

# Evaluation of orange-fleshed sweet potato (*Ipomoea batatas* L.) genotypes for salt tolerance through shoot apex culture under in vitro NaCl mediated salinity stress conditions

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Received: 30 December 2007 / Accepted: 19 May 2008 / Published online: 20 June 2008  
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**Abstract** Fifteen genotypes of sweet potato were evaluated for salinity stress tolerance under in vitro NaCl mediated salinity stress conditions (MS, MS + 0.5% and MS + 1.0% NaCl). The growth parameters such as number of leaves, number of shoots, number of roots, length of plantlets and length of roots decreased significantly among the genotypes with increase in level of salinity. Of the 15 genotypes tested, six genotypes (108X1, 90/606, 90/696, CIP 8, S-30X15 and SP-61) were unable to sprout even at 0.5% NaCl and were characterized as susceptible to salt stress, three genotypes (CIP 6, 90/774 and CIP 3) which could tolerate 0.5% NaCl as moderately tolerant and six genotypes (CIP 12, CIP 13, JO 14, JP 13, SB-198/115 and Gouri) as tolerant to salinity at 1.0% NaCl. Amongst the six genotypes showing tolerance to 1.0% NaCl, the exotic genotypes—JP 13, CIP 12 and indigenous one SB-198/115 continued to exhibit

significant higher values for growth parameters over the susceptible one. Based on the performance under NaCl mediated salinity stress (1.0%), the pattern of salinity tolerance in the genotypes through shoot apex culture was JP 13 > SB-198/115 > JO 14 > Gouri > CIP 12 > CIP 13. The effect of salt stress on the activity of antioxidative enzymes was studied in leaves of 8-week-old plantlets of those six genotypes, which responded at higher NaCl stress along with a susceptible genotype 90/606. In leaves of salt stressed plants, superoxide dismutase (SOD), guaiacol peroxidase (GPX) and catalase (CAT) activities increased when compared with the stress free control. The increase was more pronounced in the tolerant genotypes than that in the susceptible one. These results indicate that oxidative stress may play an important role in salt stressed sweet potato plants and that the greater protection of tolerant plants from salt induced oxidative damage results, at least in part, through the increase in the activity of antioxidant enzymes.

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**Keywords** Antioxidative enzymes · In vitro ·  
Salt tolerance · Shoot apex · Sodium chloride ·  
Sweet potato

**Abbreviations**

BA Benzyl adenine  
CAT Catalase  
EDTA Ethylene diamine tetra acetic acid  
GA<sub>3</sub> Gibberellic acid

GPX	Guaiacol peroxidase
MS	Murashige and Skoog
NAA	$\alpha$ -Naphthalene acetic acid
NBT	Nitroblue tetrazolium chloride
ROS	Reactive oxygen species
SOD	Superoxide dismutase

## Introduction

Salinity is an important growth-limiting factor for most of the non-halophytic plants (Villa-Castorena et al. 2003). One of the most effective ways to overcome salinity problems is to identify and grow salt tolerant plants/varieties. Selection and development of suitable genotypes for this purpose requires an efficient screening method. In vitro technology offers a meaningful tool for characterizing salt tolerant plants and also for quick evaluation of germplasm against salt stress under controlled conditions with limited time and space (Gosal and Bajaj 1984). Axillary bud/shoot apex culture has been found to be an effective method for testing and selecting salt tolerant genotypes (Martinez et al. 1996). With respect to the whole plant, a similar response to salt stress could be expected in plantlets grown through in vitro shoot apex culture thereby maintaining the genetic stability. Media supplemented with various salt concentrations have been used for screening genotypes of sugar beet, tobacco, Chinese cabbage and canola (Chandler et al. 1988). Extensive research has been done on salinity tolerance of cereals (Nabors and Dykes 1985), leguminous crops (Gosal and Bajaj 1984) and field grown vegetable crops (Cano et al. 1998).

Salt inhibits plant growth by inducing oxidative stress through an increase in reactive oxygen species (ROS), such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $\cdot OH$ ), which disturb the balance between the production of ROS and the quenching effects of antioxidant enzymes, thus, imparting oxidative damage to lipids, proteins, and nucleic acids (Halliwell and Gutteridge 1984). To overcome the negative consequences of ROS, plants have evolved various protective mechanisms either to reduce or to completely eliminate it. One of the protective mechanisms is the enzymatic antioxidant system, which operates with a sequential and

