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Induction of Salt Tolerance in Two Cultivars of Sorghum (*Sorghum bicolor* L.) by Exogenous Application of Proline at Seedling Stage

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Abstract: This study was carried out to study the possibility of salt tolerance induction in two cultivars of sorghum by exogenous application of different levels (0, 50 *mM* and 100 *mM*) of proline. Salt treatments (100 *mM*) adversely affected the germination percentage, growth and chlorophyll contents of both cultivars. However, applications of proline alleviated the adverse effects of salt stress. However, high concentration of proline (100 *mM*) was not as much effective as compared to low concentration i.e. 50 *mM* in both cultivars.

Key words: Sorghum · Proline · Salt tolerance · Chlorophyll · Growth · Germination

INTRODUCTION

Sorghum is moderately salt tolerant [1]. Sorghum is a C4 grass that is well adapted to semi-arid and arid regions [2] where salinity is the major problem. Moreover this grain crop is the fifth most important cereal grown worldwide, due in large parts to its unusual tolerance to adverse environmental conditions [3-4].

Salt stress in arid and semi-arid regions is one of the major stresses that can severely limit plant growth and productivity [5-6]. Overcoming salt stress is a main issue in these regions to ensure agricultural sustainability and crop production [7]. Compatible solutes, such as proline, are known to accumulate under salt stress in many crops [8]. Their main role is probably to protect plant cells against the negative effects of salt by maintaining the osmotic balance, stabilizing subcellular structures, such as membranes and proteins and scavenging ROS [9]. Exogenous proline was found to be very effective in detoxifying H2O2 by enhancing the activities of catalase and peroxidase in tobacco under salt stress [10]. Salt stress brought about a reduction in the growth and protein content in *Pancratium maritimum* that was significantly increased by exogenous proline. In the leaves of *Pancratium maritimum*, salt stress up-regulated ubiquitin, a small protein which targets damaged proteins for degradation via the proteasome. However, salt stress resulted in a decrease in the amount of ubiquitin-congugates, particularly in the roots and this effect was reversed by peroxidase, but the activity of these enzymes was found to be significantly higher in the presence of exogenous proline. It was concluded that exogenous proline improves salt tolerance of plants by protecting the protein turnover machinery against stress damage and up-regulating stress protective proteins [10-12].

Therefore, this study was carried out to explore the efficacy of proline to minimize the adverse effects of salt stress on two cultivars of sorghum.

MATERIAL AND METHODS

Proline was applied exogenously through the rooting medium at germination stage to set up the levels of proline solute that could alleviate the adverse effects of salt stress on germination, growth and chlorophyll contents of sorghum. This experiment was conducted in growth chamber at the Department of Botany, University of Gujrat, Gujrat, Pakistan. Effect of varying concentrations (0, 50 *mM*, 100 *mM*) of proline under NaCl stress was assessed on germination, seedling growth and chlorophyll contents of two sorghum cultivars (Myco India, line brand) in this lab experiment. The experiment was arranged in a completely randomized design (CRD) with four replications. There were following four treatments.

T_0 = Distilled water
T_1 = NaCl (100 *mM*)
T_2 = NaCl (100 *mM*) + Proline (50 *mM*)
T_3 = NaCl (100 *mM*) + Proline (100 *mM*)

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Seeds of the two cultivars *Myco India* (V1) and *Line Brand* (V2) were surface sterilized in 5% sodium hypochlorite solution for 5 minutes before further experimentation. Ten seeds of each cultivar were placed in Petri plates (18 cm diameter) containing filter paper moistened with 10 ml of varying levels of proline (0, 50 *mM*, 100 *mM*) and then added in full strength Hoagland's nutrient solution containing 0 or 100 *mM* NaCl. The seeds were then allowed to germinate for 8 days in a growth room at 25°C. After 8-days of sowing, total germination percentage was recorded. After harvest, shoot and root of each seedling were separated and data for fresh biomass recorded. The chlorophyll *a, b* and total chlorophyll were determined according to the method of Arnon [13].

