



ISSN: 2348-1900

Plant Science Today

<http://horizonepublishing.com/journals/index.php/PST>



Research Article

Amelioration of salinity stress by NaCl pretreatment with reference to sugar metabolism in legumes *Cajanas cajan* L. and *Vigna mungo* L.

Paramita Chatterjee, Sabarni Biswas, and Asok K Biswas*

Department of Botany, University of Calcutta, India

Article history

Received: 16 October 2016
Accepted: 15 November 2016
Published: 01 January 2017

© Chatterjee *et al.* (2017)

Editor

K. K. Sabu

Publisher

Horizon e-Publishing Group

Corresponding Author

Asok K Biswas
✉ asokkbiswas16@gmail.com

Abstract

The effect of salinity stress and its amelioration by pretreatment with low concentration of NaCl (50mM) on growth and sugar metabolism in arhar (cv. T120) and maskalai (cv. WBU109) seedlings were studied. Salinity was found to be more toxic for root growth than shoot growth. Fresh weight and dry weight of the seedlings gradually decreased with increasing concentrations of NaCl treatment. It was demonstrated that direct germination on NaCl solution increased both reducing sugar and non-reducing sugar contents while decreased the starch contents which were to some extent decreased by pretreatment of seeds with 50mM NaCl prior to germination in salt solutions. Salinity stress also affected the activities of different sugar metabolizing enzymes. The increase in the activities of starch phosphorylase, sucrose phosphate synthase, sucrose synthase and decrease in the activities of acid invertase were observed in directly salt treated test seedlings that were altered by pretreatment with sublethal concentration of NaCl in both the cultivars arhar (cv. T120) and maskalai (cv. WBU109) seeds. Thus the application of pretreatment by sublethal concentration of NaCl in both arhar (cv. T120) and maskalai (cv. WBU109) seeds exhibited significant alteration of all the pertinent parameters tested under salinity stress and the effect of pretreatment in most of the parameters were more prominent in arhar (cv. T120) with compared to maskalai (cv. WBU109) seedlings.

Keywords

Arhar; Maskalai; NaCl; Sugar metabolism; Biochemical changes

Chatterjee, P., S. Biswas, and A. K. Biswas. 2017. Amelioration of salinity stress by NaCl pretreatment with reference to sugar metabolism in legumes *Cajanas cajan* L. and *Vigna mungo* L.. *Plant Science Today* 4(1): 28-40. <http://dx.doi.org/10.14719/pst.2017.4.1.272>

Introduction

Among all the major environmental stresses salinity is one of the most important stress affecting plant productivity and creates a problem in many areas, specially the dry and semi arid regions. Soil salinity is a complex effect causing disturbance of membrane integrity, nutrient imbalance and disturbances on general metabolic activities. Salinity exerts its undesirable effects through

osmotic inhibition and ionic toxicity. Osmotic inhibition results in the reduction of water uptake capability ability of the plant to take up water, which leads to the reduced growth rate. Salinity stress alters a wide array of metabolic processes in growing plants and induces changes in contents and activity of many enzymes (Krishnamurthy *et al.*, 1987; Richharia *et al.*, 1997). Plants usually try to cope up with this salinity stress by using different

strategies. Among these responses accumulation of compatible solutes including proline, soluble sugars, sugar alcohols and glycine betaine etc are significant. In legumes, salt stress concentrations also imposes a prominent limitation of productivity related to the adverse effects on the growth of the host plant, carbohydrate metabolism, root nodule formation as well as nitrogen fixation capacity. To provide defense of biomolecules and osmoregulation increased sugar concentration under salinity stress has been reported by many workers.

Starch is the principle carbohydrate stored in the cereal grains. Starch and sucrose are principal end products of photosynthesis (Zhou et al., 2002). Sucrose phosphate synthase (SPS, EC 2.4.1.14) catalyses the synthesis of sucrose in photosynthetic and non-photosynthetic plant tissues (Geigenberger and Stitt, 1993) and is an important control point in biosynthesis of sucrose. The last step of photosynthetic production of sucrose is catalysed by sucrose phosphate synthase (Krause et al., 1998) which converts hexose phosphates to sucrose. Prolonged water stress which limited photosynthesis, led to the loss of SPS activity as in leaves of *Phaseolus vulgaris* (Hawker et al., 1980), whereas in *Spinacea oleracea* leaves stimulation of SPS activity was observed (Quick et al., 1989). Two major enzymes- sucrose synthase (EC 2.4.1.13) and acid invertase (EC 3.2.1.26) are involved in sucrose breakdown and plays an important role in energy metabolism by metabolizing sucrose into diverse pathways relating to metabolic function of storage cells (Ranwala and Miller, 1998). Sucrose synthase is a cytosolic enzyme which catalyzes sucrose breakdown *in vivo* (Geigenberger and Stitt, 1993). Invertase is the ubiquitous enzyme with different pH optima, acid invertase is located in the vacuoles and catalyzes hydrolysis of sucrose to glucose and fructose (Van den Ende et al., 2002). Sucrose synthase and acid invertase play an important role in phloem loading and unloading by maintaining sucrose concentration gradient (Lohaus et al., 1995). Sucrose breakdown inside the tissues is accomplished by acid invertase or sucrose synthase (Pfeiffer et al., 1996).

In leaves of glycophytes, content of soluble sugars increase under salinity (Flowers et al., 1977; Chaillou and Guerrier, 1992; Evers et al., 1997; water stress (Foyer et al., 1998), chilling stress (Guy et al., 1992). The soluble sugars along with their compatible solutes contribute to osmotic adjustment (Flowers et al., 1977), and directly modulate the expression of genes involved in various metabolic processes, storage functions and defense (Hebers and Sonnewald, 1998).

To evaluate meaningful physiological and biochemical effects of salt stress in arhar (cv. T120) and maskalai (cv. WBU109), and its reversal by sub-lethal dose of NaCl pretreatment it is desirable to examine conditions relevant to salinity stress in

a system which can grow well without interference of any added nutrients.

Material and methods

Plant materials and salt treatment

Seeds of arhar (cv. T120) and maskalai (cv. WBU109) were collected from Pulse and Oil Seed Research Institute Beherampore West Bengal India. Seeds were surface sterilized with 5% sodium hypochlorite solution for 10 minutes and washed thoroughly in sterile distilled water. Nearly 25 seeds were placed on each sterilized glass plate lined by blotting papers with 3 replicas containing suitable concentration of test solutions. Different concentrations of NaCl was added in required proportion to hydroponic solution (where hydroponic solution was treated as control) to prepare the salt solutions of concentrations 50mM, 100mM and 150mM. The hydroponic solution was composed of 2mM Ca(NO₃)₂, 5mM KNO₃, 2mM MgSO₄, 0.1mM KH₂PO₄, 5mM NH₄NO₃, 0.5mM Na₂SiO₃, 0.05mM NaFe(III)EDTA, 5μM ZnSO₄, 0.5μM CuSO₄, 5μM MnCl₂, 5μM H₃BO₃ and 0.1μM NaMoO₃ (Widodo et.al., 2009). The plates containing seeds (non-pretreated) were kept at 27-30°C under the influence of 16h photoperiod at 200μmolm⁻²s⁻¹ photon irradiance for 3 weeks. The possible reversal of salt toxicity was determined by treating the seeds with 50mM of NaCl (pretreated) prior to salt treatment in salt solutions. Experiments were performed from the seedlings raised from non pretreated and pretreated seeds to characterize the toxic effects of sodium chloride and its possible reversal on growth and sugar metabolism of test seedlings.

Morphological studies

The root and shoot lengths of 21 days old seedlings raised from non pretreated and pretreated seeds were measured. The fresh weight and dry weight of those seedlings were also measured. The seedlings were harvested, weighed in equal amount for each set and stored at -40°C for further biochemical studies.

