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Isolation and functional characterization of *Salt overly sensitive 1* (*SOS1*) gene promoter from *Salicornia brachiata*

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Abstract

Soil salinity is a major abiotic stress and salt overly sensitive (SOS) pathway plays an important role in imparting tolerance to salinity by reinstating cellular ionic equilibrium. *Salt overly sensitive 1* (*SOS1*) gene of SOS pathway has been implicated in increasing salt tolerance in plants. In this study, a 734 bp fragment of *SOS1* promoter (SbUSOS1) was isolated from a halophyte *Salicornia brachiata* Roxb. *In silico* analysis of SbUSOS1 predicted several *cis*-acting regulatory elements such as DOF motif, GT elements, ABRE-like sequence, and root specific motifs. Functional validation of SbUSOS1 into tobacco stems and leaves using the *GUS* reporter gene showed that this promoter is induced by salt stress (250 mM NaCl) but not by ABA (500 μM) and cold (4 ºC) stresses. This study indicated that SbUSOS1 was functional with predicted *cis*-acting elements that could be responsible for its salt-inducible nature. It can be used for the development of salt stress tolerant transgenic plants.

Additional key words: abscisic acid, *Agrobacterium tumefaciens,* cold stress, β-glucuronidase, NaCl, salt-inducible promoter.

Introduction

Salinity is one of the major abiotic stresses decreasing the growth, productivity, and yield of the crops at an alarming rate in various parts of the world (Sarin *et al.* 1975). Various chemicals such as calcium (Ca^{2+}), cyclic nucleotides, polyphosphoinositides, nitric oxide (NO), sugars, abscisic acid (ABA), jasmonates (JA), salicylic acid (SA), and polyamines play an important role in stress signaling, modulating gene expression, regulating various transporters/pumps, and biochemical reactions (Tuteja and Sopory 2008). Of the several mineral nutrients required for growth and development of plants, Na^+ is not considered an essential mineral nutrient, however, its excess adversely affects the growth of the plant and is known to delay flowering as well as cause a yield loss (Gill 1979, Hasegawa *et al*. 2000, Zhu 2001, Chinnusamy *et al*. 2006). It also affects pollination thereby decreasing the grain yield (Maas 1986).

Saline soils usually contain very high concentration of Na^+ which disrupts K^+ and other mineral absorption (Zhu 2001). The halophytes actively transport Na^+ from root to shoot but salt sensitive glycophytes prevent Na^+ accumulation in the shoot (Flowers *et al*. 1977, Lauchli 1984). Plasma membrane Na^+/H^+ antiporters were thought to be responsible for Na^+ transport through the plants (Lacan and Durand 1996, Hasegawa *et al*. 2000). There are three known mechanisms to prevent accumulation of Na^+ in the cytosol of a plant cell: *1*) restriction of Na^+ influx, *2*) active Na^+ efflux, and *3*) compartmentalization of Na^+ in the vacuole (Niu *et al*. 1995, Blumwald *et al*. 2000, Zhu 2001).

SOS1 (*Salt overly sensitive 1*) gene plays an imperative role in germination and growth of plants in saline environment and improves salt tolerance in plants (Hasegawa *et al*. 2000). Initially, *SOS1* gene was

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Abbreviation: AtUSOS1 - *Arabidopsis thaliana SOS1* gene upstream sequence; CaM - calmodulin; EDTA - ethylene-diamine tetraacetate; EtBr - ethidium bromide; GUS - β-glucuronidase; LB - Luria broth; MEME - multiple expectation maximization for motif elicitation; MES - 2-(N-morpholino) ethanesulfonic acid; PLACE - plant *cis*-acting regulatory DNA elements; SbUSOS1 - *Salicornia brachiata* upstream sequence of *SOS1* gene; ThUSOS1 - *Thellungiella halophila SOS1* gene upstream sequence; X-gluc - 5-bromo-4-chloro-3-indolyl-β-D-glucuronide; YEMA - yeast extract mannitol agar.

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identified in *Arabidopsis thaliana* (Wu *et al*. 1996), which functions as a plasma membrane Na^+/H^+ antiporter with a very long predicted cytoplasmic tail (Shi *et al*. 2000). Mutation in *SOS1* makes plant extremely sensitive to Na^+ inhibition. The *SOS1* has been shown to be upregulated by NaCl stress but not by drought, cold, or ABA (Shi *et al*. 2000).

