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Calcium interaction with salinity-induced effects on growth and metabolism of soybean (*Glycine max* L.) cultivars

A. Arshi[1], A. Ahmad[1], I.M. Aref[2] and M. Iqbal*[1,2]

[1]Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi - 110 062, India
[2]Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, Riyadh - 11451, Saudi Arabia

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Abstract: *In the present work, hydroponic culture of JS-335 and Bragg cultivars of soybean (Glycine max) were raised to analyze changes in growth, reactive oxygen metabolism in terms of H_2O_2 content, lipid peroxidation (TBARS), free radical quenching systems (non-enzymatic and enzymatic antioxidants) and ion accumulation in different plant parts under NaCl and $CaCl_2$ stress. Fifteen-day-old seedlings were treated with solutions of 25 mM (T_1), 50 mM (T_2) and 100 mM (T_3) NaCl alone and in combination of 10 mM $CaCl_2$ i.e., 25 mM + 10 mM (T_4), 50 mM + 10 mM (T_5) and 100 mM + 10 mM (T_6). Observations recorded at 30 days after sowing displayed significant decreases in plant biomass, leaf water potential, leaf area, chlorophyll content and the contents of glutathione (GSH) and ascorbate (AsC) on application of NaCl alone. However, H_2O_2 content and lipid peroxidation (TBARS) in leaves were enhanced, consequently invoking the activities of SOD, APX, GR and CAT. Application of NaCl + $CaCl_2$ alleviated adverse effects of NaCl stress. The Na^+ and Cl^- contents in different plant parts increased with NaCl as well as with NaCl + $CaCl_2$ treatments. The maximum accumulation occurred in roots, followed by the stem and the leaves. The K^+ and Ca^{2+} contents decreased under NaCl stress; but NaCl + $CaCl_2$ treatment reduced the extent of decrease caused by NaCl. Thus, calcium ameliorated the deleterious effects of NaCl stress and stimulated plant metabolism and growth.*

Key words: *Antioxidant enzymes, Biomass accumulation, Glycine max, Ion accumulation, Salinity stress*
PDF of full length paper is available online

Introduction

Abiotic stresses pose a serious threat to agriculture and the natural status of the environment. Salinity affects plant growth and its deleterious effects are attributed to a reduced osmotic potential of the growing medium, specific ion toxicity and nutrient deficiency (Luo *et al*., 2005; Bhattacharjee, 2008). Low osmotic potential of saline solutions prevents water uptake by plants, resulting in a "physiological drought". Alterations in physiological processes due to osmotic stress cause reduction in growth. Plant dry weights may be drastically reduced (Kim *et al*., 2009; Ozdener and Kuttbay, 2008).

Salinity can cause hyperionic and hyperosmotic effects on plants, leading to membrane disorganization and metabolic toxicity, including the excessive generation of reactive oxygen species (ROS) such as the superoxide anion (O_2^-), H_2O_2 and the hydroxyl radicals, particularly in chloroplasts and mitochondria (Mittler, 2002). Generation of ROS causes rapid cell damage by triggering a chain of reactions. To protect themselves from the harmful effects of oxidative stress, plants develop ROS-scavenging mechanism that involves detoxification processes carried out by an integrated system of the non-enzymatic reduced molecules, like ascorbate and glutathione, and the enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Jaleel *et al*., 2007). Studies of antioxidant mechanisms may provide clues to enhance salt tolerance in plants.

* **Corresponding author:** iqbalg5@yahoo.co.in

Ca^{2+} sustains K^+ transport and K^+/Na^+ selectivity in Na^+ challenged plants (Rengle, 1992). In plant cells, calcium functions as a second messenger, coupling a wide range of extracellular stimuli to intracellular responses (Sneeden and Formm, 2001), and plays important role in plant growth and development (Arshi *et al*., 2006a) and may be involved in signal transduction involving new gene expression (Trofimova *et al*., 1999) under oxidative stress. It also controls guard-cell turgor and stomatal aperture and helps in turgor maintenance (Bhattacharjee, 2009). Calcium is known to increase salinity tolerance and mitigate the adverse effects of saline conditions on plant growth (Jaleel *et al*., 2007). However, reports on calcium interaction with salinity and the resultant effect on growth and metabolism of crop plants are meager. Given this, the present study was undertaken to investigate the ameliorating effect of calcium on salt-induced alterations on plant growth, ion accumulation and antioxidant activities in soybean cultivars (JS-335 and Bragg).