Estimation of reducing and non-reducing sugar

Estimation of reducing sugar was done by the method of Miller (1972). 1g each of root and shoot samples were crushed in 10 ml of 80% ethanol, centrifuged at 2000 rpm for 20 minutes and the supernatants were collected. To 1.0 ml of alcoholic extract, 0.5 ml of DNSA (3, 5-dinitrosalicylic acid) reagent was added. The tubes were allowed to stay for 5 minutes in boiling water bath. The absorbances of the samples were measured at 515 nm using spectrophotometer. A standard curve for glucose was prepared. The quantity of reducing sugar present in the samples was calculated from the standard curve and expressed as mg/g fresh

Table 1. Effect of sodium chloride (NaCl) on the growth, fresh weight and dry weight of twenty one days old non-pretreated and pretreated arhar (cv. T120) and maskalai (var. W BU109) seedlings.

Treatment	<i>Cajanus cajan</i>				<i>Vigna mungo</i>			
	Seedling length(cm)		Fresh weight (g)	Dry weight (g)	Seedling length (cm)		Fresh weight (g)	Dry weight (g)
Non-pretreated	Root	Shoot			Root	Shoot		
	Control	11.82±0.02			15.58±0.3	1.40±0.04		
50mM NaCl	8.59±0.02*	13.75±0.02*	1.23±0.036*	0.136±0.003*	7.59±0.2*	12.82±0.4*	1.71±0.02*	0.126±0.002
100mM NaCl	7.6±0.30*	11.58±0.2*	1.164±0.04*	0.102±0.006*	7.0±0.07*	11.18±0.1*	1.11±0.03*	0.098±0.002*
150mM NaCl	5.77±0.07*	9.29±0.3*	1.15±0.02*	0.096±0.002*	5.74±0.04*	9.09±0.08*	1.03±0.01*	0.081±0.004*
Pretreated (50mM NaCl)	Seedling length(cm)		Fresh weight (g)	Dry weight (g)	Seedling length (cm)		Fresh weight (g)	Dry weight (g)
	Control	11.61±0.27	17.4±0.25	1.72±0.04*	0.186±0.004*	11.11±0.1	15.4±0.25	1.51±0.02*
50mM NaCl	9.51±0.20*	14.68±0.31	1.54±0.05	0.172±0.003*	8.88±0.25*	14.01±0.07	1.36±0.06	0.152±0.001
100mM NaCl	7.9±0.80*	12.73±0.3*	1.38±0.05	0.144±0.004*	7.14±0.07*	12.15±0.06*	1.27±0.02	0.12±0.002*
150mM NaCl	6.52±0.24*	10.21±0.3*	1.09±0.03*	0.09±0.005*	6.15±0.07*	9.14±0.07*	1.09±0.02*	0.092±0.003*

Values are mean ± SE of 3 replicates. Figures in parenthesis are % increase (+) and decrease (-) over control. * indicates statistically significant at P ≤ 0.05.

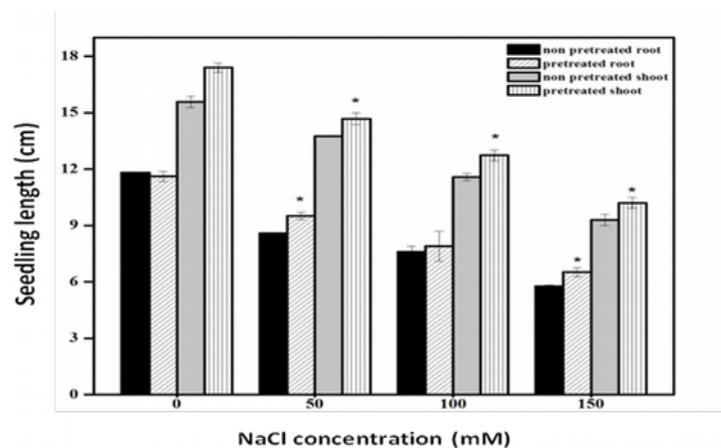
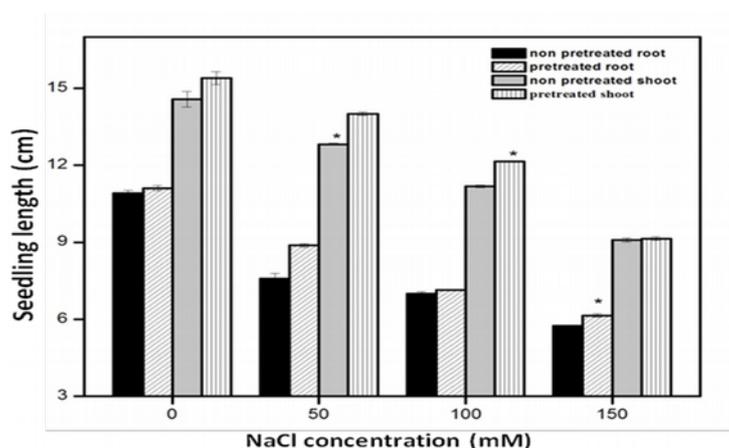
**Figure 1.** Effect of sodium chloride (NaCl) on growth of twenty one days old non-pretreated and pretreated (with 50mM NaCl) arhar (cv.T120) seedlings.**Figure 2.** Effect of sodium chloride (NaCl) on growth of twenty one days old non-pretreated and pretreated (with 50mM NaCl) maskalai (cv.WBU109) seedlings.

Table 2a. Effect of Sodium Chloride (NaCl) on the sugar and starch content of twenty one days old non-pretreated and pretreated seedlings of arhar (var. T120; *Cajanus cajan*)

Treatment	Reducing sugar (mg g ⁻¹ f.w)		Non-reducing sugar (mg g ⁻¹ f.w)		Starch (mg g ⁻¹ f.w)	
	Root	Shoot	Root	Shoot	Root	Shoot
Non-pretreated						
Control	7.63±0.33	7.63±0.32	16.9±1.05	34.36±1.88	1.95±0.03	1.96±0.03
50mM NaCl	8.88±0.3*	7.86±0.2	45.2±2.7*	36.9±1.4	1.63±0.065*	1.72±0.06*
100mM NaCl	9.44±0.28	8.07±0.07	51.1±2.5*	38.6±2.5	1.54±0.03*	1.53±0.035*
150mM NaCl	9.88±0.31	8.1±0.0.6	65.8±2.93*	41.5±2.02	1.43±0.035*	1.44±0.07*
Pretreated (50mM NaCl)						
Control	7.44±0.27	6.59±0.22	17.5±0.76	40.41±1.97	1.85±0.06	1.86±0.035
50mM NaCl	7.44±0.24*	6.64±0.18	36.3±0.9*	27.9±1.76	1.69±0.035*	1.78±0.035*
100mM NaCl	8.68±0.16*	7.61±0.11	43.1±0.95	25.4±1.76*	1.78±0.047*	1.68±0.035*
150mM NaCl	9.62±0.42*	7.91±0.05	49.4±2.34*	39.2±2.003	1.58±0.05*	1.64±0.03*

Values are mean ± SE of 3 replicates. Figures in parenthesis are % increase (+) and decrease (-) over control. * indicates statistically significant at P ≤ 0.05.

