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ORIGINAL ARTICLE

SALT STRESS MITIGATION BY CALCIUM CHLORIDE IN VIGNA MUNGO (L.) HEPPER

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Abstract:

Present study is aimed to elucidate the adverse effects of NaCl on growth and metabolism and its amelioration by $CaCl_2$ .The physiological studies were made to examine the effect of 75mM NaCl stress and its amelioration by 10 mM $CaCl_2$ and the combination of 75 mM NaCl with 10 mM $CaCl_2$ during seed germination and seedling growth from 2nd to 8th day after sowing at interval of 2 days . The physiological parameters include changes in the proline , glycine betaine contents and the activity of the antioxidant enzymes like superoxide dismutase and catalase were studied in the seedlings.

KEYWORDS:

Sodium chloride, Calcium chloride, Amelioration, Vigna mungo, Growth, Antioxidant enzymes.

INTRODUCTION:

Salinity is regarded as one of the major environmental stress which adversely affects plant growth and metabolism and limits crop production [1] , [2], [3], [4]. In saline soils plant faces major problem of obtaining water from a soil of negative osmotic potential as well as growing plant has to deal with high concentrations of potentially toxic ions of sodium, chloride etc., [5].

Agricultural Soils of arid and semi arid areas, where irrigation is employed salinity is increased [6]. Plant salt-tolerance expressed by morpho-physiological characteristics [7], molecular / biochemical [8] mechanisms that are induced under these stress conditions and delays germination and seedling emergence [9].

High sodium disrupts potassium (K+) nutrition, and when accumulated in the cytoplasm it inhibits many enzymes [10]. 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 [11]. 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 [12] , [13].

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 [14]. Little attention has been made to study growth and metabolism to elucidate the adverse effects of NaCl salinity and its amelioration by CaCl2 during early seedling growth of blackgram (Vigna mungo (L.) Hepper). Hence an attempt had been made to study the ameliorating effect of calcium on NaCl stress in Vigna mungo during seedling growth.

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MATERIAL AND METHODS

Blackgram (Vigna mungo (L.) Hepper cv. PBG-1] seeds were obtained from Regional Agriculture Research Centre, S.V. Agricultural College, Tirupati. The seeds were surface sterilized with 0.2 % $HgCl_2$ solution for 5 minutes with frequent shaking and washed thoroughly with distilled water. The seeds were presoaked in 500 ml of distilled water of 12 hours. The seeds were germinated on fluted filter paper towels in bread boxes and irrigated with different concentrations ranging from 10 to100 mM NaCl and 75 mM NaCl selected based on the minimum percentage of seed germination and control seeds were maintained irrigating with distilled water.

Similarly seeds were also grown in separate bread boxes over fluted filter paper towels and irrigated with various concentrations from 10 to 50 mM $CaCl_2$ solutions. Based on the maximum percentage of seed germination 10 mM $CaCl_2$ was selected. Ten seeds were germinated separately in distilled water Control, 75 mM NaCl, 10 mM $CaCl^2$ and combination of 75 mM NaCl + 10 mM $CaCl_2$. The maximum temperature during the experimental period varied between 30 ^{oC} to 42 ^{oC} during the experimental period.

The seedlings were harvested randomly on 2nd, 4th, 6th and 8th days after sowing. Three replicates were maintained and in each replicate five seedlings were taken for experiments.

MORPHOLOGICAL PARAMETERS

Root length, Shoot length and Fresh weight , Dry weight were calculated in the seedlings.

BIOCHEMICAL PARAMETERS

Proline and Glycinebetaine were extracted and estimated as per the methods [15] and [16] expressed as μg g -1 DW.

ANTIOXIDANT ENZYME ACTIVITIES

SOD (Superoxide dismutase E.C. 1.15.1.1)

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to [17]. 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. The values are expressed as means ± SE of five samples in each group.

