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Improvement of Salinity Tolerance Indices and regulation of Na^+ and K^+ homeostasis in Hashemi Rice Mutants (Oryza sativa L.)

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Abstract

Salt stress is a serious environmental threat reducing crop yield. Hence, developing any breeding plan requires an understanding of the basic physiology and cell molecular genetic regulation under salinity stress. In this study, we evaluated the effectiveness of gene expression changes on ion homeostasis comprising salt overly sensitive (*SOS1*) and vacuolar Na+/H+ antiporter (*NHX1*) along with ion content measurement and proline content in the rice mutants at Rice Research Institute of Iran in 2018-2019. To survey these realities, tolerant mutant genotypes (*em$_4$hs290* and *em$_4$hs84*) along with Hashemi parent cultivar, IR28 (sensitive), and FL478 (tolerant) seedlings were treated with 100 mM NaCl. Based on the results of growth indices, the seedling length of Hashemi cultivar and IR28 decreased considerably about 44.7%, and 44.2% reduction to that of the control, and the leaves progressively yellowed. Results showed that proline content and K^+ and K^+/Na^+ ratio increased about ~2–3-fold higher in the tolerant genotypes than in the susceptible ones. Also, the overall amount of the *OsNHX1* and *SOS1* expression increased in tolerant genotypes compared to the susceptible ones. Accordingly, the compatible solute accumulation significantly advanced resulting in improvement of ionic homeostasis and probably suppresses the stress. Moreover, the variable pattern of gene expression in the two salt-tolerant mutants (*em$_4$hs290* and *em$_4$hs84*) and Hashemi parent showed that the induced mutation could increase the salt-tolerant in mutant genotypes through ionic and osmotic homeostasis. Generally, these tolerant mutant genotypes could be applied to develop salt-tolerant varieties in rice breeding programs which can bring on production sustainability.

Keywords: Gene expression, Mutation, Rice, Salinity, Stress Index.

Introduction

Increased food production is undeniably necessary to meet the nutrient needs of the growing world population to 9 billion in the 2050s. Salt stress as one of the most intense environmental problems

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influences the potential of plant production and induces significant crop loss worldwide (Zhang et al., 2018). Previous studies show that climate changes and inappropriate irrigation practices enhance salt accumulation in soil (Pitman & Läuchli, 2002; Roy et al., 2014), and lead to incurring significant costs of approximately $12 billion per year globally (Pitman & Läuchli, 2002). Identifying traits related to salinity tolerance is required to improve this trait and high-yielding genotypes (Munns & Tester, 2008). Most cultivated rice varieties are susceptible to salinity stress; their salinity threshold is three dSm^{-1} (Chinnusamy et al., 2005; Munns, 2005). Despite numerous attempts and various strategies to develop salinity tolerance in rice, the achievements are relatively moderate (Hoang et al., 2016). So, the breeding cultivars of salinity tolerant with the ability to grow in salt soils is critical for sustainable agriculture and food security (Zhang et al., 2022). Though achievement of salinity-tolerant rice cultivars is time-consuming (taking at least 6 to 7 years), laborious, and incompetent through traditional breeding programs (Sun et al., 2017) (Wang et al., 2019). Therefore, mutation breeding approach is a fast and critical method for creating genetic diversity in favorite traits (Ahloowalia et al., 2004), containing the development of tolerant cultivars to biotic and abiotic stress and agronomic traits improvement (Masoabi et al., 2018). In many research, salinity-tolerant rice mutants were created using combining induction mutations and in vitro selection (Huong et al., 2020; Yunita et al., 2020; Zhang et al., 2019).

Plants have developed several mechanisms to tolerate salt stress. The most effective mechanism of salinity tolerance is selective regulation of Na^+ uptake and efflux systems with limitation in sodium ions (Na^+) admission into the cytosol (Ji et al., 2013; Zhu, 2003). Because of resemblances in ionic characteristics, Na^+ can contest with and absorb through potassium ions (K^+) uptake systems. Na^+ efflux from roots and Na^+ sequestration within vacuoles would occur if cytosolic Na^+ levels in plants decrease (CRAIG PLETT & Møller, 2010). Moreover, proline, as an important osmolyte, plays in the modulation osmotic potential of cells under drought and salinity stresses in some plants (Bagheri et al., 2023). Accumulation of proline could enhance plant salinity tolerance by decreasing the destructive effect of salinity. Many studies demonstrated that the novel salt-tolerant rice genotype increased proline content under salt stress (Nahar et al., 2022, 2023; Koc et al., 2024). The mechanism of salt tolerance is a complicated trait containing several mechanisms of physiological and biochemical (Ganie et al., 2019; Rasel et al., 2021).

