Belisle, M., Vaughan, M. M., Jullian, B., Chevone, B. I., Mendes, P., Nessler, C. L. and Lorence, A. 2013. Elevating vitamin C content via overexpression of myo-inositol oxygenase and l-gulono-1,4-lactone oxidase in *Arabidopsis* leads to enhanced biomass and tolerance to abiotic stresses. In Vitro Cell Dev Biol Plant, 49: 643-655.

[19] Lutts, S., Kinet, J. M. and Bouharmont, J. 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot, 46:1843-1852.

[20] Maathuis, F. J. M. and Amtmann, A. 1999. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. Ann Bot, 84: 123-133.

[21] Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol, 59: 651-81.

[22] Naliwajski, M. R. and Sklodowska, M. 2014. The oxidative stress and antioxidant systems in cucumber cells during acclimation to salinity. Biol Plantarum, 58: 47-54.

[23] Parida, A. K. and Das, A. B. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf, 60: 324-349.

[24] Qin, J., Dong, W. Y., He, K. N., Yu, Y., Tan, G. D., Han, L., Dong, M., Zhang, Y. Y., Zhang, D., Li, A. Z. and Wang, Z. L. 2010. NaCl salinity-induced changes in water status, ion contents and photosynthetic properties of *Shepherdia argentea* (Pursh) Nutt. Seedlings. Plant Soil Environ, 56: 325-332.

[25] Roy, S. J., Tucker, E. J. and Tester, M. 2011. Genetic analysis of abiotic stress tolerance in crops. Curr Opin Plant Biol, 14: 232-239.

[26] Schmittgen, T. D. and Livak, K. J. 2008. Analyzing real-time PCR data by the comparative C_T method. Nat Protoc, 3:1101-1108.

[27] Smirnoff, N. 2005. Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In Smirnoff, N.(eds). Antioxidants and reactive oxygen species in plants. Blackwell Publishing Ltd, pp 53-86.

[28] Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and Speleman, F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol, 3: 0034.1–0034.11.

[29] Véry, A.-A., Nieves-Cordones, M., Daly, M., Khan, I., Fizames, C. and Sentenac, H. 2014. Molecular biology of K^+ transport across the plant cell membrane: what do we learn from comparison between plant species? J plant physiol, 171: 748-769.

[30] Wang, M., Zheng, Q., Shen, Q. and Guo, S. 2013. The critical role of potassium in plant stress response. Int J Mol Sci, 14: 7370-7390.

[31] Witcombe, J. R., Hollington, P. A., Howarth, C. J., Reader, S. and Steele, K. A. 2008. Breeding for abiotic stresses for sustainable agriculture. Philos Trans R Soc Lond B Biol Sci, 363:703-716.

پاسخ ژن‌های *L-gulonolactone Oxidase* و *HKT2;1* به غلظت‌های بالای کلرید سدیم در اندام هوایی گیاه آلوروپوس لیتورالیس (*Aeluropus littoralis*)

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چکیده

شوری یکی از مهمترین تنش‌های غیرزیستی است که رشد و عملکرد گیاهان زراعی را محدود می‌سازد. تنش شوری گیاهان را از دو طریق سمیت یونی و افزایش فشار اسمزی تحت تاثیر قرار می‌دهد. حفظ تعادل یونی، دفع نمک و استفاده از سیستم‌های آنتی اکسیدانی از تدابیر اصلی تحمل به شوری در گیاهان هستند. انتقال دهنده‌های یون سدیم و پتاسیم همراه با آنزیم‌هایی که در خنثی‌سازی گونه‌های اکسیژن فعال درگیر هستند نقش‌های کلیدی در تحمل به شوری گیاهان دارند. در این تحقیق پاسخ‌های ژن *HKT2;1* درگیر در حفظ تعادل یونی و ژن *L-gulonolactone oxidase* درگیر در چرخه سنتز اسید آسکوربیک در اندام هوایی گیاه آلوروپوس لیتورالیس در غلظت‌های مختلف کلرید سدیم و زمان‌های متفاوت ارزیابی شد. افزایش غلظت‌های درونی و بیرونی سدیم و غلظت درونی پتاسیم بیان هر دو ژن را تحت تاثیر قرار دادند. افزایش معنی‌داری در بیان ژن *AlHKT2;1* با افزایش غلظت سدیم در همه زمان‌ها مشاهده شد و بیشترین سطح بیان در روز ششم در غلظت ۳۰۰ میلی‌مولار رخ داد و به ۲۵ برابر سطح بیان در شاهد رسید. افزایش بیان در ژن *L-gulonolactone oxidase* نیز بعد از ۵۴ ساعت از آغاز تنش مشاهده شد. نتایج بدست آمده اثبات نمود که ژن‌های *HKT2;1* و *L-gulonolactone oxidase* در مواجهه با غلظت‌های مختلف شوری پاسخ‌های بیانی مناسبی دارند و احتمالا قسمتی از مکانیسم‌های درگیر در تحمل به شوری در گیاه آلوروپوس لیتورالیس هستند.

کلمات کلیدی: اسید آسکوربیک، انتقال دهنده یون، بیان ژن، تنش شوری