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Salicylic Acid Prevents the Deleterious Impact of Salt Stress on *Vigna Unguiculata* L.

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ABSTRACT

Plant growth was reduced significantly by salt stress which is a serious environmental problem. Plants have different metabolites that are working in response of different biotic and abiotic stresses and salicylic acid is one of them that act as a vital compound in plants for response against different environmental stresses and modifications and it has also an important role in declining damages in plants that are caused by different stresses. Present project was designed to explore the same phenomenon of salicylic acid on *Vignaunguiculata* irrigated with different sea-salt concentrations. So, *Vignaunguiculata* seeds were grown in pots containing loamy soil in field conditions. Sea-salt concentration(0, 2.5 dS/m and 5 dS/m) and salicylic acid levels(0, 0.5mM and 1mM) were used in this experiment which is complete randomized design (CRD) and factorial experiment. Present investigation revealed reduction in root length, plant height, total fresh biomass, total dry biomass, relative water content (RWC), photosynthetic pigments(chlorophyll a, b, total chlorophyll) and proteins while increase in total carbohydrates as salt concentration increased. Results regarding presence of ions in different parts of plant showed that sodium ion level showed increase while potassium ion level showed decrease in different plant parts as sea salt concentration increased in irrigation water. Different doses of salicylic acid exhibited improvement in studied parameters under non-stressed and stresses environment.
KEYWORDS: Biomass, Chlorophyll, Protein, Potassium, Salinity, Salicylic acid, Sodium.

INTRODUCTION

Salt stress affects negatively the plant development, growth and productivity and it is known as serious environmental problem which badly affect most of the cultivated area of the world (20%) [1].[2] stated that physiological drought caused by high salinity as high salt concentration cause soil porosity and reduction in water potential. [3]revealed that high salt concentration also affects plants at both whole level and cellular level in terms of its physiology. High concentration of salt had negative impact on plants either by osmotic stress or toxic levels of specific ion [4]. Occurrence of high concentration of sodium and chloride ions in the soil solution cause osmotic stress which leads to decrease in availability of water to roots. During salinity stress condition presence of high salt levels in soil force the plant roots to absorbed high concentrations of sodium and/or chloride ions and then moved towards leaves where they stored at alarming level. Nutrient deficiency especially potassium ion nutrition and ion imbalances can also be occur under salt stress[5].

Different phenolic compounds that produced in plants are now considered as hormone (endogenous growth regulator)and one of them is known as salicylic acid (SA). It has been well documented that this compound has positive role in defense mechanism of plant against different types of stress factors (biotic and abiotic)[6, 7]. This compound act as antioxidant (non-enzymatic) and had vital role in different physiological processes regulation of plants including photosynthesis and known as plant growth regulator[8, 9, 10, 11]. Many researchers studied different plants under different stresses and observed the deleterious effects and then amelioration of these effects after exogenous application of salicylic acid, like [12] studied rice under heavy metal stress while [11] studied wheat under salt stress. When plants grown under different stress conditions and then foliarly applied with salicylic acid it can enhance the salinity and drought stress resistance of these plants [13, 14].

Vignaunguiculata sub sp. *unguiculata* (L.) Walp. (Cowpea) is a member of Fabaceae family and known as coope, black eyed pea, coope, southern peas, yard long pea, lobia, china pea, niebe or frijol worldwide. Height of this plant reaches up to 80cm or more and it is a glabrescent scrambling annual herb. In different regions of the world like

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Africa, Southern Europe, Central and South America and semi-arid tropics covering Asia this plant acts as an important food legume crop. This crop introduced as food crop in Pakistan and had 553 tons production from 257 hectares [15]. Human consumption of this plant based on different parts of this plant such as the immature seeds, leaves and fresh or dry seeds which contain 64% carbohydrates and 23-32% proteins. Additionally, animal feeding was also done in dry season by the pods and dry seeds of this plant[16]. So, this plant became a valuable source of income for grain traders and farmers in many countries of Africa[17, 18]. This plant acts as a fodder and considered as crude protein source[19]. Many researchers perform extensive investigations on salinity stress effects on cowpea (*Vignaunguiculata* L.) and used NaCl as a source of salt stress[20, 21, 22, 23, 24, 25]. Taking into consideration the importance of this plant to farmers and to the economy current experiment was designed to investigate the beneficial effect foliarly applied ascorbic acid in different concentrations on salt stressed cowpea (*Vignaunguiculata* L.)

MATERIAL AND METHODS

Plant biomass and growth parameters:

Cowpea (Vignaunguiculata L.) seeds were taken from Agriculture Research Instutute Tarnab, Peshawar. The experiment consisted of 36 pots divided into three groups. All groups were irrigated with three salt treatments (0, 2.5 dS/m and 5 dS/msea-salt irrigation). First group was foliarly sprayed with distilled water, second group was sprayed with 0.5 mM salicylic acid while third group was foliarly applied with 1.0 mM salicylic acid. All these 36 pots were then arranged in a completely randomized design (CRD) in the Department of Botany, University of Karachi, Karachi. 36 pots had basal outlet for leaching of solution purpose and each pot was filled with 3 kg of sandy loam soil having Hoagland's solution at saturation percentage. Same size seeds were surface sterilized with 0.1% $HgCl_2$ for 1 minute and then washed three times with distilled water and 5 seeds were sown in each pot. Seedlings were irrigated with 150 ml tap water daily. When seedlings were reached at 3-leaf stage, they were thinned to one seedling/pot. At this stage, concentration of sea-salt in the irrigation water is gradually increased until the ideal salinity for each treatment reached. Plants were irrigated with 1.5L of tap water/Sea-salt solution two times in a week. When the required salinity level was achieved then different doses of salicylic acid were applied foliarly on plants. At the end of the experiment, root length, number of leaves, number of branches, fresh biomass, dry biomass, and number of pods per plant were recorded in all harvested plants. Samples of leaves were collected for biochemical analysis and relative water content during the grand growing season.

