Proc. Indian natn. Sci. Acad. **B57** Nos. 3&4 pp. 267-269 (1991)

Amelioration of NaCl Salinity Stress by Calcium Chloride: Lipid Peroxidation, Superoxide Dismutase and Catalase Activities

P M SWAMY and C V SUBBA REDDY
Department of Botany, S V University, Tirupati 517 502

(Received 7 August 1990; after revision 19 March 1991; Acepted on 3 June 1991)

Leaf discs of *Vigna unguiculata* L., when treated with sodium chloride, showed an increase in lipid perioxidation and a decrease in superoxide dismutase and catalase activities, but a reverse trend was observed when treated with calcium chloride. The NaCl induced lipid peroxidation and the reduced activities of Catalase and SOD were mitigated by $CaCl_2$. The results suggest that the adverse effects of sodium chloride on membrane deterioration may be relieved by calcium chloride by the modulation of lipid peroxidation through maintaining higher levels of cellular scavengers such as SOD and Catalase.

Key Words: NaCl salinity, Amelioration, $CaCl_2$, Enzyme activities

Introduction

Membranes of soybean leaf tissues reportedly become increasingly leaky as the concentration of NaCl increased (Leopold & Willings 1984). $CaCl_2$ protects leaf membranes against the leakiness induced by NaCl and $(NH_4)_2SO_4$ (Levitt 1972, Poovaiah & Leopold 1976).

The mechanism by which $CaCl_2$ protects the membranes from NaCl damage is not clearly understood. It is likely that the free radical-induced lipid peroxidation mediates the membrane deterioration in plant tissues (Dhindsa et al. 1981). The present investigation was undertaken to examine whether $CaCl_2$ can inhibit the free radical-induced membrane deterioration caused by sodium chloride.

Cowpea leaf discs were selected as the experimental plant material. Effect of sodium chloride and calcium chloride individually and in combination on lipid peroxidation, SOD and catalase activities were studied.

Materials and Methods

Cowpea (*Vigna unguiculata* L.) plants were grown for 10 days in earthenware pots containing farmyard manure mixed with red soil (1:3) under natural photoperiods of 11 hr day^{-1}. The maximum temperature varied between 30 ± 2°C and the minimum between 16 and 20°C.

The primary leaves of 10-day-old plants were harvested and washed thoroughly with tap water and then blotted dry with filter paper. Leaf discs of about 5 mm diameter were taken with the help of a cork borer from either side of the midrib. Twenty leaf discs were allowed to float in each petridish of 7.5 cm diameter containing 50 ml of test solutions viz., sodium chloride (50 mM), calcium chloride (5 mM); sodium chloride (50 mM) + calcium chloride (5 mM). Glass-distilled water was used in the control set. The leaf discs of control and treatments were illuminated (500 μE sec^{-1} m^{-2}) for 6 hours.

After 6 hr of incubation, lipid peroxidation and the activities of SOD and catalase were measured.

The level of lipid peroxidation in th leaf discs was measured in terms of amalonaldehyde (MDA) a product of lipid peroxidation by thiobarbituric acid (TBA) reaction. The reactivity of TBA was determined with minor modifications (Heath & Packer 1968).

The level of superoxide dismutase was assayed by measuring its activity to inhibit the photochemical reaction of nitro blue tetrazolium (NBT)

(Beauchamp & Fridovich 1971). Catalase activity was assayed by measuring the initial rate of the disappearance of hydrogen peroxide (Chance & Maehly 1955).

Results

The level of MDA during 6 hr of the light incubation of cowpea leaf discs are shown in table 1. The NaCl-treated leaf discs showed higher level of lipid peroxidation and the increase was 13.24% over control. The lipid peroxidation in the $CaCl_2$-treated leaf discs was very low when compared to either control or the NaCl-treated leaf discs. The decrease in lipid peroxidation is 12.59% over control. NaCl in the presence of $CaCl_2$ showed a little increase in the lipid peroxidation and the per cent of increase is only 1.32.

NaCl treatment caused a large decrease in the specific activity of superoxide dismutase over control during the 6 hr of incubation (table 2). The decrease was 24.5% over the control. On the other hand, the $CaCl_2$-treated leaf discs maintained a higher level of enzyme activity over control. The increase in the enzyme level was 30.3% over control. However, the specific activity of SOD moved nearer to the control in the leaf discs treated with NaCl + $CaCl_2$ (2.42% over control).

The catalase activity showed a sharp decrease of 17.1% over the control in the leaf discs treated with NaCl. The $CaCl_2$-treated leaf discs were found to have a higher (15.6%) catalase activity over control. However, in the interaction between NaCl and $CaCl_2$, the enzyme activity was close to the control leaf discs, the increase being 1.6% over control (table 3).

Discussion

The results obtained in this study show that (i) NaCl stress of leaf discs is accompanied by an increase in lipid peroxidation, (ii) $CaCl_2$ apparently alleviated NaCl stress, atleast in part, by maintaining high levels of SOD and catalase activities which might be expected to control lipid peroxidation.

