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Changes in growth and metabolic profile of Chickpea under salt stress

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ABSTRACT

Objectives: To analyze differences in sensitivity to saline stress among kabuli and desi cultivars of chickpea.

Methodology and results: From ten genotypes of chickpea, three cultivars were screened in saline soils; two of them are kabuli (PUSA-1053, PUSA-939) and one desi types (BG-256). The seeds of the different cultivars were inoculated with Rhizobium sp. strain G-118 and the plants were grown in the greenhouse. After 12 days when symbiosis establishes, saline water of varying concentrations (0, 4, 8, 12, 16 dSm^{-1}) NaCl, Na_2SO_4, $CaCl_2$ were supplied to the seedlings. Plants were harvested at 30, 60 and 90 days after sowing (DAS) for analysis. Data were subjected to statistical analysis of variance, and the means compared by the LSD test ($p<0.05$). Results showed that plant growth, nodulation and nitrogenase activity were more severely affected in cultivar BG-256 under salinity treatments than cultivars PUSA-1053 and PUSA-939. Nodule mass and number was enhanced under salt stress in PUSA-1053 and PUSA-939. Phosphoenolpyruvate carboxylase (PEPCase) activity increased significantly in the nodules of tolerant cultivars under salt stress at all harvests, and this was clearly related to the salt treatments. Salinity reduced leaf chlorophyll and Rubisco activities in all the three cultivars.

Conclusions: Salt stress limits photosynthetic efficiency, nitrogen fixation and carbon metabolism. Alteration in the nodular metabolism seemed to be directly responsible for nitrogen fixation by the salt tolerant cultivars. The present study reveals that under saline stress tolerant cultivars are more capable of nodulation which helps to attain higher nitrogen fixation by symbiosis than the sensitive ones.

Key words: chlorophyll pigments, nitrogenase activity, nodule, PEPCase, Rubisco

INTRODUCTION

Symbiotic nitrogen fixation by legumes is sensitive to environmental stresses particularly salinity (Serraj, 2002), which can limit plant growth due to both specific ion and osmotic stress, and reduced symbiosis with bacteria (Rout & Shaw, 2001; Rao *et al*., 2002). Salt stress limits photosynthetic efficiency, nitrogen fixation and carbon metabolism (Soussi *et al*., 1999; Ferri *et al*., 2000; Mudgal, 2004). The C4 dicarboxylic acids (particularly malate and succinate) that are required for symbiotic nitrogen fixation are derived from nodule cytosolic PEPCase activity and they provide the

source of carbon and energy to the bacteroid nitrogenase for the conversion of nitrogen into ammonia. Due to reduction in the carbohydrate supply to bacteroids salinity reduces the nitrogen fixation capacity of bacteria.

Chickpea *(Cicer arietinum* L.) is one of the most important grain legumes cultivated in arid and semiarid regions of the world. The agronomical importance of chickpea is based on its high protein concentration and symbiotically fixed nitrogen with Rhizobia. Among pulse crops, Bengal gram ranks first in terms of area and production in India; it provides high quality protein to the people of developing countries. The protein content in *C. arietinum* L. is around 20% thereby the average productivity of protein is almost 125 kg/ ha. Two main types of chickpea are recognized, i.e. (a) Desi type with small and brown seed, which accounts for nearly 90%; and (b) Kabuli type with bold and cream-colored seed, which is grown in around 10% area of world production. Nearly 90% of the crop is cultivated in rain-fed lands. The average yield of chick pea in the major growing regions is around 0.5-0.7 t/ha. The realized seed yield of 850 kg/ha is a result of several abiotic and biotic stresses.

Generally the crop produces excessive vegetative growth under high input conditions and is unable to convert the biomass into high seed yield. The major abiotic constraints to production include drought, heat, cold and salinity while biotic constraints are Fusarium wilt, Ascochyta blight, Rhizoctonia dry root rot, Botrytis gray mold (BGM), chickpea stunt and Helicoverpa pod borer (HPB). Since Bengal gram is grown mostly in semi-arid regions and it is sensitive to NaCl, Na_2SO_4 and $NaHCO_3$ its growth is affected severely. The present study was undertaken to determine the impact of biotic and abiotic stress in *C. arietinum* L.

MATERIALS AND METHODS

Seeds of *C. arietinum* L were obtained from the Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttranchal, India; Chandra Shekhar Azad University of Agriculture Technology, Kanpur, India and Indian Agriculture Research Institute, Delhi, India. Seeds were inoculated with Rhizobium G-118 obtained from RBDC, Hissar, India. Inoculation was done by thoroughly mixing of soil and 5mL of 1% (w/v) gum arabic solution with 1.5 kg of seeds. To evaluate the effect of irrigating with saline water on chickpea, field experiments were conducted between years 2006-2008. The seeds were surface sterilized with 0.1% $HgCl_2$, followed by washing them 5-6 times with water to remove traces of $HgCl_2$. Plots of 1x1 m^2 area were prepared and separated from each other by polythene sheets of 0.2 mm thickness, up to depth of 30 cm to stop leaching of salts between two adjacent plots. Plant to plant distance was fixed at 15 cm and a distance of 45 cm separated the plots. The experiment was laid out in randomized block design with three replicates per treatment. Tube well water of 1 dsm^{-1} EC was used to irrigate the control plants.