Catalase activity (E.C. 1.11.1.6)

The activity of ctalase was estimated by the method of [18]. The 1g fresh material of seedlings were placed in pre – chilled mortor and pestle and homogenized with 10 ml of 0.05 M ice cold Tris-HCl buffer (PH 7.0), the extract was passed through cheese cloth and centrifuged t 3500 Xg for 20 minutes. The resultant supernatant was used as a source of enzyme. The reaction mixture contained 1 ml of enzyme, 2ml of H_2O_2 (0.005M) and 3ml of Tris-HCl buffer (PH 7.0). The rection was stopped by adding 10 ml of 2.5N H_2SO_4. After 1 min of incubation at 20 ^{o}C , the residual H_2O_2 was titrated with 0.01N $KMNO_4$. A blank was prepared by adding the extract to an acidified solution of raction mixture at zero time. Catalase activity was expressed as mg H_2O_2 oxidised / g-1 fresh weight / minute.

RESULTS AND DISCUSSION

NaCl treatment was decreased root and shoot length when compare in blackgram seedlings combination of NaCl with $CaCl_2$ increased root and shoot length when compare with NaCl separately. Maximum growth was observed in 10 mM CaCl2 treated seedlings (Table – 1). Salinity can inhibit root growth by altering the external water potential, increasing ion toxicity, or causing an ion imbalance [19].

Synergetic effect of NaCl and $CaCl_2$ reduced the salt stress and enhanced the growth.

Fresh weight of the seedlings decreased continuously from 2 nd to 8 th day after sowing in all the treatments including control (Table -2). NaCl treatment caused considerable reduction in the fresh weight than the control, $CaCl_2$ or NaCl with $CaCl_2$. The dry weight of the blackgram seedlings decreased continuously during germination. NaCl treatment caused decrease in dry weight than the other treatments including control.

Proline accumulation found in the seedlings treated with NaCl when compare to all other treatments including control. Increased proline 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 [20] and in spinach leaves [21]. NaCl treatment caused an appreciable increase in the proline content of the seedlings than the other treatments (Fig-1).

It could also be due to prevention or feedback inhibition of synthesis of the biosynthetic enzyme caused by sequestering of proline away from its site of synthesis or by relaxed feedback inhibition of regulatory step enzymes [22]. On the otherhand proline content decreased by the addition of $CaCl_2$ while $CaCl_2$ alone was similar in the effect of the control. Increase in proline content under NaCl + $CaCl_2$ treatment was much less than NaCl treatment. Increased proline may be an adaptation to overcome the salinity [23].

Proline is an important compatible solute, that plays a role in osmotic adjustment, stablizes sub-cellular structures, scavenges free radicals, nitrogen source, as well as generation of ATP [24].

The blackgram seedlings showed an increase in the glycine betaine content from 2^{nd} to 8^{th} day after sowing (Fig-2). NaCl treatment caused an increase in the glycine betaine content of the seedlings treated with calcium chloride. The increase in the glycine betaine content of the seedlings treated with 10 mM $CaCl_2$ was lower than the other treatments except control. NaCl treatment caused an increase in the glycine betaine content of the embryonic axis of the seedlings than the control and $CaCl_2$ treatments. $CaCl_2$ with NaCl treatment caused an increase in the glycine betaine content of the seedlings than the other treatments. Glycinebetaine accumulation may serve as an intercellular osmoticum, and may be closely correlated with elevation of osmotic pressure [23]. Glycine betaine content increase in addition $CaCl_2$ to NaCl stressed seedlings than NaCl alone. An increase in the glycinebetaine levels under stress conditions was found to icrease the sodiumflux from the cytoplasm to the vacuole and was also known to modify the membrane behavior in water stressed barley leaves [25]

The level of superoxide dismutase activity of the seedlings decreased gradually from 2^{nd} to 8^{th} day after sowing (Fig-3). $CaCl_2$ treatment caused greater increased in the level of superoxide dismutase when compared to the other treatments. The level of enzyme activity was lower in the seedlings treatment with NaCl. The superoxide dismutase activity was inhibited in the NaCl stressed seedlings when compared to control and $CaCl_2$ treatment. SOD catalyzes the dismulation of superoxide radical ($O_{2.}$) with great efficiency resulting in the production of H_2O_2 and O_2 (26). Salt inhibition of the superoxide dismutase activity in the salt stressed pea leaves was reported earlier [27] and [28]. The decrease in the SOD activity could activate the accumulation of Oxygen species which in turn causes oxidative damage in the salt stressed seedlings. A decrease in SOD activity could impair the $O_{2.}$ scavenging system of cells and favour accumulation of $O_{2.}$ [29]. Because the measured enzyme activity is a result of both synthesis or enhanced degradation of the enzyme. In addition the accumulation of H_2O_2 under drought also could lower SOD activity. The depression of SOD activity in plants have been observed with abiotic stresses also [30], [31], [29] .

