

1 **Improvement of Salinity Tolerance Indices and regulation of Na<sup>+</sup> and K<sup>+</sup>**  
2 **homeostasis in Hashemi Rice Mutants (*Oryza sativa* L.)**

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4 **Abstract**

5 Salt stress is a serious environmental threat reducing crop yield. Hence, developing any breeding  
6 plan requires an understanding of the basic physiology and cell molecular genetic regulation under  
7 salinity stress. In this study, we evaluated the effectiveness of gene expression changes on ion  
8 homeostasis comprising salt overly sensitive (*SOS1*) and vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter (*NHX1*)  
9 along with ion content measurement and proline content in the rice mutants at Rice Research  
10 Institute of Iran in 2018-2019. To survey these realities, tolerant mutant genotypes (*em4hs290* and  
11 *em4hs84*) along with Hashemi parent cultivar, IR28 (sensitive), and FL478 (tolerant) seedlings  
12 were treated with 100 mM NaCl. Based on the results of **growth indices, the seedling length** of  
13 Hashemi cultivar and IR28 decreased considerably about 44.7%, and 44.2% reduction to that of  
14 the control, and the leaves progressively yellowed. Results showed that proline content and K<sup>+</sup> and  
15 K<sup>+</sup>/Na<sup>+</sup> ratio increased about ~2–3-fold higher in the tolerant genotypes than in the susceptible  
16 ones. Also, the overall amount of the *OsNHX1* and *SOS1* expression increased in tolerant genotypes  
17 compared to the susceptible ones. Accordingly, the compatible solute accumulation significantly  
18 advanced resulting in improvement of ionic homeostasis and probably suppresses the stress.  
19 Moreover, the variable pattern of gene expression in the two salt-tolerant mutants (*em4hs290* and  
20 *em4hs84*) and Hashemi parent showed that the induced mutation could increase the salt-tolerant in  
21 mutant genotypes through ionic and osmotic homeostasis. Generally, these tolerant mutant  
22 genotypes could be applied to develop salt-tolerant varieties in rice breeding programs which can  
23 bring on production sustainability.

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

25 **Introduction**

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

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28 influences the potential of plant production and induces significant crop loss worldwide (Zhang et  
29 al., 2018). Previous studies show that climate changes and inappropriate irrigation practices  
30 enhance salt accumulation in soil (Pitman & Läuchli, 2002; Roy et al., 2014), and lead to incurring  
31 significant costs of approximately \$12 billion per year globally (Pitman & Läuchli, 2002).  
32 Identifying traits related to salinity tolerance is required to improve this trait and high-yielding  
33 genotypes (Munns & Tester, 2008). Most cultivated rice varieties are susceptible to salinity stress;  
34 their salinity threshold is three  $\text{dSm}^{-1}$  (Chinnusamy et al., 2005; Munns, 2005). Despite numerous  
35 attempts and various strategies to develop salinity tolerance in rice, the achievements are relatively  
36 moderate (Hoang et al., 2016). So, the breeding cultivars of salinity tolerant with the ability to grow  
37 in salt soils is critical for sustainable agriculture and food security (Zhang et al., 2022). Though  
38 achievement of salinity-tolerant rice cultivars is time-consuming (taking at least 6 to 7 years),  
39 laborious, and incompetent through traditional breeding programs (Sun et al., 2017) (Wang et al.,  
40 2019). Therefore, mutation breeding approach is a fast and critical method for creating genetic  
41 diversity in favorite traits (Ahloowalia et al., 2004), containing the development of tolerant  
42 cultivars to biotic and abiotic stress and agronomic traits improvement (Masoabi et al., 2018). In  
43 many research, salinity-tolerant rice mutants were created using combining induction mutations  
44 and in vitro selection (Huong et al., 2020; Yunita et al., 2020; Zhang et al., 2019).