Both Salt Overlap Sensitive genes in rice (*OsSOS1/OsNHX7*) and Arabidopsis (*AtSOS1/AtNHX7*) encode a plasma membrane Na^+/H^+ antiporter, which has principal roles in Na^+ extrusion in the

roots under salinity conditions (Chinnusamy et al., 2005; Ding & Zhu, 1997). Therefore, Na^+/H^+ antiporters of SOS1 and NHX1 have contained the principal role of Na^+ exclusion and sequestration, to decrease salinity toxicity in the plant. So, Na^+ uptake in plants is done using several ion channels and carrier-type transporters, which have been identified. The cation/H^+ exchange through membranes is catalyzed by identified NHX-type antiporters (Bassil & Blumwald, 2014; Jiang et al., 2010). The compartmentation of Na + ions into the vacuole is mediated conventionally by the function of tonoplast (vacuole membrane) localized NHX-type.

Six family members of rice NHX-type antiporter were recognized as associated with three subclasses with various cellular localizations: SOS1 is located in the plasma membrane (Martínez-Atienza et al., 2007) and five other intracellular members comprising *OsNHX1* up to *OsNHX4* and *OsNHX5* are located in the tonoplast and prevacuolar compartment, respectively (Fukuda et al., 2011; Fukuda et al., 1999). The previous studies revealed that some plant species advanced salt and drought stress tolerance via *NHX1* overexpression (Xue et al., 2004; Xiao et al., 2009; Zhang & Blumwald, 2001; Liu et al., 2010 & Ohta et al., 2002) and K^+ homeostasis effectively adjusts through *NHX1* and *NHX2* (Andrés et al., 2014; Barragán et al., 2012). The plasma membrane Na^+/H^+ antiporter, Salt Overly Sensitive 1 (*SOS1*), is the most characteristic Na^+ efflux protein in plants. (Shi et al., 2000). The Na^+ effluence at the root surface and Na^+ transport from root to shoot are mediated by SOS1 (Tester & Davenport, 2003). Then the K^+/Na^+ ratio is advanced appropriately in leaves as the significant site for performing metabolic activities. So, the SOS3/SOS2 complex activates the Na^+/H^+ antiporter promotion and the expression regulation of the *SOS1* gene for the activity of *SOS1* (Sánchez-Barrena et al., 2005). Mutants lacking in SOS2 and SOS3 exhibit salt-sensitive phenotypes analogous to *SOS1* plants (Zhu, 2001).

This study revealed how the selected EMS mutants improved salinity tolerance. So, the following objectives were investigated under salt stress: (i) evaluation of differences in Na^+/K^+ homeostasis among the mutant rice genotypes and control; (ii) Clarifying some of the morphological and biochemical traits at different time points and the expression levels of key genes (*NHX1* and *SOS1*) concerning ionic responses and their rules in the defense strategies in the mutant rice genotypes (*em$_4$hs290* and *em$_4$hs84*) in comparison with the control rice cultivars; (iii) Revealing the importance of mutation in improving tolerance to salt stress in mutant rice genotypes.

Material and method

Experimental materials selection

For evaluation of salinity tolerance in the mutant genotypes, we first surface sterilized the seeds of Salt-sensitive and tolerant varieties (IR28 and FL478), two EMS-derived salt-tolerant mutants (*em4hs290* and *em4hs84*) in rice (*Oryza sativa, cv. Hashemi*) at Rice Research Institute of Iran (Rasht, Guilan province, Iran) in 2018-2019. Forty healthy seeds were placed equally on filter paper in a 9-cm-diameter Petri dish. After four days, the germinated seeds were transferred on perforated Styrofoam floats with a net bottom suspended on buckets in a hydroponic system with Yoshida solution (Yoshida & Coronel, 1976) in the greenhouse. Plants were grown in a greenhouse under structured conditions (25°C, 60% humidity, 16/8 hour light/dark cycle). The nutrient solutions were exchanged every five days. After 14 days, seedlings were grown under normal conditions, then one compartment of the nutrient solutions was treated with 100 mM NaCl solutions (about ECiw 10 dS m^{-1}) based on the test results to determine the appropriate salt concentration of Hashemi rice (Khazaie et al., 2023), while control plants were supplied in a nutrient solution without NaCl (ECiw 0 dS m^{-1}). A part of the samples was immediately frozen in liquid nitrogen at four-time points 6, 24, 48, and 72h after the onset of salinity treatment, chosen to capture both early and late stress responses, and then kept at −80 °C until RNA extraction. Another part of the samples was kept for biochemical, Physiological, and growth parameters measurements. Ten seedlings were collected for each time point measurement.

Physiological and growth parameters

The root length, and also length of six rice seedlings were measured with a ruler after 14 d of salinity stress. After removing three seedlings of roots, stems and leaves, the fresh weight of each seedling and root was calculated on a scale (± 0.001 g). Besides, the seedlings were oven-dried at 40 °C for three days following measurement of dry weights of the seedlings and roots.

Proline concentration measurement

Fresh shoot tissues of the rice genotypes were collected at different time points (6, 24, 48, and 72h after salinity stress), and then proline content was measured according to the protocol instruction provided by (Bates et al., 1973). The Proline concentration was estimated by a standard curve (L-proline) and read as micrograms per gram of fresh weight.