Saline solutions of 4, 8, 12, 16 and 20 EC were prepared by mixing the salts NaCl, Na_2SO_4, $NaHCO_3$ and $CaCl_2$. Preliminary experiments were conducted on the effects of salt stress on growth, and salt tolerance index was calculated for ten different cultivars of chickpea. On the basis of the above screening, three cultivars, one of desi (BG-256) and two kabuli type (PUSA-1053 and PUSA-939) with differing salt sensitivities (the former being salt sensitive and latter two salts tolerant) were selected for a comprehensive study. The selected varieties of chick pea [PUSA-1053, PUSA-939 and BG-256] were grown in experimental plots. Weeding was done at 15 day intervals on a regular basis. Before sowing, the seeds were surface sterilized and followed by treatment with fungicide (chlorothalonil and mancozeb).

The first irrigation was done 20 days after sowing (DAS). For this purpose saline water of each EC level was prepared with tube well water and 50 liter of saline water was used to irrigate each plot. The quantity of water (controlled and saline) was determined by estimating the water holding capacity of the soil. Sowing was carried out in October. The second and third irrigation was carried out after 50 and 70 DAS. The control plots were irrigated on the same day with available tube well water. All recommended agronomic and cultural practices were followed to raise the crop.

The first plants were sampled 30 DAS for studying various growth parameters. Before uprooting the plants, soil was irrigated properly so that the root could be taken out fully without any damage. The saline solutions of different electrical conductivities were

prepared by mixing the salts NaCl, Na_2SO_4, $NaHCO_3$, $CaCl_2$ as described by USSL staff (1954). The electrical conductivity of different salinity levels was adjusted by direct measurements with a conductivity meter.

Salt Tolerance Index (STI) was calculated by comparing total plant (shoot + root) dry mass at different salt concentrations to the total plant dry mass obtained for the controls, as below:

STI = (TDW at S_x / TDW at S_i) x 100

Where: TDW (total dry weight); S_x (x treatment); Si (control treatment)

Dry mass (DM) was determined after drying the plant parts in an oven at 65°C for 72 h. Growth analysis was evaluated according to Benincasa (1988).

Relative growth rate (RGR) was calculated as: RGR = (ln DM2 - ln DM1) (t2 - t1)-1 (g.g-1.d-1)

Where: DM1 is the initial total (shoot + root) dry mass, DM2 is the final total dry mass, and (t2 - t1) the difference in time interval between the two samplings (30 d).

Nitrogenase (EC 1.7.99.2) activity (ARA) was measured by acetylene reduction with nodulated roots of plants according to the method of Herdina and Silsbury (1990). Ethylene produced in gas samples during the reaction was analyzed using a Perkin Elmer 8600 gas chromatograph with a Porapak R column (Ligero *et al*., 1986).

Nitrogen content was estimated by the colorimetric method of Snell and Snell (1955). Oven dry matter (powdered) was used for the analysis. A sample weighing (50mg) was digested with nitrogen free concentrated sulphuric acid (5mL) in a kjeldhal flask on sand bath for 30 minutes. The digested material was made carbon free by adding suitable quantity of hydrogen peroxide (H_2O_2). A colorless liquid was obtained. The digested material was neutralized using 20% NaOH, using methyl orange as indicator. The volume of neutralized extract was made up to 100 mL (w/v). An aliquot of this solution was developed with 3.5 ml of Nessler`s reagent.

Extraction of chlorophyll was carried out in dimethyl sulphoxide (DMSO) using leaf discs, following the method of Hiscox and Israelstam (1979). The absorbance of chlorophyll in DMSO was measured at two wavelengths, 645 and 663 nm, in a spectrophotometer against DMSO.

Rubisco assay was done in the leaves according to the procedure of Keys and Parry (1990). The samples were ground in 3 mL of 100 mM Bicine, pH 7.8 containing 10mM $MgCl_2$, 1mM EDTA, 5 mM DTT, and 2 % (w/v) PVP. The homogenate was filtered and centrifuged at 35,000 rpm for 10 min at 0°C. Rubisco activity was assayed by determining the incorporation of $^{14}CO_2$ into the acid stable product by liquid scintillation counting.

Phosphoenol Pyruvate Carboxylase (PEPCase EC 4.1.1.31) activity was assayed by the method of Christellar *et al.* (1977). The nodule extract was prepared in ice-cold medium containing 50mM Tris HCl, 10mM $MgCl_2$ and 5mM DTT with the pH adjusted to 8.0.The homogenate was centrifuged at 35,000 rpm for 20 min and the supernatant assayed according to the procedure of Maruyama *et al*. (1966). For the determination of *in vivo* PEPCase activity nodules were exposed to $^{14}CO_2$ in a plexiglass chamber according to the method of Kar *et al.* (1990).

All experimental data were subjected to statistical analysis of variance, and the means compared by the LSD test ($p<0.05$). All values are means ± SD of three sets of experiments with triplicates in each set.

RESULTS

Screening at seed level: STI and TDM: First experiment showed variability in total dry mass of the ten chickpea cultivars (Table1) in cooradination with salt tolerance index (Fig. 1). On the basis of STI and TDM, three cultivars were selected for a detailed study. Out of three, cultivarsPUSA-1053 and PUSA-939 were salt tolerant (Kabuli) while BG256 (Desi) is salt sensitive.

Growth parameters: Relative growth rate was maximum in cultivar PUSA-1053 (Kabuli tolerant), medium for cv. PUSA-939 (Kabuli sensitive) and minimum for cv. BG-256 (Desi sensitive) (Table 2).

