SHORT COMMUNICATION

Assessment of Physiological, Cellular and Biochemical Responses to Salt Stress in *Vigna mungo* and *Cicer arietinum* : A Preliminary Study

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Abstract

A major environmental stress that affects plant growth negatively is salt stress. It is responsible for damaging an array of commercial plants utilized as food sources in several countries. Plants being sessile, are unable to avoid the effects of salt stress. The primary goal of this specific research was to analyze the morphological, cellular, and biochemical impact of salt stress on two commercially important crops, *Vigna mungo* and *Cicer arietinum* plants by subjecting them to two concentrations of salt viz. 50 and 100 mM while maintaining control. The crops were subjected to stress and growth, biochemical and genetic parameters were assessed. The growth parameters Showed that stress inflicted by plants significantly affected shoot length and root length and the quantitative change in adventitious roots. It was also observed to destroy both species' tetrad structure of the vascular bundles. The impact was also visible on the cell division, where the mitosis was observed to decrease considerably. The biochemical assays indicated that NaCl significantly induced stress in these plants by reducing the catalase activity along with the chlorophyll pigments and causing an accumulation of proline and phenolic compounds. The comparative study showed that *V. mungo's* response to the salt stress exceeded that of *C. arietinum* at similar salt concentrations, indicating that assessing the soil for these specific stress inducing ions before crop plantation will help in enhancing the productivity of the land, thereby increasing the yield.

Keywords: Salt stress, Salt tolerance, Stress conditions, *Vigna mungo*, *Cicer arietinum*.

Highlights

- Salt stress negatively affects Vigna mungo and Cicer arietinum, key food crops.
- Salt stress alters these plants' root and shoot growth, vascular bundles, and mitosis.
- NaCl-induced stress reduces catalase activity and chlorophyll, increasing proline and phenolic compounds.
- Vigna mungo is more responsive to salt stress than Cicer arietinum, emphasizing the need for soil assessment.
- This study provides initial insights into salt stress responses, paving the way for further research.

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Introduction

Salinity stress falls under abiotic stress, which refers to any deviation from optimal extrinsic conditions like relative humidity, temperature, and salinity. It includes stress caused by drought conditions (Bray *et al*., 2000). Similar to abiotic stress, there are biotic factors known as biological stress, expressed when plants are affected by diseases or attacked by pathogens (Gull *et al*., 2019).

Salinity is the accumulation of salt in the soil due to untreated water, advanced irrigation practices, pesticides, and water with heavy metals. High salinity impacts plant growth patterns, reduces water holding capacity, and affects xylem and tracheary elements. This leads to increased transpiration and salt accumulation in the soil (Godfray *et al*., 2010). Salt stress alters various plant growth parameters, reducing crop yield (El Sabagh *et al*., 2021).

Seed germination is a complex process affected by internal factors like hormones, proteins, age, and size of the seed, as well as external factors like salinity, temperature, light, moisture, and nutrients (N, P, K, Zn, Mn) (An and Lin, 2011). Salinity in the soil delays seed germination by affecting internal factors and impacting the rate of germination (Finch *et al*., 2006).

An increased root/shoot ratio is often observed when plants are subjected to salinity conditions (Bernstein *et al*., 2002; Delane *et al*., 1982; Weimberg *et al*., 1984). As soil salinity increases, salt concentration in plants also rises, affecting root/shoot ratio. High salt concentration inhibits shoot growth and morphological traits, hindering the primary response to salinity stress (Lazof and Bernstein, 1998; Munns, 1993). Despite root tolerance, salinity stress reduces root mass, diameter, length, elongation rate, volume, and lateral root production (Jbir *et al*., 2001; Shafi *et al*., 2010; Shahzad *et al*., 2012).

Root and shoot development affect vascular bundles, and leaves show significant effects in the presence of NaCl. Chlorosis, indicated by yellowing leaves, reduces epidermal thickness with bulliform cells, impacting both adaxial and abaxial regions (Carcamo *et al*., 2012). Salt stress inhibits mitotic activity, reducing

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the number of dividing cells. This interference with mitosis involves the inhibition of cell division by the salt stress response (Fernandes *et al*., 2007).

