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# Effect of Salinity on Seed Germination, Accumulation of Proline and Free Amino Acid in *Pennisetum glaucum* (L.) R. Br

Sonam Sneha, Anirudha Rishi, Amit Dadhich and Subhash Chandra
Department of Biotechnology and Food Sciences, Jayoti Vidyapeeth Women's University, Jaipur-303007, India

**Abstract:** Salinity is a major threat to agriculture, plants exhibits a variety of responses to salt stress that enable them to tolerate and survive in such conditions. Salinity affects physiological and biochemical processes in plants. A short term salt stress induced physiological and biochemical response were observed in *P. glaucum*. The experiment was conducted to understand the influence of salinity on seed germination, proline and free amino acid accumulation in *P. glaucum*. It was observed that as the salt concentration increased the germination percentage decreased as compared to control as well as the root/shoot length also decreased. This suggests that salinity greatly influences the germination as well as the plant growth. The levels of biochemical components proline and free amino acid were measured during the salt stressed condition. The 14 days old seedlings were subjected to 4 salt treatments (50, 100, 150 and 200 mM NaCl), free proline and free amino acids was calculated at 0, 12, 24, 48, 72 and 96th hour. Proline and free amino acid content in the salt stressed tissues increased with increase in salt concentration as well as with duration of salt stress. This result suggests that proline and free amino acid acids acts as compatible solutes in *P. glaucum* to protect the cellular macromolecules, maintain the osmotic balance and also scavenge the free radicals under salt stressed condition.

**Key words:** *P. glaucum*, proline, free amino acid, salt stress

## INTRODUCTION

*Pennisetum glaucum* (L.) R. Br. is an important crop of arid and semi-arid area. Pearl millet can grow in drought, low soil fertility and low pH but salinity greatly influences the growth, development and yield. The soil salinity problem can be solved by either two methods, first is to modify the soil conditions so that, it can support the agricultural purpose and second method is to exploit the genetic potential of plants for their adaptability to adverse environmental conditions. Many plants in response to environmental stress (drought, salinity, extreme temperature and oxidative stress) condition accumulate proline which is a proteinogenic amino acid and functions as a molecular chaperone to protect protein integrity and enhance the activities of different enzymes (Choudhary *et al*., 2005; Yoshiba *et al*., 1995; Yang *et al*., 2009; Abbaspour, 2012; Rajendrakumar *et al*., 1994; Kuznetsov and Shevyakova, 1997; Durgaprasad *et al*., 1996). Several experiments using transgenic plants or mutants have shown that proline metabolism has a complex effect on development and stress responses and proline accumulation is important for the tolerance to certain adverse environmental conditions (Hong *et al*., 2000; Mattioli *et al*., 2008; Szekely *et al*., 2008; Miller *et al*., 2009). It has been found that proline have capacity to quench singlet oxygen (ROS) (Szabados and Savoure, 2010). Proline protects and stabilizes ROS scavenging enzymes and inactivates alternative detoxification pathways (Matysik *et al*., 2002). Proline enhances the activities of glutathione-S-transferase, superoxide dismutase and catalase in tobacco under salt stress (Hoque *et al*., 2008). Proline catabolism in mitochondria is connected to oxidative respiration and administers energy to resumed growth after stress, proline oxidation regulates mitochondrial ROS levels and influences programmed cell death. The main objective of this study is to find out the short term response of proline accumulation and seed germination in *P. glaucum* under salt stressed condition.

## MATERIALS AND METHODS

**Collection of plant material:** The seeds of *Pennisetum glaucum* (L.) R.Br. cv. WCC-75 was collected from All India Coordinated Pearl Millet Improvement Project, Jodhpur, India.

**Corresponding Author:** Sonam Sneha, Department of Biotechnology and Food Sciences, Jayoti Vidyapeeth Women's University, Jaipur-303007, India

**Germination of seeds:** An experiment was conducted to study the germination and seedling growth of *Pennisetum glaucum* (L.) R. Br cv. WCC-75 under four levels of salt stress (50, 100,150 and 200 mM NaCl). In each petriplate 25 seeds were placed on Whatman filter paper after surface sterilized with 0.1% $HgCl_2$ and washed thoroughly with autoclaved distilled water. Salt solution of specific concentration (0, 50, 100, 150 and 200 mM) was added to the petriplates. Seed germination was recorded on the 7th day to calculate their germination percentage. The seeds were considered germinated only when the radical emerged from the coleorhizae and was at least 2 mm long.

**Salt stress treatment:** In other experiment seeds of *P. glaucum* were surface sterilized with 0.1% $HgCl_2$ for 3 min and washed thoroughly with autoclaved distilled water and then the seeds were grown in hydroponic condition.

Salt stress treatment was given to the 14 days old seedling. The salt treatment was given by adding known volume of the salt (NaCl) at concentrations of 50, 100, 150 and 200 mM. The hydroponic system was kept in 16 h photoperiod at temperature 25±2°C. The hydroponic solution was aerated externally to prevent anaerobic condition.