Table 2b. Effect of Sodium Chloride (NaCl) on the sugar and starch content of twenty one days old non -pretreated and pretreated seedlings maskalai (var. WBU109; *Vigna mungo*)

Treatment	Reducing sugar (mg g ⁻¹ f.w)		Non-reducing sugar (mg g ⁻¹ f.w)		Starch (mg g ⁻¹ f.w)	
	Root	Shoot	Root	Shoot	Root	Shoot
Non-pretreated						
Control	7.95±0.25	7.1±0.23	33.1±0.64*	35.2±0.61	1.82±0.035	1.76±0.04
50mM NaCl	9.00±0.76*	7.24±0.19*	38.16±0.66*	35.9±0.35	1.49±0.04*	1.52±0.04*
100mM NaCl	9.37±0.32*	7.83±0.12*	45.42±0.84*	37.14±1.2	1.46±0.03*	1.36±0.02*
150mM NaCl	10.02±0.55	7.92±0.22*	60.61±0.75*	41.34±0.72*	1.43±0.03*	1.21±0.03*
Pretreated (50mM NaCl)						
Control	6.92±0.37	6.14±0.03	26.9±1.06*	34.02±1.00	1.77±0.035	1.69±0.02
50mM NaCl	8.86±0.18	7.09±0.11	36.2±0.67*	35.62±1.17	1.58±0.04*	1.56±0.046*
100mM NaCl	9.24±0.13*	7.21±0.11	41.1±0.91*	36.29±1.6	1.62±0.01*	1.41±0.02*
150mM NaCl	9.83±0.22*	7.68±0.08	51.14±1.5*	40.5±0.76*	1.56±0.02*	1.48±0.015*

Values are mean ± SE of 3 replicates. Figures in parenthesis are % increase (+) and decrease (-) over control. * indicates statistically significant at P ≤ 0.05.

weight. The amount of non-reducing sugar was measured by subtracting the value of reducing sugar from the value of total soluble sugar and expressed as mg/g fresh weight.

Estimation of starch

Estimation of starch was performed according to

McCready et.al., (1950). The residual mass, obtained after centrifugation for the extraction of total soluble sugar, was suspended in 2.5 ml of distilled water followed by the addition of 3.25 ml of 52% perchloric acid. The mixture was centrifuged at 2000 rpm for 20 minutes and supernatant was collected which was filtered through Whatman filter paper (No.42). 1.0 ml of

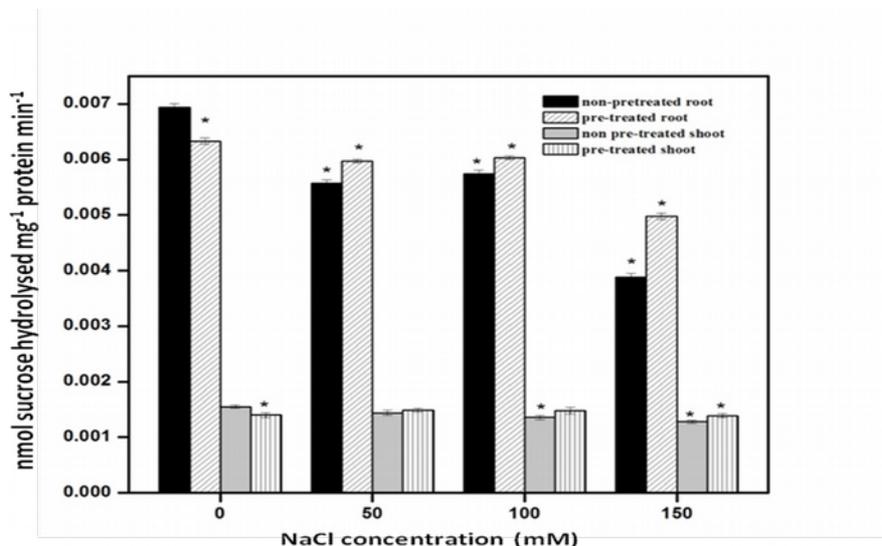


Figure 3. Effect of sodium chloride (NaCl) on acid invertase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) arhar (cv.T120) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

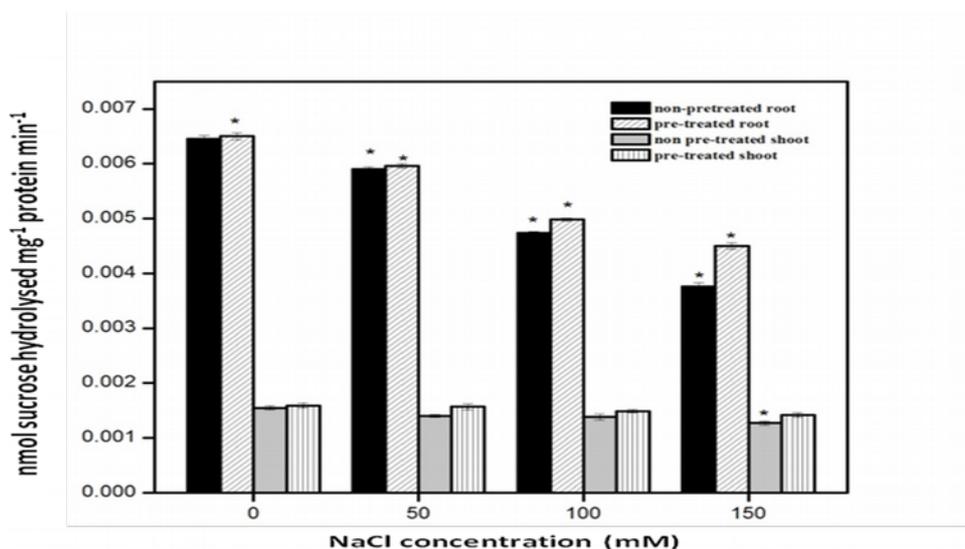


Figure 4. Effect of sodium chloride (NaCl) on acid invertase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) maskalai (cv. WBU109) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

filtrate from every set was taken and analyzed for starch content following the same procedure as the total soluble sugar. Quantity of starch was calculated in terms of glucose and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed in mg /g of fresh weight of tissue.

Assay of acid invertase activity (EC 3.2.1.26)

Acid invertase was assayed according to Borkowska and Szczerba (1991). Root and shoot samples of 1g each were homogenized in 10 ml of 10mM sodium acetate buffer (pH 4.6) containing 3.3mM $MgCl_2$, 1mM EDTA, and 1mM PMSF (phenylmethane sulfonyl fluoride). The homogenates were centrifuged at 10,000 rpm and 4°C for 20 minutes and supernatants were collected. The assay mixture contained 0.2 ml

10mM sodium acetate buffer (pH 4.6), 0.4 ml 0.4 M sucrose, and 0.4 ml enzyme and incubated at 30°C for 30 minutes. The reaction was terminated with the addition of 0.5 M Na_2HPO_4 . The resulting reducing sugars were estimated by Nelson-Somogyi method (Nelson, 1944; Somogyi, 1952). To the reaction mixture 1.0 ml DNSA (3,5-dinitrosalicylic acid) reagent was added. The tubes were kept in boiling water bath for 5 minutes immediately followed by cooling under running tap water. The content of the tubes were diluted to 10 ml by adding distilled water. The color intensity was measured at 560 nm using a spectrophotometer. Invertase activity was expressed as the nmol of reducing sugar produced by hydrolysis of sucrose $min^{-1} mg^{-1}$ of protein sample.

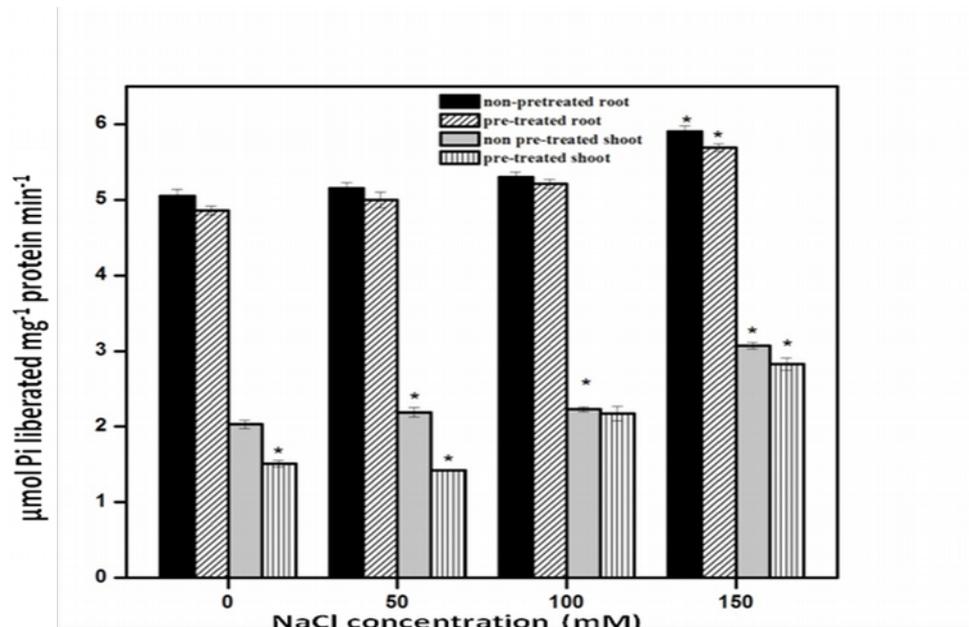


Figure 5. Effect of sodium chloride (NaCl) on Starch Phosphorylase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) arhar (cv. T120) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