Salinity alters enzyme functioning and stress biomolecule accumulation. Salt-resistant plants, like Jatropha cucas, exhibit increased catalase enzyme activity (Gao *et al*., 2008). Phenols, known to protect from ROS species, increase in areas with salt and water stress (Cvikrova *et al*., 2006). Phenol content in tissues rises with increasing soil salt concentrations (Agastian *et al*., 2000). Proline, an amino acid, accumulates in response to abiotic stress, protecting cell membranes and enzymes during salt stress (Verbruggen and Hermans, 2008; Hmidi *et al*., 2018; Qureshi *et al*., 2013; Pottosin *et al*., 2014).

Materials And Methods

The Rashmi strain of Vigna mungo plant and JG11 strain of Cicer arietinum plants were procured for the current study. They were grown in sets of three groups, and one set was maintained as control. The treatment group 1 of plants was subjected to salt stress with 50 mM NaCl and treatment group 2 with 100 mM NaCl, respectively.

Selection of Plants

The suitable plant seeds were bought from Gandhi Krishi Vigyana Kendra GKVK, Bengaluru. The experiment seeds happened to be pre-treated, hence surface sterilization was not performed. After being submerged in water for eight hours, they were subsequently placed directly into a germination tray containing a mixture of soil and coco peat in a 2:1 ratio.

Data Recording And Analysis

Germination Analysis

The germination study was conducted by growing C. arietinum and V. mungo seeds containing moist absorbent cotton on petri plates. The seeds underwent a seven-day incubation period at room temperature to facilitate germination. Salt stress was introduced 72 hours after germination had commenced. Throughout this period, moisture levels were consistently monitored, and the total number of germinated seeds was tallied to calculate the overall germination rate (Fatemeh *et al*., 2015).

In the experimental phase, the plants were germinating for 72 hours were exposed to two distinct salt concentrations through spraying. Three separate sets were kept for subsequent analysis, including a control group and groups treated with 50 and 100 mM salt concentrations. To assess the plant's growth rate, measurement details were assessed for both shoot and root length and the count of adventitious roots. These measurements were recorded on the 7^{th}, 14^{th}, and 21^{st} day after the induction of salt stress. (Kumar *et al*., 2021). Furthermore, following a 72-hour germination period, transverse sections of the germinated seed's root were prepared. Before being placed in a watch glass filled with safranin, these portions were initially rinsed with distilled water for approximately one minute. Subsequently, the sections were examined under a microscope at a 10X magnification level to observe any alterations in the vascular bundles (Jupa *et al*., 2015).

The index of mitotic activity was determined utilising plant seeds. These seeds were initially washed with distilled water to ensure removal of any impurities or contaminants, followed by washing with mercuric chloride solution (0.1%) followed by several washes using distilled water. After surface sterilization, 10 to 15 seeds were taken in three petri plates. They germinated under salt stress for about 72 hours at different concentrations (control, 50 and 100 mM). Salt stress was given to root tips, which had grown 8 to 9 cm after 72 hours. They were fixed in "farmer fixative," which is glacial acetic acid and absolute ethanol in a ratio of 1:3 for about 24 hours, followed by treatment with acetocarmine and heat fixing. They were then squashed to visualize the mitotic stage (Srabani *et al*., 2012).

Biochemical Analysis

The catalase activity was studied using plant leaves (1g), which were finely ground with 5 mL of phosphate buffer (0.1M). The paste was filtered through a muslin cloth and the filtrate was centrifuged at 6000 rpm at 4°C for 30 minutes. To the supernatant (1-mL) H_2O_2 (2 mL) and phosphate buffer (3 mL) was added. A blank was prepared with using 1-mL of distilled water. It was incubated at 20°C for 1-minute, reaction was stopped by adding 10 mL of 0.7 N H_2SO_4. Then it was titrated against 0.01 N $KMnO_4$, endpoint was the development of a permanent pale pink color. Titrations were performed repeatedly until consistent values were obtained (Thimmaiah *et al*, 2004).