Nodule number : Salt treatments significantly reduced nodule number per plant (NN) and nodule dry mass at all stages of growth in the three cultivars BG 256, PUSA-939 and PUSA-1053 but the reduction percentage was higher in the sensitive cultivar (Fig. 2 and 3)

Table 1: Effect of salt stress on dry mass of seedlings in ten chickpea cultivars at 12 days after sowing

S.No.	Varieties	Dry mass of seedlings

		Control	4 EC	8 EC	12 EC	16 EC
1	PUSA-1053	40.2 ± 0.45	30.5 ± 0.53	21.0 ± 0.52	18.1 ± 0.47	16.7 ± 0.44
2	PUSA-939	37.1 ± 0.51	25.2 ± 0.48	19.0 ± 0.45	18.0 ± 0.44	14.3 ± 0.57
3	PUSA-372	16.7 ± 0.46	10.3 ± 0.54	8.1 ± 0.47	7.3 ± 0.66	5.6 ± 0.62
4	BG-256	21.0 ± 0.55	7.3 ± 0.55	3.5 ± 0.56)	2.0 ± 0.33	ND*
5	KPG-59	14.1 ± 0.53	7.2 ± 0.34	6.6 ± 0.58	4.1 ± 0.37	4.3 ±0.63
6	BGD-72	17.3 ± 0.48	12.1 ± 0.52	8.3 ±0.36	2.0 ± 0.22	0.5 ± 0.02
7	PG-186	6.3 ± 0.53	3.7 ± 0.65	2.1 ± 0.21	0.7 ± 0.41	0.3 ±0.01
8	AVRODHI	3.0 ± 0.21	1.0 ± 0.06	0.7 ± 0.05	0.3 ± 0.04)	ND*
9	RADHA	2.5 ± 0.28	1.3 ± 0.35	0.8 ± 0.05	0.7 ± 0.42	0.3 ± 0.02
10	HC1	1.7 ± 0.35	0.9 ± 0.04	0.3 ± 0.02	0.2 ± 0.02	ND*

Values are shown as mean ± SD of three sets of experiments with triplicates in each set. ND*= Not detected.

Table 2: Effect of salt stress on relative growth rate (RGR) assessed in chickpea using dry weight of root (DWR) and dry weight of shoot (DWS) at 30 and 60 days after sowing (DAS).

Variety	EC of saline water (dsm^{-1})	DWR (mg)		DWS (mg)	
		30DAS	60DAS	30DAS	60DAS
PUSA-1053	Control	63.1 ± 1.56	75 ± 1.64	430.1 ± 6.44	535.2 ± 5.97
	4	60.0 ± 1.32	65.6 ± 1.45	300.0 ± 4.24	350.4 ± 4.33
	8	55.0 ± 1.13	58.0 ± 1.18	275.1 ± 2.39	320.0 ± 3.22
	12	40.1 ± 0.95	50.0 ± 1.06	210.4 ± 1.94	240.3 ± 2.39
	16	35.0 ± 0.72	45.1 ± 0.83	195.1 ± 1.83	220.4 ± 2.30
PUSA-939	Control	52.1 ± 1.04	72.0 ± 1.37	300.4 ± 4.38	400.4 ± 5.45
	4	50.0 ± 0.93	60.1 ± 1.36	260.2 ± 2.29	310.3 ± 3.31
	8	35.1 ± 0.62	40.3 ± 0.84	240.5 ± 2.34	280.3 ± 2.38
	12	27.4 ± 0.45	30.2 ± 0.46	180.7 ± 1.33	215.1 ± 2.24
	16	27.0 ± 0.35	35.0 ± 0.42	130.4 ± 1.13	160.2 ± 1.27
BG-256	Control	50.1 ± 0.94	68.4 ± 1.23	258.6 ± 2.24	320.0 ± 3.27
	4	36.3 ± 0.57	40.5 ± 0.93	242.1 ± 2.33	278.0 ± 2.39
	8	28.2 ± 0.38	45.6 ± 0.98	180.4 ± 1.25	280.0 ± 2.34
	12	20.2 ± 0.23	38.7 ± 0.68	150.0 ± 1.14	250.0 ± 2.23
	16	18.3 ± 0.21	25.1 ± 0.35	100.0 ± 1.34	220.0 ± 2.34

Values are shown as mean ± SD of three sets of experiments with triplicates in each set.

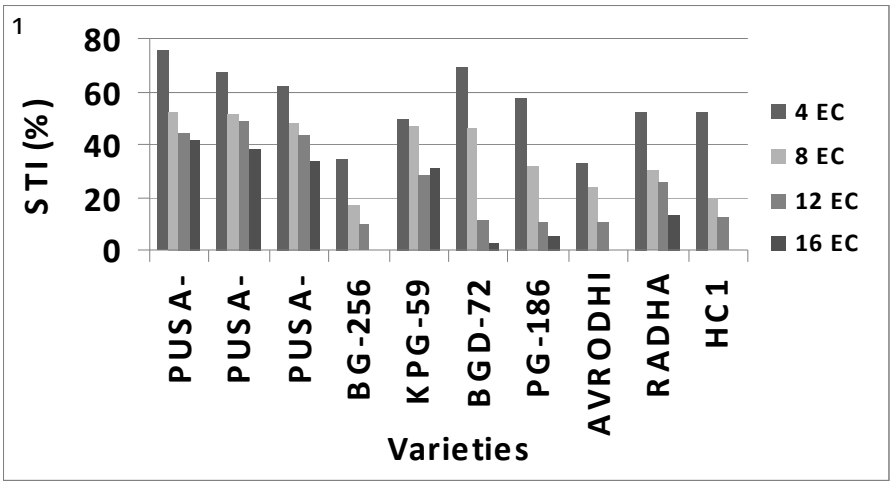

Figure 1: Salinity tolerance index of seedling at 12 Days after sowing.

