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Amelioration of Adverse Effects of Salt Stress in Okra (*Hibiscus esculentus* L.) By Foliar Application of Proline

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Abstract: The aim of this investigation was to improve salt tolerance abilities in okra (*Hibiscus esculentus* L.) by foliar application of proline. Salinity is a great threat to agriculture all over the world. Salinity is a major abiotic stress that reduces metabolism, growth and productivity of many crops. However, foliarly applied proline is considered to be fruitful solution in okra to reduce salt stress. In this regard, a potted experiment was conducted in the Department of Biological Sciences, University of Sargodha to investigate whether foliar application of proline could ameliorate the toxic effects of salinity on okra plants. Three levels of NaCl (0mmol, 40mmol, 80mmol) were applied to okra plants according to the saturation percentage of soil. Different levels of proline (0mM, 30mM and 60mM) were applied exogenously as foliar spray on okra to protect the plants against salt stress. Data for different parameters such as plant height, shoot and root fresh and dry weights, different ions accumulations, protein, amino acids, nitrate reductase, total sugar and chlorophyll contents of okra were collected. Applications of proline decreased the uptake of Na^+ and increased the concentrations of potassium and chlorophyll contents. This showed that application of proline stabilized the physiology and induced the salt tolerance in okra plants.

Key words: Okra · Salinity · Proline · Foliar spray · Amelioration

INTRODUCTION

Salinity is one of the major stress to crop that reduce plants growth and productivity [1]. The ion toxicity has negative impacts on plant growth and development due to low water potential within the local root environment [2[. Salinity also affected the photosynthetic rate, stomatal conductance and CO_2 assimilation in plants [3]. Due to the spreading effects of stress, the area for cultivation of okra been decreased [4]. Cation accumulation plays important roleon okra during salinity stress tolerance [5]. Salt stress causes a number of changes in plant metabolism. Of them, ion toxicity, osmotic stress and production of reactive oxygen species (ROS) are most prominent [6]. ROS are generally produced in mitochondria and chloroplasts. In chloroplasts, ROS are generated by direct transfer of excitation energy from chlorophyll to produce singlet oxygen, which is responsible for the reduction of photosynthesis [3]. Therefore, the role of an efficient antioxidant system (antioxidant enzymes and antioxidant compounds) is vital for scavenging ROS. While evaluating various physiological and biochemical selection criteria for salt tolerance in crops. [7] proposed that biochemical and physiological studies supply more direct and reliable information on salinity tolerance and could be used for enhancing salinity tolerance in important crops. For example, the accumulation of osmoprotectants such as glycine betaine, proline and trehalose in plants in response to salt stress has been widely reported [8, 9]. Among them, exogenous application of proline had gained considerable attention in mitigating the effect of salt stress [10]. Thus the mechanism used for salinity tolerance in okra is the foliar application of osmoprotectant such as proline, potassium etc. that stabilize proteins and membranes against denaturation effect caused due to salt stress [11]. However, increases the level of proline that acts as an osmoprotectant, membrane stabilizer and reactive oxygen species (ROS) scavenger [12]. Proline modify morphological and physiological characteristic of rice plant and may also induce better adaptation of plant to environment which improving its growth and yield [8].

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Okra (*Ablemoschus esculentus* L.) is a nutritionally important summer vegetable and belongs to family malvaceae. Being an excellent source of potassium, calcium and unsaturated fatty acids for instance, linolenic and oleic acid [13], okra is very essential for human nutrition. The main objective of this study was deterimine the ability of poline to induce salinity tolerance in okra.

MATERIALS AND METHODS

An experiment was conducted in the Department of Biological Sciences, University of Sargodha to determine the effect of exogenous application of proline on okra under saline conditions. Different levels 0, 40, 80 mM of NaCl were applied to each pot according to the saturation percentage of soil and different levels 0, 30, 60 mM of proline were applied exogenously as a foliar spray to determine whether proline application could tolerate the effect of salinity stress on okra (*Hibiscus esculentus).* The experiment was laid down on a completely randomized design with two factors factorial arrangement and with three replicates. The seeds of the cultivars were obtained from Ayub Agricultural Research Institute, Faisalabad and sown in pots. Each pot filled with 5kg well mixed soil. Different salinity levels were adjusted in accordance with saturation percentage of soil. In each pot, 15 seeds were sown. After complete germination, thinning practiced to maintain 10 plants in each pot. Three plants were harvested from each pot at the time of sampling. The data about following parameters were collected.

