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SALT STRESS MITIGATION BY CALCIUM CHLORIDE IN *VIGNA RADIATA* (L.) WILCZEK

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This work assesses the ameliorating effect of calcium chloride on sodium chloride-stressed plants of *Vigna radiata* (L.) Wilczek. Plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM $CaCl_2$, or 5 mM $CaCl_2$. Groundwater was used for irrigation as the control. Plants were harvested randomly 30 and 50 days after sowing. NaCl and $CaCl_2$-stressed plants showed reduced growth as indicated by decreased root length, stem length, total leaf area and dry weight. Proline and glycinebetaine content and the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase and catalase were increased under treatment with NaCl alone and $CaCl_2$ alone. When $CaCl_2$ was combined with NaCl, $CaCl_2$ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of the plants.

Key words: Sodium chloride, calcium chloride, amelioration, growth, antioxidant enzymes.

INTRODUCTION

Environmental factors influence the character, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceeds the optimum tolerance of a plant, it stresses the plant and in turn influences its development and structural, physiological and biochemical processes (Jaleel et al., 2007a). Soil salinity is one among the several environmental stresses causing drastic changes in the growth, physiology and metabolism of plants and threatening crop and vegetable cultivation around the globe (Jaleel et al., 2007b).

Plant growth and development are internal processes under the control of the environment. Temperature, moisture, light, nutrients and gases can either enhance or retard these processes, and sometimes may act as stressors damaging and in extreme cases killing the plant (Jaleel et al., 2007c). In many salt-sensitive plants, glycophytes, which include most crop plants, a major part of growth inhibition is caused by excess Na^+ (White and Broadley, 2003; Jaleel et al., 2007d). High sodium disrupts potassium (K^+) nutrition, and when accumulated in the cytoplasm it inhibits many enzymes (Sankar et al., 2006).

Calcium plays an important role in plant growth and development. It is implicated in the movement of cellular organelles such as the spindle apparatus and secretory vesicles, and may play a key role in integrating plant cell metabolism (Jaleel et al., 2007e). The cells of fibrous tissue need more calcium because it is required to bind the polysaccharides that form the middle lamella in the cell plates that arise between daughter cells. Adequate Ca^{2+} levels are necessary for the membrane to function normally. Most of the interest in calcium in plants has centered on its role in the cytoplasm in controlling developmental process. Free calcium in the apoplast may also influence plant growth (Lawlor, 2002; Jaleel et al., 2007f).

Legumes have long been recognized to be either sensitive or moderately tolerant to salinity. Salt tolerance varies even among legumes, and most of them respond to saline conditions by salt exclusion, that is, exclusion of NaCl from the leaves (Manivannan et al., 2007). The present study assess-

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Abbreviations: APX – ascorbate peroxidase; CAT – catalase; $CaCl_2$ – calcium chloride; DAS – days after sowing; GB – glycinebetaine; NaCl – sodium chloride; PRO – proline; SOD – superoxide dismutase.

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TABLE 1. Effect of NaCl, $CaCl_2$ and their combination on the root length, stem length, total leaf area and whole plant dry weight of *Vigna radiata*. Values are means ± SD of 7 replicates

Growth parameters	Control		100 mM NaCl		100 mM NaCl + 5 mM $CaCl_2$		5 mM $CaCl_2$	
	Days after sowing (DAS)							
	30	50	30	50	30	50	30	50
Root length (cm plant^{-1})	161.53 ±5.57	198.38 ±7.085	108.63 ±3.746	136.08 ±4.692	142.08 ±5.074	170.28 ±8.514	120.24 ±4.294	150.03 ±5.358
Stem length (cm plant^{-1})	14.42 ±0.497	23.62 ±0.844	8.03 ±0.287	17.27 ±0.596	12.09 ±0.432	20.54 ±0.708	10.05 ±0.347	19.52 ±0.697
Total leaf area (cm^2 plant^{-1})	40.58 ±1.449	94.62 ±3.263	21.30 ±0.761	57.89 ±1.996	34.18 ±1.179	81.51 ±2.911	30.28 ±1.081	70.29 ±2.244
Whole plant dry weight (mg plant^{-1})	0.520 ±0.019	1.510 ±0.054	0.238 ±0.013	0.731 ±0.026	0.408 ±0.014	1.008 ±0.035	0.330 ±0.011	0.988 ±0.033

