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Assessment of Sequence Polymorphism and Gene Expression of *OsSOS1* Gene in Two Contrasting Rice Genotypes

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Salt stress causes severe reduction in the growth and yield of rice plants. The ability to maintain cellular ion homeostasis is of importance to help the plant survive under salt stress. Salt overly sensitive 1 (SOS1), a plasma membrane Na^+/H^+ antiporter, has been proven to play critical roles in Na^+ exclusion out of the cell, hence contributing to salt tolerance in plants. In this study, we analyzed the natural nucleotide polymorphisms occuring within the entire coding sequence as well as the upstream region of the *OsSOS1* gene by comparing the sequences of two contrasting rice genotypes, namely, Nipponbare (salt-sensitive) and Pokkali (salt-resistant). In total, six nucleotide polymorphisms were identified in the coding sequence, and 44 nucleotide substitutions, 225-bp-insertion and 65-bp-deletion were observed in the upstream region of the *OsSOS1* gene. Futher *in silico* analysis revealed that two out of six nucleotide polymorphisms in the coding sequence were non-synonymous (A1600G, G2204A) which led to two amino acid substitutions (T534A, S735N, respectively) positioned in the C-terminal domain of OsSOS1 transporter, but caused no effect on protein properties. In the upstream region of *OsSOS1* gene, 44 single nucleotide polymorphisms and two INDELs were identified, in which nucleotide substitutions at position -1392, -1389, -822, -583, +57 and an insertion at position -1035 caused change in cis-regulatory elements. Analysis of *OsSOS1* expression revealed that salt induced the expression of the gene in the roots, but not in the leaves in both investigated rice cultivars.

Keywords: Polymorphism, salt stress, rice, OsSOS1, salt overly sensitive

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important cereal crops in the world. It is considered a salt-sensitive species (Akita and Cabuslay, 1990; Lee et al., 2003). Salinity severely affects both the ability of plants to take up water and mineral nutrients. The high Na^+ concentration relative to other cations is the main factor affecting plant growth by interfering with vital Na^+-sensitive enzymes (Tester and Davenport, 2003; Munns et al., 2006) and affecting ion transport (Rains and Epstein, 1965; Rubio et al., 1995). Efflux of excessive Na^+ out of the cells is an important salt tolerance mechanism in plants.

The plasma membrane Na^+/H^+ antiporter encoded by *salt overly sensitive 1 (SOS1)* has been proven to be involved in Na^+ extrusion and hence in salt tolerance in plants (Shi et al., 2000, 2002; Martinez-Atienza et al., 2007; Wu et al., 2007; Maughan et al., 2009; Olías et al., 2009). The reduction in *SOS1* expression using RNAi results in high levels of ion accumulation and causes injure by salt stress in *T. salsuginea* (Oh et al., 2009), and in tomato (Olías et al., 2009). On the other hand, *SOS1*-overexpressing plants could exclude more Na^+ and showed enhancement of salt tolerance (Shi et al., 2003; Li et al., 2014; Chen et al., 2017). SOS1 plays a role in Na^+ efflux from the cytosol to the surrounding medium in root epidermal cells and from the surrounding parenchyma to the vascular tissues and in controlling long-distance Na^+ transport (Shi et al., 2002; Olías et al., 2009). *SOS1* is expressed in epidermal cells at the root tip and in parenchyma cells at the xylem symplast boundary of roots, stems, and leaves (Shi et al., 2002). Shi et al. (2000) reported that

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the expression of *AtSOS1* was upregulated by NaCl treatment in both roots and shoots, but higher in roots than in shoots. A similar expression pattern was observed in *TaSOS1* (Xu et al., 2008), *ThSOS1* (Oh et al., 2009) and *PtSOS1* (Guo et al., 2012).

Investigation of natural genetic variation is an alternative means to elucidate the functional role of genes (Baxter et al., 2010). In the study of Ren et al. (2005), six nucleotide polymorphisms leading to four amino acid changes were identified, which were thought to contribute to differences in Na^+ transport efficiency between the salt-tolerant cultivar Nona Bokra and the salt-sensitive cultivar Koshihikari. In another study, using a rice diversity panel for genome wide association mapping, Campbell et al. (2017) could identify three non-synonymous variants within *OsHKT1;1* gene that were associated with altered Na^+ accumulation in the root.

