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Effect Of Calcium Chloride On Growth And Biochemical Changes Of Black Gram (vigna Mungo L .) Under Salt Stress

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Abstract: The present study was aimed to observed the ameliorative effect of Ca^{2+} on the seedling growth of mung bean under salinity stress. Seedlings were exposed to various concentrations of $CaCl_2$ and NaCl separately and standard concentrations were selected based on the growth. The experiments were carried out in the seedlings exposed to 75 mM NaCl, 10 mM $CaCl_2$ treatments along with the combination of 10 mM $CaCl_2$ and 75 mM NaCl. The control was maintain with distilled water. Experiments were carried out in two days interval upto 8th day from the transfer of seedlings to treatments. The seedlings exposed to 75 mM NaCl concentration showed slow growth (both shoot and root length) when compare to other treatments. Biomass production and Relative Water Content was found to be higher in $CaCl_2$ treated seedlings. The seedlings under salt stress maintained less proteins and nucleic acids than that of $CaCl_2$ and water and combination of NaCl with $CaCl_2$. The experimental results indicate that the $CaCl_2$ ameliorate the effect of NaCl stress by maintaining the Relative Water Content , protein and nucleic acids. Seedlings were treated with solutions of 75 mM NaCl, 10 mM $CaCl_2$ and the combination of 75 mM NaCl with 10 mM $CaCl_2$ during seedling growth from 2^{nd} to 8^{th} day after transferring at interval of 2 days. The growth and biochemical parameters include changes in the protein, DNA and RNA contents were studied in the seedlings.

Keyword: Sodium Chloride, Calcium Chloride, Vigna mungo, Growth, Protein, DNA and RNA.

INTRODUCTION:

Salinity stress represents a worldwide increasing environmental problem for crop production. Approximately 6% of the world's total land area and 23% of the cultivated lands are characterized by various salt-degraded soils (FAO, 2005) and the continuous accumulation of salt in cultivated soils as a result of irrigation and climate warming increases the importance of this stressful factor (Szabolcs, 1994). Saline impact on the osmotic potential of plants and soils subsequently affects water availability due to the limitation of water uptake of the plants. In addition, excessive uptake of Na and Cl may result in a limited assimilation, transport and distribution of mineral nutrients, as well as nutrient imbalances within the plants (Lauchli and Epstein, 1990; Marschner, 1995). Elevated NaCl levels in the root medium reduce the nutrient assimilation, especially of K and Ca, resulting in ion imbalances of K, Ca and Mg compared to Na (Gabr, 1999; Khan et al., 2000a).

Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behaviour (Marschner, 1995; Rengel, 1992). Because calcium appears to be readily displaced from its membrane binding sites by other cations, these functions may become seriously impaired by reduced calcium availability. Increasing the external concentration of calcium largely counteracted this displacement (Lynch and Lauchli, 1988). Maintaining an adequate supply of calcium in saline solutions is an important factor in controlling the severity of specific ion toxicities, particularly in crops which are susceptible to sodium and chloride injury (Grattan and Grieve, 1999; Maas, 1993). When plants are challenged with salinity stress, an increase in the concentration of Ca2+ often can ameliorate the inhibitory effects on growth. The role of Ca^{2+} as a second messenger in many biological systems, coupled with these observations, indicates that plants are able to adjust to high salt environments by activating a signal transduction system involving Ca^{2+} (Hasegawa et al., 2000). To overcoming the negative impact of salinity, addition of supplemental Ca^{2+} to the growth medium as an ameliorative agent could be necessary. Number of experiments were conducted to assess the effectiveness of supplemental calcium on mitigating the effect of salinity stress in various plants, but the role of supplemented Ca^{2+} in amelioration of NaCl stress in mung bean during seedling growthis scanty. Hence the present study was undertaken to test the growth and some biochemical changes during stress conditions. The aim was to determine if this would correct Ca^{2+} deficiencies in the presence of high NaCl and also to assess effects of supplemental Ca^{2+} on some key growth and biochemical parameters.

