Impact of Exogenously Applied Ascorbic Acid on Growth, Some Biochemical Constituents and Ionic Composition of Guar (*Cymopsis Tetragonoloba*) Subjected to Salinity Stress

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ABSTRACT

The present study was undertaken to examine the effects of exogenous application of 0.5mMascorbic acid (AA) on growth, associated biochemical parameters and ionic composition in (*Cymopsis tetragonoloba)* grown under different doses of seasalt irrigation. In a pot experiment, AA was applied through foliar-spray at the concentrations of 0 and 0.5mM with or without 0, 2.5 dS/m^{-1} and 5 dS/m^{-1} seasalt concentration. Vegetative and reproductive growth measurements (plant height, root length, number of leaves, leaf area, fresh and dry biomass, number of pods per plant, pod weight per plant, seed number per plant and seed weight per plant), chlorophyll a, b, total chlorophyll, protein, carbohydrates, sodium and potassium ions indifferent plant parts were recorded to study the effects of these treatments. Thepresence of salt reduced the vegetative and reproductive growth parameters, chlorophyll a, b, total chlorophyll, proteins and potassium ions concentration in different parts of *Cymopsis tetragonoloba* plants. Total carbohydrates and sodium ion concentration in different plant parts showed increase while increasing in sea salt concentration in irrigation water. The AA application not only mitigated the inhibitory effects of salt stress but also induced a stimulatory effect on all the studied growth parameters.

Key words: Salinity, Ascorbic acid, yield, chlorophyll, protein, carbohydrates, sodium, potassium.

1. Introduction

Salt in soil and irrigation water is one of the most important factors that limit plant growth and productivity [1] [2]. FAO estimated that more than 800 million hectares of world's agricultural land are seriously affected by salinity [3]. Salt stress may affect plant growth and development directly through its potential toxic effects, and indirectly by way of its osmotic effects [4]. Ion imbalances, osmotic stresses and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress [5].

Ascorbic acid ubiquitously present in plants and has been reported to play a vital role in alleviating the adverse effects of salt on plant growth and metabolism in many crop plants [6]. It is an abundant small molecule in plants. It is a major substance in the network of antioxidants that include ascorbate, glutathione, a-tocopherol, and a series of antioxidant enzymes. It has also been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes [7] [8] [9] [10]. It is also believed to detoxify 1O2 and OH. [7] [11] [8]. Generally the effects of ascorbic acid in mitigating the adverse effects of salt stress have been ascribed to activation of some of the enzymatic reactions [12]. These positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage[13] [14] [6].

Guar (*Cymopsis tetragonoloba* (L.) Sis an annual summer legume crop grown in Asia as a vegetable for human consumption, forage for cattle, and as a green manure. Highly refined guar gum which is present in endosperm of seed is used as a stiffener in soft ice cream, instant puddings and whipped cream substitutes, while lower grade guar gum is used in the textile, paper, petroleum, mining, pharmaceutical and cosmetic industries [15]. Information on how ascorbic acid regulates physiological/biochemical processes in guar plants subjected to salt stress is not much available in the literature. Thus, the main objective of the present study was to examine whether the adverse effects of salt stress on wheat plants could be mitigated by exogenous application of ascorbic acid as a foliar spray.

2. Material and Methods

2.1. Plant material and growth conditions

Seeds of Guar (*Cymopsis tetragonoloba)* (L.) were obtained from the International Center for Biosaline Agriculture, Dubai. This experiment was comprised of 24 clay pots which were divided into two sets. Treatment sets were as follows:

I - Control and salt treatments: The first set was subjected to 0, 2.5 dS/m^{-1} and 5 dS/m^{-1} seasalt irrigation.

II - Salt treatment and Ascorbic acid foliar application: The second set was subjected to 0, 2.5 dS/m^{-1} and 5 dS/m^{-1} seasalt irrigation and after two weeks from sowing guar seedling were sprayed with 0.5mM ascorbic acid and then sprayed again after 45 days from sowing.