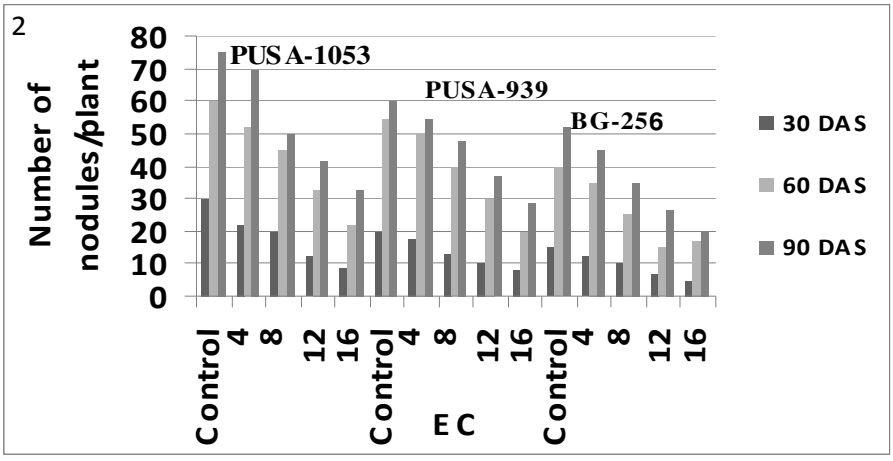

Figure 2: Effect of salinity on number of nodules/plant in three gram genotypes (DAS-days after sowing).

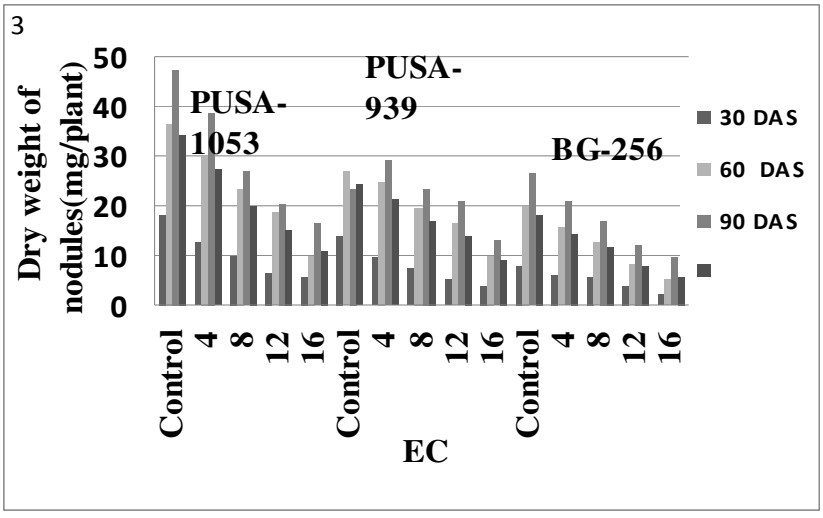

Figure 3: Effect of saline water irrigation on dry weight of nodules (data are expressed in mg/plant) (DAS-days after sowing

Nodule nitrogenase activity (ARA): Nodule nitrogenase activity (ARA) was reduced in all cultivars under different levels of salinity (Table 3) and the adverse effects increased as the duration of stress increased. Cultivars PUSA-1053 and PUSA-939 showed significantly higher nitrogenase activity compared to BG 256. The higher nitrogen fixation in the salt tolerant Desi and Kabuli chickpea cultivars resulted in higher total nitrogen content in leaves of chickpea (Table 4).

Chlorophyll contents: Leaf chlorophyll contents (chlorophyll a and b) were reduced significantly in all the chickpea cultivars as salinity increased (Fig. 4A, 4B), the decrease being greater in cultivar BG-256 (sensitive genotype).

Table 3: Effect of salt stress on nitrogenase activity (ARA) μ mole ethylene/mg nodule dry wt. /hr.) in three cultivars of chickpea.

Variety	EC of saline water (dsm^{-1})	30-DAS	60-DAS	90-DAS
PUSA-1053 (Kabuli tolerant)	Control	0.104.± 0.004	0.125 ± 0.003	0.083 ± 0.005
	4	0.103 ± 0.003	0.122 ± 0.004	0.075 ± 0.005
	8	0.098 ± 0.005	0.112 ± 0.005	0.073 ± 0.003
	12	0.093 ± 0.004	0.109 ± 0.004	0.067 ± 0.005
	16	0.081 ± 0.005	0.103 ± 0.005	0.060 ± 0.003
PUSA-939 (Kabuli sensitive)	Control	0.075 ± 0.005	0.088 ± 0.004	0.060 ± 0.003
	4	0.063 ± 0.003	0.069 ± 0.005	0.045 ± 0.003
	8	0.058 ± 0.003	0.065 ± 0.004	0.035 ± 0.002
	12	0.054 ± 0.005	0.058 ± 0.005	0.030 ± 0.002
	16	0.051 ± 0.003	0.053 ± 0.005	0.027 ± 0.002
BG-256 (Desi sensitive)	Control	0.051 ± 0.004	0.066 ± 0.003	0.043 ± 0.003
	4	0.047 ± 0.002	0.057 ± 0.004	0.040 ± 0.003
	8	0.035 ± 0.003	0.048 ± 0.003	0.030 ± 0.002
	12	0.032 ± 0.004	0.040 ± 0.002	0.025 ± 0.002
	16	0.030 ± 0.003	0.038 ± 0.002	0.022 ± 0.003

Values are shown as mean ± SD of three sets of experiments with triplicates in each set.

