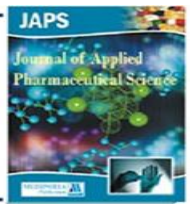
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 29-08-2011
Revised on: 15-09-2011
Accepted on: 26-09-2011

Salt stress tolerance and stress proteins in pearl millet *(Pennisetum glaucum (L.) R. Br.)*

R. Johnsi Rani

R. Johnsi Rani
Department of Chemistry,
Holy Cross College,
Tiruchirappalli, Tamil Nadu, India

ABSTRACT

Salt stress as a major adverse factor can lower leaf water potential, leading to reduced torgor and some other responses, and ultimately lower crop productivity in arid and semi arid zone. Plant responses to salt stress have much in common. Salt stress reduces the ability of plants to take up water and this quickly causes reductions in growth rate. The initial reduction in shoot growth is probably due to salt effects. If excessive amounts of salt enter into the plant, salt will eventually rise to toxic levels and reduce the photosynthetic leaf area of the plant that cannot sustain growth. In order to understand the processes that give rise to tolerance of salt and to identify the salt stress proteins in the salt stress effect of on plant growth was studied using different salt solutions like Copper sulphate, Cadmium chloride and zinc sulphate with different concentrations like 200µM, 150µM, 100µM.

Key words: $CuSO_4.5H_2O$-Copper sulphate, $CdCl_2.H_2O$-Cadmium chloride, $ZnSO_4.7H_2O$-Zinc sulphate, growth, salt tolerance, pearl millet.

INTRODUCTION

One of the unique properties of living organisms is growth. It is a complex phenomenon and represents the end result of metabolic pathways bringing about an overall irreversible change. Growth is the final morphological expression of various metabolic activities taking place in the plant. Temperature and salinity are major factors which significantly affect plant productivity in arid and semi arid regions (Bray *et al.*, 2000). If plants exposed to high light intensity at very low temperature or under drought stress, development of photo oxidative damage and generation of reactive oxygen species is very common (Foyer *et al*., 1997). Exposure of plants to the abiotic stresses results in production of reactive oxygen species as byproducts, which damage the cellular components (Noctor and Foyer, 1998). Abiotic stress mediated gene expression is regulated via different transcription factors of which drought responsive element binding (DREB) proteins play an important role (Parimita *et al.*, 2007). When exposed to salt stress, leaves from dark brown wheat seedlings showed reduced accumulation of chlorophyll during irradiation (Abdulkader *et al*., 2007). The ability of induced systems to tolerate severe levels of stress signifies the importance of stress proteins (Uma *et al*., 1995). Salinity impairs seed germination, reduces nodule formation retards plant development and reduces crop yield (Greenway and Munns, 1980). Salinity is the process of accumulation of soluble slats, by which saline soils are produced. The composition of salts in large amounts mostly is calcium, sodium, Magnesium, chloride and sulphate ions and in relatively small amounts are potassium, carbonates, bicarbonates, borate and lithium salts (Zhu, 2001). Accumulation of these salts increases the osmotic pressure of the soil solution because of restricted water intake by plants(Cramer et al. 1999).Several reports appearing

For Correspondence:
Dr. R. Johnsi Rani
Email: r_jrani@yahoo.co.in

in the literature revealed that salinity causes many adverse effects on the morphology, anatomy and physiology of pearl millet (Hussain et al., 2010). For instance, percent germination, height, grain and straw yield of pearl millet decreased with increasing concentration of salinity (Hussain et al., 2008). When plants are exposed to salt stress, they adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly
(Hasegawa et al., 2000).

Pearl Millet is a member of the gramineae family and it is the staple food and fodder crop of millions of poor rural families in the hottest and driest dryad agricultural environments of India. Although grain and Stover of this crop are not commercially important commodities; as most are consumed in the homesteads where they are produced, crop losses are economically important. Indeed in some of the hottest, driest regions of India, pearl millet is the only cereal that can be grown and so plays a critical role in food security. In these harshest of environments, grains yields are severely limited by drought and disease (Fao and ICRISAT, 1996). The objective of the present study was to evaluate the growth rate on metal stress and to identify and characterized the salt stress proteins synthesized on metal stress in pearl millet.

MATERIALS AND METHODS

The seeds of pearl millet *[Pennisetum glaucum [L.] R. Br.]* Were obtained from Tamil Nadu Agricultural University, Coimbatore for the present investigation and were surface sterilized with 0.1% mercuric chloride and washed thoroughly with double distilled water and germinated on moistened Whatmann number-1 filter paper in Petri dishes for 72 hours maintained at 28^{o}C.

Salt Stress

The effect of salt stress on plant growth was studied using different salt solution concentrations like 200µM, 150µM, and 100µM-Copper Sulphate, Cadmium chloride and Zinc sulphate.

