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Amelioration of Salt Stress in Cowpea (*Vigna unguiculata* L.) through Potassium Nitrate Application

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ABSTRACT

Presence of salt in water and soil considered as an important stress factor of environment that severely reduced development of plant and its growth. When plants grow under such stress condition, adequate and appropriate fertilizers and good application methods can minimize such hazardous effects of stress and increased productivity of plants. Keeping such consequences in mind field trial has been taken and pot experiment was designed to examine the potassium nitrate effects (foliar) on the response of cowpea (*Vigna unguiculata* L.) grown under different levels of salts. Three levels of irrigation water salinity (0, 2.5 dS/m and 5 dS/m seasalt irrigation) and three levels of potassium nitrate (0, 250 ppm and 500 ppm) were applied in complete randomized block design with three replicates. Results exhibited reduction effect of salinity on growth (plant height, root length, fresh and dry biomass) and biochemical parameters (Chlorophyll a, b total chlorophyll, and protein) and relative water content and potassium ion concentration in different plant parts while carbohydrate and potassium ion concentration showed increase as seasalt salinity increase in irrigation water. Potassium nitrate application significantly improved abovementioned growth and biochemical parameters, thus helps in alleviation of stress condition.
KEYWORDS: Salinity, Potassium, Chlorophyll, Proteins, carbohydrates.

INTRODUCTION

Plant expose to different stresses in the environment and salt stress either it is present in soil or in water is the major form that cause reduction in plant development and growth all over the world [1, 2]. Sodicity and salinity cause billions of financial loss and reduced in crop yield every year. In the field of agriculture presence of soluble salts in high concentration in the soil moisture of root zone is known as soil salinity. One of the major causes in the increase in soil salinity is usage of contaminated and poor quality water which then results in reduction in the yield of salt sensitive crops [3]. Presence of high amount of soluble salts in saline water/soil produced high osmotic pressure which ultimately restricted the uptake of water through roots and results in reduction of plant growth. High concentration of salt in soil solution also disturbed the balanced absorption of essential elements/nutritional ions by plants [4]. When we compared normal soil conditions with stressed ones absorption of toxic ions in higher concentration under high salt level results in the increased ions level in the tissue inside, ultimately more negative osmotic potential was observed in the tissue and cause reduced development and growth of plant [5]. Presence of high NaCl concentration results in nutritional imbalance in plants and it also reduces the ionic concentration of magnesium, calcium and potassium [6, 7].

Rate of photosynthesis had great effect on plant growth and development. This phenomenon was influenced negatively under different abiotic stresses and ultimately reduced plant development and growth of plant occur [7]. Soil and water salinity had more severe effect on plants during vegetative stage, so this stage considered more sensitive [8]. When we applied salt to any plant it either showed quick response and within few minutes it showed reduction (when plants in first growth phase) or it showed its effect within few days or weeks (second phase of growth) [7]. Many researchers concluded and stated that salt presence exhibited reduction in total dry biomass, root length and shoot length of different plants [9]. Presence of salt disturbed water potential and caused ionic imbalance in soil and cell which resulted in reduced developmental growth and productivity of most agricultural crops. Growth of plant and its yield depends on the presence different levels of salinity and its severity [10].

Potassium is very essential and abundant cation present in plants and its concentration in the cytoplasm was found between 100 and 200 mM [11] and in apoplastic region it may vary between 10-200 and sometimes reached upto 500 mM [12]. This nutrient had played an essential role in Osmoregulation, photosynthesis, phloem transport, stress

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resistance, enzyme activation, protein synthesis, energy transfer, stomatal movements and cation-anion balance [13]. Potassium is also required for different physiological processes such as tropism, regulation of osmotic pressure in cell and maintenance of cell membrane turgor and potential [14]. Efficient use of iron depends on adequate supply of potassium as application of higher concentration of potassium cause its competition with iron [15]. Present experiment was designed with the hypothesis whether potassium in the form of potassium nitrate had any beneficial and significant effect on the growth, biochemistry and elemental status of cowpea grown under different levels of root zone salinity.

MATERIAL AND METHODS

Plant biomass and growth parameters:

Vigna unguiculata L. seeds were purchased from Dubai International Bio-salt Agriculture Center. In this experiment 36 pots were used and divided into three groups (12pots/treatment). The three treatments were irrigated with water (control), 2.5 dS/m and 5dS/m in seawater and potassium nitrate was used as:

1st treatment: Without potassium nitrate.

2nd treatment: Potassium provided as potassium nitrate @ 250 ppm.

3rd treatment: Potassium provided as potassium nitrate @ 500 ppm.

The sandy loam soil was thoroughly washed and each pot having basal out let was approximately filled with 3 Kg of sandy loam. Full strength Hoagland's solution was used to fully saturate the soil. Healthy seeds were surface sterilized with mercuric chloride (0.1%) for 1 minute and then washed thrice with autoclaved distilled water. In each pot 5 seeds were sown and were irrigated with tap water i.e., 150 ml everyday. At three leaves stage the plants were thinned and only one plant was left in each pot. All the pots were arranged in a completely randomized design (CRD) in the Department of Botany, University of Karachi, Karachi. Sea salt treatment begins at this stage and irrigation with 1.5L tap water/Sea salt solution is done twice a week for each pot. Different concentrations of copper sulphate, molybdenum oxide and zinc sulphate were foliar applied at different concentrations when salinity was maintained in the pot. At termination of experiment, root length, number of leaves and branches, fresh and dried biomass, and number of pods per plant were recorded in harvested plants. Leaf samples were collected for biochemical analysis and relative water content during their splendid growth season.

