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Foliar Application of Proline for Salt Tolerance of Two Wheat (*Triticum aestivum* L.) Cultivars

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Abstract: This experiment was conducted to determine that whether the foliar spray of proline induce the salt tolerance in wheat cultivars or not. On the basis of findings of the present studies it can be concluded that salt stress negatively affects the germination %, growth and chlorophyll contents of both wheat cultivars but the exogenous application of proline significantly ameliorates the harmful effects of salt stress. However, high concentration of proline (100mM) application was more affective than 50mM. As whole the proline induce the salt tolerance in both cultivars of wheat.

Key words: Foliar Application % Proline % Salt Tolerance % Wheat

INTRODUCTION

Wheat (*Triticum* spp.) is one of the world's major cereal crops [1]. Wheat is the major cereal crop of Pakistan, which is grown all over the country. It is grown to meet the food demand of over growing population of Pakistan [2].

Salt stress adversely affects physiological and biochemical processes and hence significantly reduced the yield. This is the most serious threat to agriculture and major environmental factor that limits crop growth and productivity [3-4]. Salinity stress is known to affect various growth processes including photosynthesis, ion regulation, water relations etc. [5].

Plants have defense mechanisms that allow them to acclimatize in saline environment. One of them is the accumulation of certain organic metabolites/osmolytes. These are also collectively known as compatible solutes [6-9]. Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain the cell turgor [10]. Proline which is usually considered as an osmoprotection agent is also known to be involved in reducing the oxidative damage by scavenging and/or reducing the free radicals.Proline accumulation was proposed to be associated with tolerance to osmotic and saline stress [11-13]. Exogenous application of proline is known to induce abiotic stress tolerance in plants [14-16]. The most studied compound under salinity stress is proline. The amount of proline usually increases under salinity [17]. During osmotic adjustment, many plants accumulate proline in response to salt stress widely believed to function as a protector against salt damage [18].

Hence the present studies were conducted to improve the salt tolerance of two wheat cultivars by exogenous application of proline at seedling stage.

MATERIALS AND METHODS

In this experiment the salt tolerance in two wheat cultivars was induced by the exogenous application of proline. The experiment was conducted in Botany Lab, University of Gujrat, Punjab, Pakistan during March, 2011.

The seeds of two wheat cultivars (Seher and Lasani) were obtained from Botany department of University of Gujrat, Punjab, Pakistan. River sand was used as growth medium. Five seeds were sown in each pot; before sowing the sand was washed with adequate amount of distill water to eradicate salts previously present in sand. Then seeds were sown and 20ml Hoagland's solution was given to each pot. After full germination the plants were thinned and only healthy four plants were selected.

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Salt (NaCl) treatment (10 ds m^{-1}) was applied after 8 days of germination.Three levels (0mM, 50mM and 100mM) of proline were applied as foliar spray after two weeks of germination. Completely Randomized Design (CRD) with four replicates was used for this study/experiment. There were following four treatments.

T_o = Control (distill water)
T_1 = 100mM NaCl
T_2 = 100Mm NaCl + 50mM Proline as foliar Spray
T_3 = 100Mm NaCl + 100mM Proline as foliar spray

To calculate the plant biomass the plants were carefully uprooted from the pots and then washed with distills water and separated in to roots and shoot, after that fresh and dry the weights (g) of root and shoot were recorded. The root length was measured from base to the tip of the longest root and shoot length is measured from the base of the shoot to the youngest leaf. The measurement is done with the help of scale. The chlorophyll *a,b* and carotenewere determined with the method as described by [19]. Na^+, K^+cations were determined with a flame photometer (Spectrolab S20-4) graded series of standards (ranging from 10 to 100 mg/L) of Na^+, K^+ were made and standard curve for each element was drawn. The values of Na^+, K^{++} from the flame photometer were compared for standard curve and total quantities were calculated.

