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RESEARCH ARTICLE

IN VITRO, ASSESS THE IMPACT OF SALT STRESS ON GERMINATION AND PHYSIOLOGICAL RESPONSE OF COWPEA

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Abstract: Soil salinity is the primary constraint on plant growth and affects sustainable agriculture. This study examined the impact of salt stress (NaCl) on cowpea germination and nutritional qualities at varying NaCl concentrations (50 mM,100 mM,150 mM, and 200mM NaCl concentrations) by laboratory bioassay. Germination of cowpea was observed after seven days of bioassay. The study's findings indicate that both seedling growth and germination rates were impacted by an increase in salt concentrations. Viable and surface-sterilized seeds were chosen to germinate in varying NaCl concentrations and distilled water as a control. The experiment was conducted in a triplet with a control. Final observations were recorded after fifteen days of germination of cowpea. Experimental findings revealed that salt concentration significantly affects the germination and physiological growth of cowpea. Increasing salt concentration also significantly affects protein and total sugar content.

Keywords: Germination, Bio-assay, Salt stress, Seedlings, Nutrients

INTRODUCTION

Soil salinity is a significant abiotic stress that leads to soil degradation and decreased crop yields (Ben Gaid *et al*. 2024). According to U. Nachshon *et al*. (2018), soil salinization is a significant problem for agriculture that is expected to worsen due to the impact of climate change on crop yields (Chaudhry *et al*. 2022). Salinized agricultural soils are increasing annually on a global scale (Gupta *et al*. 2021), particularly in arid and semi-arid regions characterised by low precipitation and elevated temperatures and evaporation rates. A significant portion of agricultural land accumulates salt over time, primarily as a result of intensive agricultural practices and poor irrigation facilities (Maatallah *et al*.2023). Salt stress affects almost all aspects of plant development, including symbiosis, transpiration, photosynthesis, germination, root and shoot growth, and blooming (Negrão *et al*. 2017). An optimal concentration of NaCl maximises plant growth; however, elevated concentrations entirely inhibit seed germination and the growth and development of plants in saline soils (Gupta *et al*. 2021). Salinity stress inhibits seed germination, delays germination time, and reduces both germination percentage and speed (Tomar and Singh 2006; Tomar 2008; Malik *et al*. 2022).

Vigna unguiculata (L.) Walp., more often known as cowpea, is a significant legume and an essential crop in the context of global climate change and food security (Carvalho *et al*. 2019). All stages of development and growth of cowpea are affected by salinity, with the germination and seedling stages being the most sensitive stages (Dong *et al*. 2019). In cowpea seedlings, salinity can entirely inhibit the germination process, which ultimately results in the death of the plant (Ravelombola *et al*. 2017). The purpose of this study was to assess *Vigna unguiculata* (L.) Walp's tolerance to salt stress. The current study evaluated how salt stress affects *Vigna unguiculata* seed germination, seedling growth, and development. For the test species, the chosen parameters were used to assist in identifying the harmful and crucial salt concentration values.

MATERIALS AND METHODS

In-vitro analysis experiments were conducted with three replicates at the Department of Botany, C.C.S. University (29º18' N and 78º47' E), Meerut, Uttar Pradesh, India. Seeds of *Vigna unguiculata* were obtained from the Indian Agricultural Research Institute (IARI), New Delhi, India. Salt stress was induced using various concentrations of NaCl saline solutions. Double-distilled water was used to make saline solutions along with control. Sodium hypochlorite (0.1%) was used to disinfect the seeds for fifteen minutes. They were repeatedly washed to get rid of any remaining particles and dried on blotting paper. Ten healthy seeds were put into Petri dishes that had been sterilized and contained filter

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paper. Each treatment had three replications. Double-distilled water (10 ml) served as the control. For two weeks, the corresponding salt concentrations were used to keep the filter sheets moist. The following analysis was done to assess the impacts of treatments and control:

Control: 10 seeds with 10 ml of double-distilled water.