On the other hand NaCl caused a greater decrease in the level of enzyme activity at all stages of seedlings growth. Addition of $CaCl_2$ to the stressed seedlings caused an increase in the level of activity than control seedlings. $CaCl_{2.}$ treated seedlings maintain higher levels of SOD and catalase activity and lower levels of lipid peroxidation and peroxidase activity [32].

The catalase activity dcreased from 2^{nd} to 8^{th} day during seedling growth (Fig - 4). The higher level of catalase activity found in the $CaCl_2$ treated seedlings than that of other two treatments. However the lower level of catalase ativity was found in NaCl treated seedlings than the $CaCl_2$ and NaCl with $CaCl_2$ treated seedlings.

The $CaCl_2$ treated seedlings showed higher level of catalase activity when compared to other treatments including control seedlings. The $CaCl_2$ treated seedlings showed higher levels of catalase activity in wheat and maize seedlings [29], [33]. Free radical induced lipid peroxidation is involved in NaCl stress and that Ca^{2+} alleviating the effect of NaCl salinity, at least in part by modulating lipid peoxidation and by maintaining higher levels of superoxide dismutase and catalase activities [34]. The decrease in SOD and catalase activities can be seen as weakening factor of the scavenger mechanism of the plants. The two

enzymes are no longer able to oppose efficiently in the increased radical production under stress.

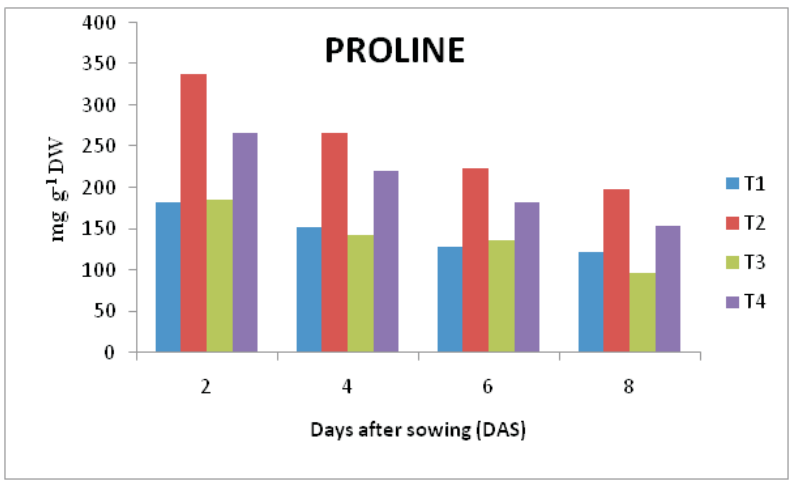
Table – 1. Effect of NaCl (75mM), CaCl2 (10 mM) and their combination on the root and shoot length (in cms) of Vigna mungo seedlings during germination.
(Values are mean of 3 replications ± SE)

Treatments	Days after sowing (DAS)							
	2		4		6		8	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
Control	0.301 ± 0 .005	2.523 ± 0 020	2.140 ± 0.015	5.260 ± 0.011	3.653 ± 0.018	6.41 ± 0.12	4.42 ± 0.017	6.616 ± 0.012
75 mM NaCl	0.080 ± 0 .007	0.356 ± 0.012	2.626 ± 0 .069	3.840 ± 0.014	3.150 ± 0.005	4.21 ± 0.04	3.843 ± 0 .048	5.320 ± 0.052
10mM $CaCl_2$	0.453 ± 0 .008	3.133 ± 0.011	2.396 ± 0.012	5.900 ± 0.057	4.120 ± 0.015	7.10 ± 0.06	6.33 ± 0.04	8.716 ± 0.014
75mM NaCl + 10mM $CaCl_2$	0.276 ± 0.006	1.136 ± 0.012	2.850 ± 0.037	3.466 ± 0.683	4.536 ± 0.038	6.30 ± 0.05	5.65 ± 0.04	6.875 ± 0.365

