

Physiological and biochemical characterization of *Sesamum* germplasms tolerant to NaCl

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ABSTRACT

Sesamum indicum L. (family-Pedaliaceae) is an economically important oil seed crop grown in tropical and sub-tropical countries. It is widely used in food, nutraceutical, pharmaceutical industries. Salinity is considered as the most important abiotic stress limiting to crop production. In this context, the present study was to evaluate the *Sesamum* genotypes for salinity tolerance. Germinated seedlings (15-d-old) were used to screen the germplasm at different concentrations (0, 25mM, 50mM, 75mM, 100mM) of NaCl and observation was taken after 15th, 30th and 45th days of treatment. Ion content (Na⁺, Cl⁻, Ca⁺⁺, Mg⁺⁺ and K⁺⁺) were measured after 15 days of treatment. Fresh and dry weight was less in salt sensitive genotypes than tolerant genotypes. During increase of salinity concentration, all the genotypes had a negative impact on roots. The seedlings showed reduced growth and displayed variation in ion uptake thus accumulating Na⁺ and Cl⁻ in the roots. At higher concentration of salt treatment showed the more dry weight and displayed more effective ion regulation by manipulating low Na⁺/K⁺ and Na⁺/Ca⁺⁺ ratio. The tolerant genotypes exhibited the lowest shoot Na⁺ content under salinity conditions. Higher proline accumulation was observed at 100 mM after 15 days of NaCl treatments in 'KM-13' genotype. After 15 days of treatment, the genotype 'ES 2138-2' showed maximum proline accumulation. The total carbohydrates contents increased in all the ten genotypes in presence of NaCl. Highest carbohydrate content was found in genotype 'SI-1926' grown in 100 mM NaCl. Enzyme activities are variable in different genotypes with different concentration of NaCl. This study will help in *Sesamum* improvement programme.

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) family Pedaliaceae, is one of the oldest high-value, multipurpose oil seed crop grown widely in tropical and subtropical areas [1, 2]. The average yield of sesame on global scale is 5.1 quintals/ha while, current world production is estimated at about 4.04 million tons annually [3]. India placed second in the world after Myanmar with 18.20 lakh ha and 6.10 lakh tons production respectively. The average yield of sesame on global scale is 5.11 q/ha, while in India, it is 3.30 q/ha which is very low [3]. It is widely used against various diseases including cancer, cold, colic etc [4]. *Sesame* oil contains an unique compound known as lignans. Lignans comprises sesamin, sesamol, and a small amount of sesamol [5]. Lignans

are also phytoestrogens and their conversion to enterolactone is very important in preventing hormone-dependent cancers (like breast and prostate) and cardiovascular diseases. Soil salinity is one of the most important problems for irrigated agriculture, which drastically affect crop productivity throughout the world. It is mainly due to low precipitation and high transpiration causing disturbance in salt balance in the soil and also renders ground water brackish and affects plant growth adversely [6,7]. Nearly, 80 million hectares of arable lands of the world are estimated to be affected by salt [8]. Salinity effects are more noticeable in arid and semiarid regions, mainly due to the acceleration of salinity by a deficit of precipitation and high temperature coupled with a high evaporation demand [9].

Salt stress changes the morphological, physiological and biochemical responses of plants [10]. High salinity causes lower water potential and induces both hyper osmotic and ionic stress and results in alteration in plant metabolism including ionic imbalances, water potentials and specific ion toxicity [6].

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About 15% of the total land area of the world has been degraded by soil erosion and physical and chemical degradation including soil salinization. High salt concentrations cause an imbalance in cellular ions, resulting in ion toxicity and osmotic stress, which leads to the generation of reactive oxygen species (ROS). These cytotoxic ROS's are highly reactive and when the ability of plant for scavenging is less than the ROS production, they can seriously disrupt normal metabolism through oxidative damages to the photosynthetic pigments, proteins, nucleic acids and lipids [11,12]. NaCl is the most abundant salt found in environments effected by salinity because of its ability to compete with various nutrients resulting in nutrient deficiency and specific toxicity. Many crops tolerate salinity to a threshold level and above which yield decreases as the salinity increases. Screening of genotypes is necessary to identify the salt tolerant germplasm for breeding programme to evolve the salt tolerant and high yielding crop varieties. In this context, the present study was carried out to screen the ten *Sesamum* genotypes under salinity stress with physiological and biochemical mechanism.

2. MATERIALS AND METHODS

2.1. Plant material and treatments

The genetically pure seed material of 33 *Sesamum* genotypes representing different geographical locations in India was collected from the germplasm centre of AICRP in *Sesamum* at Orissa University of Agriculture & Technology, Bhubaneswar, India. Out of them, 10 genotypes (SI-2138-2, S-0140, Prachi, Kanaka, SI-1025, SI-205, KM-13, IS-607-1-84, SI-1926 and ENT-78-301) were selected basing on their germination and growth rate under different concentration of NaCl (0, 25 mM, 50 mM, 75 mM, 100 mM) treatments. Seeds were sown into the pots (20 cm × 30 cm) filled with 5 kg soil (soil and sand in the ratio 3:1), pH of soil ranges from 5.96 to 6.35 and temperature of soil is 89 to 91°F and grown under the green house. Soil electrical conductivity was measured of the amount of salts in soil (salinity of soil). It is an important indicator of soil health. It is commonly expressed in units of milliSiemens per meter (mS/m). The seedlings were watered regularly with half strength Hoagland solution [13]. Different concentrations of NaCl (0, 25mM, 50mM, 75mM and 100mM) was added into 15-day-old plant in every 2 days intervals upto 45 days. The morphological observation was taken after 15th, 30th and 45th days after the treatment. Salt stress in change of proteins, antioxidant enzymes (POD, SOD, GPX), chlorophyll content, proline and polyphenol contents and total carbohydrates contents were analyzed. Ten seedlings from each treatment were sampled randomly at 15th, 30th and 45th days after NaCl treatment. Data on root and shoot length and plant fresh and dry weight were determined. All the plant samples were dried at 65 ± 2°C for 2 days in hot air oven to a constant weight as dry weight was determined. The ion content analysis was made after 15 days of the growth. After drying, the shoots were grounded and analysed for Cl⁻, Mg⁺², Na⁺, K⁺⁺, Ca⁺ and Na⁺/K⁺ and Na⁺/Ca⁺. The content of Cl⁻, Mg⁺², Na⁺, K⁺⁺, Ca⁺ and Na⁺/K⁺ and Na⁺/Ca⁺ (mg/g

dry weight) of shoot was determined from 500 mg digested sample (5 ml sulphuric acid + 5 ml perchloric acid) using the Atomic Spectrophotometer (M/S Thermo Fischer, Germany). A stand curve was drawn based on a graded series of standards (ranged from 10 to 100 mg/l) of Cl⁻, Mg⁺², Na⁺, K⁺⁺, Ca⁺ and Na⁺/K⁺ and Na⁺/Ca⁺.

2.2. Determination of total carbohydrate

Five hundred milligrams of fresh leaves were taken into a tube and hydrolyzed with 5 ml 2.5N HCl for 3 hrs by the hot water bath. Further, the test tubes were cooled at room temperature and the sample was neutralized by adding solid sodium carbonate till the effervescence ceases. The volume was made to 100ml and centrifuged at 5000 rpm for 15 min. The supernatant was collected and 0.1 ml aliquots from each sample were taken for analysis. The volume was made up to 1ml in all test tubes by adding distilled water. Four milliliter of anthrone reagent was added to each tube and heated for 8 min in a boiling water bath. Then, it was cooled rapidly and optical density (OD) was taken at 630 nm.

2.3. Proline content

To determine the proline level, 0.5 g of leaf samples from each treatment were homogenized in 3% (w/v) sulphosalicylic acid and then filtered through filter paper [14]. Mixture was heated at 100°C for 1h in a hot water bath after addition of ninhydrin and glacial acetic acid. Reaction was stopped by putting in ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined by using calibration curve and expressed as µg proline per gm fresh weight basis.

2.4. Estimation of protein

1 gm fresh leaves of *Sesamum* were homogenized in ice cold trichloroacetic acid (TCA) buffer and incubated overnight at 4°C. Then, the sample was centrifuged at 8000 rpm for 20 mins. Supernatant was discarded and pellet was washed with acetone to remove the pigments. The sample was again centrifuged at 10,000 rpm for 10 mins and washed with 80% alcohol to remove phenolic compounds. This was centrifuged at 5000 rpm for 10 mins and the pellet was suspended in known volume of extraction buffer and kept in boiling water bath for 2 mins. 100µl aliquot was taken and volume was made up to 1ml. Five milliliter of Reagent-C (50 ml of 2% sodium carbonate dissolved in 0.1N NaOH + 1 ml of 0.5 % copper sulphate dissolved in 1% sodium potassium tatarate) was added, mixed well and kept for 10 mins. Further, 0.5 ml of Folin-Ciocalteu reagent was added, mixed well and incubated in the dark for 30 mins at room temperature. After blue colour developed, the OD was taken at 660 nm. The protein content was calculated against the standard graph.

2.5. Enzyme assays

500 mg leaf tissues were grinded by using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium

phosphate buffer pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 g for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C. The superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT), as described by Giannopolitis and Ries [15]. The reaction solution consisted of 50 µl of the enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium and 2 µM riboflavin in a total volume of 1.5 ml. After adding riboflavin, the test tubes were shaken manually and placed under fluorescent lighting from two 20W lamps. The reaction was allowed to proceed for 15 min, after which the lights were switched off. Reduction in NBT was estimated by reading the absorbance of the reaction mixture at 560 nm, and one unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction and the results were expressed as Unit/ mg.

Peroxidase (POD) (EC 1.11.1.7) activity was determined with guaiacol as reducing substrate in a reaction mixture containing 0.1M K-phosphate buffer, pH 6, 20 mM guaiacol, and 30 mM H₂O₂. The oxidation of guaiacol was assessed by recording the absorbance increase at 470 nm and 25 °C [16]. The enzyme unit was expressed as the absorbance change at 470 nm, per minute, under the above conditions.

Glutathione peroxidase (PXR) was analysed by the method of Wendel [17]. 1 mg of NADPH, 9.2 ml of 1 mM sodium azide solution (in 50 mM sodium phosphate buffer with 0.4 mM EDTA), 0.1 ml of glutathione reductase enzyme solution (100 U/ml), and 0.05 ml of glutathione reduced (GSH) were added and mixed by inversion.

