Oryza Vol. 52 No.1, 2015 (1-9)

Expression analysis of *Arabidopsis thaliana sos101* gene and its orthologues in three rice genotypes with differing salt tolerance

V Kumar[1], MS Nimmy[1], SK Lenka[1, 2], AK Singh[3] and KC Bansal[1, 4*]

[1]National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, NewDelhi -110 012, India

[2]Current Address: Reliance Industries Limited, Reliance Technology Group, Reliance Corporate Park, Thane Belapur Road, Navi Mumbai- 400701, India

[3]Indian Agricultural Research Institute, New Delhi -110 012, India

[4]National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi- 110 012, India

**Email :kailashbansal@hotmail.com; kcbansal@nbpgr.ernet.in*

ABSTRACT

Salinity is one of the major abiotic stresses which adversely affect crop plants limiting growth and yield potential. In the present study, a salt induced sos101 gene from Arabidopsis thaliana (Atsos101) which encodes WD40 repeat containing protein has been identified. Furthermore, four candidate orthologues of Atsos101 gene in rice (Ossos101) have also been identified by database searching. Salt responsive temporal gene expression was analyzed by quantitative real-time RT-PCR for the candidate orthologues in three rice genotypes with differing salt tolerance at two different salt concentrations (50 mM and 100 mM NaCl). In order to understand the evolutionary relation between the Arabidopsis sos101 gene and its orthologues from rice and other crop plants a phylogenetic analysis was performed. Analysis of the amino acid sequence revealed highly conserved WD40 repeat domain in SOS101 protein and its orthologues in rice. This study revealed the salt inducible regulation of WD40 repeat proteins in both Arabidopsis and rice.

Key words: *sos101, WD40 repeat protein, salt stress, arabidopsis, rice*

Plants survive in an environment which is mostly unfavorable for their growth and thus prevent them from achieving full yield potential. The changing global climate conditions will continue to affect the immotile plants in a number of ways leading to reduction in crop production. The knowledge about the mechanisms of plant adaptation to these stress conditions such as biotic as well as abiotic provides ways to improve the crop species and to increase the productivity in adverse environmental conditions (Hirayama and Shinozaki, 2010; Boyer, 1982). Drought, high salinity, high and low temperature are the common abiotic stresses that negatively affect the plant growth and crop production. By various biochemical and physiological processes plants respond and adapt to these stresses. Many stress responsive genes alter their expression leading to stress response and tolerance (Shinozaki *et al.*, 2003). Gene expression profiling using cDNA microarrays or gene chips has identified many genes that are regulated by cold, drought or salt stress (Kawasaki *et al.,* 2001; Seki *et al*., 2001; Zhu *et al.,* 2002; Rabbani *et al*., 2003; Lenka *et al*., 2011).

Soil salinity is a serious abiotic stress limiting plant growth and productivity. Salinity results in water deficit conditions in the plants and leads to physiological drought and ion toxicity. Worldwide, more than 20% of the cultivated land is affected by saline conditions (Gupta and Huang, 2014). Plant adaptation to salinity is dependent upon the activation of cascades of molecular networks involved in stress perception, signal transduction and expression of specific stress-responsive genes (Tuteja *et al*., 2008). A major advance in the knowledge and understanding of plant salt tolerance has been the discovery of the SOS signalling

pathway in which the calcium-binding protein SOS3 senses salt-induced Ca^{2+} signal and activates SOS2 which is a protein kinase. SOS2 and SOS3 regulate the expression of various ion transporters such as the plasma membrane Na^+/H^+ antiporter SOS1 (Zhu, 2003). Several other studies also point to the importance of the plant hormone ABA, the calcium sensor, calcineurin B-like 1 (CBL1), potassium homeostasis, and MAPK and CDPK genes in salt-stress responses that lead to protection against salt stress (Ma *et al*., 2006).

WD40 repeat proteins are a large family of proteins confined particularly to eukaryotes and contain a conserved unit which recurs 4-8 times within each polypeptide. The WD repeat comprises a 44–60 residue sequence that typically contains the glycine-histidine (GH) dipeptide 11–24 residues from its N-terminus and the tryptophan-aspartate (WD) dipeptide at the C-terminus (Neer *et al*., 1994; Smith *et al*., 1999). WD proteins have role in signal transduction, cytoskeletal dynamics, protein trafficking, nuclear export, RNA processing and chromatin modification (Van Nocker and Ludwig, 2003). In *Arabidopsis thaliana*, the WD40 proteins have been identified and analyzed in detail and found to play key role in plant-specific processes (Van Nocker and Ludwig, 2003). Zhu *et al.,* (2008) reported that an Arabidopsis WD40-repeat protein (HOS15) has role in abiotic stress tolerance. Rice is one of the major staple foods worldwide and also a model for genomic research in cereals (Zhang *et al*., 2008). There are many reports of QTLs as well as genes identification associated with salinity tolerance in rice. There are reports correlating WD40 proteins with salt stress in rice such as a genome-wide survey of stress-responsive genes revealed five genes encoding WD40 proteins in response to salt stress (Huang *et al*., 2008). Another gene encoding a putative WD40 protein, in foxtail millet was also highly induced by various abiotic stresses (Mishra *et al*., 2014).