Relative water content: (RWC)

To analyze the relative water contents method developed by [16]. The fresh weight (FW) of the leaves was measured and the leaves were then allowing to rehydrate in distilled water for two hours and their turgor weight (TW) was measured. To obtain dry mass (DM) leaf samples were placed in a pre-heated oven at 80°C for 48 hrs. The relative water content (RWC) were measured while using the following formula

Relative Water Contents (%) = (Fresh Weight-Dry Weight)/ (Total Weight-Dry Weight) *100

Chlorophyll content:

Chlorophyll contents (Chl) was measured in fresh leaves according to the protocol of [17].

Estimation of total Carbohydrate Content:

Carbohydrate estimation was performed in plant extracts by the method of [18] using an Anthrone reagent.

Estimation of total Protein contents:

Total protein contents were extracted and analyzed by the Bradford reagent method [19].

Mineral Estimation of Vegetative Parts

Samples of root, shoot and leaves were taken for analysis of different cations (Na^+ and K^+) during their grand growth period. The sample was dried and the weight of ash of 0.5 grams of each dry sample were taken. The ash solution was then prepared in 50 ml of deionized water and then diluted in deionized water for mineral analysis. The PFP1 flame photometer was used to measure the concentration of cations in the sample.

Experimental design and data analysis

The completely randomized design (CRD) was used for experimental purposes with three salt concentration and three replicas. Statistical analysis using SPSS software for analysis of variance (ANOVA), Duncan's multiple comparison using mean ($P < 0.05$).

RESULTS AND DISCUSSION

Growth

Data for different growth parameters (root length, plant height and fresh and dry biomass) presented in figures 1-4 revealed that plants treated with salinity exhibited significant ($P<0.001$) reduction in these parameters. Salt stress produced negative effects on growth of plants and this is a common phenomenon and serious problem which was reported by different workers [20, 4]. Main factors that affect growth of plant in salt stressed condition are imbalanced nutrition and specific ion toxicity. Plant exhibited reduction in leaf growth as most earlier and common salt stress response [21, 22]. At highest salinity level (0.6% sea salt solution) increase in dead leaves and leaf growth reduction both occur which further cause leaf area reduction in plant. When plant expose to salt stress, reduction in growth mainly caused due to the accumulation of different ions in young and especially in old leaves [20].

Potassium performs major role in balancing different ions in plants and this phenomenon is reflected in metabolism of nitrogen [23]. Foliar application of different potassium nitrate concentrations showed significant ($P<0.0001$) improvement in above mentioned growth parameters in saline as well as non-saline environment. Foliar application of potassium nitrate to salt treated cause improvement in the status of N and K and also cause reduction in uptake of toxic ions. Application of potassium caused an increase in leaf potassium content which followed by RuBP carboxylase activity, photorespiration and photosynthesis. In present investigation considerable improvement can be seen even under salinity stress condition. [24] investigated that when NaCl grown guava seedling supplemented with 10 mM calcium nitrate it exhibited beneficial effect on metabolism and growth. [25] grown sunflower under salt concentration (150 mM NaCl) and beneficial effect was observed when same plants were foliarly applied with 1.25% potassium nitrate and potassium sulfate. When vegetable crops grown and irrigated with saline water, this act lead to gradual increase of salinity stress in plant root zone. Increase in salinity cause decrease in phosphorus uptake and also cause reduction in its availability to plants [26] and it happens to Calcium and potassium as well [27], which results in reduced growth of roots and plants, while foliar application of potassium results in obtaining the desirable K/Na ratio [28].

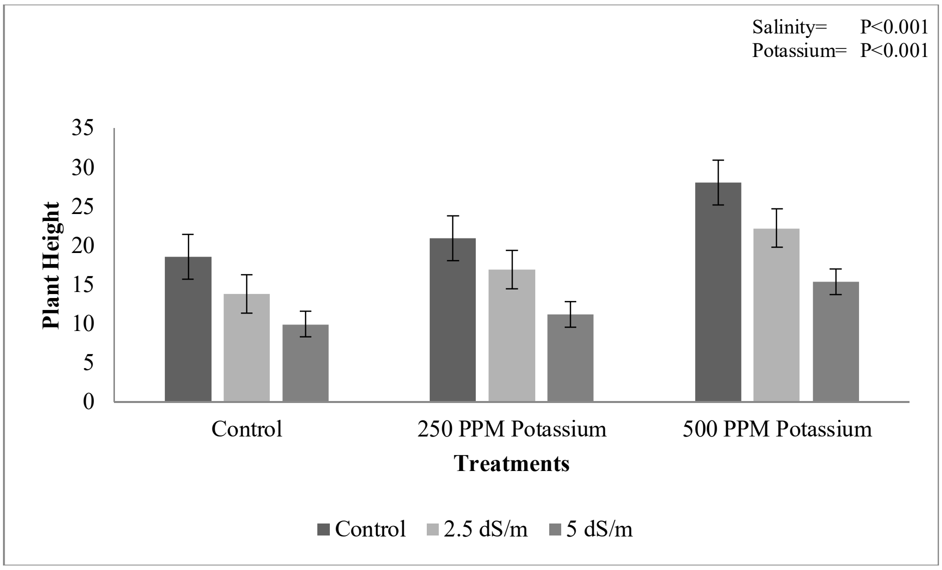

Figure 1. Influence of foliar application of potassium nitrate on plant height (cms) on *Vigna unguiculata* grown under seasalt stress.