Relative water content: (RWC)

Determination of relative water content was done by method of Mata and Lamattina [26]. First, fresh weight (FW) of leaves was taken then these leaves were placed separately in distilled water for rehydration for 2 hours and then turgid weight (TW) of leaves was measured. In the last step all leaves were dried by keeping them in preheated (80°C) oven for 48 hours and then dry weight of leaves was measured. Relative water content of different leaves was calculated using following formula.

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

BIOCHEMICAL ANALYSIS:

Photosynthetic pigments:

Chlorophyll concentration (Chl) was determined in fresh leaves following the protocol of[27].

Total Carbohydrate Determination:

Total carbohydrate estimation was performed in plant extracts by the method of[28] using an anthrone reagent.

Total Protein Determination:

Total protein contents were extracted and estimated using method described by[29].

Mineral Analysis of Different Vegetative Parts

Samples of leaves, stems and roots were taken for analysis of different cations (Na+ and K+) during the developmental period. The sample were dried and the weight of ash of 0.5 grams of each dry sample was 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.

Statistical Analysis of Data and Experimental design

The experiment was a completely randomized design (CRD) having different salicylic acid treatments and three salt treatments with three replicates. Statistical analysis was done using SPSS (Ver. 21) software for analysis of variance (ANOVA), Duncan's multiple comparison using mean ($P < 0.05$).

RESULT AND DISCUSSIONS

Plant growth

Data represented in figures 1-4 exhibited that plants treated with salt exhibited significant ($P<0.001$) reduction in root length, shoot length, fresh and dry biomass. [30] treated sugar beet plants to different concentrations of salt and observed marked reduction in different growth parameters. Different researchers observed same reduction phenomenon in different plants after treatment with salt, e.g. Cotton [31], Tomato [32, 33], Corn [34], pepper and cucumber [35]. Salicylic acid (SA) known as antioxidant and important plant growth regulator [36]and it had importance in plant to allay the harmful effects that induced by salinity on different crops development and growth[37, 11, 38]. Results of the present investigation also provide the evidence of same earlier observations that foliar supply of salicylic acid showed significant ($P<0.0001$) improvement in above mentioned growth parameters in non-saline and saline environment. [39]treated soybean plants with salicylic acid and conclude that roots and shoots of that plant showed increase in growth under normal conditions. Wheat plants also showed enhanced growth under drought stress when treated with salicylic acid[8] maize [40] and barley [37] under NaCl stress.

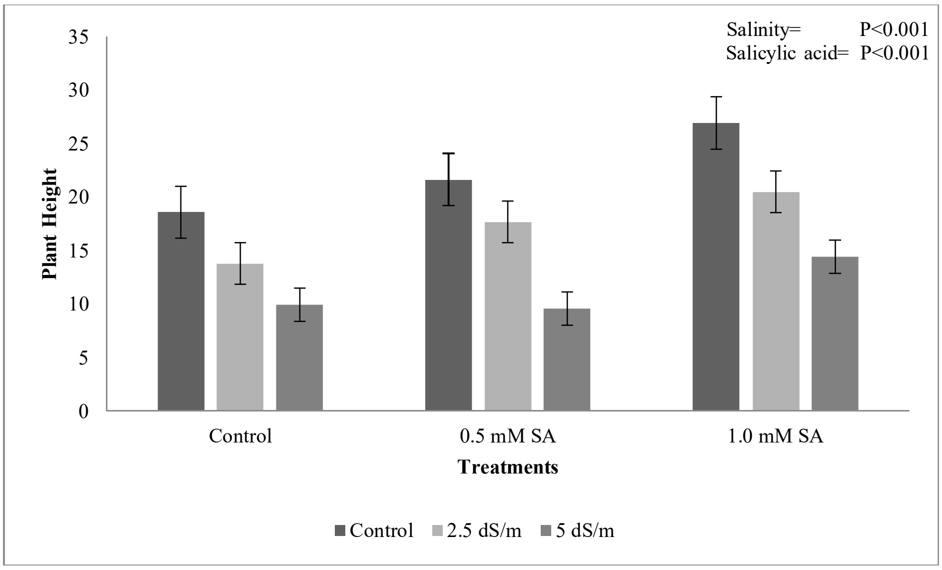

Figure 1. Effect of foliarly applied salicylic acid on plant height (cms) of *Vignaunguiculata* grown under seasalt salinity.