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

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

59 roots under salinity conditions (Chinnusamy et al., 2005; Ding & Zhu, 1997). Therefore,  $\text{Na}^+/\text{H}^+$   
60 antiporters of SOS1 and NHX1 have contained the principal role of  $\text{Na}^+$  exclusion and  
61 sequestration, to decrease salinity toxicity in the plant. So,  $\text{Na}^+$  uptake in plants is done using  
62 several ion channels and carrier-type transporters, which have been identified. The cation/ $\text{H}^+$   
63 exchange through membranes is catalyzed by identified NHX-type antiporters (Bassil &  
64 Blumwald, 2014; Jiang et al., 2010). The compartmentation of  $\text{Na}^+$  ions into the vacuole is  
65 mediated conventionally by the function of tonoplast (vacuole membrane) localized NHX-type.  
66 Six family members of rice NHX-type antiporter were recognized as associated with three  
67 subclasses with various cellular localizations: SOS1 is located in the plasma membrane (Martínez-  
68 Atienza et al., 2007) and five other intracellular members comprising *OsNHX1* up to *OsNHX4* and  
69 *OsNHX5* are located in the tonoplast and prevacuolar compartment, respectively (Fukuda et al.,  
70 2011; Fukuda et al., 1999). The previous studies revealed that some plant species advanced salt  
71 and drought stress tolerance via *NHX1* overexpression (Xue et al., 2004; Xiao et al., 2009; Zhang  
72 & Blumwald, 2001; Liu et al., 2010 & Ohta et al., 2002) and  $\text{K}^+$  homeostasis effectively adjusts  
73 through *NHX1* and *NHX2* (Andrés et al., 2014; Barragán et al., 2012). The plasma membrane  
74  $\text{Na}^+/\text{H}^+$  antiporter, Salt Overly Sensitive 1 (*SOS1*), is the most characteristic  $\text{Na}^+$  efflux protein in  
75 plants. (Shi et al., 2000). The  $\text{Na}^+$  effluence at the root surface and  $\text{Na}^+$  transport from root to shoot  
76 are mediated by SOS1 (Tester & Davenport, 2003). Then the  $\text{K}^+/\text{Na}^+$  ratio is advanced  
77 appropriately in leaves as the significant site for performing metabolic activities. So, the  
78 SOS3/SOS2 complex activates the  $\text{Na}^+/\text{H}^+$  antiporter promotion and the expression regulation of  
79 the *SOS1* gene for the activity of *SOS1* (Sánchez-Barrena et al., 2005). Mutants lacking in SOS2  
80 and SOS3 exhibit salt-sensitive phenotypes analogous to *SOS1* plants (Zhu, 2001).  
81 This study revealed how the selected EMS mutants improved salinity tolerance. So, the following  
82 objectives were investigated under salt stress: (i) evaluation of differences in  $\text{Na}^+/\text{K}^+$  homeostasis  
83 among the mutant rice genotypes and control; (ii) Clarifying some of the morphological and  
84 biochemical traits at different time points and the expression levels of key genes (*NHX1* and *SOS1*)  
85 concerning ionic responses and their rules in the defense strategies in the mutant rice genotypes  
86 (*em4hs290* and *em4hs84*) in comparison with the control rice cultivars; (iii) Revealing the  
87 importance of mutation in improving tolerance to salt stress in mutant rice genotypes.

88

89

90 **Material and method**

91 **Experimental materials selection**

92 For evaluation of salinity tolerance in the mutant genotypes, we first surface sterilized the seeds of  
93 Salt-sensitive and tolerant varieties (IR28 and FL478), two EMS-derived salt-tolerant mutants  
94 (*emahs290* and *emahs84*) in rice (*Oryza sativa*, cv. *Hashemi*) at Rice Research Institute of Iran  
95 (Rasht, **Guilan** province, Iran) in 2018-2019. Forty healthy seeds were placed equally on filter  
96 paper in a 9-cm-diameter Petri dish. After four days, the germinated seeds were transferred on  
97 perforated Styrofoam floats with a net bottom suspended on buckets in a hydroponic system with  
98 Yoshida solution (Yoshida & Coronel, 1976) in the greenhouse. Plants were grown in a greenhouse  
99 under structured conditions (25°C, 60% humidity, 16/8 hour light/dark cycle). The nutrient  
100 solutions were exchanged every five days. After 14 days, seedlings were grown under normal  
101 conditions, then one compartment of the nutrient solutions was treated with 100 mM NaCl  
102 solutions (about EC<sub>iw</sub> 10 dS m<sup>-1</sup>) based on the test results to determine the appropriate salt  
103 concentration of Hashemi rice (Khazaie et al., 2023), while control plants were supplied in a  
104 nutrient solution without NaCl (EC<sub>iw</sub> 0 dS m<sup>-1</sup>). A part of the samples was immediately frozen in  
105 liquid nitrogen at four-time points 6, 24, 48, and 72h after the onset of salinity treatment, chosen  
106 to capture both early and late stress responses, and then kept at -80 °C until RNA extraction.  
107 Another part of the samples was kept for biochemical, Physiological, and growth parameters  
108 measurements. Ten seedlings were collected for each time point measurement.