Even though Arabidopsis is not a predominantly stress-tolerant plant, it does have the ability, like all plants, to sense and respond adaptively to abiotic stresses (Zhu, 2002) allowing its formidable molecular genetic attributes to be utilized to some degree. In a study of screening of Arabidopsis T-DNA insertion mutant lines to isolate mutants impaired in salt (NaCl) stress response, a salt stress hypersensitive mutant salt overly sensitive 101 (*sos101*) was identified, which showed higher shoot growth and produced 2-3 fold higher root length as compared to wild type Arabidopsis plants on NaCl media, and further the T-DNA insertion was mapped and found to be in the promoter region of *sos101* (AT1G64610) in chromosome 1. *sos101* encodes a WD40 repeat family protein. In wild type Arabidopsis, salt stress enhanced the expression of *sos101* two fold, suggesting role of *sos101* in salt tolerance (Rai, 2008). *Atsos101* gene expression data from publicly available sources (TAIR) suggests that the gene is associated with abiotic stress, particularly, in regulating salt tolerance.

We report here the cloning of *Atsos101* (AT1G64610) from Arabidopsis encoding WD40 repeat protein, expression analysis of the gene under salt stress, identification and salt responsive expression analysis of the *Atsos101* putative rice orthologues in three rice genotypes differing in salt tolerance. Quantitative expression analysis of *sos101* genes by real time qRT-PCR in both Arabidopsis and rice suggests their role in salt tolerance. Structural and phylogenetic analysis was also carried out to study the evolutionary relationship between the Arabidopsis *sos101* gene and its orthologues from rice and other crop plants.

MATERIALS AND METHODS

Arabidopsis thaliana wild-type (ecotype Columbia) seeds were surface sterilized, placed in Petri dishes containing solid Murashige and Skoog (MS) medium, and stratified for 48 h at 4^0C. Fourteen day-old seedlings were subjected to salt stress (150 mM NaCl) and whole plant samples were collected at 6 and 24 h post-treatment (hpt), flash frozen in liquid nitrogen and preserved at -80^0C until RNA isolation. Unstressed plants were maintained as controls. Rice *(Oryza sativa* L. ssp. *indica*) seeds of three varieties IR29 (highly salt sensitive), FL478 (salt tolerant NIL of IR29), PB1121 (salt sensitive Basmati variety) used in this experiment were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi, India. Initially, 50 seeds each were kept on wet germination paper in petriplates under controlled environmental conditions at National Phytotron Facility. Four days after germination, seedlings were transferred to polystyrene foam floats with holes stitched by nylon wire mesh and made to float on trays filled with Yoshida nutrient solution (Yoshida *et al*., 1976).

Fourteen day old seedlings were exposed to salt stress by supplementing nutrient solution with 50 mM and100 mM NaCl and leaf samples were collected at 0 (control), 3, 12, 24, 72 and 144 hpt and flash frozen in liquid nitrogen and stored at -80 ^{0}C till RNA isolation. At least three biological replicates of each tissue sample were harvested. The primers used in the present study were designed using Primer3 software and details are given in Table 1.

The expression analysis of *Atsos101* (WD40) gene and its putative orthologues from rice at different time intervals post salinity stress treatment was performed. Total RNA was isolated from fourteen day-old Arabidopsis seedlings after 0 (control), 6 and 24 hpt with 150 mM NaCl and from rice shoot samples after 0 (control), 3, 12, 24, 72 and 144 hpt with 50 mM and100 mM NaCl using Trizol (Invitrogen) reagent. The integrity and quality of RNA samples was assessed through agarose gel (1%w/v) and Nano drop ND-1000 spectrophotometer, respectively. 10 µg of RNA was used for the DNase I treatment. Then 1 µg DNase treated RNA was used for the first strand cDNA synthesis using superscript III Reverse transcriptase (Invitrogen) following manufacturer's instructions. The qRT-PCR analysis was conducted using gene specific primers (Table 1) in a reaction mix of KAPA SYBR FAST qRT-PCR Master mix (Kapa Biosystems, Inc. USA). Primers specific to *Actin8* (AT1G49240) gene of Arabidopsis and *25S rRNA* (AK119809) gene of rice were used as internal control in the qRT-PCR experiment. Aliquots of 2µl undiluted cDNA were used as template for each sample. Reaction mix of standard reaction volume 20 µl was prepared according to manufacturer's instruction and reaction was performed using Light Cycler 480 II PCR system (Roche). For each time interval, three biological replicates were used. The data was normalized by the value of Arabidopsis *Actin8* gene and rice *25S rRNA* gene using the ΔΔ CT method (Livak and Schmittgen, 2001) and the corresponding fold change in the expression level at each time interval was calculated compared with that of the unstressed sample and the standard error of the mean was calculated.