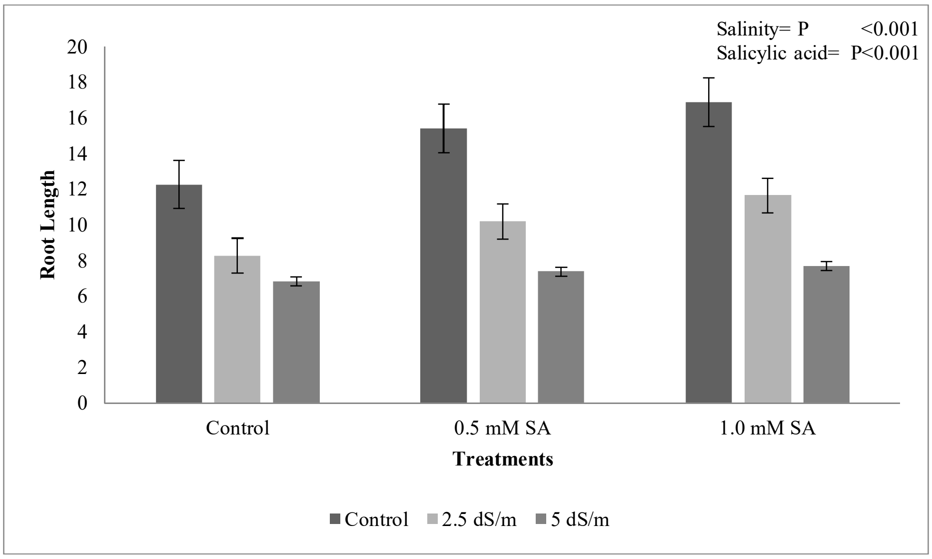

Figure 2. Effect of foliarly applied salicylic acid on root length (cms) of *Vignaunguiculata* grown under seasalt salinity.

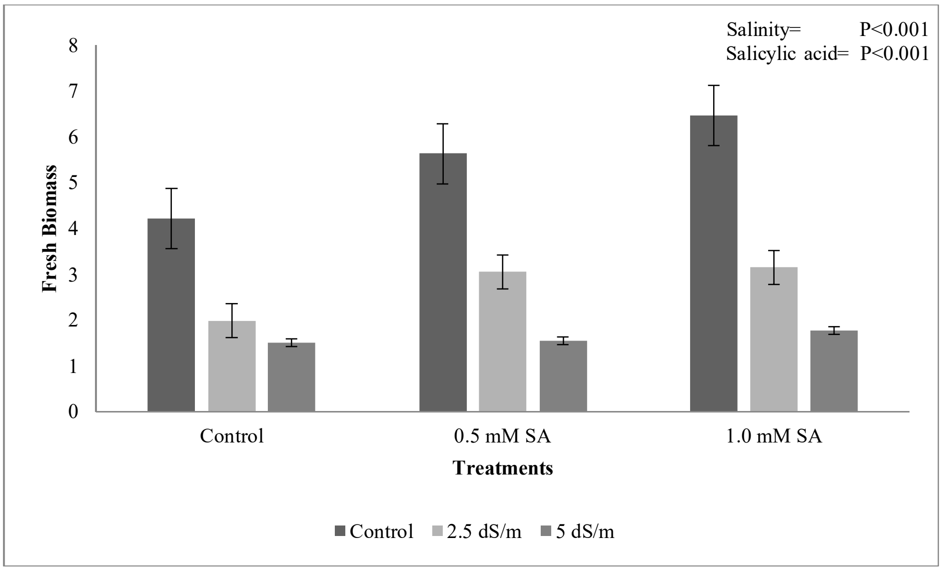

Figure 3. Effect of foliarly applied salicylic acid on fresh biomass (gms) of *Vignaunguiculata* grown under seasalt salinity.

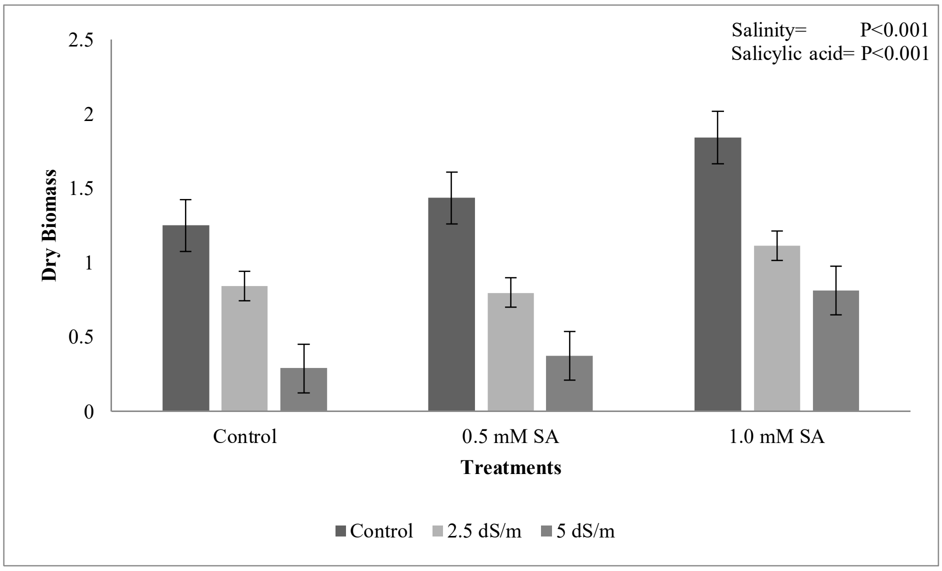

Figure 4. Effect of foliarly applied salicylic acid on dry biomass (gms) of *Vignaunguiculata* grown under seasalt salinity.