Table 4: Effect of salt stress on total nitrogen (mg/100 mg dry weight) in leaves of three chickpea cultivars.

Variety	EC of saline water (dsm^{-1})	Days after sowing		
		30-DAS	60-DAS	90-DAS
PUSA-1053 (Kabuli tolerant)	Control	11.3 ± 0.24	13.0 ± 0.29	15.3 ± 0.34
	4	10.2 ± 0.20	12.2 ± 0.25	14.0 ± 0.30
	8	9.0 ± 0.19	11.0 ± 0.23	13.3 ± 0.31
	12	8.5 ± 0.18	10.5 ± 0.21	12.0 ± 0.26
	16	5.5 ± 0.14	7.5 ± 0.16	10.5 ± 0.23
PUSA-939 (Kabuli sensitive)	Control	9.5 ± 0.19	11.7 ± 0.25	13.0 ± 0.29
	4	7.3 ± 0.16	9.3 ± 0.20	12.0 ± 0.27
	8	5.5 ± 0.15	7.5 ± 0.17	10.5 ± 0.24
	12	4.0 ± 0.13	6.5 ± 0.15	9.3 ± 0.18
	16	3.5 ± 0.12	5.2 ± 0.14	7.0 ± 0.16
BG-256 (Desi sensitive)	Control	7.5 ± 0.43	10.0 ± 0.21	12.0 ± 0.25
	4	5.3 ± 0.14	8.0 ± 0.17	11.5 ± 0.23
	8	4.2 ± 0.13	7.2 ± 0.15	9.0 ± 0.18
	12	3.4 ± 0.11	6.3 ± 0.15	8.5 ± 0.17
	16	2.1 ± 0.09	5.0 ± 0.13	6.0 ± 0.13

Values are shown as mean ± SD of three sets of experiments with triplicates in each set.

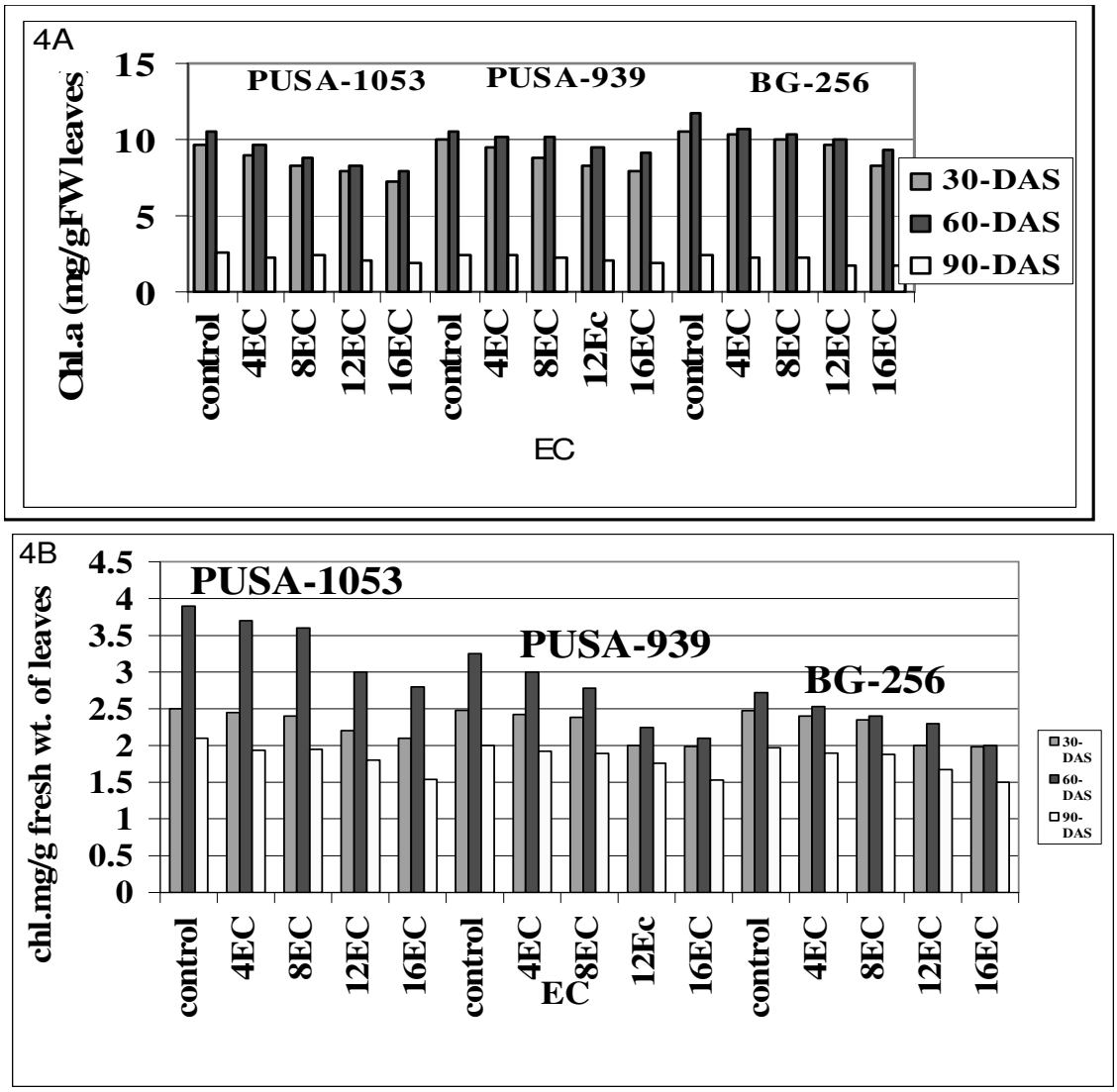

Figure 4: Variation in Chlorophyll a [4A] and Chlorophyll b [4B] content in three cultivars of chickpea at various growth stages under salt stress (DAS-days after sowing).

