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Cloning, Structural and Expression Analysis of *OsSOS2* in Contrasting Cultivars of Rice under Salinity Stress

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ABSTRACT

Salinity is one of the major environmental factors limiting growth and productivity of crop plants in coastal areas and irrigated farmlands. Salinity tolerance is a very complex trait. Plants adapt to salinity stress by coordinated and orchestrated functioning of various complex mechanisms. In *Arabidopsis thaliana*, SOS (Salt Overly Sensitive) pathway has been established as a major player in ion homeostasis and salt tolerance. The SOS pathway has recently been shown to be conserved in rice as well. In the present study, we have isolated and characterized the *OsSOS2* full-length cDNA from a salt sensitive *Oryza sativa* L. cv 'IR64', which encodes 50.65 KD protein. It was observed that *OsSOS2* transcripts are induced by salinity and further showed differential accumulation at different time intervals at seedling stage in contrasting cultivars of rice i.e. 'IR64' (salt sensitive) and 'Pokkali' (naturally salt tolerant). We have also observed tissue specific expression for *OsSOS2* in field grown mature plants of these contrasting cultivars. With the use of molecular modeling techniques, we have modeled OsSOS2 protein and present a comparative structural analysis with respect to its ortholog from model plant - *Arabidopsis thaliana*. Comparison of various orthologous sequences has shown high level of similarities between SOS2 members isolated from *Arabidopsis thaliana* and *O. sativa*. Experiments have established that the SOS3 protein senses Ca^{2+} and regulates SOS2 activity. Therefore, we have carried out the analysis of conserved binding site for SOS3 protein in SOS2 protein which can give an insight to the probable mechanism of the functioning of OsSOS2 protein. We propose that OsSOS2 is one of the important members of salinity stress response in rice functioning towards ion homeostasis.

Keywords: abiotic stress, *Arabidopsis thaliana*, ion homeostasis, *Oryza sativa*, osmotic stress, salinity, salt overly sensitive

INTRODUCTION

Crop productivity has been greatly affected due to increasing salt concentration in the soil. Salts when present in excess in soil, interfere with the mineral nutrition and water uptake, and lead to accumulation of toxic ions (Hasegawa *et al.* 2000). Excess of salt accumulation in cell leads to membrane disorganization, impaired nutrient and water acquisition, metabolic toxicity, inhibition of photosynthesis and production of reactive oxygen species. The homeostasis of intracellular ion concentrations is a fundamental property of living cells. Due to their sessile nature, plants have evolved several mechanisms to cope up with the varying salt concentrations in the environment. Re-establishment of the proper cellular ion homeostasis along with other concomitant processes is necessary for the plant growth under salt stress conditions.

Salinity tolerance is a very complex trait in plant species, since there are numerous mechanisms operating at cellular, tissue, organ, or whole plant level (Yeo 1998). In saline soils, sodium ions (Na^+) are found in abundance which are cytotoxic for plants as they accumulate in high concentrations and lead to deficiency of essential ions, such as K^+ (Hasegawa *et al.* 2000; Hernández *et al.* 2001). Several ion transporters have been reported earlier that facilitate Na^+ entry and exit in plant cells. It has been observed that Na^+ can also be compartmentalized in the vacuole through tonoplast-localized Na^+/H^+ antiporters (Apse *et al.* 1999). Earlier analysis has shown that the uptake of Na^+ into plant cells appears to occur at least partly through the transporter HKT1 (Rus *et al.* 2001; Laurie *et al.* 2002; Maser *et al.* 2002) and through nonselective cation channels (Amtmann and Sanders 1999). In *Arabidopsis thaliana*, an ionic homeostasis regulatory pathway activated by salt stress has been identified through molecular and genetic characterization of several salt overly sensitive (*sos*) mutants that are defective in K^+/Na^+ homeostasis (Liu and Zhu 1998; Liu *et al.* 2000; Shi *et al.* 2000). The salt overly sensitive (SOS) pathway was found essential for maintaining favorable ion ratios in the cytoplasm and for tolerance towards salt stress (Zhu *et al.* 1998; Zhu 2000).

The SOS pathway is known to be defined by three protein components namely SOS1, SOS2 and SOS3. SOS1 was considered as the first putative plant Na^+/H^+ antiporter to be described in *A. thaliana* (Shi *et al.* 2000, 2002). The *sos1* mutant in *Arabidopsis* was isolated in a genetic screen for plants hypersensitive to NaCl, together with *sos2* and *sos3* mutants (Zhu 2000). Analysis of SOS3 gene product has revealed that it shares substantial sequence similarity with the regulatory subunit of yeast calcineurin (CNB) (Liu and Zhu 1998). Sequence analysis has predicted that SOS1 protein is a 127-kDa membrane protein with 12 putative membrane-spanning domains and a long hydrophilic tail at the C-terminal end of the protein (Shi *et al.* 2000). It is proposed that SOS3 is a myristoylated calcium binding protein that senses calcium signal (Liu and Zhu 1998; Ishitani *et al.* 2000). Further, SOS3 physically interacts with the protein kinase SOS2 and activates the substrate phosphorylation activity of SOS2 in a calcium dependent manner (Halfter *et al.* 2000; Liu *et al.* 2000). The SOS2-SOS3 complex phosphorylates and thus activates a plasma membrane localized Na^+/H^+ antiporter, SOS1 (Shi *et al.* 2000; Qiu *et al.* 2002; Quintero *et al.* 2002). Any mutation in SOS1, SOS2, or SOS3 reduces the Na^+/H^+ exchange activity and a constitutively active SOS2 enhances Na^+/H^+ exchange activity in a SOS1-dependent and SOS3-independent manner (Qiu *et al.*

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2002).