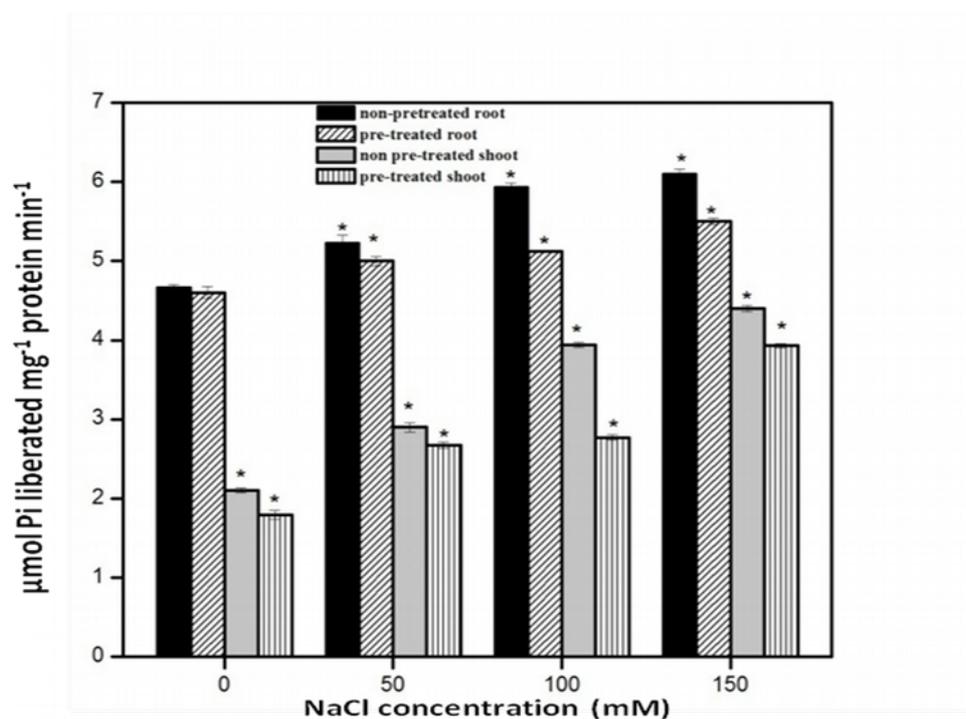


Figure 6. Effect of sodium chloride (NaCl) on Starch Phosphorylase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) maskalai (cv. WBU109) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

Assay of starch phosphorylase activity (EC 2.4.1.1)

For determination of starch phosphorylase activity 1g each of root sample and shoot samples obtained from each set were homogenized in 10 ml 50 mM citrate buffer (pH 6.0) containing 1mM EDTA, 5mM β -mercaptoethanol and 1mM PMSF. After centrifugation at 10000 rpm for 20 minutes at 4°C, supernatants were collected for enzyme assay. The assay mixture contained 2.0 ml 50 mM citrate

buffer (pH 6.0), 0.5 ml 5% soluble starch (m/v), 1.0 ml 0.1mM glucose-1-phosphate and 0.5 ml enzyme extract. The reaction was initiated with the addition of glucose-1-phosphate and was stopped after 15 minutes by adding 1.0 ml 5% TCA. The content was centrifuged and from the supernatant phosphorus was estimated following the method of Fiske and Subarow (1925). The specific activity of the enzyme was calculated as μmol of Pi liberated min^{-1} mg^{-1} of protein sample.

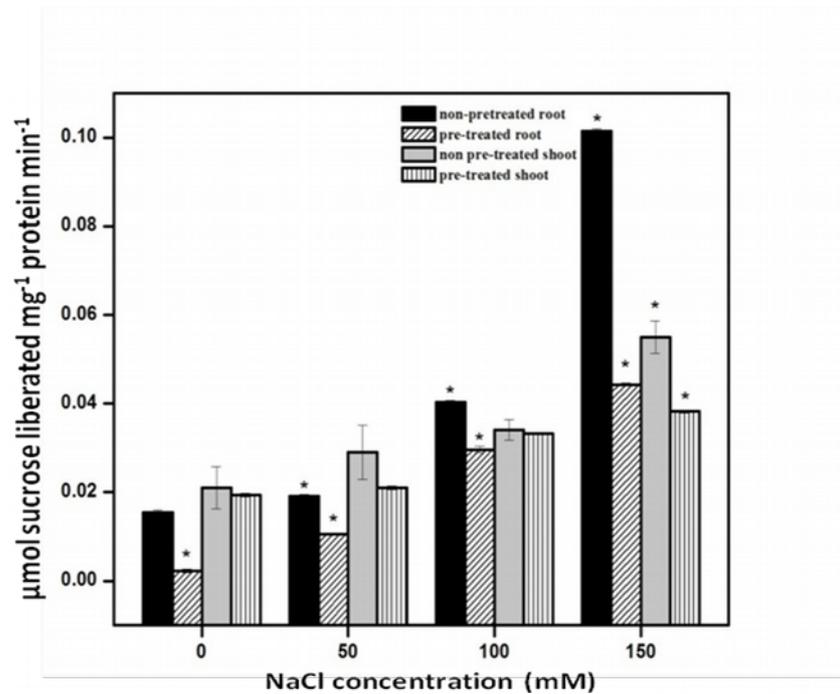


Figure 7. Effect of sodium chloride (NaCl) on Sucrose Synthase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) arhar (cv.T120) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

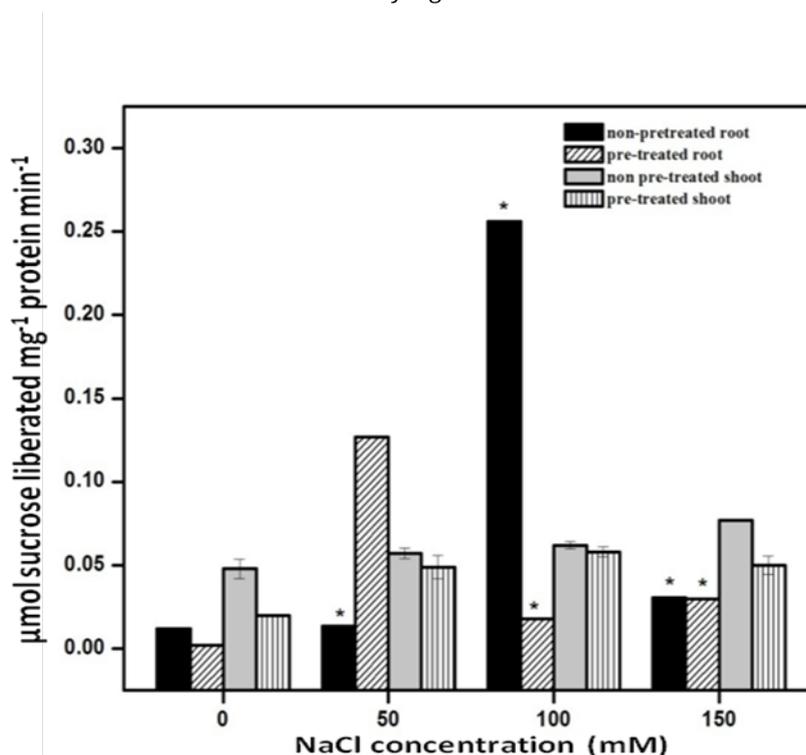


Figure 8. Effect of sodium chloride (NaCl) on Sucrose Synthase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) maskalai (cv.WBU109) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