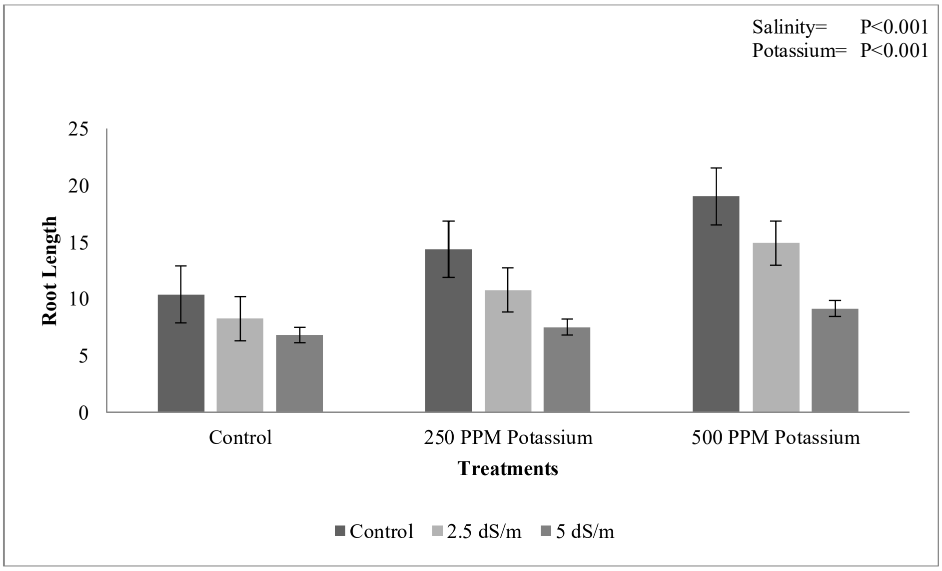

Figure 2. Influence of foliar application of potassium nitrate on root length (cms) on *Vigna unguiculata* grown under seasalt stress.

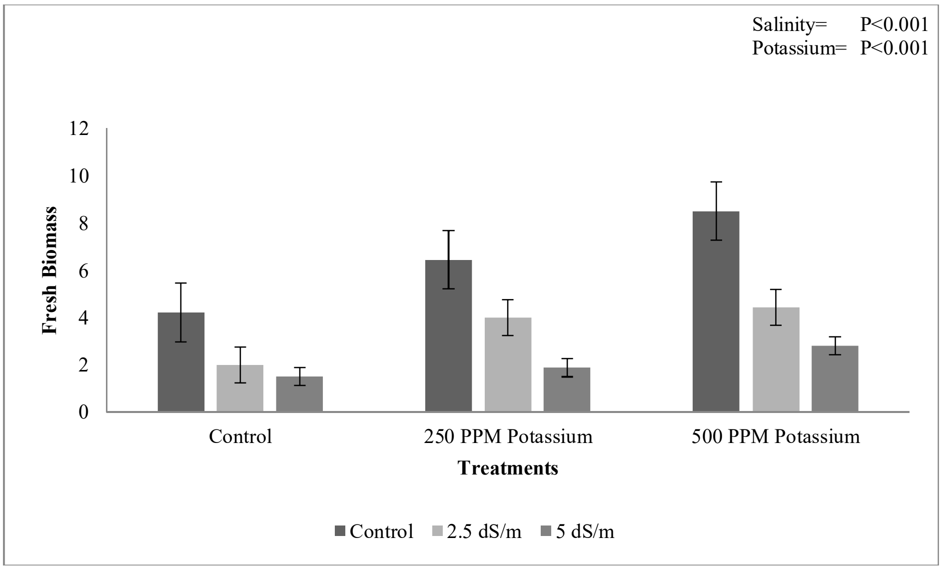

Figure 3. Influence of foliar application of potassium nitrate on fresh biomass (gms) on *Vigna unguiculata* grown under seasalt stress.

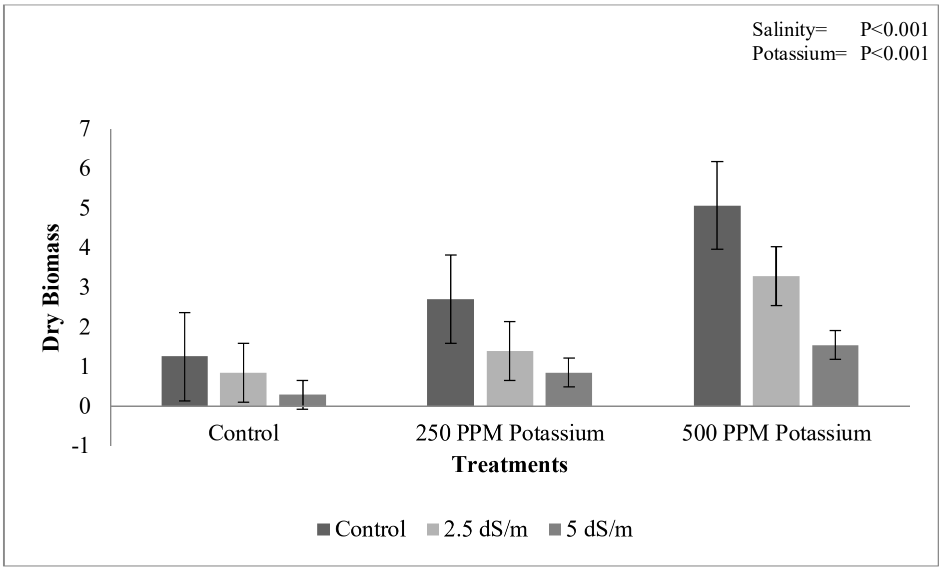

Figure 4. Influence of foliar application of potassium nitrate on dry biomass (gms) on *Vigna unguiculata* grown under seasalt stress.