Determination of Na^+ and K^+ content

The potassium (K^+) and sodium (Na^+) concentration in shoot tissues was determined using the method developed by (Isaac & Johnson Jr, 2019). After drying and grinding of plant samples, each sample was digested on the digestion unit including a di-acid mixture (20 ml) containing HNO3 and HClO4 acid (9:4) (Turbotherm, Gerhardt analytical systems, Germany) according to the established procedure by (Tandon, 1995). The concentration of K^+ and Na^+ samples and the standard solutions were determined by a flame photometer (Systronics FF128).

RNA extraction and semi-quantitative RT-PCR

14-old-day leaf samples were collected after 6, 24, 48, and 72h for gene expression analysis. Total RNA was extracted by utilizing RNX-plus TM (Synaclone) and measured using Thermo Scientific NanoDrop 2000 (USA). cDNA synthesis was constructed according to the instructions of Thermo Scientific™ Fermentas First Strand cDNA Synthesis Kit. The housekeeping gene UBQ10 in rice (accession no. AT4G05320) was used as the reference gene (Yang et al., 2012).

The specific gene primers related to ionic homeostasis were designed by Primer3 Input (version 0.4.0) (Table 1). The Real-Time PCR reactions were performed in the iQ5 (Bio-Rad, Palo Alto, USA), and PCR programs were done as follows: at first, an initial denaturation at 95℃ for 4 min, then samples were located in a cycling regime of 45 cycles at 95 ℃ for 30 s, 58-60 ℃ for 30 s and 72 ℃ for 30 s. The Quantitative real-time PCR (qRT-PCR) method and data analysis were performed by the method provided by Pfaffl and colleagues (Pfaffl et al., 2002).

Table 1. The applied specific primers for Q Real-time PCR in rice genotypes.

Gene	Primer sequence (5'→3')	Product length (bp)	Melting temperature	Tm (ºC)	NCBI accession number
Ubiquitin10	F- TGGTCACTAATCAGCCAGTTTGG R- CACCACAAATACTTGACCAACAG	81	60.65 61.01	59	XM_015769228.1
SOS1	F- ACTTGGACGATGAGCCTGTG R- ATTTAGAAGCCGCACACGGA	98	60.04 60.04	58	XM_015763865.2
OsNHX1	F- TCCAGCCTCCGGATGCT R- ATCAGCGCGTCGTCGAA	77	60.00 59.46	60	XM_006658017.2

Data analyses

Statistical analysis was performed in a random complete factorial with three repeats using one-way ANOVA followed by Tukey's HSD test to determine significant differences between treatment groups ($p < 0.05$) through SAS $_{ver9.2}$ software.

Results

Effects of salt stress on rice seedling growth

After treating the two-old-week seedlings with 100 mg NaCl, the onset of morphological damage was observed after three days. On the seventh day, morphological changes such as a rolling leaf, whitening of the leaf tip, growth limitation, and finally death were found in the plants, while the plants grew normally under control conditions. The lentgh of IR28 and Rice Hashemi seedlings was considerably decreased (respectively about 44.7%, and 44.2% reduction to that of the control), and the leaves progressively yellowed. Nevertheless, the mutant genotypes (*em$_4$hs290* and *em$_4$hs84*) and FL478 were not almost affected after 3 to 5 days. IR28 and Hashemi genotypes gradually died after 3 to 5 days of salt stress, whereas the older leaves of the mutant genotypes and FL478 just started to yellow. The mutant genotypes and FL478 were able to grow and produce new leaves after seven days of the salinity stress (Fig. 1). Thus, the genotype's survival was graded as *em$_4$hs290* > *em$_4$hs84* > FL478 > Hashemi cultivar > IR28 (Table 2). These results illustrated that the mutant genotypes and FL478 are more tolerant than the Hashemi cultivar and IR28.

Table 2. Effect of salt stress on morphological traits of the mutant rice genotypes and control

Rice genotypes	Stem length (cm)		Root length (cm)		Stem fresh weight (g)		Root fresh weight (g)		Stem dry weight (g)		Root dry weight (g)	
	Control	10 dS	Control	10 dS	Control	10 dS	Control	10 dS	Control	10 dS	Control	10 dS
Fl478	51.9	24.75	10.5	11	0.46	0.125	0.51	0.185	0.07	0.03	0.02	0.015
IR28	42.5	19	8	6.3	0.405	0.07	0.34	0.115	0.11	0.02	0.03	0.01
Hashemi	48.4	21.5	9.1	7.5	0.43	0.1	0.475	0.125	0.065	0.02	0.02	0.01
em3hs84	55.5	30	12.95	10.75	0.9	0.185	0.835	0.275	0.135	0.03	0.04	0.02
em3hs290	54.2	37.5	13.8	13.2	1.11	0.33	0.81	0.645	0.15	0.06	0.03	0.025

Fig. 1. Growth and developmental status of the studied genotypes under salinity stress. A: The rice genotypes at normal conditions B: The mutant rice genotypes under salinity stress C: IR28 and Hashemi under salinity stress.