T1: 10 seeds with 10 ml of 50mM NaCl solution.

T2: 10 seeds with 10 ml of 100mM NaCl solution.

T3: 10 seeds with 10 ml of 150mM NaCl solution.

T4:10 seeds with 10 ml of 200mM NaCl solution.

Germination percentage

Developing a new plant from a seed is called seed germination. It was calculated by given formula-

Germination percentage =

$$\frac{\text{no. of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

Seedling measurement:

After 15 days of seed germination, some physiological parameters were recorded, such as the length of the shoot and root, the fresh and dry weights of the root and shoot. A measuring scale was used to determine the length of the seedlings. After the sample was freshly weighed, it was left in the oven at 72°C for 48 hours, and the seedling's dry weight was measured.

Tolerance index:

It was measured by given formula-

Tolerance index

$$= \frac{\text{Value in treatment x 100}}{\text{Value in control}}$$

Biochemical parameters

Protein estimation:

The protein content of seedlings was measured using the Bradford method (1976). Fifty milligrams of fresh material were pulverised in five millilitres of Tris-EDTA Buffer using a mortar and pestle. The crushed extract was centrifuged for 10 minutes at 5000 rpm. Subsequently, 1 ml of supernatant and 5 ml of Coomassie Brilliant Blue dye were added, followed by a 5-minute incubation at 25°C. As a control, 1 ml of Tris-EDTA buffer was combined with 5 ml of Coomassie Brilliant Blue dye. Measure the absorbance at 595 nm.

Protein (mg/g) =

$$\frac{\text{O.D*Factor*Dilution*1000}}{\text{100xTotal volume}}$$

Total and reducing sugar:

This experiment was performed by Nelson's (1944) method. For the standard curve, 1.0 mg/ml stock solution of D-glucose was used.

Statistical analysis

All experiments were performed in triplicate. All the data collected from observations obtained from the use of different concentrations of Na Cl were analyzed statistically. One-way ANOVA analysed the data across many groups for a single variable at a specific time point. Each mean was derived from three distinct values, with $p < 0.05$ deemed indicative of a significant difference. Data were presented as mean ± standard deviation (SD) and standard error derived from triple experiments.

RESULTS AND DISCUSSION

The impact of salt stress on cowpea seed germination and early seedling growth was assessed in this study. This study aimed to determine the resistance of cowpeas to salt stress.