These clay pots have basal outlet for drainage. Out of 24 pots 12 pots present in each set and 4 replicates were maintained for each treatment i) control (non-saline), ii) 2.5 dS/m^{-1} and iii) 5 dS/m^{-1}seasalt concentrations. Every pot was filled with 3 Kg of thoroughly washed sandy loam soil. Soil in each pot was saturated with full strength Hoagland's solution. Approximately uniform size and equal number of seeds were surface sterilized with 0.1% mercuric chloride for one minute and then washed with distilled water. 5 seeds were sown in each pot. They were then daily irrigated with an equal amount i.e., 150 ml of tap water. When seedlings were reached at 3 leaves stage they were thinned out as one seedling per pot. All these 24 pots were then arranged in a completely randomized design (CRD) in the Department of Botany, University of Karachi, Karachi. Seasalt treatment was started at this stage and concentrations of seasalt were gradually increased in irrigation water till it reached to the desired salinity of each treatment. Each pot was irrigated with 1.5L of tap water / seasalt solution twice a week. Root length, number of leaves and branches, fresh and dry biomass, number of pods per plant were recorded in harvested plants at termination of experiment. Leaf samples were collected at grand period of growth for biochemical analysis.

2.2. Chlorophyll content

Chlorophyll concentration (Chl) was determined in fresh leaves following the protocol of [16].

2.3. Total Carbohydrate Content

Estimation of carbohydrate was done in plant extracts by [17] method using Anthron reagent.

2.4. Analysis of total Protein

Total protein was extracted and analyzed by [18].

2.5. Mineral Estimation of Vegetative Parts

Samples of leaf, stem and root were taken at grand period of growth for the analysis of different cations ($Na^+$and K^+). Samples were dried and 0.5gm of each dry sample was taken for ash weight. Then solution of ash was made in 50ml of de-ionized water, then dilutions were made in de-ionized water for mineral analysis. Concentration of cations in samples was measured using a PFP1 flame photometer.

2.6. Experimental design and statistical analysis

The experimental design was completely randomized Design (CRD) with three salt levels and three replicates. Collected data was analyzed statistically by using SPSS to analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($P < 0.05$).

3. Results and Discussion

3.1. Plant Growth

In present investigation plants treated with seasalt exhibited significant ($P<0.001$) decrease in plant height, root length, number of leaves, leaf area, fresh and dry biomass, number of pods per plant, seed number per plant and seed weight per plant as compare to non-treated plants (Figures 1-9). It is well established fact that Na is a toxic element whose higher concentration disturbs the different metabolic activities. The varieties which were successful in retaining the Na in the root were tolerant [19].

Reductions in dry matter of salt-stressed maize plants are expected in view of some earlier studies that show that salinity stress results in a marked stunting of plants [20] [21] [22]. Salt stress also results in a considerable decrease in the fresh and dry weights of plants [23] [24]. Turgor pressure reductions in expanding tissues, reductions in the photosystem activity, and direct effects of accumulated salt on critical metabolic steps in dividing and expanding cells are 3 key physiological mechanisms responsible for the growth inhibition induced by salinity [25].

Decrease in plant height with increasing salinity is typical effect of the toxic ions accumulation in cells which severely affect cell division and expansion [26]. Reduction of leaf area per plant at higher sodium chloride concentrations (80 and 160 mM) is

due to severe foliar injuries (chlorosis and necrosis) and shedding of leaves [27]. Therios and Misopolinos [28] studied salt stressed olive trees (*Oleaeuropaea L.*) and found to drop leaves of all size, age and from all positions while seedlings of Z. spina-christi only shed their basal old leaves. This phenomenon also reported that such differentiation may reflect removal of salts from the more active young tissues towards older ones, a typical trait of species that remove toxic salts from their transpiration stream [29].

Hakim *et al.*, [30] observed that shoot and root dry weights of rice significantly deceased with the increase in the salinity levels. This decrease of dry weight might be due to some reasons such as (i) salt stress reduced photosynthesis per unit leaf area which turned into limited supply of carbohydrate needed for shoot growth, (ii) reduced turgor resulting in lower water potential and (iii) disturbance in mineral supply might have directly affected growth. In addition, salinity affected final cell size as well as rate of cell production and thereby resulting in reduced shoot and root dry weight. Similar results are also documented by [31] and [32].

The yield reduction of plant might be due to the reason that salts modify the metabolic activities of the cell wall which limit the cell wall elasticity. As a result cell walls become rigid and consequently the turgor pressure efficiency in cell enlargement is decreased. Presence of salt also disturbed the photosynthesis, the shrinkage of cell contents, reduced development and differentiation of tissues, unbalanced nutrition and damage of membranes. As a result it affected the growth and also yield contributing characters resulted grain yield [30]. Many research findings also support this phenomenon [33] [32] [34].