Okcu (2005) and Naveed Khalid et al., (2001)

reported that high salinity reduced root and shoot length and seedling fresh weight and dry weight in chickpea.

A relationship between protein metabolism and NaCl stress in plants were well documented (Levitt, 1972). Salinity affects the metabolism of nitrogen containing compounds eg. Protein synthesis and free amino acid pool composition (Poljakoff – Mayber, 1982). In general, rates of protein synthesis are lower in NaCl treated plants (Aspinall, 1986). Sodium chloride salinity decreases protein synthesis and increases its hydrolysis in many crop plants for instance, in pea roots (Klyshev and Rakova, 1964; Sulochana et al.,2002), grape leaves (Saakyan and Petrosyan, 1964), Phaseolus aconitifolius (Huber et al.,1977), Phaseolus vulgaris (Younis et al., 1993), brinjal (Maliwal and Nanawati, 1974), tomato (Maliwal, 1975), chick pea (Kumar et al., 1982) and cotton (Pessarakli and Tucker, 1985).

DNA and RNA contents to various degrees (Ashraf et al., 1996). The oxidative stress may lead to DNA damage and mutagenesis, protein and carbohydrate oxidation and metabolic disorders (Sies, 1985; 1986; Pryor and Godber, 1991).

MATERIAL AND METHODS

Black gram [Vigna mungo (L.) Hepper cv. LBG-623] is a salt sensitive variety. 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. The two day old seedlings when exposed to different concentrations of NaCl ranging from 10 to100 mM and 75 mM NaCl was selected based on the minimum growth of seedlings , same way seedlings also exposed to various concentrations of $CaCl_2$ ranging from 10 to 50 mM, based on the maximum growth of seedlings 10 mM $CaCl_2$ was selected. Then the two day old seedlings were transferred separately in distilled water as the Control, 75 mM NaCl, 10 mM $CaCl_2$ and combination of 75 mM NaCl + 10 mM CaCl2. The experiments were carried out 2 day interval upto 8^{th} day. The maximum temperature during the experimental period varied between 30°C to 42°C during the experimental period.

GROWTH PARAMETERS

Root length and Shoot length were calculated in the seedlings during growth. Relative water content (RWC) was calculated by using the formula. Fresh weight and dry weight of t seedlings were measured in intravels of in all concentrations and results were tabulated.

RWC = Fresh weigh-Dry weight / Turgid weight-Dry weight X 100

Biochemical parameters

The protein content was estimated as per the method of Bradford (1976) and total contents of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were estimated by using the method of Jayaraman (1981).

RESULTS AND DISCUSSION

Screenig of seedlings growth in different concentrations of NaCl (Table-1) and different concentrations of CaCl2 (Table-2). Apart from based on the maximum growth of seedlings 10 mM $CaCl_2$ was selected. 75 mM NaCl was selected based on the minimum growth of seedlings compare with other treatments.

NaCl treatment seedlings showed slow root and shoot length when compare seedlings combination of NaCl with $CaCl_2$ increased root and shoot length, when compare with NaCl separately. Maximum growth was observed in 10 mM $CaCl_2$ treated seedlings (Table – 3). Salinity can inhibit root growth by altering the external water potential, increasing ion toxicity, or causing an ion imbalance (Evans, 1969; Savithramma, 2003). Synergetic effect of NaCl and $CaCl_2$ reduced the salt stress and enhanced the growth. NaCl treatment caused reduction in the shoot and root length. $CaCl_2$ of the other hand caused increase in the length of shoot and root. Addition of $CaCl_2$ stressed seedlings caused ameliorative effect of NaCl stress by increasing the shoot and root length during seedling growth.

Increase in the fresh weight might be due to enhanced ell division and differentiation. It is well known that Ca^{2+} could modulate developmental metabolisms, membrane conductance and some hydrolytic enzymes and proteins functioning in cell division and development (Clarkson and Hanson, 1980) (Table- 4).