es the ameliorating effect of calcium on salt stress in *Vigna radiata* (L.) Wilczek plants, with specific emphasis on growth, biochemical parameters and antioxidant enzyme activity.

MATERIALS AND METHODS

Greengram [*Vigna radiata* (L.) Wilczek cv. ADT 3] seeds were surface-sterilized with 0.2% $HgCl_2$ solution for 5 min with frequent shaking and then thoroughly washed with deionized water. The seeds were sown in plastic pots (300 mm diam) filled with 3 kg of a 1:1:1 soil mixture containing red soil, sand and farmyard manure (FYM). Two seeds per pot were sown, and all the pots were watered with tap water up to 19 days after sowing (DAS). On day 20 the pots were irrigated with groundwater as control or with 100 mM NaCl, 100 mM NaCl with 5 mM $CaCl_2$, or 5 mM $CaCl_2$ solutions. The plants were uprooted randomly 30 and 50 DAS and used for growth assessment, biochemical and antioxidant enzyme assays.

MORPHOLOGICAL PARAMETERS

Root length, stem length and dry weight (DW) were calculated from plant samples. Total leaf area of the plant was measured with a LICOR photoelectric area meter (Model L 1–3100, Lincoln, U.S.A.) and expressed as cm^2 plant^{-1}.

BIOCHEMICAL PARAMETERS

Proline (PRO) was extracted and estimated according to Bates et al. (1973), and glycinebetaine (GB) by the method of Grieve and Grattan (1983). Both PRO and GB content were expressed as μg g^{-1} DW.

ANTIOXIDANT ENZYME ACTIVITIES

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to Beauchamp and Fridovich (1971). The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M potassium cyanide (KCN) and 5.6×10^{-5} M nitroblue tetrazolium salt (NBT) dissolved in 3 ml 0.05 M sodium phosphate buffer (pH 7.8); 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for 1 h. Identical solutions kept in darkness served as blanks. Absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units (U) defined as the amount of change in absorbance as 0.1 h^{-1} mg^{-1} protein. Ascorbate peroxidase (APX; EC 1.11.1.1) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 μl enzyme extract. Absorbance was read as the decrease at 290 nm against the blank, corrected for the low nonenzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9 mM^{-1} cm^{-1}). Catalase (CAT) (EC 1.11.1.6) was measured according to Chandlee and Scandalios (1984), modified. The assay mixture contained 2.6 ml 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml 15 mM H_2O_2 and 0.04 ml enzyme extract. Decomposition of H_2O_2 was followed by decline of absorbance at 240 nm. Enzyme activity was expressed in U (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein). The values are expressed as means ± SD of seven samples in each group. Enzyme protein was determined (Bradford, 1976) for all three enzymes to express specific enzyme activity.