Analysis of variance technique of the data was computed for all parameters by using the COSTAT for DOS version 3.03 Computer Program. Completely Randomized Design was followed for this experiment. Four factors include varieties, salt treatments and different levels of proline. Bar graph using mean + S.E values was drawn by using Microsoft Excel software.

RESULTS

Salt stress reduced the germination %, plant biomass and chlorophyll contents of both cultivars.However, exogenous application of proline significantly ameliorates the harmful effects of salt stress by keeping the growth, germination % and pigmentation of sorghum to same level to some extent as compared to control.

Germination %: Analysis of Variance (ANOVA) Fig. 1 for data of germination % presented in tableshows that salt stress reduces the germination % of both wheat cultivars. But the foliarly applied two levels of proline i.e. 50mM and 100mM showed improvement in germination % under salt stress and increase germination. Both levels of proline were almost equally effective.

Root fresh Weight (g): Data presented in Fig. 2 shows that salinity or salt stress has a negative effect on root fresh weight of both wheat cultivars and significantly reduced it. By the application of two levels of proline i.e. 50mM and 100mM root fresh weight of both cultivars increasedunder unfavorable environment. 50mM was more effective than 100mM in ameliorating the adverse effects of salinity.

Shoot Fresh Weight (g): Analysis of variance (ANOVA) of data presented in Fig.3 shows that salt stress significantly reduces the shoot fresh weight of both cultivars. By the application of proline shoot fresh weight of both cultivars showed improvement under salt stress and improvement in this attribute was almost same in both cultivars.

Root Length (cm): Analysis of variance (ANOVA) of data presented in Fig.4 shows that salt stress reduced the root length of both wheat cultivars. Application of two levels of proline i.e. 50mM and 100mM in both cultivars showed non-significant improvement in root length under salt stress.

Shoot Length (cm): Analysis of variance (ANOVA) of data presented in Fig.5 shows that salt stress significantly reduces the shoot length of both wheat cultivars. But the application of two levels of proline i.e. 50mM and 100mM improve the shoot length of both cultivars under salt stress.100mM level of exogenous proline was more efficient than other levels.

Plant Dry Weight (g): Analysis of variance (ANOVA) of data presented in Fig. 6 shows that salt stress significantly reduces the plant dry weight of both wheat cultivars. But application of two levels of proline i.e. 50mM and 100mM was helpful in encountering the adverse effects of salt stress.50mM was more beneficial than 100mM in both cultivars.

Chlorophyll a: ANOVA Fig. 7 shows that salt stress reduces the chlorophyll ‘a’ contents of both wheat cultivars. However, exogenous application of two levels

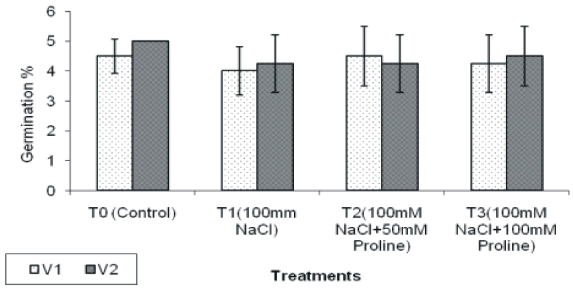

Fig. 1: Effect of Exogenous application of proline on germination % of two wheat cultivars under saline conditions.

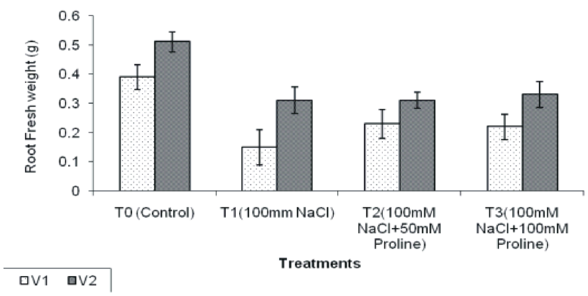

Fig. 2: Effect of Exogenous application of proline on root fresh weight of two wheat cultivars under saline conditions.

