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Gene expression dynamics of *HKT* family genes in salt-tolerant and salt-sensitive indica rice cultivars

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Abstract

Soil salinity is one of the major constraints limiting productivity of crop plants. In present study, effect of NaCl (150mM) on high-affinity potassium transporters (*HKT*s) were investigated into four contrasting rice genotypes salt-tolerant (CSR11, CSR27) and salt-sensitive (MI48 and VSR156). Salinity stress significantly reduced growth of VSR156 and MI48 whereas, only slight reduction was observed in CSR11 and CSR27. Under NaCl stress, *OsHKTs* group I transcripts were significantly up-regulated in salt-sensitive varieties in comparison with salt-tolerant. However, *OsHKT*s group II showed higher expression in salt-tolerant one in comparison with sat-sensitive. These results suggested the differential expression of *OsHKT* group I and II genes and hence, their differential behavior in response to salt stress. Our study, elucidate the role of seven *OsHKT* genes in shoot and root for salt tolerance in rice plant.

Key words: Sodium, rice, *HKT* transporters, salinity tolerance

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal and major staple food crop worldwide. It is consumed by more than 50% of the world population (Mohanty 2013). There are several abiotic stresses limiting productivity of the rice, among them salinity stress is one of the major abiotic stress (Kadar et al. 2006). Salinity stress occurs due to the presence of different soluble ions such as sodium (Na^+), potassium ion (K^+), chloride (Cl^-), calcium (Ca^{+2}), sulphate (SO_4^{-2}), magnesium (Mg^{+2}) and bicarbonate (HCO^{-3}) in the soil that adversely affects plant growth and development (Lewis 1984). Due to high osmolarity of soil solution, cellular damage was increased by accumulation of Na^+, production of free radicals and eventually affects plant growth and development leading to significant loss of grain yield (Munns et al. 2006). Primary, salt defensive mechanisms such as sequestration and exclusion of ions from root and leaves maintain the Na^+/K^+ homeostasis for survival of the plant (Munns and Tester 2008). Thus, expression of the ion transporter genes were likely to play a significant role in providing effective tolerance to salt stress (Mishra et al. 2016).

The high affinity potassium transporter (*HKTs*) genes are a group of potassium transporters further classified into two main groups according to their amino acid sequence similarity, and differences in Na^+ and K^+ ions selectivity (Horie et al. 2001). Group I is uniport transporters (Na^+ specific) which includes *HKT1;1, HKT1;2, HKT1;3, HKT1;4, HKT1;5* and Group II is Na^+/K^+co-transporters or symporter which includes *HKT2;1, HKT2;2, HKT2;3, HKT2;4* (Horie et al. 2001; Garciadeblas et al. 2003; Maser et al. 2002). The hybrid *HKT2;2/2;1* showed strong permeability in salt-tolerant variety Nona Bokra for Na^+ and K^+ ion at high sodium concentrations (Oomen et al. 2012). The maintenance of low Na^+ ion in cytoplasm is major important strategies for salt tolerance in plants (Gupta and Huang 2014; Tester and Davenport 2003). The group I have Serine-G-G-G and group II have G-G-G-G signature (Jabnoune et al. 2009; Maser et al. 2002). All of *HKT* encode proteins with specific transport activities that expressed in various tissues and organs. *OsHKT1;5* and *OsHKT2;3* showed its role in Na^+ transport that attributes to high level of salt tolerance by maintaining K^+ homeostasis in the shoot under salt stress (Ren et

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al. 2005; Mickelbart et al. 2015). The compartmentalization of Na^+ into vacuoles is resulting in the maintenance of K^+/Na^+ ratio in the cytoplasm of shoot cell and the uptake of K^+ ion occurs into root from the soil and discriminated the K^+/Na^+ uptake (Leidi et al. 2010).