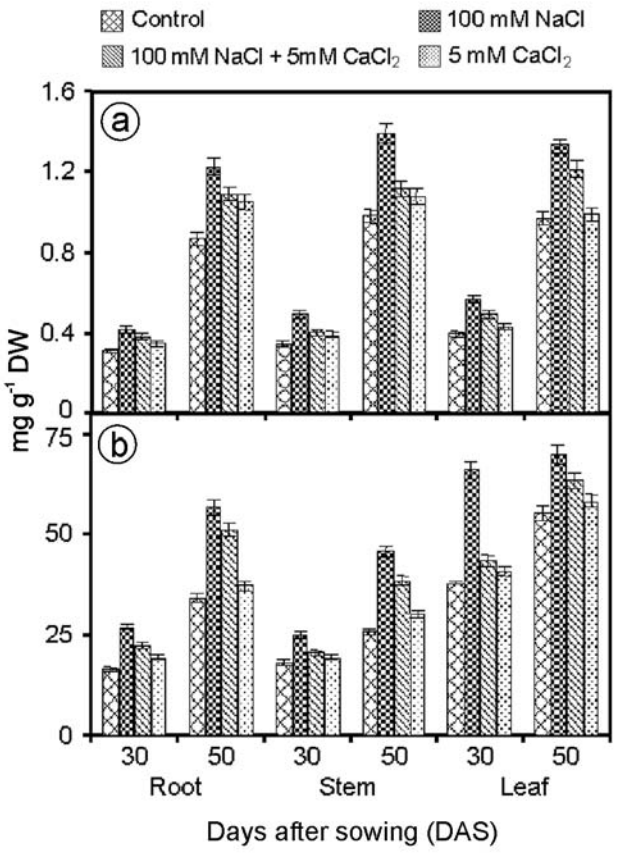

Fig. 1. Effect of NaCl, $CaCl_2$, and NaCl and $CaCl_2$ combined on (**a**) proline and (**b**) glycinebetaine content in *Vigna radiata*. Values are means ± SD of 7 replicates.

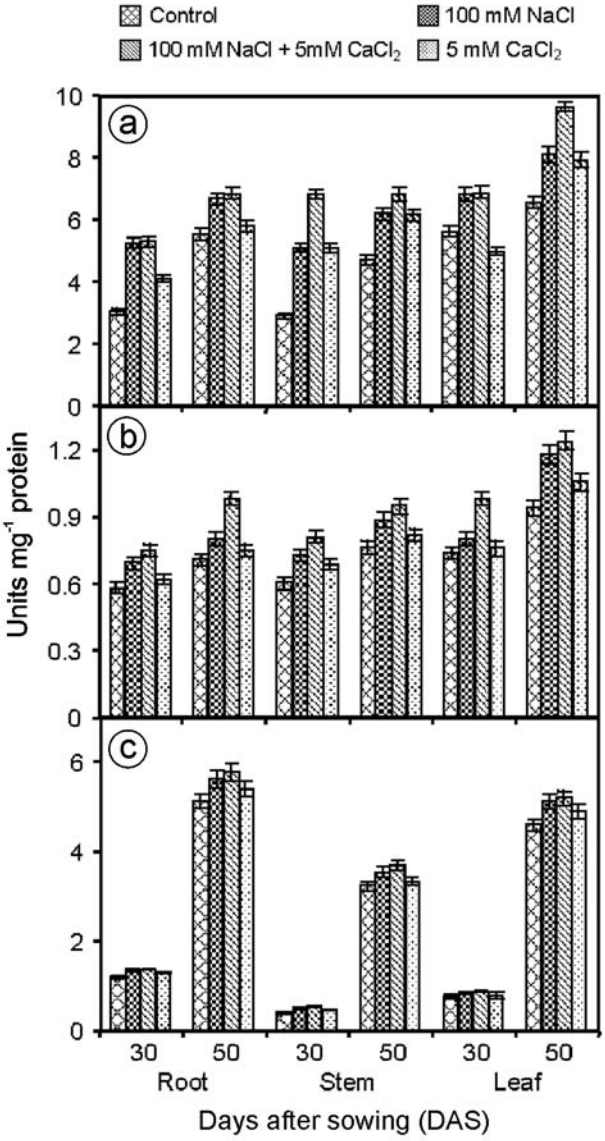

Fig. 2. Effect of NaCl, $CaCl_2$, and NaCl and $CaCl_2$ combined on (**a**) superoxide dismutase, (**b**) ascorbate peroxidase and (**c**) catalase activity in *Vigna radiata*. Values are means ± SD of 7 replicates.