Ion content changes in shoots of the genotypes under salt stress

The K^+ and Na^+ content and K^+/Na^+ ratio displayed a significant ($P<0.01$) difference among the mutant genotypes and the control cultivars under the salinity stress. Regardless of the type of tissues and genotypes, salinity stress reduced K^+ content. The K^+ content in the leaves of *em$_4$hs290* and *em$_4$hs84* reached from 4.05 % to 4.25 %, which is higher than IR28 and Hashemi cultivars (2.81 and 3.11%) (Fig. 2a). The Na^+ content in both mutants was 0.23%, which is lower than that of IR28 and Hashemi cultivar (0.59 and 0.48%) (Fig. 2b). The Na^+ accumulation in leaves and stems of all evaluated genotypes was extremely higher or lower under stress conditions. Plants may develop different approaches to achieve salinity tolerance by regulating via regulation of osmotic adjustment, tissue tolerance adaptation, restriction in Na^+ ion loading and accumulation in tissues, or Na^+ exclusion from the cytosol (Shabala et al., 2010; Cuin et al., 2011; Shahzad et al., 2022). Moreover, the potassium/sodium (K^+/Na^+) ratio in different parts of all genotypes reduced under salinity stress. The K^+/Na^+ ratio in R28 and Hashemi cultivars (0.22 and 0.15% respectively) was higher than that of the mutant genotypes (0.051 to 0.054) and FL478 (0.062). The ability of salinity tolerant genotypes to decrease Na^+ net uptake and maintain K^+ uptake triggered desirable K^+/Na^+ratio in all tissues (Fig. 2c).

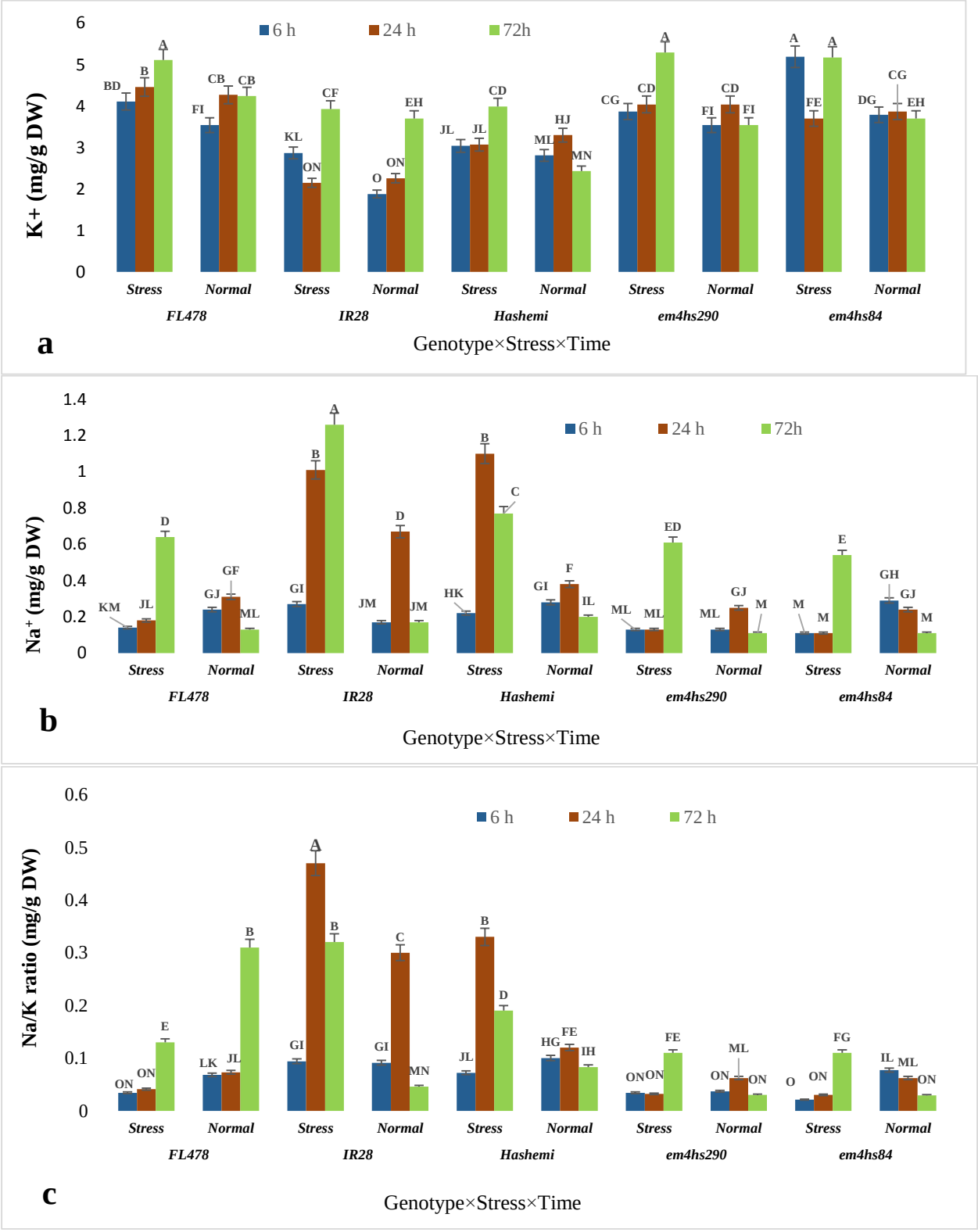

Fig. 2. Mean comparison of salt stress effects on cellular ion and mineral accumulation in shoot. Changes in Na concentration (a), K concentration (b), and Na/K ratio (c) in rice genotypes under 100 mM NaCl treatment over time using Tukey's test (P< 0.05).