In this study, we assessed the presence of natural polymorphism in *OsSOS1* gene sequence, including both the coding sequence and the upstream region of the gene in two contrasting rice cultivars, the well-known salt-sensitive cultivar Nipponbare and the salt-tolerant cultivar Pokkali. The detected nucleotide polymorphisms in the coding sequence and upstream sequence were further analyzed *in silico* to elucidate the potential effect on either protein properties or transcriptional regulatory by cis-elements, respectively. The expression level of *OsSOS1* gene in the roots and leaves of both cultivars were investigated in response to salt stress by using real-time RT PCR.

MATERIALS AND METHODS

PLANT MATERIAL, CULTIVATION AND SALT TREATMENT

Seeds of two rice (*Oryza sativa*) cultivars (Nipponbare, Pokkali) were kindly provided by Vietnam National University of Agriculture (Hanoi, Vietnam). The seeds were germinated for 4 days. The seedlings were then transferred to boxes containing Yoshida medium (Yoshida, 1976) and placed in a growth chamber (12 h days with 500 $\mu E\ m^{-2}\ s^{-1}$ at 26°C and 12 h night at 22°C) for gene expression analysis. The seedlings were fixed to holes in the lid with a piece of foam material. The growth medium was renewed every week. After 14 days of normal growth, the medium was exchanged for medium with the appropriate salt concentration (0 mM, 50 mM and 100 mM NaCl). Stress treatment was performed for 7 days.

MESUREMENT OF NA^+ AND K^+ CONTENT

The leaves of both control and salt-stressed seedlings were harvested at day 7 of salt treatment. The leaves were oven-dried at 60°C, and then dry weight was determined for each sample. The samples were extracted in concentrated nitric acid in a microwave oven for 5 min at 650 W. The Na^+ and K^+ contents were measured using the inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan 9000, Perkin-Elmer Inc., USA).

DNA EXTRACTION

The DNA extraction was performed by using the cetyl trimethylammonium bromide (CTAB) method. About 200 mg leaf powder was mixed with 500 µL CTAB buffer and incubated at 65°C for 20 min. Then 500 µL CI 24:1 (chloroform : isoamylalcohol) was added and centrifuged at 14 000 rpm at 4°C for 15 min. The supernatant was transferred into a new tube, and the DNA was precipitated by cold isopropanol for 15 min. The DNA pellet was collected by centrifuging at 10 000 rpm at 4°C for 5 min and washed with 70% ethanol. Finally, after drying at room temperature, the pellet was dissolved in Tris-EDTA buffer and kept at -20°C. The quality and quantity of extracted DNA were estimated by visualizing the band of total DNA on ethidium bromide-stained 1% agarose gel.

RNA ISOLATION AND FIRST-STRAND cDNA SYNTHESIS

The leaves and roots of both control and salt-stressed seedlings were harvested at day 7 of salt treatment and immediately frozen in liquid nitrogen. The leaf/root material of 5 different plants per cultivar and treatment was homogenized using a ball mill (Mixer Mill MM 400, Retsch, Germany) and equal fractions were pooled. Total RNA was isolated using GeneJET Plant RNA purification Kit (Thermo Fisher Scientific, USA). RNA concentration was determined photometrically (NanoDrop ND-1000 UV-Vis spectrophotometer, Nanodrop Technologies, Wilmington, DE). Purified RNA was treated with DNase I (Thermo Fisher Scientific, USA) and the absence of genomic DNA was confirmed by PCR using primers for amplification of an intron of *OsHKT2;1* gene sequence (FW: 5'-ATCATCAGGTGTGTTCCTCTCTC-3', RW: 5'-CATTGGCTTGATGCCCAGTGT-3'). The integrity of the final RNA samples was checked on 2% (w/v) agarose gel. 1 µg of total RNA was transcribed into cDNA using Revert-Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA).