Serine threonine kinase (*SOS2*) plays an important role by transmitting signals to downstream transcriptional regulators. *SOS3* gene encodes a calcineurin B like protein involved in Ca^{2+} signaling which can interact and activate SOS2 protein kinase (Gong *et al*. 2004). *SOS4* gene was reported to be associated with root hair development in plants and controlled by genetic, hormonal, and environmental factors (Shi and Zhu 2002). A systematic study of wild relatives and extremophiles is a good source of information and genes/enzymes for development of salt tolerant crops. For example, superoxide dismutase (SOD) from *Potentilla atrosanguinea* (Kumar *et al*. 2012) and anti-freeze proteins (AFP) from *Ammopiptanthus mongolicus* (Yong *et al*. 1999) can be used for improving tolerance to various stresses.

Salicornia brachiata Roxb. (family *Amaranthaceae*) is an extreme halophyte and it is distributed in various parts of coastal India, such as Gujarat, West Bengal, and Tamil Nadu. It is a small bushy, leafless plant with tiny flowers on the succulent stems. It grows on saline soil and accumulates 40 - 50 % of NaCl in its dry mass. *S. brachiata* could serve as the useful source of genes related to salt stress (Yadav *et al*. 2011). However, there are no attempts to clone/isolate salt stress inducible promoters from this plant till date.

Over-expression of a transgene using strong constitutive promoters could improve resistance of transgenic plants under abiotic stresses. Number of crops have been developed using CaMV35S promoter which sometime may cause diminutive growth and reduction of yield in transgenic plants (Kasuga *et al*. 1999, Karim *et al*. 2007). Therefore, stress inducible promoters, which are expressed only when exposed to stresses, are being preferred over constitutive promoters in developing stress tolerant transgenics (Rai *et al*. 2009, Zhu *et al*. 2010). The tissue-specific and stress inducible promoters are potentially powerful tool for improving plant tolerance to abiotic stresses in a tissue-specific manner. Promoters contain important *cis*-regulatory elements which play an important role in gene expression and regulation.

A salt-responsive promoter for *vacuolar H^+ pyrophosphatase (TsVP1*) gene, which was induced under salt stress especially in the root tips, was isolated from *Thellungiella halophila* (Sun *et al*. 2010). There are few reports on abiotic stress related promoters like *OsDREB1B* (Gutha *et al*. 2008), *rab16A*, *OsABA2*, and *HP1* (Rai *et al*. 2009), oxidative stress-inducible peroxidase (Kim *et al*. 2003), and *SOS1* (Shi *et* al. 2002). *FRY1* locus in *A. thaliana* encodes an inositol polyphosphate-1-phosphatase which catabolizes inositol-3-phosphate (IP3) and is known to be involved in ABA, salt, and cold stress signaling (Xiong *et al*. 2001).

Keeping in view the role of *SOS1* gene in improving plant salt tolerance, we aimed to clone promoter (SbUSOS1) of *S. brachiata* and validate its function. The isolated stress-inducible promoter can be used for the development of salt stress tolerant transgenic plants.

Materials and methods

Seeds of *Salicornia brachiata* Roxb. were kindly provided by Dr. Bhavnath Jha, Discipline of Marine Biotechnology and Ecology, Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India. Seedlings of *S. brachiata* and *Nicotiana tabacum* L. were raised in plastic pots (13 cm top and 9.5 cm bottom diameter, and 15.5 cm depth) containing garden soil under a 16-h photoperiod, irradiance of 300 $\mu mol\ m^{-2}\ s^{-1}$, temperature of 25 ºC, and air humidity of 60 % at National Phytotron Facility (IARI Campus, New Delhi).

The *S. brachiata* genomic DNA was extracted from 100 mg leaves of 8-week-old plant using *DNeasy*® plant extraction kit (*Qiagen*, Hilden, Germany) as per the manufacturer's protocol. The *GenomeWalker*™ universal kit (*Clontech*, Palo Alto, CA, USA) was used to amplify *SOS1* gene promoter as per the protocol of manufacturer. *SOS1* specific primers (SOS1-GSP1 and SOS1-GSP2) were designed from nucleotide sequence available in *NCBI* GenBank database under accession No. EU879059. The PCR was performed using *Advantage 2* polymerase mix (*Clontech*) and the primers AP1 (provided in the *GenomeWalker*™ universal kit), SOS1-GSP1 (for the primary PCR, Table 1), AP2 (provided in the *GenomeWalker*™ universal kit), and SOS1-GSP2 (for secondary PCR, Table 1) as per the manufacturer's protocol.