simultaneous action of many enzymes such as superoxide dismutase, peroxidase, and catalase (Mittler 2002) that reacts with ROS and keeps them at low levels. The salt tolerant plants should, therefore, have an efficient antioxidant system for effective removal of the ROS (Rout and Shaw 2001). Superoxide dismutase (SOD, EC 1.15.1.1) is the major  $O_2^-$  scavenger and its enzymatic action results in  $H_2O_2$  and  $O_2$  formation. The  $H_2O_2$  produced is then scavenged by catalase (CAT) and several classes of peroxidases (POX). Catalases (EC 1.11.1.6) are tetrameric homoproteins that exist as multiple isozymes, found in peroxisomes, cytosol and mitochondria, and dismutate  $H_2O_2$  into  $H_2O$  and  $O_2$ . Peroxidases are homoproteins of approximately 50 kDa that are present as multiple isozymes in plant tissues and are distributed throughout the cell and catalyze the reduction of  $H_2O_2$  to  $H_2O$ . The levels of these antioxidant enzymes increase in plants under salt stress and a correlation of these enzyme levels and salt tolerance exists (Hernandez et al. 2000; Mittova et al. 2003).

Sweet potato (*Ipomoea batatas* L.) is a herbaceous dicotyledonous species of the family Convolvulaceae, grown in the tropics of the world. Tubers of this crop are rich in carbohydrates and the orange-fleshed tubers are ready and cheap source of  $\beta$ -carotene, a precursor of Vitamin A. Despite its wide adaptability to diverse agro-ecological conditions and calorie yield, the productivity of sweet potato is affected to a greater extent due to salinity. Intensification of breeding in sweet potato for tolerance to salinity stress through a sustainable way would help obtaining stress tolerant lines. So far, sweet potato has not been subjected to intense breeding program as well as biotechnological research for salinity stress tolerance. Screening of available germplasm would be the first step for salinity stress breeding. In vitro screening through shoot apex culture would provide the most systematic, quick and efficient routes to isolate stress tolerant plants. Information on salt tolerance in this nutrition rich crop is limited (Ekanayake and Dodds 1993; Mukherjee 2001).

The objective of this investigation was to evaluate and characterize 15 orange-fleshed sweet potato genotypes of indigenous and exotic background under NaCl mediated salinity stress conditions through in vitro shoot apex cultures and also to study salinity induced changes in antioxidant enzymes.

## Materials and methods

### Study site and plant materials

The study was undertaken at the Regional Centre of Central Tuber Crops Research Institute (RCCTCRI), Bhubaneswar (20°15' N latitude, 85°52' E longitude and altitude of 26 m above sea level), India. Forty-seven diversified genotypes were screened under NaCl mediated hydroponics cultures (Dasgupta et al. 2006) out of which, 15 orange-fleshed genotypes were selected as the source materials for the present study. Of the 15 genotypes, five (CIP 3, CIP 6, CIP 8, CIP 12 and CIP 13) were introduced from International Potato Centre (CIP), Lima, Peru, two genotypes (JP 13 and JO 14) were collections of Japan and rest eight genotypes (Gouri, SB-189/115, 90/606, 90/696, 90/774, SP-61, 108X1 and S-30X15) were collected from different regions of India and were maintained at RCCTCRI, Bhubaneswar, India.

### Culture medium and incubation condition

Culture medium included the MS basal medium (Murashige and Skoog 1962) supplemented with NAA (2.7  $\mu\text{M}$ ), BA (4.4  $\mu\text{M}$ ) and GA<sub>3</sub> (1.45  $\mu\text{M}$ ) (Mukherjee 2001) and 30 g l<sup>-1</sup> sucrose. Various concentrations of NaCl (0, 0.5 and 1.0% w/v) were added to the medium before the adjustment of pH to 5.7  $\pm$  1 and then 0.8% Difco-Bacto agar (Hi-Media, India) was dissolved. The medium was sterilized at 105 kPa for 15 min in a steam autoclave (REMI, India). Shoot apexes of all the 15 genotypes were surface sterilized with 0.1% mercuric chloride (Merck, India) solution for 3–5 min, followed by thorough rinsing in sterilized distilled water thrice and inoculated in the test tubes (25  $\times$  100 mm) containing MS medium with different levels of NaCl. The cultures were maintained at 25  $\pm$  2°C with 16/8 h light/dark cycle and 45  $\mu\text{mol m}^{-2} \text{s}^{-1}$  illumination level provided by cool/white fluorescence tubes (Phillips, India) with 55–60% relative humidity for 8 weeks. Each treatment included ten replicates (tubes) of each genotype, of which three replicates were used for antioxidant enzyme assays. The experimental set up was factorial experiment in completely randomized design (CRD).