Relative Water Content

Leaf's relative water content significantly ($P<0.001$) reduced at the highest level of salinity (Fig. 5). [29] grown sorghum plants under sodium chloride stress and studied their water relation, he observed significant reduction in osmotic potential of leaf, water pressure of leaf and relative water content. This reduction in water relation was basically due to restricted availability of water to cells which ultimately cause reduction of turgor in cell. Relative water content of increased Ca2+ level was almost constant at high salt level, and it showed advanced level and ability for osmotic adjustment of cell. Foliar application of potassium showed significant ($P<0.0001$) improvement in relative water content. [30] studied red beat with potassium application and observed significant reduction in relative water content at low potassium level. When maize [31] and *Vigna radiata* [32] plants grown under water stressed conditions and foliarly supplemented with potassium then increase in relative water content and leaf turgor was observed.

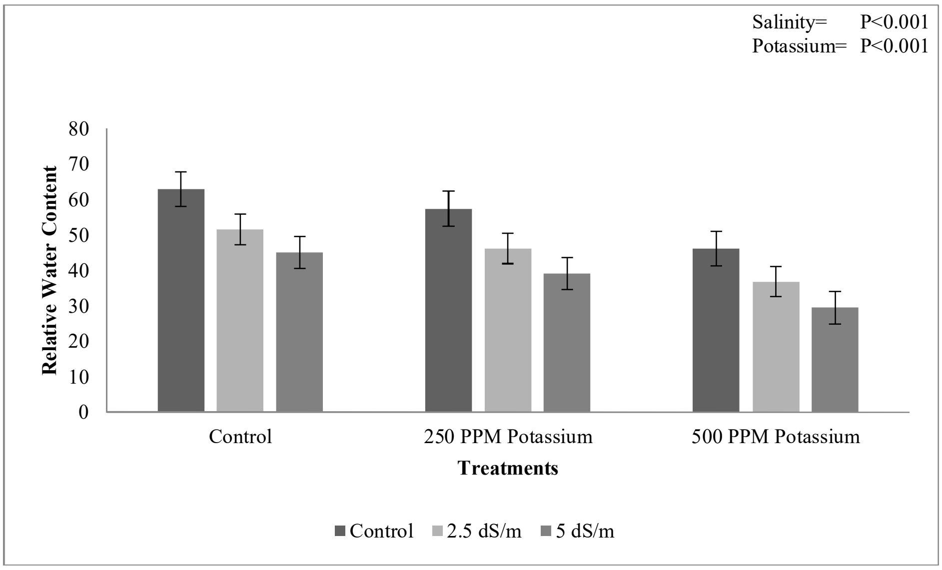

Figure 5. Influence of foliar application of potassium nitrate on relative water content on *Vigna unguiculata* grown under seasalt stress.

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Chlorophyll Content

In the present study, saline stress led to significant (P<0.001) decrease in the chlorophyll b, *a* and total chlorophyll (Figures 6-9). Exogenous spraying of KNO_3 causes beneficial effect and salt stress grown plants showed ameliorative effect in leaf chlorophyll content. Abiotic stressed conditions cause decrease in gas exchange and Rubisco activity which ultimately result in limited and decrease photosynthesis [33]. [34] studied barley plants that grown under salt stress and foliarly applied with potassium nitrate and salicylic acid. Results showed that photosynthetic pigments showed improvement after application of salicylic acid and potassium nitrate. It is also observed in barley plants under stress conditions that decreased sodium and increased potassium concentrations after application of potassium nitrate gives positive response and may be responsible for maintenance of photosynthesis. [35] stated that potassium application cause promotion in photosynthetic activity.