The salt stress induced plant leaves (0.5 g) were ground with 10 times the volume of 80% ethanol to estimate the phenol content. The homogenized mixture was subjected to centrifugation at a speed of 10,000 revolutions per minute (rpm) for a duration of 20 minutes. The supernatant was preserved, and the remaining residue underwent another round of extraction with 80% ethanol. The mixture was centrifuged, and the resulting supernatant was collected. It was then placed in a hot air oven set at 64°C to allow for the evaporation of the supernatant until it became dry. The resulting residue was reconstituted by dissolving it in a known volume of 5 mL of distilled water. Subsequently, aliquots of this dissolved residue ranging from 0.1 to 0.5 mL were adjusted to a final volume of 3 mL with water. To this solution, 0.5 mL of Folin-Ciocalteu reagent was added, and after 3 minutes, 2 mL of 20% Na_2CO_3 was introduced. The mixture was then briefly subjected to boiling water for 1-minute. After cooling, the tubes were used to measure absorbance at 650 nm. By utilizing different concentrations of standard catechol, standard curve was created. (Goldblith and Proctor, 1951).

For determination of proline content, the plant samples (0.2 g) were homogenized using 10 mL sulpho-salicylic acid. The plant extracts were run in a centrifuge at 6000 rpm for 7 minutes. The supernatant was collected, equal volume of ninhydrin reagent and glacial acetic acid mixture, and 20 mL of phosphoric acid were added. It was heated at 100°C for an hour, vivid red hue was observed and they were further kept in a cold water bath for 2 minutes. To this equal volume of toluene was mixed. The upper layer containing the toluene was used to read the absorbance at 520 nm. (Bates *et al*., 1973)

To estimate the chlorophyll content, 0.25 grams of plant sample were utilized. This sample was homogenized in 80% acetone (10 mL). Subsequently, the plant extracts underwent

centrifugation at 3000 rpm for a duration of 10 minutes. The resulting supernatant was collected and standardized with 25 mL of acetone. Chlorophyll a and chlorophyll b levels were determined by measuring the absorbance at 645 and 663 nm, respectively (Mackinney 1941).

Results And Discussion

Effect of NaCl stress on morphological parameters

The results indicated that V. mungo had higher germination rate and longer roots compared to C. arietinum for both treatments (Table 1). The shoot length increased as an outcome of the salt stress till 21 days. However, the growth rate of the experimental plants was reduced comparatively to that of the control plants (Tables 2a and 2b). The shoot length showed a similar trend in both *V. mungo* and *C. arietenum* plants. *C. arietenum* plants in treatment group 2 showed lesser root growth of an average of 27.4 cm as compared to treatment group 1 where the average was 28 cm. The *V. mungo* plants in treatment group 1 followed a similar pattern by showing an average growth of 84.2 cm and treatment group 2 was 55.6 cm. In *V. mungo* plants, the root length in treatment group 1 showed higher growth with an average of 7.7 cm compared to treatment group 2 where the average growth was 6.6 cm. *C. arietinum* followed the same trend in both the treatment groups as that of *V. mungo* (Table 3a and 3b). These findings are consistent with prior research demonstrating how salt stress can impact plant growth, either directly or indirectly, and these effects can vary among plant species (Munns *et al.,* 2008). The observation of quantification of adventitious roots in the experimental plants of C.arietinum revealed a decrease as salt stress levels increased, in contrast to the control group. The notable trend becomes evident from the second week onwards, as the plants in the two groups consistently exhibited a declining number of adventitious roots compared to the control group. (Control: 18.2 ± 3.033, 50 mM: 17.8 ± 4.969, 100 mM: 15.4 ± 2.701). This pattern also continued in the third week of the treatment (Control: 20.4 ± 3.209, 50 mM: 20.2 ± 3.271, 100 mM: 18.6 ± 4.393). Conversely, the number of adventitious roots in V. mungo displayed a variable pattern in response to salt stress. During the first week of treatment, plants in treatment group 1 exhibited a slight increase in the number of adventitious roots (10.2 ± 2.774), which was significantly higher than both the control and treatment group 2 (Control: 7.8 ± 3.271, 100 mM: 10 ± 1.581). In the second week of exposure to salt stress, plants in treatment group 2 had a notably higher number of adventitious roots (19.8 ± 3.492) compared to the control group and treatment group 1 (Control: 17 ± 4.472, 50 mM: 15.2 ± 4.494). This pattern persisted into the third week, where again, the number of adventitious roots in treatment group 2 (20.8 ± 3.271) exceeded that of the control group and treatment group 1 (Control: 17.2 ± 1.303, 50 mM: 15.6 ± 4.979) (Table 4a and 4b). The findings are similar to the previous finding that salt concentration in soil makes it difficult for the seed to extract the water and utilize it for its growth through the process of osmosis (Pantola *et al*., 2017). Moreover, the reduction in the number of adventitious roots from 20.2 to 18.6 with increasing salt stress in C. arietenum plants also corresponds to the previous findings where vegetative growth was Showed to be significantly affected due to salt stress (Blaylock *et al*., 1994). Under salt stress subjection, both plant species deteriorated in the vascular bundle's group. (Figs 1 and 2). This phenomenon is similar to a previous report concerning Zea mays. (Farhana

