Research Article

# Ameliorating Effects of Salt Stress (KCl, NaCl) on Growth and Germination Parameters of Pearl Millet (*Pennisetum americanum*)

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**Abstract** | Plants facing many abiotic stresses in which salinity is considered as most pronounced factor to effect the plant growth. This research was performed to assess the effects of NaCl, KCl, a mixture of NaCl + KCl in various concentrations on the growth and germination of Pearl millet (*Pennisetum americanum*), locally named as Bajra. Five seeds sown in petri dishes to observe the radical, plumule length and germination percentage after treatments to 100, 150, 200 and 250mM concentration of above mentioned salts. Control petri dishes/ pots were irrigated with distilled water. The experiment was extended to study growth (number of leaves, leaf length, stem breadth, stem length, number of tillers) and yield (spike length, total seeds on single spike, whole plant weight, weight of 50 seeds per spike) parameters in the field, which was performed in completely randomized design (CRD). 39 pots lined with polythene bags were used to avoid percolation of water. Data was analyzed by variance (ANOVA). Salinity effects on the test species were analyzed by (LSD) Least Significant Differences method. 100mM concentrations of the said two salt and a mixture of two salts concentrations was less stress causing on all parameters studied. The degree of toxicity of the salts decreases as NaCl<KCl<NaCl+KCl.

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## Introduction

Among all the abiotic stresses, salinity is considered as the most toxic one to affect the quality and quantity of the crops (Ouda *et al.*, 2008) thus highly affecting the health of economy and farming circles. World has faced issues for healthy soil resources for upcoming generations (Munns, 2003). Halophytes and glycophytes are very sensitive to salt stress and hence they adopt certain mechanisms to resist salinity in order to function properly (Zhu, 2003). In short salinity highly affects main physiological processes of plant viz photosynthesis, growth, metabolism of lipid and protein synthesis (Parida and Das, 2005).

Plants facing abiotic stress of salinity produce various products in which Reactive Oxygen Species (ROS) is very important. ROS destroys components of cell and eventually influence the metabolic processes of the plant (Foyer *et al.*, 1997). Salinity is accumulation of various salts which may be present in combinations, such as, soluble forms of Sodium, Chloride ions, Calcium, Sulphate and Magnesium and the trace salts including Borate, Bicarbonates, Carbonates, Lithium and Potassium (Zhu, 2001). Some of the main reasons for destruction of soil with salt stress include entry of irrigation water with poor drainage system, removal of natural plant cover, overuse of fertilizers, industrial effluents, salt rich water flooding, raised water table and poor quality of groundwater (Parida and Das, 2005) and this has resulted in a serious threat to advance agriculture causing loss and imbalance in


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development of crops (Isayenkov and Maathuis, 2019). Plants facing salt stress become sensitive to salinity and in turn check its growth (Rani, 2011). It has been reported that all the developmental stages of Pearl millets are checked by salinity (Ashraf and Idress, 1992; Ashraf *et al*., 2003). Increase in the levels of salinity reduces the percent germination, height of plant, area of leaf, biomass of the whole plant and productivity of the Pearl millet (Khan *et al*., 2002). Almost all stages (vegetative and reproductive) are stressed and declined in pearl millet (Kafi *et al*., 2018; Radhouane, 2013; Dadar *et al*., 2014).

Cereals are significant food crops and utilized universally. Millet is sixth most important crop (Shahidi and Chandrasekara, 2013). *Pennisetum* is considered as one of the most popular cereal crop of drought area of Pakistan, despite its economic importance this crop has received little attention as compared to other crops and is being grown in different areas of Pakistan (Siddig *et al*., 2013; Sarwar *et al*., 2013).

*Pennisetum* (Pearl millet) is considered to be salt bearing crop when compared to other crops (Krishnamurthy *et al*., 2007) and maximum stress tolerance has been observed in several species of *Pennisetum* (Muscolo *et al*., 2003).

Pakistan, Algeria, Iran and India facing extreme environmental conditions like salinity, drought and extreme temperature favors growth of millets. Due to their $C_4$ photosynthetic pathway and brief life cycle millet is considered as a favorable crop of stressed environments (Blastenspergo *et al*., 2000; Blatensperger, 2002). Tolerance may be maintained by subjecting the seeds to osmotic stress before its germination (Idress and Ashraf, 1992).

