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Research Article

Development of Finger Millet (*Eleusine coracana* (L.) Gaertn.) Transgenic for Salt Tolerance by Overexpressing Antiporter Gene *OsSOS1* Involved in Sodium Extrusion

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ABSTRACT

Salinity stress is one of the abiotic stress that adversely affect the plant growth and development. To maintain growth and productivity under salt stress conditions plants must exercise specific tolerant mechanisms. The OsSOS1 plasma membrane gene plays a crucial role in sodium extrusion from root epidermal cells under salinity. To develop salinity tolerant finger millet (GPU28) plants, OsSOS1 gene from Oryza sativa, was overexpressed by Agrobacterium tumerfaciens – mediated transformation. Direct plant regeneration by culturing shoot apical meristems (SAMs) was employed to generate transgenic plants as it is a simple and promising tool for plant regeneration because of rapid and effective regeneration capacity. The functional significance of OsSOS1 gene in transgenic plants (T_1) was demonstrated invitro and also by invivo using Hydroponics system. Overexpressing the OsSOS1 gene in finger millet conferred high salt tolerance, promoted seed germination and increased root length, shoot length, chlorophyll, membrane stability index and reduction in reactive oxygen species (ROS) relative to wild type plants. These results clearly demonstrate that transgenic rice plants overexpressing OsSOS1, plasma membrane Na^+/H^+ antiporter have better salt-tolerance. This could have been mediated by the proton gradient generated by SOS1, which drives Na^+/H^+ antiporter which carries out extrusion of excess Na^+ from cytosol into the apoplast and there by reducing the toxic effect of Na^+ in the cell.

Keywords: *Salinity, OsSOS1, SAMs, Transgenic plants*

INTRODUCTION

Saline soils is an increasing problem in current agriculture since, salinity decreases crop yield and quality. High saline soils cause tremendous yield losses especially in arid and semi-arid regions (Hasanuzzaman et al., 2014). Salinity is defined as a soil condition with a high concentration of soluble salts, with EC (Electric conductivity) >4 dS/m or ~40 mM NaCl (Munns & Tester, 2008).

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The problem of soil salinity is further increasing due to climate change and poor drainage. In 2003, it was expected that by next 25 years, salinization can lead to 30 per cent loss of total arable lands and could even increases up to 50 per cent by 2050 (Wang et al., 2003). Moreover, 45 million ha of irrigated land is affected by salt which is 19.5per cent of total irrigated land (FAO, 2016). The productivity of irrigated land is two times higher than that of rain fed land and it means that 1/3rd of the world's food is produced on 15per cent of the total cultivated land (Munns & Tester, 2008). Hence, salt stress has been considered as one of the most serious limiting factor in crop growth and production.

There are several factors responsible for soil salinity. Natural or primary salinity results from the accumulation of salts over long periods of time through natural processes in the soil or groundwater. Human activities can also leads to soil salinization which is called as secondary salinity that results in to change in hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops transpiration (Garg & Manchanda, 2008). Irrigation water adds appreciable amounts of salt even, good quality irrigation water contains about 200–500 mg/kg of soluble salt. Since crops require 6,000–10,000 m^3 of water per hectare each year, 1 ha of land will receive 3–5 tons of salt. Hence, the amount of salt removed by crops is negligible and salt will accumulate in the root zone which must be leached by supplying more water than actual water required by the crops and it ultimately affects normal crop growth and development.

A major challenge towards world agriculture includes production of 70% more food for an additional 2.3 billion people by 2050 (Gupta & Huang, 2014). Salinity stress lead to a series of morphological, physiological and molecular changes in plants that negatively influences plant growth and productivity (Wang et al., 2003). Finger millet is the staple food crop that supply a major portion of calories and proteins to people in tropical and sub-tropical areas. It is grown world wide, with an annual production of 4.5million tons. Millets are native of semi-arid tropics, where salinity is most common phenomena. Finger millet plants are not resistance to high salinity at germination and early growing stages. Salinity reduces grain yield through reduction both in shoot biomass and harvest index. Satish et al., (2016) reported that increased salinity from 100-200mM affects the vegetative growth, which eventually reduces production even in low saline concentrations.

Salt tolerance of plant is a complex phenomenon. To maintain growth and productivity under salt stress condition, plants have adapted various mechanisms includes osmotic stress tolerance, ion exclusion and Tissue tolerance (Roy et al., 2014).

Antiporters are an important group of genes that play a pivotal role in ion homeostasis in plants. The Salt Overly Sensitive 1 (*SOS1*) gene encodes a plasma membrane Na^+/H^+ antiporter that play an important role in imparting salt stress tolerance to plants. In addition to its role as an antiporter *SOS1* , having 10 to 12 transmembrane domains and a long cytoplasmic tail that act as a Na^+ sensor (Zhu, 2003), it plays a crucial role in sodium extrusion from root epidermal cells under salinity.

Sodium efflux by *SOS1* is also vital for salt tolerance of meristem cells such as growing root-tips and shoot apex as these cells do not have large vacuoles for sodium compartmentation (Shi et al., 2000 & 2002). Sodium efflux through *SOS1* under salinity is regulated by *SOS3–SOS2* kinase complex. Isolated plasma membrane vesicles from *sos1* mutants show significantly less inherent as well as salt stress-induced Na^+/H^+ antiporter activity than vesicles from wild-type plants (Qiu et al., 2002). The expression of *SOS1* is ubiquitous, but stronger in epidermal cells surrounding the root-tip, as well as parenchyma cells bordering the xylem. Thus, *SOS1* functions as a Na^+/H^+ antiporter on the plasma membrane and plays a crucial role in sodium efflux from root cells and the long distance Na^+ transport from root to shoot (Shi et al., 2002).

