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Calcium chloride priming reverses the effect of salinity stress on α-amylase activity during seed germination in *Vigna radiata*

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Abstract
Farmland salinization is a global issue that is causing significant agricultural losses. Seeds of *Vigna radiata* were treated 200 mM NaCl, $CaCl_2$ + 200 mM NaCl and control (distilled water). 200 mM NaCl treatment had a considerable negative impact on seed germination and seeds were unable to germinate. Reduction in seed germination was correlated with low α-amylase activity after 96 h of incubation as compared to control seeds. Seed priming with $CaCl_2$ increased seed germination upto 84% after 96 h of incubation under salinity stress. $CaCl_2$ treatment increasesα-amylase activity by 45% as compared to control at 96 h of incubation under salinity stress at 200 mM of NaCl. $CaCl_2$ treatment rescue seed germination in *Vigna radiata* under salinity stress by increasing α-amylase activity.

Keywords: *Vigna radiata*, salinity, calcium chloride, seed priming, seed germination, α-amylase

Introduction
Food habits are changing rapidly around the world in the recent decades. Non-vegetarian food *Vigna radiata* also known as green gram or mung is a major crop plant grown in many Asian countries with life cycle of 60-90 days. It is high in nutrients such as proteins, vitamins and minerals and part of daily diet in many countries.

Seed germination is an important step in plant development that is required for seedling establishment (Bewley *et al*., 1997) [1]. All plants require healthy seed germination which shows dynamic biomechanical changes along with adjustments in transcript, protein and hormone levels. An essential enzyme called α-amylase hydrolyses stored reserves and provides energy to the developing embryo during seed germination (Damaris *et al*., 2019) [3]. The key enzymeinvolved in starch degradation in germinating rice (Murata *et al*., 1968) [9], maize (Helland *et al*., 2002) [3], and peas (Juliano *et al*., 1969) [4], 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.

Agriculture is threatened by changing climatic and soil conditions. Mung bean seedlings are significantly affected by salinity in terms of growth and development (Saha *et al*., 2010) [13]. Different seed priming techniques can reverse inhibitory effect of salinity stress. Priming of seeds with calcium chloride $CaCl_2$ is known to mitigate inhibitory effect of salinity stress in green gram (Sharma and Dhanda, 2015) [14]. The purpose of this research is to determine the effect of $CaCl_2$ on α-amylase activity during seed germination in *Vigna radiata* under salinity stress.

Materials and Methods
Plant material
Collection of seeds: The *Vigna radiata* seeds were collected from the local market of Pune, Maharashtra.

Germination treatment
The seeds of *Vigna radiata* were rinsed with autoclaved distilled water and the surface sterilization was done with 70% ethanol for 2 min before being rinsed

distilled water) and priming solutioni. e PS (200 mM NaCl+ 20 mM $CaCl_2$) for 16 h at 30 °C to initiate germination process and then transferred to sterile petri plates layered with tissue paper moistened with above mentioned treatment solutions.

Seed germination assay

At 30 °C in an incubator treated seeds were transferred to autoclaved petriplates layered with tissue paper and moistened with the aforementioned treatment solutions. The germination analysis was performed by observing the seed growth daily for up to four days and seeds were considered as germinated when radicle showed growth up to 1 cm. Germination percentage was calculated for each treatment every 24 h until day 4.

Preparation of crude extract

Treated seeds were incubated for 24, 48, 72 and 96 h, slightly modified method of Sottirattanapan *et al*. (2017) [15] was used to prepare crude extract. 10 ml of 0.1 M sodium acetate buffer pH 5.6 was used to homogenize five seeds followed by soaking of homogenate for 30 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 measure α-amylase activity.

Quantitative assay for α-amylase

The treated and control *Vigna radiata* seeds were collected after incubation at 30 6 for 24, 48, 72 and 96 h. Quantification of α-amylase was done by DNS method of Miller (1959) [8] with little modifications. After treatment at 70 °C for 15 min in water bath, 1 ml of 0.1% soluble starch was added in the crude enzyme extract and again the mixture was kept for 10 min at 60 °C in water bath. After addition of 2 ml of DNS this mixture was placed in boiling water bath for 5 min. Maltose was used as a standard to estimate the reducing sugars released due to starch degradation by α-amylase enzyme in crude extract using a spectrophotometer at 540 nm.

Qualitative assay for α-amylase

The embryoless half seeds were used for starch plate test to assess qualitatively the effect of $CaCl_2$ on α-amylase activity under salinity stress according to the method of Liu *et al*., (2018) [6]. Treated and control seeds of *Vigna radiata* as mentioned above were taken. Embryos were removed by cutting seeds and embryolesss cut seeds were adjusted on the plate with cut edge on the 2% agar including 0.2% soluble potato starch, 20 mM sodium succinate pH 5.0 in 9 cm petridishes. After incubation for 72 h at 28 °C, significant volume of I_2/KI solution was added.