RESULTS

Salt stress (100 *mM*) reduced the biomass and chlorophyll contents of both sorghum cultivars. However, exogenous application of proline ameliorated the adverse effects of salt stress by keeping the growth, germination and pigmentation of sorghum to same level to some extent as compared to control.

Germination Percentage: The increase concentration (100 *mM*) of NaCl negatively affected the germination percentage of both cultivars (Fig. 1). Seed germination of *Line Brand* (V2) was more affected by salt stress than *Myco India* (V2). Exogenous application of proline ameliorated the effects of salt stress at germination stage.

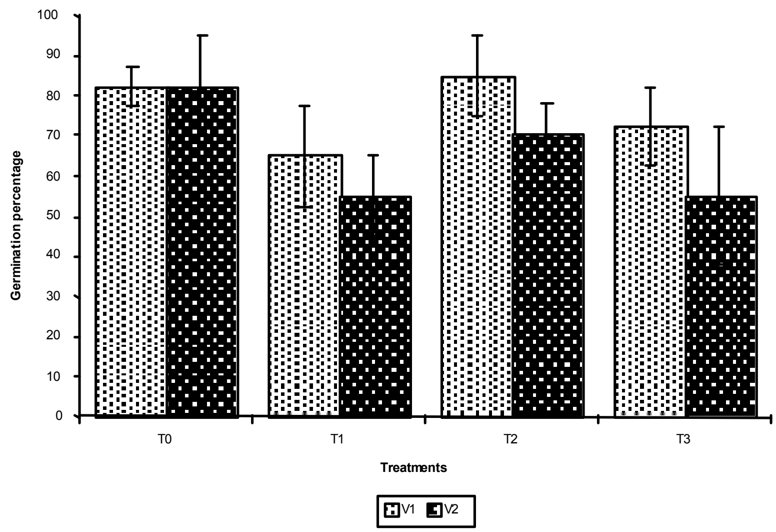

Fig. 1: Effect of exogenous application of proline on germination percentage of two sorghum cultivars under saline conditions

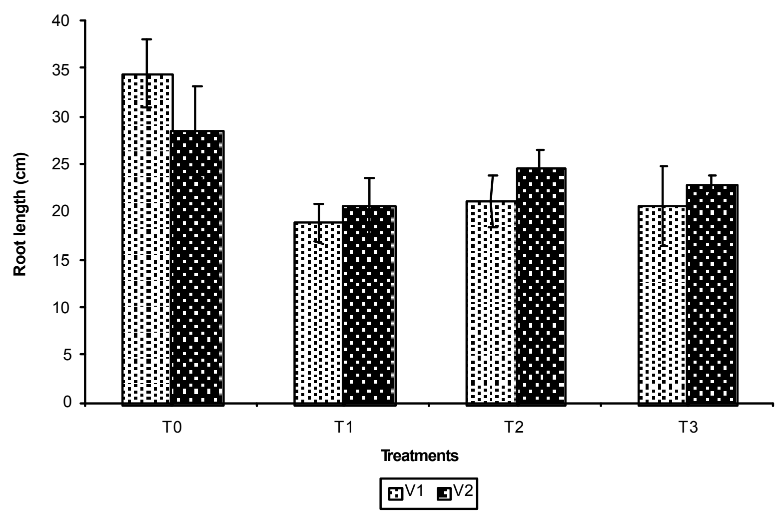

Fig. 2: Effect of exogenous application of proline on root length (cm) of two sorghum cultivars under saline conditions