The *HKT* transporter was thought to be analogous to the function of the *AtHKT*1 gene in *Arabidopsis*, that was a Na^+-transporter and played a crucial role in controlling cytosolic Na^+detoxification (Berthomieu et al. 2003; Rus et al. 2004; Sunarpi et al. 2005). Recently *HKT2;3* gene was reported to be involved in salt tolerance through allele mining of wild rice (Mishra et al. 2016). In this study, we discussed the role of seven *OsHKT* expression in root and shoot at seedling stage in different salt tolerant and salt sensitive rice varieties under salt stress condition to see the differential behavior of *HKT* family transporters.

Materials and methods

Plant material and growth condition

In present study uniform and healthy seeds of two each of salt tolerant (CSR27 and CSR11) and salt-sensitive (MI48 and VSR156) rice varieties were surface sterilized with 0.1% mercuric chloride then washed several times with sterile distilled water. For experiment, seeds of each cultivar were sown in the petri dishes (10 cm diameter, Axygen, India) containing germination paper. For germination percentage, the seeds of varieties put it in various concentrations of sodium chloride (50, 100, 150 and 200 mM) were tested along with control (0 mM) their four biological replicates. The plants were grown in a culture room at 25 ± 1°C, relative humidity 70-80% with 16 h light and 8h dark periodicity. For growth experiment, seeds were germinated and seedlings were transferred to hydroponic medium. Hydroponic solution was renewed every two days.Tenth day after germination, seedlings were treated with 150 mM NaCl for 24h to the hydroponic solution. The shoot and root samples were collected after 24h NaCl treatment. Quantitative Real Time PCR experiments were performed in four biological replicates with two technical replicates.

RNA isolation and qRT PCR

Total RNA was isolated from the freshly harvested shoot and root samples of all the four rice varieties under control and treated conditions. Total RNA was extracted and RT PCR were performed as described by Mishra et al. 2016. In brief the RNA integrity was checked in 1.2 % formaldehyde denaturing agarose gel and quantified using Nanodrop Spectrophotometer (Thermo Scientific, USA). RNA (1µg) was used for reverse transcribed using Affinity Script QPCR cDNA Synthesis Kit (Stratagene USA). The cDNA was diluted and used as a template in quantitative real-time PCR analysis. The diluted cDNA was amplified using SYBR Green PCR Master Mix (Agilent USA) on the Stratagene Mx3005P detection unit. Primers for the actin gene were used as an internal control to normalize the expression data for each gene. The different *OsHKT* primer sequences were used (Table 1) for qRT PCR. PCR conditions were set: denaturation at 95^{o}C for 10 min followed by 40 cycles each cycle at 95^{o}C for 30

Table 1. A list of OSHKT primer sequence is used in the stury

Sequence Name 5'	Sequence 3'	MSU Rice Locus	Alternative gene name
OsHKT1;1 - Na+ transporter-F	TTGGACCTATTGTTCACCTCTG	LOC_Os04g51820	KT4
OsHKT1;1 - Na+ transporter-R	TGTTGGCATTGGCATTGTTG3		
OsHKT1;3 - Na+ transporter-F	CCTCAAGCCACGAGACAAG	LOC_Os02g07830	HKT6
OsHKT1;3 - Na+ transporter-R	AATACCTCACCACCAATCAGC 5		
OsHKT1;4 - Na+ transporter-F	GGAGAAGGTGGTGAACGC	LOC_Os04g51830	HKT7
OsHKT1;4 - Na+ transporter-R	CGAAGAGCACGAGGATGG		
OsHKT1;5 - Na+ transporter-F	GACGACGAGGCTACTGATG	LOC_Os01g20160	HKT8~ SKC1
OsHKT1;5 - Na+ transporter-R	GAGACGACGGTGAAGATGG		
OsHKT2;1 - Na+ transporter-F	CCACTCAACTTCTCTGCTCTG	LOC_Os06g48810	HKT1
OsHKT2;1 - Na+ transporter-R	CCTTCATCACTCCACCATCC		
OsHKT2;3 - Na+ transporter-F	GTCACCTGCTGCCTAACTTG	LOC_Os01g34850	HKT3
OsHKT2;3 - Na+ transporter-R	TTCTCCTGCCTGCCTTGC		
OsHKT2;4 - Na+ transporter-F	TGTCGGTTCATCGCATTCC	LOC_Os06g48800	HKT9
OsHKT2;4 - Na+ transporter-R	CATTTGTGTGGTGGCTAATCC		

sec, 55°C for 40 sec, 72°C for 1 min, and final extension at 72°C for 10 min.

Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data set using SAS 9.3 software and significant difference (P <0.05) were noted. Further Duncan's multiple range tests (DMRT) was done for mean significant differences among treatments and the effect which are significantly different were represented by different alphabets. The means with different letter grouping are significantly different.

Results and discussion

Effect of salt stress on per cent germination, shoot and root length, fresh and dry weight of plants

This study revealed a significant physiological difference among rice cultivars exposed to (50, 100, 150 and 200 mM) NaCl stress (Fig. 1). Seed

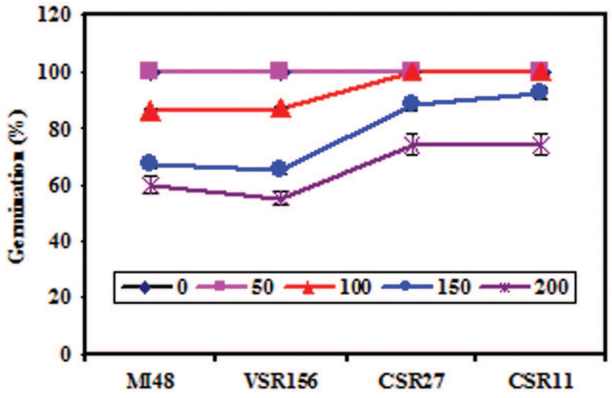

Fig. 1. Effect of NaCl on germination percentage

germination is a critical stage for seedling establishment to determine successful crop production (Danai-Tambhale et al. 2011). The seedling germination of all varieties were significantly decreased with 100-200 mM NaCl concentration however, at 50 mM NaCl 100% germination was observed (Fig. 1). At 150 mM NaCl, seed germination of VSR156 and MI48 was reduced by 35 and 33% respectively whereas, CSR27 and CSR11 showed only a slight reduction of 12 and 8% showed its tolerant nature. According to Akbar and Ponnamperuma (1982), the osmotic stress by salinity was the major factor which reduced the seed germination. Salinity significantly affects plant height of different rice cultivars. Control plant showed maximum shoot as well as root length (Fig. 2). Anbumalarmathi and Mehta (2013) also reported