MATERIALS AND METHODS

Construction of plant transformation vector and finger millet transformation

The *OsSOS1* cDNA clone was obtained from Rice Genome Research Centre (RGRC), Japan (Fig.1). To perform plant transformation *OsSOS1* was amplified with High fedility DNA polymerase in conjunction with full length SOS1 F (GGGGTCGACTTGGT TTTGACCGTCATACAC) and SOS1 R (GGGGTACCCAAAAATATATATCTATAA AT) primers with *KpnI* site. Amplified product was cloned into T/A vector (pTZ57R/T, MBI Fermentas). *OsSOS1* was released from pTZ57R/T by digesting with *KpnI* and cloned into the linearised pB4NU plant binary vector under ubiquitin promoter and terminator to generate Pubi::*OsSOS1*::Tnos. The resulting vector was mobilized into *Agrobacterium tumerfaciens* (EHA105) and used to transform finger millet (*Eleusine coracana* (L.) Gaertn.).

In present study a modified medium proposed by Satish et al., (2017) was following for *Agrobacterium* mediated transformation and rapid regeneration of finger millet. The entire transformation procedure, from initiating SAMs (Shoot apical meristems) to planting putative transgenic plantlets to the green house has been completed within 52 days. Putative transgenic plants regenerated directly from SAM in the presence of Hygromycin (30 mg/L). Histochemical GUS assay was carried out in a subset of finger millet shoots (transformed by *Agrobacterium* mediated method) that survived on the selection medium after culturing in shoot proliferation medium. The putative primary transformants obtained were hardened and transferred to the transgenic facility and allowed to grow to maturity. Putative transformants were analysed and selected by PCR amplification. To select transgenic T_1 seedlings, seeds from the parental plants (T_0 seeds) were germinated on Hygromycin-supplemented MS medium.

Gus histochemical analysis

The*Gus*histochemical assay was performed with 1 mM X-gluc (5-bromo-4-chloro-3-indolyl-*β*-d-glucuronide), in 50 mM sodium phosphate buffer and 1% Triton X-100 at 37 °C overnight in dark to confirm *Agrobacterium*-mediated transformation. The transformed SAMs were incubated with X-gluc substrate solution for the analysis of *gus*A expression and fixed in 0.3% formaldehyde in 10 mM MES (pH 5.6), 0.3 M mannitol for 45 min at room temperature, followed by several washes in 50 mM NaH_2PO_4 (sodium phosphate buffer; pH 7.0). The explants were washed thoroughly with a series of 70% ethanol for 15min each to clear chlorophyll, and examined for the presence of bluecolor spots and photographed.

Extraction of genomic DNA and PCR analysis

Genomic DNA was isolated from T_0 primary transforments of finger millet of pB4NU-Ubi::*OsSOS1* lines via the CTAB (N-cetyl-N,N,N-trimethylammonium bromide) method. To confirme the presence of the transgene, PCR was conducted with ubiquitin forward and SOS1 reverse and HptII selectable primers.

Selection of putative transforments in the presence of antibiotics

De-husked seeds from putative, *OsSOS1* transgenic finger millet were sterilized and germinated in petridishes for one week on hormone free half strength MS medium supplemented with sucrose (3%); pH – 5.8 and agar 0.8% with antibiotic selection. Three plates consisting of 20 seeds each were maintained for each transgenic line. The total number of seeds (out of 60 seeds), germinated and survived in antibiotic selection for one week were recorded as resistant to antibiotics. Seedlings that failed to continue growing on antibiotics and turned brown were counted as non transgenic lines.

Selection of transgenic plants exposed to salt stress (NaCl)

The T_1 seeds from transgenic and WT (Wild type) finger millet seeds, were surface sterilized. The seeds were placed on half strength MS medium containing NaCl (200 mM and 300 mM), solidified with phytagel (as the solidifying agent) in petridish and cultured in a growth room for 5 days at 25°C and the

per cent germination was recorded at the end of stress period (Verslues et al., 2006).

Imposition of salt stress using hydroponic system

Wild type and transgenic plants were grown in 1/2 strength hoagland's solution for 21 days. The seedlings (Wild Type, *OsSOS1*- 4, 5, 6 and 8)were then grown in 50 mM NaCl for a period of 24 hours to acclimatize plants to new environment. After 24 h of induction period, Hoagland's solution was decanted and the treatment was imposed with 1/2 strength hoagland's (Table 1) solution containing different concentration of NaCl which served as different treatment. The three treatments are as follows: T1: Control + Hoagland's solution, T2: 150 mMNaCl + Hoagland's solution, T3: 250 mMNaCl + Hoagland's solution (3 Replications). These solutions were changed once in 5 days and maintained for 25 days and During the growth period different parameter were recorded during the growth period.

Chlorophyll Stability Index (CSI)

Chlorophyll stability index was estimated according to Luna et al., (2000) in leaf punches collected from both stressed and non stressed plants. 100 mg of leaf sample was weighed and incubated in acetone (80 %): DMSO (1:1) solution (10 mL) in dark for 24hours. The supernatant was collected and OD values were determined at 652 nm using UV-Visspectrophotometer.

$$\text{The CSI was calculated using the formula (mg/g FW):} = \frac{A652}{34.5} X \frac{\text{Volume}}{\text{Fresh Weight}}$$

$$\text{Total chlorophyll percent reduction over control} = \frac{\text{Total chlorophyll of control} - \text{Total chlorophyll of stress}}{\text{Total chlorophyll of control}} \text{ X } 100$$

$$\text{Chlorophyll Stability Index (CSI)} = 100\text{-R}$$

Cell Membrane stability (CMS)

Leaf samples were collected from transgenic lines and wild type plants in to a test tubes containing deionised/distilled water. Sample taken out was blot on tissue properly and record thefresh weight and tissue was shade dried, till there is 50% loss in fresh weight. After recording the values 10 mL of distilled water was added to the tissue and incubated for 30-45 min. Electrical conductivity (EC) (T_1) was recorded. Test tube were wrapped withparafilm and autoclaved for 15 min this to kill tissue and the final electrical conductivity(EC) (T2) was recorded.