Based on the available cDNA sequence information in the TAIR (http://arabidopsis.org) database the following gene specific oligonucleotide primers were designed using Primer 3 software. The forward primer R1, ATGGGTACTCCTGGTGATGAAGA, and the reverse primer R1, TTAAACCCGAATTGGCAAACCATAG were used to amplify the coding region of *sos101* gene of Arabidopsis using cDNA from salinity stress treated sample prepared in the previous step as template. PCR mixture contained 100 ng of cDNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1 U of Taq DNA polymerase and 0.15 µM of each primer in a total volume of 20 µl. A PCR was performed with cycling parameters of 94ºC for 5 min for initial denaturation followed by 33 cycles of 94ºC for 1 min (denaturation), 55ºC for 1 min (annealing) and 72ºC for 1 min (extension), a final extension at 72ºC was performed for 10 min in an Eppendorf Thermal Cycler. Amplified PCR product was gel purified and cloned in pGEMT-Easy vector. Cloned amplified PCR product was sequence confirmed.

The comparative multiple sequence alignment of *Atsos101* and its orthologues from rice was performed to understand their sequence similarity. To understand the evolutionary relation of WD40 gene from Arabidopsis, we performed phylogenetic analysis. For this, the amino acid sequences of genes from

Table 1. List of genes and corresponding primers used for expression analysis by qRT-PCR

Gene	Forward primer	Reverse primer
Os03g0115400	GAAGGAGGAGGAGATTTGGAT	G CAGAGCAACTCCGACAAGAA
Os04g0568400	CCAGCACCAGTTCTTCAGATG	TGATACACGGCGGACAATTC
Os02g0539900	CAGTAGCATCTGCTCTTCCTTC	CCACATCCCTTGTGCAGTTA
OSIGBa01396	ATGGCTGACGACGAGGA	ACCATCTCGAACAGCACAAA
AT1G64610(*Atsos101*)	ATGGGTACTCCTGGTGATGAAGA	TTA AAC CCG AAT TGG CAA ACC ATAG
AT1G49240(*Actin8*)	ATGAAGATTAAGGTCGTGGCA	GACATCTCTCCAAACGCTGT
AK119809(*25S-rRNA*)	AAGGCCGAAGAGGAGAAAGG	CGTCCCTTAGGATCGGCTTAC

different plants which showed significant match (>30% similarity) with predicted *Atsos101* (WD40) of Arabidopsis were downloaded from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). The phylogenetic tree was generated using the Neighbor-Joining method with 1000 replicates in the Molecular Evolutionary Genetic Analysis 5 (MEGA5) program (Kumar *et al*., 2008). Structural annotation was done with FGENSH (http://linux1.softberry.com/). The amino acid sequence of predicted ORFs was utilized for motif scanning using ExPASy (http://www.expasy.ch/tools/) tools. For motif scan, PROSITE, HAMAP profile, Pfam, HMMS (local and global models) were used as parameters. The domains which play vital role in salt stress were predicted from the deduced amino acid sequences of Arabidopsis and four rice WD40 orthologue genes using SMART software (Letunic *et al*., 2012).

RESULTS AND DISCUSSION

Arabidopsis seedlings under a high salt stress (150 mM NaCl) showed visible symptoms of leaf shrinking or loss of turgor at 6 and 24 hpt as compared to control (0 h) plants. No visible symptoms were observed in seedlings of rice lines IR 29, FL478 and PB1121 subjected to 50 mM NaCl stress treatment. However, in response to 100 mM NaCl treatment, the leaves of rice line IR 29 turned white and exhibited leaf rolling at 10 days post treatment (dpt) whereas both FL478 and PB1121 showed no visible symptoms at this stage. At 16 dpt with 100 mM NaCl, complete mortality was observed in both IR29 and PB1121 seedlings while FL478 seedlings remained healthy thus exhibiting salinity stress tolerance.

The qRT-PCR analysis revealed 3.3 fold induction of *Atsos101* gene at 24 hpt with 150 mM NaCl when compared to control plants at 0 hpt. This result indicates a probable role for *Atsos101* gene in salt tolerance mechanism of *Arabidopsis thaliana* (Fig.1). There was no significant up regulation of *Atsos101* gene at 6 hpt.

PCR amplification from cDNA of salinity stress subjected plant tissue revealed that the *Atsos1* gene has a single exon of 1944 bp (Fig. 2) which codes for 647 amino acids (Suppl. Fig. 1). Based on tBLASTx and BLASTp analysis we found four candidate orthologue genes in rice. The selected candidate genes

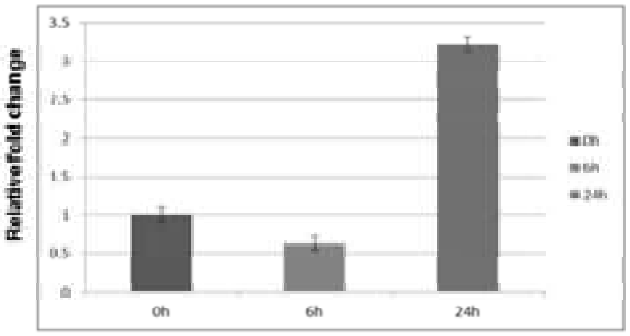

Fig. 1. qRT-PCR analysis of *Atsos101* gene under salt stress (150 mM NaCl) at 0,6 and 24h time points in 14 day old seedlings of *Arabidopsis thaliana*. Error bars represent standard deviation