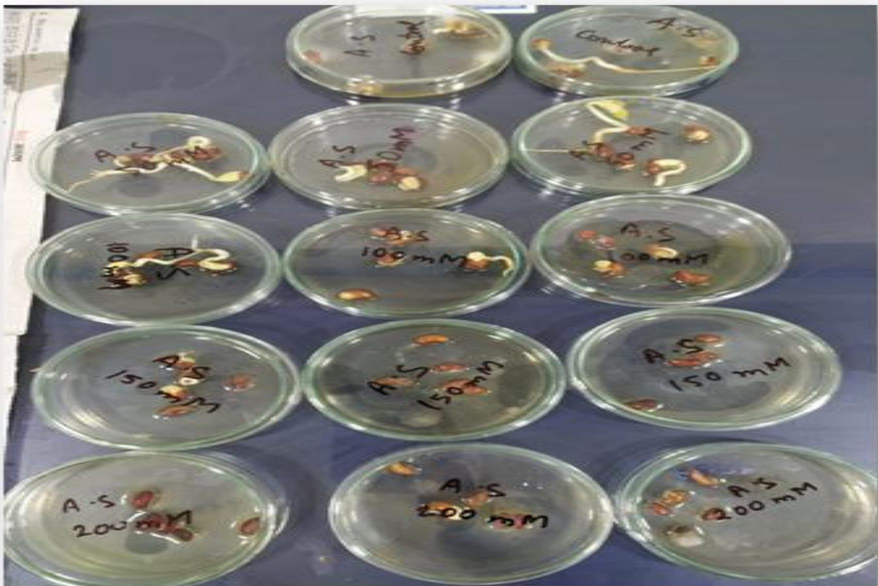

Fig 1. shows a bioassay of cowpea grown under different concentrations of NaCl

Germination percentages

Seed germination percentages of cowpea showed an inhibitory effect of salt concentrations. As the concentration was increased, germination percentages decreased. According to a study by Zahedi *et al*. (2012), salt stress reduced the rate of germination in cowpea, and poor seed germination led to a considerable decrease in yield. The highest germination percentage was observed at 50 mM NaCl concentration, and as the concentration of salt increased, the germination percentages decreased significantly (Table 1). A similar reduction in the percentage of germination under salt stress was reported by Dsouza *et al*. (2024).

Seedling measurement

The length of the radical and plumule decreased significantly ($p < 5\%$) as the salt concentration increased compared to the control. Radicle length was more suppressed compared to plumule as the saline concentration increased (Table 1).Maximum seedling was observed in distilled water (control), and minimum growth was observed in 200mM NaCl concentration. As salt stress increased, the results indicated that shoot length and root lengths were significantly reduced (Jamil and Rha, 2007). The steady decrease in root length that was observed with the increase in salinity could be attributed to the fact that the inhibitory impact of NaCl salt on root growth is greater than the inhibitory effect on shoot growth (Zahedi *et al*. 2012).

The steady decrease in fresh weight was observed as salt concentration increased (Thiam *et al*. 2013). No significant results were found in dry weight as salt concentration increased. Cowpea seedlings' dry weight was reduced significantly compared to the control. Similar results were observed by Zahedi *et al*. (2012) and Thiam *et al*. (2013).

Figure 2. shows the measurement of cowpea seedlings

Tolerance Index

A cowpea's sensitivity to salt stress is modest. The growth and physiological functions of cowpeas are affected by salt stress, which eventually lowers their Tolerance Index (Table 1, Fig. 3). The tolerance index was significantly decreased as the salt concentrations increased. Similar results were observed by Sing *et al*. (2020).

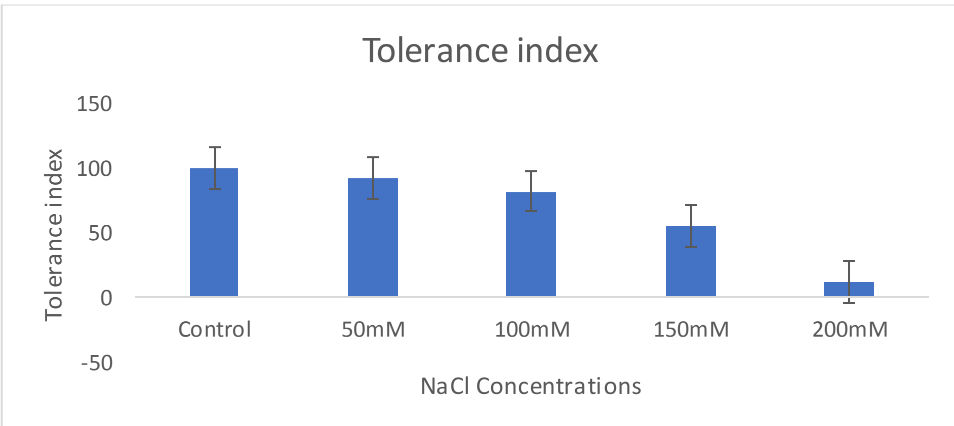

Fig 3. shows the tolerance index of cowpea under different concentrations of NaCl

Table 1. Shows mean with standard deviation of germination percentages, shoot and root length (cm), fresh and dry weight (mg) and tolerance index of cowpea seedings under different concentration of NaCl.