Data Collection: The data collection for plant height, shoot length, root length, shoot and root fresh and dry weight, nitrate reductase, protein, amino acids, total sugar, chlorophyll a and b and total chlorophyll contents. Potassium, calcium, sodium, magnesium and phosphorus contents in shoot and root of okra plants were collected.

Plant Height:
Height of three randomly selected plants from each proline level of different field was measured in centimeters from soil surface to top of the plants and their mean were calculated.

Shoot & Root Fresh Weight: For each plant, shoot and root were separated and fresh weights were measured separately with the help of a digital electrical balance and their mean were calculated.

Shoot and Root Dry Weight: The plants were dried in an oven at 60°C for 24 hour and their dry weights were determined and their mean were calculated.

Chlorophyll Contents Determination: The chlorophyll a & b were determined with the method as described by Arnon [14].

Determinations of Ions
Digestion: For ions detection ground material (0.01g) was digested according to the method of wolf [15].

Sodium, Potassium and Calcium, were determined with help of Flame photometer (JenewaPFP7).

Protein: Protein was determined by the method as described by Lowry *et al*., [16].

Total Amino Acids: Total amino acids were estimated as described by Hamilton and Van Slyke [17].

Total Sugars: Total Sugars were determined according to the method of Yemm and Willis [18].

Statistical Analysis: The data for all the parameters were analyzed by analysis of variance technique [19]. Differences for various characters were compared by using Least Significant Differences test at 0.05 level of probability.

RESULTS

Saline growth medium significantly reduced the plant height, shoot length, root length, shoot fresh weight, root fresh weight and root dry weight (Table. 1). Application of proline as a foliar spray enhanced the growth of the plants. At 40 mmol of salinity, among different concentration of proline 30 mmol of proline was more effective to enhance the plant height, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight, root dry weight, chlorophyll b, total chlorophyll and calcium in shoot of Okra (Fig. 1, 3, 4, 5, 6, 7, 8, 9, 10) and at 80 mmol of salinity 60 mmol of proline was more effective to enhance root length, chlorophyll b and total chlorophyll of okra Fig. 9, 10.

Saline growth medium significantly reduced the Application of proline enhanced the growth of the okra plant. At 40 mmol salinity, application of 30 mmol of proline was more effective to enhance calcium in shoot,

Table 1: Analysis of variance of the data of plant height when different concentrations of proline applied as a foliar spray on okra (*Hibiscus esculentus* L.) under saline and non saline conditions

Source	DF	Plant height	Shoot length	Root length	Shoot fresh weight	Shoot dry weight	Root dry weight	Chlorophyll a	Chlorophyll b	Total chlorophyll
Salinity	2	0.88451*	76.6759 *	37.8426*	64.9605	0.7673*	0.8845 *	0.00648*	0.0270**	0.0139 *
Proline	2	0.11351**	7.0093*	0.4537 *	10.2239	0.062341	0.11351*	0.0067*	0.03417*	0.13589*
Interaction	4	0.076344	75.9954	2.0370	2.7664	0.063056	0.076344	0.024127	0.022589	0.19163
Error	18	0.268815	17.3889	7.0463	32.9575	0.858474	0.268815	0.0070472	0.05048	0.041341
Total	26									

Table 2: Analysis of variance of the data of plant height when different concentrations of proline applied as a foliar spray on okra (*Hibiscus esculentus* L.) under saline and non saline conditions

Source	DF	Shoot K	Root K	Shoot Ca	Root Ca	Nitrate reductase height	Protein	Total Amino acid	Total sugar
Salinity	2	8.03156 *	0.82206 *	15.8216*	5.64926*	0.00548**	1.8538 **	71.553**	0.012689*
Proline	2	4.2169*	2.89482 *	8.7305 *	3.89703	0.005963*	1.2667 **	81.193**	0.02183**
Interaction	4	2.70980	3.41495	6.2315	5.55073	0.0062921	13.2084	40.8427	0.0317041
Error	18	7.99711	2.71478	6.1220	8.97731	0.0136969	12.5942	45.4705	0.689109
Total	26								