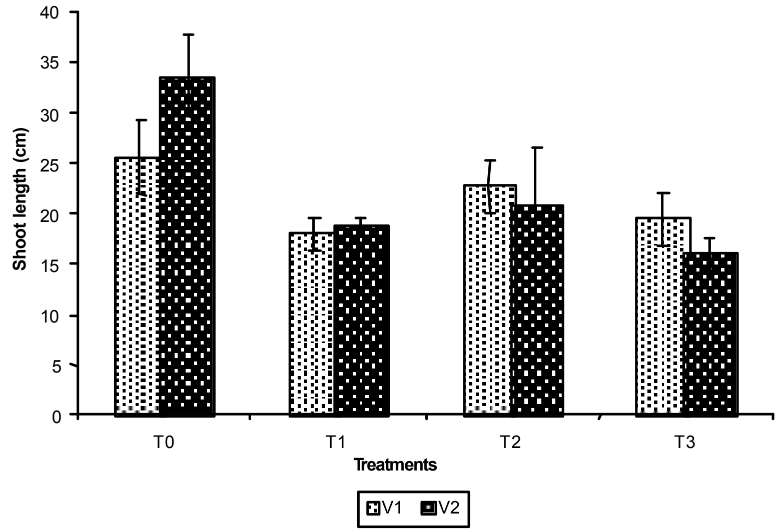

Fig. 3: Effect of exogenous application of proline on shoot length (cm) of two sorghum cultivars under saline conditions

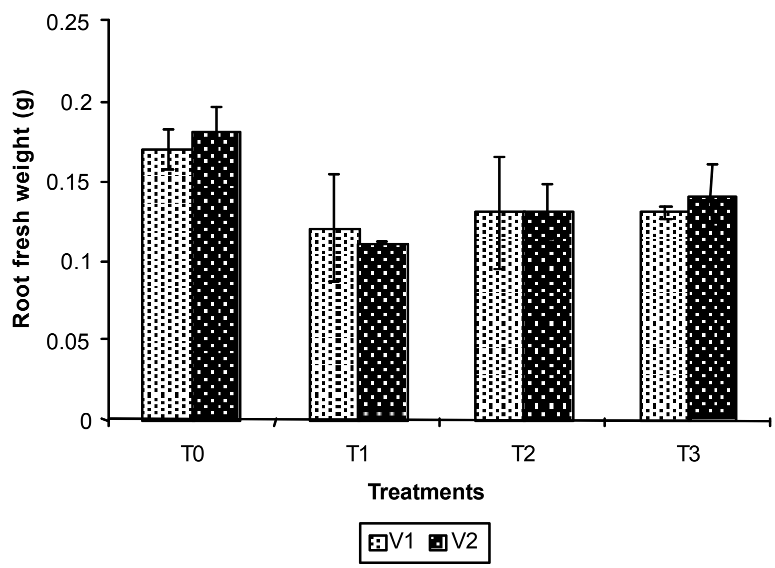

Fig. 4: Effect of exogenous application of proline on root length (cm) of two sorghum cultivars under saline conditions

Both levels of proline (50 *mM* and 100 *mM*) improved the germination in sorghum as it was present in control. However, proline level of 50 *mM* was more effective than that of 100 *mM*.

Root Length (cm): Root length was decreased plants treated with NaCl of both cultivars than that in non-salinized plants (Fig. 2). Exogenous of Application proline maintained the growth attribute in both cultivars. In both cultivars, application of 50 m*M* proline was more effective in keeping constant root fresh weight under saline conditions as comparison to control.

Shoot Length (cm): Applications of salt in the growth medium caused reduction in shoot length of both sorghum cultivars (Fig. 3). Under saline conditions 50 *mM* proline was more effective to reduce the effect of NaCl than 100 *mM* proline in both cultivars. Proline level, 50 *mM* showed 26.58% and 11.78% increased shoot length as compared to NaCl stresses plants. However, high concentration of proline (100 *mM*) was not so much effective as compared to low concentration i.e. 50 *mM* in both cultivars.

Root Fresh Weight (g): Data presented in Fig. 4 showed that NaCl in growth medium caused a drastic reduction