Then, into 3 ml of the mixture 0.05 ml of enzyme extract was added. It was vortexed and incubated for 5 min at room temperature. After the incubation, 0.05 ml of H₂O₂ was mixed immediately and measured at 340 nm after every 30 s over a period of 5 min. GPX activity was calculated from the change in optical density per minute in the maximum linear rate range using a molar extinction coefficient for NADPH of $6.22 \times 10^3/\mu\text{mol}$ and assuming 2 mol of GSH formed for each mole of NADPH consumed. One unit activity was defined as 1 µmol NADPH oxidized per minute.

2.6. Statistical Analysis

Each data point represents the mean of three samples \pm SE. Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test, and the significance level of $P < 0.05$ was employed.

3. RESULTS AND DISCUSSION

Soil salinity is the major limiting factor for crop yield and productivity. Intensive agronomic practices, poor water management, irrigation without sufficient drainage systems, long periods of hot and dry seasons and high levels of evaporation lead

to the salinization of millions of hectares of agricultural land [5]. The present study showed that there was distinct growth effect of *Sesamum* genotypes in various concentrations of NaCl. The growth attributes were decreased with increase of NaCl concentration. The results showed the both shoot and root fresh weight decreased in all genotypes but more prominent in one genotype SI-1926 (Table. 1).

Table 1: Fresh and dry weight of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl after 15 days of treatment (*20 replicates/treatment; repeated twice).

Genotypes	NaCl concentration (mM)	Fresh weight (g) (Mean \pm SE)*		Dry weight (g) (Mean \pm SE)*	
		Shoot	Root	Shoot	Root
SI-2138-2	0	22.5 \pm 1.7 m	1.32 \pm 0.03 j	4.12 \pm 0.9 h	0.23 \pm 0.01 f
	25	7.64 \pm 1.2 e	0.72 \pm 0.05 g	0.34 \pm 1.1 a	0.08 \pm 0.06 b
	50	6.09 \pm 1.1 d	0.39 \pm 0.07 d	2.08 \pm 0.9 f	0.09 \pm 0.04 b
	75	3.72 \pm 1.6 b	0.29 \pm 0.09 c	0.55 \pm 0.2 b	0.08 \pm 0.03 b
	100	2.60 \pm 1.2 a	0.26 \pm 0.07 c	0.35 \pm 0.5 a	0.04 \pm 0.07 a
S-0140	0	23.4 \pm 1.5 n	1.51 \pm 0.03 l	4.25 \pm 1.1 h	0.29 \pm 0.05 h
	25	14.6 \pm 1.2 i	0.83 \pm 0.07 h	3.31 \pm 0.9 g	0.18 \pm 0.02 e
	50	12.3 \pm 1.6 h	0.76 \pm 0.05 g	0.68 \pm 0.9 c	0.09 \pm 0.09 b
	75	10.6 \pm 2.5 g	0.52 \pm 0.07 e	0.54 \pm 0.2 b	0.08 \pm 0.07 b
	100	9.50 \pm 1.8 f	0.46 \pm 0.09 d	0.41 \pm 0.8 a	0.07 \pm 0.04 b
Prachi	0	20.3 \pm 1.2 l	1.55 \pm 0.03 l	4.00 \pm 0.6 h	0.30 \pm 0.06 a
	25	8.00 \pm 2.3 e	0.37 \pm 0.07 d	3.43 \pm 0.4 g	0.20 \pm 0.09 a
	50	18.9 \pm 1.8 k	0.95 \pm 0.02 i	3.02 \pm 0.8 g	0.22 \pm 0.07 a
	75	13.9 \pm 1.1 i	0.60 \pm 0.04 f	1.38 \pm 0.5 e	0.09 \pm 0.06 b
	100	5.43 \pm 1.2 c	0.18 \pm 0.04 a	0.62 \pm 0.2 c	0.03 \pm 0.02 a
Kanaka	0	25.0 \pm 1.5 o	1.94 \pm 0.02 m	3.88 \pm 0.9 h	0.31 \pm 0.03 h
	25	22.4 \pm 1.1 m	1.58 \pm 0.03 l	2.63 \pm 0.2 f	0.26 \pm 0.06 g
	50	20.8 \pm 1.0 l	1.41 \pm 0.04 k	2.52 \pm 0.5 f	0.22 \pm 0.07 f
	75	12.4 \pm 1.6 h	0.75 \pm 0.07 g	1.49 \pm 0.4 e	0.12 \pm 0.09 c
	100	12.2 \pm 1.5 h	0.86 \pm 0.05 h	1.23 \pm 0.8 e	0.20 \pm 0.87 e,f
SI-1025	0	16.8 \pm 1.0 j	1.06 \pm 0.02 i	1.72 \pm 0.2 f	0.13 \pm 0.03 c
	25	11.3 \pm 1.7 g	0.62 \pm 0.07 f	1.29 \pm 0.7 e	0.12 \pm 0.09 c
	50	7.37 \pm 1.1 e	0.43 \pm 0.04 d	0.64 \pm 0.4 c	0.09 \pm 0.03 b
	75	6.48 \pm 1.7 d	0.50 \pm 0.05 e	0.57 \pm 0.8 b	0.08 \pm 0.04 b
	100	6.10 \pm 1.0 d	0.53 \pm 0.04 e	0.91 \pm 0.2 d	0.07 \pm 0.06 b
SI-205	0	14.2 \pm 1.6 i	0.74 \pm 0.05 g	1.54 \pm 0.9 f	0.10 \pm 0.02 b, c
	25	10.4 \pm 2.2 g	0.39 \pm 0.07 d	0.92 \pm 0.9 d	0.11 \pm 0.01 c
	50	5.87 \pm 1.0 c	0.30 \pm 0.09 c	0.98 \pm 0.5 d	0.12 \pm 0.04 c
	75	7.61 \pm 1.1 e	0.28 \pm 0.05 c	0.56 \pm 0.2 b	0.05 \pm 0.03 a
	100	8.18 \pm 1.2 e	0.60 \pm 0.04 f	0.31 \pm 0.9 a	0.07 \pm 0.07 b
KM-13	0	15.3 \pm 1.1 i, j	0.88 \pm 0.07 h	1.77 \pm 0.8 f	0.21 \pm 0.05 f
	25	15.0 \pm 1.2 i, j	0.77 \pm 0.05 g	1.25 \pm 1.1 e	0.12 \pm 0.09 c
	50	14.6 \pm 1.3 i	0.45 \pm 0.09 d	0.59 \pm 0.8 c	0.09 \pm 0.07 b
	75	7.98 \pm 2.5 e	0.37 \pm 0.07 d	1.03 \pm 0.1 d	0.05 \pm 0.06 a
	100	3.13 \pm 1.3 b	0.31 \pm 0.16 c	0.97 \pm 0.5 d	0.10 \pm 0.09 b, c
IS-607-1-84	0	11.2 \pm 1.2 g	0.73 \pm 0.03 g	1.37 \pm 0.8 e	0.12 \pm 0.07 c
	25	7.90 \pm 1.0 e	0.18 \pm 0.09 a	0.52 \pm 0.4 b	0.12 \pm 0.02 c
	50	6.83 \pm 1.2 d	0.13 \pm 0.07 a	0.62 \pm 0.8 c	0.13 \pm 0.07 c
	75	9.94 \pm 1.1 g	0.65 \pm 0.02 f	1.26 \pm 0.2 e	0.11 \pm 0.09 c
	100	6.80 \pm 2.2 d	0.13 \pm 0.05 f	0.83 \pm 0.4 d	0.03 \pm 0.50 a
SI-1926	0	17.9 \pm 1.1 k	1.29 \pm 0.01 j	0.59 \pm 0.8 c	0.28 \pm 0.12 h
	25	16.7 \pm 1.3 j	0.85 \pm 0.04 h	0.54 \pm 0.5 b	0.20 \pm 0.87 e, f
	50	15.3 \pm 1.2 i, j	0.75 \pm 0.03 g	0.38 \pm 0.2 a	0.12 \pm 0.05 c
	75	7.09 \pm 0.9 e	0.44 \pm 0.05 d	0.43 \pm 1.1 a	0.06 \pm 0.01 a, b
	100	5.21 \pm 1.2 c	0.12 \pm 0.07 a	0.29 \pm 0.4 a	0.02 \pm 0.06 a
ENT-78-301	0	12.2 \pm 1.1 h	1.46 \pm 0.02 k	1.24 \pm 0.8 e	0.13 \pm 0.04 c
	25	10.4 \pm 1.3 g	0.39 \pm 0.07 d	0.92 \pm 0.9 d	0.11 \pm 0.03 c
	50	8.87 \pm 1.1 f	0.30 \pm 0.09 c	0.98 \pm 0.2 d	0.12 \pm 0.07 c
	75	7.61 \pm 1.2 e	0.28 \pm 0.07 c	0.56 \pm 0.5 b	0.05 \pm 0.05 a
	100	6.77 \pm 1.2 e	0.20 \pm 0.02 a	0.34 \pm 0.1 a	0.11 \pm 0.09 c

* Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

Shoot and root growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by various researchers [18 - 21]. This shows that a mild salinity can adversely affect the growth of *Sesamum*. However, the genotypes 'Prachi', 'Kanaka' and 'KM-13' showed higher dry weight of shoot and root in the presence of NaCl and, therefore, were categorized as salt-tolerant (Table 1).

Although information is lacking on sesame, the adverse effect of salinity on plant biomass has earlier been observed in a number of plant species e.g. cotton [22], linseed [23], bean [24], maize [25] tetraploid wheat [26], pea [27], alfalfa [28] and sorghum [29]. Munns and Tester [19] reported that the salinity reduces the ability of plants to uptake water which subsequently leads to a reduction in growth rate along with a chain of metabolic changes. There are also reductions in plant biomass attributes under stressful conditions because of a reduced photosynthetic activity per unit leaf area [24], additional cost to exclude or compartmentalize salts within the cells and the salt-induced damage to the plant cells and tissues [27].

The protein, proline and total carbohydrate content were increased in seedlings grown in the soil having NaCl as compare with seedlings grown without application of NaCl (Tables 2-4).