SOS2 encodes a 446-amino acid Ser/Thr protein kinase and can be classified as a member of the SnRK3 subgroup of SNF1-related protein kinases. SOS2 has a highly conserved N-terminal catalytic domain similar to that of *Saccharomyces cerevisiae* SNF1 and animal AMPK (Liu *et al.* 2000). Earlier, SOS2 in *Arabidopsis* was found to be a Ser/Thr protein kinase with two functional domains (Guo *et al.* 2001). The N-terminal region of SOS2 contains the kinase catalytic domain, which has a sequence similar to the SNF1/AMP kinases and C-terminal region which has a regulatory function and contains an autoinhibitory domain (the FISL domain) that interacts with SOS3 (Guo *et al.* 2001). In *Arabidopsis*, SOS2 was found to be expressed in both roots and shoots and is up-regulated under salt stress in the roots (Liu *et al.* 2000). Autophosphorylation assays demonstrate that SOS2 is an active protein kinase. It has been observed that SOS2 is active in substrate phosphorylation only when plants are exposed to salt stress. Further, SOS2 activity depends on SOS3 and calcium (Halfter *et al.* 2000).

Recently NaCl-induced activity of plasma membrane Na^+/H^+ antiporter has been reported in rice (Martínez-Atienza *et al.* 2007). Until now, there has been no report about the characterization of SOS2 gene from rice. In order to understand the role of SOS2 protein in salt stress tolerance in *O. sativa*, we have isolated full length cDNA for OsSOS2, from *O. sativa* cv. 'IR64'. Comparison of nucleotide and amino acid sequences of various OsSOS2 genes suggest a high degree of sequence conservation and thus the possible, functional conservation among plant species. We have also attempted to analyze OsSOS2 protein using homology modeling in order to understand the various conserved structural features of the protein and compared with that of *Arabidopsis* SOS2 (AtSOS2) protein. Also, the expression patterns for SOS2 under high salt conditions in different tissues of mature plants grown under standard agronomic practices have been investigated.

MATERIALS AND METHODS

Experiment to analyse transcript accumulation of *OsSOS2* in rice genotypes 'IR64' and 'Pokkali'

1. Plant materials and growth conditions

Seeds of rice genotypes ('IR64' and 'Pokkali') were washed with de-ionized water and allowed to germinate in ½ Yoshida medium (Yoshida *et al.* 1976) under hydroponic system for 48 h in dark and then transferred to light for further growth under control conditions (28 ± 2°C, 12h light and dark cycle). For experiments pertaining to mature plants, seeds of 'IR64' and 'Pokkali' were directly sown in microplots and brought to maturity employing standard agronomic practices and experimental tissues were harvested from various organs of plants.

2. Stress treatments

For salinity stress treatment, 6 d old seedlings of rice ('IR64' and 'Pokkali') as well as plants at tillering stage were treated with 200 mM NaCl for analysis of very early (10, 20, or 30 min), and late (24, 48 or 72 h) response. Similarly, for organ specific transcript analysis from mature plant, tissues were harvested from both vegetative (upper, middle and lower leaf and stem) and reproductive (panicle) parts of mature plant and subjected to salinity stress. Essentially, leaves were cut and allowed to float in ½ Yoshida medium or medium supplemented with 200 mM NaCl, in glass Petri dishes and kept in the culture room for 30 min and 24 h. Similarly, panicle was subjected to the stress treatment in Petri dishes and kept in the culture room for 30 min and 24 h after which the tissue was harvested and used for analysis of spatial distribution (constitutive and stress-induced abundance) of *OsSOS2* transcripts employing RNA gel blot analysis.

3. RNA extraction and Northern blot analysis

Total RNA was extracted from tissue using TRIzol method as per the manufacturer's instructions (Invitrogen, USA). Northern blots were prepared using 20 µg total RNA. OsSOS2 probes were prepared by labeling the PCR-amplified fragments of OsSOS2 cDNA clones with α^{32} P-dATP using HexaLabel DNA labeling kit (Fermentas Life Sciences) and purified using PCR purification kit (Qiagen). Northern blots were hybridized at 65°C in 5X SSC, 5X Denhardt's reagent, 0.1% SDS and 100 µg/ml denatured salmon sperm DNA for 16-18 h. Membrane was washed twice in 0.5X SSC, 0.1% SDS and 0.1X SSC, 0.1% SDS for 15 min each at 65°C and scanned on a phosphorimager using the software Fujifilm Image Reader. High stringency was maintained during hybridization as well as washing to ensure specificity of signal on membranes. The relative transcript abundance was calculated using the Image Gauge (Fuji Photofilm Co. Ltd., Japan).