Physiological modulations under salt stress

The compatible solute accumulation such as proline, is one of the most important mechanisms involved in crop plant response to abiotic stresses like drought and salinity (Singh et al., 2018). The results illustrated that IR28 and Hashemi cultivars accumulated lower proline in comparison to the mutant genotypes and FL478 (Fig. 3a). After applying the stress, the proline concentration increased in *em4hs290* (0.96) and *em4hs84* (0.77) rather than IR28 (0.42) as compared to the control plants (Fig. 3a). Therefore the tolerant plants accumulated proline to survive against the salt stress, compared to the respective controls (Fig. 3b).

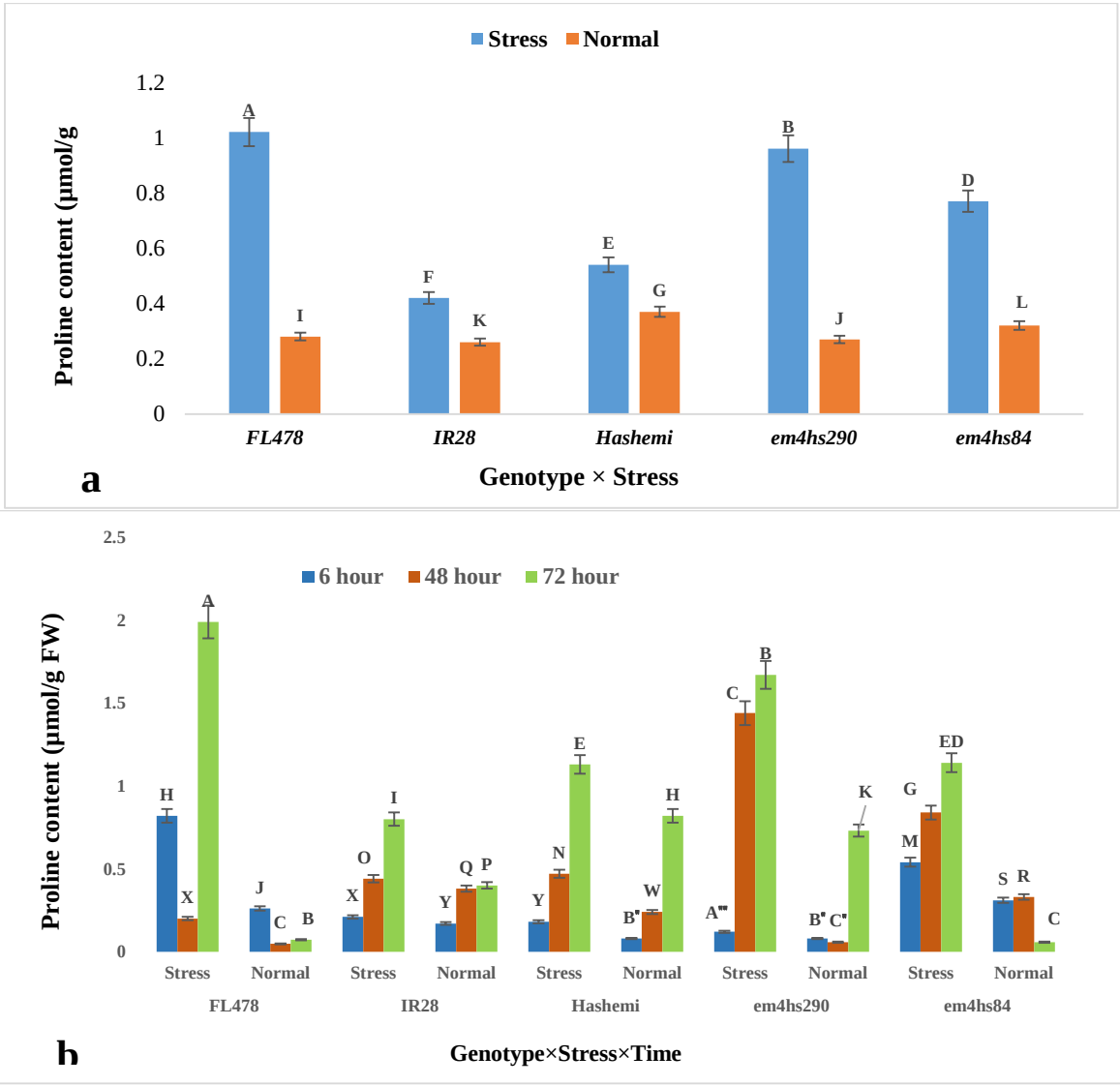

Fig. 3. Mean comparison of proline content in the rice genotypes (a) and the interaction of genotype×stress×time (b). Different letters in each rice line show significant differences using Tukey's test at (P< 0.05).