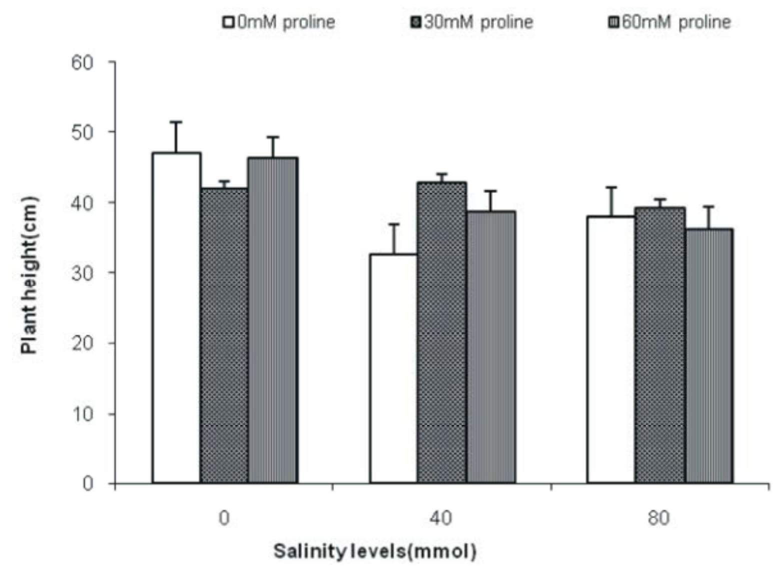

Fig. 1: Effect of foliarly applied proline on plant height of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

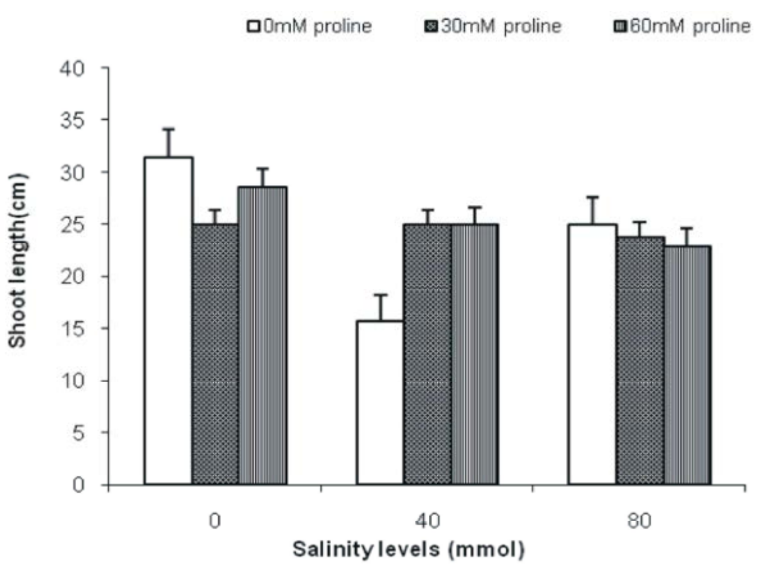

Fig. 2: Effect of foliarly applied proline on shoot length of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

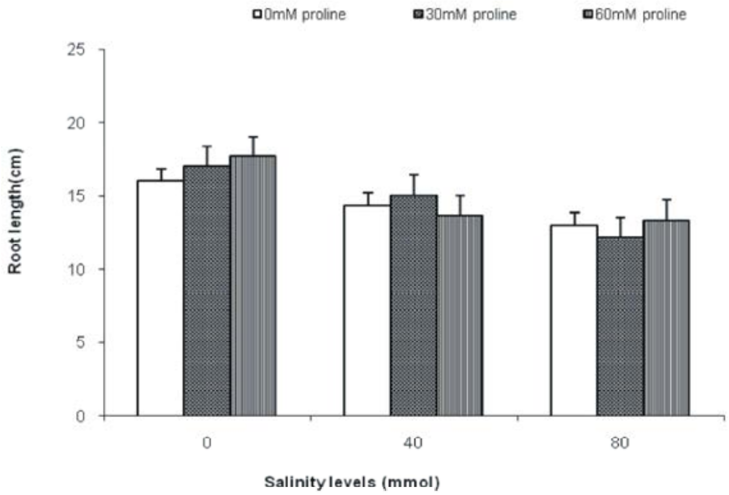

Fig. 3: Effect of foliarly applied proline on root length of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