The Relative Water Content is high level in $CaCl_2$ treated seedlings. NaCl treate seedlings showed lower level of Relative water content. Comparing with $CaCl_2$ or combination with NaCl treated seedlings (Table- 5)

NaCl treatment caused slower decrease in the seedlings than the seedlings treated with either $CaCl_2$ and its combination with NaCl. However, the disappearance was more rapid in the seedlings treated with NaCl during seedling growth (Fig-1).

Sodium chloride stress caused retention of more protein in the seedlings than that of the control and other treatments. The slow decrease of protein content in the seedlings of NaCl treated seedlings may be related to the inhibition of hydrolytic enzymes caused by sodium chloride ions.

Reduction in protein content under salinity have been well documented by various workers in different crop plants like peas (Uprety and Sarin, 1975), rice (Krishnamurthy, 1991), Pigeon Pea (Gill and Sharma, 1993), Phaseolus vulgaris (Younis et al, 1993), tomato (Perez - Alfocea et al, 1993), Crotalaria striata (Chandrashekar and Sandhya Rani, 1996). Salinity adversely affected the protein metabolism. Protein degradation under saline environment have been reported due to decrease in protein synthesis, accelerated proteolysis, decrease in the availability of amino acids and denaturation of enzymes involved in protein synthesis (Levitt, 1972; and poljakoff - Mayber, 1982). It was observed that the protein content of the black gram decreased with increasing concentration of NaCl (Anilkumar et al, 1996). However, the protein content slightly increased in the

salt resistant varieties of barley, wheat and sunflower (Helal et a!., 1975; Saha and Gupta, 1993). Black gram, a salt sensitive species showed a reduced protein content under salt stress.

The protein content of the seedlings treated with $CaCl_2$ was higher in the seedlings growth than that of the other treatments. This may be due to the activation of certain enzymes which are under the control of calcium. The enzymes that are known to be under the control of calcium are α -amylase (Chrispeels and Varner, 1967), pyruvate kinase (Mildvan and Cohn, 1965), threonyl SRNA synthase (Allande et al, 1965), Phosphate dehydrogenase (Marquet, 1964) Polygalacturonic transaminase (Starr and Moran, 1962) Phospholipase (Davidson and Long, 1958).

The seedlings treated with NaCl caused a lower level of DNA content. Seedlings treated with either $CaCl_2$ or the combination of $CaCl_2$ and NaCl caused a decline, but the decline was in between the control and NaCl treatments of black gram seedlings (Fig-1).

The RNA content of the seedlings showed slow in the control and treated seedlings (Fig-1). The decline was at different levels based on the nature of treatment. NaCl treatment caused a slower decline than the other two treatments ($CaCl_2$ and $CaCl^{2+}$ NaCl).

In the present study DNA and RNA contents showed lower level during seedling growth from 2nd to 8th day after transferring. Such changes in DNA and RNA contents are reported by earlier workers (Udavenko and Gogoleva, 1974; Lamathia et al., 1985; Lalonde and Dhindsa, 1990; Bates et al., 1990; Momotaini et al., 1991; Balaska and Kubica, 1992; Sreenivasulu, 1996; Basha, 2011). The NaCl treated seedlings showed lower levels of DNA and RNA content than the $CaCl_2$ and combination of both $CaCl_2$ and NaCl treated seedlings. The DNA and RNA contents of leaves as well as roots have shown to decline during water and salt stress (Khan and Garg, 1981). Shah and Loomis (1965) reported that DNA content per cell which remained relatively constant during water stress. Decrease in DNA is typically smaller than that of RNA (Thiman, 1980). The salt stress appears to increase in RNAse activity and decrease in RNA content. The oxidative stress may lead to DNA damage and mutagenesis, protein and carbohydrate oxidation and metabolic disorders (Sies, 1985; 1986; Pryor and Godber, 1991).