Effect of salinity stress on the expression of ion transport-related genes

Based on QRT-PCR results, the changes in relative gene expression levels confirmed the relationship between ion transport regulation and salt stress (Fig. 4).

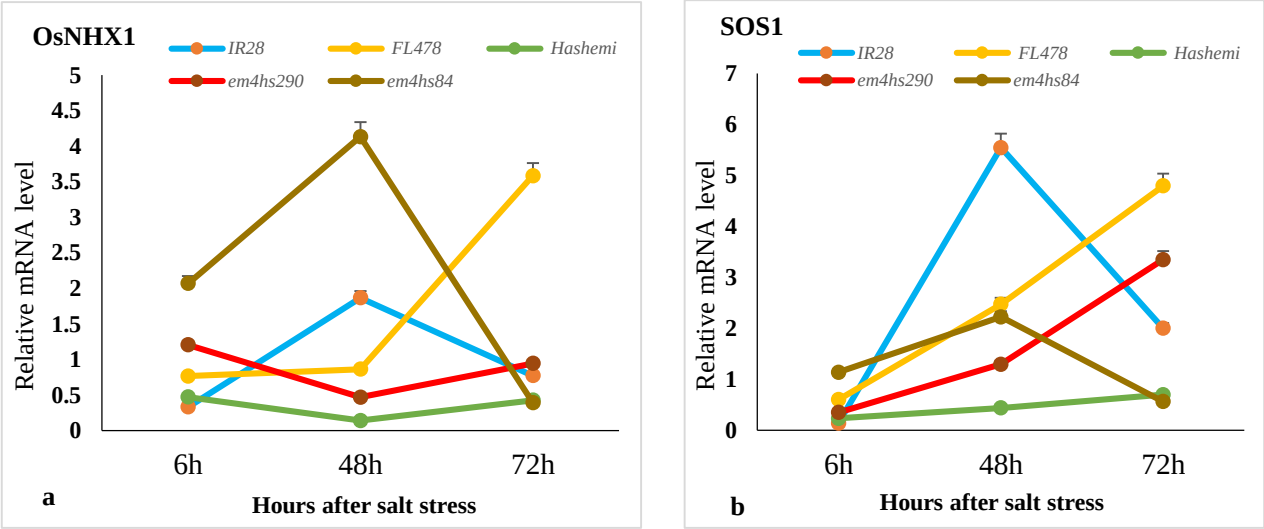

Fig . 4. Relative gene expression analysis of the ion transport-related (a: *OsNHX1* and b: *SOS1*) genes by Q real-time PCR among three biological replicates in the rice genotypes. The studied reference gene was Ubiquitin10. Expression levels of genes in salt-stressed plants were normalized concerning those in non-stressed plants.

The induction of *OsNHX1* and *SOS1* expression was slightly higher in the tolerant genotypes, and reached a peak at 72 h after stress initiation (Fig. 4). The expression of *SOS1* increased in IR28 and Hashemi rice cultivars during 48h and 72h. However, the expression of *OsNHX1* elevated in IR28 at 48 h after the stress induction (Fig. 4a). The expression of *OsNHX1* and *SOS1* enhanced in tolerant genotypes compared to the susceptible genotype after 6 h of salinity stress. Despite this, after prolonged stress, a significant difference between tolerant and susceptible genotypes was detected. However, the expression levels of *SOS1* increased in *em4hs290* and FL478 (Fig. 4b). The tolerant genotypes also demonstrated early and higher expression in ion transport-related genes (*NHX1* and *SOS1*) compared to the sensitive genotypes. After 6 h, the expression of genes (*OsNHX1* and *SOS1*) started to increase and presented the most meaningful increase in SOS1 expression, with above a 10-fold increase in shoot tissue after 48 h of salt stress (Fig. 5). After applying salt, the expression levels of *OsNHX1* and *SOS1* genes significantly up-regulated in all genotypes. Remarkably, expression levels of *SOS1* and *OsNHX1* were considerably up-regulated

under 48 h salt treatment in all genotypes. It is generally recognized that Na^+ and K^+ transporter gene families such as *SOS* and *NHX* play a significant role in cellular or whole plant Na^+ exclusion, sequestration, and planta movement (El Mahi et al., 2019; Martínez-Atienza et al., 2007; Shabala & Munns, 2017; Shabala et al., 2010).