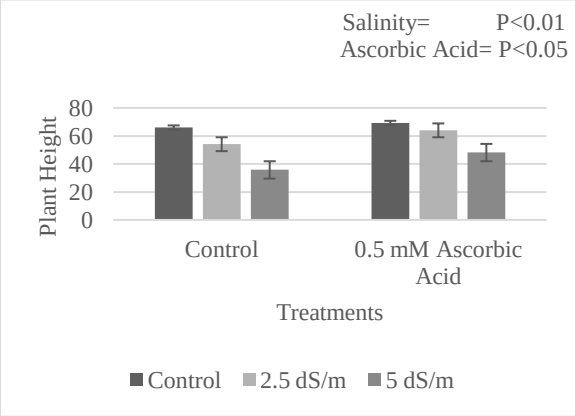

Figure 1. Influence of foliar application of ascorbic acid on plant height (cms) of *Cymopsis tetragonoloba* grown under seasalt salinity.

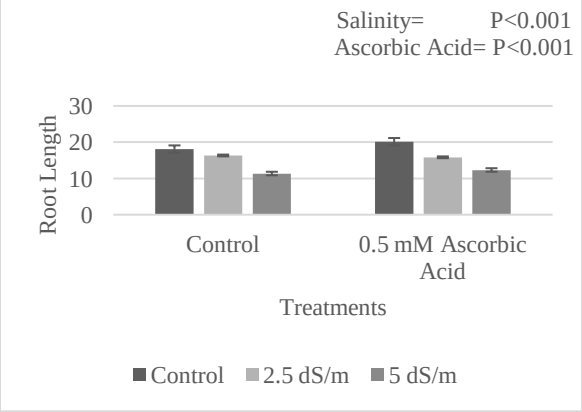

Figure 2. Influence of foliar application of ascorbic acid on root length (cms) of *Cymopsis tetragonoloba* grown under seasalt salinity.

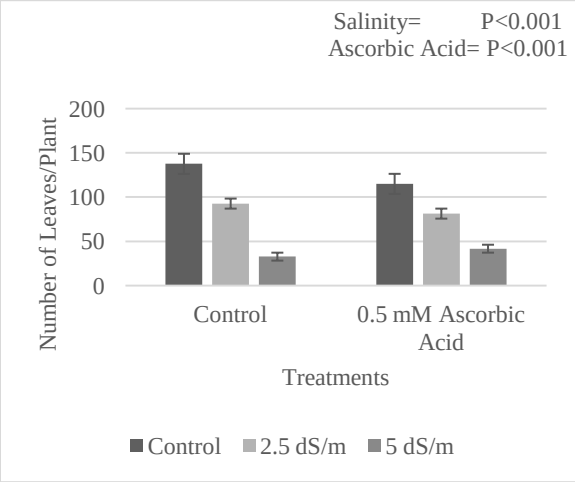

Figure 3. Influence of foliar application of ascorbic acid on number of leaves/plant of *Cymopsis tetragonoloba* grown under seasalt salinity.

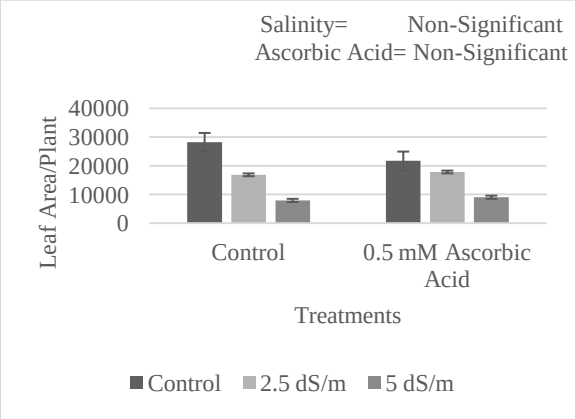

Figure 4. Influence of foliar application of ascorbic acid on leaf area/plant (mm^2) of *Cymopsis tetragonoloba* grown under seasalt salinity.