Millet being considered as favourable crop in the stressed areas of Pakistan, the present study was conducted to assess the effects of NaCl, KCl, a mixture of NaCl + KCl in various concentrations on the growth and germination of Pearl millet (*Pennisetum americanum*). Further the capacity of Pearl millet to cope the salt stress is assessed in this study.

## Materials and Methods

### *In vitro test*

Pearl millet (*Pennisetum americanum*) seeds were collected from Department of Botany Islamia College Peshawar. Surface of seeds were sterilized by $HgCl_2$ (0.1%) for 30 seconds and different parameters like germination, radical and plumule length of seeds were recorded. Petri dishes after sterilization were lined with blotting paper and a layer of cotton, salt solution concentrations (100,150, 200 and 250mM) of KCl, NaCl and mixed (NaCl+KCl) was used to moisten the petri dishes. Replicate petri plates (three) of each treatment consisted of 5 seeds. Seeds soaked with distilled water were considered as control, dishes were incubated for 3 to 5 days at 27° C.

### *Screen house experiment*

An experimental trial was performed in pots in the screen house bench at the Islamia College Peshawar in CRB design (Sam *et al*., 2014). Sandy loam soil collected from Botanical garden field, Botany Department, Islamia College Peshawar, Pakistan. The sterilized soil with pH 8.0 was used for pot experiment. For the experiment, total 39 pots (32 cm diameter) were used, 3 replicates for each treatment were done and filled with 4 kg sterilized fertile soil in each pot @ 10 seeds of pearl millet/pot were sown. After 5days of germination only five seedlings were left and extra were removed from each pot by thinning. The treatment was applied when seedlings were at two leaf stage. Concentrations of different salt included NaCl, KCl and NaCl+KCl and were prepared st concentrations of 100mM, 150mM, 200mM and 250mM from each salts. While distilled water was used for control. Solution of 10ml from each salt concentrations were added in each pot at different interval of time, that is, 15, 30 and 45days. After 90 days of growth of pearl millet, different growth parameters like radical length, Plumule length, number of tillers, stem breadth, stem length, number of leaves, leaves length, spike length, total seeds on single spike, weight of whole plant taken, weight of 50 seeds from each concentration were taken and control were recorded.

### *Statistical study*

The data was analyzed of variance (ANOVA). Different growth parameters and germination of pearl millet (*Pennisetum americanum*) were subjected to one-way ANOVA followed by the least significant difference LSD test at P=0.05. All analysis was performed using IBM-SPSS STATISTICS program (Sokal and Rohlf, 1995).

*In vitro test*

Different concentrations of various salt solutions (100mM, 150mM,200mM and 250mM) showed significant indirect effect on length of radicle and Plumule of millet plant compared to control. Extreme stress was produced on Plumule and radical length at highest concentration (250mM) of salt solution. Minimum length of plumule and radical were produced when mix (NaCl+KCl) concentrations were observed. Percent germination was also affected when concentration of different salts was applied at different time period (Figure 1).

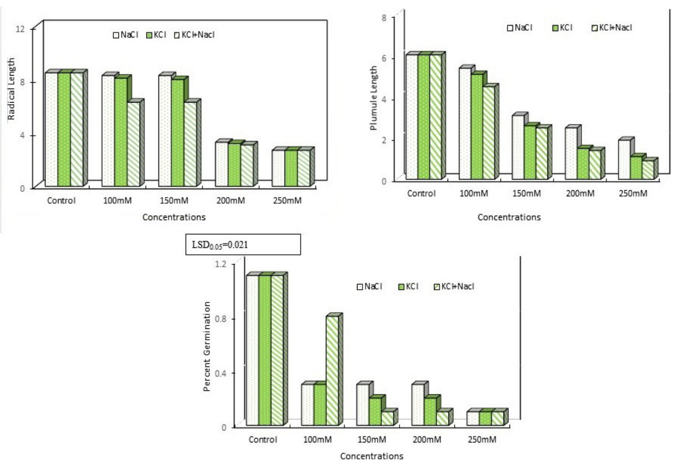


**Figure 1:** *Effect od salt stress (100 mM, 150mM, 200mM, 250mM) on germination of Pearl millet (Pennisetum americanum).*