**Estimation of proline:** The proline content of the seedlings was estimated at 12, 24, 48, 72 and 96 h after the salt treatments (50, 100, 150 and 200 mM) following the modified protocol of Bates *et al.* (1973). For proline estimation fresh material was homogenized in 3% aqueous Sulphosalicylic acid. The homogenate was centrifuged at 6,000 rpm for 15 min at 4°C. In a test tube 2 mL aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin and incubated for 1 h at 100°C in water bath. The reaction was terminated by incubating the tubes in an ice bath and extracted with 10 mL of toluene. The chromatophore-containing toluene was then aspirated from the aqueous phase and its absorbance was noted at 520 nm using uv-visible spectrophotometer (Shimadzu, UV-1800) and toluene was taken as blank.

**Estimation of free amino acid:** The free amino acid content of the seedlings was estimated at 12, 24, 48, 72 and 96 h after the salt treatments (50, 100, 150 and 200 mM) following the modified protocol of Sugano *et al.* (1975). For free amino acid estimation the frozen plant material was homogenized with 70-80% ethanol, the homogenate was centrifuged at 5,000 rpm for 10 min, extraction was repeated 3-4 times and supernatant was combined. Above sample was taken in test tube and 1 mL of ninhydrin reagent and 0.2 M citrate buffer was added to it and boiled for 10 min and cooled under running water. Absorbance was noted at 570 nm using a uv- visible spectrophotometer (Shimadzu, UV-1800), total free amino acids were calculated form a standard curve prepared against glycine.

## RESULTS AND DISCUSSION

In present study, effect of sat stress on germination of *P. glaucum* has been investigated. As the salt concentration increases the rate of germination decreased significantly as shown in Fig. 1. The results observed are similar as observed in Bread wheat, Spinach and Fenugreek (Al-Saady *et al*., 2012; Keshavarzi *et al*., 2011; Akbarimoghaddam *et al*., 2011).A considerable decrease in root and shoot length was also observed with increasing salinity as shown in Fig. 2 and 3.The reduction in root/shoot length is due to excessive accumulation of salt in the cell wall which affects the metabolic activities

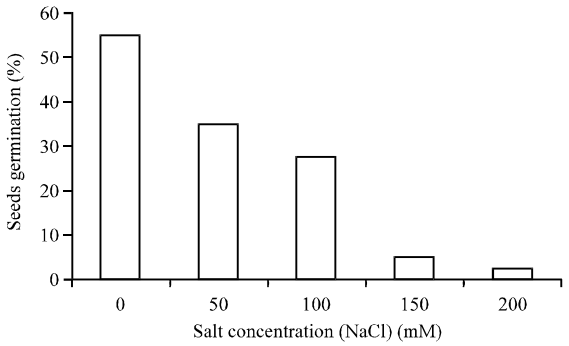


Fig. 1: Effect of Salt stress (NaCl) on germination efficiency under different salt concentration on 7 days after sowing. (The values are mean of 3 replicates)

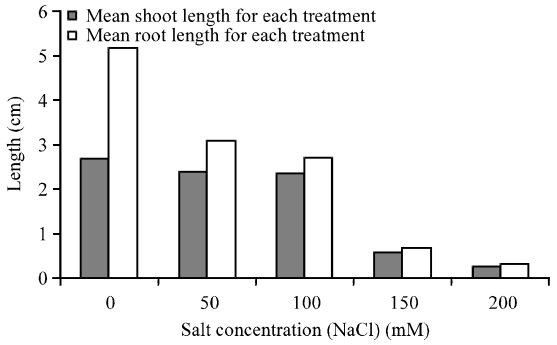


Fig. 2: Effect of Salt stress (NaCl) on mean shoot and root length under different salt concentration on 7 days after sowing. (The values are mean of 3 replicates)

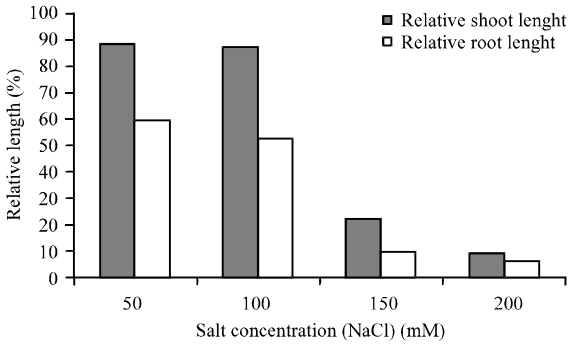


Fig. 3: Effect of Salt stress (NaCl) on relative shoot and root length under different salt concentration on 7 days after sowing. (The values are mean of 3 replicates)