The amplified product (734 bp), corresponding to *Dra*I library, was ligated into a *pGEM*®*-T Easy* vector (Promega, Madison, USA) and transformed into DH5α *Escherichia coli* cells using standard protocol (Sambrook and Russell 2001). The transformed cells were screened by blue-white screening and colony PCR method and sequenced on an automated DNA sequencer (*ABI PRISM*™ *310* and *3130 xl* genetic analyzer, *Applied Biosystems*, Carlsbad, USA).

Sequence was analyzed to identify *cis*-acting regulatory elements using an online *PLACE/Signal Scan* database (http://www.dna.affrc.go.jp/). The conserved motifs in the promoter were identified using multiple expectation maximization for motif elicitation (MEME; http://meme.sdsc.edu/meme/cgi-bin/meme.cgi). For MEME analysis, the upstream sequences (734 bp) from translational start sites (TSS) of *SOS1* gene from *Arabidopsis thaliana, Vitis vinifera, Oryza sativa,* and

Triticum aestivum were retrieved from online databases http://www.arabidopsis.org/, http://www.ncbi.nlm.nih.gov/, http://rapdb.dna.affrc.go.jp/, and http://www.plantgdb.org/TaGDB/, respectively. To compare the sequences from the above mentioned plants, *Motif Alignment & Search Tool*, (*MAST*; http://meme.sdsc. edu/) was carried out.

SbUSOS1 was amplified by PCR using forward primer (FP) and reverse primer (RP) containing *Hin*dIII and *Nco*I sites for facilitating sub-cloning into the appropriate site of pCAMBIA 2301 at their 5′ end (Table 1). In the binary vector pCAMBIA 2301 (*Cambia*, Canberra, Australia), the CaMV35S promoter corresponding to GUS was removed (Fig. 1). The amplified product and the pCAMBIA 2301 vector were simultaneously double digested using *Hin*dIII and *Nco*I restriction enzymes. The digested product was purified and ligated using T4 DNA ligase (*Fermentas*, Hanover, USA), *SbUSOS1::GUS*.

Table 1. Primers used in the present study.

Primer	Sequence
AP1	5′-GTAATACGACTCACTATAGGGC-3′
AP2	5′-ACTATAGGGCACGCGTGGT-3′
SOS1-GSP1	5′-ACGCGAGTACCTCGAAGAAA-3′
SOS1-GSP2	5′-CACCTCAATTCGAGATGCTG-3′
FP	5′-GCGAAGCTTCCTCAACTATCATCT-3′
RP	5′-CGCCATGGGGCTGCCCTGGCTG-3′

SbUSOS1::GUS was transformed into DH5α *E. coli* cells using the standard protocol (Sambrook and Russell 2001). The plasmid construct (*SbUSOS1::GUS*) isolated from DH5α *E. coli* cells was further transformed into *Agrobacterium tumefaciens* GV3101 cells by freeze-thaw method (Sambrook and Russell 2001). The transformed cells were spread on *YEMA* plates (yeast extract + mannitol + agar) containing kanamycin (50 μg cm^{-3}) and rifampicin (25 μg cm^{-3}). Further, colony PCR was performed to identify the positive clones.

A. tumefaciens strain GV3101 containing the construct (*SbUSOS1::GUS*) was inoculated on Luria brot (LB) medium and grown at 28 ºC overnight (Voinnet *et al*. 2000). From the overnight grown culture, 0.1 cm^3 was inoculated in 20 cm^3 of LB containing 10 mM MES (pH 5.7), 150 μM acetosyringone (3,5-dimethoxy-4′-hydroxy-acetophenone), 25 μg cm^{-3} rifampicin, and 50 μg cm^{-3} kanamycin and grown at 28 ºC overnight. Cells were harvested by centrifugation at 3 000 *g* for 15 min and resuspended in agroinfiltration buffer (10 mM MES, 150 μM acetosyringone, and 10 mM $MgSO_4$ solution). Agrobacterial suspension was adjusted to a final absorbance (A_{600}) of 0.8 for agroinfiltration.

In planta agroinfiltration into the stems and leaf base of 6-week-old tobacco plants was performed as per the protocol of Jefferson *et al.* (1987). The agrobacterial suspension (0.1 cm^3) was infiltrated into tobacco plants. They were used for treatments (mentioned below) or as control 1 (without stress). Control 2 was set up as a negative control, *i.e*., plant infiltrated with infiltration buffer. After agro-infiltration, tobacco plants were sprayed with water and covered with transparent plastic covers to maintain high air humidity. The plants were kept in a growth chamber for 48 h. Then, three independent stresses, *i.e.*, salt (250 mM NaCl; Yadav *et al*. 2011), ABA (500 μM; Zahur *et al*. 2009), and cold (4 ºC; Yadav *et* al. 2011) were imposed on plants. The plants were then incubated in the growth chamber for 16 h (salt and cold stress) and 6 h (ABA at 22 ºC). The whole set of the experiment was conducted using three biological replicates and repeated twice independently.