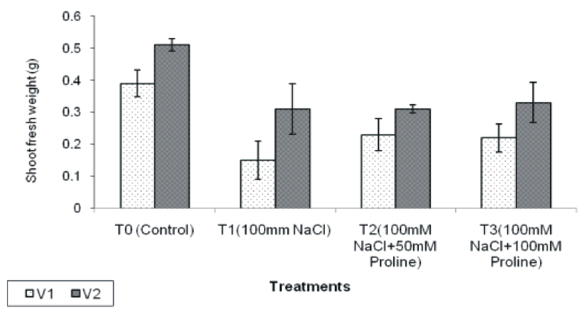

Fig. 3: Effect of Exogenous application of proline on shoot fresh weight of two wheat cultivars under saline conditions.

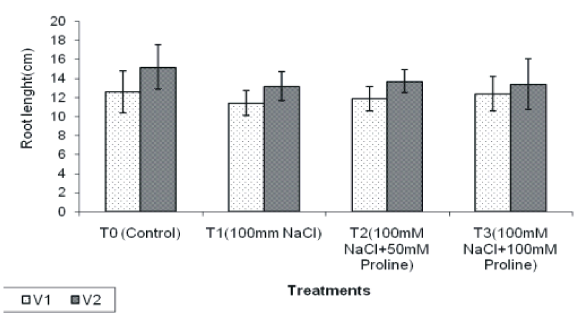

Fig. 4: Effect of Exogenous application of proline on root length of two wheat cultivars under saline conditions.

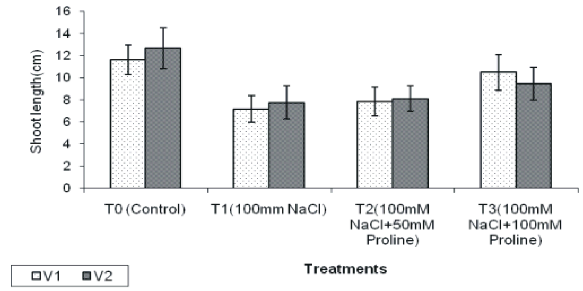

Fig. 5: Effect of Exogenous application of proline on shoot length of two wheat cultivars under saline conditions.

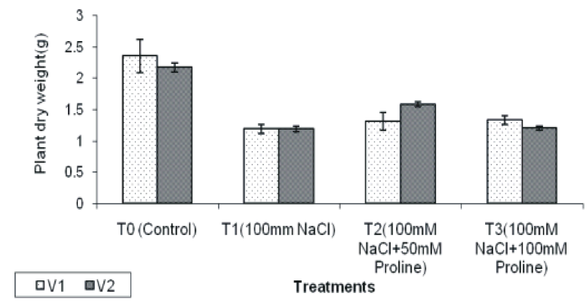

Fig. 6: Effect of Exogenous application of proline on plant dry weight of two wheat cultivars under saline conditions.

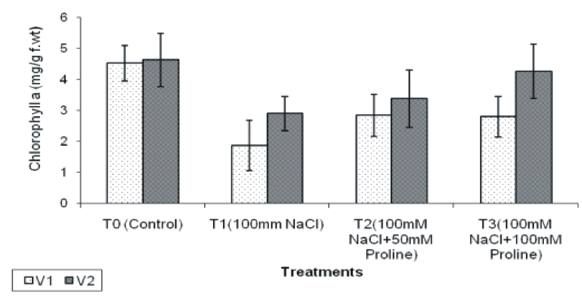

Fig. 7: Effect of Exogenous application of proline on chlorophyll ‘a’ of two wheat cultivars under saline conditions.