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 radicle which is reversed by treating seeds with 20 mM $CaCl_2$ (Fig. 1). On day 4 seed germination at 200 mM NaCl was significantly less as compared to control but seed priming with $CaCl_2$along with 200 mM NaCl increase seed germination up to 84% (Fig.2).

Quantitative assay

Quantitative assay for α-amylase reveals that 200 mM NaCl treatment significantly decreased α-amylase activity as compared to control and PS treated seeds. 24, 48 and 72 h after treatment no significant differences were observed between α-amylase activity of control, PS and 200 mM NaCl treated seeds. As compared to the control, seeds treated with 200 mM NaCl showed reduction in α-amylase activity by 52% while PS treated seeds show increased α-amylase activity by 45% compared to control seeds after 96 h of incubation. (Fig.3).

Qualitative assay

Qualitative assay was performed to assess the effect of $CaCl_2$ on the enzyme activity under salinity stress after incubation of 72 h. The control and PS treated seeds showed more transparent area around them as compare to seeds treated with 200 mM Figure – 1 NaCl (Fig. 4).

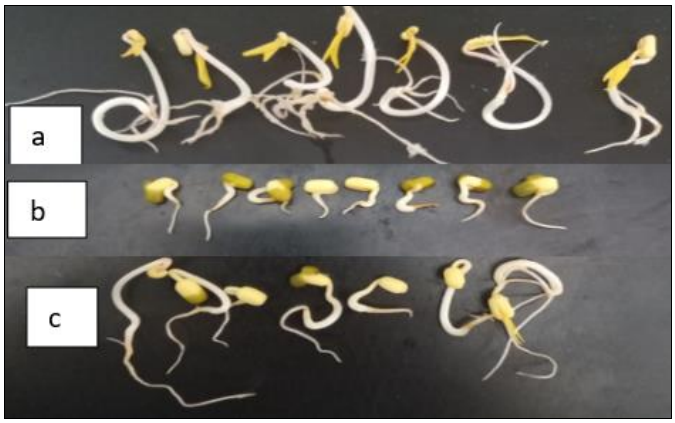

Fig 1: Effect of $CaCl_2$on *Vigna radiata* seed germination under salinity stress

Data for day 4 after treatment show reduction in radicle length at 200 mM NaCl. This inhibitory effect of salinity was reversed by $CaCl_2$ treatment. a. Control, b. 200 mM NaCl, c. 20 mM $CaCl_2$ + 200 mM NaCl

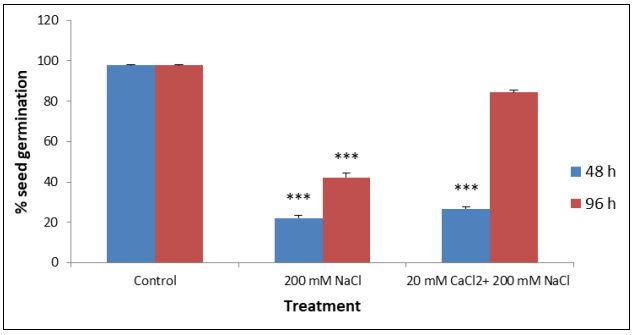

Fig 2: Graph showing effect $CaCl_2$ on *Vigna radiata* seed germination under salinity stress

$CaCl_2$ treatment reverse inhibitory effect of salinity on seed germination after 96 h of incubation. Data are presented for 3 replicates as a means ± SD. *** (p<0.001) for same time indicates significant differences compared to control

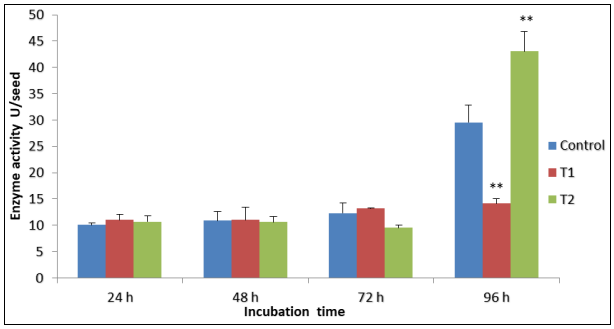

Fig 3: Graph showing effect of $CaCl_2$ on α-amylase activity under salinity stress

Reduction in α-amylase activity due to 200 mM NaClat 96 h incubation is recovered by $CaCl_2$ treatment under salinity stress. 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). T1. 200 mM NaCl, T2. 20 mM $CaCl_2$ + 200 mM NaCl

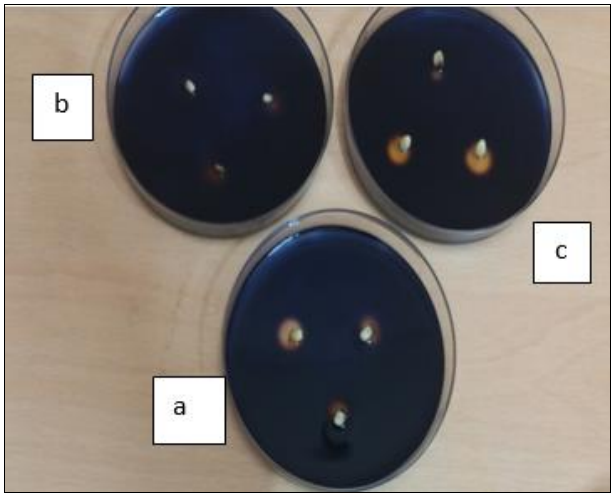

Fig 4: Image showing effect of $CaCl_2$ treatment on α-amylase activity under salinity stress