109

110 **Physiological and growth parameters**

111 The root length, and also length of six rice seedlings were measured with a ruler after 14 d of  
112 salinity stress. After removing three seedlings of roots, stems and leaves, the fresh weight of each  
113 seedling and root was calculated on a scale ( $\pm 0.001$  g). Besides, the seedlings were oven-dried at  
114 40 °C for three days following measurement of dry weights of the seedlings and roots.

115

116 **Proline concentration measurement**

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

121 **Determination of Na<sup>+</sup> and K<sup>+</sup> content**

122 The potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>) concentration in shoot tissues was determined using the  
 123 method developed by (Isaac & Johnson Jr, 2019). After drying and grinding of plant samples, each  
 124 sample was digested on the digestion unit including a di-acid mixture (20 ml) containing HNO<sub>3</sub>  
 125 and HClO<sub>4</sub> acid (9:4) (Turbotherm, Gerhardt analytical systems, Germany) according to the  
 126 established procedure by (Tandon, 1995). The concentration of K<sup>+</sup> and Na<sup>+</sup> samples and the  
 127 standard solutions were determined by a flame photometer (Systronics FF128).

128

129 **RNA extraction and semi-quantitative RT-PCR**

130 14-old-day leaf samples were collected after 6, 24, 48, and 72h for gene expression analysis. Total  
 131 RNA was extracted by utilizing RNX-plus TM (Synaclone) and measured using Thermo Scientific  
 132 NanoDrop 2000 (USA). cDNA synthesis was constructed according to the instructions of Thermo  
 133 Scientific™ Fermentas First Strand cDNA Synthesis Kit. The housekeeping gene UBQ10 in rice  
 134 (accession no. AT4G05320) was used as the reference gene (Yang et al., 2012).

135 The specific gene primers related to ionic homeostasis were designed by Primer3 Input (version  
 136 0.4.0) (Table 1). The Real-Time PCR reactions were performed in the iQ5 (Bio-Rad, Palo Alto,  
 137 USA), and PCR programs were done as follows: at first, an initial denaturation at 95°C for 4 min,  
 138 then samples were located in a cycling regime of 45 cycles at 95 °C for 30 s, 58-60 °C for 30 s and  
 139 72 °C for 30 s. The Quantitative real-time PCR (qRT-PCR) method and data analysis were  
 140 performed by the method provided by Pfaffl and colleagues (Pfaffl et al., 2002).

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

Gene	Primer sequence (5'→3')	Product length (bp)	Melting temperature	T <sub>m</sub> (°C)	NCBI accession number
<i>Ubiquitin10</i>	F- TGGTCACTAATCAGCCAGTTTGG	81	60.65	59	XM_015769228.1
	R- CACCACAAATACTTGACCAACAG		61.01		
<i>SOS1</i>	F- ACTTGGACGATGAGCCTGTG	98	60.04	58	XM_015763865.2
	R- ATTTAGAAGCCGCACACGGA		60.04		
<i>OsNHX1</i>	F- TCCAGCCTCCGGATGCT	77	60.00	60	XM_006658017.2
	R- ATCAGCGCGTCGTCGAA		59.46		

142

143 **Data analyses**

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

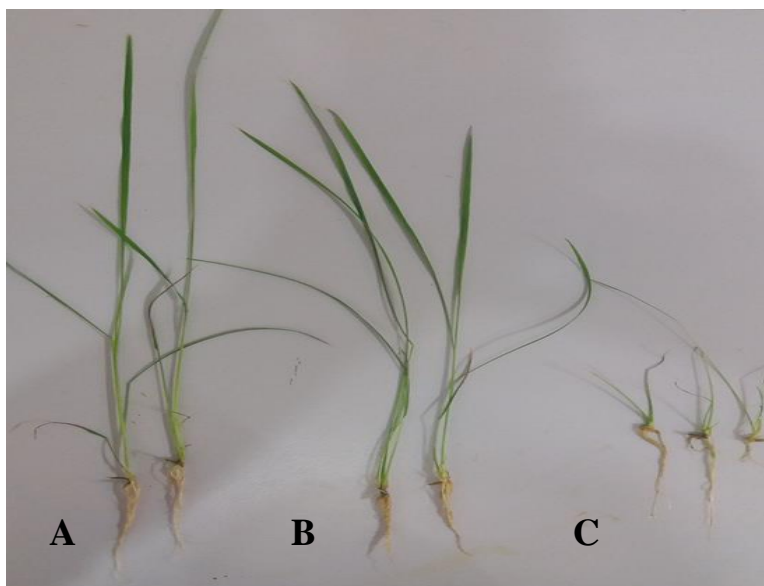
147 **Results**148 **Effects of salt stress on rice seedling growth**