Assay of sucrose synthase (SS) (EC 2.4.1.13) and sucrose phosphate synthase (SPS) (EC 2.4.1.14) activity

Sucrose phosphate synthase and sucrose synthase were extracted from the test samples following the method of Hubbard et.al. (1989). Samples of each set were extracted in 5.0 ml of 50 mM HEPES-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1mM EDTA, 2.5 mM DTT and 0.05 % (v/v) Triton X-

100, at 4°C. The homogenates were centrifuged at 10000 rpm and 4°C for 10 minutes. SPS and SS ACTIVITY were assayed according to the method of Miron and Schaffer (1991). After incubation at 30°C for 30 minutes, the reaction was stopped by adding 70 μ l of 30% KOH. The hexoses were destroyed by placing the tubes in boiling water bath for 10 minutes. After cooling, 140 μ l of DNSA reagent was added in each reaction tube. The reaction tubes

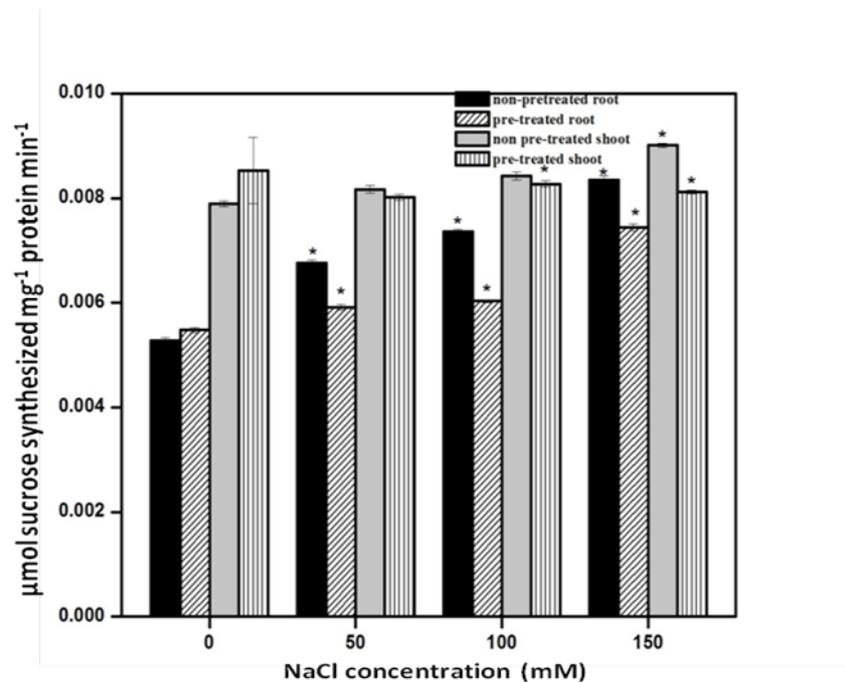


Figure 9. Effect of sodium chloride (NaCl) on Sucrose Phosphate Synthase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) arhar (cv.T120) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

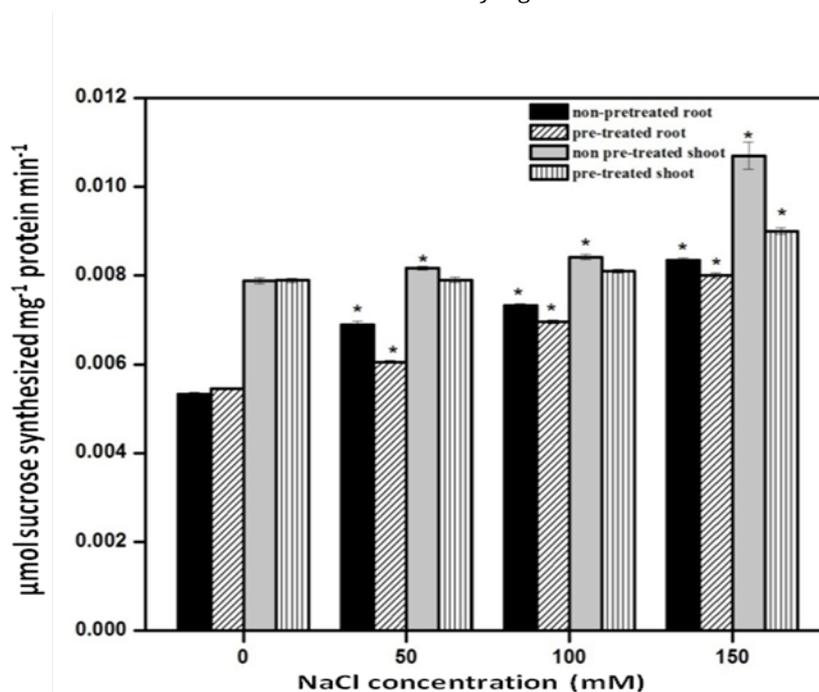


Figure 10. Effect of sodium chloride (NaCl) on Sucrose Phosphate Synthase activity of twenty one days old non-pretreated and pretreated (with 50mM NaCl) maskalai (cv.WBU109) seedlings. Each data point is the mean of 3 replicates \pm SE. *indicates statistically significant at $P \leq 0.05$.

were again boiled in the water bath for 5 minutes and cooled subsequently. The volume of each reaction tube was made 1ml. The amount reducing sugar formed, was measured by taking the value of absorbance at 515 nm. Specific activities of the enzymes were calculated as nmol of sucrose formed $s^{-1} mg^{-1}$ of protein sample.

Statistical analysis

The experiments were carried out in a completely randomized design (CRD) with three replicated;

each replication comprised a single plate containing of 25 seeds. The data and significant values were compared by descriptive statistics (\pm SE).

Results

Effect of NaCl on seedling growth and fresh and dry weight

The effect of different concentrations of NaCl on the reduction of root and shoot growth was significant in 21 days old seedlings of *Cajanas*

cajan cv. T120 and *Vigna mungo* cv. WBU109 (Table 1, Figure 1 and Figure 2) The effect was more prominent in root rather than shoot. The maximum reduction in root and shoot growth was observed in 150mM NaCl treatment in both legume cultivars. The rate of reduction in growth was about 27%, 36%, 51% in root; and 12%, 26% and 40% in shoot under 50mM, 100mM and 150mM NaCl treatments respectively of *Cajanas cajan* var. T120; about 31%, 36%, 47% in root and about 12%, 23%, 38% in shoot under same concentrations of NaCl treatment respectively in case of *Vigna mungo* var. WBU109 in respect to non pretreated control.

On the other hand seeds pretreated with sublethal (50mM) concentration of NaCl exhibited partial overcome of inhibition of seedling growth in comparison to respect of non pretreated control. After pretreatment the reduction in root growth was slowed down on an average to about 33% in root and 20% in shoot in *Cajanas cajan* var. T120, where as in *Vigna mungo* var. WBU109 after pretreatment the rate of reduction was checked to on an average about 19% and 24% in shoot and root respectively with respect to non pretreated control.

The effect of NaCl was also significant in the fresh and dry weight of both *Cajanas cajan* and *Vigna mungo* var. WBU109 seedlings (Table 1). Due to salinity stress the reduction in fresh weight and dry weight of *Cajanas cajan* var. T120 seedlings were on an average about 15% and 30% respectively. In case of *Vigna mungo* var. WBU109 seedlings the rate of reductions on an average were about 23% and 26% in fresh weight and dry weight respectively. Pretreatment of *Cajanas cajan* var. T120 and *Vigna mungo* var. WBU109 seeds with 50mM NaCl prior to germination in different concentrations of NaCl, appreciably narrowed down the rate of reduction in fresh weight on an average about 4% and 5% in *Cajanas cajan* var. T120 and *Vigna mungo* respectively. Reduction in dry weight in both *Cajanas cajan* var. T120 and *Vigna mungo* var. WBU109 seedlings was narrowed down by about 12% and 13% respectively over non-pretreated control.