Table 2: Protein content (mg per g fresh weight basis) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl Concentration (mM)	Protein content (mg per g fresh weight basis) (Mean \pm SE) ⁺		
		15 days of treatment	30 days of treatment	45 days of treatment
SI-2138-2	0	6.4 \pm 0.4 a	8.8 \pm 0.5 a	10.0 \pm 0.4 b
	25	8.0 \pm 0.5 b	10.4 \pm 0.8 b	13.7 \pm 0.8 d
	50	12.0 \pm 0.6 d	17.8 \pm 0.3 e	*
	75	18.6 \pm 0.5 g	26.2 \pm 0.6 i	*
	100	24.0 \pm 0.7 j	31.6 \pm 1.2 k	*
S-0140	0	8.8 \pm 0.2 b	19.0 \pm 0.7 f	8.0 \pm 0.7 a
	25	8.0 \pm 0.4 b	17.2 \pm 0.8 e	14.6 \pm 0.8 d,e
	50	17.2 \pm 0.8 g	23.6 \pm 1.4 h	*
	75	18.4 \pm 0.9 g	28.6 \pm 0.7 j	*
	100	29.6 \pm 0.8 m	30.0 \pm 1.0 k	*
Prachi	0	8.6 \pm 0.3 b	14.0 \pm 0.8 d	21.7 \pm 0.5 h
	25	17.4 \pm 0.9 g	24.6 \pm 1.2 h	29.6 \pm 0.7 j
	50	21.2 \pm 1.0 h	25.8 \pm 1.1 h,i	*
	75	30.4 \pm 1.2 m	34.8 \pm 1.2 m	*
	100	37.6 \pm 1.2 n	40.4 \pm 1.0 n	*
Kanaka	0	7.8 \pm 0.6 b	12.8 \pm 0.8 c	17.0 \pm 0.6 f
	25	9.4 \pm 0.7 c	14.4 \pm 0.7 c,d	22.2 \pm 0.9 h
	50	9.8 \pm 0.6 c	15.6 \pm 0.6 d	*
	75	12.4 \pm 0.8 d	19.0 \pm 1.6 f	*
	100	26.4 \pm 1.7 k	35.8 \pm 0.7 m	*
SI-1025	0	6.6 \pm 0.7 a	9.8 \pm 0.8 b	10.8 \pm 0.6 b
	25	13.2 \pm 1.0 d	21.2 \pm 2.3 g	25.6 \pm 1.2 i
	50	18.0 \pm 0.8 g	28.6 \pm 1.0 j	*
	75	26.4 \pm 0.9 k	32.8 \pm 1.2 l	*
	100	28.8 \pm 1.1 l	33.2 \pm 0.9 l	*

SI-205	0	8.4 \pm 0.5 b	13.2 \pm 0.7 c	12.2 \pm 0.6 c
	25	10.6 \pm 0.9 c	16.8 \pm 0.8 e	19.4 \pm 0.8 g
	50	16.8 \pm 1.2 f	31.2 \pm 1.0 k	*
	75	26.6 \pm 1.5 k	32.2 \pm 1.3 l	*
	100	29.4 \pm 1.4 m	33.4 \pm 1.2 l	*
KM-13	0	5.8 \pm 0.7 a	7.7 \pm 0.6 a	10.2 \pm 0.5 b
	25	6.4 \pm 0.5 a	8.2 \pm 0.7 a	9.6 \pm 0.6 b
	50	11.6 \pm 0.8 d	18.2 \pm 0.6 f	*
	75	11.8 \pm 0.7 d	23.6 \pm 0.8 h	*
	100	19.6 \pm 0.9 h	24.0 \pm 1.0 h	*
IS-607-1-84	0	10.6 \pm 0.8 c	14.2 \pm 0.4 c,d	14.4 \pm 0.7 d
	25	16.4 \pm 0.6 f	19.6 \pm 0.6 f	7.0 \pm 0.7 a
	50	20.4 \pm 0.7 h	27.6 \pm 1.1 j	*
	75	22.4 \pm 0.9 i	29.6 \pm 1.4 k	*
	100	28.0 \pm 1.2 l	34.0 \pm 0.8 m	*
SI-1926	0	7.5 \pm 0.4 b	9.4 \pm 0.6 b	13.8 \pm 0.8 d
	25	8.6 \pm 0.6 b	11.6 \pm 0.7 c	15.0 \pm 0.6 e
	50	8.8 \pm 0.8 b	14.6 \pm 0.6 d	*
	75	10.8 \pm 0.8	15.1 \pm 0.4 d	*
	100	18.6 \pm 0.7 g	24.4 \pm 0.8 h	*
ENT-78-301	0	8.4 \pm 0.6 b	12.2 \pm 0.7 c	14.4 \pm 0.7 d,e
	25	10.2 \pm 0.5 c	12.4 \pm 0.8 c	13.8 \pm 0.8 d
	50	12.6 \pm 0.8 d	14.2 \pm 0.8 c,d	*
	75	16.8 \pm 0.7 f	23.2 \pm 0.9 h	*
	100	27.4 \pm 0.9 l	34.7 \pm 0.7 m	*

⁺10 replicates / treatment; repeated twice. *Plant did not survived, Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

Table 3: Proline content (μ g per gm fresh weight basis) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl concentration (mM)	Proline content (μ g per gm fresh weight basis) (Mean \pm SE) ⁺		
		15 days of treatment	30 days of treatment	45 days of treatment
SI-2138-2	0	3.10 \pm 0.5 b	6.16 \pm 0.4 b	9.50 \pm 0.5 c
	25	3.46 \pm 0.3 b	10.4 \pm 0.8 d	13.4 \pm 0.8 e
	50	7.73 \pm 0.6 f	16.2 \pm 0.7 f	*
	75	14.2 \pm 0.4 i	19.5 \pm 0.6 h	*
	100	27.0 \pm 1.1 n	35.5 \pm 1.1 p	*
S-0140	0	5.16 \pm 0.3 d	5.20 \pm 0.4 a	8.07 \pm 0.7 b
	25	6.92 \pm 0.7 e	5.54 \pm 0.7 a	12.2 \pm 0.8 d
	50	8.46 \pm 0.4 g	12.3 \pm 0.6 e	*
	75	12.6 \pm 0.8 h	23.2 \pm 0.8 j	*
	100	25.8 \pm 0.7 m	30.6 \pm 0.7 n	*
Prachi	0	2.24 \pm 0.6 a	8.00 \pm 0.5 c	9.42 \pm 0.8 c
	25	3.80 \pm 0.5 b	8.31 \pm 0.8 c	13.5 \pm 0.6 e
	50	8.16 \pm 0.6 g	12.5 \pm 0.4 e	*
	75	14.2 \pm 0.8 i	18.0 \pm 0.3 g	*
	100	21.1 \pm 0.6 l	27.6 \pm 0.5 l	*
Kanaka	0	2.08 \pm 0.4 a	8.65 \pm 0.2 c	12.2 \pm 0.5 d
	25	4.16 \pm 0.8 c	10.4 \pm 0.9 d	17.6 \pm 0.7 f
	50	12.4 \pm 0.6 h	17.6 \pm 1.2 g	*
	75	17.8 \pm 0.7 j	21.8 \pm 0.8 i	*
	100	22.2 \pm 0.9 l	33.2 \pm 1.0 o	*
SI-1025	0	3.46 \pm 0.8 b	4.80 \pm 0.8 a	5.54 \pm 0.8 a
	25	5.07 \pm 0.4 d	6.93 \pm 0.7 b	16.9 \pm 0.7 f
	50	12.0 \pm 0.8 h	15.2 \pm 0.6 f	*
	75	18.1 \pm 0.5 k	24.3 \pm 0.3 k	*
	100	21.2 \pm 0.7 l	29.7 \pm 1.3 m	*
SI-205	0	4.16 \pm 0.6 c	6.58 \pm 0.8 b	10.0 \pm 0.6 c
	25	5.88 \pm 0.8 d	7.27 \pm 0.9 b	16.9 \pm 0.7 f
	50	6.23 \pm 0.5 d	15.5 \pm 0.6 f	*
	75	13.3 \pm 0.4 h	17.6 \pm 0.7 g	*

KM-13	100	17.6±0.8 j	27.6±0.9 l	*
	0	4.16±0.4 c	6.92±0.7 b	9.4 ±0.8 c
	25	6.42±0.6 d	7.27±0.5 c	14.2±0.9 e
	50	12.8±0.4 h	16.5±0.6 f	*
	75	19.4±0.8 k	24.8±0.8 k	*
IS-607-1-84	100	27.1±0.9 n	32.9±0.7 o	*
	0	7.80±0.6 f	10.7±0.6 d	12.1±0.7 d
	25	12.8±0.8 h	15.2±0.7 f	23.4±0.9 h
	50	14.8±0.7 i	20.4±0.8 h	*
	75	21.2±0.4 l	27.3±1.2 l	*
SI-1926	100	24.5±0.8 m	33.3±1.0 o	*
	0	4.16±0.6 c	10.0±0.6 d	13.7±0.9 e
	25	9.35±0.7 g	12.7±0.7 e	23.2±0.8 h
	50	7.61±0.8 f	15.5±0.5 f	*
	75	16.8±0.7 j	21.8±0.6 i	*
ENT-78-301	100	26.2±0.9 n	29.0±1.3 m	*
	0	7.16±0.8 f	9.10±0.7 d	11.7±0.7 d
	25	7.61±0.5 f	10.7±0.8 d	18.3±0.6 g
	50	14.5±0.5 i	18.3±0.7 g	*
	75	18.2±0.8 k	27.3±0.9 l	*
	100	25.5±1.1 m	31.0±1.2 n	*

*Plant did not survived, [†]10 replicates / treatment; repeated twice. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

Higher proline accumulation (27.12 mg/gm) was observed in genotype 'KM-13' grown at 100 mM of NaCl treatment. This genotype successfully tolerate at higher salinity level by accumulating more proline in leaves. Protein content was higher in genotypes 'IS-607-1-84' (10.6 mg per gm), 'S-0140' (8.8 mg per gm) and 'Prachi' (8.6 mg per gm) as compared to other genotypes. Furthermore, increase of protein content with increase of NaCl concentration in all the genotypes. After 45 days of treatment, all the plants were died except in plant grown in soil without treatment of NaCl as well as 25 mM NaCl. The total carbohydrates contents increased in all the ten genotypes of *Sesamum* in presence of NaCl (Table. 4).