### Preparation of enzyme extract and enzyme assays

For antioxidant enzyme assays, the in vitro leaves (fresh weight 250 mg) of 8-week-old culture, were ground in liquid nitrogen to fine powder and were homogenized with 50 mM NaPO<sub>4</sub> buffer (pH 7.8) containing 1 mM EDTA, 0.1% Triton X-100, 1 mM ascorbate and 10% sorbitol. The homogenate was centrifuged at 15,000 rpm at 4°C for 20 min and the supernatant was used for the following enzyme assays. Total protein concentrations were determined by spectrophotometric method of Bradford using bovine serum albumin as the standard (Bradford 1976).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction mixture (1.5 ml) contained 50 mM phosphate buffer (pH 7.8), 0.1  $\mu\text{M}$  EDTA, 13 mM methionine, 75  $\mu\text{M}$  NBT, 2  $\mu\text{M}$  riboflavin and 50  $\mu\text{l}$  enzyme extract. Riboflavin was added last and tubes were shaken and illuminated with two 20 W fluorescent tubes. The reaction was allowed to proceed for 15 min after which, the illuminating tubes were switched off. Non-illuminated and illuminated tubes without enzyme extract served as control and the absorbance of the reaction mixture was taken at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme required for 50% inhibition of the NBT photoreduction rate and the results were expressed as unit per milligram of protein.

Catalase (CAT, EC 1.11.1.6) activity was assayed from the rate of H<sub>2</sub>O<sub>2</sub> decomposition as measured by the decrease of absorbance at 240 nm, following the method of Aebi (1983). The reaction mixture (1.5 ml) comprised 100 mM phosphate buffer (pH 7.0), 60 mM H<sub>2</sub>O<sub>2</sub> and 50  $\mu\text{l}$  enzyme extract. The decrease of H<sub>2</sub>O<sub>2</sub> was monitored at 240 nm for 1 min and quantified by its molar extinction coefficient (40.0 mM<sup>-1</sup> cm<sup>-1</sup>) and the results were expressed as  $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1}$  (1 unit) mg<sup>-1</sup> of protein.

Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was determined as described by Urbanek et al. (1991) in a reaction mixture (2.0 ml) containing 100 mM phosphate buffer (pH 7.0), 0.1  $\mu\text{M}$  EDTA, 5.0 mM guaiacol, 15.0 mM H<sub>2</sub>O<sub>2</sub> and 50  $\mu\text{l}$  enzyme extract and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar

extinction coefficient ( $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The results were expressed as  $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} (1 \text{ unit}) \text{ mg}^{-1}$  of protein taking into consideration that 4 mol  $\text{H}_2\text{O}_2$  are reduced to produce 1 mol tetraguaiacol. All the spectrophotometric assays were performed using a UV–Visible spectrophotometer at room temperature.

#### Scoring of data and statistical analyses

Data on growth parameters were recorded after eight weeks of inoculation on all the ten replicates under control and NaCl mediated salinity stress conditions while that on enzyme assays were recorded in triplicate. Statistical analyses were carried out using analysis of variance (ANOVA) for two factors in CRD, after square root transformation wherever required (Gomez and Gomez 1984).

## Results

### Shoot apex culture

A wide variation in the studied parameters was observed among the genotypes tested with respect to different levels of salinity. The analysis of variance for growth parameters in vitro (Table 1) indicated that there were significant effects of NaCl treatment, genotypes and NaCl  $\times$  genotypes interaction for all the studied parameters. The result revealed that the growth parameters decreased significantly with increase in salinity stress. Plantlet growth was reduced remarkably in all the genotypes under 0.5 and 1.0% NaCl stress, compared with their control. Particularly, survivability of the explants was reduced to a great extent under salinity. Out of 15 genotypes tested, nine could survive at 0.5% NaCl. However at higher (1.0%

NaCl) stress, growth was completely suppressed and only six genotypes viz., CIP 12, JP 13, Gouri, JO 14, CIP 13 and SB-198/115 could survive, while rest of the genotypes necrosed and ultimately died in between 2–3 weeks of inoculation. Therefore, induction of 1.0% NaCl may be considered for stringent selection in stress tolerance studies in sweet potato.

### Effect of sodium chloride on growth parameters

Days to bud break was 2–5 days, 8–14 days and 12–22 days in stress free control condition, 0.5% NaCl and 1.0% NaCl, respectively. At higher stress of 1.0% NaCl delay in sprouting was observed i.e., JP 13 sprouted at 12.06 days followed by SB-198/115 (12.83 days), CIP 12 (13 days), JO14 (15.25 days), Gouri (15.56 days) and CIP 13 (22.33 days).

At higher stress (1.0% NaCl), no shoots emerged even after 21 days of culture and the explant consequently necrosed in nine genotypes (Table 2). Mean number of shoots per explant at 1.0% NaCl stress was 1.3 to 1.6 in the six responded genotypes. The decrease in shoot number due to salinity varied from 12.00 to 100% under 0.5% NaCl stress and 36.36 to 100% at higher dose of 1.0% NaCl. Growth in terms of shoot length varied from 6.54 cm (CIP 6) to 9.06 cm (90/606) under non-stress control; 5.15 cm (CIP 3) to 6.81 cm (Gouri) in the nine responded genotypes at 0.5% NaCl; and 1.86 cm (CIP 13) to 5.46 cm (CIP 12) amongst six responded genotypes under 1.0% NaCl (Fig. 1a). Shoot length was decreased by 12.98% (SB-198/115) to 33.11% (90/774) under 0.5% NaCl and 25.95% (SB-198/115) to 75.46% (CIP 13) among the responded genotypes under 1.0% NaCl.