Experiment to study the three dimensional structure of OsSOS2

1. Homology modeling and analysis

The three-dimensional structure of OsSOS2 (DQ298963) was modeled in a stepwise procedure, starting with the identification of templates. BLAST search against the PDB database (www.rcsb.org) identified structures of SOS2 bound with calcium sensor SOS3 in *Arabidopsis* (2EHB.pdb), phosphorylated SNF1 kinase domain in *E. coli* (3EAE.pdb), AMPK fragment from *Schizosaccharomyces pombe* (3H4J.pdb) and protein kinase domain of yeast amp-2 activated protein kinase snf1 from *Saccharomyces cerevisiae* (3HYH.pdb) as potential template structures for modeling OsSOS2 protein. These template structures were aligned using STAMP (Russell and Barton 1992). These aligned structures were used as a profile for aligning the target sequence using ClustalX (Thompson *et al.* 1997). The automated comparative protein modeling program MODELLER9v7 (Sali and Blundell 1993; Fiser *et al.* 2000) was then used to generate a 100 all-atom model by alignment of the target sequence with the selected template sequence in an alignment file. The best model was chosen on the basis of stereochemistry quality report generated using PROCHECK (Morris *et al.* 1992; Laskowski *et al.* 1993) and side chains were optimized using SCWRL 4.0 (Canutescu *et al.* 2003). The bond distance and dihedral angle restraints on the target sequence were derived from its alignment with the template three-dimensional structures. The spatial restraints and the energy minimization steps were performed within Modeller using the CHARMM22 force field for proper stereochemistry of proteins. More than one template has been chosen for modeling SOS2 protein, as it has been suggested to improve the quality of the model (Sánchez and Sali 1997). The presence of conserved structural motifs was studied using STRIDE (Frishman and Argos 1995) on modeled OsSOS2 in comparison to motifs present in template structures. Molecular visualization and analysis of the final model were carried out with Visual Molecular Dynamics (VMD) (http://www.ks.uiuc.edu/Research/vmd/) (Humphrey *et al.* 1996). Further, secondary structure was predicted using JNET (Cuff and Barton 1999; Cole *et al.* 2008), SABLE (Adamczak *et al.* 2004, 2005; Wagner *et al.* 2005), PREDATOR (Kabsch and Sanders 1983; Frishman and Argos 1995, 1996, 1997), PSIPRED (Jones 1999) and SAM (Hughey and Krogh 1996). Fold-recognition analysis was carried out using FUGUE (Shi *et al.* 2001), mGENETHREADER (Jones 1999; McGuffin and Jones 2003) and 3DPSSM (Fischer *et al.* 1999; Kelley *et al.* 2000). The architectural motifs and the topology of proteins with known three-dimensional structure were analysed according to SCOP (Murzin *et al.* 1995) and CATH (Orengo *et al.* 1997) classifications.

RESULTS AND DISCUSSION

SOS pathway and ion homeostasis in plants

Arabidopsis has served as a model system for analyzing salinity stress response because of the availability of its complete genome sequence, stable transformation protocols,