S.N.	Treatments	Germination percentages	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)	Tolerance index
1	Control	82±2.5	9±3.5	2.21±0.24	0.726±0.12	0.09±0.00	100±00
2	50mM	64±1.7	4.76±2.4	1.74±0.37	0.318±0.12	0.063±0.00	52.8±2
3	100mM	46±1.5	3.22±1.93	1.64±0.28	0.379±0.09	0.156±0.03	35.7±2
4	150mM	32±2.5	1.77±0.63	0.96±0.30	0.2±0.05	0.121±0.01	16.6±1
5	200mM	5±0.57	0.7±0.17	0.26±0.05	0.05±0.02	0.008±0.02	7.7±2

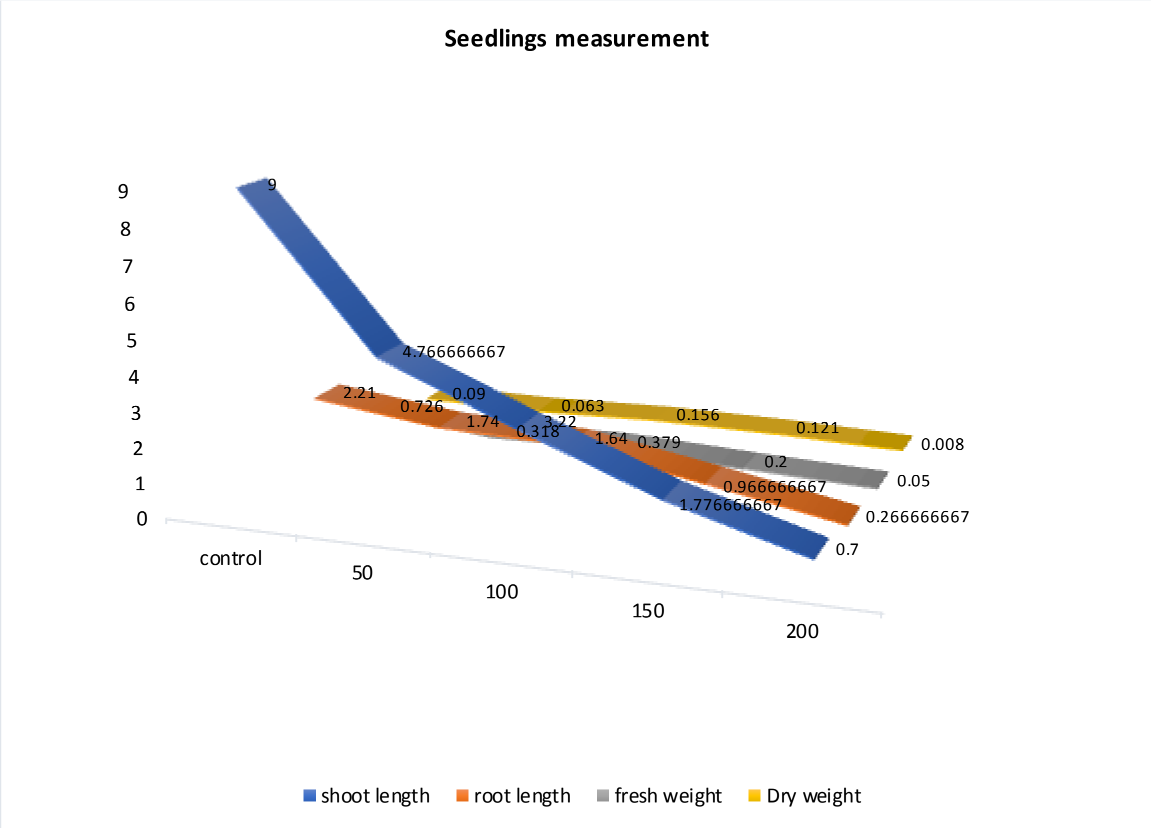

Fig 4. Shows a graphical representation of seedling measurement of cowpea under different concentrations of NaCl