*Screen house experiment*

Salinity is known to induce stressed condition in plant. In present study different concentration of salt solutions were applied to observe the adverse effect on the growth of plant in contrast with control, such as stem length and stem breadth were gradually decreased by increasing concentration of treatments (KCl and NaCl and by mixed combination of NaCl+KCl), it is very interesting to note that at 100mM concentration of NaCl and KCl no significant effect was found (Table 1). This depicts that plant can tolerate the salinity at 100mM saline solution whereas significant ($p<0.05$) decrease observed in the length and breadth of plant at concentration higher than 100mM of NaCl and KCl. Similarly, number of tillers, numbers of leaves and length of leaves were also affected significantly ($p<0.05$) with the intensification of concentration of salt solutions of NaCl, KCl and mixed (NaCl + KCl) salt concentration at different intervals (Table 1). Spike length and weight of whole plant also depicted indirect significant ($p<0.05$) effect by decreasing the length of spike, weight of whole plant and grain weight (Figure 2). This shows that yield of the plant reduced by intensification in concentration of the salts i.e, the yield reduced by salt stress in following pattern control> 100 mM > 150 mM > 200 mM > 250 Mm (Figure 2). In all figures the control plants showed good growth which is followed by 100 mM concentration salt stress. This shows the said concentration (100 mM NaCl) soils can be used for growing the pearl millet.