Table 4: Total carbohydrate content (mg per gm fresh weight basis) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl Concentration (mM)	Carbohydrate content (mg per gm fresh weight basis) (Mean ± SE) [†]		
		15 days of treatment	30 days of treatment	45 days of treatment
SI-2138-2	0	26.2±0.9 j	34.6±1.1 e	24.2±0.9 a
	25	29.4±1.3 k	34.2±0.9 e	46.4±1.5 e
	50	36.4±1.2 n	42.4 ±1.5 h	*
	75	39.1±0.8 p	43.8±1.8 i	*
	100	42.4±1.1 q	48.6±1.4 j	*
S-0140	0	12.2±0.5 c	58.2±1.3 m	56.7 ±1.2 f
	25	24.2±0.6 i	42.6±1.7 h	42.8±1.5 d
	50	26.3±0.7 j	56.4±1.5 l	*
	75	20.2±0.8 g	62.0±1.3 o	*
	100	18.4± 0.7 f	34.2±1.2 e	*
Prachi	0	20.6±1.1 g	28.4±1.3 c	37.4±1.8 c
	25	12.4±0.5 c	32.5±1.7 d	42.2±1.4 d
	50	24.1±0.6 i	36.6±1.2 f	*
	75	24.8±0.8 i	36.2±1.6 f	*
	100	26.5±0.9 j	38.4±1.3 g	*
K an ak a	0	16.2±0.6 e	56.5±1.7 l	34.5±1.7 b
	25	26.3±0.5 j	62.2±1.5 o	54.3±1.6 f

SI-1025	50	24.3±0.7 i	65.4±1.4 p	*
	75	28.2±0.8 k	68.2±1.8 q	*
	100	32.5±0.7 l	76.4±1.4 s	*
	0	4.5±0.7 a	38.2±1.5 g	48.6±1.3 e
	25	6.6±0.6 b	56.0±1.3 l	62.0±2.2 g
SI-205	50	14.2±0.5 d	62.4±2.0 o	*
	75	32.1±0.8 l	62.6±1.5 o	*
	100	32.5±0.9 l	64.5±1.3 p	*
	0	18.4±0.6 f	28.2±1.4 c	48.2±1.7 e
	25	21.2±0.8 g	32.6±2.6 d	55.0±1.8 f
KM-13	50	28.4±0.7 k	62.6±1.5 o	*
	75	32.2±0.9 l	82.2±1.2 u	*
	100	36.4±1.1 n	52.6±1.6 k	*
	0	22.8±0.6 h	52.8±1.4 k	56.2±1.4 f
	25	18.4±0.8 f	62.2±1.8 o	38.4±1.6 c
IS-607-1-84	50	14.2±0.7 d	62.5±1.3 o	*
	75	24.5±0.8 i	73.4±2.1 r	*
	100	32.3±1.0 l	78.4±1.8 t	*
	0	28.2 ±0.7 k	34.6±1.3 e	46.1±1.7 e
	25	33.5±1.2 m	24.8±1.4 l	78.5±1.4 h
SI-1926	50	24.2±0.7 i	29.2±1.5 c	*
	75	27.4±0.8 k	36.4±1.3 f	*
	100	28.2±0.6 k	48.6±1.1 j	*
	0	20.4±0.5 g	34.2±1.4 e	38.6±1.6 c
	25	32.2±1.2 l	44.4±1.3 i	62.5±1.4 g
ENT-78-301	50	32.6±1.0 l	52.4±1.5 k	*
	75	38.4±1.2 o	58.8±1.2 m	*
	100	42.8±1.3 q	60.4±1.4 n	*
	0	18.4±0.6 f	22.7±1.6 a	34.6±1.2 b
	25	26.2±0.8 j	42.5±1.3 h	56.5 ±2.1 f
ENT-78-301	50	28.3±0.5 k	28.4±1.7 c	*
	75	24.5±0.7 i	38.6±1.5 g	*
	100	21.5±0.6	42.2±1.4 h	*

*Plant did not survived, [†]10 replicates / treatment; repeated twice. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

Proline and total carbohydrates help osmotic adjustment during stress and protect native structure of macromolecules and membranes during extreme dehydration. Rise in total carbohydrates levels in salt-treated genotypes may contribute towards better adaptation to salinity. The proline is one of the prevalent osmolytes that are commonly found in high concentrations when plants are exposed to salt stress [19]. The exact role of proline with regard to plant's response to environmental stresses is rather controversial [30]. Proline accumulation in plant cells might be due to an increase in proteolysis or a decrease in protein synthesis [31]. Accumulation of proline under stress conditions can protect the cell by stabilizing sub cellular structures (e.g. proteins and enzymes) and buffering the cellular redox potential [32]. Besides its role as an osmolyte, proline can also confer enzyme protection and increase membrane stability under various conditions [23]. In present study, the higher proline accumulation is found at 100 mM of NaCl treatment for 15 days in genotype KM-13 and 30 days in genotype ES2138-2. Proline has been shown to accumulate in response to salinity in a number of plant species [8, 23, 33]. Since proline accumulation in the Sesame genotypes was well-correlated to their growth attributes and production of this free amino acid in the salt-tolerant genotypes was more notable, it seems that it plays some protective roles against salt stress in sesame, at least in the genotypes used in this experiment.

Table 5: Inorganic ion (Na^+ , Cl^- , K^+ , Ca^{2+} and Mg^{2+}) content in 15 days old seedlings of ten genotypes of *Sesamum indicum* L. treatment with different concentrations (0, 25, 50, 75 and 100 mM) of NaCl. Experiment conducted twice with three replications (*Three replicates/treatment; repeated thrice).