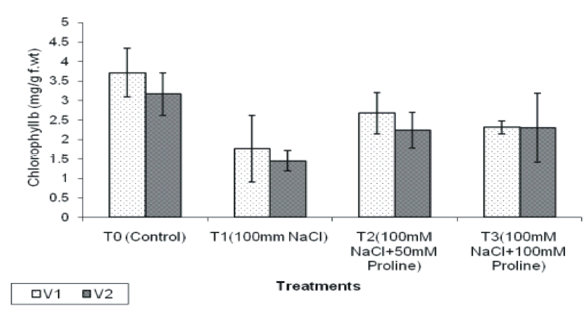

Fig. 8: Effect of Exogenous application of proline on chlorophyll b of two wheat cultivars under saline conditions.

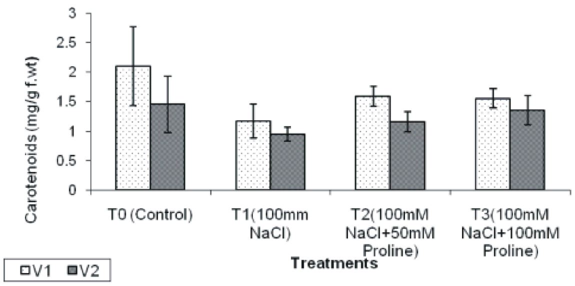

Fig. 9: Effect of Exogenous application of proline on carotenoids of two wheat cultivars under saline conditions.

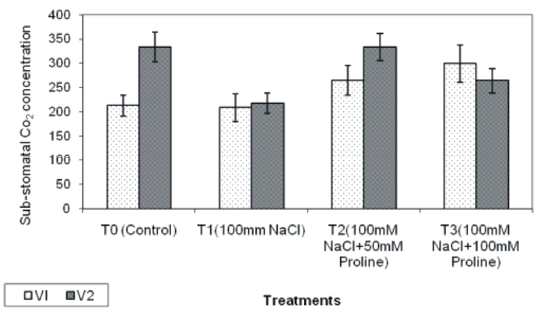

Fig. 10: Effect of Exogenous application of proline on Sub-Stomatal CO2 conc. of two wheat cultivars under saline conditions.

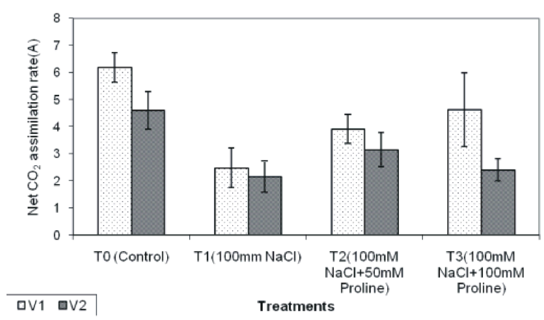

Fig. 11: Effect of Exogenous application of proline on net CO_2 assimilation rate of two wheat cultivars under saline conditions.

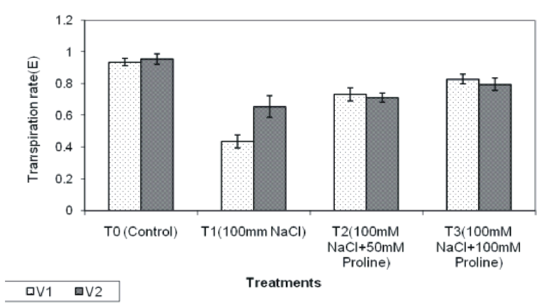

Fig. 12: Effect of Exogenous application of proline on transpiration rate of two wheat cultivars under saline conditions.

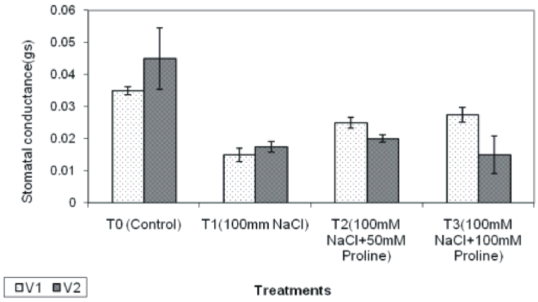

Fig. 13: Effect of Exogenous application of proline on Stomatal conductance of two wheat cultivars under saline conditions.