The histochemical GUS activity assay was performed 54 h for ABA and 64 h for cold and salt stresses after agroinfiltration. Stem and leaf bases were vacuum infiltrated in 30 cm^3 of X-gluc staining buffer [20 mM X-gluc, 0.2 M sodium phosphate buffer (pH 7), 0.5 M EDTA (pH 8), 0.1 % *Triton X-100*, 0.5 M potassium ferrocyanide, and 0.5 M potassium ferricyanide] for 10 min and then incubated at 37 °C for 48 h. The samples were rinsed in 95 % (v/v) ethanol to remove the chlorophyll. The prepared samples were then viewed and photographed using a microscope (*Wild M8*, Heerbrugg, Switzerland).

Results and discussion

Salt stress is one of the major environmental factors limiting crop productivity (Munns 1993). Salt stress causes oxidative damage, ion toxicity, and disruption of cellular homeostasis through the production of reactive oxygen species (ROS; Munns 1993, Gosset *et al*. 1996, Gomez *et al*. 1999, Savoure *et al*. 1999, Hernandez *et al*. 2000). Since the role of *SOS1* in salt stress tolerance is well known, it was decided to clone the promoter of *SOS1* from a halophyte *S. brachiata* which could be important in driving salt stress inducible genes expression in crop plants.

The upstream region of the *SOS1* gene was cloned using PCR-based directional *GenomeWalker*™ universal kit method with *SbSOS1* specific primers (Table 1). A 734 bp region upstream of *SOS1* gene was amplified, cloned, and sequenced (Fig. 2). The cloned sequence exhibited homology with 5′ end sequence of *SOS1* which confirmed that cloned sequence was the upstream region of the *SOS1*.

Analysis of SbUSOS1 was carried out to identify different *cis*-acting regulatory elements with the help of *PLACE/Signal Scan* (Higo *et al*. 1988). The sequence harboured multiple stress *cis*-acting regulatory elements including elements for early response to dehydration, light-regulated and pathogen and salt-induced gene expressions, as well as ABA responsive and root hair