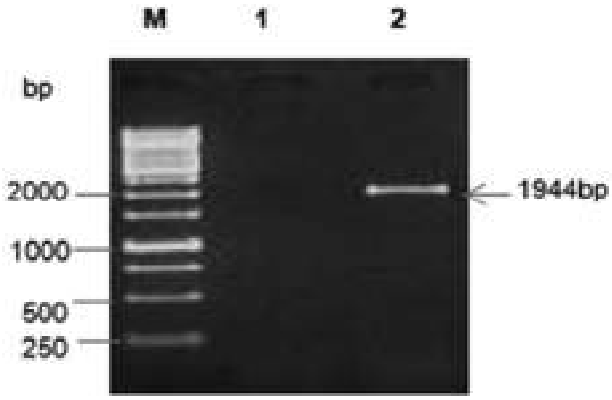

Fig. 2. Amplification of *Atsos101* gene in *Arabidopsis thaliana* seedlings. Full length cDNA of 1,944 bp was PCR amplified and cloned in pGEMT-Easy vector (M: 1kb molecular weight marker, Lane 1:Non template control, Lane 2:*Atsos101* gene

were Os04g0568400, Os02g0539900, OsIGBa0139P06.1 and Os03g0115400 and all the four genes code for WD40 repeat domain containing protein.

Real time quantitative RT-PCR analysis was carried out for three *Atsos101* orthologue genes (Os04g0568400, Os02g0539900 and OsIGBa0139P06.1) in three rice genotypes differing in salt tolerance such as salt tolerant FL478, susceptible IR29 and moderately susceptible PB1121 after exposure to salt (50 mM and 100 mM NaCl) stress to study the expression pattern of orthologues under salt stress. It is observed that in salt sensitive IR29 cultivar, Os04g0568400 gene is significantly induced by 3.2 fold at 12hpt and shown a maximum induction of 4.4 fold at

24hpt at 50mM NaCl stress whereas at 100mM NaCl stress, the maximum induction was only 2 fold at 24hpt. In salt tolerant FL478, a significant upregulation of 2.7 fold was observed immediately after stress ie, 3hpt at 50mM and 4.8 fold at 100mM. In moderately salt tolerant cultivar PB1121, Os04g0568400 showed a significant upregulation of 8.2 fold at 12hpt at 50mM and 5.7 fold upregulation at 24hpt at 100mM. Expression profiling of Os04g0568400 gene in the three rice genotypes revealed that the gene showed immediate up regulation in salt tolerant FL478. The second *Atsos101* orthologue (OSIGBa0139P06.1) from rice showed very early induction of 3.9 fold and 4 fold at 3 and 24hpt respectively at 100mM in case of resistant genotype FL478. In case of moderately tolerant and susceptible rice genotypes, the gene showed enhanced level of transcription in the later hours of stress. In case of IR29 at 50mM, a significant up regulation of 4.8 fold at 24hpt and 9.4 fold and at 100mM, 4.2 fold up regulation was observed at 144hpt. (Fig. 3). In PB1121, a significant up regulation of 4.5 fold was seen at 24hpt at 50 mM and 2.29 fold at 144hpt at 100 mM. In salt tolerant FL478, at 50mM, a significant up regulation of 2.8 fold was observed at 144hpt and at 100 mM 4 fold upregulation was seen at 24hpt. The transcript accumulation pattern of Os02g0539900 orthologue showed similar trend like Os04g0568400 (Fig. 3). Among all three rice cultivars, FL478 exhibits not only higher salt tolerance, but the expression of all three orthologues are induced at 3hpt at 100 mM NaCl stress condition. The OSIGBa0139P06.1gene is also highly induced at 3hpt in PB1121 at 100 mM salinity stress which may contribute to a moderate level of tolerance in this cultivar. In IR29, which is the most sensitive cultivar, relatively late expression of these genes has been observed. In a similar study, the WD40 gene, *BnSWD1* was found to be highly up regulated under salt stress and might be having role in ABA dependent and/or independent signaling salt stress pathways of rapeseed plant (*Brassica napus*) (Lee *et al*., 2010). In another study involving many WD40 coding genes of Foxtail millet were found to be upregulated at 6th hour during salinity stress (Mishra *et al*., 2014). SOS pathway has been established to have a major role in maintenance of cell ion homeostasis and in turn plant salt tolerance (Zhu, 2000). Salt stress induced activity of Na^+/H^+ antiporter has been reported in rice (Martinez-Atienza *et al*., 2007). In order to get insight into the role of SOS2 gene in rice, Kumar *et al*., 2012 studied the expression pattern in contrasting cultivars of rice under high salinity. In a study by Jannesar (2014) SOS pathway genes were selected as candidate genes to evaluate *A. lagopoides* salt tolerance in various conditions. By studying differential gene expression under salinity Chakraborty *et al*., 2014 determined the role of SOS pathway genes in salinity stress tolerance in *Brassica* genotypes. They observed a significantly higher induction of SOS1, a plasma membrane bound Na^+/H^+ antiporter in *Brassica* resulted in exclusion of toxic Na^+ into apoplast region from inside cell and consequently a better salt tolerance.