Table 1: Effect on Germination rate in experimental plants

Concentration/ Plant Species	*Control (%)*	*50 mM (%)*	*100 mM (%)*
Cicer arietenum	92	84	88
Vigna mungo	100	92	100

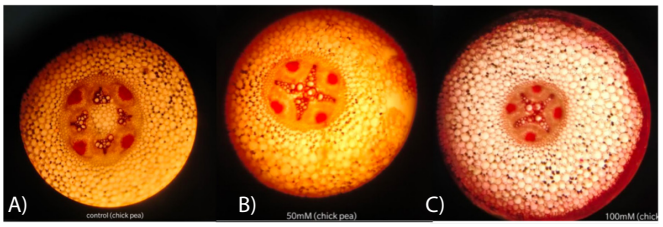

Fig. 1: Effect of salt stress on the development of vascular bundles in experimental plants of *C. arietenum* A) Control B) 50 mM C) 100 mM concentration of NaCl

Table 2a: Effect of salt stress on the shoot length in *C. arietinum*

Concentrations/ Days of salt stress	*Shoot Length of C. arietinum (cm)*		
	Control	*50mM*	*100mM*
7	28.6	11.5	8.7
14	24.2	23.2	16.2
21	30	28	27.4

Table 3a: Effect of salt stress on the root length in *C. arietinum*

Concentration/ Days of salt stress	*Root length of C. arietinum (cm)*		
	Control	*50mM*	*100 mM*
7	25.3	14.8	3.3
14	6	7.2	6.2
21	7.3	7.7	6.6

Table 2b: Effect of salt stress on the shoot length in *V. mungo*

Concentrations/ Days of salt stress	*Shoot Length of V. mungo (cm)*		
	Control	*50mM*	*100mM*
7	19.8	58.1	10.8
14	97.2	78	64.4
21	106.8	84.2	55.6

Table 3b: Effect of salt stress on the root length in V. mungo

Concentration/ Days of salt stress	*Root length of V. mungo (cm)*		
	Control	*50mM*	*100 mM*
7	4.1	4.8	3.3
14	6	7.2	6.2
21	7.3	7.7	6.6

Table 4a: Effect of salt stress on the adventitious roots in *C. arietinum*

Concentration/ Days of salt stress	Number of adventitious roots in C. arietinum		
	Control	50mM	100 mM
7	7.4	10	10
14	18.2	17.8	15.4
21	20.4	20.2	18.6

Table 4b: Effect of salt stress on the adventitious roots in *V. mungo*

Concentration/ Days of salt stress	*Number of adventitious roots in V. mungo*		
	Control	*50mM*	*100 mM*
7	7.8	10.2	10
14	17	15.2	19.8
21	17.2	15.6	20.8