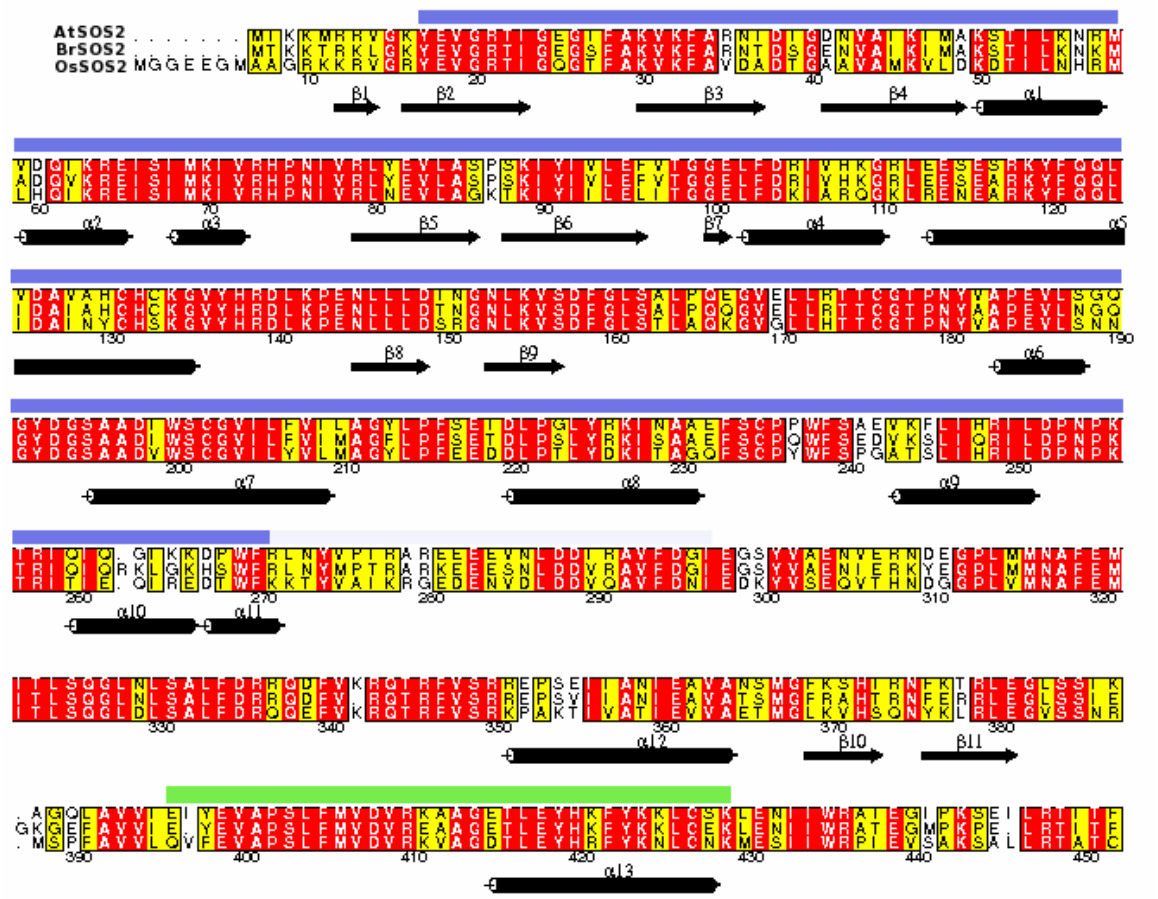

Fig. 1 Alignment of SOS2 protein sequences, showing structural motifs in the modeled OsSOS2. Secondary structure elements are indicated (α-helices as cylinders and β-strands as arrows). The sequences are numbered with respect to OsSOS2. The figure was prepared using Alscript program (Barton 1993). The colored boxes above the alignment show the presence of conserved kinase (Blue) and NAF (Green) domain.

short generation time, expressed sequence tags and mutant lines, etc. (Rhee *et al.* 2003). However, it is also suggested that experiments using the *Arabidopsis* model must now be designed in a rational way to increase the possibility of identifying target genes with the potential for engineering salt tolerance in crop plants (Denby and Gehring 2005). Further challenge is to select and characterize target genes with an important function in stress tolerance and biotechnological potential. In this regard, 'salt overly sensitive' (*SOS*) pathway has been well explored in *Arabidopsis* (Zhu 2003) and the engineering of SOS1 has resulted in enhanced salt tolerance (Shi *et al.* 2002). The *SOS* pathway has been found to be conserved in rice as well (Martínez-Atienza *et al.* 2006). In recent years, detailed investigations have been carried out on the three major genes of this pathway namely *SOS*1, *SOS*2 and *SOS*3 (Liu and Zhu 1998; Halfter *et al.* 2000; Shi *et al.* 2000; Gong *et al.* 2002; Qiu *et al.* 2002; Quintero *et al*. 2002). SOS3 is a calcium sensor, SOS2 is a serine/threonine protein kinase and SOS1 is a plasma membrane associated Na^+/H^+ antiporter. Fine regulation of this pathway brings out ion-homeostasis in *Arabidopsis* system and mutation in any of these genes renders plant more sensitive towards salinity stress (Zhu 2003). With this viewpoint, our laboratory is engaged in isolating and characterizing *SOS* pathway members from *Oryza sativa* L. cv. 'IR64'. For this purpose, primers have been designed and amplicons thus obtained have been sequenced and compared with *Arabidopsis SOS* members.

Cloning of full length cDNA for OsSOS2

We isolated a 1.4 kb full length cDNA coding for *OsSOS2* (DQ298963) which possess typical features such as kinase, FISL and regulatory domains (**Fig. 1**). *SOS*1 has been documented in literature to be a single copy gene in A*rabidopsis* and rice (Martínez-Atienza *et al.* 2006). *SOS*2 seems to be multigene family in Arabidopsis, as 25 SOS2 like protein kinase (PKS/CIPKs) have been reported and 10 SOS3 like calcium binding proteins (designated as SCaBPs/CBLs) have been indicated in *Arabidopsis* (Guo *et al.* 2001; Kolukisaoglu *et al.* 2004).

Identification of three dimensional folds in OsSOS2 protein

To create model of OsSOS2, we first performed BLAST searches against Protein Data Bank (PDB) for proteins with similar sequence and known 3D structure using 453 residue long sequence of OsSOS2 from *O. sativa* (DQ298963). The identified templates were then used to model the OsSOS2 protein using threading approach (see Methods). The threading approach helps to assess the compatibility of the target sequence with the available protein folds based not only on the sequence similarities but also on structural considerations (Bujnicki 2003; Godzik 2003). The conserved domain in both the sequences was identified using Pfam database (Finn *et al.* 2008). Analysis of results obtained from Pfam showed the presence of two conserved domains kinase (PF00069) and NAF/FISL domain (PF03822) in the OsSOS2 protein sequence (**Fig. 1**). The presence of conserved domains identified in Pfam searches were also confirmed in searches against CDD (conserved domain database). The major secondary structure and fold region of OsSOS2 protein sequence were found to be well conserved.