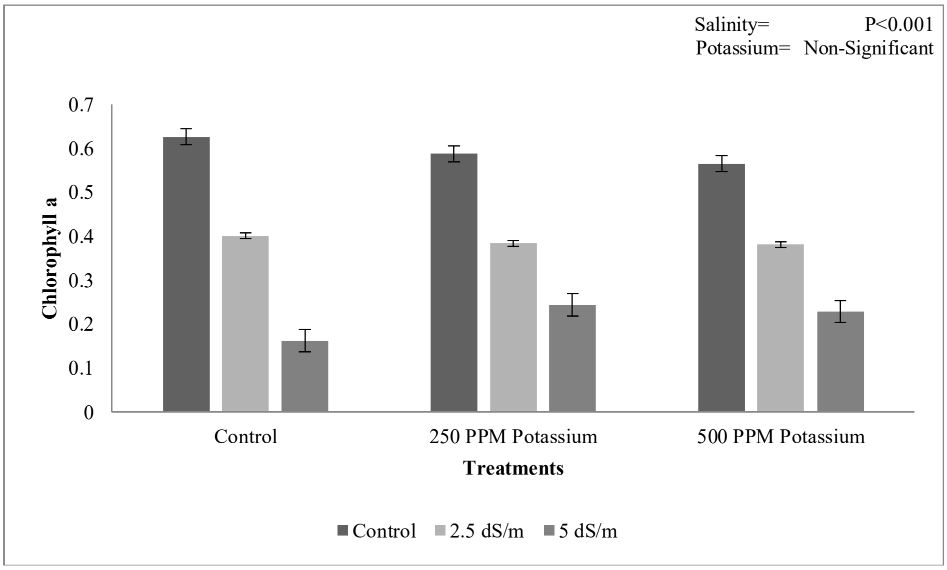

Figure 6. Influence of foliar application of potassium nitrate on chlorophyll a (mg/gm fr. wt.) on *Vigna unguiculata* grown under seasalt stress.

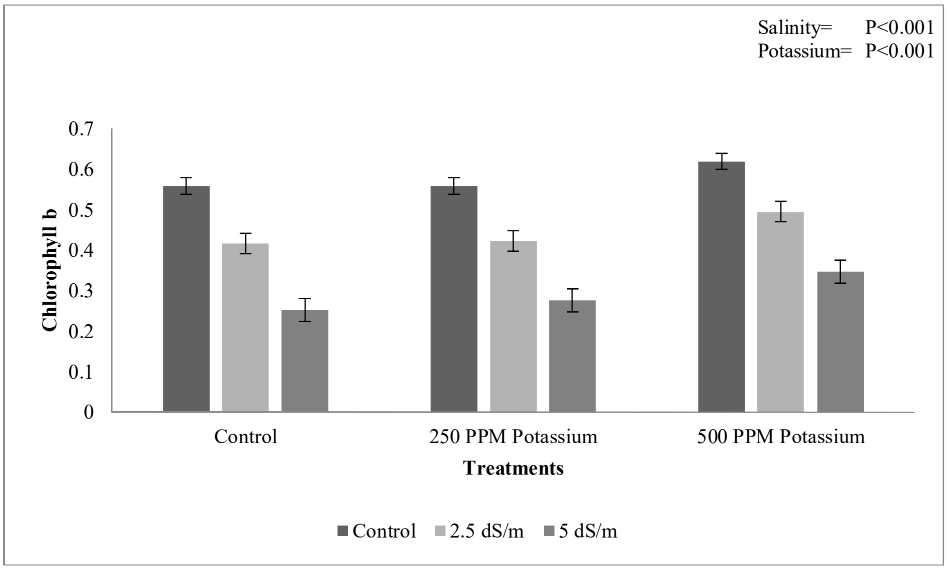

Figure 7. Influence of foliar application of potassium nitrate on chlorophyll b (mg/gm fr. wt.) on *Vigna unguiculata* grown under seasalt stress.

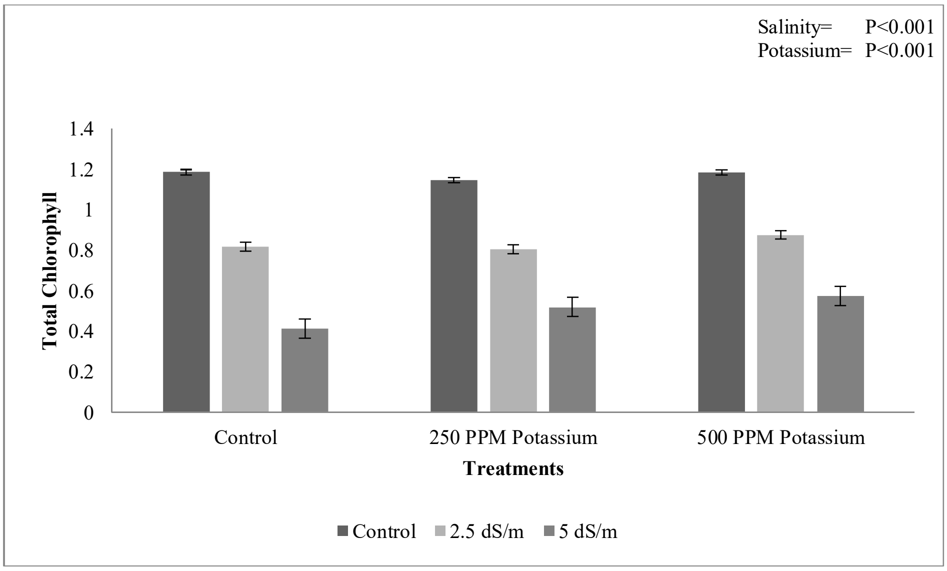

Figure 8. Influence of foliar application of potassium nitrate on total chlorophyll on *Vigna unguiculata* grown under seasalt stress.