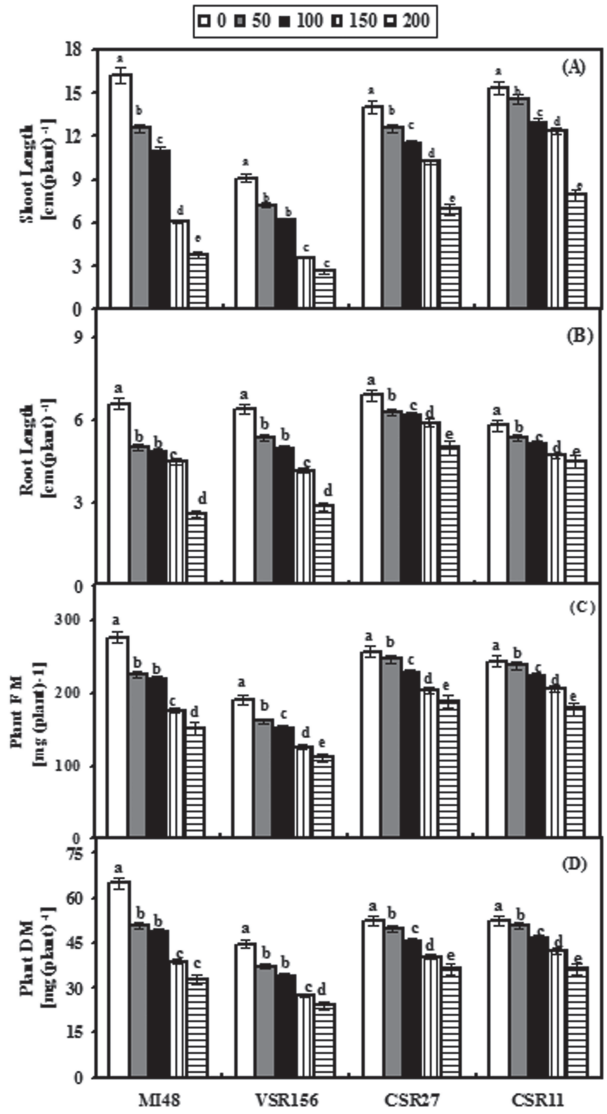

Fig. 2. Impact of NaCl on various morphological traits. (a) shoot length (b) root length, and (c) plant dry weight of germinated seedlings after 10th day of germination at various NaCl levels in salt-sensitive (VSR156, MI48) and salt-tolerant (CSR11, CSR27) used in Figure 1. All values are means of triplicates (± SD). Values with different superscripts are significantly (*P*< 0.05) different from each other (Duncan's multiple range test)

reduction of seed germination, shoot- root length, fresh and dry mass of rice cultivars under NaCl stress. Increasing salt concentration leads to reduction in shoot/root length in which shoot length showed higher reduction compare to root (Figs. 2A and 2B). Among these varieties, CSR27 and CSR11 showed 26 and 21% reduction compared to MI48 and VSR156 which

exhibited 2 fold more reduction in shoot length at 150 mM NaCl. Root length also showed reduction in dose dependent manner. The dry mass depicted higher reduction in comparison with fresh weight (Figs. 2C and 2D). Seeds germinated on 200 mM NaCl were unable to survive, and at 150 mM NaCl maximum difference appeared between all varieties. Therefore, expression analyses were performed after exposing plants at 150 mM NaCl concentration.

Expression of OsHKT genes

Expression of *OsHKT* genes at transcript levels displayed variable expression. The transcript level of different OsKHT1;1, OsKHT1;3, OsKHT1;4, OsKHT1;5 were quantified after NaCl stress for four contrasting rice varieties (Fig. 3). The analysis revealed induced expression of *OsHKT1.1* in shoot and root of all the genotypes.

Maximum expression was observed in the roots of VSR156 root (1.5 fold) followed by MI48, CSR11 and CSR27. In shoot also similar trend was noticed with highest expression in VSR156 (4 fold) followed by MI48, CSR11 and CSR27. Higher expression of *OsHKT*1 in root indicated its role in Na^+ uptake by root and transport to shoot, where Na^+ caused damage to the shoot. Similarly, the expression of *OsHKT*1;1, *OsHKT*1;3 and *OsHKT*1;4 also showed similar response in root however, more than 2 fold higher expression was observed in shoot compare to root (Fig. 3B). The, expression analysis of *OsHKT*1 family genes showed higher expression in MI48 and VSR156 in comparison with CSR27 and CSR11. Induced expression of *OsHKT*1 in rice under salt stress was also reported (Mishra et al. 2016; Kader et al. 2006). In K^+ deficient condition, *OsHKT*1 specifically induced Na^+ uptake in rice root (Garciadeblas et al. 2003). Under salinity stress condition, Na^+ competes at K^+ binding sites and excess Na^+ entering the cytosol increased Na^+/ K^+ ratio in cytosol that resulted in K^+ deficiency (Maathuis and Amtmann, 1999; Horie et al. 2001) and thus, leads to *OsHKT*1induction in all genotype. The higher uptake of Na^+ into the cytosol of sensitive genotype (VSR156 and MI48) may leads to higher expression of *OsHKT*1 than in tolerant genotype (CSR27 and CSR11) as reported earlier (Kader and Lindberg, 2005).