The nucleotide and amino acid sequences of *Atsos101* gene and its rice orthologues downloaded from Arabidopsis and rice databases were used for *in silico* analysis. The multiple sequence alignment of these five genes revealed considerable variation among them. The results also revealed that the Arabidopsis *Atsos101*gene showed 59.4, 59.3, 59.1 and 66.1% sequence similarity with candidate rice orthologues Os04g0568400, OsIGBa0139P06.1, Os03g0115400 and Os02g0539900, respectively (Suppl. Fig. 2). Further at the amino acid sequence level the Arabidopsis WD40 repeat protein showed 59.4, 59.0, 67.1 and 54.2% similarity with WD40 repeat proteins from Os04g0568400, OsIGBa0139P06.1, Os03g0115400 and Os02g0539900, respectively (Suppl. Fig. 3). Further phylogenetic analysis was performed to understand the evolutionary relationship between the Arabidopsis *Atsos101* gene and its orthologues from rice and other crop plants. The results revealed that *sos101* gene from Arabidopsis was most divergently related to its orthologues from rice and formed separate cluster with all the dicot plants. Whereas among the four rice orthologues, Os04g0568400 and OsIGBa0139P06.1 belong to same sub-cluster and both together were more closely related to Os03g0115400 orthologue. The rice orthologue Os02g0539900 was distantly related to all the rice orthologues and formed a separate cluster (Fig. 4). The rice *sos101* genes belonged to the cluster containing *sos101* orthologues from wheat, maize, barley, brachypodium, sorghum and setaria. This can be attributed to different evolutionary pathways the dicot and monocot plants have followed over millions of years. In a similar study involving 200 putative rice WD40 genes, the phylogenetic analysis found that all WD40