The reduction in leaf number was observed at 0.5% NaCl and the same was pronounced at 1% NaCl

**Table 1** ANOVA (mean sum of squares) for different growth parameters in vitro from a  $15 \times 3$  factorial experiment in complete randomised design

Source	df	Growth parameters in vitro				
		Shoot number	Leaf number	Root number	Shoot length	Root length
Genotype	14	1.71**	5.20**	1.31**	4.51**	1.94**
Salinity	2	22.49**	88.08**	21.07**	249.23**	146.60**
Genotype $\times$ salinity	28	0.56**	1.93**	0.47**	2.73**	1.12**
Error	405	0.04	0.07	0.04	0.79	0.51

\*\* Significant at  $P < 0.01$

**Table 2** Growth performance of sweet potato genotypes under different levels of NaCl mediated salinity stress in vitro

Genotypes	Number of shoots/explant			Number of leaves/explant			Number of roots/explant		
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
108X1	2.30 (1.66)	0.00 (0.71)	0.00 (0.71)	7.00 (2.74)	0.00 (0.71)	0.00 (0.71)	2.00 (1.56)	0.00 (0.71)	0.00 (0.71)
90/606	2.10 (1.60)	0.00 (0.71)	0.00 (0.71)	6.40 (2.62)	0.00 (0.71)	0.00 (0.71)	2.00 (1.57)	0.00 (0.71)	0.00 (0.71)
90/696	2.50 (1.71)	0.00 (0.71)	0.00 (0.71)	6.40 (2.62)	0.00 (0.71)	0.00 (0.71)	2.10 (1.60)	0.00 (0.71)	0.00 (0.71)
90/774	2.10 (1.60)	1.40 (1.37)	0.00 (0.71)	6.20 (2.59)	3.20 (1.92)	0.00 (0.71)	2.50 (1.70)	1.60 (1.43)	0.00 (0.71)
CIP 12	3.10 (1.87)	2.40 (1.69)	1.60 (1.36)	6.70 (2.68)	4.00 (2.11)	3.70 (1.87)	2.60 (1.74)	2.00 (1.57)	1.20 (1.25)
CIP 13	2.40 (1.69)	1.80 (1.50)	0.60 (0.99)	6.50 (2.64)	4.00 (2.12)	1.00 (1.11)	2.20 (1.64)	1.70 (1.47)	0.40 (0.91)
CIP 3	2.20 (1.62)	1.30 (1.33)	0.00 (0.71)	6.30 (2.60)	3.70 (2.03)	0.00 (0.71)	2.30 (1.66)	1.40 (1.34)	0.00 (0.71)
CIP 6	2.70 (1.75)	1.80 (1.50)	0.00 (0.71)	6.40 (2.62)	3.80 (2.07)	0.00 (0.71)	2.30 (1.66)	1.40 (1.37)	0.00 (0.71)
CIP 8	2.40 (1.69)	0.00 (0.71)	0.00 (0.71)	6.30 (2.61)	0.00 (0.71)	0.00 (0.71)	2.40 (1.69)	0.00 (0.71)	0.00 (0.71)
Gouri	2.60 (1.74)	2.10 (1.59)	1.40 (1.33)	6.80 (2.70)	4.00 (2.11)	4.00 (2.00)	2.10 (1.60)	1.70 (1.47)	1.00 (1.19)
JO 14	2.20 (1.63)	1.70 (1.47)	1.40 (1.34)	6.60 (2.66)	4.40 (2.20)	3.50 (1.92)	2.30 (1.67)	1.90 (1.52)	1.20 (1.28)
JP 13	2.50 (1.70)	2.00 (1.56)	1.50 (1.40)	6.80 (2.70)	4.00 (2.12)	4.30 (2.16)	2.30 (1.66)	1.50 (1.40)	1.30 (1.33)
S-30X15	2.60 (1.73)	0.00 (0.71)	0.00 (0.71)	6.20 (2.59)	0.00 (0.71)	0.00 (0.71)	2.30 (1.66)	0.00 (0.71)	0.00 (0.71)
SB-198/115	2.50 (1.72)	2.20 (1.62)	1.30 (1.33)	6.70 (2.68)	4.30 (2.18)	4.40 (2.19)	2.30 (1.65)	1.80 (1.50)	1.20 (1.30)
SP-61	2.80 (1.81)	0.00 (0.71)	0.00 (0.71)	6.50 (2.64)	0.00 (0.71)	0.00 (0.71)	2.40 (1.69)	0.00 (0.71)	0.00 (0.71)
Mean	1.85	0.84	0.39	4.89	1.77	1.05	1.71	0.75	0.32
	G	T	G*T	G	T	G*T	G	T	G*T
SE m	0.04	0.02	0.06	0.05	0.02	0.08	0.04	0.02	0.06
LSD (0.05)	0.10	0.05	0.18	0.13	0.06	0.22	0.10	0.04	0.17
LSD (0.01)	0.13	0.06	0.23	0.17	0.08	0.29	0.13	0.06	0.22