Relative water content

Optimal plant growth maintenance is a result of osmoregulation or plant water status maintenance and it is regarded as key physiological process[41]. In present investigation relative water content of cowpea leaves was significantly ($P<0.001$) decreased at the highest concentration of salinity (Figure 5).[42] studied linblack gram (*Vignamungo*) under salinity stress observed increase in leaf water potential with decrease in osmotic potential. Accumulation of organic and or inorganic solute is the main reason for decrease in osmotic potential. Soluble sugars, amino acids mainly proline, and glycinebetain are different organic solutes that had an important role in adjusting different metabolites osmotically in the cell[43, 44, 45]. Sodium and potassium are inorganic solutes and they are also considered important in osmoregulation but sodium is damaging the cell as compared to potassium and other organic solutes[46, 47]. Increase in salinity in plants results in the more negative values of relative water content, water potential and osmotic potential[48]. Foliar application of salicylic acid showed significant ($P<0.0001$) improvement in relative water content. Increase in RWC of wheat plants treated with salicylic acid was reported.

Experiments on different crops including tomato [14, 49], barley [37], and cucumber [50] also showed the same phenomenon when grown under salt stress. This fact was also hypothesized that application of salicylic acid on leaves results in reduction of transpiration rate and improvement in leaf diffusive resistance.

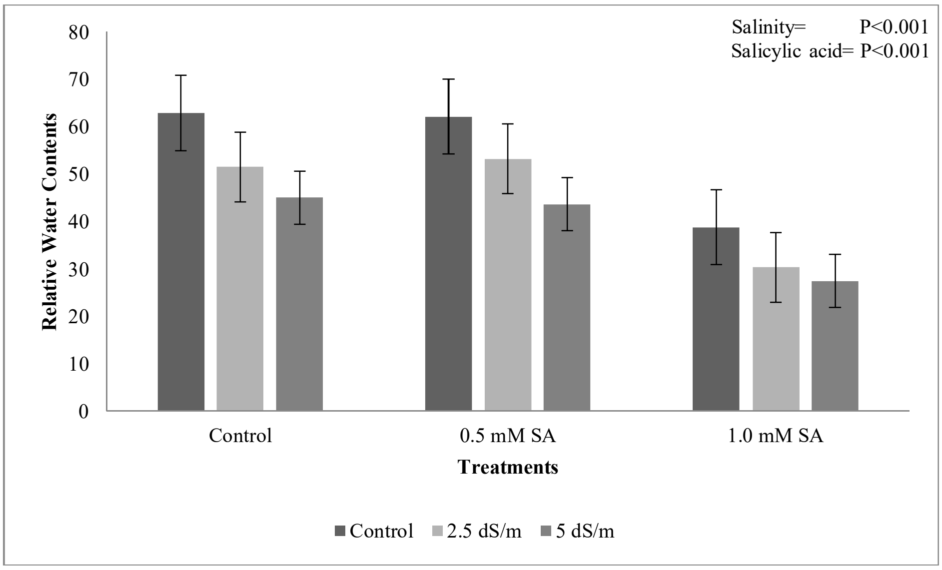

Figure 5. Effect of foliarly applied salicylic acid on relative water content of *Vignaunguiculata* grown under seasalt salinity.

Chlorophyll

In the present study, saline stress cause significant ($P<0.001$) reduction in the chl *a*, *b*, and total chlorophyll contents (Figures 6-9). [50] for cucumber and [51] for wheat concluded the same results when plants exposed to salt stress. When [52] exposed sorghum plants to different concentrations of salt chlorophyll biosynthesis in leaves showed reduction. [53]also observed same negative effect on chlorophyll content in strawberry plants when treated with different concentrations of salt. [48]also studied the same effect of salt stress on chlorophyll in many crops. When barley plants treated with NaCl leaves carotenoids and chlorophyll a and b showed significant reduction in under stressed plants as compare to normal ones [37]. In another study on bean plants these all parameters showed significant promotion in NaCl treated plants as compared to non-saline ones[54]. According to[55] chlorophyll concentration in salt treated plants depends on concentration and type of salt, biological processes going on in plants and developmental stages of plants. In present investigation foliar application of salicylic acid showed significant ($P<0.001$) improvement in chlorophyll (a, b and total chlorophyll). Rate of photosynthesis increased in soybean plants which is a result of high pigment contents in the leaves after application of salicylic acid[56]. According to[57] when they worked on maize plants and treated them with salicylic acid carotenoid and chlorophyll content exhibited increase.

Carbohydrates

Reduced water to plant results in increased carbohydrate concentration and lower water potential which helps in maintenance of protein structure and prevent oxidative losses. At molecular level for the activity of sugar responsible genes carbohydrates play key role and as a result plant give different response like expansion of cells and defensive response[58]. Data presented in figure 10 explained that plants subjected to different salinity levels exhibited significant ($P<0.001$) promotion in total carbohydrates. When *Zea mays* treated with different NaCl concentrations plant exhibited promotion in soluble sugar contents with increase in NaCl concentrations while polysaccharides showed opposite effect in salt treated plants. Maria *et al.,* [59] treated tomato plants to salinity stress and observed increase in soluble sugar content. It is evident that in the presence of salts promotion in soluble carbohydrates in the root region helpful in maintaining balance against osmotic pressure. During salinity stress conditions for escaping from plasmolysis condition plant cell should change macro molecules to micro molecules. Conversion of starch to glucose and decomposition of sucrose to glucose and fructose helps to enhance osmotic pressure of cell[60].