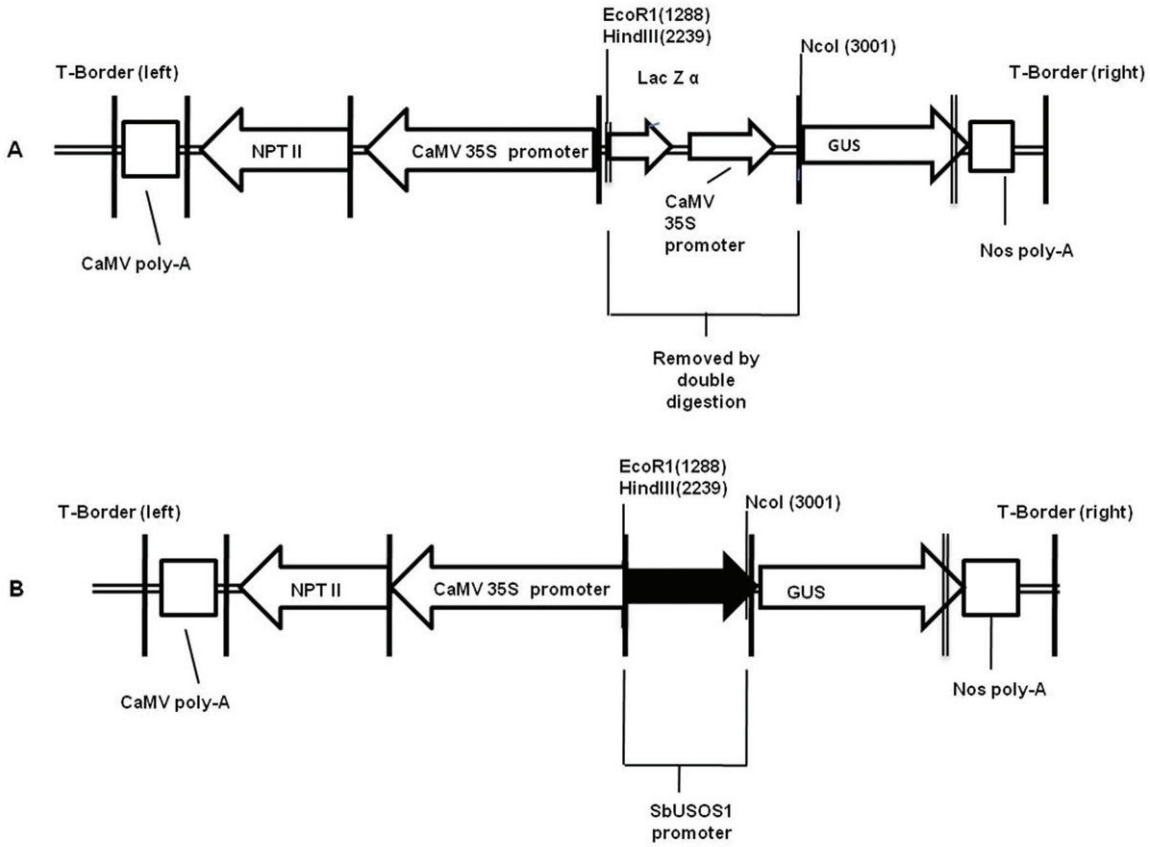

Fig. 1. Linear map of T-DNA of pCAMBIA 2301. *A* - complete T-DNA region; *B* - the modified T-DNA region (CaMV 35S promoter immediate to GUS replaced with SbUSOS1).

and pollen specific elements (Fig. 3). The SbUSOS1 sequence was submitted to GenBank under acc. No. JQ658427. The complete sequence of SbUSOS1 along with all the *cis*-acting regulatory elements are shown in Table 2 and Fig. 3.

Functional *SOS1* gene promoters are reported from *A. thaliana* (AtUSOS1; Shi *et al*. 2002) and *Thellungiella halophila* (ThUSOS1; Oh *et al*. 2010). We compared the upstream (734 bp) regions of SbUSOS1, AtUSOS1, and ThUSOS1 using *PLACE/Signal Scan* and found that few *cis*-acting regulatory elements functionally related to storage-protein, light regulation, A/T rich core, abiotic and biotic stresses or those nodule specific, hormone responsive, root-hair specific, and guard-cell specific were present only in SbUSOS1 and ThUSOS1 but not in AtUSOS1. This may be because, unlike *S. brachiata* and *T. halophila*, *A. thaliana* is not a halophyte. Some of the common regulatory elements are DNA binding with one zinc finger transcriptional factors (Dof) protein, GT element, and abscisic acid responsive element (ABRE). Dof factors play an important role for the genes stimulated by plant hormones and stress signals (Yanagisawa and Sheen 1998). GAAAAA having GT-1 motif was reported from *Glycine max* and calmodulin (CaM) isoform (SCaM-4) was found to be involved in pathogen- and salt-induced *SCaM-4* gene expression (Park *et al*. 2004). ABRE-like sequence (ACGTG) is required for etiolation-induced expression of *erd1* (early responsive to dehydration) gene in *Arabidopsis* (Simpson *et al*. 2003).

It has been reported that the drought-induced dehydration responsive element (DRE) usually exists

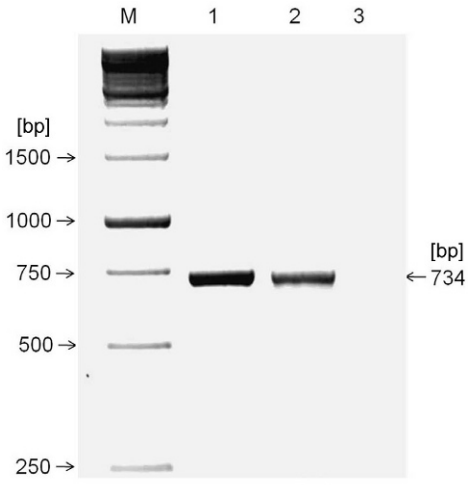

Fig. 2. Agarose gel electrophoresis. *Lane* M - 1 kb DNA marker, *lanes* 1 and 2 - PCR positive clones with FP and RP primers (Table 1) confirming the SbUSOS1 insert in *A. tumefaciens*, *lane* 3 - pCAMBIA 2301 vector.

