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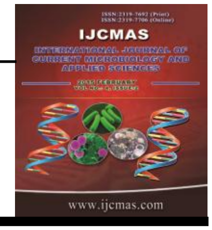

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Original Research Article

Application of Calcium Chloride to Mitigate Salt Stress In *Vigna Radiata* L. Cultivars

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A B S T R A C T

The growth of *Vigna radiata L.* is drastically effected under salinity which is mostly seen in arid and semiarid soil. It's important to understand the salt tolerance among different varieties of mungbean, combat the problem and to improve the Quality and production of the crop. Thus present study was framed to study mitigation of saline stress on mungbean by $CaCl_2$ treatment in pot culture experiments. It was observed that in the presence of individual NaCl and $CaCl_2$ treatment, the growth of plant was reduced with its some major changes in important stress related physiological contents (chlorophyll and carotenoids). In contrast combined treatment of NaCl and $CaCl_2$ reduced saline stress in these plants, increased their growth and positively altered physiological contents like Proline content increased during stress and decreased during combined treatments of NaCl and $CaCl_2$. Decrease in chlorophyll and carotenoids level was seen in the plants under separate NaCl and $CaCl_2$ treatments.

Keywords

Vigna radiata L., Salt Stress, Calcium Chloride, Chlorophyll, Carotenoid, Proline.

Introduction

Mung bean, *Vigna radiata (L.)* is one of the most valuable and popular crops of the world. Also known as green gram or golden gram, it is mainly cultivated in India and others Asian countries. It is greatly consumed in sprouts or dry seed form because of its high protein content. Germinated seeds of Mung bean contain anti-carcinogenic, antibacterial and antifungal properties which neutralize the toxicity, thus it is used for the welfare of human beings. It has a fantastic property to fix the atmospheric nitrogen by forming symbiotic relation with *Rhizobium* bacteria (Zahir *et al.*, 2010). Hence, it also enhances the productivity of soil which promotes the cropping system. The agricultural productivity of this crop is drastically limited due to salinity stress. One of the most atrocious environmental factors for almost all salt-sensitive legume crops and resulted >70% yield loss, even under mild stress conditions in arid and semi-arid regions. The available land is continuously transforming into saline (1-3% per year) land either due to natural salinity or induced by human. The increased salinity is expected to have devastating global effects, resulting in up to 50% land loss by the year 2050.

Because of continuous use of traditional methods of irrigation, the harmful ground water rises up, damaging the upper soil level which is majorly utilized for agriculture. Alike acts would totally put in danger the agricultural capability of fertile soil in salinity prone areas which may result in damaging effects. It is observed in most of the crops of Rajasthan where soil is highly saline in nature which may inhibits the seedling growth and germination of the plant at quite initial stage. This can lead to poor yield of crop and their quality.

Mungbean is highly sensitive to salinity stress, mainly it affect the root emergence compared to shoot. A very little amount of saline water can induce high response in Mungbean (Saha *et al.,* 2010). Salt is one of the most predominant factors which affect the growth of the crop. During salinity conditions the length of the root, branches and number of root hairs decreases. Mainly the chlorophyll content was found to be reduced in plants under salt stress. Mostly, they appear in brown in salinity conditions and the proline content gradually increases (Friedman *et al.,* 2006). If salt stress affects physiological pathways, biochemical metabolism, then in that case morphologically the plant will be abnormal with effected yield. Different types of methods to reduce saline stress have been studied from time to time which also includes application of $Cacl_2$ (Jaleel *et al.*, 2007). In response of salt treatment many metabolite's and solutes concentration get increased in roots and shoots of Mungbean such as proline, reducing sugar, starch, proteins etc. After studying various types of methods to reduce saline stress, calcium chloride was selected to conduct the research study.

Different pre decided doses of NaCl and $CaCl_2$ were given to three variety of *Vigna radiata* viz. T 44, SML 66, Sarif and results were observed after 30 days and 60 days. It was observed that individual application of NaCl and $CaCl_2$ induced the stress where as combined treatments of NaCl with $CaCl_2$ decreased the stress. The aim of this study was to mitigate the saline stress on mungbean by understanding the response of plant against different salt treatments and to enhance the growth and yield of the crop. As *Vigna radiata L.* is an important pulse crop widely cultivated here in Rajasthan it was taken as the study plant and here the following objective were framed.(i) Screening of most salt tolerant variety of *Vigna radiata* L. by studying germination and seedling behavior. (ii) Application of calcium chloride to reduce sodium chloride stress on all three varieties of *Vigna radiata L.*