Comparative modeling of SOS2 sequences

To generate three dimensional model structure of OsSOS2 protein, a set of respective 100 all atom structure had been generated using Modeller9v7. Ramachandran plots were generated for SOS2 protein structures in OsSOS2 to determine deviations from normal bond lengths, dihedrals and no

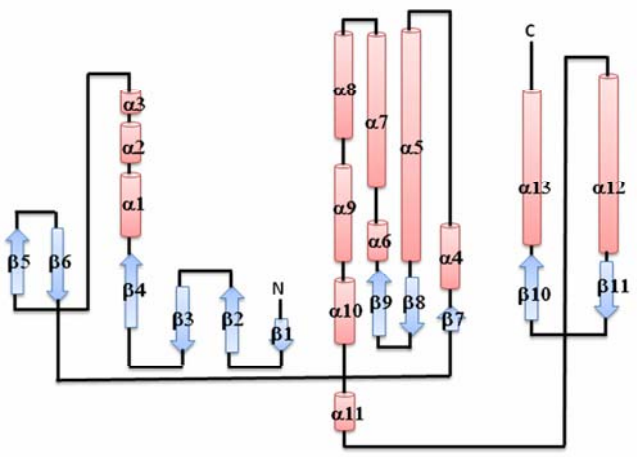

Fig. 2 Secondary structure topology of OsSOS2 protein showing position of various structure motifs. The secondary structures were named according to the sequence of appearance from N-terminal to C-terminal.

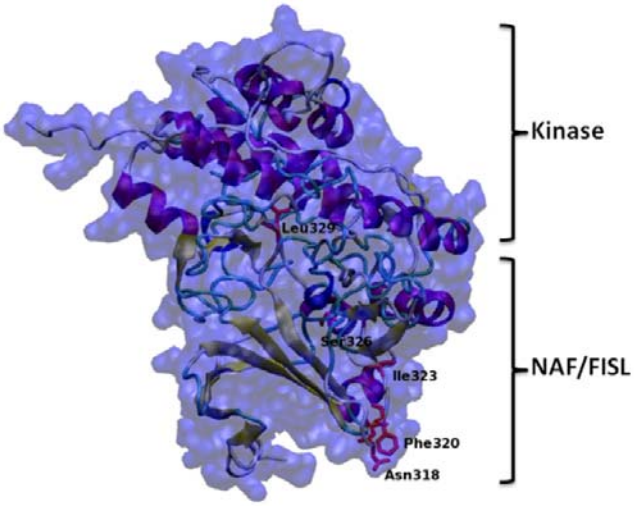

Fig. 3 New cartoon view diagram of modeled OsSOS2 protein shown as a transparent MSMS surface. The figure was prepared using Visual Molecular Dynamics (VMD) (http://www.ks.uiuc.edu/Research/vmd/)

bonded atom-atom distances, and also with a viewpoint to compare the SOS2 protein model against the SOS2 structure solved by X-ray crystallography. Analysis for modeled OsSOS2 protein showed 97% residues in allowed regions while 2 and 1% residues were observed in generously allowed and disallowed region respectively. The procheck results summary showed 10 residues out of 326 as labeled in OsSOS2. The torsion angles of the side chain designated by $\chi1-\chi2$ plots showed no residues in the labeled region in both the models. All main-chain and side-chain parameters were found to be in the 'better' region. G-factor is essentially a log odds score based on the observed distribution of stereochemical parameters such as main chain bond angles, bond length and phi-psi torsion angles. The score for G-factors should be above -0.50 for a reliable model. The observed G-factor scores of the present model were found to be -0.06 for dihedral bonds, -0.38 for covalent bonds and -0.14 overall in OsSOS2 protein. The distribution of the main chain bond lengths and bond angles were observed to be 95% within limits for OsSOS2 protein structure. Conclusively, predicted structure of OsSOS2 protein was observed to be reliable model for analysis with various structural motifs conserved. Regions of the secondary structure were also verified using PREDATOR and STRIDE software.

Three dimensional structure of OsSOS2 protein

The comparison of OsSOS2 and AtSOS2 protein sequence with that of SOS2 proteins sequences in other plant species revealed the presence of conserved residues. OsSOS2 protein was observed to possess 11 β-strands and 13 α-helices (**Fig. 2**). SOS2 protein consists of two distinct structural domains namely kinase domain and NAF domain (as shown in **Fig. 1**). Analysis of kinase domain of SOS2 revealed that kinase domain of SOS2 resembles that of Snf1 domain of members of the Snf1/AMP-activated kinase (AMPK) family. The Snf1/AMPK family was found to be conserved in all eukaryotes and members of this family play fundamental role in cellular responses to metabolic stress (Hardie *et al.* 1998; Carling 2004). The N-terminal domain of the SOS2 protein was found to be a β-rich segment (**Fig. 2**). The kinase domain showed the presence of ATP binding conserved residues Lys88 and Glu117 in β6 and α5 secondary structure motif. The high degree of sequence conservation with respect to the other kinase proteins shows that kinase domain of the SOS2 protein has highly homologous structure. Earlier analysis has revealed that the phosphorylation of activation loop of SOS2 protein leads to its activation (Hanks and Hunter 1995; Johnson *et al.* 1996). The activation loop was found to be conserved in OsSOS2 protein as in various other kinase proteins. Recent analysis of OsSOS2 protein has identified conserved Ser228 in its sequence (Fujii and Zhu 2009) which was found to be regulated by phosphorylation. Thus, the autophosphorylation on Ser228 was suggested to be involved in SOS2 function under salt stress (Fujii and Zhu 2009).