Sesamum <i>indicum</i> Vars.	Diff. Conc. NaCl (mM)	Na ⁺ Content (mmol/g dry weight) (Mean \pm SE)*		Cl ⁻ Content (mmol/g dry weight) (Mean \pm SE)*		K ⁺ Content (mmol/g dry weight) (Mean \pm SE)*		Ca ⁺⁺ content (mmol/g dry weight) (Mean \pm SE)*		Mg ⁺⁺ content (mmol/g dry weight) (Mean \pm SE)*	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
SI-2138-2	0	1.1 \pm 0.1	3.8 \pm 0.5	0	0	0.3 \pm 0.01	0.9 \pm 0.02	0.01 \pm 0.01	0.01 \pm 0.00	0.15 \pm 0.04	0.35 \pm 0.02
	25	1.4 \pm 0.2	4.0 \pm 0.6	0.4 \pm 0.01	1.2 \pm 0.2	0.2 \pm 0.01	0.8 \pm 0.01	0.01 \pm 0.00	0.01 \pm 0.00	0.16 \pm 0.06	0.41 \pm 0.04
	50	1.2 \pm 0.3	3.7 \pm 0.7	0.3 \pm 0.01	1.0 \pm 0.1	0.2 \pm 0.01	0.7 \pm 0.02	0.01 \pm 0.00	0.02 \pm 0.01	0.16 \pm 0.04	0.61 \pm 0.04
	75	1.0 \pm 0.1	3.0 \pm 0.3	0.2 \pm 0.01	0.8 \pm 0.01	0.1 \pm 0.00	0.6 \pm 0.02	0.02 \pm 0.01	0.02 \pm 0.00	0.19 \pm 0.05	0.63 \pm 0.05
	100	0.9 \pm 0.2	2.6 \pm 0.5	0.1 \pm 0.01	0.5 \pm 0.01	0.1 \pm 0.00	0.4 \pm 0.01	0.02 \pm 0.00	0.04 \pm 0.01	0.21 \pm 0.03	0.58 \pm 0.04
S-0140	0	1.5 \pm 0.1	4.9 \pm 0.6	0	0	0.6 \pm 0.02	1.0 \pm 0.3	0.01 \pm 0.00	0.01 \pm 0.00	0.10 \pm 0.03	0.53 \pm 0.05
	25	1.8 \pm 0.3	5.2 \pm 0.8	0.5 \pm 0.02	1.5 \pm 0.3	0.4 \pm 0.01	0.9 \pm 0.04	0.02 \pm 0.01	0.01 \pm 0.00	0.12 \pm 0.04	0.62 \pm 0.04
	50	2.1 \pm 0.2	5.5 \pm 0.4	0.4 \pm 0.01	1.6 \pm 0.4	0.6 \pm 0.02	0.9 \pm 0.05	0.02 \pm 0.01	0.05 \pm 0.01	0.12 \pm 0.05	0.91 \pm 0.06
	75	2.5 \pm 0.5	5.8 \pm 0.6	0.4 \pm 0.02	1.4 \pm 0.3	0.5 \pm 0.02	0.7 \pm 0.03	0.03 \pm 0.01	0.08 \pm 0.02	0.14 \pm 0.02	1.03 \pm 0.22
	100	2.0 \pm 0.2	5.0 \pm 0.4	0.2 \pm 0.01	1.0 \pm 0.2	0.3 \pm 0.01	0.6 \pm 0.02	0.02 \pm 0.00	0.10 \pm 0.03	0.13 \pm 0.03	0.80 \pm 0.07
Prachi	0	2.2 \pm 0.1	5.6 \pm 0.5	0	0	0.4 \pm 0.01	1.2 \pm 0.4	0.01 \pm 0.00	0.01 \pm 0.00	0.14 \pm 0.02	0.61 \pm 0.06
	25	2.0 \pm 0.3	6.2 \pm 0.6	0.5 \pm 0.01	1.5 \pm 0.2	0.5 \pm 0.02	0.8 \pm 0.02	0.02 \pm 0.00	0.02 \pm 0.01	0.16 \pm 0.01	0.81 \pm 0.05
	50	2.2 \pm 0.3	6.7 \pm 0.7	0.6 \pm 0.02	1.6 \pm 0.3	0.6 \pm 0.02	0.9 \pm 0.03	0.02 \pm 0.00	0.04 \pm 0.01	0.23 \pm 0.02	1.0 \pm 0.23
	75	2.1 \pm 0.2	6.9 \pm 0.7	0.4 \pm 0.02	2.3 \pm 0.6	0.8 \pm 0.03	1.0 \pm 0.5	0.03 \pm 0.01	0.08 \pm 0.02	0.25 \pm 0.04	0.92 \pm 0.14
	100	1.8 \pm 0.1	6.4 \pm 0.5	0.3 \pm 0.01	1.6 \pm 0.5	0.6 \pm 0.02	0.9 \pm 0.03	0.03 \pm 0.01	0.08 \pm 0.04	0.21 \pm 0.06	0.72 \pm 0.11
Kanaka	0	2.8 \pm 0.2	6.7 \pm 0.4	0	0	0.5 \pm 0.02	1.5 \pm 0.2	0.01 \pm 0.00	0.01 \pm 0.00	0.15 \pm 0.04	0.56 \pm 0.09
	25	2.6 \pm 0.2	6.9 \pm 0.3	0.6 \pm 0.02	1.8 \pm 0.2	0.6 \pm 0.03	1.3 \pm 0.5	0.02 \pm 0.00	0.02 \pm 0.01	0.16 \pm 0.02	0.87 \pm 0.08
	50	2.0 \pm 0.1	7.2 \pm 0.6	0.8 \pm 0.03	2.0 \pm 0.3	0.7 \pm 0.04	1.1 \pm 0.2	0.02 \pm 0.00	0.06 \pm 0.03	0.20 \pm 0.03	0.97 \pm 0.08
	75	1.8 \pm 0.1	7.4 \pm 0.7	0.6 \pm 0.02	2.6 \pm 0.2	0.5 \pm 0.02	1.0 \pm 0.3	0.03 \pm 0.01	0.08 \pm 0.02	0.23 \pm 0.02	1.10 \pm 0.11
	100	1.7 \pm 0.1	7.3 \pm 0.8	0.4 \pm 0.01	1.8 \pm 0.1	0.4 \pm 0.01	0.9 \pm 0.06	0.04 \pm 0.01	0.09 \pm 0.04	0.22 \pm 0.01	0.85 \pm 0.07
SI-1025	0	1.2 \pm 0.1	5.0 \pm 0.4	0	0	0.6 \pm 0.02	1.3 \pm 0.4	0.01 \pm 0.00	0.01 \pm 0.00	0.10 \pm 0.02	0.42 \pm 0.08
	25	1.4 \pm 0.2	4.9 \pm 0.3	0.5 \pm 0.01	1.2 \pm 0.2	0.6 \pm 0.03	1.2 \pm 0.5	0.01 \pm 0.00	0.01 \pm 0.00	0.13 \pm 0.03	0.66 \pm 0.05
	50	1.6 \pm 0.2	4.7 \pm 0.3	0.4 \pm 0.02	1.0 \pm 0.2	0.5 \pm 0.01	1.2 \pm 0.3	0.02 \pm 0.00	0.03 \pm 0.01	0.12 \pm 0.04	0.61 \pm 0.05
	75	1.7 \pm 0.2	3.8 \pm 0.4	0.3 \pm 0.01	0.9 \pm 0.08	0.4 \pm 0.01	1.0 \pm 0.4	0.02 \pm 0.01	0.04 \pm 0.01	0.15 \pm 0.05	0.56 \pm 0.06
	100	1.3 \pm 0.1	3.0 \pm 0.5	0.1 \pm 0.01	0.7 \pm 0.06	0.3 \pm 0.01	0.9 \pm 0.03	0.03 \pm 0.01	0.04 \pm 0.01	0.15 \pm 0.01	0.58 \pm 0.08
SI-205	0	1.3 \pm 0.1	4.7 \pm 0.4	0	0	0.5 \pm 0.02	1.1 \pm 0.3	0.01 \pm 0.00	0.01 \pm 0.00	0.11 \pm 0.02	0.45 \pm 0.04
	25	1.5 \pm 0.2	4.6 \pm 0.3	0.5 \pm 0.01	1.3 \pm 0.2	0.5 \pm 0.02	1.0 \pm 0.2	0.01 \pm 0.00	0.01 \pm 0.00	0.12 \pm 0.02	0.51 \pm 0.05
	50	1.6 \pm 0.2	4.2 \pm 0.5	0.4 \pm 0.02	1.1 \pm 0.1	0.4 \pm 0.01	1.1 \pm 0.1	0.02 \pm 0.01	0.03 \pm 0.01	0.13 \pm 0.03	0.53 \pm 0.06
	75	1.2 \pm 0.1	3.8 \pm 0.3	0.2 \pm 0.01	0.6 \pm 0.04	0.3 \pm 0.01	1.1 \pm 0.2	0.02 \pm 0.00	0.04 \pm 0.02	0.13 \pm 0.05	0.44 \pm 0.07
	100	1.0 \pm 0.1	3.2 \pm 0.2	0.1 \pm 0.01	0.4 \pm 0.03	0.2 \pm 0.01	0.8 \pm 0.05	0.03 \pm 0.01	0.04 \pm 0.01	0.15 \pm 0.04	0.40 \pm 0.04
KM-13	0	1.9 \pm 0.2	5.7 \pm 0.5	0	0	0.3 \pm 0.01	1.7 \pm 0.5	0.01 \pm 0.00	0.01 \pm 0.00	0.12 \pm 0.02	0.24 \pm 0.03
	25	2.0 \pm 0.2	6.3 \pm 0.6	0.5 \pm 0.01	1.3 \pm 0.3	0.4 \pm 0.01	1.6 \pm 0.4	0.01 \pm 0.00	0.01 \pm 0.00	0.13 \pm 0.01	0.52 \pm 0.06
	50	2.3 \pm 0.2	6.6 \pm 0.4	0.8 \pm 0.02	1.6 \pm 0.2	0.5 \pm 0.02	1.6 \pm 0.3	0.02 \pm 0.01	0.03 \pm 0.01	0.14 \pm 0.04	0.53 \pm 0.05
	75	2.1 \pm 0.1	6.2 \pm 0.3	1.0 \pm 0.01	1.8 \pm 0.4	0.4 \pm 0.02	0.9 \pm 0.02	0.03 \pm 0.01	0.05 \pm 0.01	0.13 \pm 0.03	0.82 \pm 0.07
	100	1.6 \pm 0.2	5.8 \pm 0.5	0.7 \pm 0.02	1.5 \pm 0.2	0.6 \pm 0.03	0.8 \pm 0.05	0.02 \pm 0.00	0.08 \pm 0.02	0.14 \pm 0.02	0.50 \pm 0.06
IS-607-1-84	0	1.6 \pm 0.3	4.6 \pm 0.4	0	0	0.5 \pm 0.02	1.2 \pm 0.2	0.01 \pm 0.00	0.01 \pm 0.00	0.11 \pm 0.01	0.45 \pm 0.04
	25	1.8 \pm 0.2	4.8 \pm 0.3	0.3 \pm 0.01	1.6 \pm 0.3	0.4 \pm 0.01	1.0 \pm 0.3	0.01 \pm 0.00	0.02 \pm 0.01	0.13 \pm 0.02	0.71 \pm 0.06
	50	2.0 \pm 0.3	5.0 \pm 0.4	0.7 \pm 0.02	1.3 \pm 0.4	0.3 \pm 0.01	0.8 \pm 0.03	0.03 \pm 0.00	0.03 \pm 0.01	0.14 \pm 0.03	0.82 \pm 0.08
	75	2.0 \pm 0.2	5.1 \pm 0.3	0.8 \pm 0.01	1.0 \pm 0.2	0.4 \pm 0.01	0.8 \pm 0.02	0.04 \pm 0.01	0.04 \pm 0.02	0.15 \pm 0.06	0.82 \pm 0.05
	100	1.4 \pm 0.1	4.9 \pm 0.6	0.6 \pm 0.02	0.8 \pm 0.03	0.3 \pm 0.01	0.6 \pm 0.02	0.05 \pm 0.02	0.06 \pm 0.03	0.15 \pm 0.04	0.74 \pm 0.03
SI-1926	0	2.5 \pm 0.2	6.3 \pm 0.7	0	0	0.4 \pm 0.02	1.4 \pm 0.4	0.01 \pm 0.00	0.01 \pm 0.00	0.12 \pm 0.02	0.42 \pm 0.04
	25	2.8 \pm 0.3	6.6 \pm 0.6	0.6 \pm 0.02	1.6 \pm 0.3	0.3 \pm 0.01	1.0 \pm 0.6	0.01 \pm 0.00	0.01 \pm 0.01	0.13 \pm 0.04	0.71 \pm 0.07
	50	2.4 \pm 0.2	6.8 \pm 0.5	0.8 \pm 0.03	1.3 \pm 0.2	0.7 \pm 0.03	1.2 \pm 0.5	0.01 \pm 0.00	0.03 \pm 0.02	0.15 \pm 0.06	0.81 \pm 0.06
	75	2.5 \pm 0.2	7.0 \pm 0.4	0.7 \pm 0.02	1.4 \pm 0.2	0.8 \pm 0.04	1.0 \pm 0.2	0.02 \pm 0.01	0.04 \pm 0.01	0.14 \pm 0.05	0.92 \pm 0.07
	100	2.2 \pm 0.3	5.6 \pm 0.6	0.5 \pm 0.02	1.2 \pm 0.1	0.8 \pm 0.03	0.9 \pm 0.05	0.03 \pm 0.01	0.06 \pm 0.02	0.13 \pm 0.03	0.63 \pm 0.08
ENT-78-301	0	1.9 \pm 0.1	4.6 \pm 0.4	0	0	0.6 \pm 0.02	1.3 \pm 0.2	0.01 \pm 0.00	0.01 \pm 0.00	0.11 \pm 0.01	0.51 \pm 0.05
	25	2.1 \pm 0.2	5.1 \pm 0.3	0.3 \pm 0.01	1.4 \pm 0.4	0.4 \pm 0.01	1.2 \pm 0.3	0.01 \pm 0.00	0.02 \pm 0.01	0.12 \pm 0.03	0.81 \pm 0.05
	50	2.3 \pm 0.3	5.5 \pm 0.6	0.4 \pm 0.02	1.2 \pm 0.3	0.2 \pm 0.01	1.3 \pm 0.2	0.02 \pm 0.01	0.02 \pm 0.02	0.14 \pm 0.02	0.61 \pm 0.06
	75	2.6 \pm 0.4	5.7 \pm 0.5	0.3 \pm 0.01	1.3 \pm 0.5	0.1 \pm 0.01	1.0 \pm 0.1	0.02 \pm 0.01	0.03 \pm 0.01	0.13 \pm 0.04	0.73 \pm 0.03
	100	1.6 \pm 0.2	4.2 \pm 0.4	0.2 \pm 0.01	1.0 \pm 0.2	0.1 \pm 0.01	0.9 \pm 0.05	0.04 \pm 0.01	0.01 \pm 0.00	0.12 \pm 0.04	0.56 \pm 0.05

A positive relationship between accumulation of proline and stress tolerance was found in *Cichorium intybus* [1], different cultivars of sesame [33] and tomato [34]. The ion content of the shoot and root depended on the genotype and the concentrations of NaCl in the medium. Na^+ and Cl^- content were significantly higher in root in each genotype with increasing NaCl application (Table 5). The tolerant genotypes (i.e. Vars. Kanaka, Parchi, KM-13, SI-1926) having high accumulation of Na^+ content as well as Cl^- as

compared with medium tolerant (i.e. S-0140, ENT-78-301, IS-607-1-84) and low tolerant (i.e. SI-1025, SI-205, SI-2/38-2) genotypes. Salt stress enhanced Na^+ content in shoot which resulted in decrease of K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratio in tolerant genotypes and also increase in medium and low tolerant genotypes (Figs.1A-J). Na^+ and Cl^- were also limits cell elongation and cell differentiation which may lead to the reduction of plant growth, root and shoot lengths in higher concentrations of NaCl.