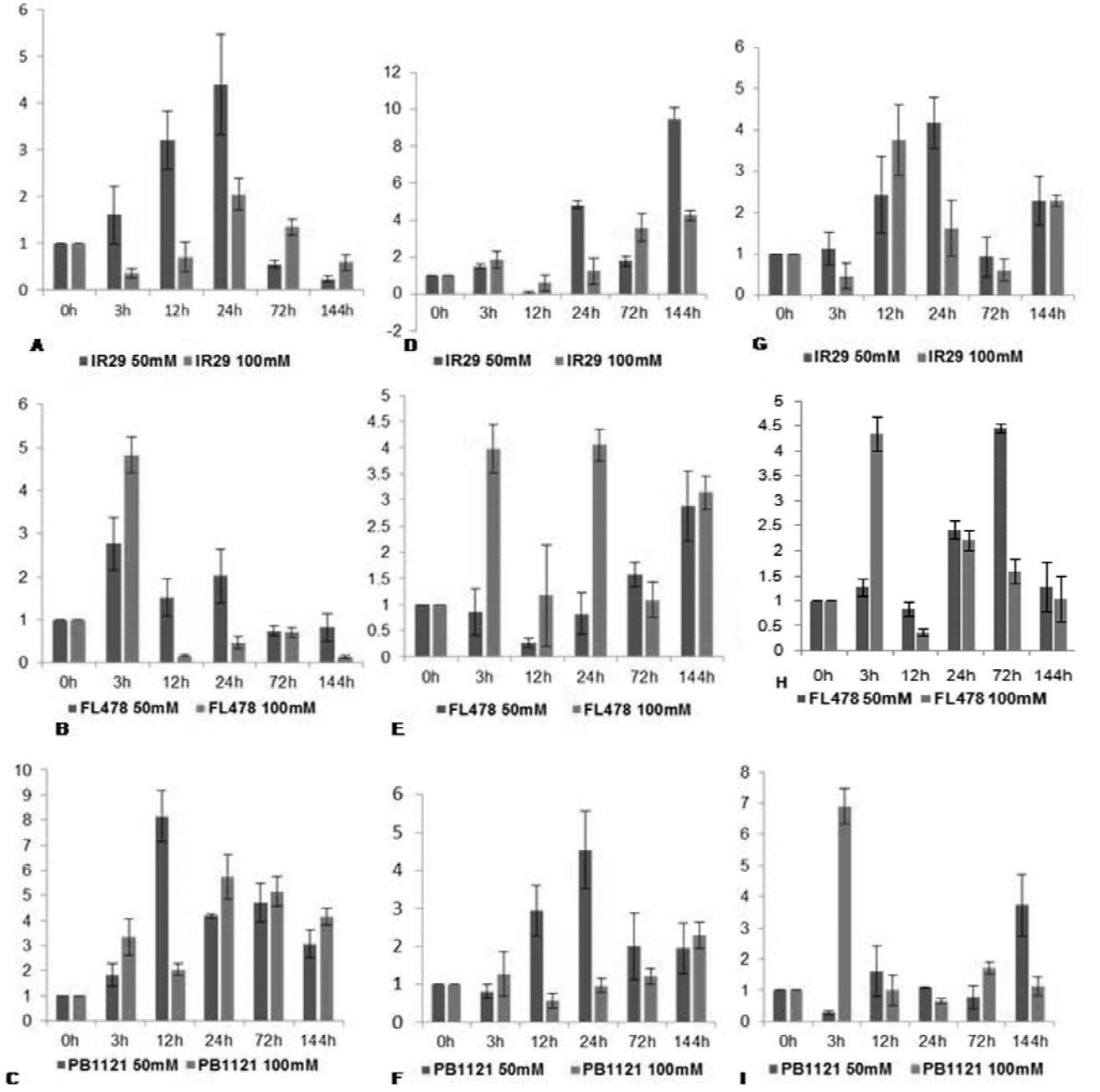

Fig.3. Expression level of rice orthologues of *Atsos101* under salt stress of 50 mM (purple) and 100 mM (green) NaCl at different time points. Os04g0568400 (A-C); Os02g0539900 (D-F) and OSIGBa0139P06 (G-I) in rice cv. IR29 (upper panel); cv. FL478 (middle panel) and cv.PB1121 (lower panel)

genes were clustered into five distinct groups and also WD40 proteins from rice and Arabidopsis were present in all groups (Ouyang *et al*., 2012). Similarly the WD40 genes of foxtail millet were found to be in different cluster and were genetically more diverse. Thus the diverse domain variation and conservations or variations which were evidenced between those proteins might specify the functional equivalence or diversification respectively, with the various aspects of their biological functions (Puranik *et al*., 2012). The phylogenetic analysis of nucleotide sequences of *Atsos101* of Arabidopsis and its orthologues from rice showed that rice orthologue Os04g0568400 was most closely related to Arabidopsis than other rice orthologues (Suppl. Fig.

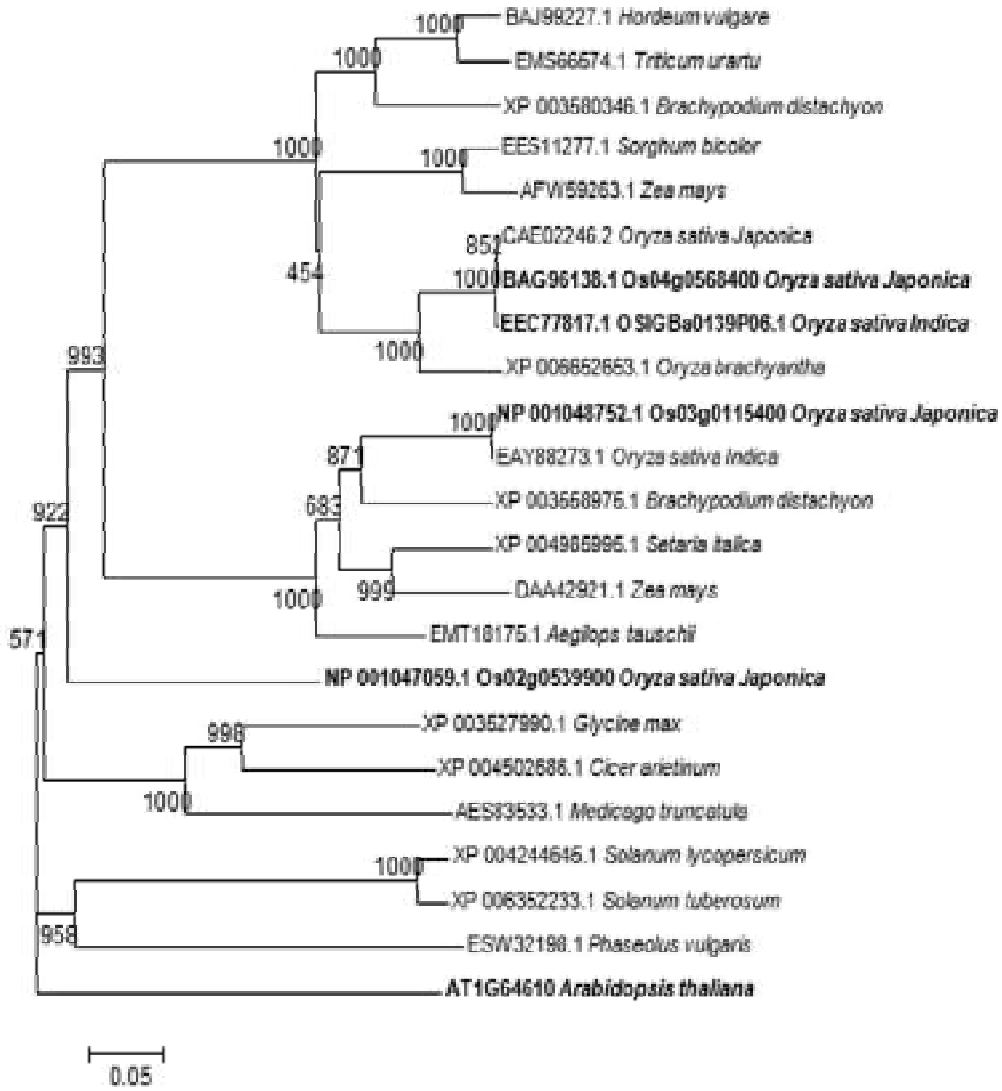

Fig. 4. Phylogenetic tree of *sos101* homologues from different plant species. Protein sequences were used to construct neighbour-joining tree in MEGA5 software with bootstrap value of 1000

4). The *in silico* domain prediction using SMART online server revealed the WD40 repeat protein from Arabidopsis and its three orthologues from rice (Os04g0568400, OsIGBa0139P06.1 and Os03g0115400) code for seven repetitive WD40 motifs, whereas the rice orthologue Os02g0539900 codes for six WD40 motifs (Fig. 5).