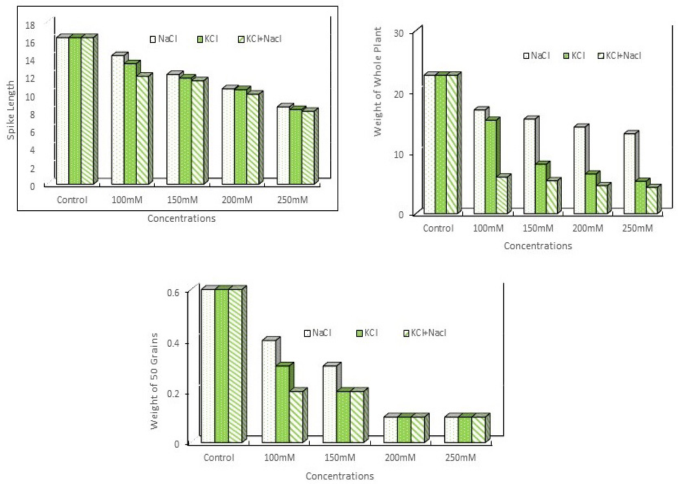


**Figure 2:** *Effect od salt stress (100 mM, 150mM, 200mM, 250mM) on yield of Pearl millet (Pennisetum americanum).*

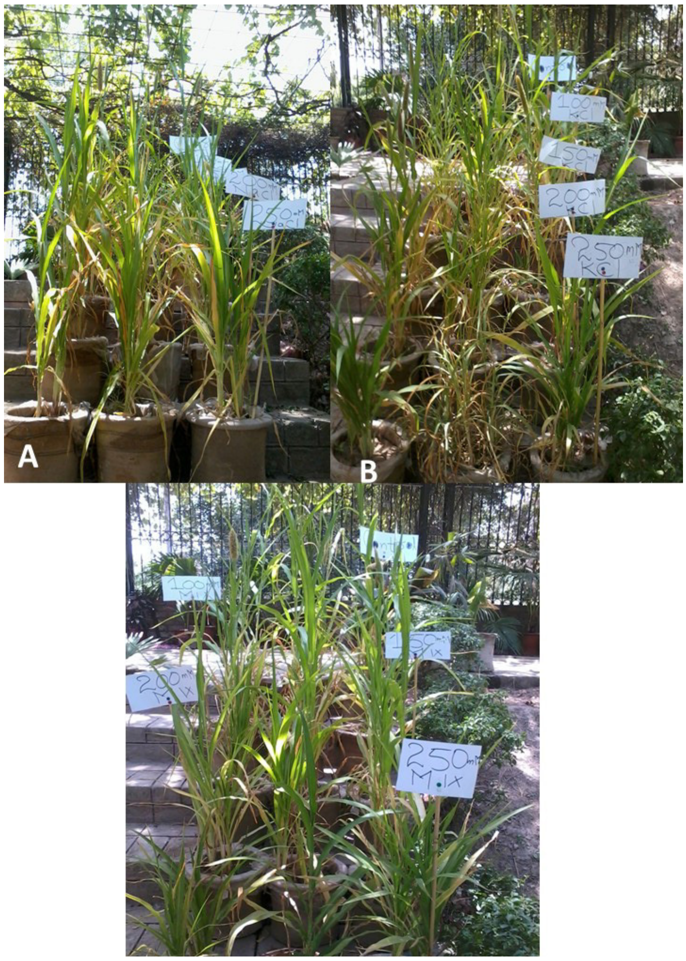


**Figure 3:** *Effect of different salt stress on the growth of Pearl millet. A: NaCl; B: KCl; C: Nacl+KCl.*

**Table 1:** *Effect of different salt stress (100 mM, 150 mM, 200 mM, 250mM) influence on vegetative growth of Pearl millet (Pennisetum americanum).*

| Vegetative Growth Parameters | Concentrations | Treatments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NaCl | | | KCl | | | NaCl + KCl | | |
| | | Dose 1 (15 days) | Dose 2 (30 days) | Dose 3 (45 days) | Dose 1 (15 days) | Dose 2 (30 days) | Dose 3 (45 days) | Dose 1 (15 days) | Dose 2 (30 days) | Dose 3 (45 days) |
| Stem length (Mean in cm) | Distilled Water | 5.1 | 6.3 | 77.3 | 5.1 | 6.3 | 77.3 | 5.1 | 6.3 | 77.3 |
| | 100mM | 3.0 | 5.4 | 76.3 | 4.4 | 6.2 | 76.3 | 4.5 | 8.3 | 73.6 |
| | 150mM | 3.0 | 5.0 | 64.2 | 4.4 | 5.2 | 64.2 | 3.4 | 6.5 | 67.3 |
| | 200mM | 5.9 | 5.0 | 27.5 | 3.4 | 5 | 27.5 | 3.0 | 6.2 | 46.3 |
| | 250mM | 2.4 | 4.7 | 2.4 | 3.3 | 4.5 | 23.7 | 3.0 | 5.3 | 45.2 |
| $LSD_{0.05}$ | | 1.479 | | | 3.917 | | | 0.132 | | |
| Stem breadth (Mean in cm) | Distilled Water | 1.06 | 1.6 | 1.6 | 1.06 | 1.6 | 1.6 | 1.06 | 1.6 | 1.6 |
| | 100mM | 0.08 | 1.5 | 1.5 | 0.08 | 0.4 | 1.1 | 0.08 | 0.08 | 0.9 |
| | 150mM | 0.08 | 1.3 | 1.3 | 0.08 | 0.4 | 0.5 | 0.08 | 0.08 | 0.7 |
| | 200mM | 0.08 | 1.1 | 1.2 | 0.08 | 0.3 | 0.3 | 0.08 | 0.08 | 0.6 |
| | 250mM | 0.08 | 0.1 | 0.1 | 0.08 | 0.08 | 0.1 | 0.08 | 0.08 | 0.5 |
| $LSD_{0.05}$ | | 0.031 | | | 0.039 | | | 0.039 | | |
| No.of tillers (Mean in cm) | Distilled Water | 1.5 | 1.5 | 1.7 | 1.5 | 1.5 | 1.7 | 1.5 | 1.5 | 1.7 |
| | 100mM | 0.08 | 1.1 | 1.5 | 0.08 | 0.08 | 0.5 | 0.08 | 0.4 | 0.2 |
| | 150mM | 0.08 | 1.1 | 1.4 | 0.08 | 0.08 | 0.5 | 0.08 | 0.2 | 0.1 |
| | 200mM | 0.08 | 0.8 | 1.3 | 0.08 | 0.08 | 0.2 | 0.08 | 0.08 | 0.1 |
| | 250mM | 0.08 | 0.4 | 0.7 | 0.08 | 0.08 | 0.3 | 0.08 | 0.08 | 0.1 |
| $LSD_{0.05}$ | | 0.028 | | | 0.044 | | | 0.052 | | |
| No. of leaves (Mean in cm) | Distilled Water | 6.1 | 10.5 | 11.2 | 6.1 | 10.5 | 11.2 | 6.1 | 10.5 | 11.2 |
| | 100mM | 5.1 | 8.4 | 10.5 | 4.6 | 7.7 | 8.5 | 5.6 | 6.2 | 7.7 |
| | 150mM | 4.3 | 7.8 | 9.1 | 4.4 | 6.0 | 8.1 | 5.3 | 6.0 | 7.7 |
| | 200mM | 4.3 | 7.0 | 8.2 | 3.8 | 5.4 | 7.5 | 4.8 | 5.5 | 6.3 |
| | 250mM | 4.0 | 6.1 | 7.1 | 2.8 | 5.3 | 6.7 | 4.1 | 5.5 | 5.9 |
| $LSD_{0.05}$ | | 0.0782 | | | 0.0706 | | | 0.0738 | | |
| Leaves length (Mean in cm) | Distilled Water | 14.3 | 18.6 | 43.8 | 14.3 | 18.6 | 43.8 | 14.3 | 18.6 | 43.8 |
| | 100mM | 12.3 | 12.5 | 36.1 | 11.6 | 16.2 | 34.6 | 16.4 | 17.3 | 42.3 |
| | 150mM | 11.1 | 12.3 | 27.3 | 11.6 | 15.5 | 29.6 | 11.1 | 13.7 | 32.7 |
| | 200mM | 10.2 | 11.3 | 25.2 | 9.3 | 14.5 | 29.1 | 10.3 | 11.1 | 29.1 |
| | 250mM | 10.03 | 11.04 | 25.2 | 8.7 | 14.4 | 21.3 | 9.2 | 10.06 | 21.2 |
| $LSD_{0.05}$ | | 0.0568 | | | 0.0146 | | | 0.0901 | | |