Table 5: Effect of salt stress on Rubisco activity (ARA µ mole CO_2/mg nodule dry wt. / hr.) in Kabuli and desi cultivars of chickpea.

Variety	EC of saline water (dsm^{-1})	Days after sowing		
		30-DAS	60-DAS	90-DAS
PUSA-1053 (Kabuli tolerant)	control	4.25 ± 0.04	4.55 ± 0.05	4.00 ± 0.03
	4	4.16 ± 0.05	4.39 ± 0.04	3.87 ± 0.03
	8	4.00 ± 0.03	4.25 ± 0.03	3.65 ± 0.04
	12	3.85 ± 0.04	4.18 ± 0.04	3.49 ± 0.04
	16	3.82 ± 0.05	4.08 ± 0.05	3.35 ± 0.03
PUSA-939 (Kabuli sensitive)	control	2.75 ± 0.02	3.25 ± 0.03	2.25 ± 0.02
	4	2.43 ± 0.02	3.15 ± 0.03	2.00 ± 0.02
	8	2.00 ± 0.01	2.55 ± 0.04	1.36 ± 0.03
	12	1.82 ± 0.03	2.00 ± 0.04	1.00 ± 0.02
	16	1.40 ± 0.02	1.45 ± 0.03	0.75 ± 0.002
BG-256	control	2.65 ± 0.04	2.88 ± 0.05	1.97 ± 0.03

(Desi sensitive)	4	2.13 ± 0.02	2.22 ± 0.03	1.32 ± 0.04
	8	1.91 ± 0.02	1.85 ± 0.04	1.01 ± 0.03
	12	1.63 ± 0.02	1.45 ± 0.03	0.77 ± 0.003
	16	1.44 ± 0.02	1.35 ± 0.03	0.43 ± 0.002

Values are shown as mean ± SD of three sets of experiments with triplicates in each set.

Rubisco activity: In the leaves of chickpea the Rubisco activity was also reduced by saline stress (Table 5). The magnitude of Rubisco suppression varied with salt concentration and cultivar.

PEPCase activity: Nodular carbon dioxide fixation was closely related to its nitrogen fixation capacity. The present study showed that PEPCase activity was increased in response to salt stress, the degree of stimulus being concentration dependent. However, in the sensitive cultivar BG-256 the PEPCase activity increased under lower saline concentrations but declined with higher salt dosages (Fig. 5A, 5B).