Biochemical parameters

Protein estimation

Cowpea is the most significant seed legume because it contains 33% seed protein (Ddamulira and Santos, 2015) and 34.9% protein in their edible leaves (Enyiukwu *et al*. 2018). Plant protein is directly impacted by salt stress because less plant development is directly correlated to plant protein results in less plant protein. Protein content was significantly decreased as the salt concentration increased. During germination, high salinity reduces water intake by causing osmotic stress. This lack of water can increase the consumption of stored proteins for osmolyte and energy production, as well as hinder metabolic processes like protein synthesis significantly (Deme *et al*. 2022) difference was found in protein content of 50mM and 100mM salt concentrations (Table 2).

Total and reducing sugars

As the concentration of salt increased, there was a decrease in the amount of total sugar, but reducing sugar content increased as the salt concentration increased (Table 2). Salinity disrupts metabolic processes essential for sugar synthesis and mobilisation in early seedlings. Total sugar decrease is mostly attributable to suppressed enzyme activity related to starch breakdown and sucrose production, along with reduced carbon absorption. Roots may undergo heightened accumulation of reducing sugars (Jayawardhane *et al*. 2022). This is regarded as a mechanism for osmotic adjustment—roots up their solute content to conserve water and maintain turgor pressure despite elevated external salinity. Similar results were found by Amirjani (2011). Whereas the concentration of salt increased, the reducing and non-reducing sugar content of rice plants increased in the roots.

Table 2. Shows the mean and standard deviation of protein, total sugar, and reducing sugar of cowpea seedlings under different concentrations of NaCl.

S.N.	Conc.	Protein	Total sugar	Reducing sugar
1	Control	0.223±0.002	0.050±0.001	0.026±0.002
2	50mM	0.168±0.003	0.023±0.001	0.015±0.001
3	100mM	0.164±0.004	0.015±0.001	0.013±0.001
4	150mM	0.152±0.001	0.008±0.001	0.007±0.001
5	200mM	0.079±0.058	0.003±0.000	0.003±0.001

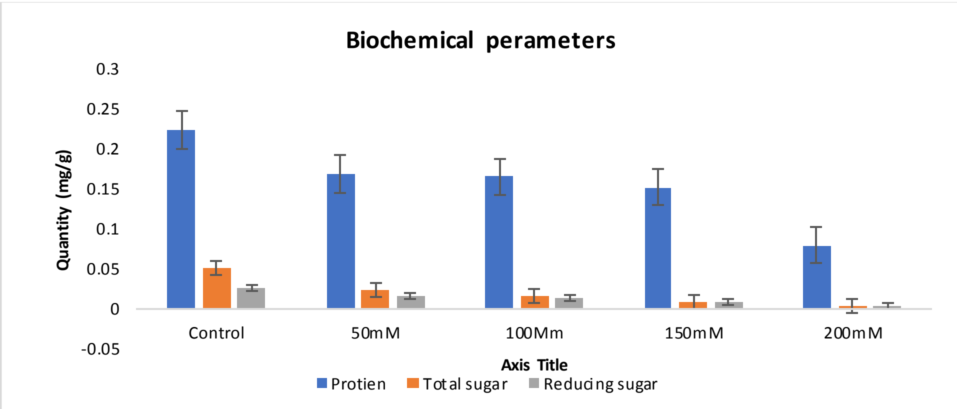

Fig 5. Shows the protein, total, and reducing sugar content of cowpea under different concentrations of NaCl.

CONCLUSION

Understanding the impact of varying salt concentrations on cowpea growth and yield is the primary goal of the research. The decrease in protein and starch content at higher salinity levels may be explained by the osmotic pressure produced by the increased salt concentration, which changed the plant cells' physiology. The results indicated a consistent decline in every parameter examined as the salinity increased. The goal of this study is to demonstrate that cowpea, a popular crop, can be cultivated as a mixed crop in low-saline areas with other crops.

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