Materials and Methods

Plant material and salt treatments: Seeds of *Glycine max* L. (cultivars JS-335 and Bragg) were obtained from the Indian Agricultural Research Institute, New Delhi. Plant culture was carried out in the hydroponics systems. Seeds were surface-sterilized with 0.1% sodium hypochloride solution, washed with distilled water for 20 min. and kept in the dark at 25°C for 48 hr to germinate in the trays with soil. After four days, the seedlings obtained were transferred to nutrient solution as per the method of Hoagland and Arnon (1950). The seedlings were grown in controlled

environmental conditions in hydroponics system at 25°C with an 11/13 hr day/night at an energy flow rate of 12.5 flux and RH of 70-80% for 15 d. The solutions were aerated continuously to provide ample O_2 and maintain the solution concentration at root surfaces. Salt treatments were imposed to 15-day-old seedlings by adding NaCl and $CaCl_2$ through the following treatments: T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 100 mM NaCl, T_4: 25 mM NaCl + 10 mM $CaCl_2$, T_5: 50 mM NaCl + 10 mM $CaCl_2$ and T_6: 100 mM NaCl + 10 mM $CaCl_2$ into half-strength nutrient solution. The nutrient solution without NaCl and $CaCl_2$ was used for the control group. Sampling was done at 30 days after sowing (DAS).

Measurement of biomass, leaf area, leaf water potential and chlorophyll: Thirty-day-old plants, treated with various NaCl and $CaCl_2$ treatments (T_1-T_6) were collected and dried in oven at 65°C for 72 hr. Dry weights of the control and the treated plants were then determined with the help of an electronic balance (Model BL 210S, Sartorius, Germany) and expressed in g per plant. The leaf area of green leaves was measured with the help of leaf area meter (Model LICOR 3000, USA). Leaf water potential (ψ_w) of the most recent fully-expanded leaves was measured using a pressure chamber. It was expressed in –Mpa. Total chlorophyll content of the leaves was estimated by the method of Hiscox and Israelstam (1979).

Determination of lipid peroxidation, H_2O_2, glutathione and ascorbate contents: Content of 2-thiobarbituric acid reactive substances (TBARS), the product of lipid peroxidation, was determined in leaves by the method of Cakmak and Horst (1991). The hydrogen peroxide content in the leaves was determined according to the method of Velikova *et al*. (2000). The glutathione content was determined by the glutathione-recycling method of Anderson (1985), whereas ascorbate content was determined by the method of Law *et al*. (1983). All the parameters were expressed in nmol g^{-1} fr wt.

Assay of superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase activities: Enzymes were extracted from 0.5 g fresh leaf tissue 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 15000 x 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. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to the method of Dhindsa *et al*. (1981), by monitoring the superoxide radical-induced nitro-blue-tetrazolium (NBT) reduction at 560 nm. One enzyme unit (EU) of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT. APX activity was determined by measuring the consumption of ascorbate, following the absorbance at 290 nm. One enzyme unit (EU) of APX activity was defined as the amount of enzyme required to consume 1 μmol ascorbate min^{-1} (Nakano and Asada, 1981), and calculated by using the coefficient of absorbance as 2.8 mM^{-1} cm^{-1}. GR activity was determined by measuring the enzyme-dependent oxidation of NADPH by the method of Foyer and Halliwell (1976). One unit of GR activity was defined as the amount of enzyme that oxidised 1 μmole NADPH min^{-1}. Catalase activity was determined by monitoring the disappearance of H_2O_2 according to the method of Aebi (1984), calculated by using the coefficient of absorbance as 0.036 mM^{-1} cm^{-1} and expressed as μmol H_2O_2 consumed per minute. One enzyme unit (EU) of CAT determines its amount necessary to decompose μmol H_2O_2 min^{-1}.

Estimation of sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-)contents: 100 mg of plant material was digested separately in the kjeldahl digestion unit with 10 ml of acid mixture (nitric acid : perchloric acid - 9:4). The sodium, potassium and calcium ion contents in the digested material were estimated photometrically using a flame photometer (Model Systronic 125, Systronic, India). Standard curves were drawn by diluting the stock solution of NaCl, KCl and $CaCO_3$ with double distilled water to obtain different concentrations of Na^+, K^+ and Ca^{2+} (5-100 ppm). The Na^+, K^+ and Ca^{2+} ion contents were estimated following the method of Tondon (1995). The content of each element in plant organs was determined using the standard curve and expressed in μmol g^{-1} dry wt.

The chloride ion content was estimated following the method of Vogel (1968), and expressed in μmol g^{-1} dry wt. Dry plant material (100 mg of each of the root, shoot and leaf taken separately) was added to a 25 ml of double distilled water, shaken for 30 min and then centrifuged for 10 min at 4,000 rpm. To a 20 ml of this extract taken in a white porcelain dish, was added 1 ml of potassium chromate. The mixture was titrated against silver nitrate solution to develop light brown colour due to the formation of silver chromate.

Statistical analysis: The SPSS statistical package software version 10.0 (Chicago, USA) was used to analyze the data obtained. Differences between varieties and treatments were determined by application of two-way analysis of variance (ANOVA). Mean separation was done by the DMRT test at $p \leq 0.05$. Linear regression was fitted with GENSTAT to find out the degree of correlation between the treatments and the different variables in the test plant.