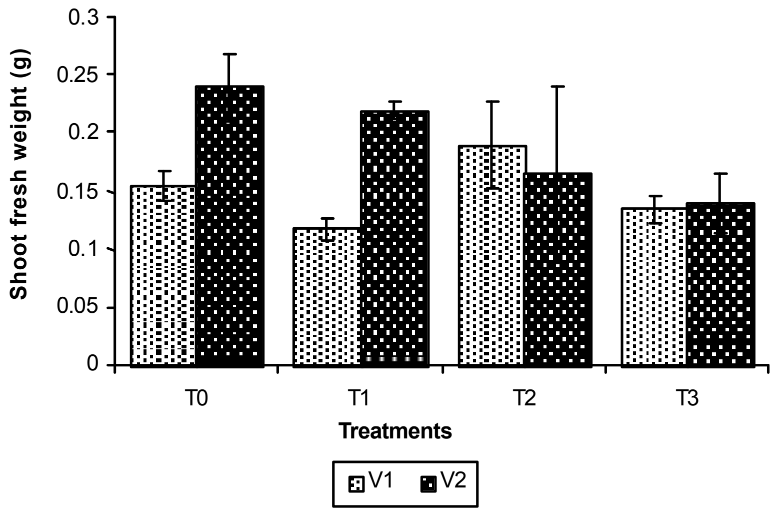

Fig. 5: Effect of exogenous application of proline on root length (cm) of two sorghum cultivars under saline conditions

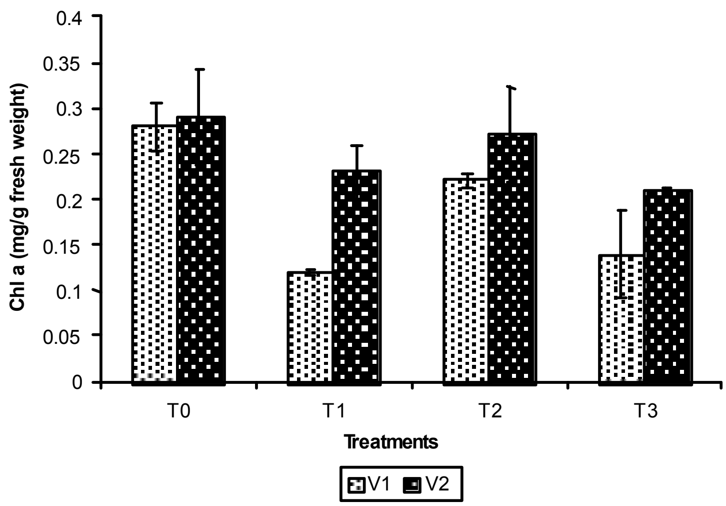

Fig. 6: Effect of exogenous application of proline on

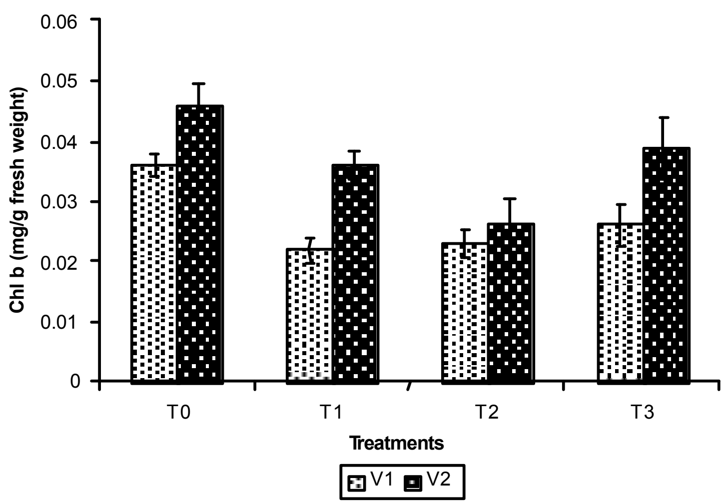

Fig. 7: Effect of exogenous application of proline on Chl 'a' of two sorghum cultivars under saline Chl 'b' of two sorghum cultivars under saline conditions conditions