$CaCl_2$ treated seedlings showed higher levels of DNA and RNA contents when compared to all other treatments during seedling growth. Calcium regulates various nuclear activities including DNA synthesis and nuclear fusion (Dauwalder et al., 1985). Gypsum treated plants in turn caused enhancement in the level of enzymes, relating to nitrogen metabolism (Boynton et al., 1980). $CaCl_2$ treatment was found to delay nuclear disintegration and maintains RNA and protein levels in Lemna (Trewaves, 1972; Poovaiah and Leopold 1973).

CONCLUSION

$CaCl_2$ treatment showed higher levels of protein content than the treatments of NaCl with $CaCl_2$, NaCl and control seedlings. Ca^{2+} exerts control over the membrane protein proteolysis and maintain the stability of membrane proteins and enzymes. This may lead to enhancement of protein content of the $CaCl_2$ treated seedlings.

Higher levels of DNA and RNA content were observed in $CaCl_2$ treated seedlings may be due to the fact that Ca^{2+} along with calmodulin regulates various nuclear activities including the initiation of synthesis and regulation of cell division.

Table 1. Effect of different concentrations of NaCl on the growth (in cms) of Vigna mungo seedlings. Values are mean of 3 replications ± SE.

Treatments	Days after treatment of seedlings			
	2	4	6	8
Control	0.31 ± 0.011	2.04 ± 0.020	3.52 ± 0.55	4.38 ± 0.49
10 mM NaCl	0.28 ± 0.017	2.7 ± 0.056	4.4 ± 0.061	5.42 ± 0.55
25 mM NaCl	0.27 ± 0.01	2.6 ± 0.046	4.32 ± .053	5.51 ± 0.53
50 mM NaCl	0.186 ± 0.003	1.92 ± 0.017	3.96 ± 0.017	5.25 ± 0.56
75 mM NaCl	0.09 ± 0.000	2.52 ± 0.011	3.24 ± 0.026	3.72 ± 0.54
100 mM NaCl	0.12 ± 0.002	2.150 ± 0.005	3.303 ± 0.012	3.84 ± .034

Table 2. Effect of different concentrations of CaCl2 on the growth (in cms) of Vigna mungo seedlings. Values are mean of 3 replications ± SE.

Treatments	Days after treatment of seedlings			
	2	4	6	8
Control	0.30 ± 0.051	2.13 ± 0.066	3.58 ± 0.568	4.40 ± 0.640
10 mM $CaCl_2$	0.46 ± 0.04	2.40 ± 0.0636	4.13 ± 0.513	6.35 ± 0.490
20 mM $CaCl_2$	0.08 ± 0.051	2.61 ± 0.051	3.14 ± 0.56	3.82 ± 0.614
30 mM $CaCl_2$	0.28 ± 0.0580	2.8 ± 0.0705	4.56 ± 0.548	5.64 ± 0.626
40 mM $CaCl_2$	0.26 ± 0.005	2.63 ± 0.0384	4.32 ± 0.5831	5.52 ± 0.5427
50 mM $CaCl_2$	0.27 ± 0.011	2.45 ± 0.058	4.21 ± 0.58	5.44 ± 0.55

Table3: Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on the Shoot length and Root length (in cms) during seedling growth of Vigna mungo. Values are mean of 3 replications ± SE.

Treatments	Days after treatment of seedlings							
	2		4		6		8	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
Control	0.30 ± 0 .005	2.523 ± 0 020	2.140 ± 0.015	5.260 ± 0.011	3.63 ± 0.061	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	3.84 ± 0 .04	5.320 ± 0.052
10 mM $CaCl_2$	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
75 mM NaCl + 10 mM $CaCl_2$	0.276 ± 0.006	1.136 ± 0.012	2.850 ± 0.037	4.20 ± 0.057	4.536 ± 0.038	6.30 ± 0.05	5.65 ± 0.04	6.875 ± 0.365