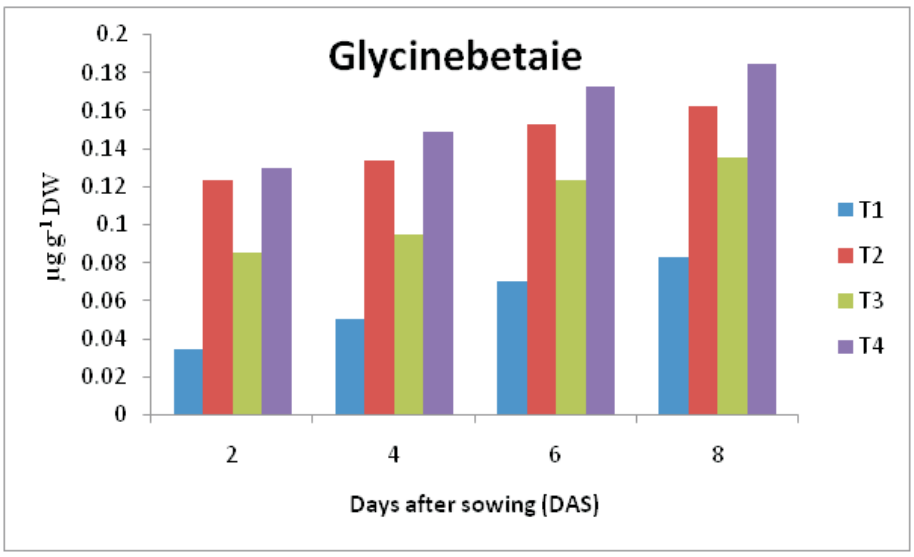
Table -2. Effect of NaCl (75mM), CaCl2 (10 mM) and their combination on the fresh weight and dry weight (in mg) of Vigna mungo seedlings during germination.
(Values are mean of 3 replications ± SE)

Treatments	Days after sowing (DAS)							
	2		4		6		8	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	0.192 ±.0046	0.032 ±.0055	0.181 ±.0058	0.031 ±.0063	0.160 ±.0057	0.028 ±.0020	0.140 ±.0057	0.023 ±.0012
75 mM NaCl	0.121 ±.0058	0.023 ±.0057	0.110 ±.0057	0.022 ±.0006	0.100 ±.0057	0.021 ±.0008	0.090 ±.0057	0.018 ±.0029
10mM $CaCl_2$	0.151 ±.0044	0.025 ±.0014	0.140 ±.0057	0.025 ±.0008	0.130 ±.0057	0.024 ±.0012	0.110 ±.0057	0.023 ±.0072
75mM NaCl + 10mM $CaCl_2$	0.141 ±.0066	0.024 ±.0008	0.130 ±.0063	0.024 ±.002	0.120 ±.0057	0.023 ±.0003	0.100 ±.0057	0.021 ±.0014

Fig- 1. Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on Proline content in Vigna mungo. Values are mean of 3 replications ± SE.