PRIMER DESIGN AND AMPLIFICATION OF *OsSOS1* GENE BY PCR

The 2388-bp upstream region and entire coding sequence (3447 bp) of *OsSOS1* were amplified separately from genomic DNA and cDNA, respectively. Primer pairs used for amplifying *OsSOS1* coding sequence are: cds1-FW (5′-AGGGGTGACAAAACAACAAG-3′) and cds1-RW (5′-TCTGGCAACCTCACTATCAC-3′); cds2-FW (5′-GTGCGGCTTCTAAATGGTG-3′) and cds2-RW (5′-AAGGGAGGATCTCACAAGGA-3′); and for amplifying *OsSOS1* upstream region: Pro-FW (5′-ACCTACGATCTGAACAATGTACTC-3′) and Pro-RW (5′-GAAGTAGTTACAGATACGAATGCC-3′). The PCR reaction mixture consisted of either genomic DNA for amplification of upstream region or cDNA for amplification of coding sequence, Dream Taq polymerase buffer (1×), $MgCl_2$ (1.5 mMol/L), dNTPs mixture (0.2 mMol/L), primers (0.4 μMol/L) and Dream Taq polymerase (1 U). The PCR reaction was performed with a thermocycle of 95°C for 5 min, 35 cycles of 95°C for 30 s, 53°C (cds1 primers)/50°C (cds2 primers)/55°C (promoter primers) for 30 s and 72°C for 2 min, and 72°C for 5 min. Then, 5 μL of PCR products were separated on 1% agarose gel for 28 min at constant 90 V in 1 × Tris-acetate-EDTA buffer. If PCR product showed only one bright single band on gel, it was purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific) and sequenced on ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA) at First BASE DNA sequencing service (Singapore). The sequences were submitted to GenBank database under accession numbers MG602252 for *OsSOS1* cds of Pokkali, MG639915 for *OsSOS1* upstream region of Pokkali, and MG657002 for *OsSOS1* upstream region of Nipponbare.

SEQUENCE ANALYSIS

The sequences were analyzed using Bioedit (Hall, 1999) and Multalin webserver (Corpet, 1988). The predicted peptide sequences were analyzed using Expasy webserver (http://web.expasy.org/translate/).

EVALUATION OF PUTATIVE CHANGES AT PROTEIN LEVEL

Several bioinformatics tools were used for evaluation of consequent effects of the polymorphisms on protein properties. The InterPro was used to identify protein domains (Finn et al., 2017; www.ebi.ac.uk/interpro/). To analyze the polymorphism influence in putative post-translational modifications, such as phosphorylation, the NetPhos3.1 was applied (Blom et al., 2004; http://www.cbs.dtu.dk/services/NetPhos/).

PUTATIVE CIS-ELEMENTS IN THE UPSTREAM REGION OF *OSSOS1*

Nipponbare 2.4-kb upstream from start codon of *OsSOS1* gene was retrieved from the MSU rice genome annotation database (Kawahara et al., 2013, http://rice.plantbiology.msu.edu/) (this region was re-sequenced in this study). The putative *cis*-elements in Nipponbare and Pokkali were predicted by submitting these upstream sequences to the PLACE database (Higo et al., 1999; http://www.dna.affrc.go.jp/PLACE/) and PlantCARE database (Lescot et al., 2002, http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

QUANTITATIVE REAL-TIME PCR FOR *SOS1* GENE EXPRESSION ANALYSIS

The transcript levels in different samples were quantified by real-time PCR analysis with specific primers for *OsSOS1* gene (FW: 5'-CTGTGGCAGGAAAGTGCTCTA-3', RW: 5'-TTCAGTGATGAGAACCCTGAGC-3') and reference gene *Actin1* (FW: 5'-CTCCCCCATGCTATCCTTCG-3', RW: 5'-TGAATGAGTAACCACGCTCCG-3') employing ABI Fast 7500 System (Applied Biosystems, Foster City, CA). 1 μL of diluted cDNAs was used as template and mixed with primers and 10 μL of SYBR Green Master Mix 2X (LuminarisHigreen low ROX qPCR master mix, Thermo Fisher Scientific, USA) in total volume of 20 μL. The thermocycle of PCR was performed as: 95°C for 10 min, 40 cycles of (95°C for 15 s, 60°C for 1 min) in 96-well optical reaction plates. The specificity of the reaction and correct size of amplified DNA were checked by agarose gel electrophoresis and melting curve analysis. Two technical replicates were performed. House-keeping gene, *Actin1* was used for normalization. The relative mRNA levels of *OsSOS1* gene (described as fold change) in different samples were computed by the 2-ΔΔCt Method (Livak and Schmittgen, 2001).