149 After treating the two-old-week seedlings with 100 mg NaCl, the onset of morphological damage  
 150 was observed after three days. On the seventh day, morphological changes such as a rolling leaf,  
 151 whitening of the leaf tip, growth limitation, and finally death were found in the plants, while the  
 152 plants grew normally under control conditions. The length of IR28 and Rice Hashemi seedlings  
 153 was considerably decreased (respectively about 44.7%, and 44.2% reduction to that of the control),  
 154 and the leaves progressively yellowed. Nevertheless, the mutant genotypes (*em4hs290* and  
 155 *em4hs84*) and FL478 were not almost affected after 3 to 5 days. IR28 and Hashemi genotypes  
 156 gradually died after 3 to 5 days of salt stress, whereas the older leaves of the mutant genotypes and  
 157 FL478 just started to yellow. The mutant genotypes and FL478 were able to grow and produce new  
 158 leaves after seven days of the salinity stress (Fig. 1). Thus, the genotype's survival was graded as  
 159 *em4hs290* > *em4hs84* > FL478 > Hashemi cultivar > IR28 (Table 2). These results illustrated that  
 160 the mutant genotypes and FL478 are more tolerant than the Hashemi cultivar and IR28.

161 **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
<i>FL478</i>	51.9	24.75	10.5	11	0.46	0.125	0.51	0.185	0.07	0.03	0.02	0.015
<i>IR28</i>	42.5	19	8	6.3	0.405	0.07	0.34	0.115	0.11	0.02	0.03	0.01
<i>Hashemi</i>	48.4	21.5	9.1	7.5	0.43	0.1	0.475	0.125	0.065	0.02	0.02	0.01
<i>em3hs84</i>	55.5	30	12.95	10.75	0.9	0.185	0.835	0.275	0.135	0.03	0.04	0.02
<i>em3hs290</i>	54.2	37.5	13.8	13.2	1.11	0.33	0.81	0.645	0.15	0.06	0.03	0.025