During saline stress condition salicylic acid is responsible for maintaining balance in the sugar level. In present investigation plants treated with salicylic acid exhibited reduction in soluble sugar content as compared to control plants. It is reported that application of salicylic acid to plants stimulates their growth by activating metabolic consumption of total soluble sugar to develop new constituents of cell. It is also evident that total soluble sugars are incorporated into polysaccharides which is accelerated by the application of salicylic acid while polysaccharide hydrolyzing enzyme system inhibited by the treatment of salicylic acid. [61] worked on ray plants and observed that application of salicylic acid reduced soluble sugar levels in plants.[40] stated that enzymatic system that is involved in polysaccharide hydrolysis deranges after application of salicylic acid.

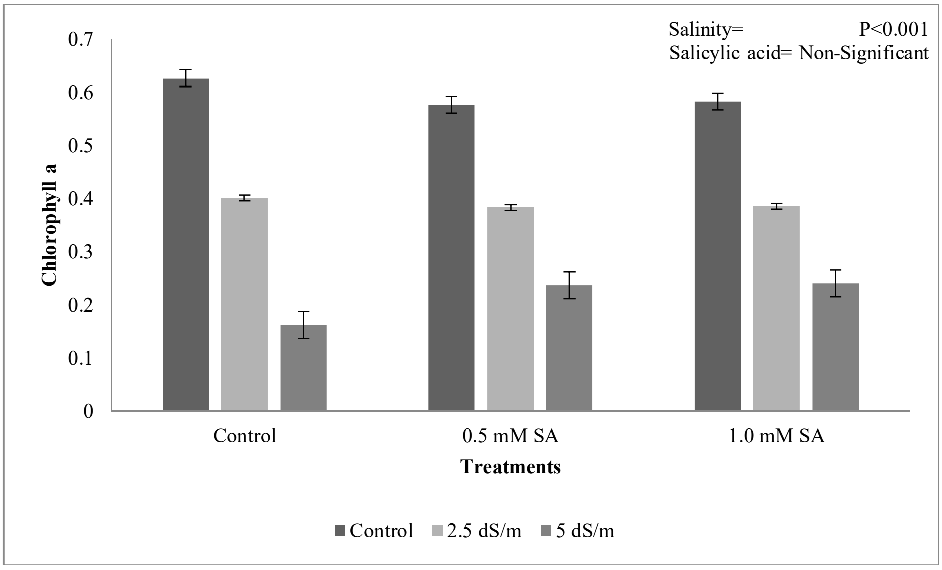

Figure 6. Effect of foliarly applied salicylic acid on chlorophyll a (mg/gmfr.wt.) of *Vignaunguiculata* grown under seasalt salinity.

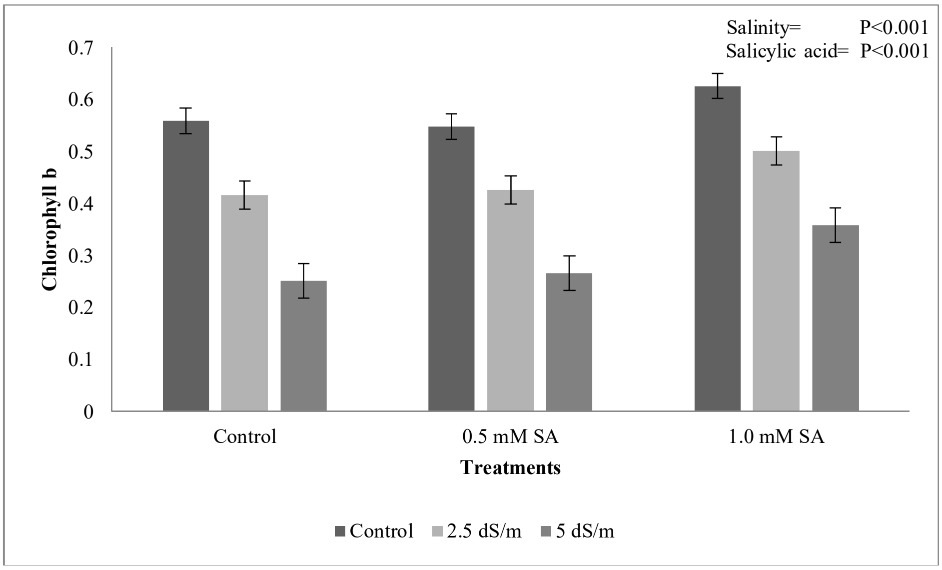

Figure 7. Effect of foliarly applied salicylic acid on chlorophyll b (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