Results and Discussion

All NaCl treatments reduced the plant biomass significantly ($p<0.05$). The maximum reduction (46% in JS-335 and 39% in Bragg with reference to control) was caused by application of 100 mM NaCl. Plant biomass decreased significantly in both the Soybean cultivars with NaCl treatments, possibly because salinity can inhibit plant growth by altering the water potential, increasing the ion toxicity, or causing an ion imbalance. Salinity can also reduce biophysical restraints to cell-wall expansion which, in turn, inhibits root growth and plant biomass (Singh *et al*., 1995; Arshi *et al*., 2002). Application of $CaCl_2$ (10 mM), together with NaCl (100 mM), reduced the plant biomass by 40% in JS-335 and 33% in Bragg with reference to control. Calcium application thus alleviated the NaCl toxicity and minimized the reduction in biomass caused by NaCl (Table 1). Supplemental calcium might have increased Ca^{2+} influx and hence

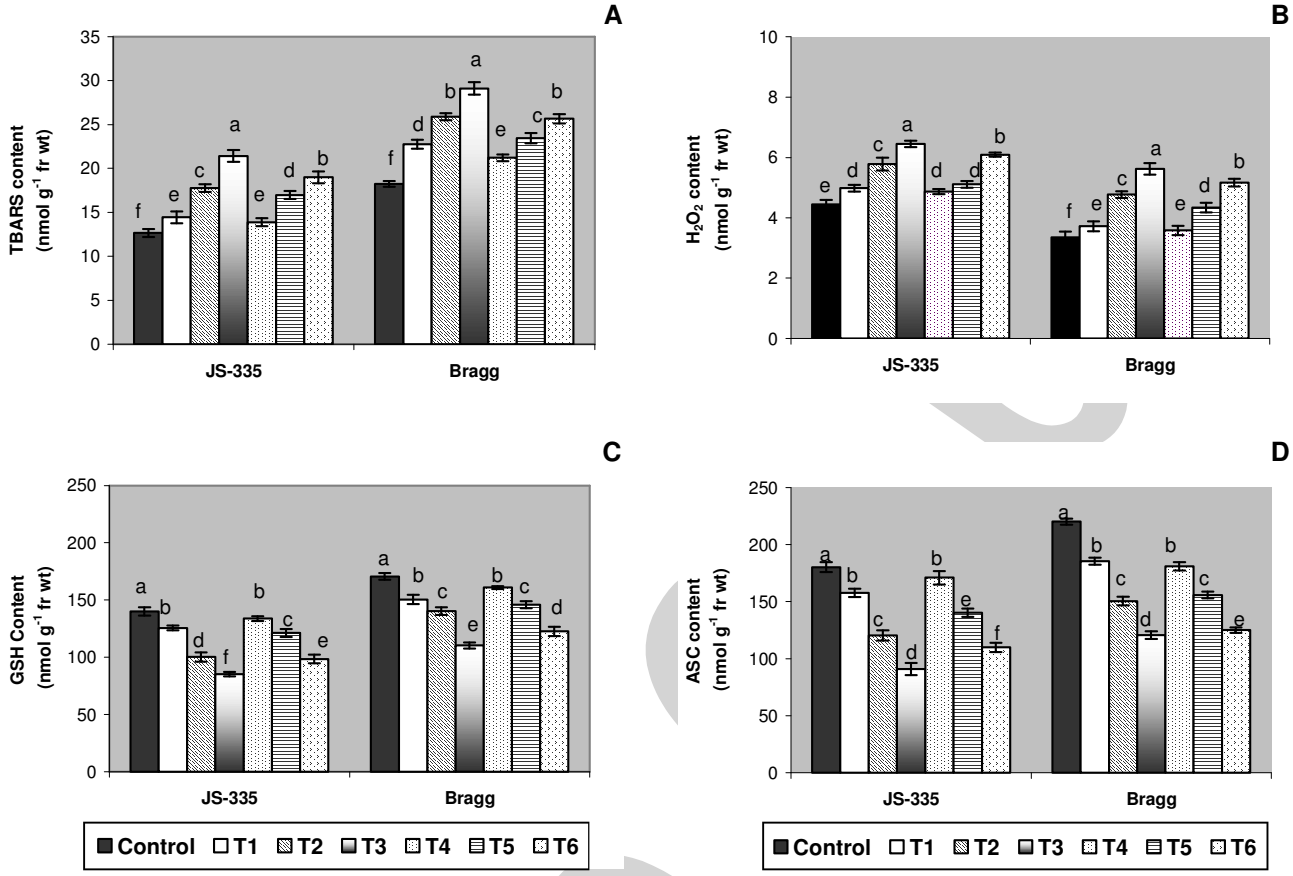

Fig. 1. Effect of NaCl, alone and in combination with $CaCl_2$ on the TBARS (A), H_2O_2 (B), GSH (C) and ascorbate (D) contents in the leaves of soybean cultivars, as observed at 30 DAS. Values represent mean ± SE (n=5). Letters at the top of bars indicate significant differences at $p \leq 0.05$ level

the calcium concentration, as was observed in the salt-stressed cotton roots (Cramer *et al*., 1986).