The other distinct domain observed in OsSOS2 was NAF/FISL domain (**Fig. 1**). Earlier analysis of AtSOS2 protein has shown that C-terminal region consist of FISL (also known as NAF) and PPI motif (Sanchez-Barrena *et al.* 2007). SOS2 protein is constitutively active when the FISL motif of the protein is removed (Guo 2001; Qiu 2002). Various binding analyses of SOS3 with SOS2 protein showed that the NAF/FISL domain plays a major role in their interaction. The NAF domain was observed to fit into the cleft formed by SOS3 (Sanchez-Barrena *et al.* 2007). In OsSOS2 protein, Asn318, Ala319, Phe320, Ile298, Ser304, and Leu325 were found to be conserved as observed in other members of the CIPK (NAF/FISL domain) family. Analysis of secondary structure revealed that Asn318 and Phe320 are involved in formation of loop connecting the N-terminus of the FISL/NAF motif. Structural analysis suggests that these residues get buried on interaction between SOS2 and SOS3 proteins (Sánchez-Barrena *et al.* 2007). The secondary structure folds present in the FISL/NAF domain of OsSOS2 protein were also found to be conserved in the other members of CIPK family. In the AtSOS2 protein structure (**Fig. 3**), the PPI domain consists of two α-helices packed against a five-stranded antiparallel β-sheet with a β1-β5-β4-β3-β2 strand order while in OsSOS2 structure three α-helices pack against five-stranded anti-parallel β-sheets (**Fig. 2**). The conservation of PPI structural motif of OsSOS2 protein with that of other SOS2 member proteins suggests that OsSOS2 proteins have similar folds that assist in phosphate binding (**Fig. 4**). In *Arabidopsis*, mutateonal analysis suggested that conserved Arg337 and Phe341 play major role in phosphatase binding (Ohta *et al.* 2003). These residues were also found conserved in OsSOS2.

Transcriptional regulation of *OsSOS2* gene in seedlings and organs of mature plants of contrasting cultivars of rice grown under standard agronomic practices

With the availability of contrasting salinity responsive rice cultivars, we were prompted to work out the fine regulation of expression of *OsSOS2* gene. For this purpose, seedlings of 'IR64' (sensitive) and 'Pokkali' (tolerant) were subjected to either very short durations (10, 20 or 30 min) or long durations (24, 48 or 72 h) of salinity stress to get an insight into regulation of *OsSOS2* gene expression in very early (≤ 30 min) and late phase (≤ 72 h) of salinity stress. This analysis indicated clear differences in regulation of *OsSOS2*

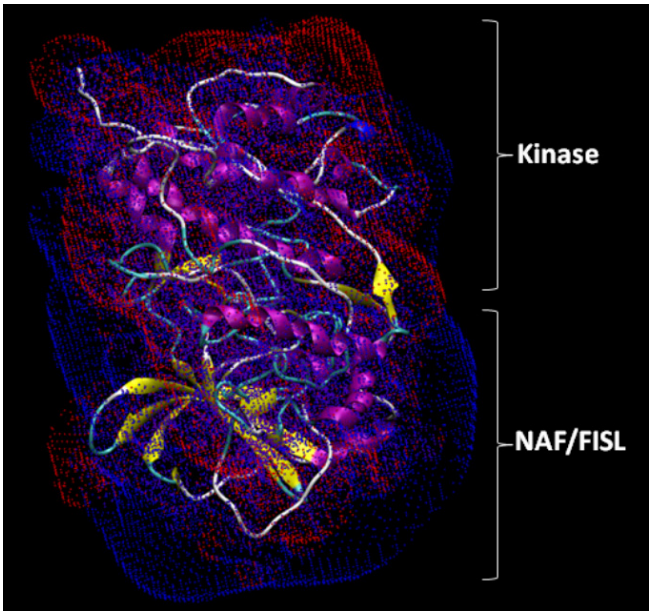

Fig. 4 The electrostatic binding energy for the OsSOS2 protein. Computing was carried out using APBS [Baker *et al*. 2001] and displayed using VMD. The OsSOS2 protein kinase is shown as a new cartoon diagram. The electrostatic binding energy is visualized by direct volume rendering and using two iso-surfaces.

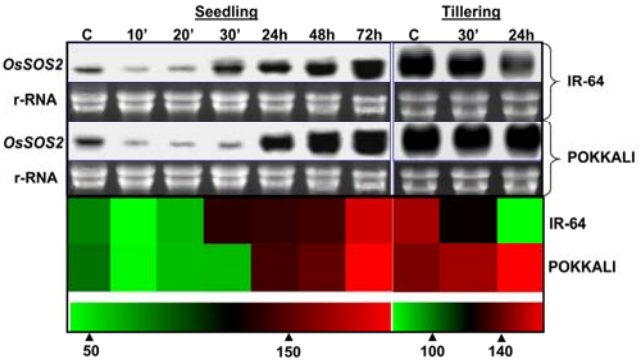

Fig. 5 Differential transcript accumulation for Os*SOS2* genes during 'very early' and 'late phase' of salinity stress in seedlings of two cultivars 'IR64' and 'Pokkali'. Northern blots probed with *OsSOS*2. Ethidium bromide (EtBr) stained RNA gel shown as the loading control. Duration of 200 mM NaCl stress has been mentioned on top of each lane. Heat map generated on the basis of signal intensity of OsSOS2 on RNA blots has been shown below the figure.