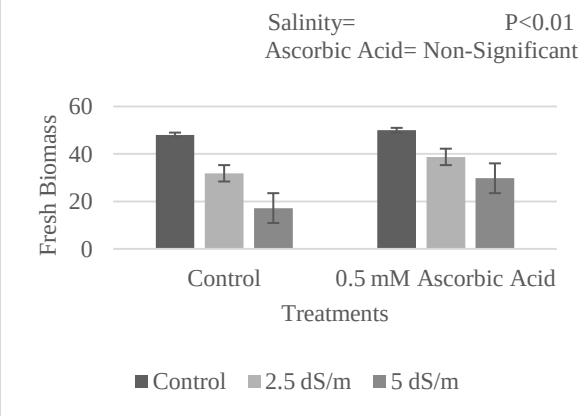

Figure 5. Influence of foliar application of ascorbic acid on fresh biomass (gms) of *Cymopsis tetragonoloba* grown under seasalt salinity.

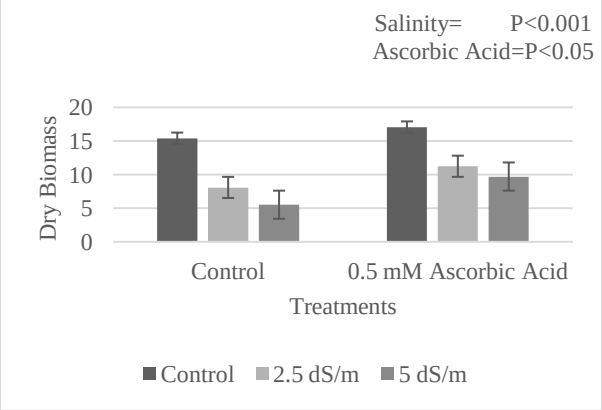

Figure 6. Influence of foliar application of ascorbic acid on dry biomass (gms) of *Cymopsis tetragonoloba* grown under seasalt salinity.

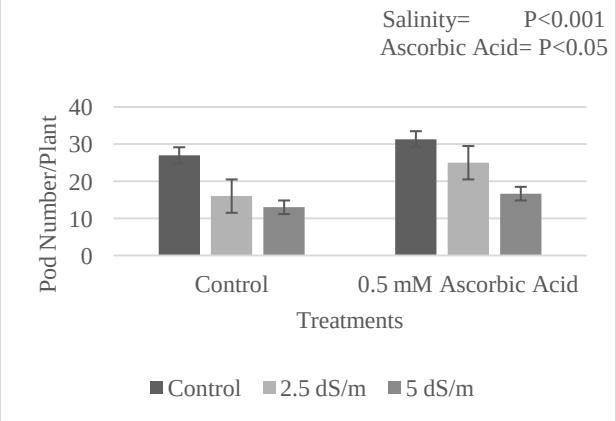

Figure 7. Influence of foliar application of ascorbic acid on pod number/plant of *Cymopsis tetragonoloba* grown under seasalt salinity.

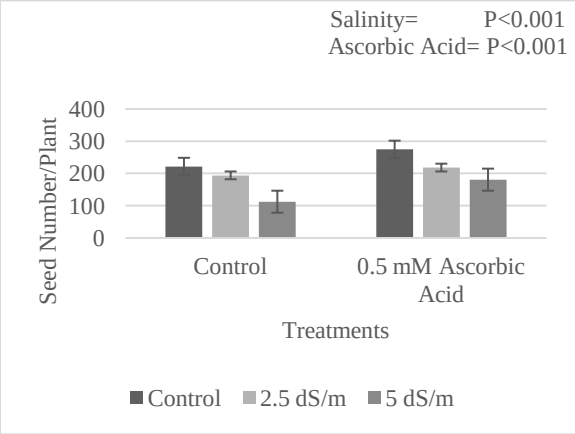

Figure 8. Influence of foliar application of ascorbic acid on seed number/plant of *Cymopsis tetragonoloba* grown under seasalt salinity.

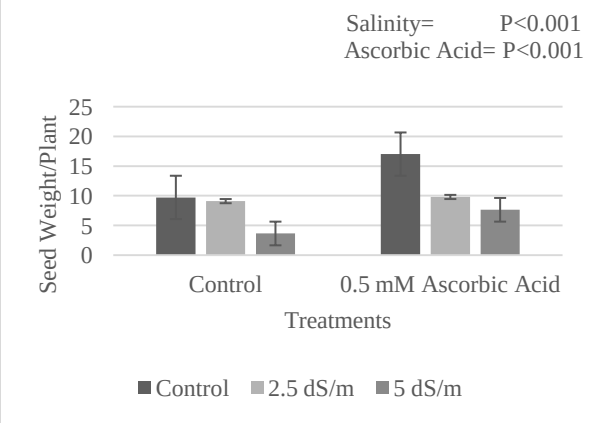

Figure 9. Influence of foliar application of ascorbic acid on seed weight/plant (gms) of *Cymopsis tetragonoloba* grown under seasalt salinity.