Table 2. Location of motifs with respect to the translational start site (TSS) detected in upstream region of SbUSOS1 using *PLACE/Signal Scan* (http://www.dna.affrc.go.jp/). L - light, T - temperature, D - drought/dehydration, BF - biotic factors, FP - fermentative pathway, BT - basic transcription, E - endosperm/embryo development, TA - transcriptional activator, H - hormone responsive, O - oxygen deficiency responsive gene, P - photosynthesis, C - *cis*-regulatory element, I - 5' upstream region for accurate initiation, B - binding site, S - NaCl induced, CS - cysteine, ET - ethylene, PC - phytochrome regulation, SS - sugar signaling, GB - gibberellin, S - tissue/organ specificity, AM - α-amylase, OP - other processes. (* - B=G/C/T; N=A/T/G/C; V=A/C/G; R=A/G; M=A/C; W=A/T; Y=C/T; K = G/T as per DNA base degeneracy codes; ** - location with respect to TSS).

Motifs		Role	Sequence*	Location**
-10 PEHVPSBD	S000392	OP	TATTCT	-698
-300 ELEMENT	S000122	OP	TGHAAARK	-21
2SSEEDPROTBANAPA	S000143	OP	CAAACAC	-561
AACACOREOSGLUB1	S000353	E	AACAAAC	-559, -569
ABRELATERD1	S000414	D	ACGTG	-491
ACGTATERD1	S000415	D	ACGT	-71,-491
ANAERO1CONSENSUS	S000477	FP	AAACAAA	-558,-568
ARR1AT	S000454	TA	NGATT	-405
ASF1MOTIFCAMV	S000024	L	TGACG	-489
BIHD1OS	S000498	BF	TGTCA	-216, -341,-433
CAATBOX1	S000028	S	CAAT	-81
CACTFTPPCA1	S000449	C	YACT	-140, -338, -16, -84,-615
CANBNNAPA	S000148	E	CNAACAC	-561
CARGCW8GAT	S000431	B	CWWWWWWWWG	-391,-447
CATATGGMSAUR	S000370	H	CATATG	-89,-286
CTACADIANLELHC	S000252	OP	CAANNNNATC	-652
CURECORECR	S000493	O	GTAC	-60, -376, -525
DOFCOREZM	S000265	OP	AAAG	-331, -373, -416, -484
EBOXBNNAPA	S000144	L	CANNTG	-89, -286, -535
GATABOX	S000039	P	GATA	-586, -600
GT1CONSENSUS	S000198	L	GRWAAW	-36, -426, -480
GT1GMSCAM4	S000453	S	GAAAAA	-36, -426
GTGANTG10	S000378	C	GTGA	-488
IBOX	S000124	L	GATAAG	-600
IBOXCORE	S000199	L	GATAA	-600
INRNTPSADB	S000395	L	YTCANTYY	-118
LECPLEACS2	S000465	CS/ET	TAAAATAT	-172
MYB1AT	S000408	D	WAACCA	-322
MYCCONSENSUSAT	S000407	T	CANNTG	-89, -286, -535
NODCON2GM	S000462	BF	CTCTT	-499, -639
NTBBF1ARROLB	S000273	S	ACTTTA	-508
OSE2ROOTNODULE	S000468	BF	CTCTT	-499, 639
POLASIG1	S000080	OP	AATAAA	-170
POLASIG3	S000088	OP	AATAAT	-688
POLLEN1LELAT52	S000245	OP	AGAAA	-425
PYRIMIDINEBOXHVEPB1	S000298	H	TTTTTTCC	-662
RAV1AAT	S000314	OP	CAACA	-253
REALPHALGLHCB21	S000362	PC	AACCAA	-323
RHERPATEXPA7	S000512	OP	KCACGW	-69
ROOTMOTIFTAPOX1	S000098	S	ATATT	-185, -392, -513, -611
TAAAGSTKST1	S000387	BF	TAAAG	-415
TATABOX3	S000110	I	TATTAAT	-393, -514
TATABOX4	S000111	I	TATATAA	-177
TGACGTVMAMY	S000377	AM	TGACGT	-489
WBOXATNPR1	S000390	BF	TTGAC	-632
WBOXHVISO1	S000442	SS	TGACT	-633
WBOXNTERF3	S000457	BF	TGACY	-633
WRKY71OS	S000447	GB	TGAC	-489, -633
WUSATAg	S000433	BF	TTAATGG	-395

upstream of the drought-induced gene promoters whereas the ABA-induced gene promoter usually harbours an ABRE (Guiltinan *et al*. 1990, Mundy *et al*. 1990). As both DRE and ABRE elements were found in SbUSOS1, it can be suggested that the respective genes might be induced by drought and salt stresses.

Previous evidence indicates a role for DNA consensus sequences for root hair-specific *cis*-elements (RHE) transcription factors (CAACA and KCACGW) as root specific *cis*-acting regulatory elements. It has also been reported that *SOS1* mRNA was more abundant in roots than in shoots (Shi *et al*. 2000). We identified that SbUSOS1 has CAACA and KCACGW motif located at -629 and -716, respectively, which predicted that *SOS1* is a tissue specific gene and SbUSOS1 is a functional promoter of *SOS1*.