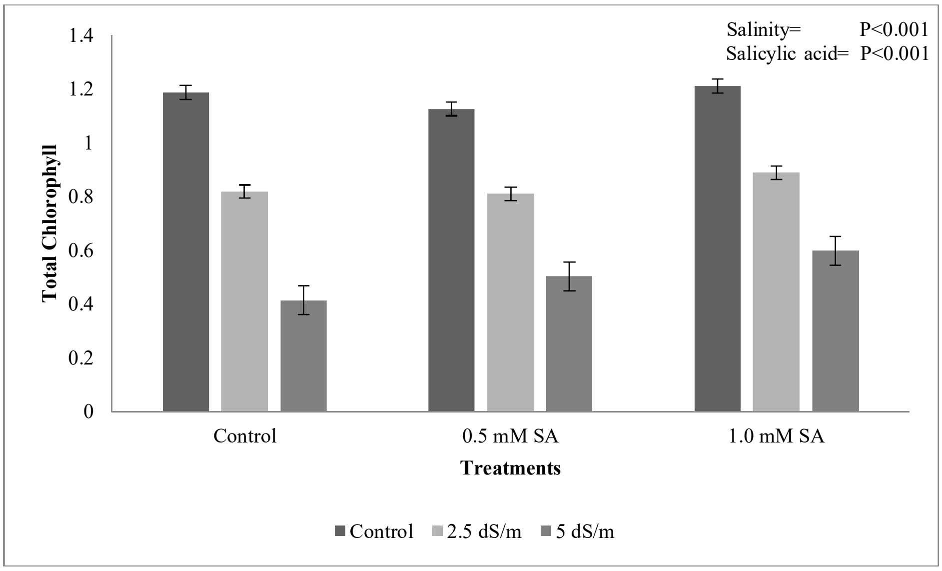

Figure 8. Effect of foliarly applied salicylic acid on total chlorophyll of *Vignaunguiculata* grown under seasalt salinity.

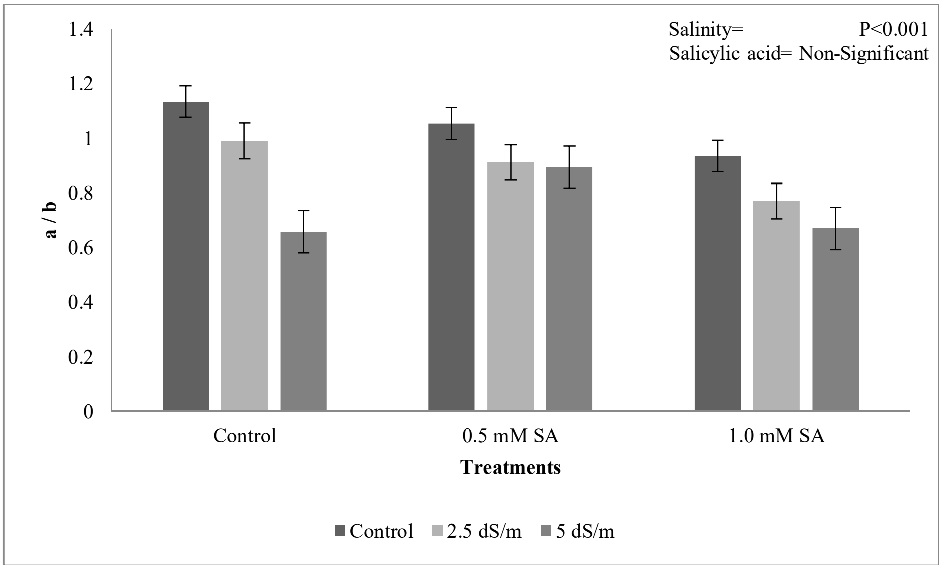

Figure 9. Effect of foliarly applied salicylic acid on chlorophyll a b ratio (a/b) of *Vignaunguiculata* grown under seasalt salinity.

Proteins

Data presented in figure 11 showed significant ($P<0.0001$) decrease in total proteins when plants subjected to different concentrations of salt. This phenomenon was also reported by [62] and [63] when they treated plants with salinity stress. When [64] treated tomato plants with salt stress they observed reduction in leaf protein level and according to [65] this effect of salt on protein level was created after reduction in the activity of nitrate reductase enzyme. It is evident that when plant treated with salt it created changes in roots and shoots proteins level but in leaf blade there is no effect. [66] also observed reduction in the total protein contents after application of salt on plants. In this study foliar application of salicylic acid exhibited significant ($P<0.01$) enhancement in protein levels in both non-saline and saline environment. [8] studied plants under water stress they observed reduced soluble protein contents and after salicylic acid application marked a slight increase. A [11] observed that saline media significantly reduce the mean protein in wheat and same plants exhibited significant promotion in total protein contents when treated with salicylic acid.

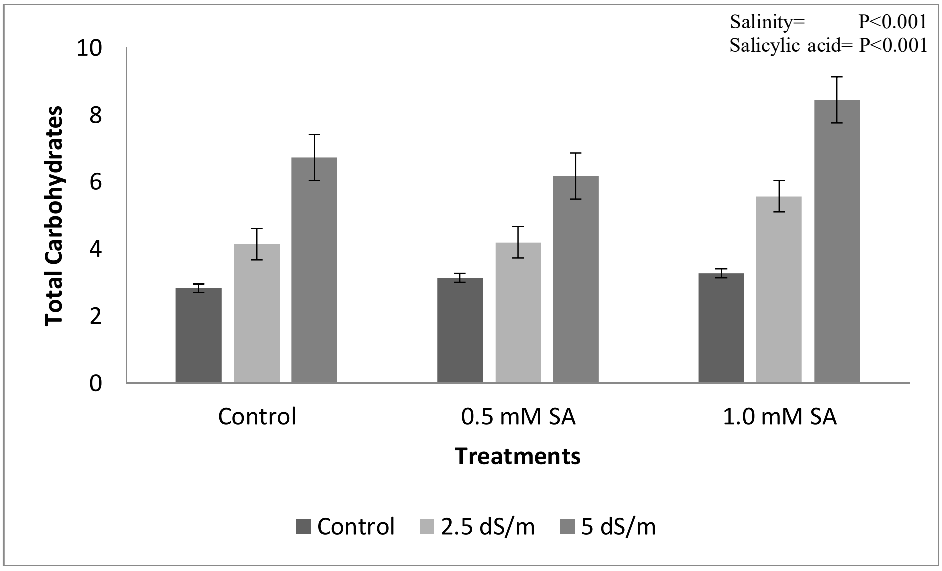

Figure 10. Effect of foliarly applied salicylic acid on total carbohydrates (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