Foliar application of ascorbic acid showed significant improvement in growth parameters in non-saline as well as salinity treated plants. These results were conformity with those reported by Biacs *et al.* [35] on tomato, Golan-Goldhirsh *et al.* [36], on soybean, Hanafy Ahmed [37] on tipburn, Tarraf *et al.* [38] and El-Shraiy [39] on lemon grass who reported that spraying acetyl salicylic acid treatments on potato plants

promoted plant growth, plant height and nu mber of leaves per potato plant. Also, [40] mentioned that SA at low concentrations affected plant size, and number of leaves of African violet plant. Moreover, [41] found that spraying ascorbic acid had favorable effects on growth characters and yield of sunflower particularly with the higher concentration. Similar results were obtained as a result to foliar spray of ascorbic acid on lettuce, [42] found that foliar application of salicylic acid gene-rally had a positive effect on vegetative growth parameters (plant height, leaves number, shoots and roots fresh and dry weight) of common bean as compared to control.

3.2. Chlorophyll

It is generally known that photosynthetic efficiency depends on photosynthetic pigments such as chlorophylls '*a*' and '*b*', which play an important role in photochemical reactions of photosynthesis [43]. Data presented in figures (10-13) showed that plant treated with sea salt showed significant (P<0.001) increase in chlorophyll a, b and total chlorophyll as compare to control plants. The changes in leaf chlorophyll content may be due to reduction in biosynthesis or increased degradation of chlorophyll under saline conditions. It is also documented that in salt stressed plants, breakdown of ultrastructure of chloroplasts including plastid envelop, thylakoids [44], and photosynthetic apparatus may result due to direct Na+ toxicity or salt-induced oxidative damage [45]. O2− radicals and singlet oxygen atoms attack double-bond-containing compounds, thus damaging the chloroplast membrane system and photosynthetic reaction centers [46]. This results in the release of chlorophyll from the thylakoid membranes. Reduction in chlorophyll content may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation [47].

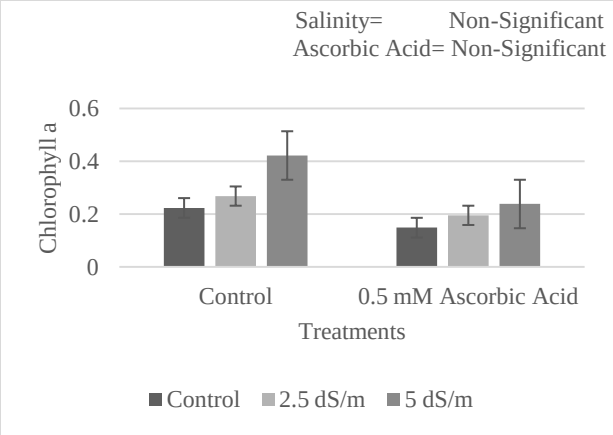

Figure 10. Influence of foliar application of ascorbic acid on chlorophyll a (mg/gm fr. wt) of *Cymopsis tetragonoloba* grown under seasalt salinity.

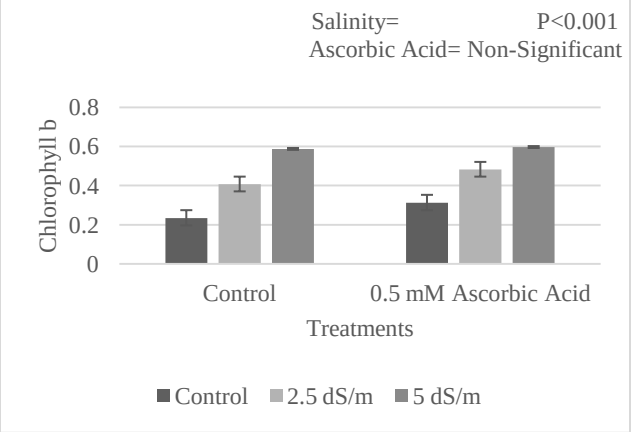

Figure 11. Influence of foliar application of ascorbic acid on chlorophyll b (mg/gm fr.wt) of *Cymopsis tetragonoloba* grown under seasalt salinity.