Calculations were done by using the formula,

$$\text{CMS (\%)} = \frac{1 - T1/T2}{1 - C1/C2} \text{ X } 100$$

Where,

C1 and T1 are treatment conductivities for control and stress before autoclave

C2 and T2 are treatment conductivities for control and stress after autoclave

Estimation of H_2O_2

The protocol of Loreto & Velikova (2001) was fallowed to quantify H_2O_2 content in the leaves. The leaf sample (0.5g) was grinded in 2.5ml of 0.1% TCA and homogenate mixture was transferred to the tubes (1.5ml falcon centrifuge tubes) and centrifuged at 10,000 rpm for 5min. The supernatant (0.5ml) was transferred to the new tube and to this 0.5ml phosphate buffer (PH 7.6) and 1ml of 1.0M potassium iodide was added and mixed will then absorbance wasmeasured at 390nM by

using H_2O_2 standard graph. H_2O_2 content was expressed as µM/g of sample.

Calculations were done by using the formula,

$$H_2O_2 \text{ content } (\mu M/g) = \frac{\text{OD of unknown}}{\text{OD of standard}} \text{ X concentration of standard}$$

RESULT AND DISCUSSION

Developing salt tolerant lines of finger millet by over expression of OsSOS1

A modified direct plant regeneration protocol by Satish et al., (2017) was followed for transformation of pB4NU-Pubi::OsSOS1::Tnos construct (Figure 1) into finger millet plants for functional validation of OsSOS1 gene. Putative transgenic plants were regenerated directly from SAM in the presence of Hygromycine (30 mg/L) (Figure 2). Putative transgenic lines were selected on Hygromycine – containing medium and were subsequently verified by GUS analysis (Figure 3). GUS positive plants were subsequently were transplanted to big earthen pots and maintained in transgenic containment facility.

PCR analysis of finger millet primary transformants

In hygromycine screening 12 transgenic lines were survived which were then established in pots and further characterized by PCR analysis. In 10 lines amplification of selectable marker HptII was noticed. It amplified 500bp in transgenic lines and no amplification was seen in wild type (WT) plant (Figure 4). Further integration of *OsSOS1* gene was analyzed by using ubiquitin forward (AACCAGATCTCCCCCAAATC) and SOS1 (CCACTTTGGGTATGGTTTGG) reverse primers. The transgenic lines 6, 8, 4 and 5 showed amplification for *OsSOS1* gene (Figure 5).

Hygromycin resistance assay

To confirm the stability and integration of Pubi::OsSOS1::Tnos cassette in the transgenics, T_1 seeds from few selected lines were used for hygromycin resistance. Hygromycine concentration was already standardized by Vasantha (2013). Since the binary vector

transformed to finger millet has HptII gene as plant selectable marker, transgenics seeds were grown on hygromycin containing medium (50 mg/L) for 7days. The transgenic seeds germinated and showed better growth while seeds did not show any growth (Figure 6).

Analysis of OsSOS1 transgenic lines for salt stress tolerance

The T_1 seeds from transgenic finger millet transformed with pB4NU-Pubi::OsSOS1::Tnos and untransformed seeds, were surface sterilized. The seeds were placed on half strength MS medium containing NaCl (200mM and 300mM). The growth of both WT as well as transgenics were inhibited under 300mM NaCl concentration. However the inhibition of shoot and root growth was more in WT seedlings compared to T1 seedlings (Table 1).

Response of transgenic finger millet to salinity stress.

The salt stress response of the transgenics and WT was assessed hydroponically. The plants were grown under this specifically designed hydroponic setup for a period of 40 days and the stress response was assessed based on several physiological screens like per cent reduction in chlorophyll, cell membrane stability, H_2O_2 content. Besides, the molecular characterization of these identified four lines was done to confirm the stabile integration of target genes.

PCR analysis of putative transformants

The PCR analysis was carried out using HptII and Gene specific primers. The result showed amplification of two genes inferring stable integration (Figure 7).

Effect of salt stress on chlorophyll content in T_1 transgenic and WT finger millet plants

To examine relevance of transgene expression in imparting overall stress tolerance, leaf bits were collected from healthy plants grown in hydroponic culture with 150mM and 250 mMNaCl and chlorophyll content was estimated. The percent reduction in chlorophyll was higher in wild type and it was relatively less in *OsSOS1* transgenics (ST)

lines. Among the *OsSOS1* lines, ST4 line showed more tolerance compared to other line (Figure 8).

Chlorophyll Stability Index (CSI)

The trends in the results on Chlorophyll stability index (CSI) were similar to that of total chlorophyll content of plants. The CSI of some of the promising T1 lines transgenics had significantly higher than wild type. The CSI% was less in wild type and is 47.84% at 150mM NaCl in *OsSOS1* transgenics (ST) lines 46.03-64.91%. At 250mM higher reduction was seen in wild type compared to transgenic lines. With increase in NaCl concentration CSI was decreased in both WT and transgenic lines but this reduction was more in WT compared to transgenic plants (Figure 9).