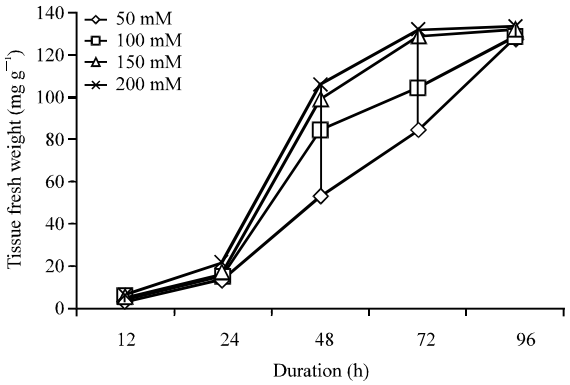


Fig. 4: Proline content (mg $g^{-1}$ fresh tissue weight) under salt stress in *P. glaucum* after exposure to 4 NaCl concentrations (50, 100,150 and 200 mM). (The values are mean of 3 replicates)

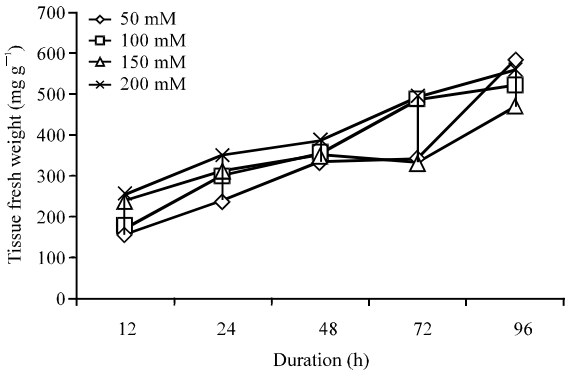


Fig. 5: Free amino acid content (µg $g^{-1}$ fresh tissue weight) under salt stress in *P. glaucum* after exposure to 4 NaCl concentrations (50, 100, 150 and 200 mM). (The values are mean of 3 replicates)

which further results in decreased cell enlargement. Decreased root/shoot length has also been reported in Wheat, Barley, Spinach and Triticale under salt stress condition (Larik and Al-Saheal, 1986; Iqbal *et al*., 1998; Keshavarzi *et al*., 2011; Naseer *et al*., 2001). The reduction in growth parameters with increasing salt concentration is due to the limited supply of metabolites to growing tissue as the metabolic productivity is significantly reduced at high salt concentration which is either due to low water uptake or toxic effect of salt as reported by Waisel (1972) and Misra and Dwivedi (2004). Various studies have reported proline accumulation in plants under saline condition which is a primary defense response to maintain the osmotic pressure in cell (Desingh and Kanagaraj, 2007; Misra and Gupta, 2005; Mansour *et al*., 2005). Under non stressed condition the level of proline and free amino acid were low but as the salt concentration increased the concentration of proline and free amino acid increased. The effects of increasing level of NaCl salinity on proline contents in *P. glaucum* increased at all levels of salinity, after the sodium chloride treatment the proline and free amino acid content first increased at 12 h as compared to that of the control and after 12 h the proline as well as free amino acid concentration showed gradual increase at all the concentrations as shown in Fig. 4 and 5. Maximum concentration of proline (133.25 mg $gm^{-1}$ tissue fresh weight) and free amino acid (560 µg $gm^{-1}$ tissue fresh weight) was observed at 200 mM of NaCl at 96th h. It has been reported that in *Brassica juncea*, *Solanum tuberosum*, *Pistacia vera* L., *Saussurea amara*, *Cajanas cajan* grasses, *Oryza sativa*, *Morus alba* and *Sapindus trifolicates* L. that as the salt concentration increases the proline content also increases (Bhamburdekar and Chavan, 2011; Madan *et al*., 1995; Rahnama and Ebrahimzadeh 2004; Wang *et al*., 2011; Mane *et al*., 2011; Unnikrishnan *et al*., 1991; Harinasut *et al*., 2000). Significant increase in proline content under salt stress is result of either due to breakdown of proline rich protein or de novo synthesis of proline or inhibition of activity of Proline dehydrogenase and Proline oxidase (Tewari and Singh, 1991; Misra and Gupta, 2006). Free amino acid accumulated in *P. glaucum* under different salt concentration suggesting they might play an important role in osmoregulation along with proline. The increased concentration of proline and free amino acid in *P. glaucum* showed a positive correlation with increasing salt concentration which maintains stabilization and osmoregulation of proteins and other macromolecules.

## CONCLUSION

From the experiments it is concluded that salinity greatly affects the morphology of plants. The seeds germination efficiency is inversely proportional to salt

concentration. The germination was also delayed in salt solution treated seeds. The shoot/root length was also affected by the salt concentration. The accumulation of osmolytes/osmoprotectants (proline and free amino acid) in *P. glaucum* suggests that it helps in osmoregulation of macromolecules in cells undress salt stressed condition. The results are similar to other studies conducted on different plants.

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