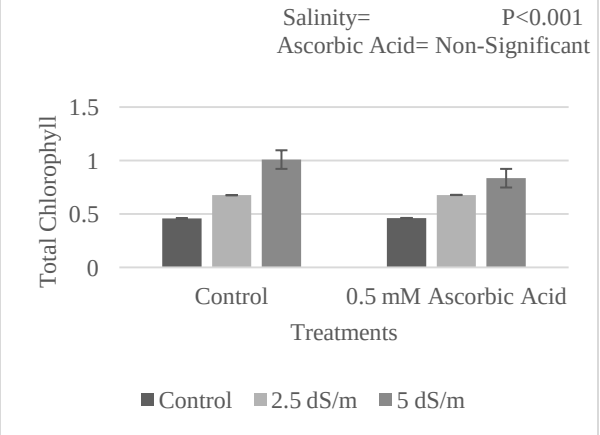

Figure 12. Influence of foliar application of ascorbic acid on total chlorophyll of *Cymopsis tetragonoloba* grown under seasalt salinity.

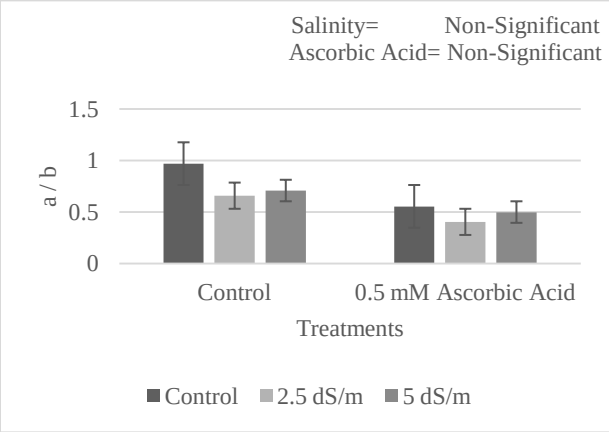

Figure 13. Influence of foliar application of ascorbic acid on chlorophyll ab Ratio (a/b) of *Cymopsis tetragonoloba* grown under seasalt salinity.

Foliar application of ascorbic acid showed significant improvement in chlorophyll a, b and total chlorophyll in non-saline as well as salinity treated plants. It is suggested that foliar applied Ascorbic acid protected photosynthetic apparatus from salt induced oxidative stress. It is further supported by the fact that chloroplast is a major source of production of reactive oxygen species (ROS) in plants [48], but it lacks catalase to scavenge ROS, therefore ascorbic acid acts as a substrate for ascorbate peroxidase (APX) to scavenge ROS produced in the thylakoid membranes [49]. Foliar application of ascorbic acid encouraged synthesis of chlorophyll involved in increases of photosynthetic metabolites, which lead to the accumulation of different fractions of soluble sugars and nitrogen content in plant tissues under saline conditions or this could perhaps alleviate the inhibitory effects of salinity on glucose incorporation to cell wall polysaccharides [50]. [51] also documented that ascorbic acid scavenged reactive oxygen species and prevented protein oxidation and degradation.

3.3. Carbohydrates

Organic acid especially sugars are the main solutes involved in osmotic adjustment in some plants when they submitted to osmotic and saline stress [52]. Data presented in figure 14 showed that plant treated with sea salt showed significant ($P<0.001$) increase in total carbohydrates as compare to control plants. It is assumed that increase in total soluble sugars under salinity stress was considered protective and adaptive functions of soluble carbohydrates under salinity stress. Under NaCl salinity, starch and soluble carbohydrates accumulated in plants [53] [54], which has been attributed to impaired carbohydrate utilization [55]. The Organic solutes accumulation (soluble and insoluble carbohydrates) might play an important role in increasing the internal osmotic pressure [56].

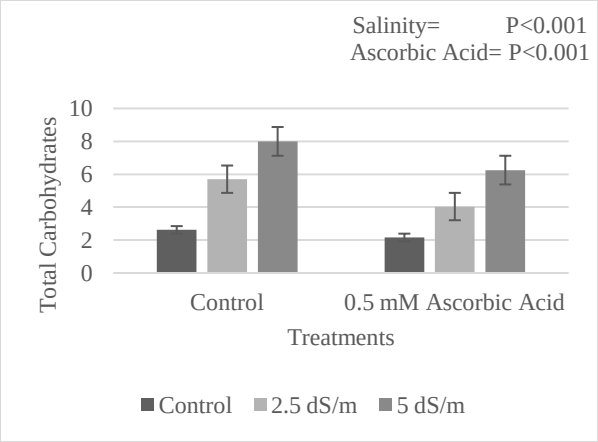

Figure 14. Influence of foliar application of ascorbic acid on total carbohydrates (mg/gm fr. wt) of *Cymopsis tetragonoloba* grown under seasalt salinity.