Protein Estimation

The protein sample of 100 µl mixed with 2.5ml of Bradford reagent which was prepared by dissolving 100 mg of coomassie blue G 250 into 50ml of 95% ethanol and 100ml of 85% orthophosphoric acid and it was allowed to stand for 5-10 minutes. Simultaneously to 0.1ml of standard solution (bovine serum albumin 0.1mg/ml) 2.5ml 0f Bradford reagent was added and kept for 5-10 minutes. The absorbance of the sample was measured at 595nm using the blank prepared.

Electrophoretic analysis

Non-denaturing, discontinues slab gel electrophoresis was carried out essentially according to the method of Davis (1964). SDS-PAGE was carried out according to Laemmli (1970), employing 10% resolving gel and 4%stacking gel.

RESULT AND DISCUSSION

Salt stress, and drought, is major ecological factors, which prevent crop plants from realizing their full genetic potential. Of the three, temperature is more pervasive and economically damaging. High temperature causes reduction in shoot dry mass, growth and net assimilation rates in a number of plants (Wahid et al., 2007). Similarly, salinity stress affects development processes such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set (Sairam and Tyagi, 2004). The growth of the seedlings of pearl millet on exposure to various concentrations of salt solution $CuSO_4.5H_2O$, $CdCl_2.H_2O$ and $ZnSO_4.7H_2O$ for the time duration of 3 days respectively are determined. The effects of different bath way solutions ($CuSO_4.5H_2O$, $CdCl_2.H_2O$ and $ZnSO_4.7H_2O$) of concentrations 200µM, 150µM, and 100µM showed a marked effect in growth. The effect of sudden verses progressive exposure to salt stress at the seedling stage was investigated in pearl millet differing in their mean level of salt and drought resistance. The results were shown in table 1, 2 and 3.

Table-1 : Effect of salt stress on pearl millet in $CuSO_4.5H_2O$.

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	9.23±0.3670
$CuSO_4.5H_2O$	100µM	3 days	5.02±0.1720
	150µM	3 days	4.10±0.3640
	200µM	3 days	2.98±0.7050

Table-2 Effect of salt stress on pearl millet in $CdCl_2.H_2O$.

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	9.23±0.3670
$CdCl_2.H_2O$	100µM	3 days	5.33±0.4359
	150µM	3 days	4.92±0.4723
	200µM	3 days	3.52±0.4621

Table-3 Effect of salt stress on pearl millet in $ZnSO_4.7H_2O$.

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	9.23±0.3670
$ZnSO_4.7H_2O$	100µM	3 days	5.78±0.5231
	150µM	3 days	4.92±0.4320
	200µM	3 days	4.02±0.1420

Influence of salt stress on protein profile of pearl millet seedlings

$CuSO_4.5H_2O$

The protein extracted from the treatment groups of pearl millet seedlings got resolved into a number of bands in 12.5% SDS-PAGE in the regions of molecular weight ranging from 100 to 10 kDa. The untreated seedlings showed greater intensity at 40

kDa figure (1) Lane (3). There is a high intensity band at 35 kDa. At 100 µM $CuSO_4.5H_2O$ (figure 1 Lane 1) there were an appearance of number of polypeptides at 20, 19, 18 and 17 kDa regions. The polypeptide at 46 kDa is of high intensity. The formation of low intensity polypeptides indicate there is protein degradation during metal stress and small polypeptides are formed. At 150 µM concentration there is a greater intensity of polypeptide at 40 kDa regions and also at regions of low intensity at 17-20 kDa region. At 200 µM concentrations, there were well defined bands at low intensities regions (Lane 4) and it is also observed that there was appearance of a new polypeptide at 50 kDa region in all treatment groups.

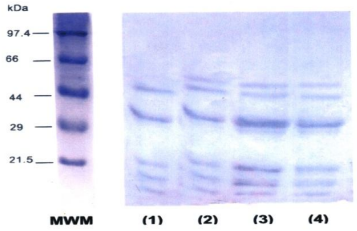


Fig. 1 Effect of Salt Stress ($CuSO_4.5H_2O$) on protein profile of Pearl Millet seedlings in 12.5% SDS – PAGE (slab gel) stained in coomassie brilliant blue. Each line was loaded with 100 µg of protein.

MWM – Molecular Weight Marker proteins (Phosphorylase – 97.4 KDa, bovine serum albumin – 66.0 KDa, Ovalbumin – 44.0 KDa, carbonic anhydrase – 29.0 KDa and soybean trypsin inhibitor – 21.5 KDa).

Lane 1 – Untreated control seedlings (28°C)

Lane 2 – Treated 100 µM $CuSO_4.5H_2O$ seedlings (28°C – 3 days)

Lane 3 – Treated 150 µM $CuSO_4.5H_2O$ seedlings (28°C – 3 days)

Lane 4 – Treated 200 µM $CuSO_4.5H_2O$ seedlings (28°C – 3 days)

$CdCl_2.H_2O$

The polypeptides resolved at 12.5% SDS-PAGE six polypeptides were clearly visualized in all the treatment groups (figure 2, Lane 1-4). These polypeptides had apparent molecular weights of 31, 44 and 47 kDa. The protein profile of the treatment groups revealed appearance of new polypeptide at 47 kDa region and at 20, 17, 18 kDa regions. Treatment at 200 µM (Lane 4) concentration there is a greater intensity of polypeptide at 31 kDa region. Non occurrence of all the polypeptides was observed at 62-97.4 kDa regions.