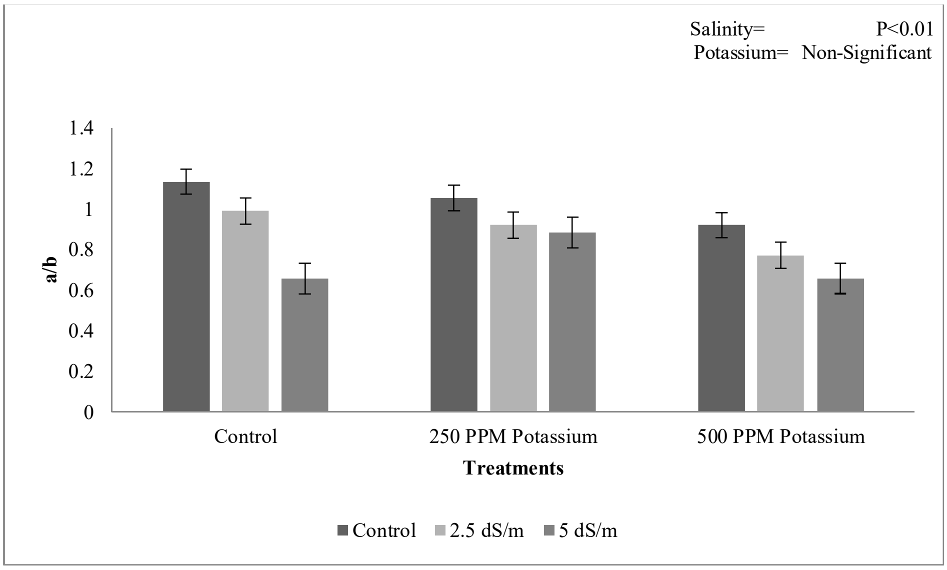

Figure 9. Influence of foliar application of potassium nitrate on chlorophyll a b ratio (a/b) on *Vigna unguiculata* grown under seasalt stress.

Total Carbohydrates

Data presented in figure 10 exhibited that plants irrigated with salt solutions showed significant ($P<0.001$) increase in total carbohydrates. When barley leaves were exposed to salinity then leaves showed increased contents of soluble carbohydrates [36]. Increase in salt concentrations soluble carbohydrate contents were also increased which indicate its importance and key role in adjustments of osmotic potential of cell. It was also assumed that under salinity stress conditions different sugars that accumulate in plants take part in osmotic adjustment [37, 38]. Interestingly, foliar application of KNO_3 to salt stressed plants exhibited decreased soluble carbohydrate. This reduction in total carbohydrate (soluble) was associated with increase in potassium and decrease in sodium ions concentrations. Potassium has important role in maintaining balance of different ions in cell and this element ionically bound to pyruvate kinase enzyme that is essential for carbohydrate metabolism and had a vital role in respiration [39]. When sugar beet plant studied under salt stressed condition this element cause improvement in salt tolerance which is due to increased plant metabolic activity and this element has important role in nitrogen and carbohydrate metabolism, transpiration and water absorption of plant [40].

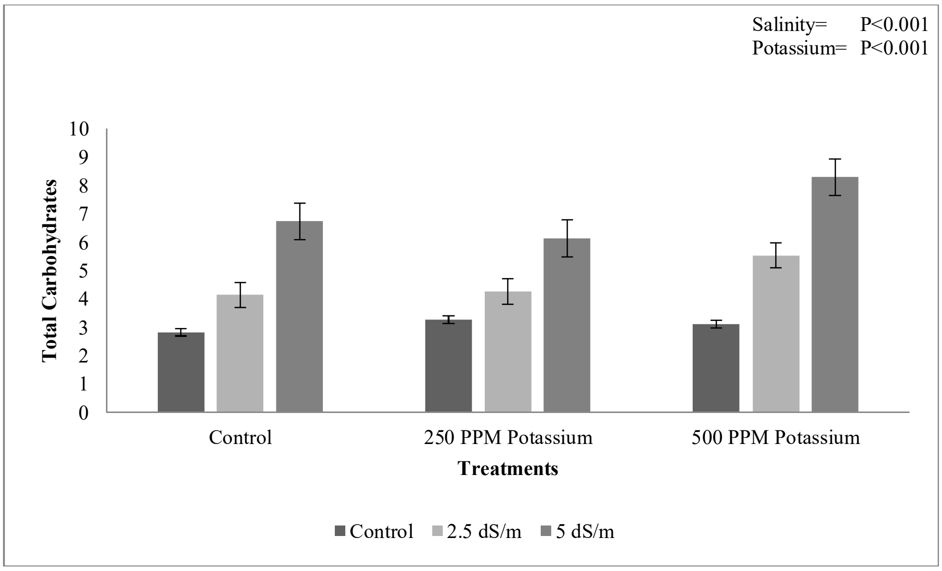

Figure 10. Influence of foliar application of potassium nitrate on total carbohydrates on *Vigna unguiculata* grown under seasalt stress.

Total Proteins

Data presented in figure 11 showed that plants irrigated with different doses of showed significant ($P<0.0001$) reduction in proteins (total). Proteins are generally formed from NO3-assimilation [41]. In safflower and sunflower leaves concentration of soluble proteins decrease when treated with salt, may be due to change in balance between proteins and soluble amino acids or high salt concentration may increase proteolytic process which results in high protein breakdown. Many workers also reported reduction in total proteins in plants when treated with salinity irrespective of their salt tolerance [42, 43, 44, 45]. Total protein concentration of plant enhanced after application of KNO_3 in the leaves while plants grown under normal and salt stressed conditions. The enhancement effect in plants may be the result of direct involvement of potassium ion when ribosome and tRNA bind with one another and different steps of translation process [46]. Foliar supply of potassium nitrate exhibited enhanced NO3- absorption, nitrate reduction and nitrogen assimilation in plants [47].