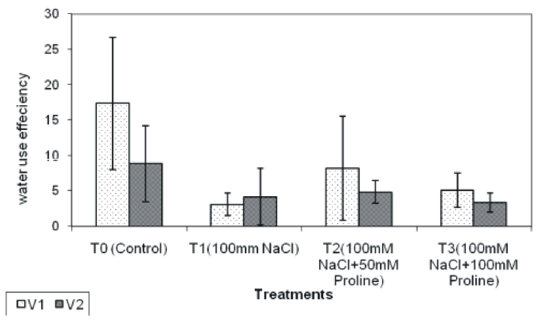

Fig. 14: Effect of Exogenous application of proline on water use efficiency of two wheat cultivars under saline conditions.

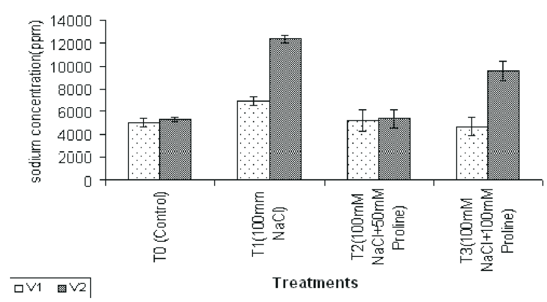

Fig. 15: Effect of Exogenous application of proline on sodium conc. of two wheat cultivars under saline conditions

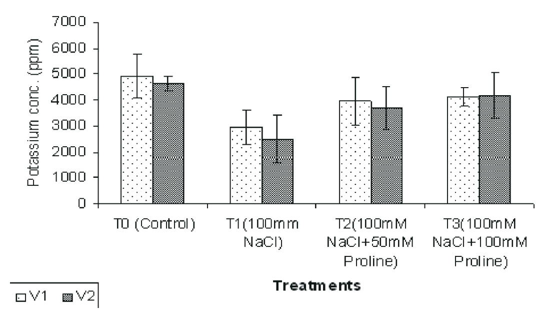

Fig. 16: Effect of Exogenous application of prolineon potassium conc.of two wheat cultivars under saline conditions.

of proline i.e. 50mM and 100mM showed improvement in this parameter of both cultivars.50mM was more effective in V1 than 100mM, whereas in V2 100mM was more effective.

Chlorophyll b: Analysis of variance (ANOVA) of data presented in Fig.8 shows that salt stress significantly reduces the chlorophyll b contents of both cultivars. Application of two levels of proline i.e. 50mM and 100mM show improvement in chlorophyll 'b' contents under salt stress.50mM was more effective than 100mM particularly in V1.

Carotenoids: Analysis of variance (ANOVA) of data presented in Fig. 9 shows that salt stress reduces the carotene contents of both cultivars. Carotenoids contents increased due to exogenous application of two levels of proline i.e. 50mM and 100mM under salt stress. Both varieties differ significantly in this attribute.

Sub-Stomatal CO_2 Concentration: ANOVA of data presented in Fig. 10 shows that salt stress reduced Sub-Stomatal CO_2 concentration of both cultivars. However, application of two levels of proline improved Sub-Stomatal CO_2 concentration. 50mM was more effective than 50mM in V2 whereas, 100mM showed more improvement in this gaseous exchange parameter in V1. Both varieties differ non-significantly for this parameter.

Net CO_2 Assimilation Rate: Analysis of variance (ANOVA) of data presented in Fig. 11 shows that salt stress significantly reduces the net CO2 assimilation rate of both cultivars. Foliar application of two levels of proline i.e. 50mM and 100mM showed improvement in net CO_2 assimilation rate under salt stress.Treatments, varieties and their interaction were significant.