A drastic decrease in leaf water potential, with reference to control, was caused by each NaCl treatment, whereas calcium application improved the leaf water potential of salinity-stressed plants in both the cultivars (Table 1). In fact, application of salt decreases the osmotic potential of the solution, creating a water stress in plants (Sairam and Tyagi, 2004). In the saline environment, sodium ions may compete with calcium ions for membrane-binding sites and, therefore, a high calcium level provides security to cell membrane from adverse effects of salinity. NaCl treatment caused a progressively increasing displacement of calcium from the membranes of isolated protoplasts, whereas supplemental calcium could restore the adequate level of calcium in the epidermal cells of maize roots (Zidan *et al*., 1990).

NaCl exhibited a dose-dependent inhibitory effect on leaf area in both the varieties. Reduction in leaf area went up to 43% in JS-335 and 36% in Bragg as observed with T_3 (100 mM NaCl) treatments, compared with controls. Similar reductions in leaf area were caused by salinity in *Cassia angustifolia* (Arshi *et al*., 2005), possibly due to inhibition of cell division and cell expansion under salt stress. A decrease in leaf size under unfavorable conditions allows conservation of energy, thereby launching appropriate defense responses and also reducing the risk of heritable damage (Chaparzadeh *et al*., 2004). The combined (NaCl + $CaCl_2$) treatment also inhibited the leaf area by 36% in JS-335 and 31% in Bragg; however, the reduction was always less than one caused by NaCl alone, suggesting that calcium mitigated the inhibitory effect of NaCl (Table 1). Salinity inhibits Ca^{2+} transport from root to shoot; a reduced Ca^{2+} availability in the leaf growth region and a high Na^+/Ca^{2+} ratio in the expanding tissue contribute to growth inhibition (Rengel, 1992). Supplemental Ca^{2+} may increase calcium concentration in the growing zone and partially restore the leaf growth rate.

NaCl treatment caused a significant reduction in the leaf chlorophyll content of both the cultivars compared with their controls. The maximum decline (34%) over the control was observed in JS-335, whereas the minimum (25%) in Bragg, with 100 mM NaCl treatment (Table 1). Reduction in chlorophyll content under salinity stress is attributed to the destruction of chlorophyll pigments and the instability of pigment-protein complex (Jaleel *et al*., 2008). A decline in the chlorophyll content may be correlated to the indirect effects of Na^+ and Cl^- ions on the contents of essential nutrients. A decline in Fe^{3+}, Mg^{2+}, Ca^{2+} and K^+ contents was observed in the aerial parts of Cd-stressed wheat plants (Ouzounidou *et al*., 1997). As both Mg^{2+} and Fe^{3+} are associated with chlorophyll formation, their