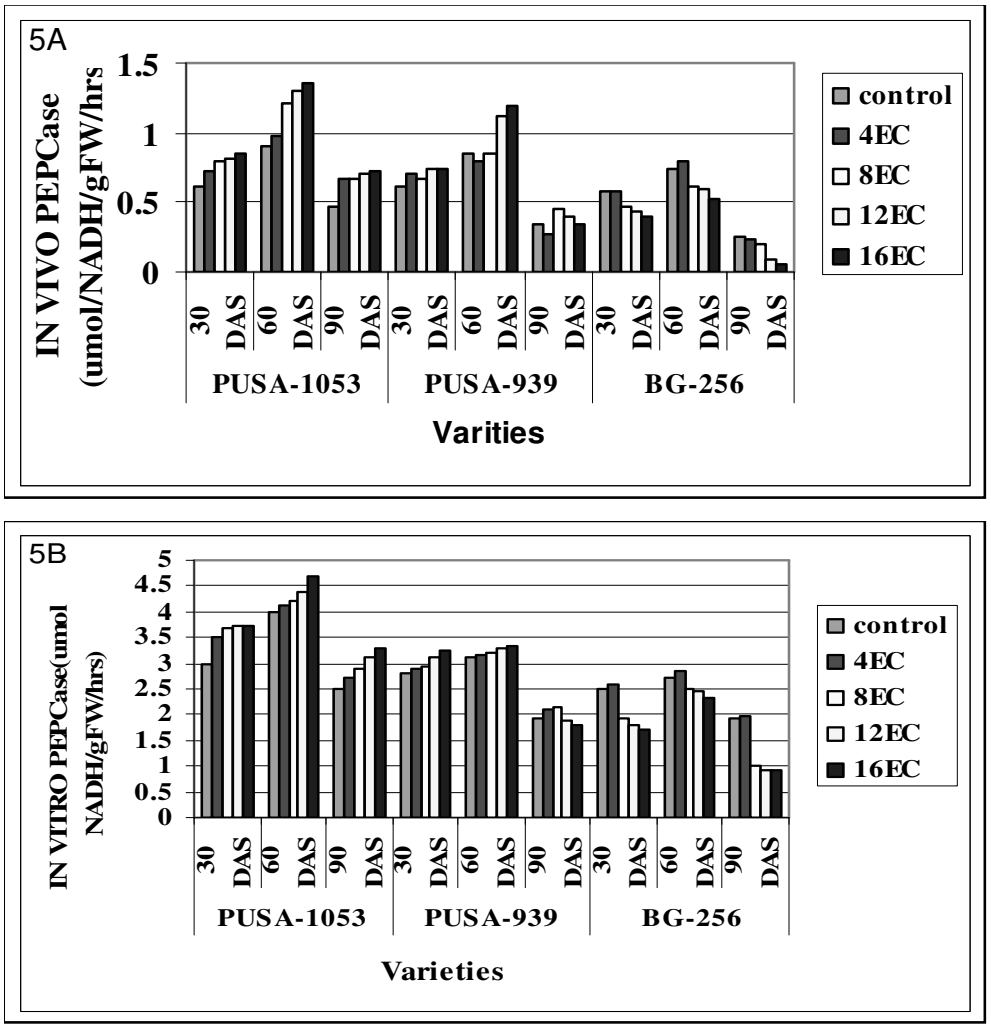

Figure 5: Variation in PEPCase activity *In Vivo* (A) and *In Vitro* (B) in three cultivars of chickpea at various growth stages under salt stress (DAS-days after sowing).

DISCUSSION

There was a significant decline in the overall growth of plants irrespective of the type of cultivar. This was

evident from the decline in relative growth rate with increasing salt stress. Decreases in root and shoot mass have been reported earlier for desi chickpea (Elsheikh & Wood, 1990). A decline in root dry mass may be caused by the reduced nutrients transportation from the soil to the growing shoots. Therefore, a decrease in shoot dry matter accompanied by a decline in root dry matter is a normal growth phenomenon under salt stress.

Sensitive cultivar BG-256) showed lower ARA values than the tolerant ones after salt treatment. Ability of nodules to reduce C_2H_2 under salinity stress declines and it has been well studied for other legumes (Serraj *et al*., 1998; Ferri *et al*., 2000; Ashraf and Harris, 2003; Sheokand, 1995).

The reduction of ARA under salt stress may be due to a restriction of oxygen diffusion in nodules or due to poisonous effects of Na or Cl accumulation (Serraj, 1998). The reasons for reduction in ARA from 60 to 90 days after sowing may have been due to the effect of salt reducing the activity of pre-formed nodules and partly due to the reduced differentiation of new pink nodules.

Disturbance in the accumulation patterns of nitrogenous fractions under salt stress might be responsible for decreased nitrogen content in the sensitive cultivars. Due to the saline stress the leaves became yellow, which ultimately resulted in significant damage to the chlorophyll pigments. Similar results have been reported for other legumes (Soussi *et al*., 1998). The inhibitory effects on chlorophyll pigments could be due to suppression of specific enzymes responsible for the synthesis of the green pigments. This effect depends on the different biological processes and growth stages of the plant and also on the type and concentration of the salts. The decrease in chlorophyll may be attributed to increased chlorophyllase activity (Mishra and Sanwal, 1994, Fang *et al.,* 1998; Soussi *et.al*., 1999, Kiani *et al.,* 2005; Mishra and Sangwan, 1996, 2006; Mishra *et al.,* 1998, 2006;). The lower levels of chlorophyll pigments in the tolerant genotypes might have been responsible for the higher dry matter accumulation in them.

Rubisco enzyme was sensitive to chloride ions thus its activity was reduced by salinity as suggested by Seeman and Critchley (1985). Drevon *et al.* (1998) suggested that PEPCases might probably be involved in the regulation of turgidity or active endosmosis of cells of the inner cortex, which is one of the proposed mechanisms of the oxygen diffusion barrier. Reduced activity of rubisco enzyme reveals that the lack of photosynthate did not inhibit the PEPCase-MDH pathway (Zhang *et al.,* 2007), which supports the hypothesis concerning the limitation in supply of energy substrates (mainly malate) to the bacteroids. The inhibition of nitrogenase activity by salt stress may be a consequence of the decrease in malate content in the nodules and it could be offset in the tolerant cultivar by an increase in the mean nodule weight (Soussi *et al*., 1999).

In the present study, the response of nitrogen fixation to salt was more pronounced than the response of photosynthesis. With the present results, it may be concluded that saline soils retard the growth, metabolic activity and symbiotic efficiency of different cultivars of chickpea. However, important variability was observed amongst different cultivars of chickpea. The data suggest that lack of photosynthates did not cause inhibition of ARA under salinity. Alteration in the nodular metabolism seemed to be directly responsible for nitrogen fixation by the tolerant cultivars. Taken together the data extracted from the present study and results output from the work of Van de Velde *et al.* (2006), the greater performance of symbiosis under saline conditions seems to be determined mainly by the tolerance of the legume host plant. Conclusively, under saline conditions tolerant cultivars should be used to get more nitrogen fixation and optimum yield.

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REFERENCES

Ashraf. M. and P. S. C. Harris, 2003. Potential biochemical indicators of salinity tolerance in plants. Plant Science, 166: 3-16.

Drevon, J.J., N. Frangne, L. Fleurat- Lessard, H. Payre, J. Ribet, V. Vadez and R. Serraj, 1998. Is nitrogenase - linked respiration regulated by osmocontractile cells in legume nodules? In: Biological Nitrogen Fixation for the 21st Century, Elmerich, C., A. Kondorosi and W.E Newton (Eds.). Kluwer Academic Press, Dordrecht, pp. 465-466.

Elsheikh, E.A.E and M. Wood, 1990. Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). Journal of Experimental Botany, 41:1263-1269.

