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CALCIUM CHLORIDE PRIMING RECOVER SEED GERMINATION UNDER SALINITY STRESS BY INCREASING REDUCING SUGAR CONTENT

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ABSTRACT

Farmland salinization is a rapidly growing global problem that causes significant agricultural losses. Seeds of Vigna radiata were treated with different concentrations of NaCl (50, 100, 200, 300, 400 and 500 mM), $CaCl_2$ + 200mM NaCl and control (distilled water). Seed germination was not affected upto 100 mM NaCl treatment but at 200 mM and above concentrations seeds were unable to germinate. Seed germinated at 100 mM NaCl showed reduction in radical growth. As concentration of NaCl increases reducing sugar decreases in treated seeds compared to control seeds. Seed priming with $CaCl_2$ increased seed germination up to 86 % and reducing sugar content.

KEYWORDS : *Vigna radiata, salinity, calcium chloride, seed priming, seed germination, reducing sugars, protein.*

RESEARCH PAPER

INTRODUCTION

Vigna radiata is a main crop plant grown in many Asian countries alternatively known as green gram or moong. It is rich in nutrients such as proteins, vitamins and minerals and part of daily diet in many countries.

All plants require healthy seed germination to begin their life cycle. Seed germination is an important step in plant development that is required for seedling establishment **(Bewley et al, 1997)**. The stage is prepared for later events during germination, which shows dynamic biomechanical changes along with adjustments in transcript, protein and hormone levels. Proteins and carbohydrates are biomolecules that play an important role in seed germination **(Mayer et al, 1974)**. During seed germination of six grassland species, it was reported that the amount of reducing sugars increased while protein content decreased with increase in germination time **(Ming Zhao et al., 2018)**. α-amylase is a critical enzyme during seed germination for the hydrolysis of stored reserves and provides energy to the developing embryo **(Damaris et al, 2019)**. In germinating rice **(Murata et al., 1968)**, maize **(Helland et al., 2002)**, and peas **(Juliano et al., 1969)**, the major enzyme involved in starch degradation is thought to be α-amylase, which increases with germination time. Deficiency of this enzyme badly affects reducing sugar content and process of seed germination.

Changing climatic and soil conditions are threat to agriculture. Salinity significantly affects growth and development of mung bean seedlings (Saha et al., 2010) .In *Jatropha curcas* seeds, carbohydrates level decreased during germination **(Lopes et al., 2013)** which indicates the maximum use of carbohydrates for germination. There are different seed priming techniques which can reverse inhibitory effect of salinity stress. Priming of seeds with $CaCl_2$ is known to alleviate inhibitory effect of salinity stress in green gram (Sharma and Dhanda ,2015). In this research we try to understand the mechanism of effect of $CaCl_2$ on seeds of *Vigna radiata* under salinity stress.

MATERIALS AND METHODS:

Plant material: *Collection of seeds:* The *Vigna radiata* seeds were procured from local market of Pune, Maharashtra.

Germination treatment: Autoclaved distilled water was used to rinse seeds of *Vigna radiata* and surface sterilization was done with 70% ethanol for 2 min and again rinsed thrice with

autoclaved distilled water to remove alcohol. These seeds were kept in different concentrations of NaCl (50, 100, 200, 300, 400, 500 mM NaCl in autoclaved distilled water), control (autoclaved distilled water) and priming solution (200 mM NaCl+ 10 mM $CaCl_2$) for 16 h at 30˚C to initiate germination process and then transferred to sterile petriplates layered with tissue paper moistened with respective solutions of NaCl.

Seed germination assay: Treated seeds were transferred to autoclaved petriplates layered with tissue paper moisten with above mentioned treatment solutions at 30˚C in an incubator. The germination analysis was done observing the seed growth daily up to 4 days and seeds were considered as germinated when radicle showed growth up to 1 cm. Germination percentage was calculated for each treatment at the interval of 24 h up to day 4.