Transpiration Rate: Analysis of variance (ANOVA) of data presented in Fig. 12 shows that salt stress reduces the transpiration rate of both wheat cultivars. However, proline mitigates the negative effects of salt stress in both cultivars. Both levels of proline i.e.50mM and 100mM were almost equally effective.

Stomatal Conductance: Analysis of variance (ANOVA) of data presented in Fig. 13 shows that salt stress reduces the Stomatal conductance of both wheat cultivars. V1 showed more improvement than V2 by the application of proline under saline environment.100mM was more effective than 50mM in V1, whereas 50mM was showed more improvement than 100mM in V2.

Water Use Efficiency: ANOVA Fig. 14 shows that salt stress significantly reduced the water use efficiency of both wheat cultivars. Both varieties differ significantly for this attribute. Application of two levels of proline i.e. 50mM and 100mM showed non-significant improvement water use efficiency of both cultivars.

Sodium Concentration: Analysis of variance (ANOVA) of data presented in Fig. 15 shows that salt stress significantly increases the sodium concentration of both wheat cultivars. Application of two levels of proline i.e. 50mM and 100mM significantly reduced the sodium concentration in both cultivars under salt stress.50mM was more effective in ameliorating the adverse effects of sodium than 100mM in both cultivars.

Potassium Concentration: Analysis of variance (ANOVA) of data presented in Fig.16 shows that salt stress reduced the potassium concentration in both wheat cultivars. Application of two levels of proline i.e. 50mM and 100mM showed improved significantly potassium concentration in both wheat cultivars under salt stress. Both levels of exogenous proline i.e. 50mM and 100mM were almost equally effective in both cultivars.

DISCUSSION

In this study salt stress significantly reduced the germination % of both wheat cultivars. These results are similar to earlier reported by [20] on barley, [21] on tomato and [22] on *Phaseolus*. Exogenous application of proline was found to be useful in alleviating the shocking effects of salt stress on seed germination of different plant species [23-27]. This reduction in seed germination and early growth i.e. seedling may be due to shortage of water and changes in the activities of certain enzyme due to intake of harmful ions [28, 29].

Salt stress reduced the plant biomass both wheat cultivars. These results are similar to earlier findings which was reported in rice [30], wheat [31] and sugar beat, cabbage, amaranth and pak-choi [32].This decrease in growth due to a reason of too much accumulation of Na^+ [33].

In the present study, photosynthetic pigments like chlorophyll ‘a’ and ‘b’ decreased in both wheat cultivars due to salt stress. Similar results were reported in different crops e.g. alfalfa [34], Sunflower [35] and wheat [36]. The decrease in Chlorophyll contents under saline conditions was also reported by [37-39]. Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions [40].

In present study gas exchange characteristics e.g. Sub-Stomatal CO_2 concentration, net CO_2 assimilation rate, stomatal conductance, transpiration rate and water use efficiency decreased in both wheat cultivars. Salt induced reduction in Sub-Stomatal CO_2 concentration occurs due to limited CO_2supply through stomata or mesophyll resistance, or efficiency of photosynthetic enzymes [41]. Exogenous application of compatible solutes e.g. proline or glycine betaine may reduce stomatal opening and reduce transpiration rate [42]. Sodium concentration increased in both wheat cultivars but K^+ concentration decreased in both wheat cultivars, however exogenous application of proline. These results similar to the earlier findings which were reported by [43]. There are various reasons for these results.[44] suggested that the association of induced increase in plant Na^+ with a decrease in K+ content may be due to the competition for sites through which influx of both ions occurs.

CONCLUSION

This experiment was conducted to determine that whether the foliar spray of proline induce the salt tolerance in wheat cultivars or not. On the basis of findings of the present studies it can be concluded that salt stress negatively affects the growth, morphology and physiology of wheat but the exogenous application of proline significantly ameliorates the harmful effects of salt.100mM Proline application was affective than 50mM produce the more positive results. As whole the proline induce the salt tolerance in both cultivars of wheat.

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