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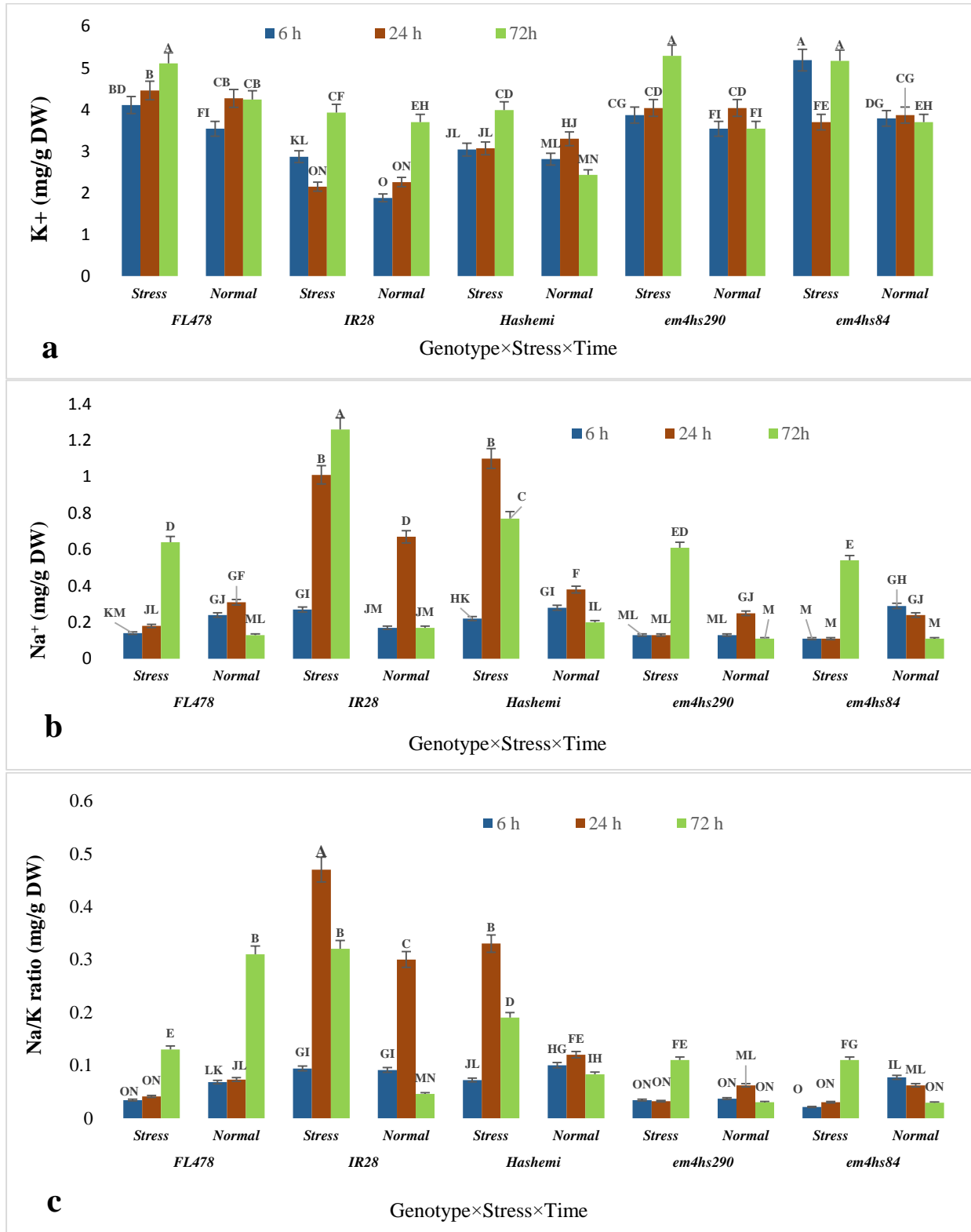


163  
164 **Fig. 1.** Growth and developmental status of the studied genotypes under salinity stress. A: The rice  
165 genotypes at normal conditions B: The mutant rice genotypes under salinity stress C: IR28 and  
166 Hashemi under salinity stress.

#### 167 Ion content changes in shoots of the genotypes under salt stress

168 The  $K^+$  and  $Na^+$  content and  $K^+/Na^+$  ratio displayed a significant ( $P < 0.01$ ) difference among the  
169 mutant genotypes and the control cultivars under the salinity stress. Regardless of the type of tissues  
170 and genotypes, salinity stress reduced  $K^+$  content. The  $K^+$  content in the leaves of *em4hs290* and  
171 *em4hs84* reached from 4.05 % to 4.25 %, which is higher than IR28 and Hashemi cultivars (2.81  
172 and 3.11%) (Fig. 2a). The  $Na^+$  content in both mutants was 0.23%, which is lower than that of IR28  
173 and Hashemi cultivar (0.59 and 0.48%) (Fig. 2b). The  $Na^+$  accumulation in leaves and stems of all  
174 evaluated genotypes was extremely higher or lower under stress conditions. Plants may develop  
175 different approaches to achieve salinity tolerance by regulating via regulation of osmotic  
176 adjustment, tissue tolerance adaptation, restriction in  $Na^+$  ion loading and accumulation in tissues,  
177 or  $Na^+$  exclusion from the cytosol (Shabala et al., 2010; Cuin et al., 2011; Shahzad et al., 2022).  
178 Moreover, the potassium/sodium ( $K^+/Na^+$ ) ratio in different parts of all genotypes reduced under  
179 salinity stress. The  $K^+/Na^+$  ratio in R28 and Hashemi cultivars (0.22 and 0.15% respectively) was  
180 higher than that of the mutant genotypes (0.051 to 0.054) and FL478 (0.062). The ability of salinity  
181 tolerant genotypes to decrease  $Na^+$  net uptake and maintain  $K^+$  uptake triggered desirable  
182  $K^+/Na^+$  ratio in all tissues (Fig. 2c).