Preparation of crude extract: Seeds treated with various concentrations of NaCl and control were incubated for 24, 48, 72 and 96 h, slightly modified method of **Sottirattanapan et al. (2017)** was used to prepare crude extract. Five seeds were ground in 10 ml of Autoclaved distilled water followed by soaking of homogenate for 10 min at 4°C with occasional agitation. The clear supernatant was used as crude extract after centrifugation of above homogenate at 10,000 x g for 10 min at 4°C and. The crude extract was stored at -80°C before use. This extract was used to estimate reducing sugars and protein content.

Estimation of reducing sugars and proteins: Reducing sugars and proteins in crude extracts were estimated by DNS (3, 5-dinitrosalicylic acid) method and Bradford method respectively.

STATISTICAL ANALYSIS:

One-way analysis of variance (ANOVA) followed by Tukey test was performed by using instat 3 software for analysis of other data. All tests are performed in at least three replicates. All data are expressed as means ± standard deviation (SD).

RESULTS

Seed germination assay: NaCl treatment affects seed germination and development of radical in concentration dependant manner (Fig. 1). After 24 h of treatment seed germination was found 0 % in all treated as well as in control group. After 48 h of treatment 97.77, 97.77, 95.56 and 22.2 % seed germination was found in control,50 mM ,100 mM and 200 mM treated seeds respectively (fig. 2). After 72 h and 96 h seed germination percentage increases upto 40 % only in 200 mM NaCl treatment. Up to 96 h of incubation 0 % seed germination was found in 300 mM and above concentrations.

Reducing sugar concentration: There was significant reduction in reducing sugar concentration in all treated groups as compared to control after 48 h of treatment (fig. 3). As concentration of NaCl increases reducing sugar decreases in treated seeds.

Protein concentration: Salinity treatment don't show any significant change in protein content up to 48 h.(Data not shown)

Seed priming with $CaCl_2$: On day 4 Seed germination at 200 mM NaCl was significantly less as compared to control but seed priming with $CaCl_2$ at 200 mM NaCl increase seed germination upto 86 %. Reducing sugar concentration was significantly increased up to 1.8 mg/ml in 200 mM NaCl treated seeds after seed priming with $CaCl_2$.

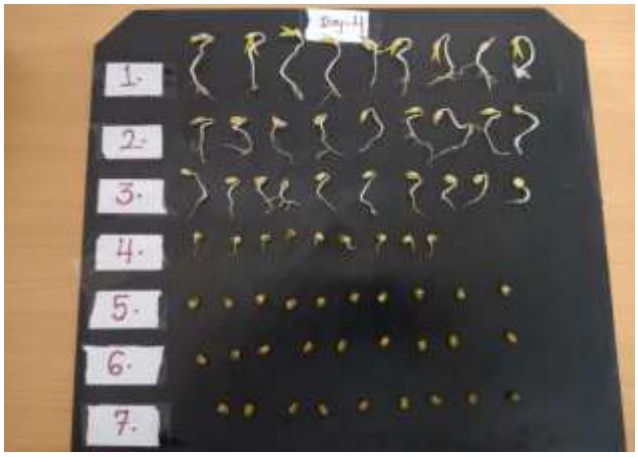

Fig.1. Effect of salinity on *Vigna radiata* seed germination. Data for day 4 after treatment show reduction in seed germination and radicle length as concentration of NaCl increase. 1. Control, 2 50 mM, 3. 100 mM, 4. 200 mM, 5. 300 mM, 6. 400 mM, 7. 500 mM NaCl.