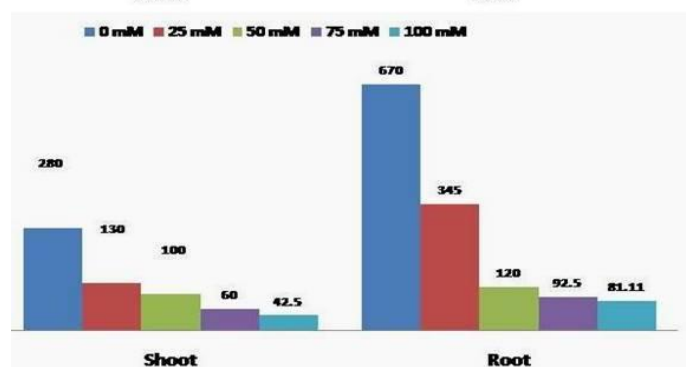
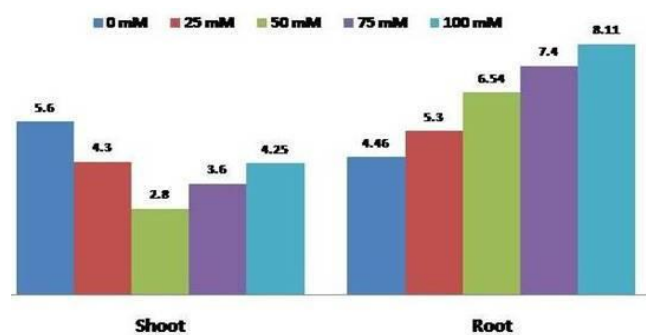


Fig. 1A:

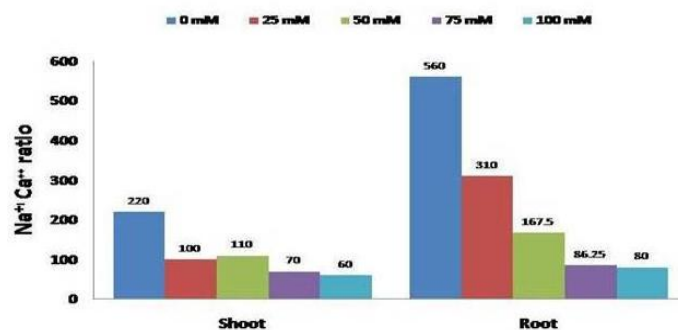
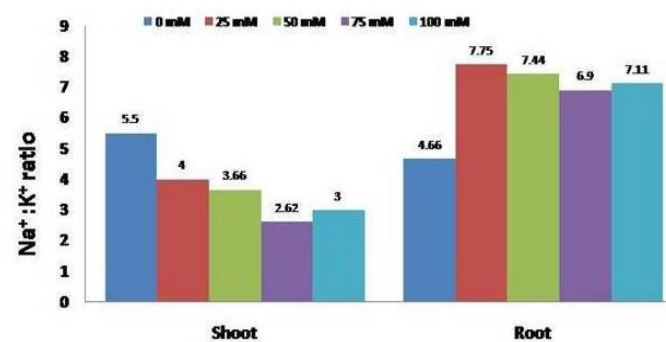


Fig. 1B:

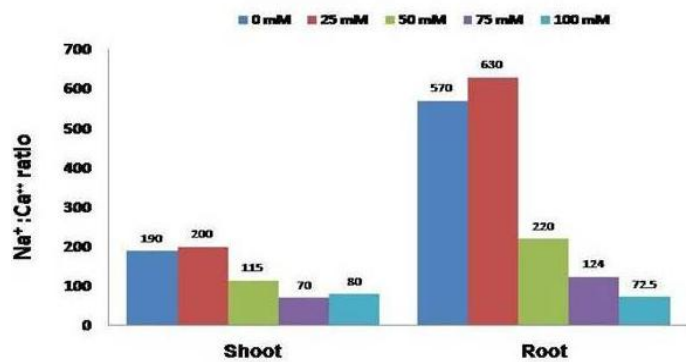
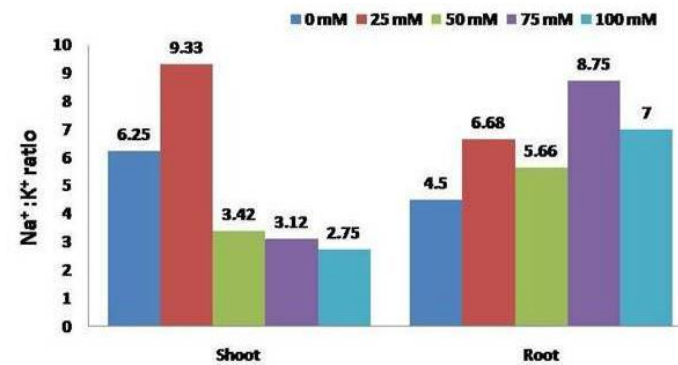
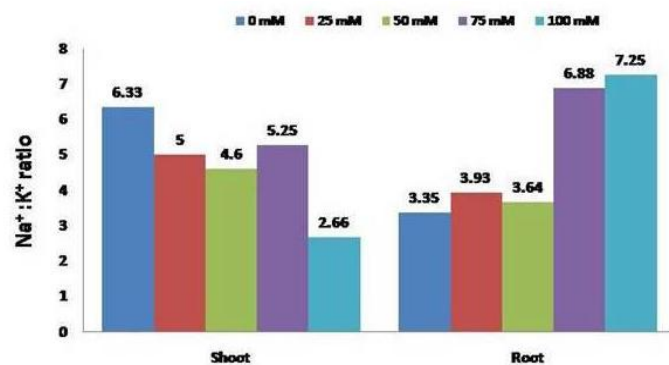


Fig. 1C:

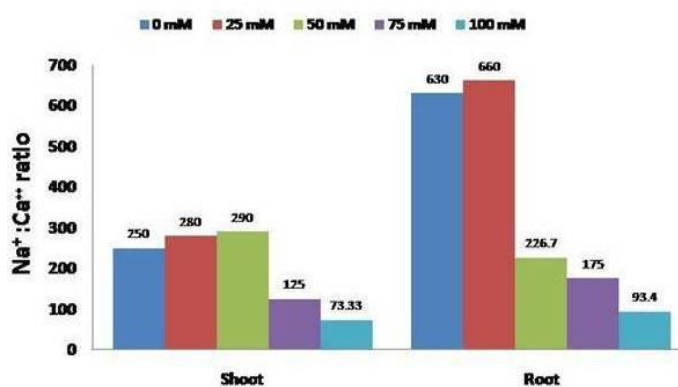


Fig. 1D:



Fig. 1E:

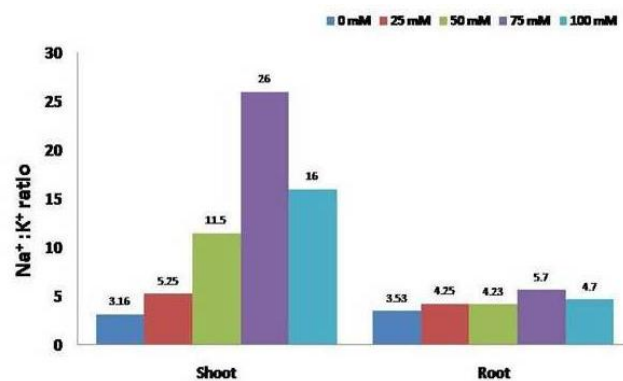


Fig. 1F:

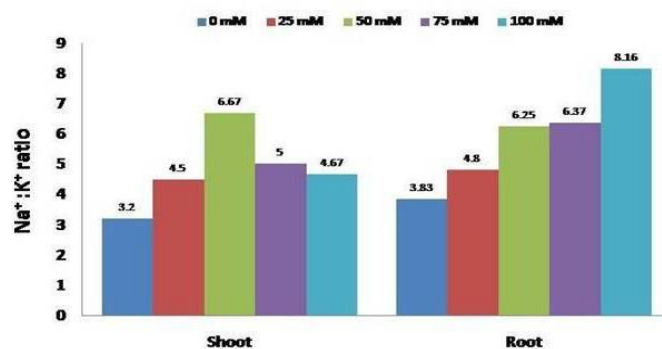
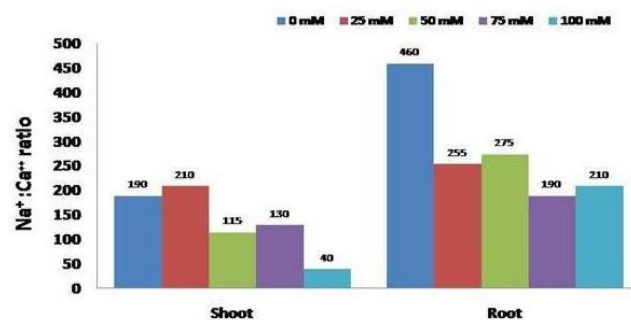
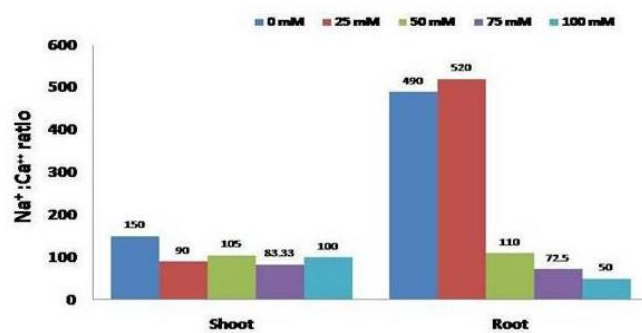