Cell Membrane Stability (CMS %)

The CMS was determined based on the leakage of electrolytes which results due to both ionic (short term) and osmotic effects (long term) of salt stress. Transgenics showed much less reduction in membrane stability compared to WT. The Cell membrane stability per cent was less in WT and is 87.48% at 250mM NaCl where as in *OsSOS1* transgenics (ST) lines it was 72.11-75.03% (Figure 10).

Quantification of H_2O_2 content

The ROS generated from NaCl stress results in accumulation of H_2O_2 leading to lipid peroxidation and MDA is formed as end product. The H_2O_2 content was quantified in transgenics as well as WT. The H_2O_2 content was higher in WT with 6.07 µM/g FW at 250mM NaCl where as in *OsSOS1* transgenics (ST) lines. It was only 3.32-4.02µM/g FW (Figure 11).

Soil salinity is a major abiotic stress for plant agriculture. Sodium ions in saline soils are toxic to plants because of their adverse effects on K^+ nutrition, cytosolic enzyme activities, photosynthesis, and metabolism (Shi et al., 2000). Three mechanisms function cooperatively to prevent the accumulation of Na^+ in the cytoplasm, i.e., salt exclusion, salt extrusion, and salt compartmentation are critical to the survival of plants under saline stress. In response to salt stress, plants maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol by regulating the expression and activity of K^+ and Na^+ transporters and H^+ pumps (Zhu, 2003). Sodium efflux from root cells prevents accumulation of toxic levels of Na+ in the cytosol and transport of Na^+ to the shoot. Molecular genetic analysis in Arabidopsis sos mutants have led to the identification of a plasma membrane Na^+/H^+ antiporter, *SOS1*, which plays a crucial role in sodium extrusion from root epidermal cells under salinity. The *SOS1* transcript level was upregulated under salt stress and sos1 mutant plants show hypersensitivity to salt stress (100 mM NaCl), by accumulating more Na^+ in shoots than wild-type plants. Sodium efflux by *SOS1* is also vital for salt tolerance of meristem cells such as growing root-tips and shoot apex as these cells do not have large vacuoles for sodium compartmentation (Shi et al., 2000 & 2002).

Many of the glycophytes are salt sensitive. Even though finger millet is C_4 plant it is relatively susceptible to salinity stress. Several finger millet growing areas are saline and the productivity is affected drastically. Though recently evolved varieties have high yield potential realized yield under saline conditions is much less. Therefore, an increased salt tolerance level in finger mille has relevance.

The plasma membrane localised Na^+/H^+ exchanger appeared (Shi et al., 2000). Later this antiporter was characterised by Zhou et al., (2006) in Arabidopsis. The first studies on sos1 mutants also started by Shi et al., (2002). Isolated plasma membrane vesicles from sos1 mutants show significantly less inherent as well as salt stress-induced Na^+/H^+ antiporter activity than vesicles from wild-type plants (Qiu et al., 2002). The expression of *SOS1* is ubiquitous, but stronger in epidermal cells surrounding the root-tip, as well as parenchyma cells bordering xylem. A key regulator *SOS3* transduces a salt stress elucidated calcium signal by activating *SOS2*, a protein kinase. The activated *SOS2* then phosporylates *SOS1* and enhances the

antiporter activity of this gene resulting in efflux of Na^+ from cytosol to apoplast.

Several transgenic studies clearly demonstrated the salt stress tolerance, can be achieved by over expressing plasma membrane antiporter *SOS1* gene involved in Na^+extrusion. Such a response was seen in tobacco by Yadav et al., (1996), Arabidopsis by Yue et al., (2012), tomato by Olias et al., (2009) and Arabidopsis by Shi et al., (2002). It is therefore evident that over-expression studies with the important *SOS1* antiporter involved in one of the key mechanisms of salt tolerance.

In the present investigation we generated transgenic finger millet plants overexpressing OsSOS1 by *Agrobacterium* mediated transformation. One of the first report of developing stable transgenics in finger millet is by Shivkumar (2006) is transgenics expressing P5Cs was developed by using callus explants. Vasantha (2013) developed transgenics by using both callus and SAM as explant. In this study transformation efficiency achieved was 19% by SAM and 6% by callus.

Satish et al., (2017) developed an improved Agrobacterium mediated transformation and regeneration system for finger millet using optimized transformation and direct plant regeneration technique. In this study they have used SAM's as explants and pCAMBI 1301 for Agrobacterium transformation. They have optimized other conditions like infection time and age of SAM to increase transformation frequency and plant regeneration in 45 days. The transformation frequency was found to be 11.8 per cent which is highest for the cultivar Co(Ra)-14.

In present study a modified medium proposed by Satish et al., (2017) was following for Agrobacterium mediated transformation and rapid regeneration of finger millet. The entire transformation procedure, from initiating SAMs to planting putative transgenic plantlets to the green house has been completed within 52 days.

Yadav et al., (2012) have developed transgenic tobacco by overexpressing the *SbSOS1* gene. SbSOS1 gene in transgenic plant promoted seed germination and increased root length, shoot length, leaf area, fresh weight, dry weight, relative water content (RWC), chlorophyll, K^+/Na^+ ratio, membrane stability index. Transgenic tobacco plants expressing SbSOS1 gene performed beter under salt stress condition compared to WT.

Olias et al., (2009) provided evidence about importance of the plasma membrane Na^+/H^+ antiporter, SOS1, in maintaining ion homeostasis in tomato by extruding Na^+ out the root. They also reported that besides its main action in extruding Na^+ out the root, *SOS1* is critical for the partitioning of Na^+ in plant organs and the ability of tomato plants to retain Na^+ in the stems, thus prevents Na^+ from reaching the photosynthetic tissues.