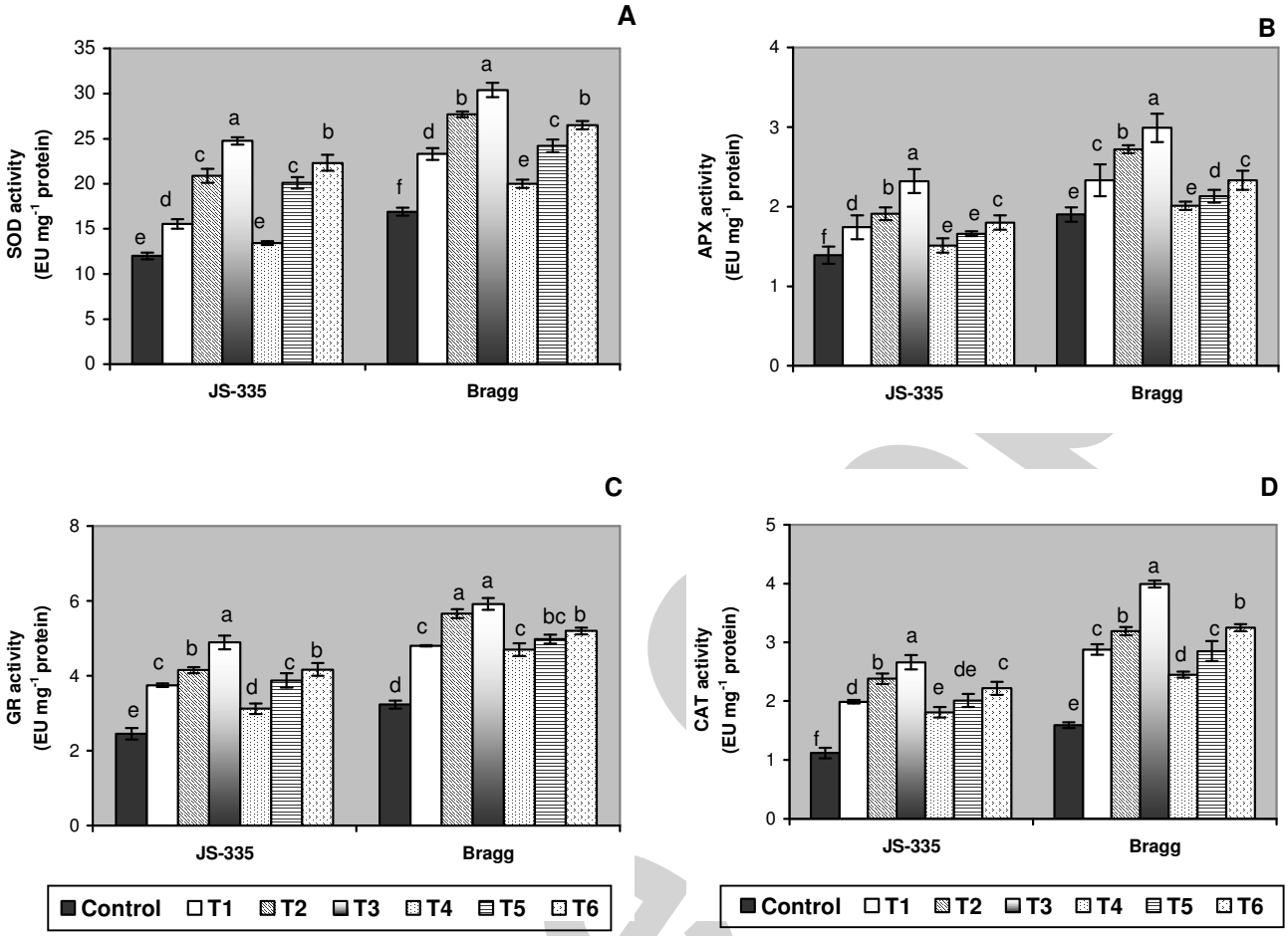

Fig. 2. Effect of NaCl, alone and in combination with $CaCl_2$ on the SOD (A), APX (B), GR (C) and CAT (D) activities in the leaves of soybean cultivars, as observed at 30 DAS. Values represent mean ± SE (n=5). Letters at the top of bars indicate significant differences at $p \leq 0.05$ level

impaired supply to leaves may inhibit the process. Similar findings as ours have been reported by Tiwari *et al*. (2009) in relation to chromium-induced oxidative stress in *Pisum sativum* L. On the contrary, combined treatments of calcium and NaCl (100 mM NaCl + 10 mM $CaCl_2$) could reduce the total chlorophyll content only by 27 and 22% in JS-335 and Bragg respectively. The extent of reduction was thus less with the NaCl + $CaCl_2$ treatment, thus showing a remedial effect of $CaCl_2$ treatment Ca might be preventing the damage from cellular dehydration by balancing the osmotic strengths of cytoplasm, as suggested earlier with reference to *Cassia angustifolia* (Arshi *et al*., 2006a,b).

To evaluate the NaCl-induced oxidative damage to membranes, the content of thiobarbituric acid reactive substances (TBARS) was determined. NaCl application was stimulatory for TBARS content (Fig. 1A), which increased in JS-335 and Bragg by 69 and 60%, respectively, over the control against the 100 mM NaCl treatment; likewise, NaCl + $CaCl_2$ treatment also increased TBARS content by 50 and 41% in the two cultivars. Calcium thus mitigated the adverse effects of NaCl. Lipid peroxidation (LPO) is associated with damages provoked by a variety of environmental stresses. Poly-unsaturated fatty acids (PUFA) are the main membrane-lipid components susceptible to peroxidation and degradation (Elkahoui *et al*., 2005). The increase in LPO, as caused by NaCl stress in the present study, can be correlated to ion accumulation and AOS production under salt stress (Hernandez *et al*., 2001). The LPO level indicates the extent of salt tolerance in the given species (Bor *et al*., 2003).

H_2O_2 content in the leaves increased with each of the NaCl treatments (Fig. 1B). Increase in H_2O_2 content was less in Bragg than in JS-335. Under NaCl + $CaCl_2$ treatment, the enhancement of H_2O_2 was relatively less. Apparently, $CaCl_2$ protected the plants from toxic effects of NaCl by reducing the H_2O_2 content; a product of peroxisomal and chloroplastic oxidative reactions (Del Rio *et al*., 1992), which itself is an active oxygen species (AOS). The H_2O_2 increase under salt stress was similar to one reported for *Cassia angustifolia* under lead stress (Qureshi *et al*., 2007) and the external supply of calcium was able to reduce it in soybean plant. Environmental stresses are known to disrupt cellular homeostasis through AOS formation (Polle, 2001). CAT removes H_2O_2 very efficiently, whereas SOD scavenges superoxide anion (Scandalios, 1993). Significantly, Ca^{2+} inhibits enhanced production of AOS.