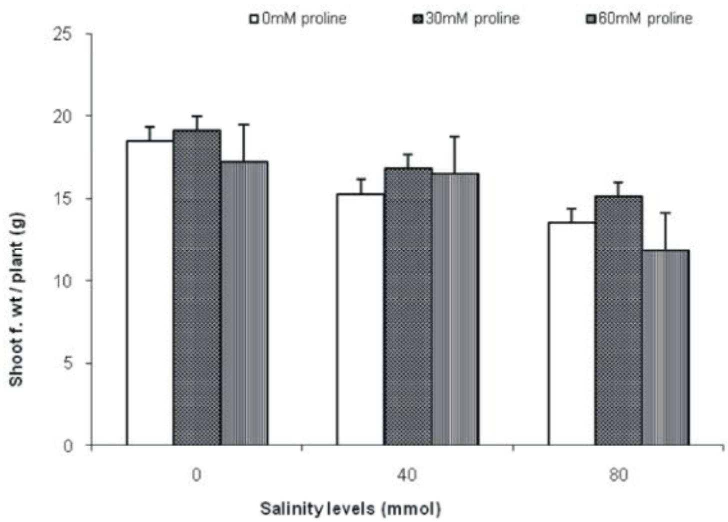

Fig. 4: Effect of foliarly applied proline on shoot f.wt of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

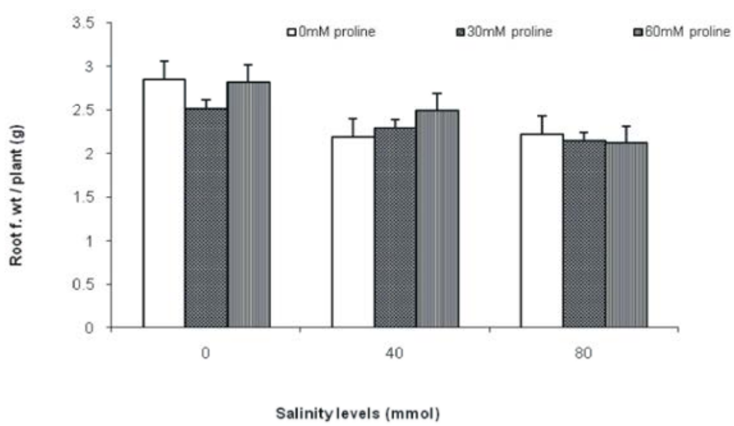

Fig. 5: Effect of foliarly applied proline on root f.wt of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

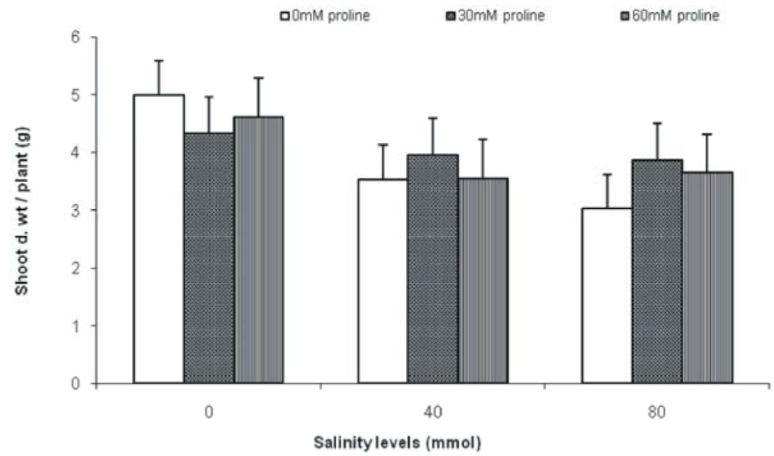

Fig. 6: Effect of foliarly applied proline on shoot d.wt of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