Foliar application of ascorbic acid showed significant improvement in total carbohydrates in non-saline as well as salinity treated plants. These results are in agreement with the findings of [57] on maize plant. According to his studies increase in total soluble sugars and sucrose in faba bean plants may indicate more stimulation in the enzymes of sugar hydrolysis.

3.4. Proteins

Data presented in figure 15 showed that plant treated with sea salt showed significant (P<0.001) decrease in total proteins as compare to control plants. Salt stress decreased the protein and chlorophyll content. Different studies have reported reduction in protein content by salinity [58] [59] [60]. [61] noticed an increase of protein content of the tomato plant *Lycopersicon esculentum* (L.) in response to salt treatment.[62] reported increased soluble protein content in the seedlings of clover plant (*Medicago citrna* L.) after treatment for 30 days with concentrations of zero, 1, 50, 100, 200 mM of NaCl. [63] reported an increase in protein content when treating barley plant (*Hordeum vulgare* L.) with 120 mM of sodium chloride. Results of [64] on *Vigna mungo* (L.) also support the previous results.

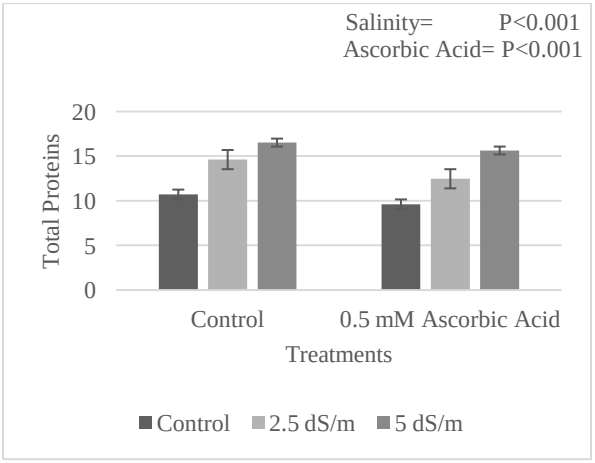

Figure 15. Influence of foliar application of ascorbic acid on total proteins (mg/gm fr. wt) of *Cymopsis tetragonoloba* grown under seasalt salinity.

Foliar application of ascorbic acid showed significant improvement in total proteins in non-saline as well as salinity treated plants. Similar results were recorded by [65] [66] [67]. [68] reported that the changes in protein profile may be due to adaptation of *Silybum marianum L.* seeds to NaCl stress. The new bands of proteins in seedlings germinated in NaCl or in combination with

vitamin C may be due to de novo synthesis of new protein [69] [65]. [70] has reported that vitamin treatments induces a significant alterations in the enzymes related to protein metabolism which indicates that vitamins might act as activators of protein synthesis.

3.5. Ions

Data presented in figures 17-19 showed that plant treated with sea salt showed significant ($P<0.001$) increase in sodium while significant ($P<0.001$) decrease in potassium as compare to control plants. Salt stress created nutritional imbalance and formed ion antagonism and caused excess accumulation of sodium ions in both shoot and roots. Sodium ion concentration increased proportionally to different levels of salinity in both root and shoot but in root, rate of increase was higher than the shoot. The higher amounts of Na+ ions in both shoot and root indicated a signal of nutritional imbalance. The sodium ions in shoots and roots were gradually accumulated and increased in salt-stressed condition but the increasing rate depends on salt concentrations [71]. In the present study, K+ concentrations decreased with the increase in salinity levels. K+ ions compete with other elements for absorption by the root [72]. This might be due to (i) high external Na negatively effects on K acquisition because of similar physiochemical properties of Na and K; (ii) KUP (potassium uptake permease) /HAK (High Affinity K) transporters are selective for K and they are blocked by Na under salt stress [73]. [74] studied that sodium ion concentration in the shoot and root increased at high salt levels. [32] reported that sodium ion increased and K ion in the shoot and root was significantly decreased with the increase of salinity levels. [75] studied that the Na+ increased and K+ ion decreased significantly in the shoot and root of two barley cultivars with the increase of salinity in the growth medium. [76] reported that Na+ ions increased and K+ ions decreased in the shoot and root of rice genotypes with the increase of NaCl levels. [77] reported that the K+ ion in seedling of Soybean significantly decreased with the increase in salinity levels. [78] also studied that sodium and potassium ion in rice significantly influenced by the effect of different levels of salinity.