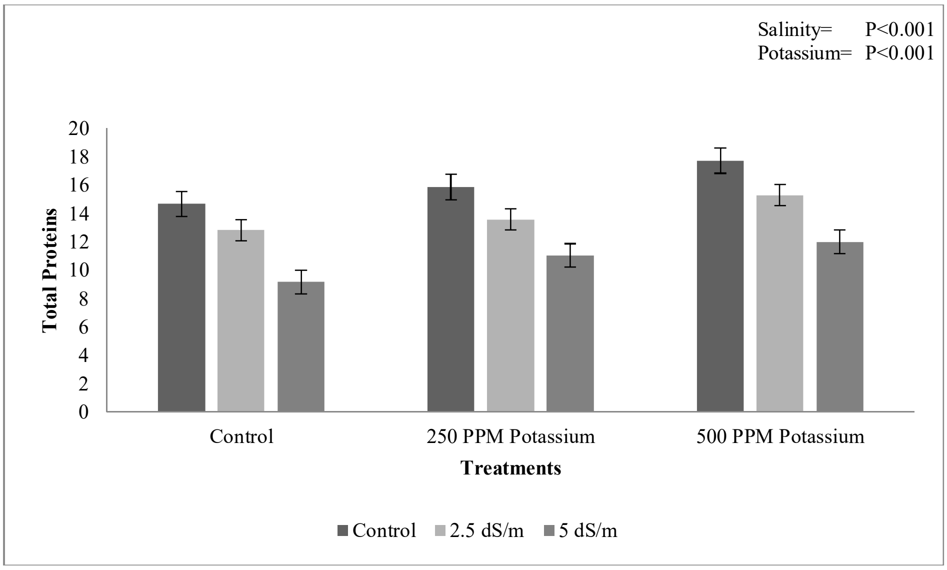

Figure 11. Influence of foliar application of potassium nitrate on total proteins on *Vigna unguiculata* grown under seasalt stress.

Ionic Composition of different plant parts

Different concentration of salt results in significant ($P<0.001$) enhancement in sodium contents and reduction in potassium contents which showed significant improvement in sodium potassium ratio (Na^+/K^+) (Figures 12-14). [36] studied barley leaves as applied with different doses of sodium chloride and concluded that sodium concentration was decreased as NaCl increased. [48] concluded that high sodium ions as a result of high NaCl concentration cause decrease in potassium concentration. [49] reported that the toxic levels of Na^+ in the plant reduced its growth as well as increase sodium potassium ratio (Na^+/K^+), while at the cell level potassium ions exchange by sodium also negatively affects plasma membrane H^+-ATPase activity. After application of potassium to salt stressed plants reduced sodium ions concentration and enhance potassium ions concentration in the leaves. At the same time sodium potassium ratio (Na^+/K^+) also exhibited improvement when salt doses increase in the medium. When barley leaves grown under salt stress and then applied with different potassium nitrate concentration it showed improvement in plant growth after improvement in sodium potassium ratio (Na^+/K^+). [50] studied sugar beet leaves under salt stress and reported main solutes for osmotic potential adjustments are sodium and potassium, so these inorganic salts also played important role in this phenomenon in addition to carbohydrates.

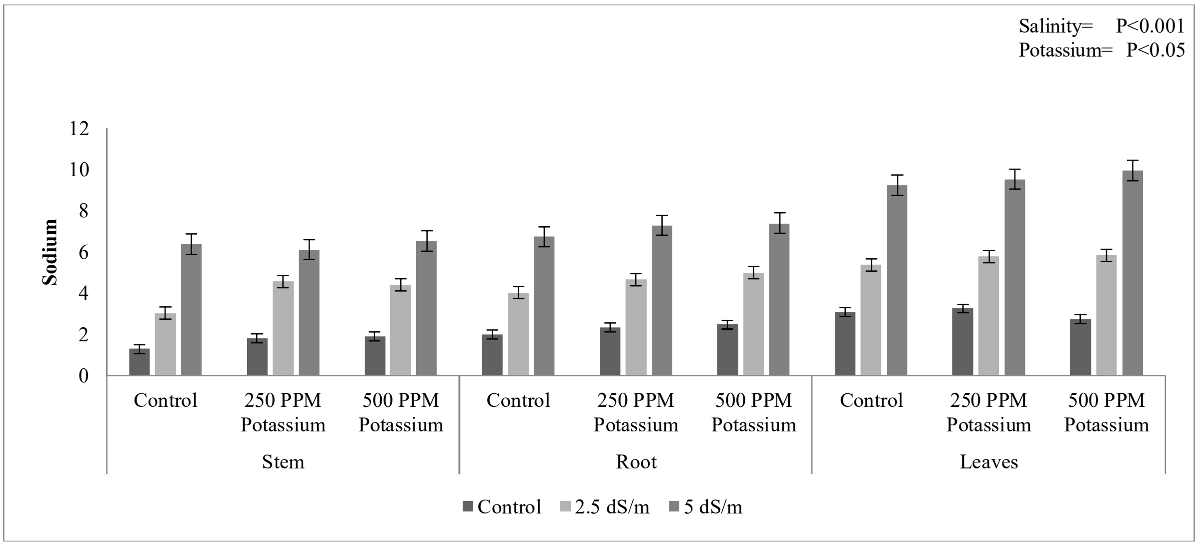

Figure 12. Influence of foliar application of potassium nitrate on sodium ion concentration of different plant parts (stem, root and leaves) on *Vigna unguiculata* grown under seasalt stress.