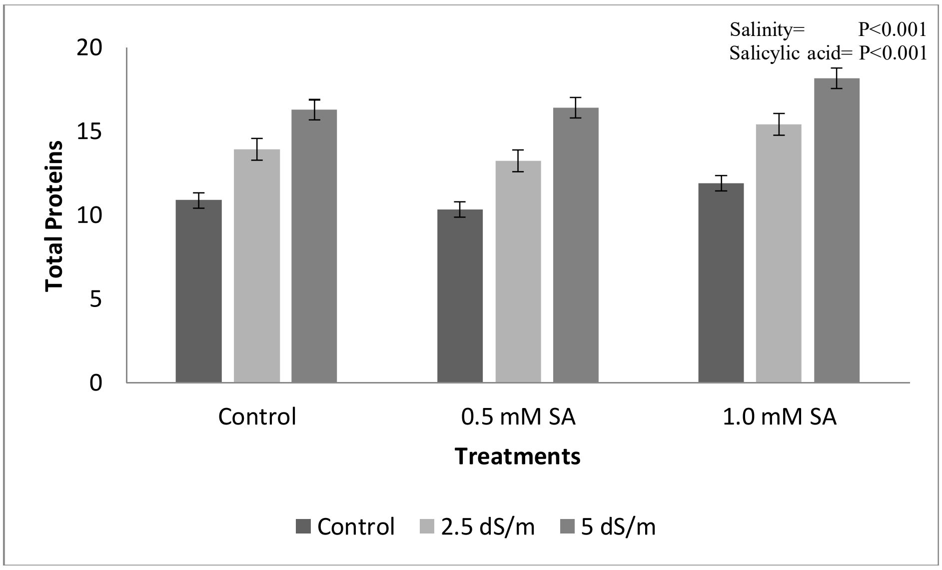

Figure 11. Effect of foliarly applied salicylic acid on total proteins (mg/gmfr.wt) of *Vignaunguiculata* grown under seasalt salinity.

Ions

When plants treated with salt it created a significant ($P<0.001$) promotion in sodium content and a considerable decrease in potassium concentration which results in a significant promotion in the Na^+/K^+ ratio (Figures 12-14). Salinity treatment in roots increased the level of sodium in the medium which reduced the uptake of potassium by root cells and as a result K+/ Na+ ratio also reduced. After application salt entry and accumulation of high levels of sodium ions in the cell will be toxic for cell and plant.[67] stated that when excess amount of sodium enters the cell it must move out of it or enter the vacuole to prevent cell death or reduced growth. Cytotoxin ions, especially Na+ and Cl- ions in salinity environments enter the vacuole, and are used as an osmotic solution [68].When salicylic acid foliarly applied on plants it exhibited beneficial effect on concentration of sodium and K^+ in different plant parts. [69]observed in tomato plant that accumulated Na+ ions in treated leaf tissue with SA and were placed in salinity and act as inorganic osmolyte.

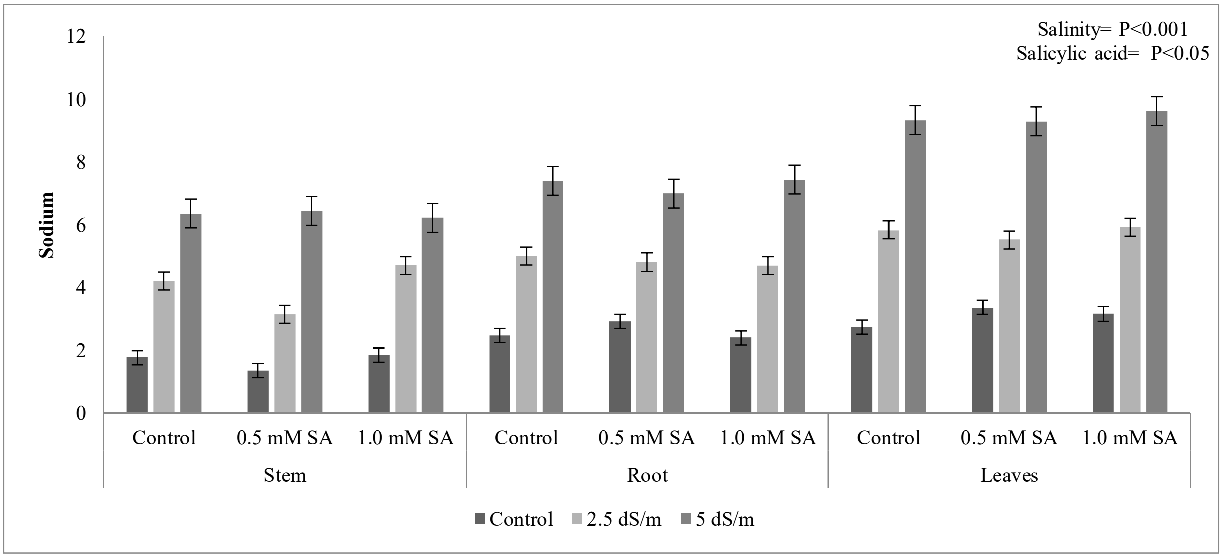

Figure 12. Effect of foliarly applied salicylic acid on Na^{+}ion concentration of different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