## Results and Discussion

In the present study all the vegetative and reproductive parameters flourished well in control (Distill water) which is followed by 100 mM salt stress and then the parameters showed decline in growth. Growth and germination of Pearl millet showed positive response in control (Distilled water) (Greenway and Munns, 1980). In the present study after the control the best growth was observed in pearl millet treated with 100mM concentrations of NaCl. This is similar to the results obtained by Takemura *et al*., 2000, i.e amelioration in growth parameters of Pearl millet at 25mM concentration of NaCl. Similarly, the reduction in the length and number of leaves with increased salinity has been observed by Bukhari *et al*., 2012. The reduced growth of leaf depicts the osmotic potential in rhizosphere of the plant (Passioura and Munns, 2000). It is observed that the weight of whole plant was significantly affected as the saline stress was increased whereas at 100mM NaCl concentration the growth of whole plant enhanced. Same result was observed by Bukhari *et al*., 2012. Seed number (yield) also reduced as the salinity (NaCl, KCl and NaCl+ KCl) increased, significant value of $LSD_{0.05}$=0.020 endorsed the effect. Same result for seed production was observed by (Ali and Idris, 2015).

Stem length, Stem breadth and number of tillers reduced as the salt stress concentrations exceeded 100Mm. The shoot growth showed significance effect as the concentrations of NaCl salt increased (Bukhari *et al*., 2012).

Pearl millet is though believed to be a halophyte, but most of the characteristics of this plant are highly affected by higher salt concentrations.

## Conclusions and Recommendations

The present study revealed that maximum levels of (NaCl, KCl, NaCl+KCl) salts adversely affected radical, plumule length, percent germination, number of tillers, stem breadth, stem length, leaf length, number of leaves, spike length, number of seeds per spike, weight of whole plant, weight of 50 seeds of spike. NaCl salt is not stress inducing salt as compared to KCl and NaCl+KCl salts. Pennisetum is proved as halophyte at certain levels of salt stress i.e, it can withstand saline stress upto 100 mM concentrations.

## Novelty Statement

Novelty of the work is to compare three different salt effects (the least stressful salt i.e., NaCl) on growth and germination (tolerance) of Pearl millet and finding tolerance index (in terms of high yield at specific concentration i.e., 100Mm of NaCl).

## Author's Contribution

**Zubia Rahim:** Conceived and designed the experiments.
**Zubia Rahim:** Performed the experiments.
**Dr. Gulnaz Parveen and Zubia Rahim:** Analyzed the data.
**Zubia Rahim, Dr. Khushnood ur Rehman:** Contributed materials/ analysis/ tools.
**Dr. Gulnaz Parveen, Zubia Rahim and Dr Salma Gul:** Wrote the paper.

*Conflict of interest*

The authors have declared no conflict of interest.

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