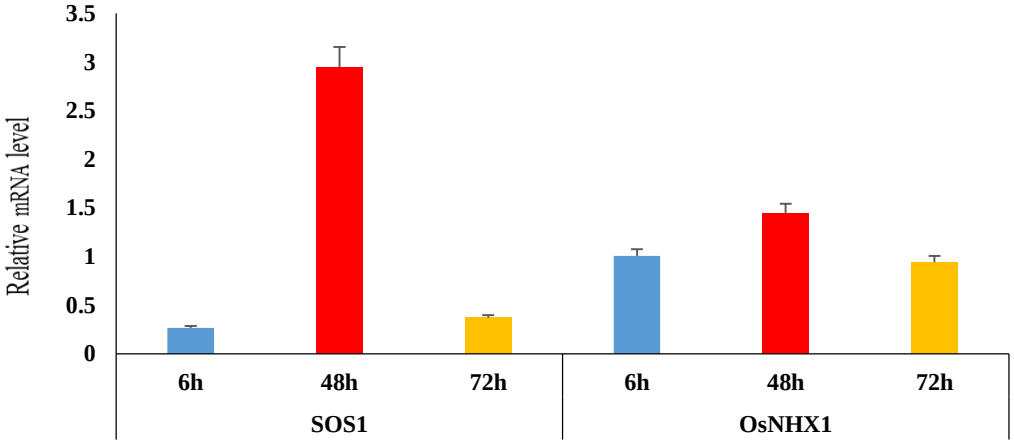

Fig . 5. The relative gene expression levels of *OsNHX1* and *SOS1* genes in the studied genotypes under salinity stress conditions by Q real-time PCR among three biological replicates in the rice genotypes. The applied reference gene was Ubiquitin10.

The results showed that the *SOS1* transcript profile was similar among all genotypes as they all appeared to have an expression peak at 48 h after stress. While a significant difference was not observed in *SOS1* expression among genotypes after 6 h salinity exposure (Fig. 4b). Salt-tolerant genotype (FL478) showed ~5–6-fold higher expression of *SOS1* when compared to Hashemi rice at 48 h and 72 h after stress. Also, *em$_4$hs290* and Fl478 reached the highest level of *SOS1* transcript ~3–4-fold transcript levels after 72 h salt treatment. However, IR28 and *em$_4$hs84* mutant genotypes showed similar expression patterns under salt stress, nevertheless, the results illustrated a peak of expression level at 48 h after stress and then showed a sharp decrease after salt treatment at 72 h. The higher expression of the *SOS1* gene was detected in the salt-resistant genotypes, while the expression was decreased considerably in the sensitive genotypes (Fig. 4b).

The results exhibited that the *SOS1* transcript levels were dissimilar among five rice genotypes. IR28 and *em$_4$hs84* showed the highest *OsNHX1* expression after salt stress, reaching a peak 48 h after stress initiation and then decreasing at 72 h after stress. Hence, *em$_4$hs290* mutant genotype, Hashemi rice, and Fl478 showed a decrease at 48 h after stress. The results illustrated that a peak in the expression levels of *SOS1* in FL478 and *em$_4$hs290* was more intense than in Hashemi cultivar

at 48 h after stress and then showed a sharp increase after salt treatment at 72 h. Moreover, the resistant genotypes under the control conditions (0 mg NaCl) indicated a higher expression in *OsNHX1* transcript levels compared to the salt-susceptible variety IR28.

In general, the mutant tolerant genotypes were able to have had different physiological and biochemical responses to salt stress with comparison to control cultivars: *em$_4$hs290* and *em$_4$hs84* showed a K^+ high content in leaves, a high proline content, and absorbed more K^+ in the response to salinity. Moreover, The two mutants (*em$_4$hs290*, *em$_4$hs84*) showed up-regulation of responsive genes and the inhibition of ion transport.

Discussion

Salt stress restrains crop production through various processes including ionic, osmotic, and oxidative stress. Its direct target is cytoplasmic concentrations via increasing sodium and chloride and disruption of membrane ion transport on cellular processes that could inhibit plant growth and development. The results exhibited that genotype and salinity stress caused meaningful effects on morphological, physiological, and molecular responses. Hence, the results indicated that the mutant genotypes and FL478 might use different mechanisms in response to stress; because each rice variety could employ one or two salt-tolerance mechanisms (Ganie et al., 2019) to decrease the damage of salt stress by adjusting physiological and biochemical mechanisms (Pental, 2019; Rasel et al., 2021; Peng et al., 2016).

The salinity stress imposed at the seedling stage led to a significant decrease in growth indices in Hashemi rice and IR28 cultivar under salinity stress intensively, while the responses of mutant genotypes and FL478 to salinity stress varied also observed by Zhang et al., (2018) and Khatun et al., (2023).

As discussed in the results section, Na^+ content significantly increased at all time points. This aligns with previous studies by Zhang et al. (2022), indicating a common stress response. The results also showed that the mutant genotypes could manage the ion uptake in the shoots by absorbing more K^+, decreasing the Na^+ concentration and the Na^+/K^+ ratio. These results are in accord with the findings of Nakhoda et al (2012) and Shahzad et al (2022) (Table 3).