RESULTS AND DISCUSSION

NaCl and $CaCl_2$ stress decreased greengram plant root and stem length and DW versus the control. NaCl combined with $CaCl_2$ increased root and stem length versus treatments with NaCl or $CaCl_2$ alone (Tab. 1). Salinity can inhibit root growth by altering the external water potential, increasing ion toxicity, or causing an ion imbalance (Jaleel et al., 2007b), and can impose biochemical restraints on cell wall expansion, which in turn can inhibit root growth (Iqbal et al., 2006). Separate NaCl and $CaCl_2$ treatments decreased the leaf area versus the control. $CaCl_2$ in combination with NaCl increased the leaf area versus NaCl-treated plants (Tab. 1). Salt stress inhibited cell division and cell expansion, and consequently leaf expansion (Hernandez et al., 2003).

PRO content increased in all parts (root, stem, leaf) of the NaCl-treated and $CaCl_2$-treated plants versus the control (Fig. 1a). Addition of $CaCl_2$ together with NaCl increased the PRO content under NaCl stress, mainly due to the breakdown of PRO-rich protein and fresh synthesis of PRO and amino acid (Jun et al., 2000). It could also be due to prevention or feedback inhibition of synthesis of the biosynthetic enzyme caused by sequestering of PRO away from its site of synthesis or by relaxed feedback inhibition of regulatory step enzymes (Kavikishore et al., 2005). Increased PRO in the stressed plants may be an adaptation to compensate the energy for growth and survival and thereby help the plant tolerate stress, as reported in *Crotalaira striata* (Chandrasekar and Sandhyarani, 1996) and in spinach leaves (Ozturk and Demir, 2003). GB

content was higher in both the NaCl-treated and $CaCl_2$-treated plants than in the control. Plants stressed with combined $CaCl_2$ and NaCl had higher GB than NaCl-stressed plants (Fig. 1b). GB accumulation may serve as an intercellular osmoticum, and may be closely correlated with elevation of osmotic pressure (Girija et al., 2002). Being a quaternary ammonium compound, GB acts as an osmotic solute. Subcellular compartmentation of GB biosynthesis is important for increased salt tolerance (Sakamoto et al., 1998).

SOD activity was increased in all parts of the NaCl-stressed and $CaCl_2$-stressed plants versus the control. Treatment with combined $CaCl_2$ and NaCl increased the plants' SOD activity versus NaCl-stressed plants (Fig. 2a). SOD activity directly modulates the amount of ROS (Hasegawa et al., 2000; Jaleel et al., 2007g). APX activity was higher in all parts of the NaCl-treated and $CaCl_2$-treated plants than in the control. NaCl together with $CaCl_2$ increased the APX activity versus the separate treatments with NaCl and $CaCl_2$ (Fig. 2b). The results were similar for CAT activity (Fig. 2c). The unique importance of Ca^{2+} for stabilization of membranes is well known (Demiral and Turkan, 2006). SOD and CAT activity has been reported to be negatively correlated with the degree of damage to plasmalemma, chloroplast and mitochondrial membrane systems, and positively correlated with stress resistance indices (Elkahoui, et al., 2005). $CaCl_2$- treated seedlings maintain higher levels of SOD and CAT activity and lower levels of lipid peroxidation and peroxidase activity (Sulochana et al., 2002). Our findings highlight the profound role of $CaCl_2$ in salt stress mitigation in crop plants like greengram, and support the efficacy of its use in crop cultivation.

REFERENCES

Bates LS, Waldern RP, and Teare ID. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil* 39: 205–207.

Beauchamp CO, and Fridovich I 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Annals of Biochemistry* 44: 276–87.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry* 72: 248–53.

Chandlee JM, and Scandalios JG. 1984. Analysis of variants affecting the catalase development program in maize scutellum. *Theoretical and Applied Genetics* 69: 71–77.