Fang, Z., J. Bouwkamp and T. Solomos, 1998. Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaeolus vulgaris.* Journal of Experimental Botany, 49: 503-510.

Ferri, A., C. Lluch and A. Ocana, 2000. Effects of salt stress on carbon metabolism and bacteroid respiration in root nodules of common bean (*Phaseolus vulgaris* L.). Plant Biology, 2: 396-402.

Herdina, J.A. and J.H. Silsbury, 1990. Estimating nitrogenase activity of faba bean (*Vicia faba* L.) by acetylene reduction (AR) assay. Australian Journal of Plant Physiology, 17:489-502.

Hiscox, T. D. and G. F. Israelstam, 1979. A method for extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany, 57:1332-34.

Kar, M., J.B. Mythili and T.V.R. Nair, 1990. Carbon dioxide fixation in the nodules of amide transporting versus ureide transporting legumes. Journal of Nuclear Agricultural Biology, 19:257-260.

Keys J.A., Parry M.A.J, 1990, Ribulose biphosphate carboxylase / oxygenase and carbonic anhydrase. In: Methods in Plant Biochemistry, Lea, P.J. (Ed.),.Vol. 3, Academic Press London, pp. 1-14.

Kiani, A.J.K, Edgar E.B. and M.J. Joseph, 2005. Mechanistic analysis of wheat chlorophyllase. Archives of Biochemistry and Biophysics, 438: 146-155.

Ligero, F., C. Lluch and J. Olivares, 1986. Evolution of ethylene from roots of *Medicago sativa* plants inoculated with *Rhizobium meliloti*. Journal of Plant Physiology, 125:361-365.

Linder, R.C., 1944. Rapid analytical methods for some of the more common inorganic constituents. Journal of Plant Physiology, 19: 76-89.

Mishra, S., A. Tyagi, I.V. Singh and R.S. Sangwan, 2006. Changes in lipid profile during growth and senescence of *Catharanthus roseus* leaf. Brazilian Journal of Plant Physiology, 18: 447-454.

Mishra, S., S. Shanker and R.S. Sangwan, 1998. Lipid profile in relation to tropnae alkaloid accumulation during leaf growth and senescence in *Duboisia myoporoides*. Fitoterapia, 59: 62-72.

Mishra, S. and G.G. Sanwal, 1994. Effect of *Cuscuta* infection on chloroplast lipid composition of *Brassica* leaves. European journal of Plant pathology, 100: 61-70.

Mishra, S. and R.S. Sangwan, 1996. Dynamics of tropane alkaloids in *Duboisia myoporoides* lead during development. Journal of Herbs Spices and Medicinal Plants, 4: 61-70

Mishra, S. and R.S. Sangwan, 2008. Changes in fatty acid composition of polar lipids associated with growth and senescence in leaves of *Catharanthus roseus*. African journal of Plant Science, 2: 34-37.

Mudgal, V., 2004, PhD thesis: Physiological Studies on Growth and Nitrogen Metabolism in *Cicer arietinum* L. Under Saline Conditions, Rohilkhand University, India

Rao, D.L.N., K.E. Giller, A.R. Yeo and T.J. Flowers, 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). Annals of Botany, 89:563-570.

Rout, N.P. and B.P. Shaw, 2001. Salt tolerance in aquatic macrophytes: Ionic relation and interaction. , Journal of Plant Physiology, 19:76-89.

Seeman, J.R. and C. Chritchley, 1985. Effect of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of salt sensitive species *Phaseolus vulgaris* L. Planta 164:151-162.

Serraj, R., 2002. Response of symbiotic nitrogen fixation to drought and salinity stresses. Journal of Physiology and Molecular Biology, 8:77-86.

Serraj, R., H. Vasquez- Diaz and J.J. Drevon, 1998.

Effects of salt stress on nitrogen fixation, oxygen diffusion, and ion distribution in soybean, common bean, and alfalfa. Journal of Plant Nutrition, 21:475-488.

Sheokand, S., S. Dhandi and K. Swaraj, 1995. Studies on nodule functioning and hydrogen peroxide scavenging enzymes under salt stress in chickpea nodules. Journal of Plant Physiology, 33:561-566.

Sleime, N., M. Lachaal and C. Abdelly, 1999. Responses of some Tunisian chickpea (*Cicer arietinum*) varieties to salinity in nutrient solution. Luter. China Pigeonpea News, 6: 23-26.

Soussi, M., C. Lluch and A. Ocana, 1999. Comparative study of nitrogen fixation and carbon metabolism in two chickpea cultivars under salt stress. Journal of Experimental Botany, 50:1701-1708.

Sudhakar, C., S. Ramanjulu, P.S. Reddy and K. Veeranjaneyulu, 1997. Response of some calvin cycle enzymes subjected to salinity shock in vitro. Indian Journal of Experimental Botany, 35:665-67.

Van de Velde, W., JCP Guerra, A. De Keyser, R. De Rycke, S. Rombauts, 2006. Aging in legume symbiosis. A molecular view on nodule senescence in *Medicago truncatula*, Plant Physiology, 141: 711-720.

Zhang, C.J., L. Chen, D.W. Shi, G.X. Chen, C.G. Lu, P. Wang, J. wang, H.J. Chu, Q.C. Zhou, M. Zuo and L. Sur, 2007.Characteristics of ribulose-1,5-carboxylase and C4 pathway key enzymes in flag leaves of a super-high yield hybrid rice and its parents during reproduction stage. South African Journal of Botany, 73: 22-28.