Figures in the parentheses indicate transformed square root values. Statistical analyses have been done on transformed values

*G* genotypes, *T* salinity levels (*T*<sub>0</sub> control, *T*<sub>1</sub> 0.5% NaCl, *T*<sub>2</sub> 1.0% NaCl), *SE m* standard error of mean difference, *LSD* least significant difference

stress over stress free control (Table 2). The rate of reduction varied from 33.33% in JO 14 to 48.39% in 90/774 at 0.5% NaCl, whereas the same was decreased by 34.33% in SB-198/115 to 84.62% in CIP 13 at 1.0% NaCl.

#### Effect of sodium chloride on rooting parameters

The result of the rooting parameters also revealed that rooting was inhibited and seized in six genotypes at 0.5% NaCl and in nine genotypes at 1.0% NaCl in vitro (Table 2). The mean number of roots at higher stress was 0.4 in CIP 13, 1.0 in Gouri, 1.2 each in CIP 12, JO 14 and SB-198/115 and 1.3 in JP 13. The decrease in number of roots among the six responded genotypes at higher stress over non-stress control was 43.48% (JP 13), 47.83% (JO 14 and SB-198/115), 52.38% (Gouri), 53.85% (CIP 12) and 81.82% (CIP 13).

Similarly, the root length was decreased with increasing level of salinity (Fig. 1b). At higher stress

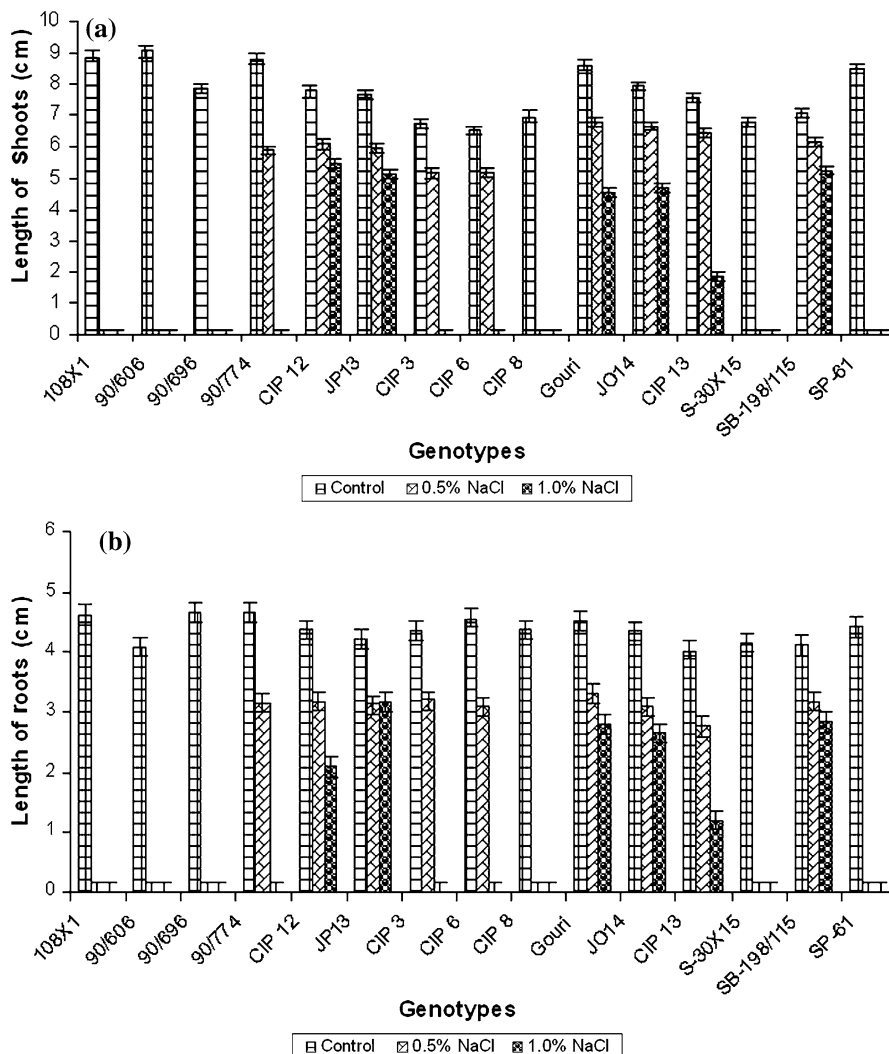
(1.0% NaCl) the length of root was decreased by 24.9, 31.3, 38.1, 38.9, 52.2 and 70.4% in six responded genotypes viz., JP13, SB-198/115, Gouri, JO14, CIP 12 and CIP 13, respectively.