In our study the finger millet transgenic plants overexpressing *OsSOS1* exhibited increased salt tolerance and seed germination under 200mM NaCl condition. Further more, in response to salt stress, transgenic plants exhibited increase in root length, shoot length, chlorophyll content and cell membrane stability relative to WT. These results clearly demonstrate that transgenic finger millet plants overexpressing *OsSOS1*, plasma membrane Na^+/H^+ antiporter have better salt tolerance.

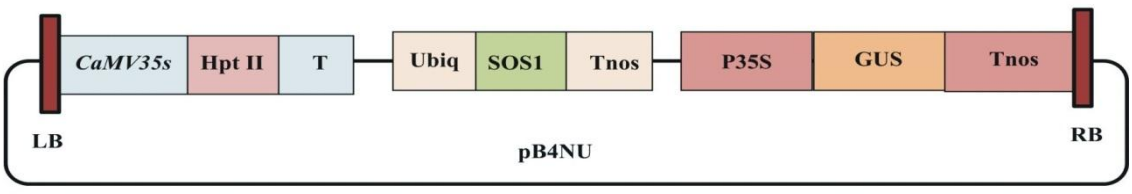

Fig. 1: Schematic representation of overexpression of *OsSOS1* gene construct in pB4NU vector

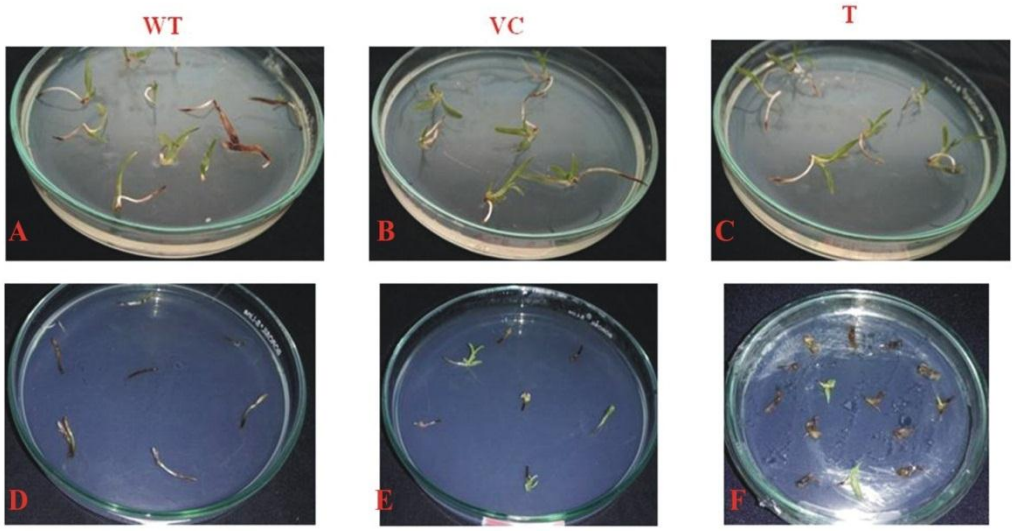

Fig. 2: Selection of transgenic pB4NU- Ubi::*OsSOS1* shoots on selection media

(A) Regeneration of shoots from the finger millet SAMs on shoot regeneration medium without hygromycin selection, absolute control; (B, C) Regeneration of shoots from the transformed finger millet SAMs on shoot regeneration medium without hygromycin selection; (E) vector control (pB4NU); (F) pB4NU- Ubi::*OsSOS1*; (D) Untransformed finger millet SAMs on the shoot regeneration medium with hygromycin selection; (E, F) Regeneration of shoots from the transformed finger millet SAMs on shoot regeneration medium with hygromycin selection; (E)vector control (pB4NU), (F) pB4NU- Ubi::*OsSOS1* for 14 days.

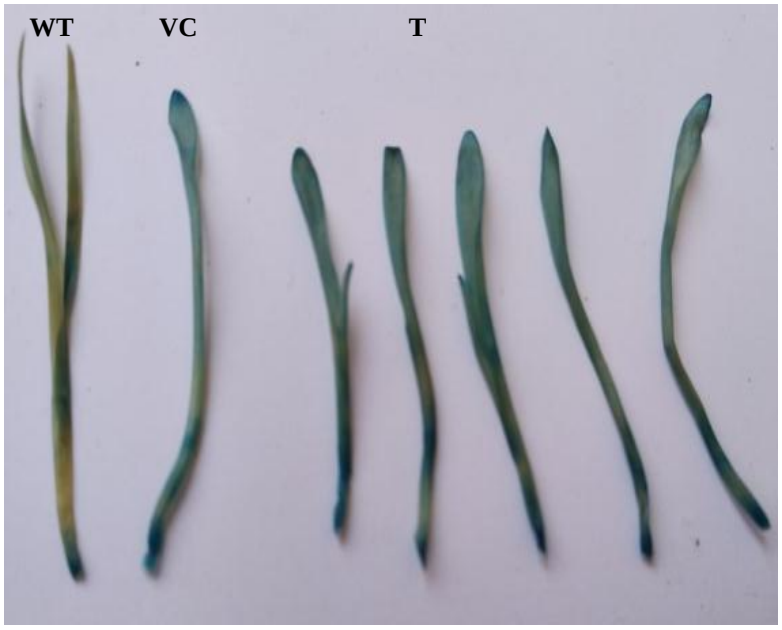

Fig. 3: Gus expression in finger millet single gene (pB4NU-Ubi:: *OsSOS1)* constructs of shoots.
WT- wild type shoots of control with no gus expression, VC - vector control shoots and T- transgenic shoots with strong gus expression after shoots recovered on the selection medium.