gene in the contrasting cultivars (**Fig. 5**). *OsSOS*2 transcripts were found to be induced within the very early phase (30 min.) in sensitive rice cultivar 'IR64'; however, the tolerant cultivar 'Pokkali' exhibited lower transcripts than 'IR64' under the conditions tested here (**Fig. 5**). It is also interesting to note that during the late phase (especially at 48 and 72 h salinity stress), the *OsSOS*2 transcripts were comparable in the two cultivars. Though, *OsSOS*2 was again found to be inducible by salinity stress in both the cultivars during the late phase, the tolerant cultivars always exhibited higher transcripts than the sensitive (**Fig. 5**). However, at tillering stage, the transcript levels were seen to decline during 24 h of salinity stress in sensitive 'IR64' cultivar but there were no significant changes in the tolerant genotype 'Pokkali'. The kinetics of induction showed a great contrast as under control conditions, 'IR64' maintained lower transcript level than 'Pokkali'. However, this analysis does not cover the differences which may exist in the two cultivars because of post-transcriptional and/or post translational regulations operative in them. Nonetheless, the fine regulation of *OsSOS2* genes in the two cultivars presents before us an interesting gene regulatory model which warrants further detailed analysis.

Most of the adaptive responses of plant towards salinity stress are observed to be controlled by their developmental status (DeRocher and Bohnert 1993). It has been established that seedling stage as well as the reproductive stage represents the two most sensitive stages in life cycle of plants (Drake and Drake 1998; Houle *et al.* 2001). In our study related to the time kinetics (very early and late) for *OsSOS2* transcript accumulation in contrasting cultivars of rice, we found *OsSOS2* up-regulation within few minutes of stress in rice salt sensitive cultivar 'IR64' but was not so in salt tolerant cultivar 'Pokkali' (**Fig. 5**). While the mechanisms that establish cell identity have been the focus of many studies, little is known in plants about how cell fate decisions came to regulate the interaction of cells with their environment. To gain an insight into how SOS pathway might be regulated in different organs of rice plant, we performed northern analysis employing tissues from mature plant. Different organs of the plant represent a unique system where gene expression pattern determines the physiological behaviour. Recent studies have documented differential gene regulation within different cell-types of an organ also (Ma and Bohnert 2007).

The contrasting genotypes of rice i.e. 'IR64' and 'Pokkali' were grown to full maturity employing standard agronomic practices. At the mature plant stage, tissue samples from various plant organs were collected and leaf discs were either incubated in nutrient solution only (control) or supplemented with NaCl (stress) before extracting total RNA for analysis. RNA gel-blot prepared from these RNA samples were successively hybridized with *OsSOS2* gene probe. For a better comparison, blots corresponding to a given organ from 'IR64' and 'Pokkali' are placed below each other (**Fig. 6**). The phosphor-images of Northern blots were visually inspected for differences in the intensity of *SOS2* transcript within various tissues. *OsSOS*2 could be detected in all the plant organs analyzed in the two cultivars of rice. Constitutive as well as salinity induced *OsSOS*2 transcripts could be detected in all organs for both the cultivars. However, as can be seen from **Fig. 6**, there are some organs which showed a contrasting pattern between the two cultivars. For example, panicles of 'Pokkali' showed relatively higher transcripts than panicles of 'IR64' under unstressed conditions. The reproductive organs (panicle) and the selected vegetative organs (leaf) showed relatively higher signal for *OsSOS*2 than other organs. 'Pokkali' showed relatively higher salinity induced transcripts for *OsSOS*2 in all tissues as compared to 'IR64'. In the case of 'IR64', lower and middle stem samples showed relatively higher salinity-induced transcripts for *OsSOS2* but in the upper and middle leaf of 'IR64', showed down regulation of OsSOS2 transcripts were observed.

Our analysis of transcript abundance for *OsSOS2* genes between various organs of field grown mature plant not only depicted differences within organs but unique differences were also noted among the two contrasting cultivars of rice (**Fig. 6**). The important observation in this regard need to be made for *OsSOS2* transcripts where only specific tissues such as panicle was documented to have higher transcripts as compared to rest of the organs in both the cultivars of rice. AtSOS1-promoter-GUS transgenic *Arabidopsis* plants showed expression in epidermal cells of the root tip and in parenchyma cells at the xylem/symplast boundary of roots, stems and leaves (Shi *et al.* 2002). In rice, it has been suggested that at the reproductive stage, it undergoes a genome expression reprogramming under stresses such as salinity and drought. Only a limited number of stress responsive genes are shared between any two organs (Zhou *et al.* 2007). Further analysis related to regulatory machinery associated with this unique organ specific expression of stress genes suggested that organ-specific transcription factor gene expression may be responsible for activating organ-specific downstream genes in secondary transcriptional response to stress. However, the real challenge would be to see how far these regulatory circuits are operational in terms of