#### Effect of sodium chloride on antioxidative enzymes

The analysis of variance (ANOVA) for different antioxidant enzymes (Table 3) revealed that mean sum of squares due to salt stress, genotypes and genotype × stress interaction were highly significant for all the enzymes studied. This indicated significant differences among the genotypes and stress conditions. The genotypes showed differential response to salt stress for the studied enzyme activities.

Stress induced increase in SOD, CAT and GPX activities were recorded in all the studied genotypes. Leaf SOD activity was greater in salt stressed plantlets than that in controls (Fig. 2a). SOD activity

Fig. 1



**Table 3** ANOVA (mean sum of squares) for antioxidant enzymes from a  $7 \times 3$  factorial experiment in complete randomised design

Source	df	SOD	GPX	CAT
Genotype	6	1459.29**	0.54**	0.101**
Salinity	2	29767.86**	0.827**	0.949**
Genotype $\times$ salinity	12	226.61**	0.007**	0.011**
Error	42	20.53	0.002	0.002

\*\* Significant at  $P < 0.01$

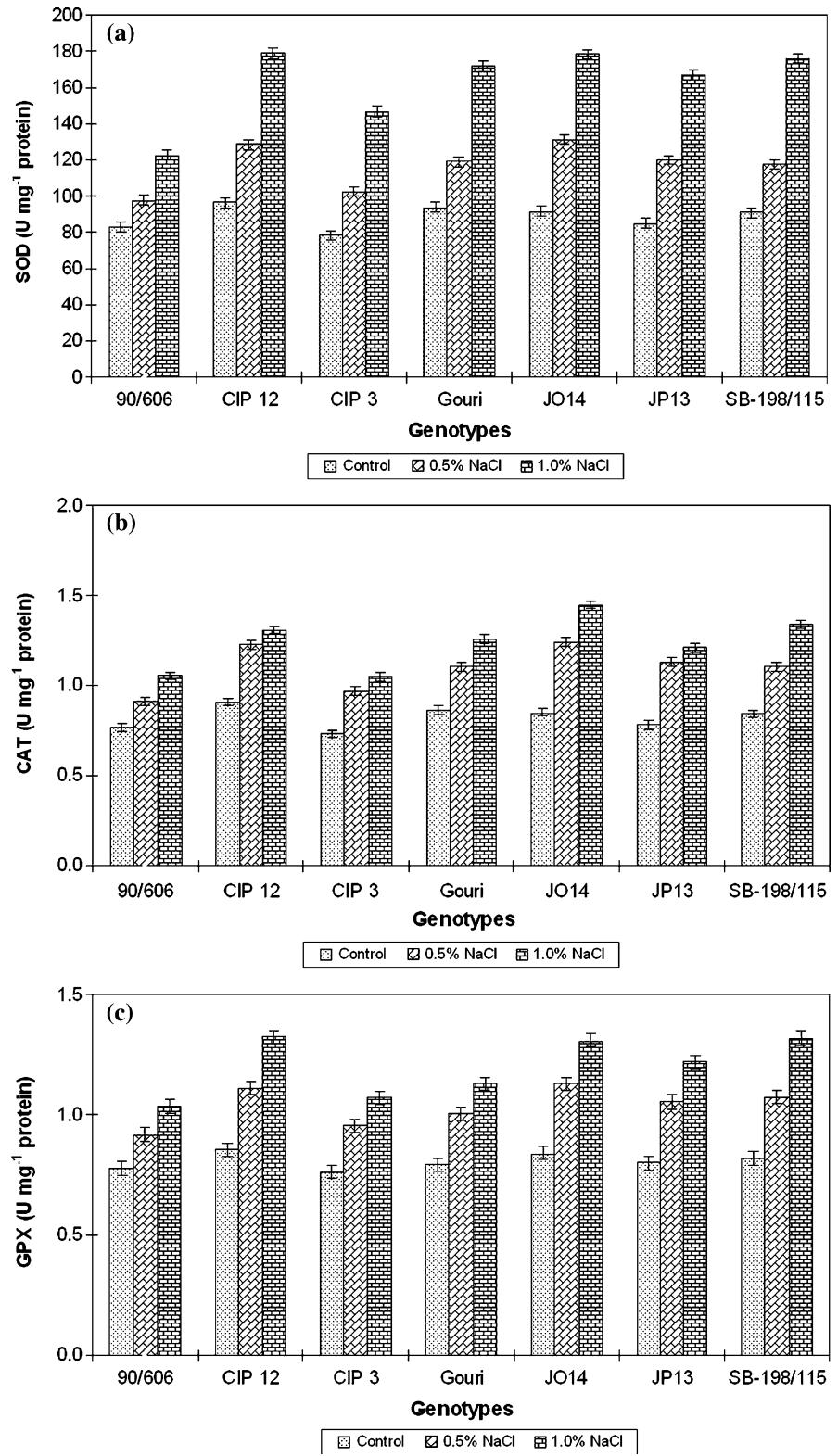
(U mg<sup>-1</sup> protein) was in the range of 82.87 (90/606) to 96.54 (CIP 12) under stress free control, 97.93 (90/606) to 131.24 (JO 14) at 0.5% NaCl and 122.61 (90/606) to 178.81 (CIP 12) at 1.0% NaCl. Salt induced increase in SOD activity was more conspicuous in