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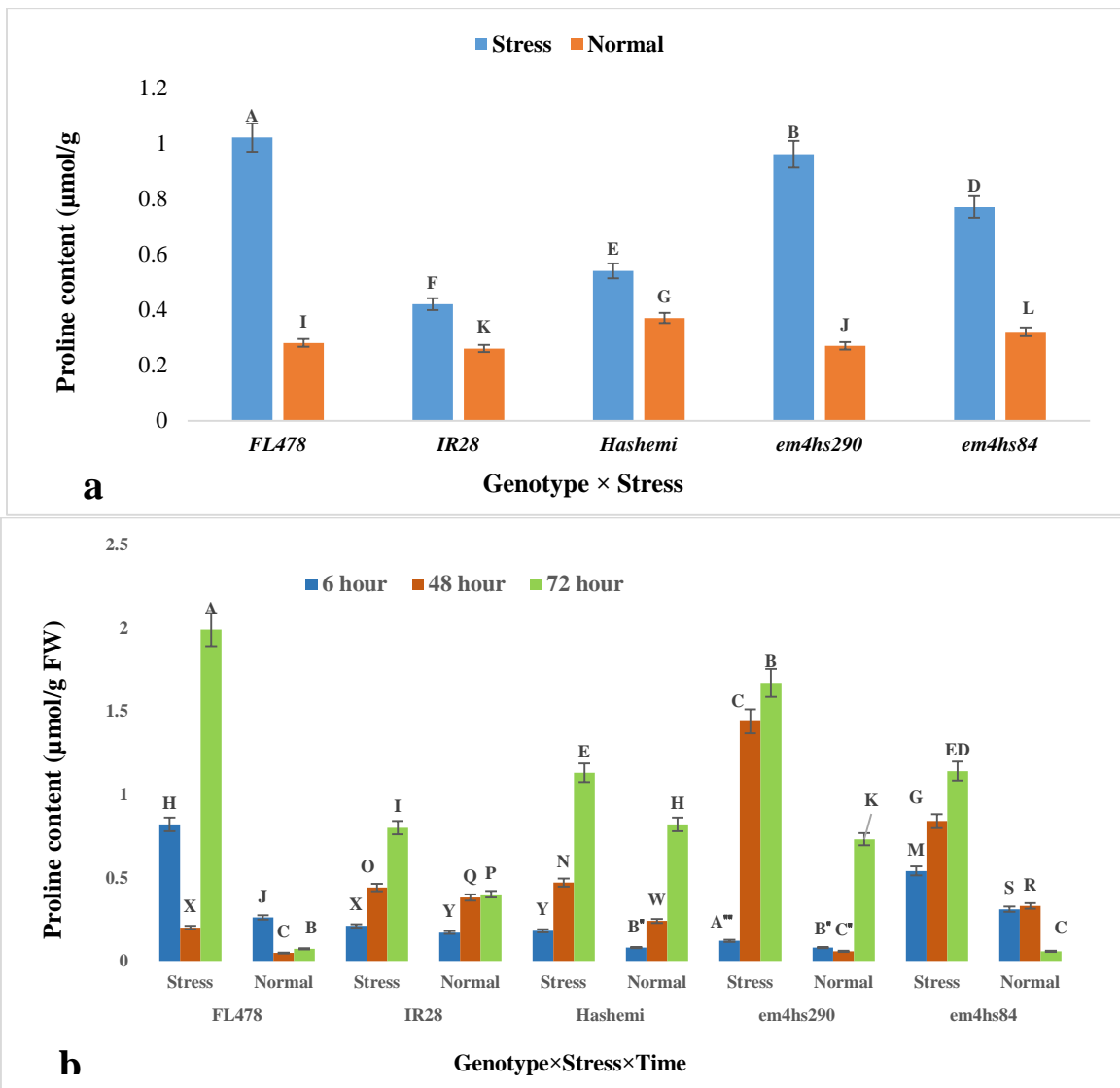
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**Fig. 2.** Mean comparison of salt stress effects on cellular ion and mineral accumulation in shoot. Changes in Na concentration (a), K concentration (b), and Na/K ratio (c) in rice genotypes under 100 mM NaCl treatment over time using Tukey's test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