RESULTS

DETERMINATION OF NA^+, K^+, AND K^+/NA^+ RATIO IN THE TWO INVESTIGATED GENOTYPES

In order to investigate whether changes of Na^+ and K^+ contents are related to the underlying salinity tolerance in the two investigated rice genotypes, the contents of Na^+ and K^+ were measured. The effect of NaCl on the contents of Na^+ and K^+ in the leaves of the two investigated genotypes is shown in Table 1. With NaCl treatment, Na^+ content increased compared with the control (0 mM NaCl). The K^+ content decreased with increasing concentration of NaCl in the nutrient solution. The salt-sensitive cultivar Nipponbare showed higher

TABLE 1. Effect of different salt conditions (0 mM, 50 mM, and 100 mM NaCl) on the contents of Na^+, K^+; and K^+/Na^+ ratio in the leaves of two rice genotypes.

Cultivar	Na^+ (mg g^{-1} DW)			K^+ (mg g^{-1} DW)			K^+/Na^+		
	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
Nipponbare	2.2±0.4	7.6±1.8	13±0.5	38.9±2.8	32±1.6	31.6±1.0	17.4	4.2	2.4
Pokkali	1.6±0.2	4.4±0.4	8.9±0.1	40.7±7.2	38.9±2.8	37.1±3.8	24.5	8.9	4.2

Na^+, but lower K^+ contents than the salt-tolerant cultivar Pokkali, resulting in a lower K^+/Na^+ ratio in Nipponbare than in Pokkali.

POLYMORPHISMS IN THE CODING SEQUENCE OF *OSSOS1*

OsSOS1 gene is 14,451 bp in length with 23 exons and 22 introns. To investigate the polymorphisms within the coding region of *OsSOS1* gene of Pokkali compared to that of Nipponbare, the entire coding sequence of *OsSOS1* gene was amplified from synthesized cDNA of Pokkali by PCR technique. The amplicon was then sequenced. The entire coding sequence of Nipponbare *OsSOS1* gene was obtained from both the MSU rice genome annotation database and by re-sequencing the PCR amplicon which used Nipponbare cDNA. By alignment of the two sequences, six nucleotide polymorphisms were detected, in which two are non-synonymous substitutions (A1600G, G2204A) and four are synonymous substitutions (A172C, C1449T,C1869A, A2214G) (Table 2, Supplementary Figs. S1 and S2). The two non-synonymous substitutions A1600G, G2204A led to the amino acid changes T534A and S735N, respectively. To elucidate the potential effect of these non-synonymous substitutions on protein properties, the putative structural domains of the OsSOS1 protein were predicted using InterPro and the position of these non-synonymous substitutions on protein domains was analyzed. It revealed that the OsSOS1 consists of an N-terminal transmembrane region (from amino acid 1 to 430) followed by a large cytoplasmic region (from amino acid 431 to 1148) with a putative cyclic nucleotide binding domain centrally located in the C-terminal tail (from amino acid 721 to 823) (Fig. 1). The phosphorylation sites were predicted by the NetPhos 3.1 program and the serine-rich site was predicted to locate at the end of the C-terminal from amino acid 1025 to 1148 (Fig. 1). The two amino acid variants T534A and S735N located in the C-terminal region (Fig. 1).

OSSOS1 GENE UPSTREAM REGION POLYMORPHISMS

The 2388-bp upstream region from ATG (consisting of also 5'UTR) of *OsSOS1* was amplified by PCR and sequenced in both cultivars. In addition, the upstream sequence of Nipponbare *OsSOS1* gene was obtained also from the MSU rice genome annotation database. Sequence alignment revealed 46 polymorphic sites, consisting of 44 single nucleotide polymorphisms and 2 INDELs (Table 2, Supplementary Fig. S3). The consequent influence of these polymorphisms on the transcriptional regulatory functions of the promoter and the cis-regulatory elements present in the promoter were predicted using PLACE and PlantCare. The complete cis-regulatory elements are shown in Table S1. In total, six polymorphic sites at -1392, -1389, -1035, -822, -583, +57 caused changes in cis-regulatory elements (Fig. 2 and Table 3). The nucleotide substitutions C-1392T and A-1389G caused an addition of MYBCORE and ACGTATERD1, respectively; while both nucleotide substitutions T-822G and C-583T caused the loss of GT1GMSCAM4 and the substitution G57T gave a deletion of MYCCONSENSUSAT (Table 2). The insertion at position -1035 led to the addition of several cis-regulatory elements, consisting of ABRERATCAL, DPBFCOREDCDC3, MYB1AT, and MYCCONSENSUSAT (Table 3).