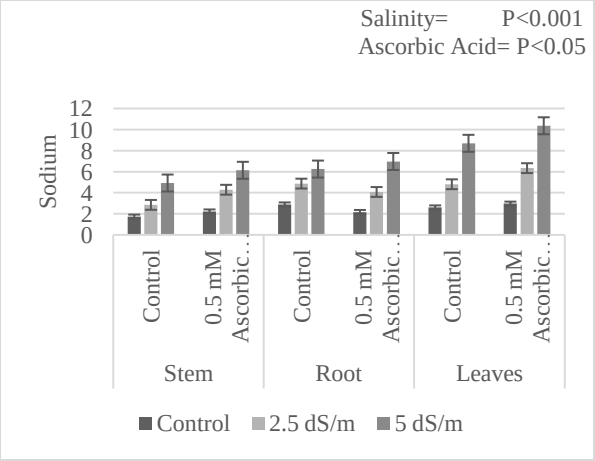

Figure 16. Influence of foliar application of ascorbic acid on sodium ion concentration of different plant parts (stem, root and leaves) of *Cymopsis tetragonoloba* grown under seasalt salinity.

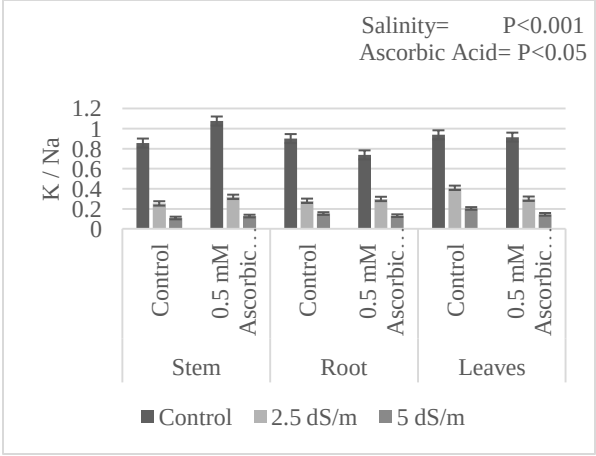

Figure 18. Influence of foliar application of ascorbic acid on potassium sodium ratio (K+/Na+) of different plant parts (stem, root and leaves) of *Cymopsis tetragonoloba* grown under seasalt salinity.

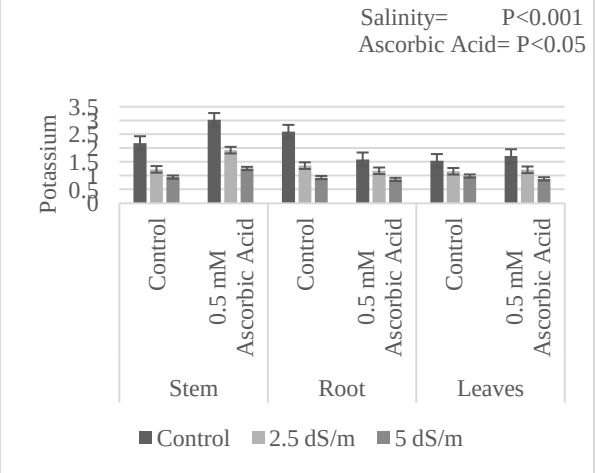

Figure 17. Influence of foliar application of ascorbic acid on potassium ion concentration of different plant parts (stem, root and leaves) of *Cymopsis tetragonoloba* grown under seasalt salinity.

Foliar application of ascorbic acid showed significant improvement in sodium and potassium in non-saline as well as salinity treated plants. [79] reported that foliar application of ascorbic acid significantly increased P and K content in wheat grains up to 400 mg L-1 relative to their untreated controls. Ascorbic acid application significantly increased N, P, and K content in leaves and grains of Ber [80], cotton, [81], wheat [82], and sunflower plants [41] compared with their controls. [83] reported that foliar application of ascorbic acid significantly increased P and K contents in wheat grains at 150 mg L -1 relative to their untreated controls.

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