Chandrasekar KR, and Sandhyarani S. 1996. Salinity induced chemical changes in *Crotalaria striata* DC. plants. *Indian Journal of Plant Physiology* 1: 44–48.

Demiral T, and Turkan I. 2006. Exogenous glycine betaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. *Environmental and Experimental Botany* 56: 72–79.

Elkahoui S, Hernandez JA, Abdelly C, Ghrir R, and Limam F. 2005. Effect of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. *Plant Science* 168: 607–613.

Girija C, Smith BN, and Swamy PM. 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environmental and Experimental Botany* 47: 1–10.

Grieve CM, and Grattan SR. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil* 70: 303–307.

Hasegawa PM, Bressan RA, Zhu JK, and Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* 51: 463–499.

Hernandez JA, Aguilar AB, Portillo B, Lopez-Gomez E, Beneyto JM, and Legaz MFG. 2003. The effect of calcium on the antioxidant enzymes from salt-treated loquat and anger plants. *Functional Plant Biology* 30: 1127–1137.

Jaleel CA, Gopi R, Manivannan P, and Panneerselvam R. 2007a. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. *Turkish Journal of Botany* 31: 245–251.

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, and Panneerselvam R. 2007b. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South African Journal of Botany* 73: 190–195

Jaleel CA, Gopi R, Manivannan P, and Panneerselvam R. 2007c. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiologiae Plantarum* 29: 205–209.

Jaleel CA, Manivannan P, Lakshmanan GMA, Sridharan R, and Panneerselvam R. 2007d. NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus. Comptes Rendus Biologies* 330: 806–813.

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, and Panneerselvam R. 2007e. Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus, Comptes Rendus Biologies* 330: 674–683.

Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, and Panneerselvam R. 2007f. Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation, *Colloids and Surfaces B: Biointerfaces* 60: 110–116.

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, GOPI R, Somasundaram R, and Panneerselvam R. 2007g. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit, *Colloids and Surfaces B: Biointerfaces* 59: 150–157.

Jun HR, Adam LH, Rozwadowski KL, Hammerlineli JL, Keller WA, and Selvaraj G. 2000. Genetic engineering

of glycinebetaine production towards enhancing stress tolerance in plants. *Plant Physiology* 122: 747–756.

LAWLOR DW. 2002. Limitation to photosynthesis in water stressed leaves: Stomata vs. metabolism and the role of ATP. *Annals of Botany* 89: 1–15.

MANIVANNAN P, JALEEL CA, KISHOREKUMAR A, SANKAR B, SOMASUNDARAM R, SRIDHARAN R, and PANNEERSELVAM R. 2007. Propiconazole induced changes in antioxidant metabolism and drought stress amelioration in *Vigna unguiculata* (L.) Walp. *Colloids and Surfaces B: Biointerfaces* 57: 69–74.

OZTURK L, and DEMIR Y. 2003. Effects of putrescine and ethephon on some oxidative stress enzyme activities and proline content in salt stressed spinach leaves. *Plant Growth Regulation* 40: 89–95.

SAKAMOTO A, MURATA A, and MURATA, N. 1998. Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. *Plant Molecular Biology* 38: 1011–1019.

SANKAR B, SOMASUNDARAM R, MANIVANNAN P, KISHOREKUMAR A, JALEEL CA, and PANNEERSELVAM R. 2006. Enhanced salinity tolerance of tomato (*Lycopersicon esculentum* (L.) Mill.) plants as affected by paclobutrazol treatment. *Journal of Current Science* 9(2): 917 – 920

SULOCHANA CH, SREENIVASA RAO TJV, and SAVITHRAMMA N. 2002. Effect of calcium on water stress amelioration through calmodulin and scavenging enzymes in groundnut. *Indian Journal of Plant Physiology* 7: 151–158.

WHITE PJ, and BROADLEY MR. 2003. Calcium in plants. *Annals of Botany* 92: 487–511.