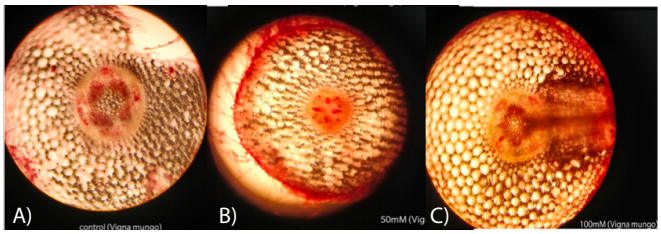

Fig. 2: Effect of salt stress on the development of vascular bundles in experimental plants of *V. mungo* A) Control B) 50 mM C) 100 mM concentration of NaCl

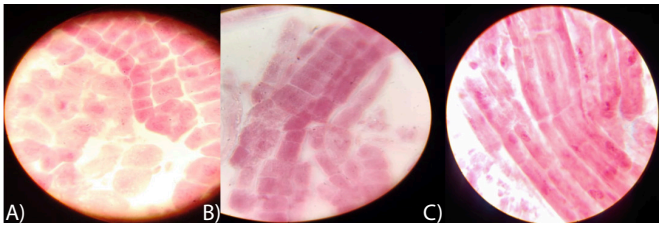

Fig. 3: Effect of salt stress on the mitotic index in experimental plants of *C. arietenum* A) Control B) 50 mM C) 100 mM concentration of NaCl

et al., 2014).

In *C. arietinum*, the mitotic index was observed to decrease in the plants grown in treatment group 1 (34.09) compared to the control (66.66). However, the mitotic index was seen to increase in the plants grown in treatment group 2 (74.28) than the control (66.66) (Fig. 3).

Similar pattern was observed in *V. mungo* where the mitotic index in plants grown in treatment group 1 was less (28.57) than the control (51.56). It was again seen to increase in the plants grown in treatment group 2 (41.86) was lesser than in control (51.56) (Fig. 4).

Effect of Nacl on Biochemical Parameters

The catalase activity in the experimental plants of both C. arietenum and *V. mungo* was observed to decrease with the increasing salt concentration. In *C. arietenum*, the catalase

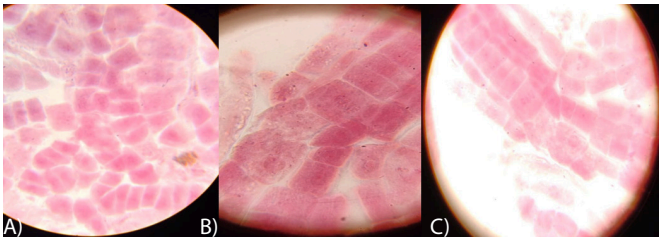

Fig. 4: Effect of salt stress on the mitotic index in experimental plants of *V. mungo* A) Control B) 50 mM C) 100 mM concentration of NaCl

Table 5: Effect of salt stress on the catalase activity in experimental plants

Concentration	*C. arietenum µmol/ml/min*	*V. mungo µmol/ml/min*
Control	15	10
50 mM	10	7.5
100 mM	8.75	6.25

Table 6: Effect of salt stress on the total phenolic content in the experimental plants

Concentration	*C. arietenum mg/ml*	*V. mungo mg/ml*
Control	0.00367	0.114
50 mM	0.1625	0.482
100 mM	0.506	0.273

activity decreased from 10 mol/ml/min in treatment group 1 to 8.75 mol/ml/min in treatment group 2. This trend was Showed to be similar for V. mungo. The catalase activity decreased from 7.5 µmol/mL/min in test group 1 to 6.25 µmol/mL/min in test group 2 (Table 5). Catalase is one of the ROS enzymes that gets affected due to salt stress. It is known to play a primary role in providing salt tolerance (Gao *et al*., 2008). The previous report concluded that wheat plants showed a decline in catalase activity with increasing soil salinity (Hameed *et al*., 2008). However, in grass species, an initial decrease of the activity of catalase was observed but with higher concentrations of salt stress the enzymatic capability catalase increased, which represents the development of salt tolerance (Mane *et al*., 2010).