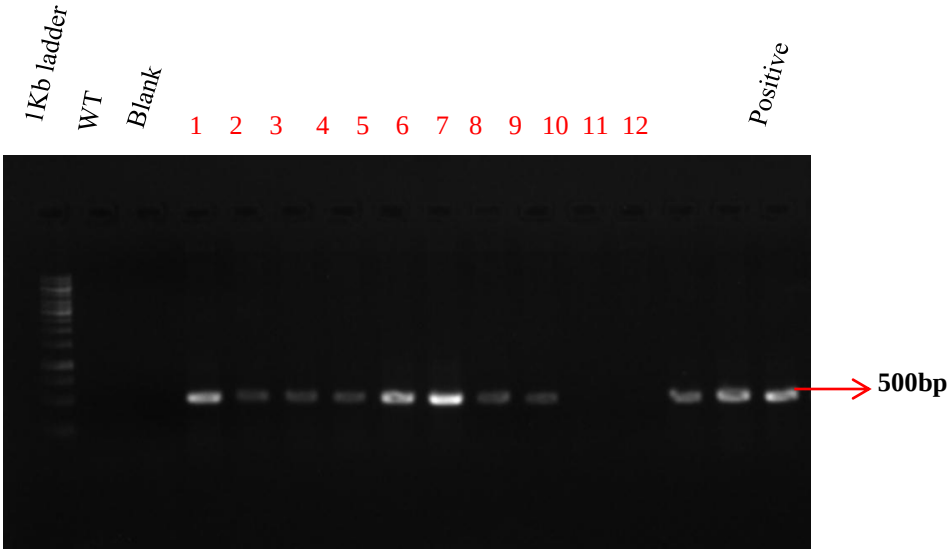

Fig. 4: Agarose gel electrophoresis of PCR amplified genomic DNA of T_0 generation finger millet single gene transgenic (*OsSOS1*) plants with HptII forward and HptII reverse primers. 1-12 DNA

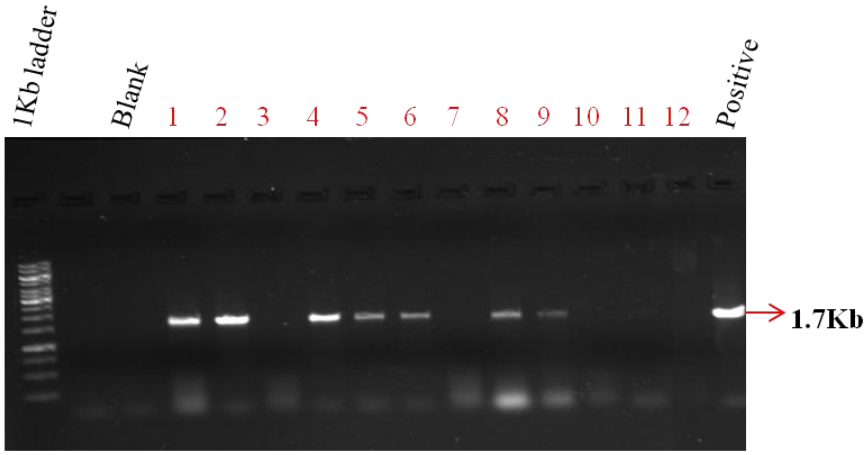

Fig. 5: Agarose gel electrophoresis of PCR amplified genomic DNA of T_0 generation finger millet single gene transgenic (*OsSOS1*) plants with ubiquitin forward and SOS1 reverse primers. 1-12 DNA

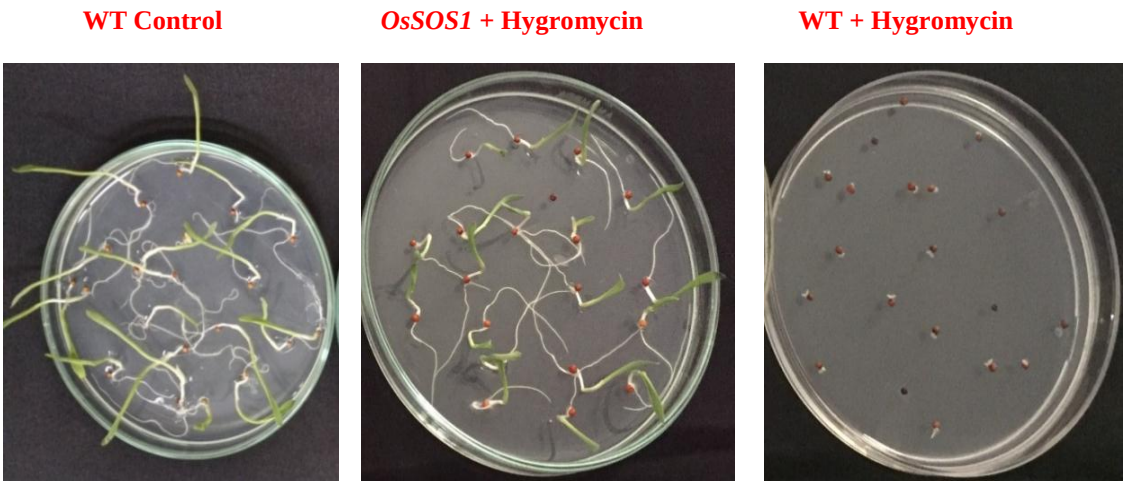

Fig. 6: Untransformed and transformed finger millet seeds were cultured on half strength MS Medium supplemented with 50 mg/L hygromycin for one week