tolerant genotypes, JP 13 (96.15%), JO 14 (94.88%), SB-198/115 (93.74%), CIP 3 (87.03%), CIP 12 (85.21%) and Gouri (83.14%) than in susceptible genotype 90/606 (47.96%).

CAT activity in leaves of control and salt stressed plants varied significantly (Fig. 2b). The CAT activity (U mg<sup>-1</sup> protein) was in the range of 0.73 (CIP 3) to 0.91 (CIP 12) in control, 0.91 (90/606) to 1.24 (JO 14) under 0.5% NaCl and 1.05 (90/606 and CIP 3) to 1.45 (JO 14) under 1.0% NaCl. The magnitude of increase was 70.20% in JO 14 as compared to 37.99% in 90/606 at 1.0% NaCl stress over control. The same was in the range of 43.38–58.89% in rest of the tested genotypes.

A similar pattern was observed for GPX activity (Fig. 2c), which also increased significantly in all the

**Fig. 2**



tested genotypes under induced salt stress condition. GPX ( $\text{U mg}^{-1}$  protein) under control condition was in the range of 0.76 (CIP 3)–0.85 (CIP 12) while the same was 0.92 (90/606) to 1.13 (JO 14) under 0.5% NaCl and 1.04 (90/606) to 1.32 (CIP 12 and SB-198/115) under higher salt stress. The increase in GPX was in the range of 18.03 (90/606) to 34.52% (JO 14) and 33.48 (90/606) to 60.98% (SB-198/115) under 0.5% and 1.0% NaCl mediated stress, respectively over control. At higher stress, tolerant genotypes (SB-198/115, JO 14, CIP 12, JP 13, Gouri and CIP 3) possessed 40–60% increase in GPX over control.

## Discussion

Different strategies are in progress for the development of NaCl tolerant plants. In vitro selection procedure either through shoot apex culture or through callus culture offers a meaningful tool for development of such tolerant plants. The shoot culture approach, which is based on intact tissue and is, therefore, less prone to result in somaclonal variation, appears to offer a better system for testing and selecting for salt tolerance. The methodology employed in present study allows to correlation of growth and activity of antioxidant enzymes of leaves with the salt tolerance of plantlets. Besides, sweet potato shoot tips are easy to propagate in vitro and salt tolerant plant materials thus obtained can be used for growing under field condition.

The results on sprouting are in accordance with the similar observations on reduction in sprouting of mulberry genotypes with increasing concentration of NaCl from 0.5 to 1.0% (Vijayan et al. 2003). Mukherjee (2001) reported that the response of four genotypes of sweet potato were considerably stable up to 0.5 g/l of NaCl and decreased at 1.0 g/l of NaCl. The delay in sprouting could be attributed to the increased osmotic potential of the saline medium affecting water and nutrients uptake, which in turn inhibited the metabolic activities necessary for bud break and further growth (Vijayan et al. 2003). The increase in salinity level was accompanied by significant decrease in the mean shoot number, which could be attributed to the negative effect of salt on bud formation and differentiation (Roussos et al. 2006). Similar trend in reduction of shoot number and shoot length has been recorded in mulberry (*Morus*

sp.) by Tewary et al. (2000) and Vijayan et al. (2003) and in wild and cultivated species of tomato (*Lycopersicon esculentum*) by Cano et al. (1998). Mercado et al. (2000) observed shoot necrosis in tomato at NaCl concentrations higher than 86 mM, which also supported the present findings. Martinez et al. (1996) reported that shoot length of potato was negatively affected by NaCl supply and concluded that the detrimental effect of salt on the plantlet growth was directly related to its concentration and exposure time.