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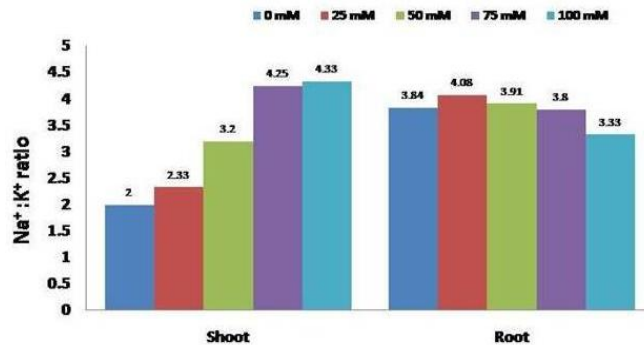
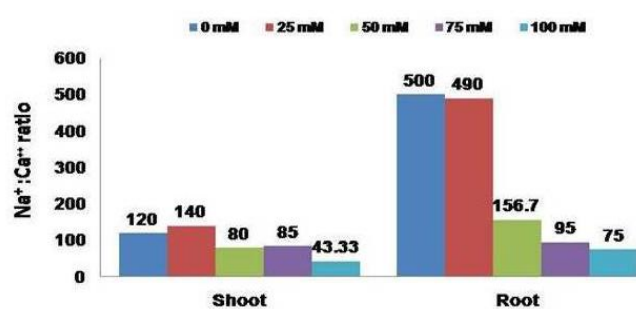
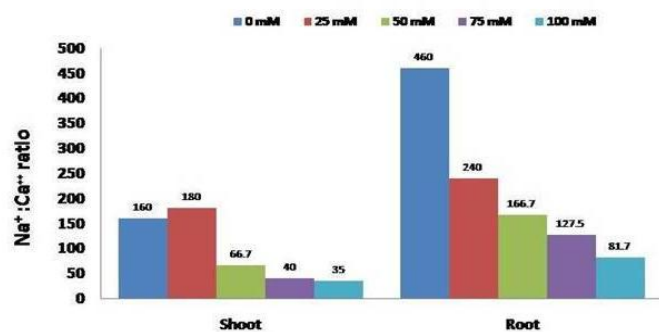


Fig. 1H:



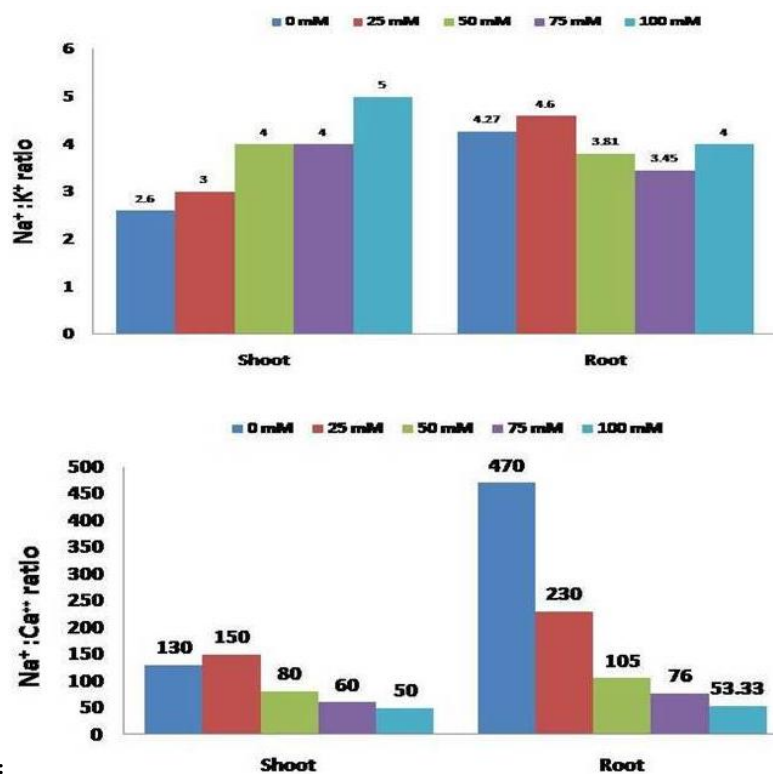


Fig. 1I:

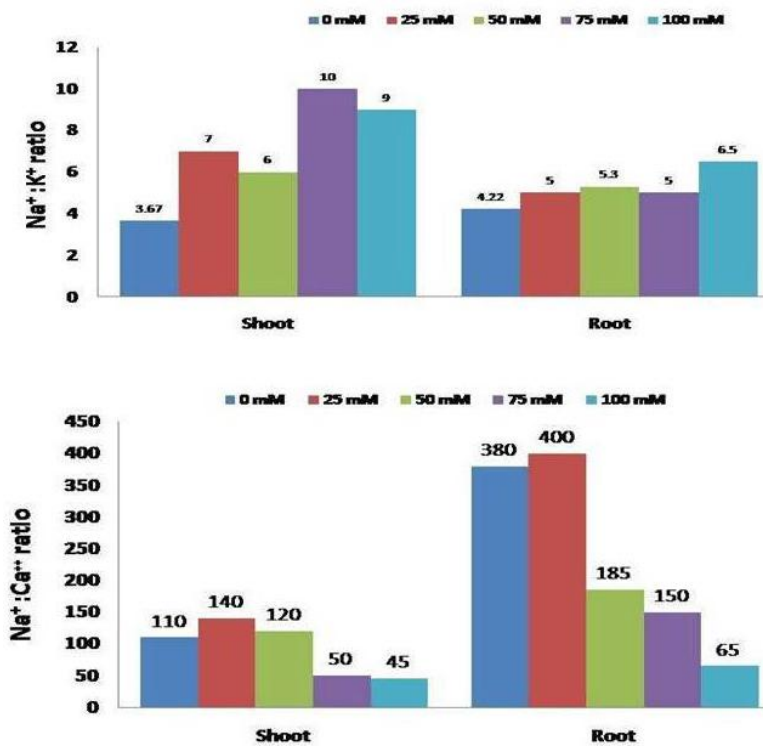


Fig. 1J:

Fig 1: Ion ratios in different genotypes of *Sesamum* after 15 days of application with different concentrations of NaCl. A: Kanaka, B: Parchi, C: KM-13, D: SI-1926, E: S-0140, F: ENT-78-301, G: IS-607-1-84, H:SI-1025, I: SI-205, J: SI-2138-2.

The present study showed a significant observation on Na^+ content in shoot and root. The tolerant genotypes maintained higher K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios which indicate of salt tolerance. Salinity also has a great impact on the nutritional status of the plant. Nutrient regulation was a vital process which was very closely linked with the salt tolerance potential. Our results indicate that the salt stress elevates the Na^+ ion content in shoots and roots while suppresses the content of cations K^+ and Ca^{2+} [35]. Both K^+ and Ca^{2+} were key ions necessary for various physiological mechanisms under saline conditions. Khayat *et al* [36] reported that the salt tolerance potential was highly associated with the concentrations of inorganic osmolytes (Na^+ , K^+ , Ca^{2+}) which can be used as screening tools for salinity tolerance. The genotypes showed higher Na^+ and Cl^- content with increasing NaCl concentrations, it indicate that the genotypes may have used the ions (Na^+ and Cl^-) to adjust its osmotic potential [37]. The growth of low and medium tolerant genotypes were reduced at higher NaCl levels may be partly due to excessive toxic ions in the cytoplasm and losing high energy through the accumulation of these ions in the vacuole. Marcum *et al* [38] reported that the high Na^+/K^+ ratio can disturb various enzymatic processes in the cytoplasm. Salt tolerant plants respond to elevated Na^+ content by maintaining low cytosolic Na^+ concentrations with high cytosolic K^+/Na^+ ratios through the extrusion or intracellular compartmentalization [39].

There was a significant difference in enzymes [Superoxide dismutase (SOD), Peroxidase (POX) and Glutathione reductase (GSH)] activities in plants grown in different NaCl treatment (Tables 6 – 8). The genotypes ‘SI-1926’ and ‘S-0140’ exhibited high activity of SOD as compared to other genotypes (Table 6).

Table 6: Superoxide dismutase (SOD) activity (U/mg protein) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl concentration (mM)	Superoxide dismutase (U/mg protein) (Mean±SE) +		
		15 days of treatment	30 days of treatment	45 days of treatment
SI-2138-2	0	65.0±1.2 i	88.3±1.4 m	52.6±1.4 f
	25	85.0±2.8 m	50.2±1.5 e	24.4±1.2 b
	50	86.0±2.5 m	35.4±1.2 c	*
	75	88.3±2.6 m	25.0±1.6 b	*
	100	106.6±3.4 p	18.3±0.8 a	*
S-0140	0	83.3±1.8 m	91.6±1.5 m	51.4±1.6 f
	25	90.6±2.1 n	52.2±1.8 e	28.5±1.3 c
	50	93.6±2.5 n	58.6±1.6 g	*
	75	95.0±2.0 o	66.7±1.4 h	*
	100	99.3±2.4 p	58.3±1.3 g	*
Prachi	0	58.3±1.8 h	64.6±1.5 h	65.0±2.6 i
	25	66.6±2.5 i	52.4±1.8 e	25.0±1.2 b
	50	75.0±2.7 k	56.0±1.4 f	*
	75	58.3±1.9 h	72.7±1.9 i	*
	100	79.3±2.5 l	75.5±1.3 j	*
Kanaka	0	25.0±1.9 b	58.3±1.4 g	58.3±1.5 h
	25	38.3±3.7 d	334.6±1.6 c	22.3±1.8 a
	50	66.6±2.6 i	36.7±1.1 c	*

SI-1025	75	58.3±3.2 h	35.3±1.2 c	*
	100	69.3±3.4 j	16.3±1.2 a	*
	0	25.0±1.8 b	58.3±1.4 g	33.3±1.2 d
	25	28.3±1.2 b	68.3±1.7 i	41.6±1.6 e
	50	33.3±1.4 c	62.7±1.5 g	*
SI-205	75	51.0±1.5 f	34.3±1.2 c	*
	100	55.0±2.8 g	17.8±1.4 a	*
	0	66.6±2.5 i	88.4±1.6 m	38.3±1.8 e
	25	73.3±2.9 k	54.6±1.4 f	66.6±2.2 j
	50	81.6±2.7 l	41.6±1.7 d	*
KM-13	75	58.3±2.6 h	42.4±1.4 d	*
	100	25.6±1.2 b	25.2±1.2 b	*
	0	30.6±1.8 c	51.6±0.6 e	28.7±2.7 c
	25	26.7±1.5 b	36.3±1.8 c	51.4±2.3 f
	50	25.0±1.6 b	62.4±1.2 g	*
IS-607-1-84	75	48.3±1.7 f	25.0±0.9 b	*
	100	58.4±2.6 h	19.6±1.0 a	*
	0	46.7±2.4 e	68.2±1.7 i	26.6±1.4 b
	25	55.0±1.3 g	53.2±1.0 f	41.7±1.5 e
	50	75.6±2.4 k	38.3±1.6 d	*
SI-1926	75	38.3±1.7 d	28.4±1.1 b	*
	100	25.0±1.1 b	18.3±0.9 a	*
	0	91.6±1.5 n	28.8±0.6 b	32.4±2.7 d
	25	66.6±2.7 i	36.7±1.1 c	56.0±2.8 g
	50	41.6±1.4 d	41.7±1.6 d	*
ENT-78-301	75	28.6±1.6 c	67.5±1.2 h	*
	100	20.7±1.5 a	68.3±2.1 i	*
	0	21.3±1.2 a	83.3±1.6 l	52.4±2.8 f
	25	26.8±1.5 b	58.3±1.5 g	22.3±1.6 a
	50	30.7±1.7 c	48.3±1.3 e	*
	75	38.3±1.2 d	34.3±1.1 c	*
	100	58.3±1.8 h	23.6±0.7 b	*

*Plant did not survived, +10 replicates / treatment; repeated twice. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

The maximum activity of SOD was recorded in ‘SI-2138-2’ (106.66 % of inhibition) at 100 mM NaCl after 15 days of application. POX activity increases with increase of NaCl concentration in all genotypes grown under 15 & 30 days (Table 7).