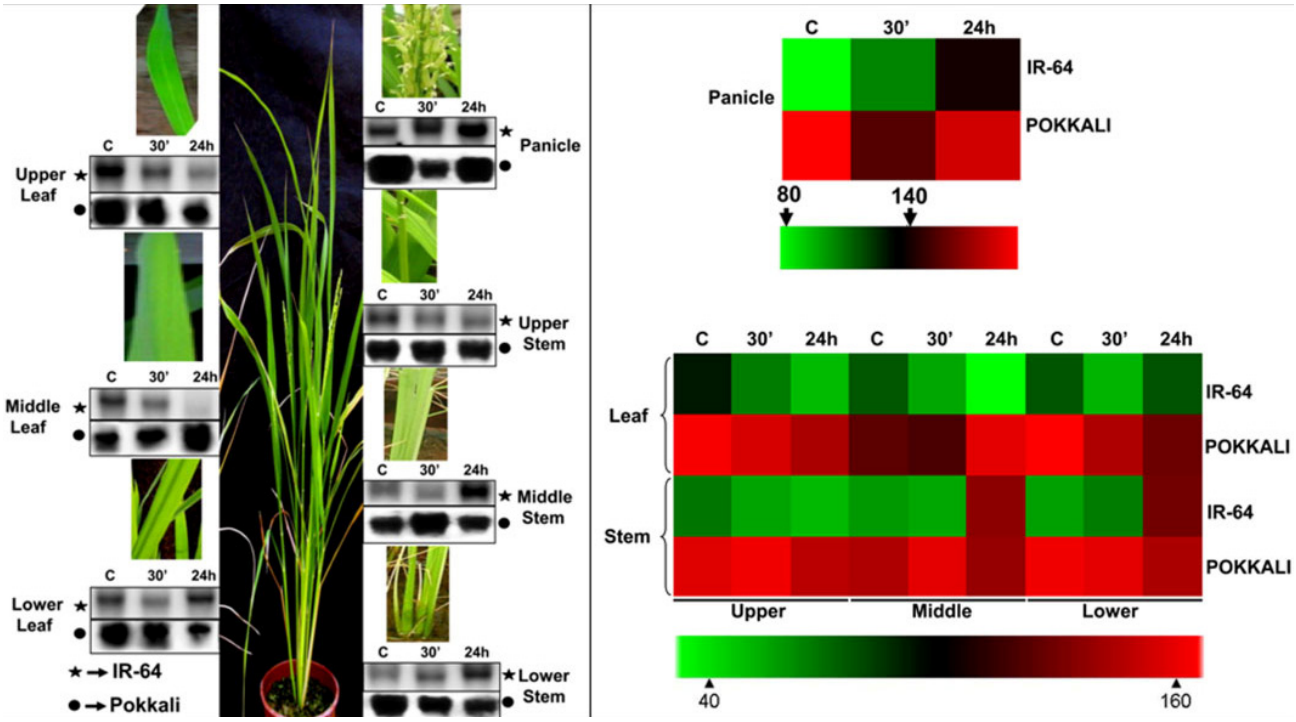

Fig. 6 OsSOS2 transcripts accumulation is tissue specific in various vegetative and reproductive organs of field grown mature plants of salt sensitive 'IR64' and salt tolerant 'Pokkali' cultivar. Analysis was performed on stem (upper, middle and lower), leaf (upper, middle and lower), panicle of the two cultivars under control (C) and 200 mM NaCl, 30 min and 24 h stress conditions. Northern blots prepared from various samples were probed with Os*SOS*2. The right panel shows the heat map generated on the basis of signal intensity of OsSOS2 on RNA blots.

specific capability of different organs in these cultivars in controlling the cell physiology or organ physiology. This study opens up several issues which warrant analysis of these two contrasting cultivars employing tools related to proteome analysis, metabolism analysis and electrophysiological analysis.

Availability of contrasting cultivars for salinity response in crop species such as *Oryza* is highly advantageous for obvious reasons. Additionally, the information reported in this study may also work as a platform for the identification and eventual manipulation of genes involved in natural variation in salinity response in *Oryza*.

CONCLUSIONS

SOS pathway has been documented as one of the major signaling pathway required for maintaining ionic homeostasis in various plant species. SOS2 protein is one of the three members of SOS pathway and is a member of the SNF1-related protein kinase 3 (SnRK3) families. SOS2 has also been observed as one of the pivotal kinases active under salt stress (Fujii and Zhu 2009). Because SOS2 protein is not constitutively active in substrate phosphorylation *in vitro* (Gong *et al.* 2002), its activation is a key signaling event under salt stress. Analysis of OsSOS2 protein structure revealed catalytic domain to be located in the N-terminal region, and the FISL motif located in the C-terminal regulatory region which serves as an autoinhibitory domain. Analysis of kinase domain has revealed several conserved residues which were suggested to play a major role in its activation. NAF/FISL domain at C-terminal was found to be well conserved in OsSOS2 protein which was suggested to mediate binding with SOS3 protein, thus activating the SOS pathway in Ca^{2+} dependent manner. Analysis of SOS2 protein has shown that the conserved PPI domain is blocked due to binding with SOS3 and therefore the kinase activity and the phosphatase binding cannot occur simultaneously (Sánchez-Barrena *et al.* 2007). Structure of OsSOS2 was found to have similar folds as that of AtSOS2 protein. Thus, the mechanism of action of OsSOS2 protein is proposed to be similar to the AtSOS2 protein. Analysis of spatial distribution of *OsSOS2* gene in two contrasting cultivar of rice revealed that reproductive parts like panicle showed a higher transcript accumulation for OsSOS2 while there was not much accumulation of OsSOS2 transcripts in other parts of mature plant. The transcripts of OsSOS2 were higher in shoots of seedlings as well as shoots at tillering stage of salt tolerant cultivar 'Pokkali' as compared to sensitive cultivar 'IR64'. We are raising transgenic plants which are either overexpressing OsSOS2 or where the gene has been knocked out in order to comment further for suitability of this gene in raising crop plants with improved salinity tolerance.

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