Effect of NaCl on the starch content

In both root and shoot of *Cajanas cajan* var. T120 and *Vigna mungo* var. WBU109 seedlings the amount of starch contents was found to be decreased under NaCl stress compared to (Table 2a and Table 2b). The salt treatment on an average showed decrease in starch contents to about 21% and 20%, in root and shoot respectively of arhar (cv. T120) seedlings. Whereas, the decrement in starch contents in maskalai (cv. WBU109) seedling on an average was about 20% in root and 23% in shoot over non pretreated control. This effect was recovered and the decrement was narrowed down to about 14% in root and 13% in shoot of arhar (cv. T120) seedlings and 13% in root and 16% in shoot

of maskalai (cv. WBU109) seedlings by the application of treatment, by the application of pretreatment of seeds with 50mM NaCl solution.

Effect of NaCl on the reducing sugar content

In both root and shoot of arhar (cv. T120) and maskalai (cv. WBU109) seedlings, the amount of reducing sugar contents found to be increased by NaCl treatment than that of control (Table 2a and Table 2b). The NaCl treatment showed, increase in reducing sugar contents in arhar (cv. T120) seedlings on an average to about 23% and 5% in root and shoot respectively. Whereas the reducing sugar contents in maskalai (cv. WBU109) seedlings increased about 19% in root and 10% in shoot over non pretreated control. This effect was recovered and narrowed down to about 14% in root and 3% in shoot of arhar (cv. T120) seedlings and 17% in root and 3% in shoot of maskalai (cv. WBU109) seedlings compared to non pretreated control, by the application of pretreatment of seeds with 50mM NaCl solution.

Effect of NaCl on the non-reducing sugar content

The amount of non-reducing sugar in root and shoot of arhar (cv. T120) and maskalai (cv. WBU109) seedlings were found to be increased by NaCl treatment compared to control (Table 2a and Table 2b). In non-pretreated arhar (cv. T120) seedlings the increments in non reducing sugar contents were about 167%, 202% and 289% in root and 7%, 12% and 20% in shoot under 50mM, 100mM and 150mM NaCl treatment respectively. Whereas, in maskalai (cv. WBU109) seedlings the increments were about 15%, 37% and 83% in root and 2%, 5% and 17% in shoot under 50mM, 100mM and 150mM NaCl treatment respectively. On the other hand the pretreatment with 50mM NaCl solution narrowed down the non reducing sugar contents to about 14%, 155%, 192% in root and 18%, 26% and 14% in shoot of arhar (cv. T120) seedlings under 50mM, 100mM and 150mM NaCl pretreated sets respectively. In case of maskalai (cv. WBU109) seedlings it was narrowed to about 9%, 24% and 55% in root and 1%, 3% and 15% in shoot of maskalai (cv. WBU109) seedlings in 50mM, 100mM and 150mM NaCl treatment respectively over non pretreated control.

Effect of NaCl on Sucrose degrading enzyme activities

Sucrose synthase (EC 2.4.1.13)

The activity of sucrose synthase (SS) increased in root and shoot of both of the test seedlings (Figure 7 and Figure 8). In roots of arhar (cv. T120) seedlings, about 24%, 161% and 558% increase in enzyme activity was recorded at 50 mM, 100 mM and 150 mM NaCl treatments respectively while in shoots, the SS activity was increased by about 38%, 61% and 161% over control at the same levels of

NaCl treatment. While, in roots of maskalai (cv. WBU109) seedlings, about 11%, 111% and 152% increase in enzyme activity was recorded at 50 mM, 100 mM and 150 mM NaCl treatments respectively while in shoots, the activity of the said enzyme was increased by about 18%, 29% and 60% over non pretreated control at the same levels of NaCl treatment. On the contrary, pretreatment of seeds with 50 mM NaCl solution followed by germination in 50 mM, 100 mM and 150 mM NaCl lowered down the increase in enzyme activity to about 32%, 92% and 188% in roots and by about 4%, 58% and 81% in shoots respectively of arhar (cv. T120) seedlings. Whereas in maskalai (cv. WBU109) seedlings after pretreatment the increase in enzyme activity was lowered to about 4%, 47% and 146% in roots and 3%, 21% and 4% in shoots in 50 mM, 100 mM and 150 mM NaCl solution respectively.

Acid invertase (EC 3.2.1.26)

In both root and shoot, the activity of acid invertase was found to be decrease by NaCl treatment over control in both the test seedlings (Figure 3 and Figure 4). The NaCl treatment showed, decrease in enzyme activity in arhar (cv. T120) seedlings to about 22% and 12% on an average in root and shoot respectively. Whereas the rate of decrements in enzyme activity in maskalai (cv. WBU109) seedling were about 26% and 13% in root and shoot respectively on an average over non pretreated control. The decrements of enzyme activity were recovered and narrowed down in arhar (cv. T120) seedlings to about 18% and 7% in root and shoot respectively. Whereas, in maskalai (cv. WBU109) seedlings the decrements of enzyme activity were narrowed down to about 20% and 3% in root and shoot respectively with compared to non pretreated control, by the application of pretreatment of seeds with 50mM NaCl solution.

Effect of NaCl on Sucrose synthesizing enzyme activities

Sucrose phosphate synthase (EC 2.4.1.14)

The activity of sucrose phosphate synthase was found to be increased in both root and shoot on NaCl exposure in twenty-one days old arhar (cv. T120) and maskalai (cv. WBU109) seedlings (Figure 9 and Figure 10). In root and shoot of arhar (cv. T120) seedlings the increase in enzyme activity on an average was about 42% and 8% respectively compared to non pretreated control, whereas in root and shoot of maskalai (cv. WBU109) seedlings the increments in enzyme activity by NaCl pretreatment were about 41% and 16% on an average respectively with respect to non pretreated control. After the application of 50mM NaCl pretreatment the increments in the activity of the enzyme were lowered down in both root and shoot of arhar (cv. T120) seedlings on an average

to about 22% and 3% respectively with respect to non pretreated control. In case of maskalai (cv. WBU109) seedlings the increments in enzyme activity were narrowed down to an average about 31% and 6% in root and shoot respectively after pretreatment with 50mM NaCl solution.

Effect of NaCl on Starch hydrolyzing enzyme activities

Starch phosphorylase (EC 2.4.1.1)

The effect of NaCl on twenty-one days old arhar (cv. T120) seedlings showed increased activity of starch phosphorylase in both root and shoot of both the samples (Figure 5 and Figure 6). The activity was increased by 8% on an average in root due to NaCl treatment over non-pretreated control in arhar (cv. T120) seedlings. When the seedlings were pretreated with 50mM NaCl solution, the increment in enzyme activity in root was found to be lowered down around to 5% on an average. In case of maskalai (cv. WBU109) seedlings the increment of said enzyme activity was about 23% in root on an average which was found ameliorated to about 12% on an average under pretreatment with 50mM NaCl. While in shoot of arhar (cv. T120) seedlings, about 20% increase in enzyme activity was recorded under same concentrations of NaCl treatment, and was ameliorated to about 5% by pretreatment with 50mM NaCl of test seeds. The NaCl treated shoot of maskalai (cv. WBU109) seedlings the rate of increment in enzyme activity was 78% on an average while the NaCl pretreated seedlings showed least increment in enzyme activity to the extent of 49% on an average with respect to non pretreated control.