Table 7: Peroxidase (POX) activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl concentration (mM)	Peroxidase ($\mu\text{mol}/\text{min}/\text{mg}$ protein) (Mean±SE) +		
		15 days of treatment	30 days of treatment	45 days of treatment
SI-2138-2	0	0.45±0.08 c	1.21±0.4 b	2.14±0.6 b
	25	0.39±0.05 b	1.88±0.6 d	3.29±0.4 c
	50	0.44±0.02 b	2.82±0.4 f	*
	75	0.49±0.04 c	2.87±0.6 f	*
	100	0.91±0.05 g	2.92±0.7 f	*
S-0140	0	0.56±0.08 c	0.79±0.04 a	2.36±0.3 b
	25	0.30±0.03 a	0.72±0.02 a	1.61±0.2 a
	50	0.47±0.06 c	0.83±0.09 a	*
	75	0.83±0.04 f	0.99±0.06 a	*
	100	1.05±0.4 h	1.78±0.4 d	*
Prachi	0	0.35±0.08 a	0.81±0.06 a	1.98±0.4 b
	25	0.38±0.02 b	0.95±0.03 a	2.04±0.3 b
	50	0.48±0.04 c	0.99±0.06 a	*
	75	0.73±0.09 e	1.04±0.5 b	*
	100	0.85±0.05 f	1.86±0.7 d	*
Kanaka	0	0.31±0.04 a	1.44±0.3 c	3.77±0.2
	25	0.33±0.03 a	2.03±0.6 d	2.24±0.8 b

SI-1025	50	0.47±0.06 c	2.26±0.2 e	*
	75	0.78±0.08 f	2.86±0.8 f	*
	100	1.56±0.4 j	3.97±0.4 h	*
	0	0.51±0.03 c	2.44±0.9 e	2.24±0.5 b
	25	0.89±0.09 g	2.97±0.4 f	2.82±0.4 c
SI-205	50	1.13±0.5 h	3.28±0.7 g	*
	75	1.32±0.7 i	0.81±0.02 a	*
	100	1.94±0.4 l	3.76±0.8 g	*
	0	0.29±0.07 a	1.76±0.7 d	2.94±0.5 c
	25	0.38±0.05 b	2.08±0.6 e	1.42±0.6 a
KM-13	50	0.64±0.08 d	2.69±0.4 f	*
	75	0.84±0.02 f	3.27±0.6 g	*
	100	1.29±0.06 i	3.56±0.8 g	*
	0	0.28±0.07 a	1.86±0.9 d	2.34±0.2 b
	25	0.46±0.09 c	2.79±0.4 f	3.05±0.7 c
IS-607-1-84	50	0.62±0.05 d	1.51±0.5 c	*
	75	0.77±0.03 f	1.49±0.9 c	*
	100	0.92±0.08 g	2.70±0.2 f	*
	0	0.37±0.02 b	0.84±0.07 a	1.54±0.5 a
	25	0.38±0.05 b	1.73±0.3 d	1.83±0.3 b
SI-1926	50	0.49±0.04 c	1.87±0.8 d	*
	75	1.01±0.06 h	2.15±0.5 e	*
	100	0.77±0.02 f	2.72±0.6 f	*
	0	0.63±0.05 d	3.07±0.2 f	2.12±0.9 b
	25	0.72±0.09 e	2.01±0.5 d	2.02±0.4 b
ENT-78-301	50	0.85±0.05 f	1.93±0.8 d	*
	75	1.24±0.4 i	1.42±0.5 c	*
	100	1.47±0.8 j	0.97±0.07 a	*
	0	0.81±0.06 f	2.24±0.4 e	1.27±0.5 a
	25	0.84±0.07 f	2.35±0.5 c	1.45±0.4 a
	50	0.92±0.03 g	2.96±0.9 g	*
	75	1.04±0.6 h	3.22±0.8 g	*
	100	1.73±0.5 k	3.56±0.6 h	*

*Plant did not survive +10 replicates / treatment; repeated twice. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

Table 8: Glutathione peroxidase (PXR) activity (Unit / mg protein) of ten genotypes of *Sesamum indicum* L. grown in soil with application of different concentrations of NaCl.

Genotypes	NaCl concentration (mM)	Glutathione peroxidase (Unit / mg protein) (Mean±SE) +		
		After 15 days of treatment	After 30 days of treatment	After 45 days of treatment
SI-2138-2	0	0.18±0.05 a	0.74±0.04 b	2.75±0.8 b
	25	0.50±0.06 b	1.55±0.3 e	1.64±0.6 a
	50	0.59±0.03 b	4.00±0.8 h	*
	75	1.24±0.4 c	5.56±0.7 i	*
	100	0.47±0.02 b	7.09±0.4 k	*
S-0140	0	1.94±0.6 e	5.16±0.8 i	3.51±0.5 c
	25	1.89±0.5 e	5.36±0.7 i	6.73±0.8 e
	50	1.52±0.7 d	6.53±0.9 j	*
	75	0.70±0.06 b,c	5.01±0.6 i	*
	100	0.57±0.08 b	1.07±0.4 d	*
Prachi	0	0.32±0.09 a,b	1.47±0.7 e	4.00±0.2 c
	25	0.52±0.06 c	1.21±0.4 d	5.16±0.7 d
	50	1.89±0.7 e	1.36±0.3 d,e	*
	75	0.70±0.04 b,c	0.85±0.08 c	*
	100	0.47±0.06 b	0.63±0.07 b	*
Kanaka	0	1.89±0.5 e	5.20±0.6 i	10.00±0.8 g
	25	0.32±0.08 a	6.74±0.9 j	10.53±1.2 g
	50	0.57±0.09 b	8.91±0.4 l	*
	75	0.79±0.05 b,c	4.10±0.8 h	*
	100	0.52±0.07 b	1.34±0.3 d,e	*
SI-1025	0	0.49±0.04 b	0.74±0.07 b	5.76±0.5 d
	25	0.73±0.03 b,c	8.07±0.4 l	8.07±0.7 f

SI-205	50	1.52±0.5 d	6.33±0.6 b	*
	75	0.60±0.06 b	2.67±0.2 f	*
	100	0.54±0.04 b	0.74±0.06 b	*
	0	3.27±1.2 g	4.74±0.9 h	6.74±0.5 e
	25	2.81±0.8 f,g	1.63±0.5 e	5.16±0.6 d
KM-13	50	2.16±0.7 e	0.88±0.04 d	*
	75	2.27±0.8 e,f	0.56±0.03 b	*
	100	1.34±0.4 c,d	0.46±0.06 b	*
	0	4.00±0.9 i	4.74±0.2 b	8.07±0.5 f
	25	3.65±0.5 h	2.99±0.4 g	5.16±0.7 d
IS-607-1-84	50	2.67±0.6 f,g	2.67±0.4 g	*
	75	2.14±0.8 e	1.52±0.6 e	*
	100	1.56±0.5 d	0.57±0.08 b	*
	0	0.74±0.05 b,c	4.00±0.6 h	6.74±0.5 e
	25	0.49±0.05 b	2.39±0.3 f	8.26±0.8 f
SI-1926	50	0.52±0.06 b	1.67±0.6 e	*
	75	0.32±0.04 a,b	1.53±0.4 e	*
	100	0.18±0.06 a	0.68±0.02 b	*
	0	1.18±0.4 c	0.57±0.06 b	8.67±0.6 f
	25	1.54±0.6 d	0.67±0.07 b	10.0±1.1 g
ENT-78-301	50	1.67±0.7 d	0.49±0.05 a	*
	75	1.24±0.5 c	0.47±0.02 a	*
	100	1.34±0.7 c	0.45±0.03 a	*
	0	0.17±0.03 a	0.78±0.04 b	6.75±0.6 e
	25	0.40±0.09 b	0.83±0.06 c	8.54±0.9 f
	50	1.10±0.8 c	0.69±0.07 b	*
	75	1.52±0.7 d	0.54±0.06 b	*
	100	0.56±0.03 b	0.51±0.07 b	*

*Plant did not survive, +10 replicates / treatment; repeated twice. Means having the same letter in a column were not significantly different by Duncan's multiple range test $P < 0.05$ level.

The genotype 'SI-1025' showed highest activity (1.941 $\mu\text{mol}/\text{min}/\text{mg}$ protein) at 100 mM after 15 days of treatment. The GPX activity decreased with increase of NaCl application. SOD is one of the most important antioxidant enzymes and is the first line of cellular defense against the oxidative stress [25, 32]. SOD plays an important role in modulating the relative amount of O_2^\bullet – and H_2O_2 in plants and hence, performs a key role in the defense mechanism against ROS toxicity [22].

In the present study, the higher SOD activity is found in the genotype 'SI-2138-2' (106.66 Unit per mg protein) grown at 100 mM NaCl for 15 days. It was also observed that the activity declined after 30 days of treatment.

The observed depression in SOD activity could be regarded as the lack of an ability to scavenge O_2^\bullet – radicals in prolonged stress which could lead to cellular damage and suppression of plant growth.

Many studies have found positive correlations between salt stress tolerance and the level of SOD activity in different plant species [33,40].

The major function of GSH in plants appears to be the scavenging of phospholipid hydroperoxides and thereby the protection of cell membranes from peroxidative damage [6, 41, 42]. The expression of many GPX is enhanced in response to abiotic and biotic stresses, including salinity, heavy metal toxicity and infection with bacterial or viral pathogens. This study will help in *Sesamum* improvement program.

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