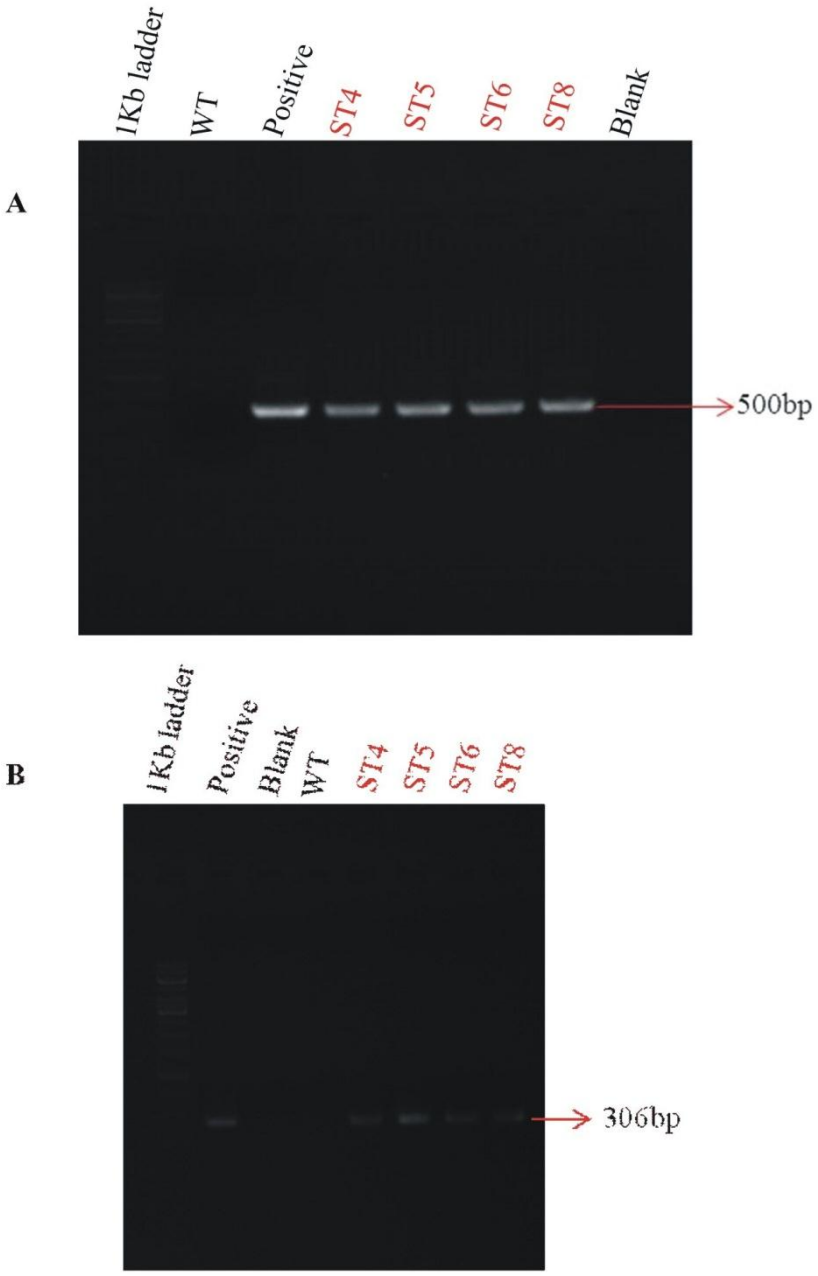

Fig. 7: Agarose gel electrophoresis of PCR amplified genomic DNA of T_1 generation finger millet single gene transgenic (*OsSOS1*) plants

(A) PCR amplification of *Hpt II* with gene specific primers;(B) PCR amplification of *OsSOS1* with gene specific primers

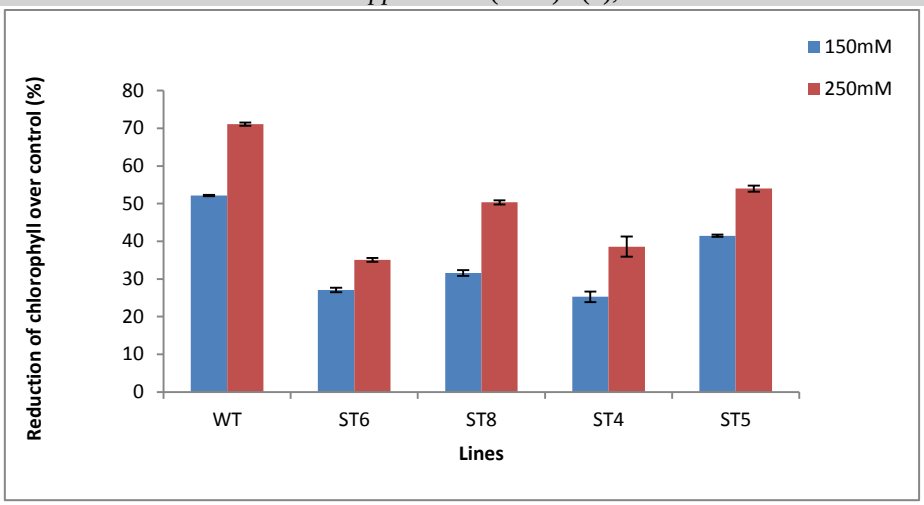

Fig. 8: Effect of salt stress on chlorophyll reduction in T_1 transgenic finger millet plants and WT. *OsSOS1* – ST