Discussion

Effect of NaCl on the growth and development

Salt treatment affected the normal growth and development of both the cultivars of legume seedlings. The rate of reduction of root growth was much more than that of shoot growth whereas on treatment with higher concentrations of NaCl the shoots became reddish in color and leaves became small. But the effect was found to be ameliorated by hardening of arhar (cv. T120) and maskalai (cv. WBU109) seeds with 50mM NaCl concentration for two hours. In pretreated seedlings, increase in shoot and root growth was observed than that of the control sets although they were under the same 50mM, 100mM and 150mM concentrations of NaCl treatment. Application of NaCl also led to the reduction of both fresh weight and dry weight. The rate of reduction of dry weight was much more than that of the fresh weight. But the toxicity effect was reduced to appreciable amount when the seeds were pretreated with 50mM concentration of NaCl followed by growing under the conc. of 50mM, 100mM and 150mM concentrations of NaCl for 21 days. Similar type of results were reported

by Misra and Dwivedi (2004) in green gram; Devi et al. (2008) in wheat seedlings and in mungbean (Saha et al., 2010) (Table 1, Figure 1 and Figure 2).

Effect of NaCl on the carbohydrate metabolism

The rate of transport of photoassimilates into sink organs is essential for the growth and development of plant organs and crop productivity. Sucrose and starch are the two major forms of photosynthetically fixed carbon in the photosynthetic cells. During photosynthesis, starch is formed as a temporary storage product and is deposited as starch granules in the chloroplast whereas sucrose is translocated to different organs and is the most commonly used photoassimilate in plants. Glucose and fructose are synthesized by photosynthesis in leaves and are then transported to roots, fruits and other sink organs where, these are utilized for intermediary and respiratory metabolism and for synthesis of complex carbohydrates like starch (Furuichi et al., 2001).

The present results indicate increase in the level of both soluble reducing sugar and non-reducing sugar in non-hardened salt treated seedlings as compared to water control. Due to hardening, the accumulation of higher level of sugars get decreased to some extent in NaCl stressed arhar (cv. T120) and maskalai (cv. WBU109) seedlings, may provide an adaptive mechanism in maintaining the favourable osmotic potential and in protecting the biomolecules and membranes. Accumulation of sugars has been associated with drought and salinity tolerant mechanisms in many plant species that helps to regulate osmotic stress in plant cells leading to protection of biomolecules and membranes (Foyer et al., 1998). Therefore, sugar accumulation leading to metabolic alterations could contribute to salt sensitivity that limits growth of the arhar (cv. T120) and maskalai (cv. WBU109) seedlings under salt stressed conditions.

A decrease in starch contents was observed in arhar (cv. T120) and maskalai (cv. WBU109) seedlings under NaCl stress. Dubey and Singh (1999) obtained the similar kind of results and concluded that the starch contents were reduced with higher magnitude in salt concentration to sensitive rice cultivars than salt tolerant cultivars under salinity stress. Starch is the principal component of dry mass accumulated in mature leaves. Although starch may not play an important role in salt-tolerance mechanism, it is suggested that the ability of plants to partition sugars into starch may help to avoid metabolic alterations by lowering feedback inhibition caused by excess amount of sucrose in cytoplasm. Decrease in starch contents and increase in reducing and non-reducing sugar content were noted in leaves of *Bruguiera parviflora* under NaCl stress (Parida et al., 2004).

Sucrose phosphate synthase (SPS) catalyses the synthesis of sucrose phosphate

during the last step of dark CO₂ fixation in photosynthetic and non-photosynthetic plant tissues and is an important control point in biosynthesis of sucrose. The activity of SPS is induced under osmotic stress (Krause et al., 1998) and salinity (Dubey et al., 1999). Sucrose contents were found to increase in tomato under salinity due to increased activity of SPS (Gao et al., 1998). The present study reveals the activity of SPS with NaCl treatment that finds support in the increase in non-reducing sugar contents like sucrose as reported earlier (Figure 9 and Figure 10). But pretreatment with 50mM NaCl is found to ameliorate the effect of NaCl on SPS activity along with the decrease of sucrose contents in both arhar (cv. T120) and maskalai (cv. WBU109) seedlings. The observed increase in sucrose contents is due to the activity of the sucrose synthesizing enzyme SPS is induced under salt stress.

Starch phosphorylase catalyzes the phosphorolytic degradation of starch by transfer of glucosyl units from glucose-1-phosphate to the non-reducing end of α -1,4-D glucan chains with the release of phosphate. The present result shows the increase in starch phosphorylase activity by NaCl treatment that correlated with the decreased starch contents in both root and shoot of the test cultivars. Similar result was reported in rice (Das et al., 2016). However, the activity of starch phosphorylase was reversed with the application of 50mM NaCl as pretreating agent. This caused decrease in starch phosphorylase activity and the level of starch contents was found to be increased (Figure 5, Figure 6, Table 2a and Table 2b). Increased activity of starch phosphorylase under salinity might help starch degradation and mobilisation of sugars.

Sucrose is the major carbohydrate imported by many plant sink tissues. Conversion of sucrose to hexoses often is the primary starting point for sink metabolism. Huber and Akazawa (1986) proposed the theory of two different pathways for sucrose degradation; one carried out by sucrose synthase (SS) and other by acid invertase. Sucrose synthase is a cytosolic enzyme that catalyzes sucrose breakdown *in vivo*. Acid invertase is located in the vacuoles and catalyzes hydrolysis of sucrose to glucose and fructose. During anoxia of rice plants, there is an increase in the activities of SS (Livingston et al, 1998). Decrease in acid invertase activity was noted in rice plants under salinity (Dubey et al., 1999; Pattanagul et al., 2008). The present results also demonstrate an increase in sucrose synthase activity and decrease in activity of acid invertase under NaCl treatment. The results also indicate that decrease in acid invertase activity does not affect the reducing sugar contents which are mainly controlled by sucrose synthase activity. (Figure 3, Figure 4, Figure 7 and Figure 8).

Conclusion

From the present study it can be concluded that, the alteration of carbohydrate metabolism and activities of some major enzymes involved in carbohydrate metabolism under salt stress may impair the growth and metabolism of the arhar (cv. T120) and maskalai (cv. WBU109) seedlings which was relevant from the morphological data as well as from the measurement of biomass. Our study demonstrates that application of NaCl in growing arhar (cv. T120) and maskalai (cv. WBU109) seedlings induced accumulation of reducing and non-reducing sugars with a concomitant increase in the activities of starch phosphorylase, sucrose phosphate synthase, sucrose synthase and decrease in the activities of acid invertase. Increased concentrations of NaCl may impair carbohydrate mechanism leading to reduced growth which may be ameliorated to some extent by pretreatment of test seeds with 50mM NaCl for two hours. Increased concentrations of NaCl led to reduced growth, development and metabolic impairment which are the very serious problem that hinders the productivity of crop plants. The pretreatment with sublethal concentration of salt solution (50mM NaCl concentration) ameliorated the damaging effect to salt toxicity and the effect of pretreatment was more effective and prominent in case of arhar (cv. T120) seedlings with compared to maskalai (cv. WBU109) in most of the considered parameters, viz. substrate contents and enzymatic activities. Therefore, the pretreatment of arhar (cv. T120) and maskalai (cv. WBU109) seeds with sublethal concentration of salt solution may improve the growth and development of test plants in saline prone soils, which is may be beneficial and cost effective in terms of productivity to farmers.

Competing Interest

The authors declare that they have no competing interests.

Authors' contributions

PC carried out the required experiments, participated in data acquisition, did statistical analysis and drafted the manuscript. SB participated in making the tables and graphs, statistical analysis and helped in drafting the manuscript. AKB conceived of the study, and participated in its design and coordination and interpretation of data. All authors read and approved the final manuscript.

Acknowledgement

The authors are grateful to the Department of Science and Technology, Government of West Bengal for financial assistance under grant No. 1012 (Sanc.)/ST/P/S&T/2G-2/2013 in completion of the work.