The expression of *SOS1* was up-regulated under salinity in tolerant genotypes in leaf tissues, which could be associated with simplifying the exclusion of toxic Na^+ into root apoplast and their ability to maintain a higher K^+/Na^+ ratio of leaves (Figs. 2, 3a) (Shahzad et al, 2022). An increase in the

relative expression of *OsNHX1* was observed in mutant genotypes and a decrease in IR28 relative expression at 6 h after stress. The high reaction was observed in the tolerant genotypes at the earliest hours after stress, whereas the sensitive genotype response varied with time. Therefore, these results revealed that the sensitive genotype takes a longer time to operate stress-responsible genes, which could be a factor for delayed hemostasis and high damage due to salinity stress. Nevertheless, it has already been demonstrated that the expression patterns of NHX-type genes in salt-sensitive and salt-tolerant plants are varied mainly, rather than probable differences in gene sequences (Hamada et al., 2001; Gong et al., 2005; Zhang et al., 2008; Xia et al., 2002;).

Therefore, the results of the experiments obviously demonstrated that mutant genotypes could perform better than IR28 and Hashemi rice under salinity level of 10 dSm-1 at early seedling stage. In rice, the salt tolerance in the seedling stage varies with the salinity tolerance during the other growth periods and may not be associated with each other (Jenks et al., 2007; Singh et al., 2010). For this reason, we need to characterize the rice salinity tolerance during the entire growth period in the field. These results contribute to understanding salinity tolerance mechanisms in rice by highlighting the role of SOS1 and NHX1 gene expression in Na+ and K+ homeostasis. However, further research is needed to elucidate these pathways fully. The results of this research and other studies illustrated that the breeding by mutation method has the potential to create new cultivars with desirable morphological characteristics. This study highlights the potential of Hashemi rice mutants in improving salinity tolerance. Considering the results from all of the experiments, EMS effectively induced variation in salt tolerance in Hashemi rice and was a successful method for developing salt-tolerant varieties and yield sustainability in rice.

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بهبود شاخص‌های تحمل به شوری و تنظیم هموستازی یون‌های Na^+ و K^+ در موتانت‌های برنج هاشمی

لیلا خزائی، و رضا شیرزادیان خرم‌آباد

چکیده

تنش شوری یک تهدید بزرگ زیست محیطی برای توسعه عملکرد محصول است. از این رو، توسعه هر طرح اصلاحی نیاز به درک اولیه فیزیولوژی و ژنتیک سلول‌های تحت تنش شوری دارد. در این مطالعه، ما پروفایل بیان ژنهای موثر بر هموستازی یونی شامل حساسیت بیش از حد نمک (SOS1) و آنتی پورتر Na^+/H^+ واکوئلی (NHX1) همراه با اندازه‌گیری محتوای یون و محتوای پرولین در ژنوتیپ‌های جهش یافته متحمل در موسسه تحقیقات برنج کشور در سال‌های 1397-1398 مورد بررسی قرار گرفت. به‌منظور بررسی این واقعیت‌ها، ژنوتیپ‌های جهش‌یافته متحمل (*em4hs290* و *em4hs84*) همراه با رقم والد هاشمی، IR28 (حساس) و FL478 (مقاوم) تحت تنش 100 میلی‌مولار NaCl قرار گرفتند. بر اساس نتایج شاخص‌های رشد، طول ساقه رقم هاشمی و IR28 به طور قابل‌توجهی حدود 44/7 درصد و 44/2 درصد نسبت به شرایط شاهد کاهش داشتند و برگ‌ها به تدریج زرد شدند. نتایج نشان داد که محتوای پرولین و نسبت K^+ و K^+/Na^+ در ژنوتیپ‌های متحمل حدود ۲ تا ۳ برابر بیشتر از ژنوتیپ‌های حساس با قرار گرفتن در معرض تنش شوری افزایش یافت. همچنین، میزان کل بیان OsNHX1 و SOS1 در ارقام متحمل بیشتر از ارقام حساس بود. بنابراین، بیان بالای ژن‌های مرتبط با گروه یونی (OsNHX1 و SOS1) در برگ ژنوتیپ‌های جهش یافته *em4hs290* و*em4hs84* تجمع املاح سازگار به‌طور قابل‌توجهی افزایش می‌دهد و هموستازی یونی را ارتقاء می‌دهد و احتمالاً تنش را مهار می‌کند. الگوی متغیر بیان ژن‌های مورد مطالعه در دو ژنوتیپ جهش‌یافته متحمل به نمک (*em4hs290* و *em4hs84*) و والد هاشمی نشان داد که جهش می‌تواند توانایی

ژنوتیپ جهش‌یافته متحمل به نمک را در استفاده از هموستاز یونی و اسمزی در پاسخ به تنش شوری تغییر دهد. به‌طور کلی، این ژنوتیپ‌های جهش‌یافته متحمل را می‌توان برای توسعه واریته‌های متحمل به شوری در برنامه های اصلاحی برنج انتخاب کرد که می‌تواند پایداری تولید را به همراه داشته باشد.