Table - 1: Plant biomass (g plant^{-1}), water potential (-MPa), leaf area (cm^2 plant^{-1}) and total chlorophyll (chl) content (mg g^{-1} fr wt.) in soybean cultivars (JS-335 and Bragg) leaves as influenced by NaCl and NaCl + $CaCl_2$ at 30 DAS

Treatments (mM)	Plant biomass		Water potential		Leaf area		Total chl content	
	JS-335	Bragg	JS-335	Bragg	JS-335	Bragg	JS-335	Bragg
Control	$6.22^{a} \pm 0.22$	$6.52^{a} \pm 0.25$	$-0.02^{f} \pm 0.01$	$-0.03^{f} \pm 0.01$	$50.22^{a} \pm 3.22$	$60.55^{a} \pm 2.22$	$1.914^{a} \pm 0.14$	$2.250^{a} \pm 0.16$
T_1	$5.32^{b} \pm 0.20$	$5.45^{b} \pm 0.82$	$-0.27^{e} \pm 0.08$	$-0.20^{e} \pm 0.08$	$40.32^{b} \pm 2.20$	$53.20^{b} \pm 3.20$	$1.749^{b} \pm 0.18$	$2.055^{b} \pm 0.18$
T_2	$4.33^{c} \pm 0.18$	$4.53^{c} \pm 0.51$	$-0.59^{c} \pm 0.05$	$-0.51^{c} \pm 0.05$	$33.88^{c} \pm 3.18$	$43.33^{c} \pm 3.18$	$1.491^{c} \pm 0.18$	$1.850^{cd} \pm 0.26$
T_3	$3.33^{d} \pm 0.25$	$3.98^{d} \pm 0.55$	$-1.13^{a} \pm 0.05$	$-0.95^{a} \pm 0.05$	$28.78^{d} \pm 3.25$	$38.81^{d} \pm 3.25$	$1.262^{d} \pm 0.15$	$1.680^{d} \pm 0.13$
T_4	$5.66^{b} \pm 0.11$	$5.77^{b} \pm 0.99$	$-0.20^{e} \pm 0.04$	$-0.15^{e} \pm 0.04$	$44.90^{b} \pm 4.11$	$54.54^{b} \pm 2.11$	$1.759^{b} \pm 0.28$	$2.069^{ab} \pm 0.33$
T_5	$4.49^{c} \pm 0.92$	$4.81^{c} \pm 0.88$	$-0.47^{d} \pm 0.08$	$-0.40^{d} \pm 0.08$	$36.44^{c} \pm 3.22$	$44.90^{c} \pm 3.92$	$1.522^{c} \pm 0.33$	$1.990^{bc} \pm 0.39$
T_6	$3.70^{d} \pm 0.65$	$4.537^{d} \pm 0.52$	$-0.91^{b} \pm 0.06$	$-0.82^{b} \pm 0.06$	$31.88^{d} \pm 4.65$	$39.17^{d} \pm 1.65$	$1.279^{d} \pm 0.28$	$1.780^{d} \pm 0.25$

The values (Mean ± SE) are based on five individual readings. Data followed by the same letters are not significantly different at $p \leq 0.05$ level

Table - 2: Sodium (Na^+), chloride (Cl^-), potassium (K^+) and calcium (Ca^{2+}) contents (µmol g^{-1} dry wt.) in different plant parts of soybean cultivars (JS-335 and Bragg) as influenced by NaCl and $CaCl_2$ treatments at 30 DAS