Salinity reduced the leaf number severely from 33.33% (JO 14) to 48.39% (90/774) at 0.5% NaCl and 34.33% (SB-198/115) to 84.62% (CIP 13) at 1.0% NaCl. Decrease in leaf number was reported in seedlings of two tomato cultivars (Mills and Tal 2004) and *Populus euphratica* (Watanabe et al. 2000). Roots are among the first organs affected by salt stress and are most sensitive. Rooting was inhibited and seized in six genotypes at 0.5% NaCl and in nine genotypes at 1.0% NaCl in vitro. The decrease in root number and length varied from 43.48 to 81.82% and 24.88 to 70.40%, respectively, among the tested genotypes at 1.0% NaCl. The previous results obtained with shoot apex growth test showed that rooting was one of the parameters most affected by salt in cultivars of tomato by Mercado et al. (2000), in potato by Martinez et al. (1996) and in mulberry (Vijayan et al. 2003). Similarly Cano et al. (1998) found that no shoots developed roots after 32 days of culture even at NaCl dose of 105 mM. However, in this study, the six genotypes, which produced shoots at higher salt stress, were able to develop roots even when cultured at 1.0% NaCl mediated salinity stress.

Based on the growth responses, six genotypes (108X1, 90/606, 90/696, CIP 8, S-30X15 and SP-61) were categorized as susceptible to salt stress, three genotypes (CIP 6, 90/774 and CIP 3) as moderately tolerant as they could tolerate 0.5% NaCl and six genotypes (CIP 13, JO14, JP13, CIP 12, SB-198/115 and Gouri) as tolerant because they could tolerate up to 1.0% NaCl under in vitro conditions. The data indicated that, although sprouting and other growth parameters viz., number of shoots, leaves and roots along with length of shoots and roots decreased with increasing concentration of salt stress, the exotic genotypes—JP 13, JO 14, CIP 12 and indigenous one SB-198/115 continued to exhibit substantially higher



values compared with the rest of the responded genotypes under 1.0% NaCl stress.

Antioxidative enzyme activities increase in diverse environmental stress situations (Mittler 2002), a response related to ROS detoxification. In this study, salt stress increased SOD activity in leaves of all studied genotypes. But the magnitude of increase was more conspicuous in tolerant genotypes than that in the susceptible one suggesting that the salt tolerant genotypes have a better  $O_2^-$  radical scavenging ability. Elevated SOD activity in salt tolerant cultivars, as compared with salt sensitive ones, has been reported in crops like rice (Dionisio-Sese and Tobita 1998), pea (Hernandez et al. 2000), wheat (Sairam and Srivastava 2002). The product of SOD activity is  $H_2O_2$ , which is still toxic and must be eliminated by conversion into  $H_2O$  in subsequent reactions. Among the many enzymes regulating the intracellular level of  $H_2O_2$ , peroxidases and catalases are considered to be most important. In the present study GPX and CAT activities increased in tolerant lines under salinity stress compared with susceptible one. It is noteworthy that salt induced SOD activity in leaves of genotypes JO 14, JP 13 and CIP 12 was accompanied by a greater increase in GPX and CAT activities. Thus, the results suggest that CAT and GPX activities, in coordination with SOD activity, play a central protective role in the  $O_2^-$  and  $H_2O_2$  scavenging process (Mittova et al. 2003) and the active involvement of these enzymes is related, at least in part, to salt induced oxidative stress tolerance in sweet potato plants. When the per cent increase in activity values of  $H_2O_2$  scavenging enzymes were compared, it was observed that CAT had a much higher  $H_2O_2$  scavenging activity than that of GPX in leaves of both control and salt-stressed sweet potato plants. Therefore, it could be presumed that CAT is more important  $H_2O_2$  scavenging enzyme in leaves than GPX. Rout and Shaw (2001) suggested that CAT and GPX were the most important  $H_2O_2$  scavenging enzymes leading to salt tolerance in aquatic macrophytes.

The overall results suggests that the genotypes JO 14, JP 13 and SB-198/115 have genetic potential to withstand salt stress as revealed by their better growth and enhanced activity of antioxidant enzymes. However, salt tolerance of these genotypes could be best judged by yield tests from farmer's field, affected by salinity. Testing of these genotypes under in vivo salt stress conditions revealed less variation in yield

parameters of the genotypes JO 14, JP 13, Gouri and SB-198/115 over non stress control (Dasgupta et al. 2007) confirming their salt tolerance. The results presented here agree with reports that the activity of antioxidant enzymes enhances in response to salt stress. This supports the hypothesis that scavenging of ROS through higher activity of antioxidant enzymes in leaves of sweet potato plantlets provides a mechanism of tolerance to the short-term salt stress. The possible involvement of ROS in salt stress tolerance of sweet potato would provide an insight into the molecular mechanism of salt induced oxidative stress in plant.

**Acknowledgements** The first author gratefully acknowledges the financial support of ICAR Ad hoc Scheme to carry out the present investigation. The authors are also thankful to the Director, Central Tuber Crops Research Institute, Trivandrum and Head, Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar for infrastructure facilities.

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