The role of *OsHKT*2;1, *OsHKT*2;3 and *OsHKT*2;4 (act as an K^+-Na^+ co-transporter) for salt-stress response in CSR27, CSR11, VSR156 and MI48 were also investigated. Expression of *OsHKT2* family genes

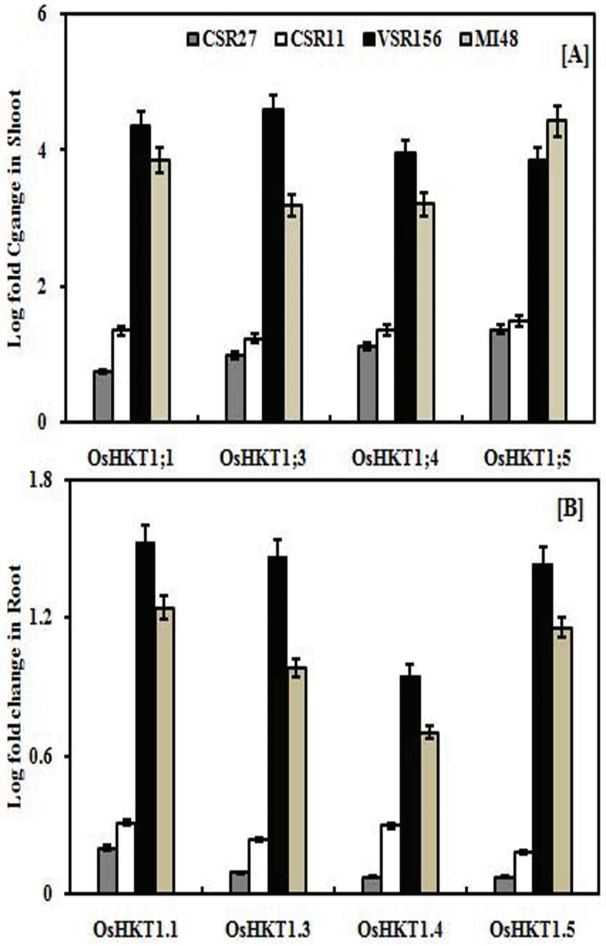

Fig. 3. Quantitative expression analysis of high-affinity potassium transporter (*OsHKT*1;1, *OsHKT*1;3, *OsHKT*1;4 and *OsHKT*1;5) genes amplification of RNA from root and shoot tissues of rice cultivars CSR27, CSR11, VSR156 and MI48 at 150 mMNaCl for 24h 10 day after germination [The results are represented as mean fold change in relative expression over four biological and two technical replicates, normalized with respect to the Actin reference gene expression. Bar represent standard deviation]

indicated a substantial increase in the tolerant rice varieties in comparison with sensitive ones (Fig. 4). The expression of *OsHKT2; 1* was observed maximum in roots of CSR27 followed by CSR11, VSR156 and MI48, a similar trend was also noticed for shoot. However, in shoot, the expression level was much higher than that of roots. The expression of *HKT2;3* was highest for CSR11 (10 fold) followed by CSR27, VSR156 and MI48 in shoot. However, the expression of *HKT2;3* was similar in CSR27 and CSR11 and less

in VSR156 and MI48. The expression of *HKT*2;4 was maximum in CSR27 (11 fold) followed by CSR11, MI48 and VSR156. However, in roots its expression was highest in CSR11 followed by CSR27, MI48 and VSR156 (Fig. 4B).