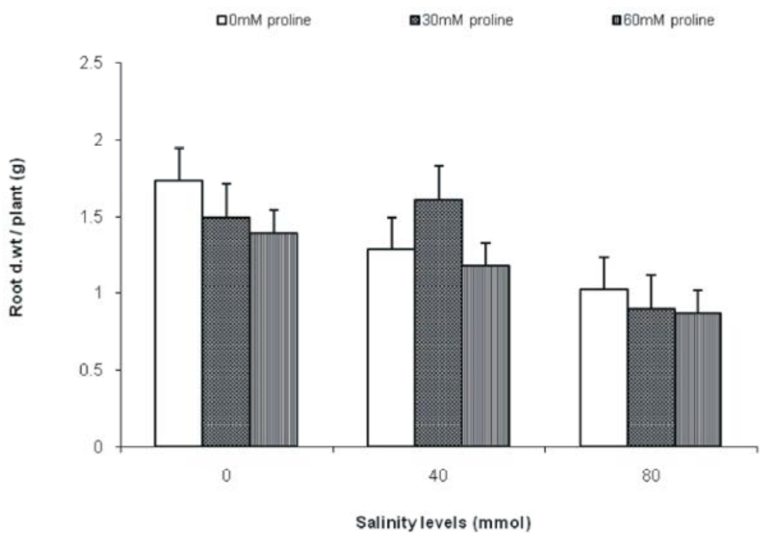

Fig. 7: Effect of foliarly applied proline on root d.wt of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

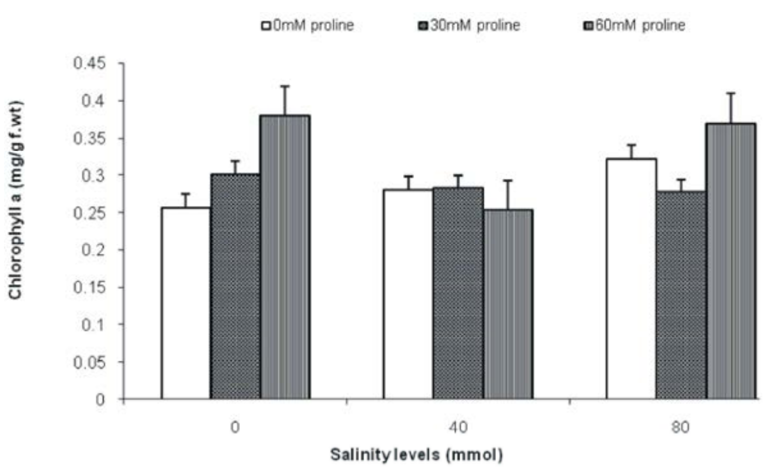

Fig. 8: Effect of foliarly applied proline on chlorophyll a of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

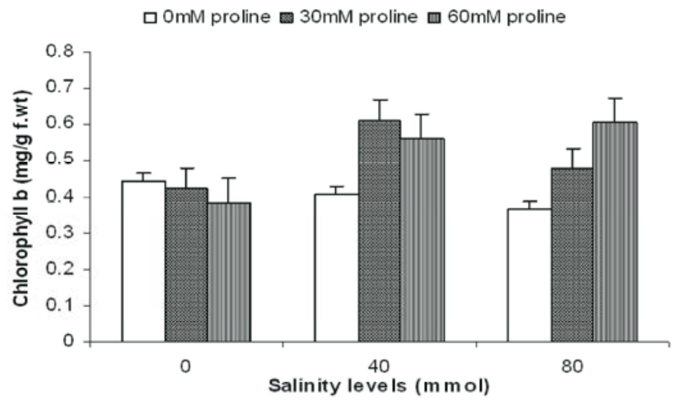

Fig. 9: Effect of foliarly applied proline on chlorophyll b of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