The present study revealed that there is a significant up regulation of *sos101* gene under salinity stress in Arabidopsis and rice with possible role in salt tolerance. We also found that the *sos101* is highly conserved across different plant species and its evolutionary pattern is conserved within monocot and dicot plants. The data obtained from this study contribute to a better understanding of the complexity of the *sos101* gene in rice, and provide the basis for further studies to dissect *sos101* function under salinity.

ACKNOWLEDGEMENTS

VK thanks the University Grants Commission for the UGC Research Fellowship and Dr. K V Prabhu, National Phytotron Facility, IARI, New Delhi, for providing Phytotron facility.

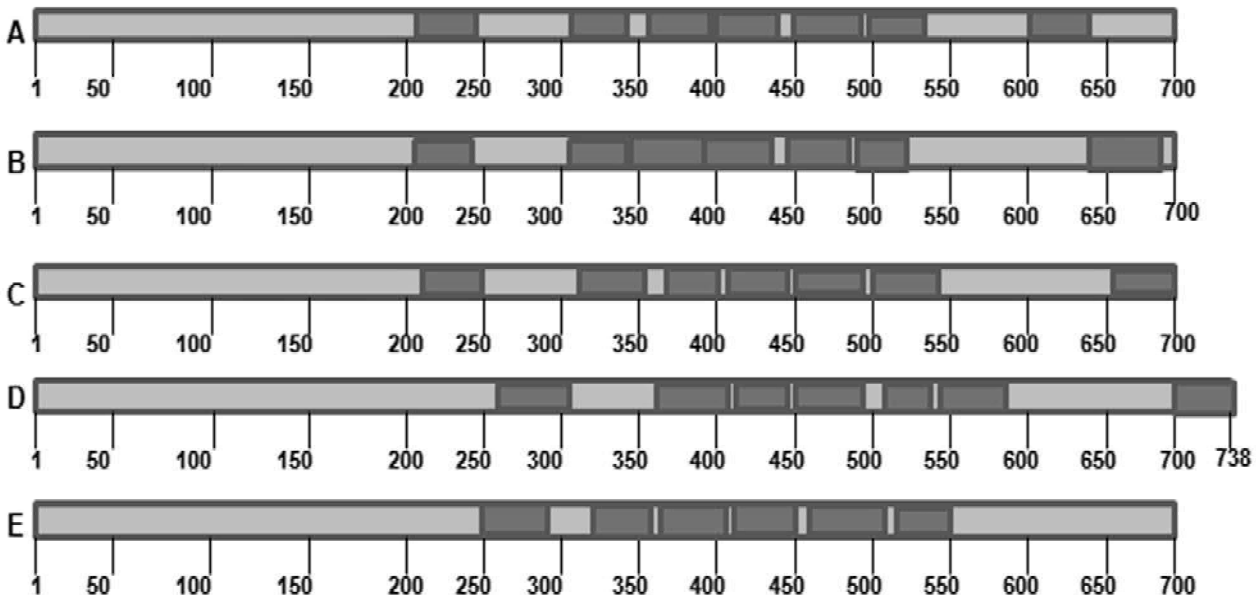

Fig. 5. Schematic representation of various WD40 motifs found in *SOS101* from Arabidopsis (A) and its putative rice orthologues OSIGBa0139P06 (B); Os04g0568400(C); Os03g0115400 (D); Os02g0539900 (E). The green boxes indicate the relative position of WD40 motif

REFERENCES

Boyer J S 1982. Plant productivity and the environment. Science. 218: 443–448.

Chakraborty K, Sairam R K, Bhattacharya R C 2012. Differential expression of salt overly sensitive pathway genes determines salinity stress tolerance in Brassica genotypes. Plant Physiol. and Biochem. 51 90e101

Gupta B, Huang B 2014.Mechanism of salinity tolerance in plants physiological, biochemical, and molecular characterization. Int. J. Genomics. 1: 1-18.

Hirayama T, Shinozaki K 2010. Research on plant abiotic stress responses in the post-genome era past present and future. The Plant J. 61 104–105.

Huang J, Wang MM, Bao YM, Sun SJ, Pan LJ, Zhang HS (2008) SRWD: a novel WD40 protein subfamily regulated by salt stress in rice (*Oryza sativa* L.). Gene 424:71–79

Jannesar M, Razavi K, Saboora 2014. A Effects of salinity on expression of the salt overly sensitive genes in *Aeluropus lagopoides*. Austr.J. Crop Sci. 8(1):1-8.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert H J 2001. Gene expression profiles during the initial phase of salt stress in rice. Plant Cell. 13**:** 889-905.