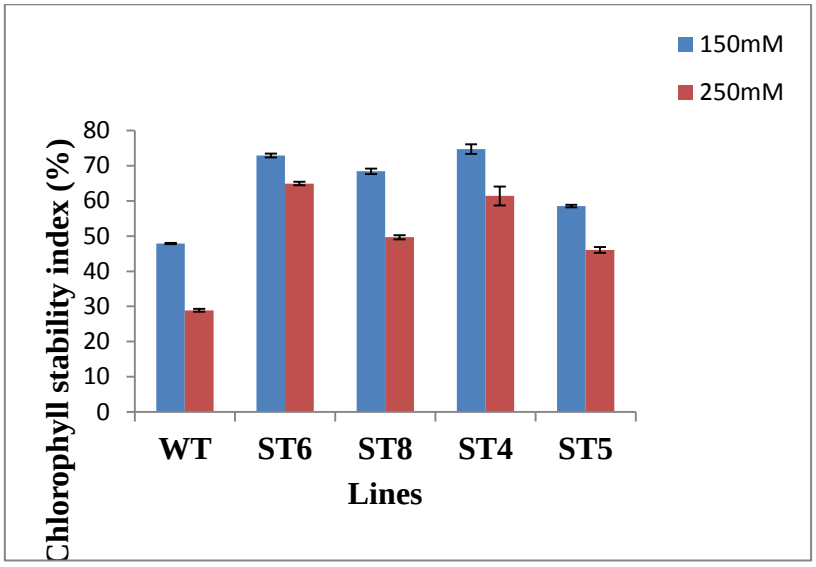

Fig. 9: Effect of salt stress on chlorophyll stability index in T_1 transgenic finger millet plants and WT

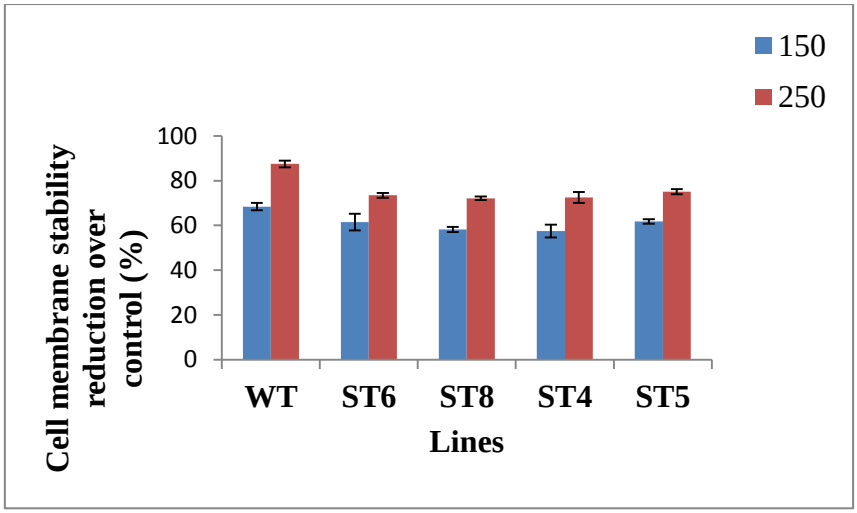

Fig. 10: Effect of salt stress on cell membrane stability reduction in T_1 transgenic finger millet plants and WT

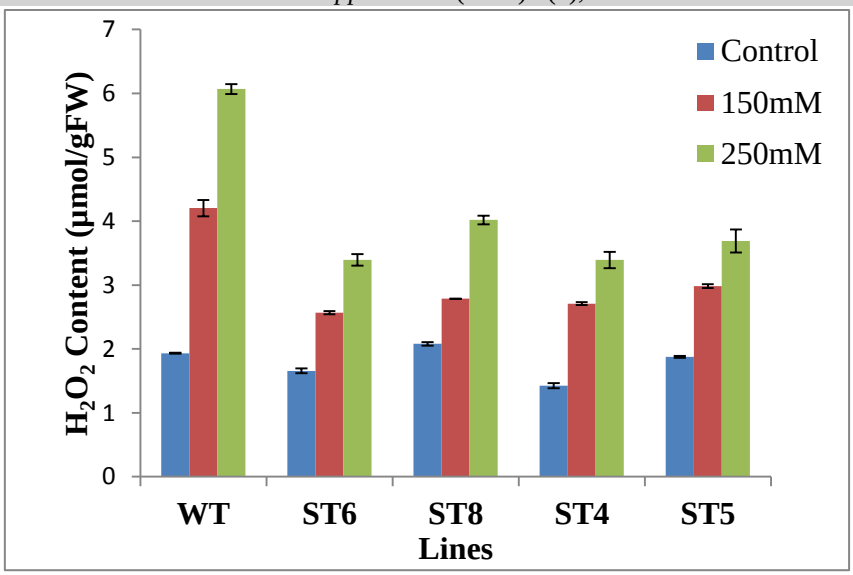

Fig. 11: Quantification of H_2O_2 content in T_1 transgenic finger millet plants and WT.

Table 1: Root and shoot length of transgenic (T1) finger millet plants under hyper osmotic stress

	Root length(Cm)			Shoot length(Cm)		
	Control	**250mM NaCl**	**300mM NaCl**	**Control**	**250mM NaCl**	**300mM NaCl**
WT	4.07±0.35	2.95±0.12	0.00±0.00	2.41±0.23	2.00±0.10	0.00±0.00
ST4	3.46±0.30	3.74±0.15	1.68±0.35	2.32±0.05	2.23±0.16	1.27±0.16
ST5	3.50±0.27	3.98±0.04	1.93±0.56	2.46±0.21	2.29±0.19	1.40±0.05
ST6	3.32±0.10	3.70±0.40	2.23±0.66	2.32±0.02	2.13±0.30	1.37±0.21
ST8	3.91±0.42	4.08±0.07	2.17±0.38	2.46±0.22	2.24±0.05	1.17±0.16

CONCLUSIONS

Overexpression of *OsSOS1* in finger millet conferred a high degree of salt tolerance, enhanced plant growth and altered physiological and biochemical parameters in response to salt stress. These results broaden the role of *OsSOS1* in planta and suggest that this gene could be used to develop salt-tolerant transgenic crops.

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