OSSOS1 GENE EXPRESSION UNDER SALT STRESS

Quantitative real-time PCR analysis was conducted to investigate the expression of *OsSOS1* gene in response to salt stress with total RNA purified from pooled roots and shoots of rice plants subjected to 50 mM or 100 mM NaCl salinity stress for 7 days. The results of two real-time PCR runs were almost identical. In general, the expression level of *OsSOS1* was highly upregulated in roots, but not in leaves under salinity (Fig. 3). After 7 days of salt treatment, the expression level of *OsSOS1* in Nipponbare root increased 4-fold under 50 mM NaCl and 2-fold under 100 mM NaCl, as compared to the control (0 mM NaCl); while the transcript accumulation in Pokkali

TABLE 2. Nucleotide polymorphisms in *OsSOS1* gene upstream sequence and coding sequence.

Position	Polymorphic type	Effect of variance	Position	Polymorphic type	Effect of variance	Position	Polymorphic type	Effect of variance
-1454	T/C		-1068	T/A		-506	T/A	
-1444	T/C		-1058	T/C		-503	C/T	
-1436	T/C		-1048	A/T		-392	C/T	
-1414	C/T		-1035	225 bp insertion	Change in cis-element	-385	C/T	
-1410	C/T		-1033	G/A		-368	G/T	
-1392	C/T	Change in cis-element	-834	C/T		-336	G/C	
-1389	A/G	Change in cis-element	-822	T/G	Change in cis-element	-326	G/A	
-1317	65 bp deletion		-718	G/A		-311	G/T	
-1273	C/T		-655	A/T		+57	G/T	Change in cis-element
-1236	G/A		-638	C/T		+92	G/C	
-1216	T/G		-621	T/C		+365	T/A	
-1152	A/G		-608	G/A		+172*	A/C	Synonymous
-1133	A/C		-583	C/T	Change in cis-element	+1449*	C/T	Synonymous
-1140	A/G		-580	G/A		+1600*	A/G	Non-synonymous (T534A)
-1118	T/C		-559	C/T		+1869*	C/A	Synonymous
-1113	G/A		-556	A/C		+2204*	G/A	Non-synonymous (S735N)
-1104	T/C		-523	A/T		+2214*	A/G	Synonymous
-1078	T/C							

Positions marked with asterisk are the polymorphic sites in the coding sequence

roots increased 1.5-fold (50 mM NaCl) and 1.6-fold (100 mM NaCl), compared to the control (0 mM NaCl) (Fig. 3a, b). In contrast, the expression level of *OsSOS1* in leaves remained unchanged in both cultivars (in Pokkali 0.9-; 1.2-fold; in Nipponbare 1.4-; 1.3-fold under 50 mM NaCl; 100 mM NaCl, compared to the control, respectively) (Fig. 3c, d).

DISCUSSION

Rice is considered a salt-sensitive species, but some subspecies such as Pokkali and Nona Bokra are able to grow in a relative high concentration of salt, which might result from natural variations in genetic makeup and regulatory mechanisms. In the current study, we assessed the natural variation in *OsSOS1* gene sequence, which encodes the Na^+/H^+ antiporter, in two well-known contrasting rice cultivars, namely, the salt-sensitive Nipponbare and the salt-tolerant Pokkali. By analyzing the Na^+, and K^+ contents we detected the higher Na^+, but lower K^+ contents in Nipponbare than in Pokkali, resulting in a lower K^+/Na^+ ratio in Nipponbare than in Pokkali (Table 1). It has been shown that the ability to maintain high K^+ levels and a high K^+/Na^+ ratio in the cytoplasm could be essential for salt tolerance (Maathuis and Amtmann, 1999; Sun et al., 2015).