Kumar S, Nei M, Dudley J, Tamura K 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform. 9:299–306.

Kumar G, Kushwaha HS, Purty RS, Kumari S, Singla-Pareek SL, Pareek A (2012) Cloning, structural and expression analysis of OsSOS2 in contrasting cultivars of rice under salinity stress. Genes Genome Genomics 6:34–41.

Lee S, Lee J, Paek K H, Kwon S Y, Cho H S, Kim S J , Park J M 2010. A novel WD40 protein, BnSWD1, is involved in salt stress in Brassica napus. Plant Biotech. Rep. 4: 165-172.

Lenka S K, Katiyar A, Chinnusamy V, Bansal K C 2011. Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. Plant Biotechnol. J. 9: 315-327.

Letunic I, Doerks T, Bork P 2012. SMART 7: recent updates to the protein domain annotation resource. Nucleic Acids Res. 40: 302–305.

Livak K J, Schmittgen T D 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. Methods. 25: 402–408.

Ma S, Gong Q, Bohnert H J 2006. Dissecting salt stress pathways. J. Exp. Bot. 57: 1097–1107.

Martinez-Atienza J, Jiang X, Garcia deblas B, Mendoza I, Zhu J K, Pardo J M, Quintero F J 2007. Conservation of the salt overly sensitive pathway in rice. Plant Physiol. 143: 1001-1012.

Mishra A K, Muthamilarasan M, Khan Y, Parida S K, Prasad M 2014.Genome-Wide Investigation and Expression Analyses of WD40 Protein Family in the Model Plant Foxtail Millet (*Setaria italica* L.). PLoS ONE. 9: e86852.

Neer E J, Schmidt C J, Nambudripad R, Smith T F 1994. The ancient regulatory-protein family of WD repeat proteins. Nature. 371: 297-300.

Ouyang Y, Huang X, Lu Z, Yao J 2012.Genomic survey, expression profile and co-expression network analysis of *OsWD40* family in rice. BMC Genomics. 13: 100.

Puranik S, Sahu P P, Srivastava P S, Prasad M 2012. NAC proteins: regulation and role in stress tolerance. Trends Plant Sci .17:1360–1385.

Rabbani M A, Maruyama K, Abe H, Khan M A, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K 2003.Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol. 133: 1755-1767.

Rai P 2008. Isolation and characterization of abiotic stress responsive mutants of *Arabidopsis*. Ph. D. Thesis, Indian Agricultural Research Institute, New Delhi.

Ruiz-Lozano J M, Collados C, Barea J M, Azcón R 2001. Arbuscular mycorrhizal symbiosis can alleviate drought induced nodule senescence in soybean plants .Plant Physiol. 82: 346–350.

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carninci P, Hayashizaki Y, Shinozaki K 2001.Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell. 13: 61-72.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M 2003. Regulatory network of gene expression in the drought and cold stress responses .Curr. Opin. Plant Biol. 6: 410–417.

Smith T F, Gaitatzes C, Saxena K, Neer E J 1999. The WD repeat: a common architecture for diverse functions. Trends Biochem. Sci. 24: 181-185.

Tuteja N, Sopory S K 2008.Chemical signaling under abiotic stress environment in plants. Plant Signal Behav. 35: 25–536.

Van Nocker S, Ludwig P 2003. The WD-repeat protein superfamily in *Arabidopsis*: conservation and divergence in structure and function. BMC Genomics. 4: 50.

Yoshida S, Forni D A, Cock J H, Gomez K A 1976. Laboratory manual for physiological studies of rice, 3rd edn. International Rice Research Institute. Manila, Philippines.

Zhang Q, Li J, Xue Y, Han B, Deng X W 2008. Rice 2020: A call for an international coordinated effort in rice functional genomics. Mol. Plant. 1: 715–719.

Zhu J K 2000. Genetic analysis of plant salt tolerance using *Arabidopsis thaliana*. Plant Physiol. 124: 941–48.

Zhu J K 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Physiol. 53: 247-73.

Zhu J K 2003.Regulation of ion homeostasis under salt stress. Curr.Opin. Plant Biol. 6: 441-445.

Supplementary information

Suppl. Fig. 1. Pairwise alignment of amino acid sequence of the cloned Atsos101 of Arabidopsis with the TAIR database protein sequence

Suppl. Fig. 2. Multiple sequence alignment of nucleotide sequences from Arabidopsis sos101 and its rice orthologues using CLUSTALX software. The identical sequences are in the boxes.

Suppl. Fig. 3. Multiple sequence alignment of amino acid sequences from Arabidopsis SOS101 and its rice orthologues using CLUSTALX software. The identical sequences are in the boxes.

Suppl. Fig. 4. Phylogenetic tree of Arabidopsis S0S101 and its putative orthologues from rice. Neighbour-joining tree was generated using MEGA5 software with 1000 bootstraps using the nucleotide sequences

Note: All the supplementary information are available with the corresponding author and are available on demand.