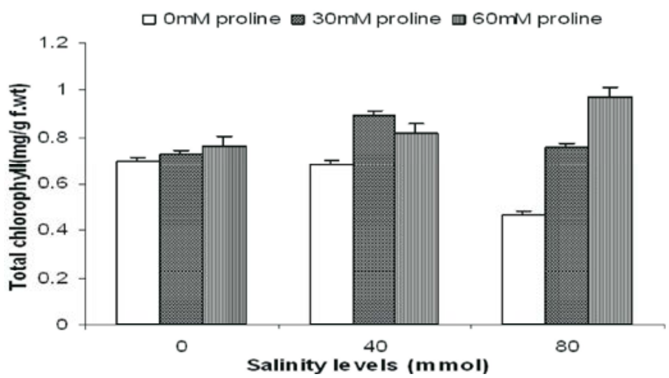

Fig. 10: Effect of foliarly applied proline on total chlorophyll of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

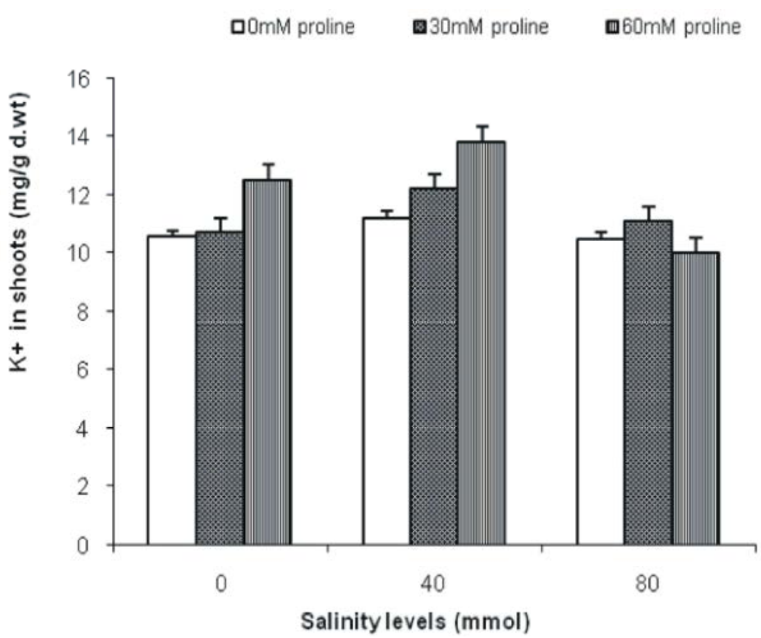

Fig. 11: Effect of foliarly applied proline on K+ in shoots of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

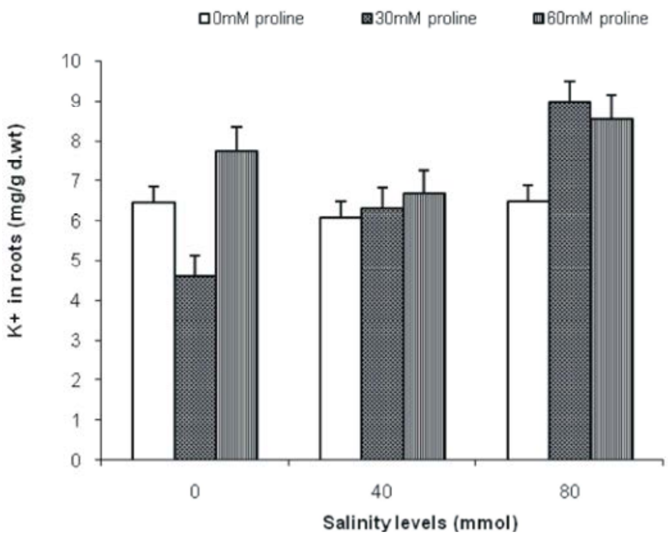

Fig. 12: Effect of foliarly applied proline on K+ in roots of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