Treatments (mM)	Genotypes					
	JS-335			Bragg		
	Root	Stem	Leaves	Root	Stem	Leaves
			Na^+ content			
Control	$20^{g} \pm 3.69$	$15^{g} \pm 7.22$	$10^{f} \pm 8.99$	$18^{g} \pm 10.12$	$15^{g} \pm 5.89$	$10^{f} \pm 5.23$
T_1	$155^{e} \pm 15.63$	$125^{e} \pm 19.63$	$40^{d} \pm 15.63$	$240^{e} \pm 15.89$	$120^{e} \pm 16.63$	$44^{d} \pm 15.58$
T_2	$550^{c} \pm 18.85$	$230^{c} \pm 13.63$	$74^{c} \pm 17.75$	$565^{c} \pm 15.22$	$330^{c} \pm 20.11$	$84^{c} \pm 13.25$
T_3	$750^{a} \pm 14.74$	$520^{a} \pm 18.88$	$108^{a} \pm 14.44$	$965^{a} \pm 18.23$	$610^{a} \pm 13.36$	$130_{a} \pm 12.22$
T_4	$110^{f} \pm 10.63$	$95^{f} \pm 10.25$	$25^{e} \pm 9.98$	$180^{f} \pm 11.20$	$85^{f} \pm 10.23$	$30^{e} \pm 5.22$
T_5	$410^{d} \pm 15.41$	$160^{d} \pm 16.21$	$45^{d} \pm 12.36$	$480^{d} \pm 15.32$	$250^{d} \pm 14.75$	$50^{d} \pm 6.63$
T_6	$660^{b} \pm 16.63$	$410^{b} \pm 18.11$	$80^{b} \pm 10.10$	$810^{b} \pm 14.45$	$525^{b} \pm 16.63$	$100^{b} \pm 12.22$
			Cl^- content			
Control	$10^{f} \pm 3.69$	$20^{f} \pm 7.22$	$10^{e} \pm 8.99$	$15^{f} \pm 10.12$	$15^{f} \pm 5.89$	$10^{f} \pm 5.23$
T_1	$100^{d} \pm 15.63$	$91^{d} \pm 19.63$	$80^{c} \pm 15.63$	$90^{d} \pm 15.89$	$80^{d} \pm 16.63$	$70^{d} \pm 15.58$
T_2	$135^{c} \pm 18.85$	$125^{c} \pm 13.63$	$115^{b} \pm 17.75$	$120^{c} \pm 15.22$	$110^{c} \pm 20.11$	$100^{c} \pm 13.25$
T_3	$210^{a} \pm 14.74$	$170^{a} \pm 18.88$	$166^{a} \pm 14.44$	$230^{a} \pm 18.23$	$210^{a} \pm 13.36$	$135^{a} \pm 12.22$
T_4	$80^{e} \pm 11.56$	$60^{e} \pm 10.11$	$40^{d} \pm 10.11$	$50^{e} \pm 9.12$	$40^{e} \pm 10.22$	$40^{e} \pm 5.89$
T_5	$110^{d} \pm 14.88$	$90^{d} \pm 9.96$	$77^{c} \pm 12.20$	$95^{d} \pm 8.88$	$80^{d} \pm 10.44$	$73^{d} \pm 4.89$
T_6	$175^{b} \pm 16.33$	$140^{b} \pm 13.22$	$110^{b} \pm 14.77$	$180^{b} \pm 7.75$	$170^{b} \pm 9.23$	$115^{b} \pm 8.56$
			K^+ content			
Control	$85^{a} \pm 4.44$	$105^{a} \pm 7.23$	$105^{a} \pm 6.66$	$85^{a} \pm 9.96$	$110^{a} \pm 6.11$	$110^{a} \pm 5.56$
T_1	$52^{c} \pm 6.32$	$88^{b} \pm 4.44$	$90^{b} \pm 4.86$	$65^{b} \pm 6.23$	$90^{b} \pm 5.22$	$100^{b} \pm 8.88$
T_2	$40^{d} \pm 4.12$	$70^{d} \pm 5.56$	$70^{c} \pm 9.33$	$50^{d} \pm 9.21$	$75^{c} \pm 7.47$	$80^{d} \pm 8.71$
T_3	$30^{e} \pm 2.23$	$40^{f} \pm 6.33$	$44^{d} \pm 5.86$	$40^{e} \pm 4.11$	$55^{d} \pm 9.96$	$66^{e} \pm 4.63$
T_4	$60^{b} \pm 6.66$	$80^{c} \pm 4.45$	$92^{b} \pm 8.11$	$70^{b} \pm 6.45$	$94^{b} \pm 7.02$	$105^{ab} \pm 7.77$
T_5	$48^{c} \pm 7.11$	$75^{cd} \pm 6.12$	$77^{c} \pm 5.33$	$56^{c} \pm 5.96$	$80^{c} \pm 9.12$	$88^{c} \pm 6.36$
T_6	$38^{d} \pm 8.22$	$50^{e} \pm 3.88$	$50^{d} \pm 4.66$	$48^{d} \pm 7.03$	$62^{d} \pm 8.42$	$72^{de} \pm 7.32$
			Ca^{2+} content			
Control	$110^{a} \pm 4.58$	$140^{a} \pm 8.21$	$150^{a} \pm 5.45$	$105^{a} \pm 5.53$	$130^{a} \pm 8.23$	$140^{a} \pm 6.11$
T_1	$80^{b} \pm 6.66$	$90^{b} \pm 6.11$	$110^{b} \pm 3.88$	$90^{bc} \pm 5.11$	$110^{b} \pm 9.11$	$130^{ab} \pm 4.33$
T_2	$60^{d} \pm 4.88$	$75^{c} \pm 4.33$	$90^{cd} \pm 4.44$	$80^{de} \pm 4.44$	$85^{cd} \pm 5.96$	$120^{bc} \pm 5.23$
T_3	$50^{e} \pm 7.12$	$44^{e} \pm 5.22$	$75^{e} \pm 6.11$	$70^{f} \pm 6.63$	$75^{e} \pm 4.44$	$100^{d} \pm 6.11$
T_4	$84^{b} \pm 4.63$	$97^{b} \pm 4.00$	$117^{b} \pm 7.24$	$94^{b} \pm 8.22$	$115^{b} \pm 5.22$	$136^{a} \pm 4.10$
T_5	$67^{c} \pm 6.12$	$81^{c} \pm 7.11$	$99^{c} \pm 5.45$	$86^{cd} \pm 7.11$	$92^{c} \pm 4.10$	$128^{b} \pm 2.99$
T_6	$57^{d} \pm 5.45$	$52^{d} \pm 5.33$	$86^{d} \pm 3.78$	$77^{ef} \pm 4.96$	$83^{de} \pm 3.63$	$109^{cd} \pm 8.11$