Materials and Methods

The three different locally available varieties of mungbean were taken Viz. T-44, Sarif and SML-66. These were cultivated in sterilized soil in 15 cm earthen pots for 30 days and 60 days. Four replicates were taken of each and every variety. The seeds were surface sterilized by 0.1% $Hgcl_2$ for 2 minutes, washed thrice with distilled water and then sown in 15cm earthen pots containing autoclaved soil. Five ml of Hoagland's complete nutrient solution was also added to pots once a week. All three varieties were treated with different molar concentration of different salts such as sodium chloride (NaCl), calcium chloride ($CaCl_2$) and combination of both (NaCl + $CaCl_2$) in pot. All replicates of three varieties were watered with normal tap water for 15 days and after 16 days different treatments of varied salt concentration were given. Control was treated with normal tap water only. After 30 and 60 days of sowing (DAS) four replicates were observed for important morphological parameters and physiological parameters. Study of morphological parameters was done to understand the salt tolerance in mungbean and the Parameters were germination percentage, root length, shoot length and seedling behavior. Physiological study included three major parameters i.e., proline and chlorophyll (a, b and total) and Carotenoids contents. Estimation of proline was done by method given by Bates *et al.*, (1973) where as for chlorophyll a, b, total and carotenoids, it was done by using methods given by Arnon, (1949).

Result and Discussion

The present study focused on the response of different mungbean varieties to salt stress and in particular, the relationship between morphological, physiological traits and yield under salt stress conditions. It was expected that findings from the study could enable the identification of factors and traits that could be used to identify the most salt tolerance varieties of mungbean under study. The effects of salt stress like all other stresses depend on the plant developmental stage at which the stress is applied as well as the degree and the duration of the stress too. In this study, plants were subjected to three different concentrations of two salts for 60 days. Prior to stress treatment, plants were treated with normal tap water for 15 days. It was seen in this study that subjecting the mungbean to different salt stress reduced growth. The results showed wide variation in the responses of the all parameters in three different varieties of mungbean in terms of morphological and physiological traits, growth and yield components. Salt stress had a negative impact on important morphological and physiological traits in all the tested varieties. A one-way ANOVA was conducted to compare the effect of various salts concentration on three varieties of *Vigna radiata L.* to understand the significant relation between them. The significant effect were observed within various treatments at the $p<0.01$ and $p<0.05$ level. Taken together, these results suggested that SML-66 variety could withstand salt stress whereas; T-44 is relatively sensitive. Particularly, our results suggest that even a small concentration of salt can affect morphological and physiological parameters of *Vigna radiata L.* However, it should be noted that combined treatment of $CaCl_2$ and NaCl can reduce the stress developed in mungbean plants.

The study conducted on three different varieties of *Vigna radiata L.* includes few major morphological parameters viz. root, shoot, seedling length and pod number; five physiological parameters, viz. proline, chlorophyll (a, b and total) and carotenoids content. The results revealed that all three varieties were different in growth response, morphological and physiological traits. One can also say that in many previous studies done by various scientists, the persisting differences indicated the existence of varied factors. Ozturk and Demir, (2003) reported that Plants treated with NaCl showed increased activity of peroxidase versus the control. Parallel results have been reported in spinach leaves and *Vigna radiata* (Manivannan *et al.,* 2007). These factors include different stress types, variation in environment, presence of genetic variation for these traits etc. Salinity stress was found as one of the major cause which initiated variations among these traits. Salt stress during the growth stage of mungbean reduced most of the parameters assessed; overall growth, carotenoids, chlorophyll a, chlorophyll b and total chlorophyll content, number of pods per plant, shoot length and root length. Similar results were observed in *Phyllanthus amarus* (Arulbalachandran *et al.*, 2009).

Table.1 Physiological parameters studied in different varieties of mungbean for salt stress mitigation by calcium chloride:

Treat -ments	A		B		C		D		E	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60DAS
T_1V_1	16.05±0.30*	31.02±0.49*	1.15±0.0*	0.57±0.0*	0.77±0.0*	0.39±0.0*	1.29±0.0**	0.65±0.0*	0.61±0.0*	0.30±0.0*
T_2V_1	33.05±0.44*	62.97±0.05*	0.91±0.0*	0.44±0.0*	0.39±0.0*	0.20±.0.0*	0.87±0.0*	0.44±0.0*	0.31±0.0*	0.16±0.0*
T_3V_1	29.02±0.56*	57.00±0.75*	0.96±0.0*	0.48±0.0*	0.43±0.0*	0.21±0.0*	0.76±0.0*	0.49±0.44*	0.39±0.0*	0.20±0.01*
T_4V_1	21.00±0.40*	42.02±0.09*	1.11±0.0*	0.50±0.0*	0.56±0.0*	0.29±0.0*	1.19±0.0*	0.59±0.0*	0.57±0.0*	0.28±0.0*
T_1V_2	9.0±0.11*	22.0±0.57*	1.08±0.0*	0.60±0.01*	1.23±0.01*	0.75±0.01*	0.86±0.01*	0.43±0.01*	0.27±0.01*	0.12±0.01*
T_2V_2	21.0±0.16*	40.0±0.08*	0.53±0.01*	0.27±0.01*	0.76±0.01*	0.40±0.01*	0.41±0.01*	0.20±0.01*	0.13±0.01*	0.07±0.0*
T_3V_2	13.0±0.54*	28.0±0.0*	0.76±0.08*	0.38±0.01*	0.89±0.01*	0.49±0.01*	0.57±0.01*	0.29±0.01*	0.17±0.01*	0.08±0.0*
T_4V_2	11.0±0.24*	23.0±0.40*	0.98±0.01*	0.50±0.0*	1.01±0.0*	0.63±0.0*	0.67±0.0*	0.34±0.0*	0.25±0.0*	0.10±0.0*
T_1V_3	20.0±0.08*	42.0±0.04*	1.20±0.0*	0.61±0.0*	1.52±0.0*	0.76±0.0*	1.03±0.0*	0.52±0.0*	0.33±0.0*	0.52±0.0*
T_2V_3	32.0±0.25*	67.0±0.09*	0.69±0.0*	0.33±0.0*	0.81±0.0*	0.40±0.0*	0.47±0.0*	0.22±0.0*	0.17±0.0*	0.23±0.0*
T_3V_3	29.0±0.25*	57.0±0.11*	0.75±0.0*	0.37±0.0*	0.97±0.0*	0.49±0.0*	0.61±0.0*	0.30±0.0*	0.23±0.0*	0.30±0.0*
T_4V_3	21.0±0.08*	48.0±0.12*	1.07±0.0*	0.52±0.0*	1.23±0.0*	0.63±0.0*	0.89±0.0*	0.45±0.0*	0.29±0.0*	0.45±0.0*

DAS= days after sowing, * and ** = significant at $p<0.05$ and $p<0.01$ respectively, T_1-plants treated with normal water (Control), T_2- plants treated with 10mM NaCl solution, T_3- plants treated with 10mM $CaCl_2$ solution, T_4- plants treated with 10mM NaCl + $CaCl_2$ solution, A – Concentration of Proline, B– Concentration of Carotenoids, C– Concentration of Chlorophyll a, D– Concentration of Chlorophyll b, E– Concentration of Total chlorophyll, V_1 – T-44 variety of mungbean, V_2 – Sarif variety of mungbean, V_3 – SML-66 variety of mungbean.

During high salt stress increased proline content in three different varieties were noticed with higher in Sarif and SML-66 and lower in T-44 (Table-1). Significant differences were found in carotenoids, chlorophyll a, chlorophyll b, total chlorophyll contents, shoot length, root length, proline content and number of pods per plant among all varieties tested. All three varieties are salt sensitive but among this T-44 were highly sensitive while Sarif variety and SML-66 variety were tolerable to the salt.

NaCl treatment reduced the growth of *Vigna radiata L.* plants as compared to control plants. Analogous results have been reported in sorghum (Azooz *et al.*, 2004). Separate NaCl treatments increased the free proline content in *Vigna radiata* L. plants versus the control, and at the same time decreased the chlorophyll (a, b and total) and carotenoids content. In the same way increase in proline and amino acid content under NaCl stress have been reported in *Catharanthus roseus* plants (Jaleel *et al.*, 2007). NaCl displayed greater noxious effects on growth and metabolism of *Vigna radiata L.* plants than $CaCl_2$.

The previous was dosed at a higher concentration than the latter. Combining $CaCl_2$ with NaCl treatment increased growth as compared to alone NaCl applied plants. Merging both the treatments increased the chlorophyll (a, b and total) and carotenoids content and decreased proline levels. Thus, over all reducing the stress developed in plants as reported in earlier studies also (Levitt, 1980).

NaCl stressed plants simultaneously treated with $CaCl_2$ had lower proline content than plants treated with NaCl only. When $CaCl_2$ was combined with NaCl the plants showed an increase growth and vigour of plants. Related results were observed in *Cajanus cajan* as reported by Gill and Sharma, (1993). In this work, addition of calcium increased the protein content and decreased the free amino acid content of the salt-stressed plants.

On Joint treatment of sodium chloride and calcium chloride salt varieties were identified as salt stress tolerant. Calcium chloride combined with sodium chloride reduces the stress developed in the plants. Hence, $CaCl_2$ has been proved to be an efficient stress reducing agent. A stepwise ANOVA showed that seed weight, carotenoids, chlorophyll a, chlorophyll b, shoot length, root length and number of pods per plant contributed significantly to the total variation of mungbean growth.

Salt stress imposes substantial undesirable effects on physiology and performance of the crop plants which eventually pilots to plant decease. Nevertheless, the intensity of adverse and detrimental effects of salinity stress depends upon the involved plant species, interrelationships between plant, soil, and environmental factors and lot of other parameters too. Sodium chloride is one of the main important components of saline soils, which affects agricultural soils worldwide. These results suggest that a selection method based on them can be used in breeding for salt stress tolerant mungbean varieties. Based on these studies, some varieties can be recommended for production under salt stressed conditions. However, vigilance should be kept and related extensive studies are desirable.

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