SbUSOS1 and *SOS1* upstream sequences from *A. thaliana, T. aestivum, O. sativa* and *V. vinifera* were

Fig. 3. *S. brachiata SOS1* promoter (SbUSOS1) sequence (acc. No. JQ658427) showing various *cis*-acting elements as determined by *PLACE* algorithm. *Highlighted boxes* represent different *cis*-acting elements. In this study, A of ATG (translational start site) was used as +1 for counting the number of nucleotides in SbUSOS1 sequence.

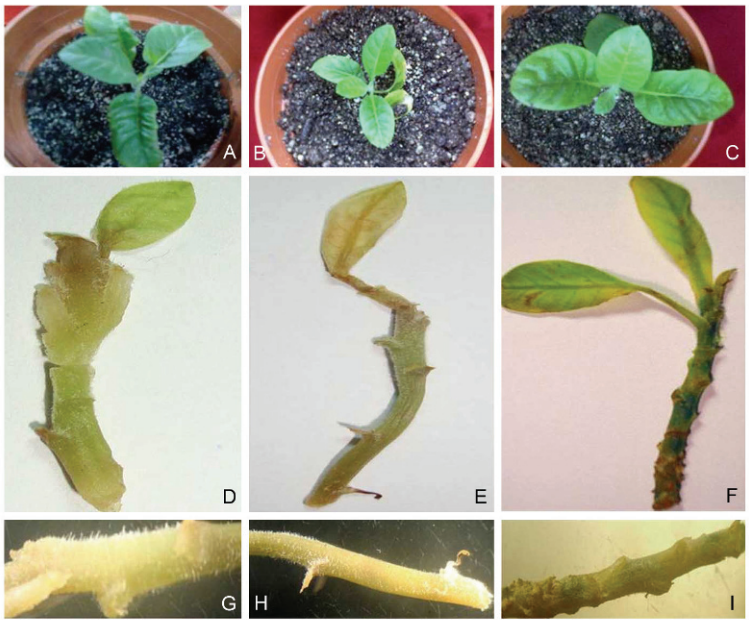

Fig. 4. *Agrobacterium*-mediated transient GUS assay in tobacco. *A,D,G* - control 1, *B,E,H* - control 2, *C,F,I* - transgenic plant, *D,E* - no *SbUSOS1::GUS* expression observed by naked eye, *F* - *SbUSOS1::GUS* expression observed by naked eye, *G-H* - no *SbUSOS1::GUS* expression observed in the stem, *I* - *SbUSOS1::GUS* expression in the stem.

used for MEME analysis to show the common motifs among them. Of the twenty motifs identified in these promoters, ten motifs were found in SbUSOS1 which were common in one or more promoter sequences. The predicted functions of these motifs, using *GOMO-MEME* database, showed similarity with defense protein, integral plasma membrane protein, and G-protein coupled receptor. The *PLACE/Signal Scan* of sequences of these motifs revealed that they consist of ABREs, GT1GMSCAM4, NOD, and DRE boxes. The above analysis also showed that most elements existing in SbUSOS1 were mainly environment or hormone responsive motifs.

Therefore, SbUSOS1 could be predicted as an inducible promoter and regulated by multiple abiotic factors and hormones. We conjecture that expression of the *SOS1* gene under normal conditions might be lower than that under stress conditions.

Further, to know the sequences that match with motifs, *MAST-MEME* database was used. *MAST* is a web-based tool used to find whether the motif of interest is present in other genes or genomes and to search for sequences that match one or more motifs (Bailey *et al*. 2006). *MAST* identified 20 different motifs which showed similarity with others.

To investigate the impact of abiotic stresses on the promoter activity of SbUSOS1 in tobacco, the histochemical GUS activity was analyzed (Figs. 4 and 5). Blue spots were observed in stems and leaf bases of NaCl treated plants. No GUS staining was observed in tissues of ABA and cold treated plants. This indicated that SbUSOS1 is a salt-inducible promoter. Our result was in line with the earlier report by Shi *et al*. (2000) who state that *SOS1* gene was induced only under salt stress but not under ABA and cold stress. *PLACE/Signal Scan* showed the presence of *cis*-acting regulatory elements related to ABA, dehydration and temperature stresses, ABRE, DOF, and MYCCONSENSUSAT, respectively, in the upstream sequences of *SOS1* of *S. brachiata* as well as *A. thaliana.* Systematic investigation in this direction is needed to reveal the exact role of these elements in salinity stress response in *S. brachiata*.