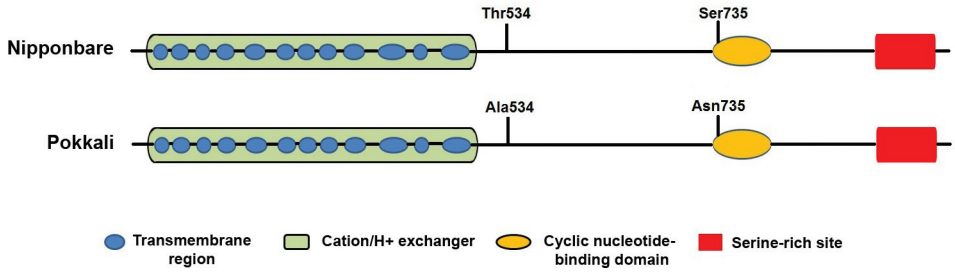

Fig. 1. Schematic representation of the domains present in *OsSOS1* proteins. All domains, except the serine-rich site, were identified using InterPro. The serine-rich site was predicted based on phosphorylation probability data obtained with NetPhos 3.1.

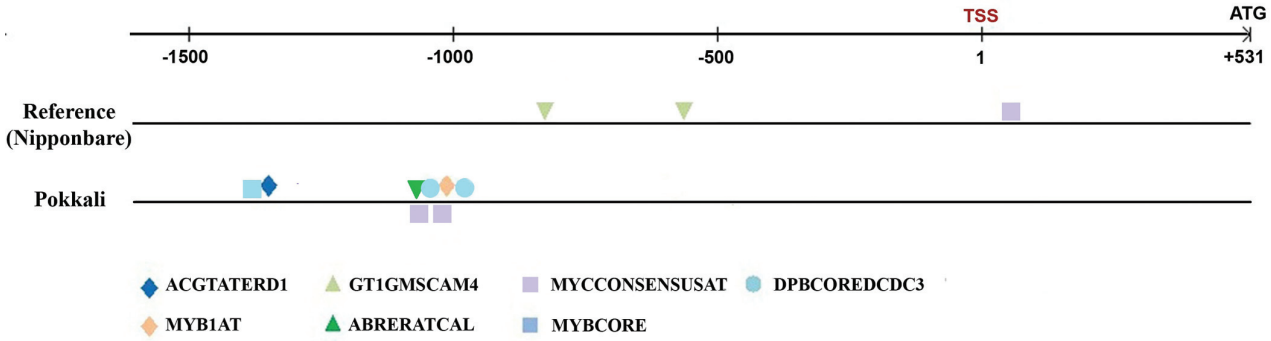

Fig. 2. Map of the upstream sequences of *OsSOS1* transporter. Schematic representation of differences in cis-regulatory elements within the upstream regions of Pokkali compared to that of Nipponbare. Positions are relative to the first base of the transcriptional start site (TSS).

This is consistent with our results; the maintenance of a high K^+/Na^+ ratio in Pokkali contributed to its salinity tolerance.

We could identify six nucleotide polymorphisms in the coding sequence, including two non-synonymous (A1600G, G2204A) leading to the amino acid changes T534A and S735N, respectively. Structural analysis showed that OsSOS1 has 12 transmembrane domains in its N-terminal region, and a long cytoplasmic tail at the C- terminus with a putative cyclic nucleotide binding domain centrally located (Fig. 1). The transmembrane region functions as a pore domain for ion transport and the long cytoplasmic tail functions as a regulatory domain (Quintero et al., 2011). This putative structure of OsSOS1 is highly homologous to SOS1 from other plant species (Shi et al., 2000; Maughan et al., 2009; Guo et al., 2012). It is well-known that the activity of SOS1 is activated through protein phosphorylation (at C-terminal domain of SOS1) by the SOS2-SOS3 complex (Qiu et al., 2002; Quintero et al., 2002) or SCaBP8–SOS2 (Quan et al., 2007). SOS3 is a calcium binding protein (Liu and Zhu, 1998), SOS2 is a protein kinase (Liu et al., 2000) and SCaBP8 is SOS3-like Calcium Binding Protein 8 (Quan et al., 2007). SOS3–SOS2 or SCaBP8–SOS2 interactions recruit SOS2 to the plasma membrane, which leads to activation of the downstream target SOS1. The two amino acid variants (T534A, S735N) are located in the cytoplasmic domain at C-terminal (Fig. 1). The phosphorylation analysis by NetPhos3.1 revealed that threonine 534 and serine 735 are not phosphorylated; instead, the phosphorylation site is a serine-rich region (Fig. 1). Thus, it is unlikely that the two amino acid substitutions influence the OsSOS1 protein properties.