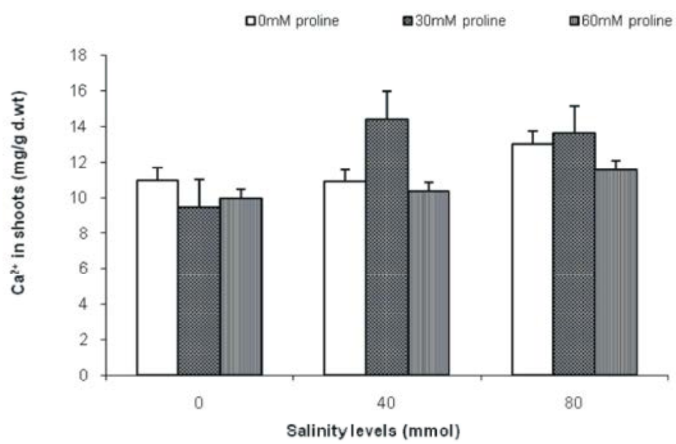

Fig. 13: Effect of foliarly applied proline on Ca^2 + in shoots of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

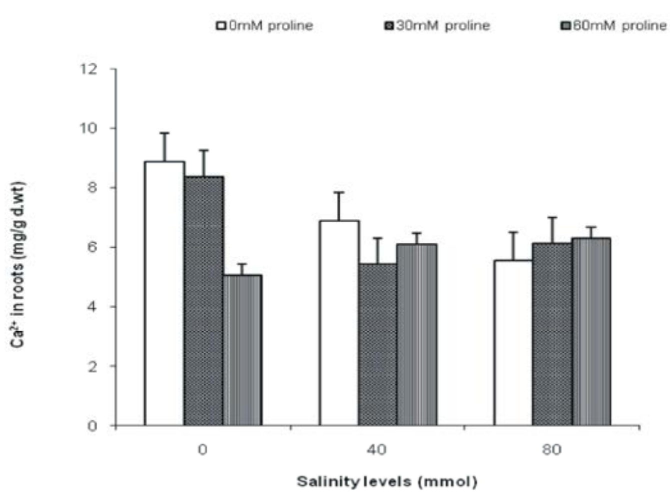

Fig. 14: Effect of foliarly applied proline on Ca^2+ in roots of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

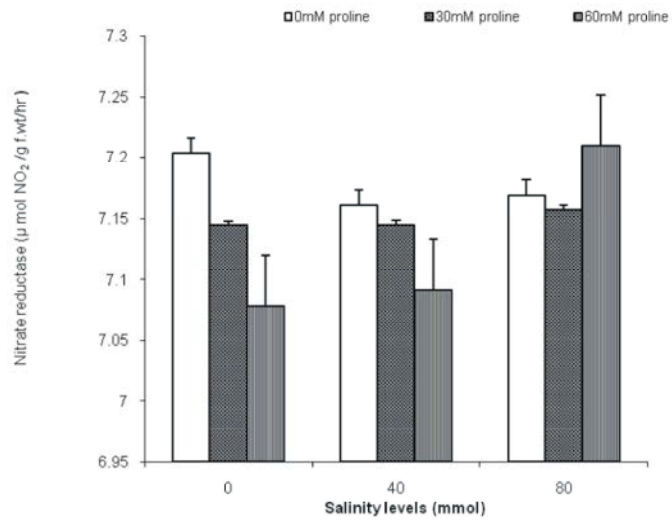

Fig. 15: Effect of foliarly applied proline on nitrate reductase a of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

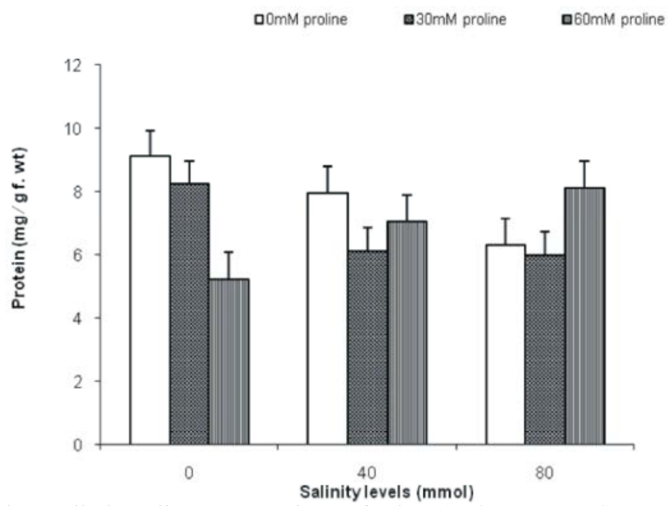

Fig. 16: Effect of foliarly applied proline on protein a of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