Figure – 1

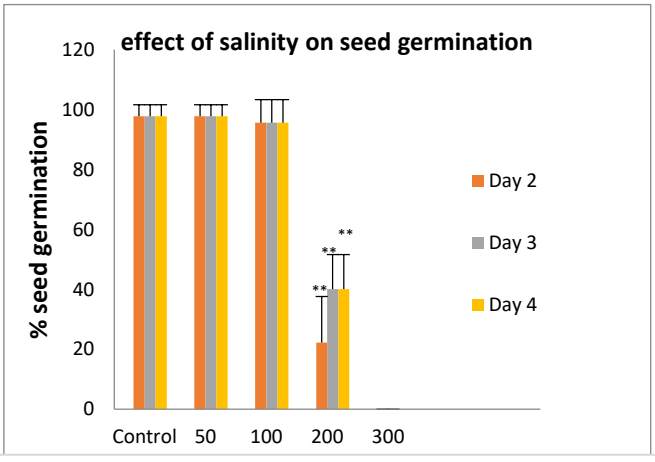

Fig.2 .Graph showing effect of salinity on seed germination in *Vigna radiata*. Reduction in % seed germination is NaCl concentration dependent .200 mM NaCl and above conc. are highly toxic. Data are presented for 3 replicates as a means ± SD. Significant difference compared to control for same time period are indicated by **(P<0.01)

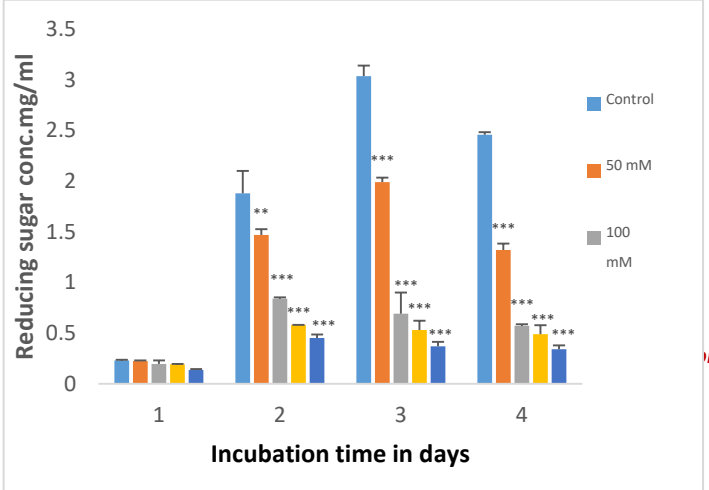

Figure - 2

Fig.3 Graph showing effect of salinity on reducing sugar content.

Reduction in reducing sugar content is parallel with increase in salinity. Data are presented for 3 replicates as a means ± SD. Significant difference compared to control for same time period are indicated by **(P<0.01).

Figure - 3

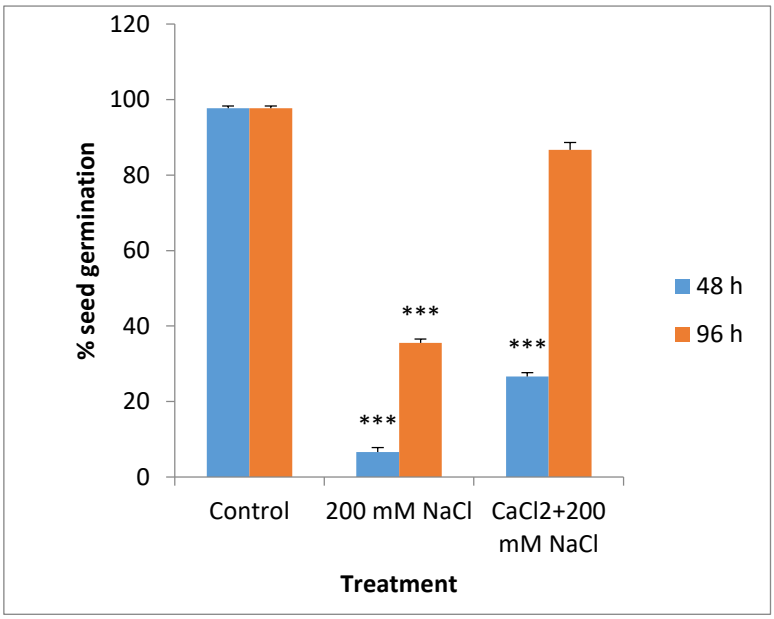

Fig.4. Effect of $CaCl_2$ on seed germination in *Vigna radiata*. Priming with $CaCl_2$ alleviate effect of salinity and restored seed germination. Data are presented for 3 replicates as a means ± SD. Significant difference compared to control for same time period are indicated by ***(P<0.001).