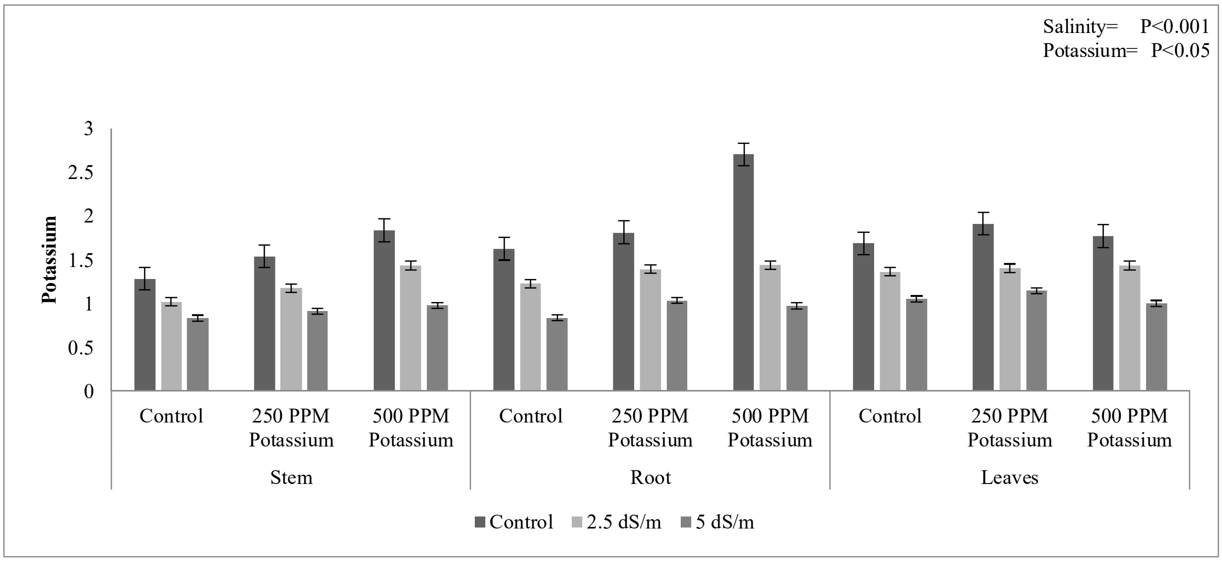

Figure 13. Influence of foliar application of potassium nitrate on potassium ion concentration of different plants parts (stem, root and leaves) on *Vigna unguiculata* grown under seasalt stress.

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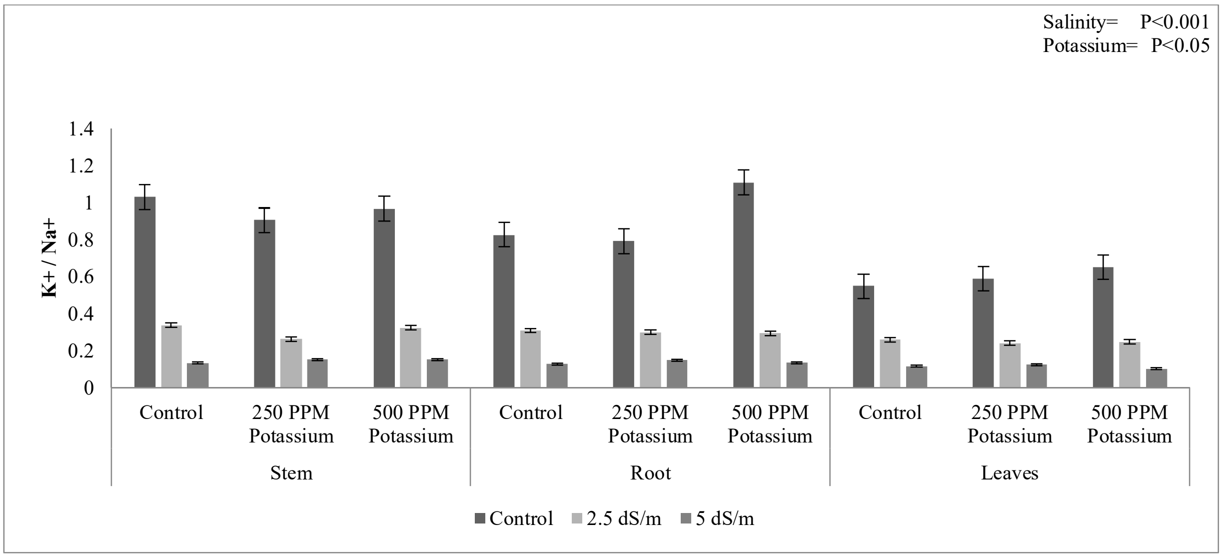

Figure 14. Influence of foliar application of potassium nitrate on potassium sodium ratio (K+/Na+) of different plant parts (stem, root and leaves) on *Vigna unguiculata* grown under seasalt stress.

CONCLUSION

Different doses of salt application significantly reduced different growth parameters, chlorophyll, total proteins and potassium ion concentration in plants while exhibited increase in total carbohydrates, sodium ion concentration and Na+/K+ ratio. Foliar application of potassium in the form of KNO3 exhibited improvement in observed parameters in normal and saline conditions and played its role in mitigating salt stress effects in studied plant.

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