The phenol content was observed to increase with the rise in salt concentration. In C. arietinum plants, it reached 0.16 mg/ml in treatment group 1 and 0.5 mg/mL in treatment group 2. Conversely, in V. mungo, the highest phenol content was recorded in treatment group 1 (0.482 mg/mL) as compared to treatment group 2 (0.273 mg/mL) (Table 6). The results do not follow the previous findings in which plants from different species of artichoke show a decrease in phenolic compounds after a certain concentration of salt stress (Rezazadeh *et al*., 2012).

The proline content, another stress-related compound, increased in experimental plants with the increase in salt concentration. In the control group, both C. arietinum and V. mungo showed the same value, which was 0.052 mg/mL. Nevertheless, it's noteworthy that the experimental plants of C. arietinum exhibited a notable rise in proline content as

Table 7: Effect of salt stress on the total proline content in in the experimental plants

Concentrations	*C. arietenum mg/ml*	*V. mungo mg/ml*
Control	0.052	0.052
50 mM	0.26	0.364
100 mM	0.469	0.625

*(cp – C. arietenum, vm – V. mungo)

Fig. 5: Absorption spectra of chlorophyll a and b in the experimental plants

the salt concentration increased (0.26 mg/mL in treatment group 1 and 0.469 mg/mL in treatment group 2). V. mungo experimental plants also showed a similar trend wherein the amount of proline content increased in subsequent testing groups (0.364 mg/mL in treatment group 1 and 0.625 mg/mL in treatment group 2) (Table 7). The previous findings reported that proline is one of the biomolecules that accumulates in plant tissues when exposed to salt stress, as it plays a protective role against damage or injury to the plant (Gratão *et al*., 2005). The current results align with these findings. Chlorophyll content, an indicator of photosynthetic activity, peaked in the respective control groups. The absorbance of C. arietinum control group was observed to be at 1.427 at 645 nm and 2.716 at 663 nm. In the control group of V. mungo, the absorbance was measured at 1.081 for 645 nm and 2.057 for 663 nm. Interestingly, in the experimental groups of C. arietenum, the peak absorbance was observed to be higher in treatment group 2 (0.837 at 645 nm and 1.310 at 663 nm) than treatment group 1 (0.778 at 645 nm and 1.118 at 663 nm). In the experimental sets of V. mungo plants, a comparable trend was noted where the absorbance was higher in treatment group 2 (0.684 at 645 nm and 1.038 at 663 nm) than that of the treatment group 1 (0.562 at 645 nm and 0.865 at 663 nm) (Fig. 5). This implies that stress by higher salt levels negatively affected the photosynthetic capacity of both V. mungo and C. arietinum. This is in accordance to the findings in the previous research, where it was Showed that sodium chloride reduces the total amount of chlorophyll through an increase in the chlorophyllase activity (Rao and Rao, 1981).

Conclusion

In summary, this study exhibits that higher salt concentration has a profound impact on various morphological and biochemical characteristics of plants. Salt stress affects shoot length, root length, and the number of adventitious roots, with an interesting finding in V. mungo, where 100 mM of salt stress actually led to increased adventitious root growth.

The mitotic index value exceeded its regular amount in Cicer arietinum plants under 100 mM salt concentration. However, both plant species exhibited a significant decline in catalase activity in response to salt stress. Additionally, the aggregation of proline and phenols in plant tissue, indicative of salt stress, showed an increase. The absorption spectrum of chlorophyll pigments revealed that control plants had higher absorption compared to experimental plants, suggesting a potential decrease in photosynthetic activity.

This study also demonstrates how V. mungo displays better tolerance to salt stress compared to C. arietinum, evident through its higher germination rate, longer roots, and more stable formation of adventitious roots. These differences in physiological and biochemical responses underscore the importance of understanding plant-specific mechanisms in response to salt stress. Overall, this research confirms that NaCl induces significant morphological and biochemical changes in plants, and further studies could provide valuable insights for addressing this agricultural challenge.

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Author's Contributions

Dr. Suba G A Manuel and Dr. Padmashree Kulkarni designed the experiment and conceptualised the idea. Ms. Bhoomika Sharma performed the experiments, collected data, analysed the data, and drafted the manuscript. All the authors have critically read the drafted manuscript and approved it for publication.

Conflict Of Interest

None

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