References

- Borkowska, B. and J. Szczerba. 1991. Influence of Different Carbon Sources on Invertase Activity and Growth of Sour Cherry (*Prunus cerasus* L.) shoot cultures. *J. Exp. Bot.* 42: 911-915. doi:10.1093/jxb/42.7.911
- Chaillou, P. B. and G. Guerrier. 1992. Salt responses in *Lycopersicon esculentum* calli and whole plants. *J. Plant Physiol.* 140: 494-501.
- Das, P., P. Seal and A. K. Biswas. 2016. Regulation of Growth, Antioxidants and Sugar Metabolism in Rice (*Oryza sativa* L.) Seedlings by NaCl and Its Reversal by Silicon. *Am. J. Plant Sci.* 7: 623-638. doi:10.4236/ajps.2016.73055
- Devi, S., R. Angrish, K. S. Datta and B. Kumar. 2008. Antioxidant defence system in wheat seedlings under sodium chloride stress: An inductive role of hydrogen peroxide. *Indian J. Plant Physiol.* 13: 22-26.
- Dubey, R. S. and A. K. Singh. 1999. Salinity Induces Accumulation of Soluble Sugars and Alters the Activity of Sugar Metabolising Enzymes in Rice Plants. *Biol. Plant.* 42: 233-239. doi:10.1023/A:1002160618700
- Evers, D., C. Schmit, Y. Mailliet and J. F. Hausman. 1997. Growth characteristics and biochemical changes of poplar shoots in vitro under sodium chloride stress. *J. Plant Physiol.* 151: 748-753.
- Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375-400.
- Flowers, T., P. F. Torke and A. R. Yeo. 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 28: 89-121.
- Foyer, C. H., M. H. Valadier, A. Migge and T. W. Becker. 1998. Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Plant Physiol.* 117: 283-292.
- Furuichi, T., I. C. Mori, K. Takahashi and S. Muto. 2001. Sugar-induced increase in cytosolic Ca²⁺ in *Arabidopsis thaliana* whole plants. *Plant Cell Physiol.* 42: 1149-1155. doi:10.1093/pcp/pce150
- Gao, Z., M. Sagi, and S. H. Lips. 1998. Carbohydrate Metabolism in Leaves and Assimilate Partitioning in Fruits of Tomato (*Lycopersicon esculentum* L.) as Affected by Salinity. *Plant Sci.* 135: 149-159. doi:10.1016/s0168-9452(98)00085-5
- Geigenberger, P. and M. Stitt. 1993. Sucrose synthase catalyses a readily reversible reaction *in vivo* in developing potato tubers and other plant tissues. *Planta.* 189: 329-339. doi:10.1007/BF00194429
- Guy, C. L., J. L. Huber and S. C. Huber. 1992. Sucrose phosphate synthase and sucrose accumulation at low temperature. *Plant Physiol.* 100: 502-508.
- Hawker, J. S. 1980. Invertases from Leaves of *Phaseolus vulgaris* Plant Grown on Nutrient Solutions Containing NaCl. *Aust J Plant Physiol.* 7: 67-72. doi:10.1071/PP9800067
- Hebers, K. and V. Sonnewald. 1998. Altered gene expression brought about by inter- and intracellularly formed hexoses and its possible implications for plant-pathogen interactions. *J. Plant Res.* 111: 323-328.
- Hubbard, N. L., S. C. Huber and D. M. Pharr. 1989. Sucrose Phosphate Synthase and Acid Invertase

- as Determinant of Sucrose Concentration in Developing Muskmelon (*Cucumis melo* L.) fruits. *Plant Physiol.* 9: 1527-1534. doi:10.1104/pp.91.4.1527
- Huber, S. C. and T. Akazawa. 1986. A Novel Sucrose Synthase Pathway for Sucrose Degradation in Cultured Sycamore Cells. *Plant Physiol.* 81: 1008-1013. doi:10.1104/pp.81.4.1008
- Krause, K. P., L. Hill, R. Reimholz, N. T. Hamborg, U. Sonnewald and M. Stitt. 1998. Sucrose metabolism in cold-stored potato tubers with decreased expression of sucrose phosphate synthase. *Plant Cell Environ.* 21: 285-299. doi:10.1046/j.1365-3040.1998.00271.x
- Krishnamurthy, R., M. Anbazhgan and K. A. Bhagwat. 1987. Salt responses of enzymes from rice cultivars differing in salt tolerance. *Curr. Sci.* 56: 489-490.
- Livingston, D. P. and C. A. Henson. 1998. Apoplastic Sugars, Fructans, Fructans Exohydrolase and Invertase in Winter Oat: Responses to Second-Phase Cold Hardening. *Plant Physiol.* 116: 403-408. doi:10.1104/pp.116.1.403
- Lohaus, G., H. Winter, B. Riens and H. W. Heldt. 1995. Further Studies of the Phloem Loading Process in Leaves of Barley and Spinach. The Comparison of Metabolite Concentrations in the Apoplastic Compartment with those in the Cytosolic Compartment and in the Sieve Tubes. *Bot. Acta.* 108: 270-275. doi:10.1111/j.1438-8677.1995.tb00860.x
- McCready, R. M., J. Guggolz, V. Silviera and H. S. Owens. 1950. Determination of Starch and Amylose in Vegetables. *Anal. Chem.* 22: 1156-1158. doi:10.1021/ac60045a016
- Miller, G. L. 1972. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars. *Anal. Chem.* 31: 426-428. doi:10.1021/ac60147a030
- Miron, D. and A. A. Schaffer. 1991. Sucrose phosphate synthase, sucrose synthase and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose accumulating *Lycopersicon hirsutum* Humb. and Bonpl. *Plant Physiol.* 95: 623-627. doi:10.1104/pp.95.2.623
- Misra, N. and U. N. Dwivedi, 2004. Genotypic difference in salinity tolerance of green gram cultivars. *Plant Sci.* 166: 1135-1142. doi:10.1016/j.plantsci.2003.11.028.
- Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153: 375-380.
- Parida, A. K., A. B. Das and P. Mohanty. 2004. Investigations on the Antioxidative Defence Responses to NaCl Stress in a Mangrove, *Bruguiera parviflora*: Differential Regulations of Isoforms of Some Antioxidative Enzymes. *Plant Growth Regul.* 42: 213-226. doi:10.1023/B:GROW.0000026508.63288.39
- Pattanagul, W. and M. Thitisaksakul. 2008. Effects of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Indian J. Exp. Biol.* 46: 736-742.
- Pfeiffer, I. and U. Kutschera. 1996. Sucrose metabolism and lipid mobilization during light induced expansion of sunflower leaves. *J. Plant Physiol.* 147: 553-558.
- Quick, W. P., G. Siegl, H. E. Neuhaus, R. Feil and M. Stitt. 1989. Short-Term Water Stress Leads to a Stimulation of Sucrose Synthesis by Activating Sucrose Phosphate Synthase. *Planta.* 177: 535-546. doi:10.1007/BF00392622
- Ranwala, A. P. and W. B. Miller. 1998. Sucrose cleaving enzymes and carbohydrate pools in *Lilium longiflorum* floral organ. *Physiol. Plant.* 103: 541-550. doi:10.1034/j.1399-3054.1998.1030413.x
- Richharia, A. P., K. Shah and R. S. Dubey. 1997. Nitrate reductase from rice seedlings: Partial purification, characterization and the effects of *in situ* and *in vitro* NaCl salinity. *J. Plant Physiol.* 151: 316-322.
- Saha, P., P. Chatterjee and A. K. Biswas. 2010. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). *Indian J. Exp. Biol.* 48: 593-600.
- Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* 195: 19-23.
- Van den Ende, W., A. Michiels, K. Le Roy and A. Van Laere. 2002. Cloning of a Vacuolar Invertase from Belgian endive leaves (*Cichorium intybus*). *Physiol. Plantarum.* 115: 504-512. doi:10.1034/j.1399-3054.2002.1150404.x
- Widodo, P. J. H., E. Newbigin, M. Tester, A. Bacic and U. Roessner. 2009. Metabolic responses to salt stress in barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J. Exp. Bot.* 60: 4089-4103. doi:10.1093/jxb/erp243.
- Zhou, R., R. C. Silcher and B. Quebedeaux. 2002. Apple Leaf Sucrose Phosphate Synthase is inhibited by Sorbitol-6- Phosphate. *Funct. Plant Biol.* 29: 569-574. doi:10.1071/PP01123