The values (Mean ± SE) are based on five individual readings. Data followed by the same letters are not significantly different at $p \leq 0.05$ level

As compared with the control, NaCl decreased the glutathione (GSH) and ascorbate (AsA) contents in both the cultivars (Fig. 1C,D). At a higher level of NaCl (100 mM), the maximum reduction in GSH and AsC contents was up to 39 and 50% in JS-335 and up to 35 and 45% in Bragg, respectively, against the controls. The combined treatment of NaCl + $CaCl_2$ reduced the GSH and AsC contents by 30 and 39% in JS-335 and by 28 and 43% in Bragg, respectively. Treatments of NaCl alone as well as with $CaCl_2$ decreased the antioxidant AsA and GSH contents in leaves of both the soybean varieties, as compared with control. Efficient destruction of O_2 and H_2O_2 in plant cells requires a concerted action of antioxidants; AsA has the capacity to eliminate different AOS including singlet oxygen, superoxide and hydroxyl radicals (Sakamoto *et al*., 1998). In the ascorbate-glutathione pathway, AsA together with GSH promotes plant tolerance to ROS by participating in the ROS detoxification in plant cells (Anjum *et al*., 2008a,b).

Under NaCl treatments glutathione content showed a strong positive correlation ($p<0.05$) with plant biomass ($r^2 = 0.858$ in JS-335 and 0.912 Bragg) and leaf area ($r^2 = 0.860$ in JS-335 and 0.857 in Bragg. The dose-dependent ascorbate content also showed a strong positive correlation with plant biomass ($r^2 = 0.971, 0.946$) and leaf area ($r^2 = 0.961, 0.917$) in JS-335 and Bragg, respectively.

The NaCl-treated plants showed a dose-dependent increase in SOD, APX, CAT and GR activities, the enhancement being greater in Bragg than in JS-335 variety (Fig. 2 A, B, C, D). The NaCl + $CaCl_2$ treatment also increased the above parameters but this increase was less than one caused by NaCl alone. Activities of these enzymes increased under salt stress so as to modulate the amount of ROS. The SOD and CAT activities have been reported to be correlated negatively to the degree of damage of plasmalemma, chloroplast, and mitochondrial membrane systems, and positively to the indices of stress resistance (Prochazkova *et al*., 2001). ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules (like AsA and glutathione) and the enzymatic antioxidants. As part of this, antioxidant enzymes fortify the defense mechanism against oxidative stress and may provide a strategy to enhance salt tolerance in plants (Misra and Gupta, 2006). Our results are in agreement with earlier findings in relation to mercury, arsenic and chromium-induced oxidative stress in Indian mustard (Diwan *et al*., 2007; Ansari *et al*., 2009; Khan *et al*., 2009). Calcium, a secondary messenger in signal transduction, regulates physiological and biochemical processes in plant responses to extracellular adverse abiotic environments. Calcium supply to plants can increase their tolerance capacity to adverse environments (Bowler and Fluhr, 2000). Its impact on the mechanism of SOD, CAT and GR activities is still vague.

The Na^+ content of plant tissues significantly increased with each concentration of NaCl, showing the maximum accumulation in roots, followed by the stem and the leaves. The accumulation was dose- as well as time-dependent. Bragg accumulated more Na^+ than JS-335 (Table 2). A little less Na^+ content was recorded with the combined treatment of NaCl + $CaCl_2$. Compared with the control, chloride (Cl^-) ion content was enhanced in different plant parts of both cultivars under NaCl stress. The maximum Cl^- content was observed in roots, followed by the stem and leaves. JS 335 was a better accumulator than Bragg. Calcium application minimized the Cl^- accumulation in plant parts. Salinity stress had an adverse effect on potassium and calcium ion contents of both cultivars, with 100 mM NaCl causing the maximum decline. Bragg suffered less damage than JS-335. The decline was highest in roots, followed by the stem and the leaves. The K^+ and Ca^{2+} contents decreased with NaCl + $CaCl_2$ treatments also, but the reduction was far less than one caused by NaCl alone (Table 2). Thus, calcium minimized the adverse effect of NaCl by improving the K^+ and Ca^{2+} contents. Na^+ and Cl^- ion contents increased, K^+ and Ca^{2+} contents decreased under increasing NaCl stress in the soybean cultivars. High saline concentrations reduce plant intake and transport of calcium, causing calcium deficiency and ion imbalance in the plant (Cramer *et al*., 1986). Calcium and potassium display similar attitudes in the selective transport of ions from the cell membrane (Boursier and Lauchli, 1990). Low Ca^{2+}/Na^+ ratio in the plant causes distortion of selectivity in root cell membranes just as in low K^+/Na^+ ratio, resulting in a passive intake of sodium into the cell and its toxic level accumulation in the plant.

Application of $CaCl_2$ to NaCl-stressed soybean cultivars significantly alleviated the effect of salinity stress. Bragg cultivar exhibited higher adaptive potential under salinity stress than JS-335, as adjudged by a relatively greater activation of antioxidant enzymes.

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