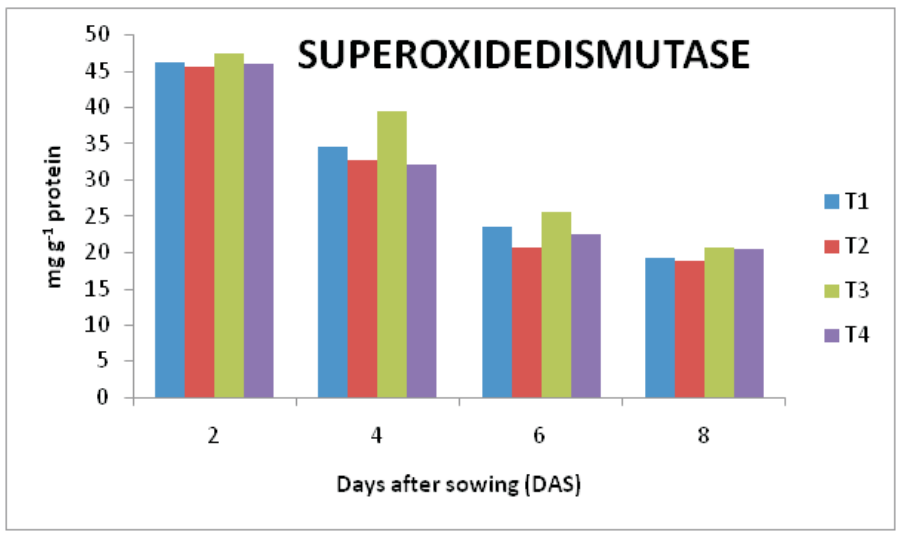


T 1 = Control, T2 = 75 mM NaCl, T3 =10mM CaCl2 , T4 = 75 mM NaCl + 10mM CaCl2
Fig- 2. Effect of NaCl (75mM), CaCl2 (10 mM) and their combination on Glycinebetaine content in Vigna mungo. Values are mean of 3 replications ± SE.



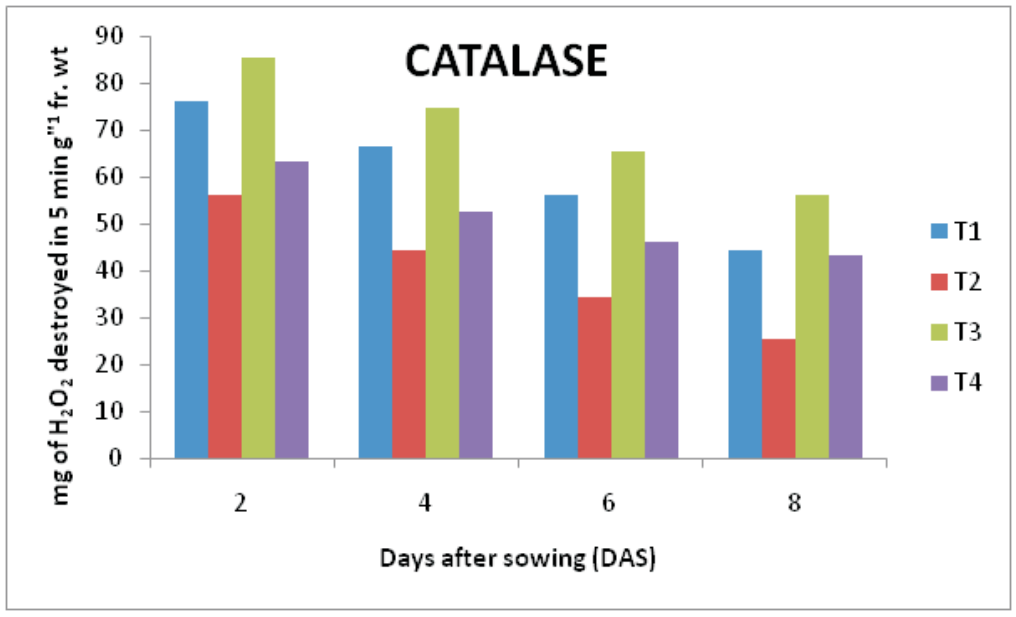
T 1 = Control, T2 = 75 mM NaCl, T3 =10mM CaCl2 , T4 = 75 mM NaCl + 10mM CaCl2

Fig- 3. Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on Superoxide dismutase activity in Vigna mungo. Values are mean of 3 replications ± SE.



T 1 = Control, T2 = 75 mM NaCl, T3 =10mM CaCl2 , T4 = 75 mM NaCl + 10mM CaCl2

Fig- 4. Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on catalase activity in Vigna mungo. Values are mean of 3 replications ± SE.



T 1 = Control, T2 = 75 mM NaCl, T3 =10mM CaCl2 , T4 = 75 mM NaCl + 10mM CaCl2

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