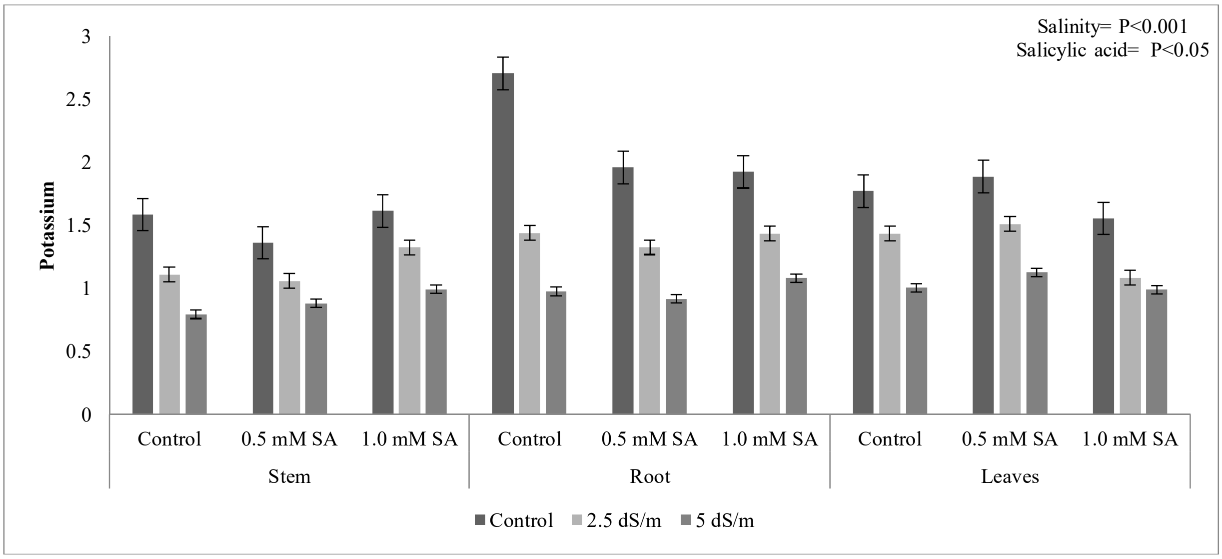

Figure 13. Effect of foliarly applied salicylic acid on K^{+} ion concentration of different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

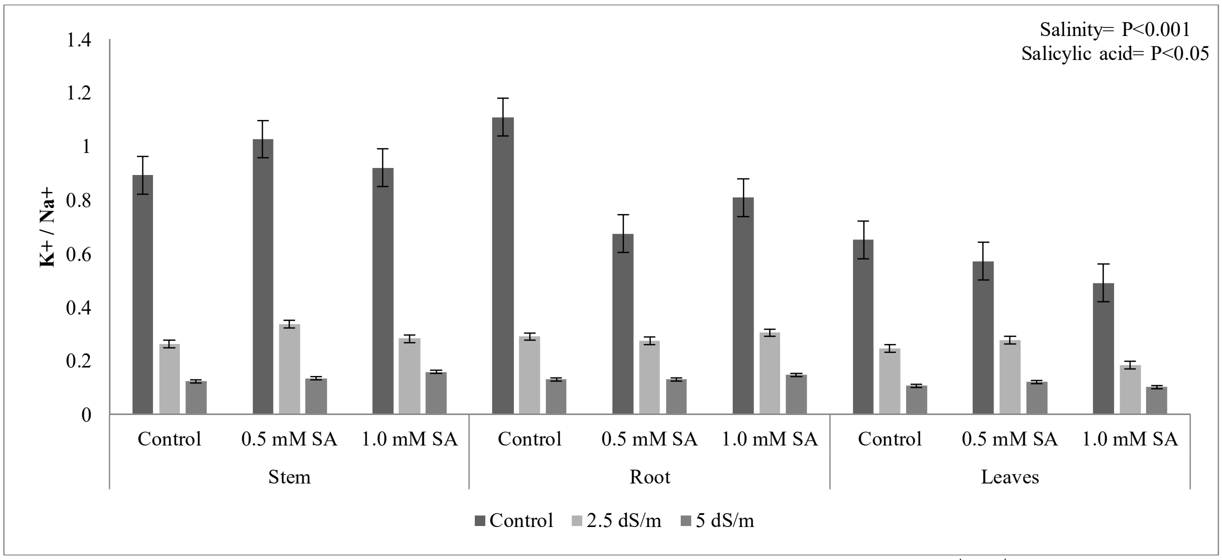

Figure 14. Effect of foliarly applied salicylic acid on potassium sodium ratio (K^+/Na^+) in different plant parts (stem, root and leaves) of *Vignaunguiculata* grown under seasalt salinity.

CONCLUSION

Collective data for vegetative growth, Primary metabolites revealed detrimental effect of salt on plant growth. Sodium also accumulated in different parts of the plant. Application of salicylic acid reduced harmful effect of salt on plant growth and improved growth under normal condition.

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