Figure - 4

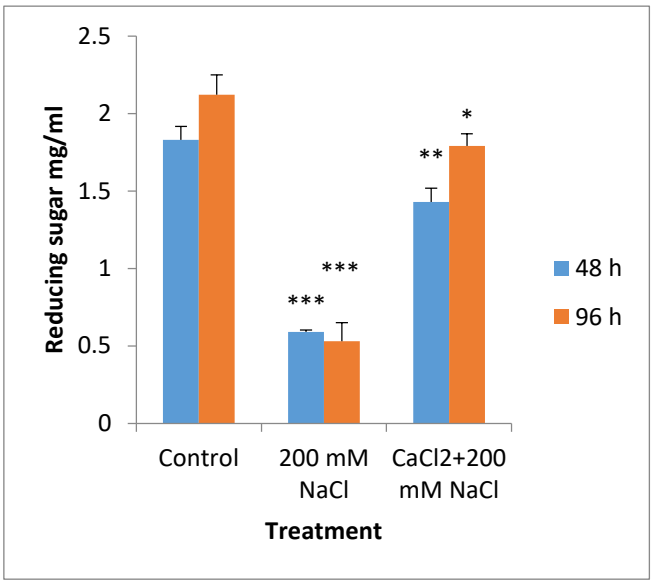

Fig.5. Graph showing effect of $CaCl_2$ Priming on reducing sugar under salinity stress. $CaCl_2$ Priming increase reducing sugar content under salinity stress. *(P < 0.05), **(P < 0.01), ***(P < 0.001) for same time indicates significant differences compared to control. Data are presented as means ± SD.

Figure - 5

DISCUSSION:

Seed germination is a crucial step in plant development which get affected by various environmental factors. One such significant factor is farmland salinisation which affect plant growth and development (Nachshon, 2018). Salinity is known to reduce seed germination various crop plants such as mung (Prakash,2017), rice (Liu et al.,2018), ground nut (Mensah et al., 2006). In many plants NaCl treatment reduces seed germination by affecting reducing sugar level due to some problems in utilisation of store food reserves (Promila and Kumar,2000). Our study also shows signifacant reduction in reducing sugar content in concentration dependant manner. NaCl treatment reduce reducing sugar in 50 mM and 100 mM treatment after 48 h of treatment compared to control by 22 % and 55 % respectively. After such significant reduction in reducing sugars seeds treated with 50 mM and 100 mM NaCl were able to show more than 95 % seed germination after 48 h of treatment.300 mM treatment show more than 75 % reduction in reducing sugars compared to control causing failure of seed germination in *Vigna radiata.*

Seed priming is a tool to enhance seed germination under various abiotic stresses. $CaCl_2$ is a chemical agent which provide calcium to developing seeds and enhance seed germination under stress condition. Caicium is known to reduce oxidative stress and maintain Na-K homeostasis under salt stress (Kamran et al., 2021). Our research on mechanism of effect $CaCl_2$ priming shows increase in reducing sugar content under salinity stress. $CaCl_2$ treatment rescue seed germination under salinity stress upto 27 % and 87 % after 48 h and 96 h of treatment respectively. This increase in seed germination was parallel with increase in reducing sugar content after 48 h and 96 h of treatment.

CONCLUSION:

Salinity stress affects seed germination in *Vigna radiata* by depleting reducing sugar content. Priming with $CaCl_2$ of seeds under salinity stress reverse inhibitory effect of NaCl treatment on seed germination by increasing reducing sugar content.

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