189 **Physiological modulations under salt stress**

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



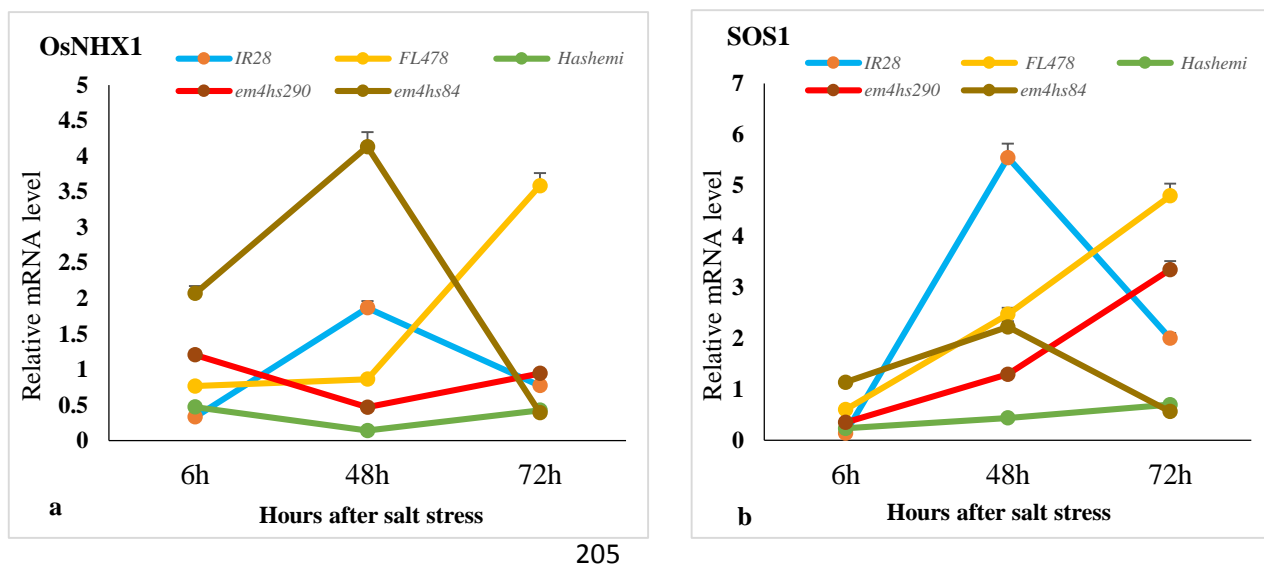
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199 **Fig. 3.** Mean comparison of proline content in the rice genotypes (a) and the interaction of  
 200 genotype×stress×time (b). Different letters in each rice line show significant differences using  
 201 Tukey’s test at (P< 0.05).

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

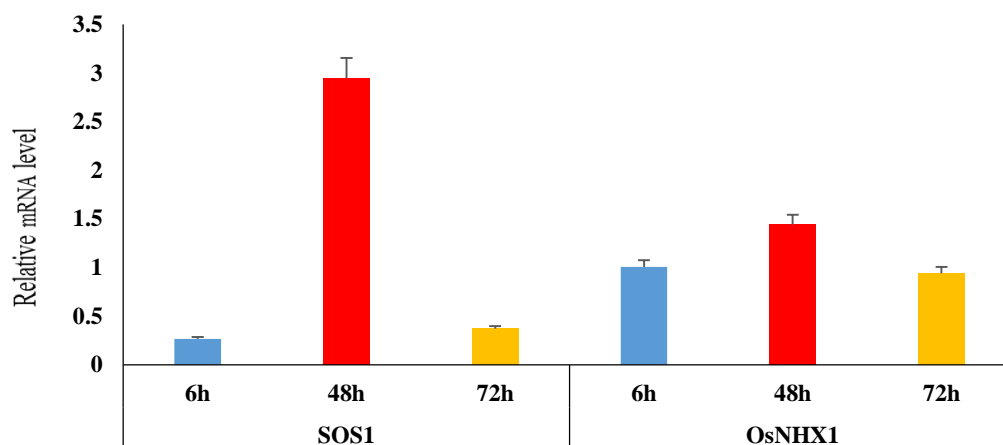
203 Based on QRT-PCR results, the changes in relative gene expression levels confirmed the  
 204 relationship between ion transport regulation and salt stress (Fig. 4).