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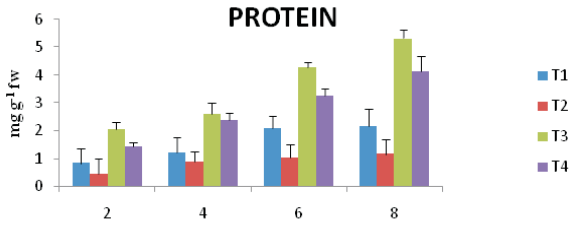
Table 4 : Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on Fresh weight and Dry weight during seedling growth of Vigna mungo. Values are mean of 3 replications ± SE.

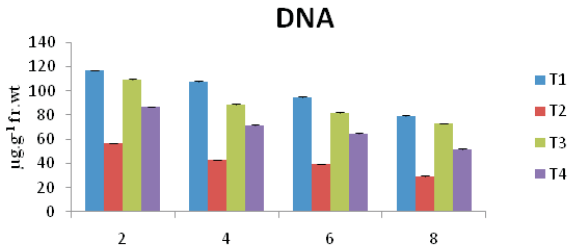
Treatments	Days after treatment of seedlings							
	2		4		6		8	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	0.126 ± 0.001	0.034 ± 0.001	0.524 ± 0.021	0.088 ± 0.033	1.460 ± 0.011	0.200 ± 0.071	2.042 ± 1.057	0.320 ± 0.052
75 mM NaCl	0.090 ± 0.001	0.030 ± 0.002	0.334 ± 0.007	0.060 ± 0.003	0.636 ± 0.031	0.116 ± 0.037	1.568 ± 0.071	0.260 ± 0.011
10 mM $CaCl_2$	0.124 ± 0.003	0.033 ± 0.002	0.510 ± 0.021	0.080 ± 0.004	1.400 ± 0.021	0.160 ± 0.017	1.850 ± 0.021	0.298 ± 0.017
75 mM NaCl + 10 mM $CaCl_2$	0.100 ± 0.007	0.032 ± 0.003	0.440 ± 0.044	0.074 ± 0.013	1.104 ± 0.017	0.155 ±0.015	1.700 ± 0.011	0.285 ± 0.028

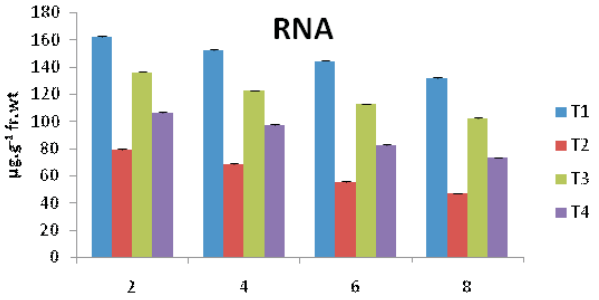
Table 5: Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on Relative Water Content (RWC) during seedling growth of Vigna mungo. Values are mean of 3 replications ± SE.

Treatment	Days after transfer of seedlings			
	2	4	6	8
Control	93.14 ± 0.008	94.26 ± 0.004	95.21 ±.0008	96.38 ± 0.007
75 mM NaCl	91.24 ± 0.074	91.43 ± 0.002	92.74 ±.002	93.56 ± 0.001
10 mM $CaCl_2$	96.28 ±.0012	97.09 ± 0.001	98.12 ± 0.001	98.53 ± 0.008
75 mM NaCl + 10 mM $CaCl_2$	94.18 ± 0.005	95.14 ± 0.005	96.57 ± 0.011	97.48 ± 0.02

Effect of NaCl (75 mM), CaCl2 (10 mM) and their combination on Protein, DNA and RNA content during seedling growth of Vigna mungo. Vlues are mean of 3 replications ± SE







Days after Treatment of Seedlings

T1-CONTROL, T2-75 mM NaCl, T3-10 mM $CaCl_2$ and T4-75 mM NaCl+10 mM $CaCl_2$

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