The Na^+/H^+ antiporter SOS1 is the Na^+ efflux protein at the plasma membrane of plants (Shi et al., 2002, 2003). It has been proven that salt upregulates SOS1 activities via increases of transcription level of *SOS1* gene (Shi et al., 2002, 2003) and enhances Na^+ exclusion from the cytosol to the extracellular space (Qiu et al., 2002; Quintero et al., 2002). The expression of *SOS1* was induced

TABLE 3. Polymorphic sites in the upstream region of *OsSOS1* and putative cis-regulatory elements.

Position	Polymorphic type	Cis-element	Change in number	Sequence (5'–3')	Function of cis-element
-1392	C/T	MYBCORE	+1	CNGTTR	Responsive to water stress
-1389	A/G	ACGTATERD1	+1	ACGT	Dehydration responsive element and dark-induced senescence
-1035	225 bp insertion	ABRERATCAL	+1	MACGYGB	ABRE-related sequence, calcium-responsive cis-element
		DPBFCOREDCDC3	+2	ACACNNG	Abscisic acid response motif
		MYB1AT	+1	TAACTG	Binding site for ATMYB2, responsive to water stress
		MYCCONSENSUSAT	+2	CANNTG	Involved in dehydration and cold response
-822	T/G	GT1GMSCAM4	-1	GAAAAA	Required for pathogen attack, salinity and salicylic acidinduced gene expression
-583	C/T	GT1GMSCAM4	-1	GAAAAA	Required for pathogen attack, salinity and salicylic acid induced gene expression
+57	G/T	MYCCONSENSUSAT	-1	CANNTG	Involved in dehydration and cold response

N= A/T/G/C; M=C/A; Y=T/C; B=T/C/G;R=A/

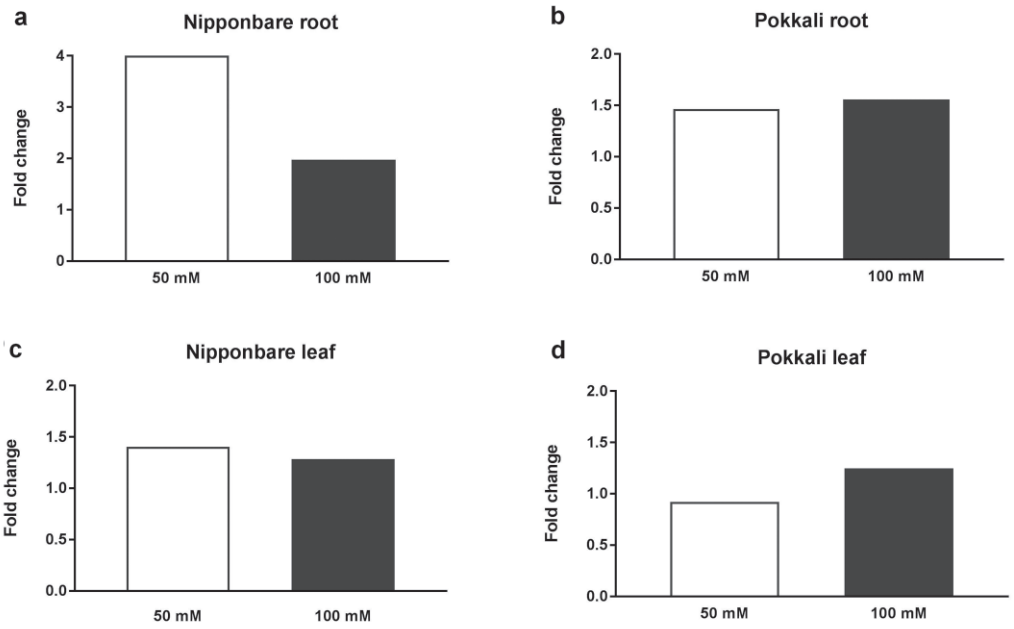

Fig. 3. The expression of *OsSOS1* gene under different NaCl concentrations (50 mM and 100 mM) for 7d in roots (A, B) and leaves (C, D) of two rice cultivars. The leaf/ root material of 5 different plants per cultivar and treatment was equally pooled for total RNA extraction. Two technical replicates of real-time PCR analysis were performed using gene-specific primers. The expression of *OsSOS1* gene in different samples was normalized with the expression of the internal control gene, actin 1. Fold changes in mRNA expression in different rice genotypes at different salt concentrations were calculated relative to its control using ddCt method (Livak and Schmittgen, 2001).