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

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

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



228 **Fig . 5.** The relative gene expression levels of *OsNHX1* and *SOS1* genes in the studied genotypes  
 229 under salinity stress conditions by Q real-time PCR among three biological replicates in the rice  
 230 genotypes. The applied reference gene was Ubiquitin10.  
 231

232  
 233 The results showed that the *SOS1* transcript profile was similar among all genotypes as they all  
 234 appeared to have an expression peak at 48 h after stress. While a significant difference was not  
 235 observed in *SOS1* expression among genotypes after 6 h salinity exposure (Fig. 4b). Salt-tolerant  
 236 genotype (FL478) showed ~5–6-fold higher expression of *SOS1* when compared to Hashemi rice  
 237 at 48 h and 72 h after stress. Also, *em4hs290* and FL478 reached the highest level of *SOS1* transcript  
 238 ~3–4-fold transcript levels after 72 h salt treatment. However, IR28 and *em4hs84* mutant genotypes  
 239 showed similar expression patterns under salt stress, nevertheless, the results illustrated a peak of  
 240 expression level at 48 h after stress and then showed a sharp decrease after salt treatment at 72 h.  
 241 The higher expression of the *SOS1* gene was detected in the salt-resistant genotypes, while the  
 242 expression was decreased considerably in the sensitive genotypes (Fig. 4b).

243 The results exhibited that the *SOS1* transcript levels were dissimilar among five rice genotypes.  
 244 IR28 and *em4hs84* showed the highest *OsNHX1* expression after salt stress, reaching a peak 48 h  
 245 after stress initiation and then decreasing at 72 h after stress. Hence, *em4hs290* mutant genotype,  
 246 Hashemi rice, and FL478 showed a decrease at 48 h after stress. The results illustrated that a peak  
 247 in the expression levels of *SOS1* in FL478 and *em4hs290* was more intense than in Hashemi cultivar

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

251 In general, the mutant tolerant genotypes were able to have had different physiological and  
252 biochemical responses to salt stress with comparison to control cultivars: *em4hs290* and *em4hs84*  
253 showed a  $K^+$  high content in leaves, a high proline content, and absorbed more  $K^+$  in the response  
254 to salinity. Moreover, The two mutants (*em4hs290*, *em4hs84*) showed up-regulation of responsive  
255 genes and the inhibition of ion transport.

## 256 Discussion

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

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

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

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

279 relative expression of *OsNHX1* was observed in mutant genotypes and a decrease in IR28 relative  
280 expression at 6 h after stress. The high reaction was observed in the tolerant genotypes at the earliest  
281 hours after stress, whereas the sensitive genotype response varied with time. Therefore, these  
282 results revealed that the sensitive genotype takes a longer time to operate stress-responsible genes,  
283 which could be a factor for delayed hemostasis and high damage due to salinity stress.  
284 Nevertheless, it has already been demonstrated that the expression patterns of NHX-type genes in  
285 salt-sensitive and salt-tolerant plants are varied mainly, rather than probable differences in gene  
286 sequences (Hamada et al., 2001; Gong et al., 2005; Zhang et al., 2008; Xia et al., 2002;).  
287 Therefore, the results of the experiments obviously demonstrated that mutant genotypes could  
288 perform better than IR28 and Hashemi rice under salinity level of 10 dSm<sup>-1</sup> at early seedling stage.  
289 In rice, the salt tolerance in the seedling stage varies with the salinity tolerance during the other  
290 growth periods and may not be associated with each other (Jenks et al., 2007; Singh et al., 2010).  
291 For this reason, we need to characterize the rice salinity tolerance during the entire growth period  
292 in the field. These results contribute to understanding salinity tolerance mechanisms in rice by  
293 highlighting the role of SOS1 and NHX1 gene expression in Na<sup>+</sup> and K<sup>+</sup> homeostasis. However,  
294 further research is needed to elucidate these pathways fully. The results of this research and other  
295 studies illustrated that the breeding by mutation method has the potential to create new cultivars  
296 with desirable morphological characteristics. This study highlights the potential of Hashemi rice  
297 mutants in improving salinity tolerance. Considering the results from all of the experiments, EMS  
298 effectively induced variation in salt tolerance in Hashemi rice and was a successful method for  
299 developing salt-tolerant varieties and yield sustainability in rice.

300  
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480  
481 بهبود شاخص‌های تحمل به شوری و تنظیم هموستازی یون‌های Na<sup>+</sup> و K<sup>+</sup> در موتانت‌های برنج هاشمی

482 لیلا خزانی، و رضا شیرزادیان خرم‌آباد

#### 483 چکیده

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

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500  
501