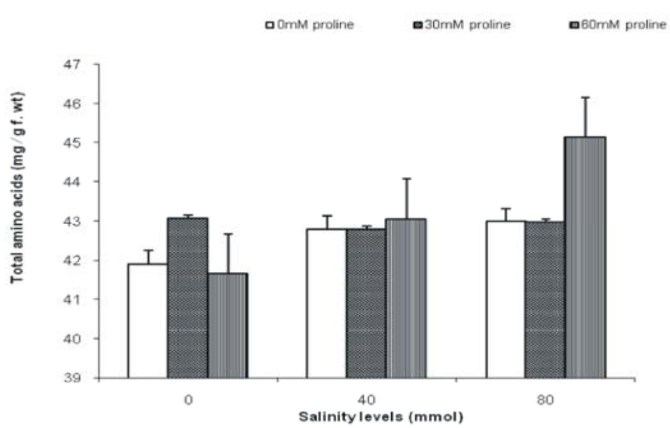

Fig. 17: Effect of foliarly applied proline on total amino acids a of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

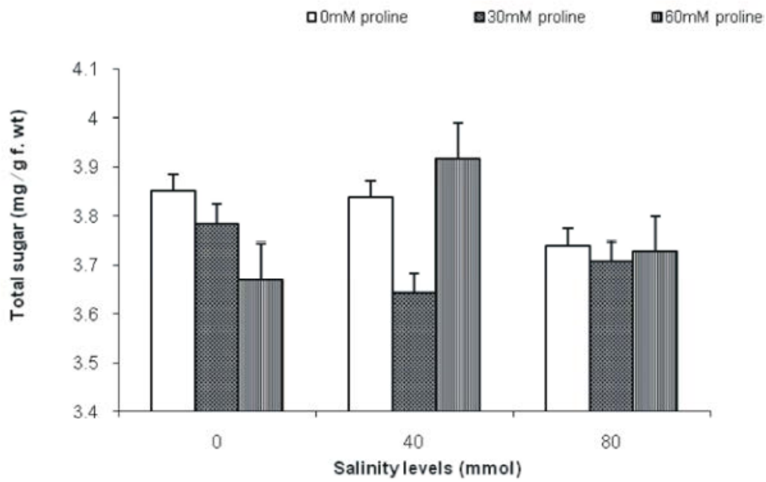

Fig. 18: Effect of foliarly applied proline on total sugar of Okra (*Hibiscus esculenus* L.) under saline and non-saline conditions

60 mmol of proline was more effective to increase K in shoot and root, total amino acid and total sugar (Fig. 11, 12, 17, 18) and at 80 mmol of salinity, application of 60 mmol of proline was more effective to enhance chlorophyll a, chlorophyll b and total chlorophyll Fig. 8, 9, 10.

DISCUSSION

Salinity affects a very large part of agricultural area across the world and is a serious problem due to rising sea-levels caused by climate change. Due to salinity stress, seed germination was delayed and germination percentage was decreased due to increasing levels of salt concentration. Under study experiment was conducted to ameliorate the effect of salinity on okra by the exogenous application of proline. Salinity can affect several physiological and biochemical processes during plant growth [20]. Decrease of plant biomass was commonly induced by environmental stress including salinity stress [21]. Salinity stress also affected the photosynthetic rate, CO_2 assimilation rate, stomatal consuctance [3]. At cellular level, dehydration due to presence of salts in leaf apoplasm [22], enhanced membrane permeability [23]. This overall salinity mediated disturbed plant physiology contributes to inhibited biomass production. The decrease in growth rates of okra in this study can be a result of a combination of above mentioned salinity mediated physiological dysfunction of okra plants. Exogenous application of potassium mitigates the effect of salinity [24]. In view of some earlier reports, it is suggested that exogenously applied proline might have caused enhanced endogenous proline accumulation under stress conditions [25, 8]. In conclusion, Application of proline as a foliar spray on okra was found to be very effective on growth and yield of rice under saline conditions. In future higher concentrations of AsA can be used as pretreatments to observe their effect on okra as well as on other crops under salinity.

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