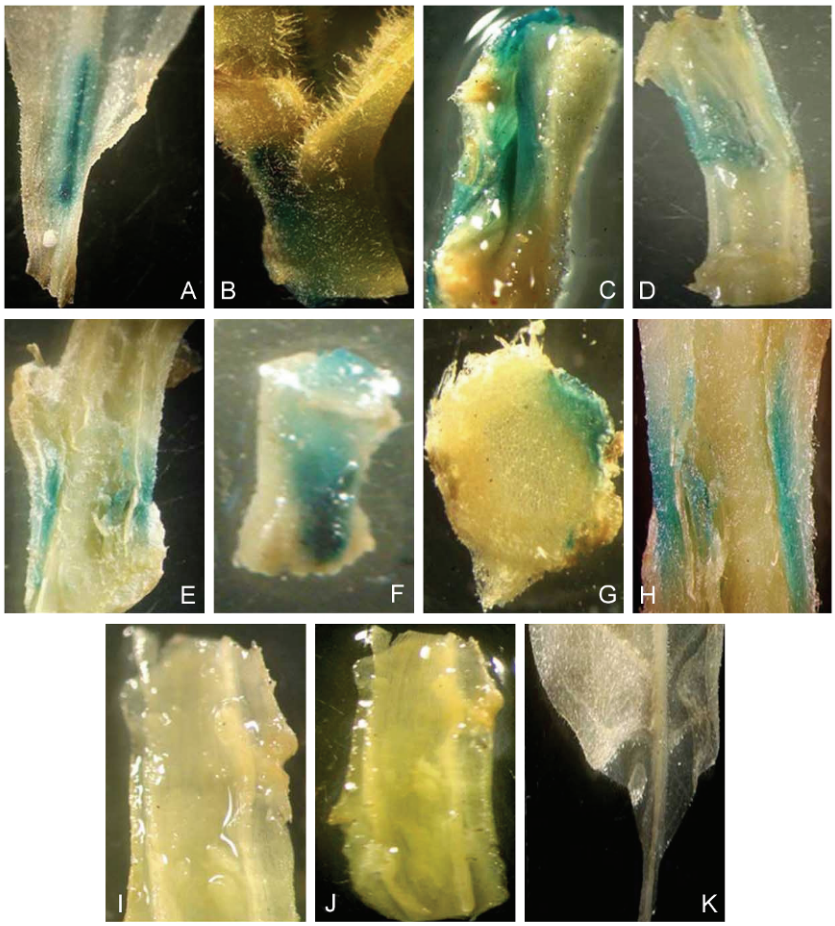

Fig. 5. Microscopic view of GUS staining of the agroinfilterated tobacco stem and leaf base. *A* - *SbUSOS1::GUS* expression in the leaf base, *B* to *F* - different parts of the stems showing the *SbUSOS1::GUS* expression under salt stress, *G* - transverse section of the stem showing *SbUSOS1::GUS* expression, *H* - vertical section of the stem showing *SbUSOS1::GUS* expression, *I* - no *SbUSOS1::GUS* expression in the control 1 stem, *J* - no *SbUSOS1::GUS* expression in the control 2 stem, *K* - leaf base of tobacco showing no *SbUSOS1::GUS* expression.

This is the first report on isolation and characterization of *SOS1* promoter (SbUSOS1) from *S. brachiata*. *In silico* analysis indicated that SbUSOS1 harbours various *cis*-regulatory elements such as AAAG (DOF motif), GAAAAA (GT elements), ACGTG/ACGT (ABRE-like sequence), KCACGW (root specific motifs for RHE transcription factors), and CAACA/CACCTG (root specific motifs RAV1-A/RAV1-B) which might enable SbUSOS1 to be a functional promoter under various abiotic stresses. The GAAAAA (GT elements) motifs are reported to play a role in salt-induced expression in plants. SbUSOS1 showed the presence of that motif, thus its role could be in the salt stress related gene expression. Histochemical GUS transient analysis validated that SbUSOS1 is a functional and a salt stress inducible promoter. Further experiments, such as electrophoretic mobility shift assay (EMSA) and deletion fragment analysis of promoter, would be required to validate the *in silico* identified motifs and a region essential for promoter activity, respectively. We also analyzed SbUSOS1 in *A. thaliana* for localization of promoter activity. This study has wide implication in engineering non-halophytes, for example, crops for salt stress tolerance.

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