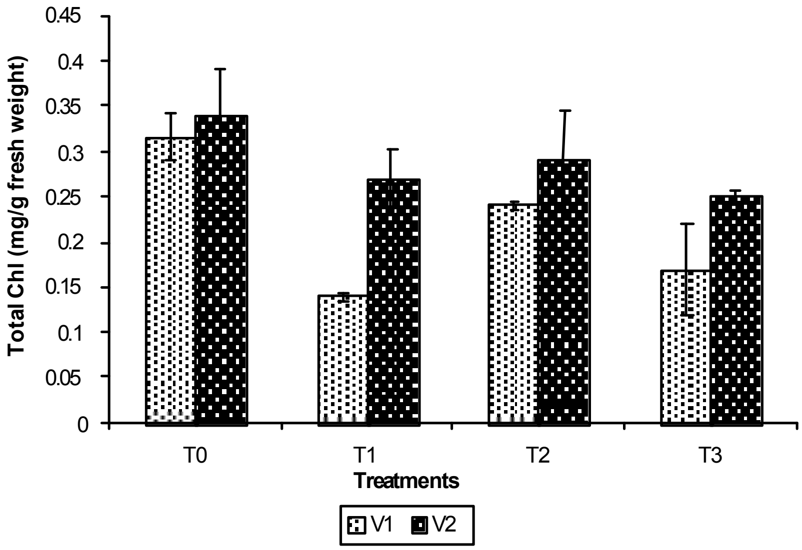

Fig. 8: Effect of exogenous application of proline on total chlorophyll contents of two sorghum cultivars under saline conditions

root fresh weight. There was no significant improvement in this attribute for V_1 by proline. However, cv. *Myco India* showed better response to exogenous applications of proline to improve growth under saline conditions.

Shoot Fresh Weight (g): Although salt stress significantly reduced the shoot fresh weight in both sorghum cultivars (Fig. 5) but exogenous applications of proline had a significant effect in maintaining the shoot fresh weight of both cultivars under NaCl. Under saline conditions, 50 *mM* proline was found to be more effective in enhancing growth parameters of both cultivars.

Chlorophyll Contents: Chlorophyll contents of both cultivars were significantly reduced by salt stress. However, exogenous applications of proline kept the leaf Chl contents constant. Chl 'a' was more significantly increased by 50 *mM* proline and 100 *mM* was more effective in maintaining the Chl 'b' contents constant in both cultivars under saline conditions (Figs. 6&7). Overall, 50 *mM* proline level was more effective in improving the total chlorophyll contents of leaf in both cultivars of sorghum (Fig. 8).

DISCUSSION

Proline, a common compatible solute accumulated in response to excessive salts in growth medium [8]. Excess of NaCl in growth medium restrict the availability of water to plant. This restriction results in dehydration of cytoplasm. This dehydration affects the metabolism of the cells and the functions of macromolecules and, ultimately reduce the of growth of plant [14-15].

Proline has role in protection of plant cell against adverse effects of salt by maintaining osmotic balance, stabilizing subcellular structures and scavenging reactive oxygen species [9]. In present study salt tolerance was induced by exogenous application of proline in sorghum and these findings of the present study are similar to some earlier studies in which it has been shown that exogenous application of proline alleviates the bad effects of salt stress on the growth and yield of different crops e.g. in groundnut [16], melon [17], *P. maritimum* [11] and tobacco BY-2 cells [10]. Exogenously applied proline was found to be effective in alleviating the adverse effects of salt stress on the seed germination of different plant species e.g. *Halogeton glomeratus, Salicornia utahensis* and *Triglochin maritima* butfailed in *Atriplex rosea, Salicornia rubra, Sarcobatus vermiculatus* and *Salsola iberica* [18-22]. Membranes become unstable in response to salt stress. This instability of membranes leads to reduction in chlorophyll reduction [23]. The decrease in chlorophyll contents under saline conditions is reported by [24-26]. Exogenous proline improved the tolerance of somatic embryos of celery to partial dehydration [27]. Exogenous proline was also effective for growth improvement of tobacco cell cultures under salt stress and this improvement was due to the role of proline as an osmoprotectant for enzymes and membranes against salt inhibition rather than as a compatible solute [28].

CONCLUSION

Salt treatments induced reduction in germination, growth and chlorophyll contents can be improved by exogenous application of proline. Exogenous application of 50 *mM* proline was more effective than 100 *mM* in sorghum. In conclusion, the adverse effects of salt stress on sorghum can be alleviated with the exogenous application of proline but there is a need to set up its levels for each crop.

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