Transparent area around seeds is correlated with α-amylase activity. a. Control, b. 200 mM NaCl, c. 20 mM $CaCl_2$ + 200 mM NaCl

Discussion

Various environmental factors affects seed germination which is a crucial step in plant development. Farmland salinisation isone such significant factor which affect plant growth and development (Nachshon, 2018) [10]. Seed germination in various crop plants such as mung (Prakash, 2017) [11], rice (Liu *et al*., 2018) [6], ground nut (Mensah *et al*., 2006) [7] is affected by salinity. In many plants such as mung (Promila and Kumar, 2000) [12], rice (Liu *et al*., 2018) [6] NaCl treatment reduces seed germination by affecting α-amylase activity. Our study also shows parallel results. 200 mM NaCl treatment significantly reduces seed germination via reduction in α-amylase activity. Under various abiotic stresses seed priming can enhance seed germination. $CaCl_2$ 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) [5]. Our research on mechanism of effect $CaCl_2$ priming shows increase in α-amylase activity under salinity stress. Qualitative assay for α-amylase activity shows more transparent area around control and PS treated seeds as compared to 200 mM NaCl treated seeds. This transparent area is formed due to starch degradation by α-amylase enzyme produced by germinating seed. Starch in presence of I_2/KI solution produces blue purple color. This α-amylase is responsible for starch degradation which provides reducing sugars for seed germination (Promila and Kumar, 2000) [12]. $CaCl_2$ treatment rescue seed germination under salinity stress up to 84% after 96 h of incubation by increasing α-amylase activity.

Conclusion

Salinity stress affects seed germination in *Vigna radiata* by reducing α-amylase activity. Priming with $CaCl_2$ of seeds under salinity stress reverse inhibitory effect of NaCl treatment on seed germination by increasingα-amylase activity.

References

1. Bewley JD. Seed germination and dormancy. The plant cell. 1997;9(7);1055.
2. Damaris RN, Lin Z, Yang P, He D. The rice alpha-amylase, conserved regulator of seed maturation and germination. International journal of molecular sciences. 2019;20(2):450.
3. Helland MH, Wicklund T, Narvhus JA. Effect of germination time on alpha-amylase production and viscosity of maize porridge. Food Research International. 2002;35(2-3):315-321.
4. Juliano BO, Varner JE. Enzymic degradation of starch granules in the cotyledons of germinating peas. Plant Physiology. 1969;44(6):886-892.
5. Kamran M, Wang D, Xie K, Lu Y, Shi C, Sabagh AE, *et al*. Pre-sowing seed treatment with kinetin and calcium mitigates salt induced inhibition of seed germination and seedling growth of choysum (*Brassica rapa var. parachinensis*). Ecotoxicology and Environmental Safety. 2021;227:112921.
6. Liu L, Xia W, Li H, Zeng H, Wei B, Han S, *et al*. Salinity inhibits rice seed germination by reducing α-amylase activity via decreased bioactive gibberellin content. Frontiers in Plant Science. 2018;9;275.
7. Mensah JK, Akomeah PA, Ikhajiagbe B, Ekpekurede EO. Effects of salinity on germination, growth and yield of five groundnut genotypes. African Journal of Biotechnology. 2006, 5(20).
8. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959;31(3):426-428.
9. Murata T, Akazawa T, Fukuchi S. Enzymic mechanism of starch breakdown in germinating rice seeds I. An analytical study. Plant Physiology. 1968;43(12);1899-1905.
10. Nachshon U. Cropland soil salinization and associated hydrology: Trends, processes and examples. Water. 2018;10(8);1030.
11. Prakash M. Effect of salinity on germination and seedling growth of green gram varieties. International Journal of Plant Sciences (Muzaffarnagar). 2017;12(1):79-84.
12. Promila K, Kumar S. *Vigna radiata* seed germination under salinity. Biologia Plantarum. 2000;43:423-426.
13. Saha P, Chatterjee P, Biswas AK. NaCl pretreatment alleviates salt stress by environment of antioxidant defense and osmolyte accumulation in mungbean (*Vigna*

radiata). Indian Journal of Experimental biology. 2010;48:593-600.
14. Sharma A, Dhanda S. Application of Calcium Chloride to Mitigate Salt Stress in *Vigna radiata* L. Cultivars. Int. J Curr. Microbiol. App. Sci. 2015;4(2):764-769.
15. Sottirattanapan P, Nantachai K, Daduang S, Funahashi T, YamadaM. Purification and characterization of amylase from roots of *Paederia Foetida* Linn. Biocatalysis and agricultural biotechnology. 2017;10:329-335.