The leaf discs treated with NaCl showed a decrease in the activities of SOD and catalase and an increase in lipid peroxidation. The increase in lipid

Table 1 *Influence of NaCl (50 mM), $CaCl_2$ (5 mM) and their interaction on malonaldehyde (MDA) content of cowpea leaf discs (MDA n mol g^{-1} fr.wt) (Values are mean of 3 replications)*

Treatment	MDA content	% Increase (+) or decrease (−) over control
Control	37.75 (±0.11)	
NaCl	42.75 (±0.22)	+13.24
$CaCl_2$	33.00 (±0.32)	−12.59
NaCl + $CaCl_2$	38.25 (±0.14)	−01.32

Values in the parentheses indicate standard error (±)

Table 2 *Influence of NaCl (50 mM), $CaCl_2$ (5 mM) and their interaction on specific activity of super oxide dismutase of cowpea leaf discs (MDA n mol g^{-1} fr wt) (Values are mean of 3 replications)*

Treatment	SOD activity	% Increase (+) or decrease (−) over control
Control	3.30 (±0.27)	
NaCl	2.49 (±0.09)	−24.55
$CaCl_2$	4.30 (±0.77)	+30.30
NaCl + $CaCl_2$	3.38 (±0.11)	+2.42

Values in the parentheses indicate standard error (±)

Table 3 *Influence of NaCl (50 mM), $CaCl_2$ (5 mM) and their interaction on catalase activity of cowpea leaf discs. Activity expressed as mg of H_2O_2 destroyed in 5 min/g fr.wt (Values are mean of 3 replications)*

Treatment	Catalase activity	% Increase (+) or decrease (−) over control
Control	0.64 (±0.06)	—
NaCl	0.53 (±0.2)	−17.19
$CaCl_2$	0.74 (±0.03)	−15.63
NaCl + $CaCl_2$	0.65 (±0.03)	−01.56

Values in the parentheses indicate standard error (±)

peroxidation was correlated with the decreased levels of both catalase and superoxide dismutase (Fridovich 1976). Superoxide dismutase and catalase remove O_2 and H_2O_2 respectively. An interaction between O_2 and H_2O_2 can generate OH, and O_2 (Kellogg & Fridovich 1975). The ability of OH, O_2 and O_2 to initiate lipid peroxidation has been demonstrated (Fang 1984, Pederson & Aust 1973). Therefore a decrease in SOD and catalse activities could result in a greater availability of the free radicals which in turn increases lipid peroxidation and result in membrane deterioration.

The unique importance of Ca^{2+} for stabilization of membranes is well known (Marinos 1962). The results of this study show that the alleviation of NaCl effect by Ca^{2+} is mediated, atleast in part, by the maintenance of high level of activities of SOD and catalase.

In conclusion, the present study shows that free radical-induced lipid peroxidation is involved in NaCl stress and that Ca^{2+} alleviating the effect of NaCl salinity, atleast in part by modulating lipid peroxidation and by maintaining higher levels of superoxide dismutase and catalase activities.

References

Beauchamp C and Fridovich I 1971 Superoxide dismutase improved assays and an assay applicable to PAGE; *Analyst. Biochem.* **44** 276-287

Chance B and Maehly A C 1955 Assay of catalases and peroxidases; *Methods Enzymol.* **2** 764-775

Dhindsa R, Dhindsa P P and Thorpe A Z 1981 Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and increased levels of superoxide dismutase and catalase; *J. Exp. Botany* **32** 93-101

Fang L Z 1984 Superoxide dismutase activity and lipid peroxidation in relation to senescence of rice leaves; *Zhiuru xuelao* **26** 605-615

Fridovich I 1976 Oxygen radicals hydrogen peroxide and oxygen toxicity; *Free Radicals in Biology* **1** 239-277

Health R L and Packer L 1968 Photoperoxidation in isolated chloroplasts I Kinetics and Stoichiometry of fatty acid peroxidation; *Arch. Biochem. Biophys.* **125** 189-198

Kellogg E W and Fridovich I 1975 Superoxide hydrogen peroxide and singlet oxygen in lipid peroxidation by the xanthine oxidase system; *J. Biol. Chem.* **250** 8812-8817

Leopold A C and Willings R P 1984 Evidence for toxicity effects of salt on membranes; in *Salinity Tolerance in Plants*, pp. 67-76 ed Richard C Staples and Gary H Toennicson (New York: John Willery and Sons Inc.)

Levitt T 1972 *Salt and Ion Stresses in Response of Plants to Saline Environments*, pp. 489-524 (New York: Academic Press)

Marinos N G 1962 Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley; *Amer. J. Bot.* **49** 834-841

Pederson T C and Aust S D 1973 The role of superoxide and singlet oxygen in lipid peroxidation promoted by xanthine oxidase; *Biochem. Biophys. Res. Com.* **52** 1071-1078

Poovaiah B W and Leopold A C 1976b Effects of inorganic salts on tissue permeability; *Pl. Physiol.* **58** 182-185