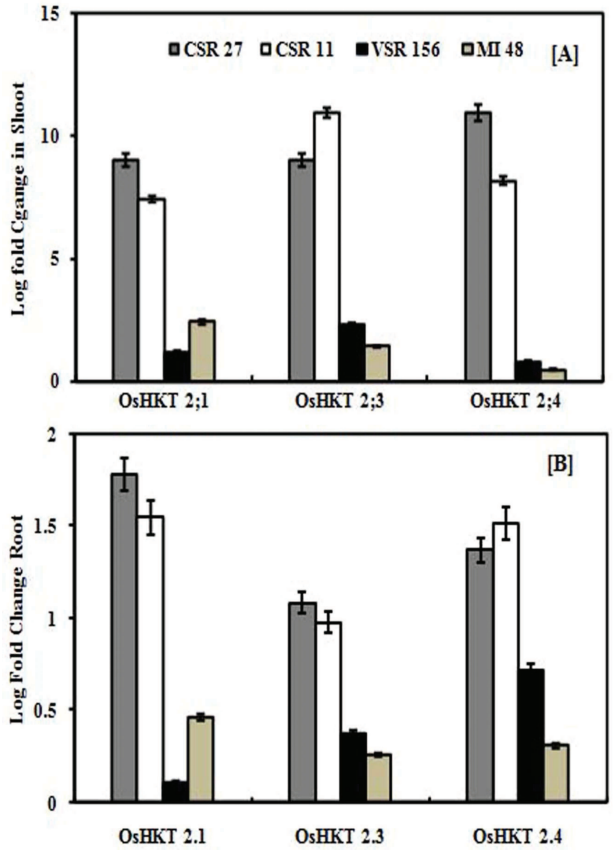

Fig. 4. Quantitative expression analysis of high-affinity potassium transporter (*OsHKT*2;1, *OsHKT*2;3 and *OsHKT*2;4) genes amplification of RNA from root and shoot tissues of rice cultivars CSR27, CSR11, VSR156 and MI48 at 150 mMNaCl for 24h 10 day after germination [The results are represented as mean fold change in relative expression over four biological and two technical replicates, normalized with respect to the Actin reference gene expression. Bar represent standard deviation]

Higher expression of *OsHKT2;1* and *OsHKT2;4* under salt stress condition leads to providing entry point of Na^+ into plant roots (Lan et al. 2010; Schachtman and Schroeder 1994). Na^+ toxicity leads to K^+ deficiency therefore, *HKT*2;1 was highly expressed in roots (Almeida et al. 2013). Uptake of Na^+ was mainly dependent on *OsHKT2;1* at concentration >30 mM NaCl and it was Na^+ selective permeability (Jabnoune et al. 2009). Other *OsHKT2* family genes *OsHKT2;3* and *OsHKT2;4* mediated N^+ independent transport of K^+ (Horie et al. 2011).

Therefore, higher expression of *OsHKT*2 in the salt-tolerant cv. CSR27 and CSR11 might conferred salt tolerance by increasing its expression, through contributing to a lower cytosolic Na^+/K^+ ratio, (Horie et al. 2001). Maathuis (2006) reported more than 3-fold change of Na^+-K^+ symport in salt stress condition. Other K^+ transporters were also observed to be up-regulated under high NaCl conditions, which reflects ability to maintain required cytosolic K^+ levels.

The ability of plants to maintain ionic homeostasis was important strategy to achieve salt-tolerance in barley in comparison with a moderate salt-sensitive (IR64) rice cultivar (Ueda et al. 2006). Present study revealed that the K^+/Na^+ homeostasis regulatory mechanism in cells of the salt-tolerant (CSR27) and salt-sensitive (CSR11) rice operated to increase K^+ uptake and on other hand Na^+ influx was reduced as determined by decrease in the expression of *OsHKT*1. Since the expression of the Na^+ transporter *OsHKT*1 was induced in CSR11 and CSR27 because of K^+ deficiency in cells (caused by Na^+ competition at transport sites), or by interruption of the cytosolic Na^+/K^+ ratio. On long term exposer of NaCl (24h) the expression of *OsHKT*1 in CSR27 and CSR11 were reduced. It is concluded that, at high NaCl conditions, CSR27 and CSR11 maintains its cytosolic Na^+/K^+ homeostasis by increasing the Na^+-K^+ coupled uptake through the induction of *OsHKT2*. CSR27 and CSR11 also maintain a low influx of cytosolic Na^+, either by conformational change of the *OsHKT1* protein and/or by post-transcriptional changes of the *OsHKT1* gene. Under stress, CSR 27 and CSR11 maintains cytosolic K^+/Na^+ homeostasis by reducing the expression of *OsHKT1*.

Authors' contribution

Conceptualization of research (NKS); Designing of the experiments (VR); Contribution of experimental materials (NKS, VR); Execution of field/lab experiments and data collection (NKS, VR); Analysis of data and interpretation (PM, VM); Preparation of manuscript (PM).

Declaration

The authors declare no conflict of interest.

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