by salt stress (NaCl) in both roots and shoots, but higher in the roots (Shi et al., 2000; Oh et al., 2009; Guo et al., 2012). In rice, the expression of *OsSOS1* was highly upregulated by salt stress in roots with the maximal 6-fold after 15 h of salt treatment, while in the leaves the expression of *OsSOS1* was reduced 5-fold after 3 h of salt treatment followed by recovery to basal level (as in control) after 48 h of salt treatment (Martinez-Atienza et al., 2007). In our study, we conducted salt stress for 7 days since salinity treatment over a period of days helps to minimize shock responses and allows plants to adapt to the saline conditions (Tester and Davenport, 2003). Our results are in agreement with the findings of Martinez-Atienza et al. (2007) with respect to high induction of *OsSOS1* gene in response to salt stress in root, but not in leaves (Fig. 3). The change pattern of *OsSOS1* expression in response to salinity was similar in Nipponbare and Pokkali, suggesting that the induced expression of *OsSOS1* gene in roots under salt stress is a general salt-adaptive response. This statement is supported by observation of salt-induced expression of *SOS1* gene in both *Arabidopsis* (salt sensitivity) (Shi et al., 2000) and its close relative *Thellungiella salsuginea* (salt extreme tolerance) (Oh et al., 2009).

The cis-regulatory elements present in gene promoter play roles in gene regulation. By comparing the upstream regions of Nipponbare and Pokkali, we could detect 44 nucleotide polymorphisms and 2 INDELs (Table 2), leading to several differences in cis-regulatory elements (Fig. 2, Table 3), in which two copies of GT1GMSCAM4 (salt-responsive element) (Park et al., 2004) were deleted, but two copies of DPBFCOREDCDC3 (ABA-responsive element) (Lopez-Molina and Chua, 2000) were added. ABA is a very important hormone involved in signaling various stresses, including salinity (Tuteja, 2007). Although two copies of salt-responsive elements (GT1GMSCAM4) were lost in Pokkali, other six of these elements are still present in the upstream region of *OsSOS1* gene of Pokkali (Table S1). Furthermore, the DPBFCOREDCDC3 (ABA-responsive element) is present in the upstream region of *OsSOS1* of Nipponbare with one copy, and with three copies in that of Pokkali (Table S1). Thus, it is likely that since the important salt-responsive cis-regulatory elements are still present in the upstream region of *OsSOS1* in both cultivars, the change pattern in gene expression in response to salt stress was similar in both cultivars.

The balance of Na^+ and K^+ contents in plants under salt stress is regulated by different transporters. It has been proven that SOS1, HKTs, and NHXs are the key transporters involved in Na^+ and K^+ homeostasis and salt tolerance (Almeida et al., 2017). Zhang et al. (2017) reported that SOS1, HKT1;5, and NHX1 synergistically regulate Na^+ homeostasis by controlling Na^+ transport systems under salt conditions in *P. tenuiflora*. In the current study, both cultivars: Nipponbare and Pokkali showed increased expression of *OsSOS1* in response to salt stress. It is worth to further investigate the expression levels of *HKT* and *NHX* genes in these two cultivars in order to understand the molecular basis of salt tolerant differences between them.

CONCLUSION

We could detect six nucleotide polymorphisms in the coding sequence, 44 single nucleotide polymorphisms and 2 INDELs in the upstream region of the *OsSOS1* gene. *In silico* analysis indicated that the variants in the coding sequence caused no effect on protein, while the variants in the upstream region led to changes in several cis-regulatory elements involving abiotic stress response. Change pattern of *OsSOS1* gene expression in response to salt stress was similar in the two rice cultivars.

AUTHORS' CONTRIBUTIONS

PTD designed experiments, prepared and revised manuscript. HQP conducted experiments and collected data. HMN conducted measurement of Na^+, and K^+ contents. DHL analyzed data. The authors declare no conflict of interest.

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