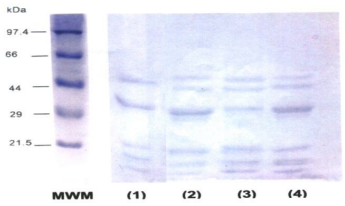


Fig. 2 Effect of Salt Stress ($CdCl_2.H_2O$) on protein profile of Pearl Millet seedlings in 12.5% SDS – PAGE (slab gel) stained in coomassie brilliant blue. Each line was loaded with 100 µg of protein.

MWM – Molecular Weight Marker proteins (Phosphorylase – 97.4 KDa, bovine serum albumin – 66.0 KDa, Ovalbumin – 44.0 KDa, carbonic anhydrase – 29.0 KDa and soybean trypsin inhibitor – 21.5 KDa).

Lane 1 – Untreated control seedlings (28°C)

Lane 2 – Treated 100 µM $CdCl_2.H_2O$ seedlings (28°C – 3 days)

Lane 3 – Treated 150 µM $CdCl_2.H_2O$ seedlings (28°C – 3 days)

Lane 4 – Treated 200 µM $CdCl_2.H_2O$ seedlings (28°C – 3 days)

$ZnSO_4.7H_2O$

Figure 3 Lane 1-3 showed the various polypeptides of treated seedlings. The polypeptide at 29 kDa was disappeared in all the treatment groups. The polypeptides at regions below 29 kDa were of high intensity. At 200 µM ther was an appearance of a new polypeptide at 50 kDa at 68 kDa regions.

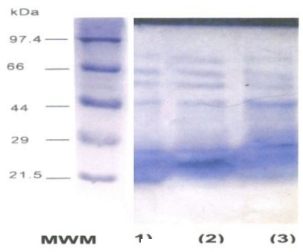


Fig 3 Effect of Salt Stress ($ZnSO_4.7H_2O$) on protein profile of Pearl Millet seedlings in 12.5% SDS – PAGE (slab gel) stained in coomassie brilliant blue. Each line was loaded with 100 µg of protein.

MWM – Molecular Weight Marker proteins (Phosphorylase – 97.4 KDa, bovine serum albumin – 66.0 KDa, Ovalbumin – 44.0 KDa, carbonic anhydrase – 29.0 KDa and soybean trypsin inhibitor – 21.5 KDa).

Lane1 – untreated control seedlings (28°C)

Lane2 – Treated 100µM $ZnSO_4.7H_2O$ Seedlings (28°C-3 days)

Lane3 – Treated 150µM $ZnSO_4.7H_2O$ Seedlings (28°C-3 days)

Lane4 – Treated 200µM $ZnSO_4.7H_2O$ Seedlings (28°C-3 days)

CONCLUSION

The above study reveals that salt stress on seeds degrades protein. The breaking up of polypeptides results in appearance of new smaller peptides. Plants have a multitude of mechanisms which help them to survive and propagate under salt stress. Salt stress proteins are believed to prevent protein denaturation. Repair of salt damaged/denatured proteins are essential for both survival and recovery from salt stress. Pretreatment of seeds could enhance salt tolerance in grains. Enhanced salt tolerance across different concentration limits can be exploited for extending cultivation of Pearl Millet beyond traditional areas where these varieties are being grown.

REFERENCES

Abdulkader, Amal F., Aronsson, Henrik, Sundqvist, Christer (2007). High salt stress in wheat leaves causes retardation of chlorophyll accumulation due to a limited rate of protochlorophyllide formation. Physiologia plantarum vol.130:1:157-166.

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses, in: W. Gruissem, B.Buchannan, R. Jones (Eds.), Biochemistry and Molecular Biology of Plants, American Society of Plant Biologists, Rockville, MD, pp. 158–1249.

Cramer GR, Basset RA, Seemann JR. Salinity calcium interaction on root growth and osmotic adjustment of two corn cultivars differencing in salt tolerance. J. Plant Nutr. 1999;13(11): 1453-1462.

Davis BJ. Disc gel electrophoresis II; Method and application to human serum proteins. Ann. Ny. Acad. Sc. 1964;121: 404-427.

FAO and ICRISAT (1996). The world sorghum and millet economies: Facis, trends and outlook. Food and Agricultural Organization of the United Nations: Rome, Italy and International Crops Research

Institute for Semi Arid Tropics: Patanchera 502324, Andhra Pradesh, India.

Foyer C.H., H. Lopez Delgado, J.F. Dat and I.M. Scot. Hydrogen peroxide and glutathione associated mechanisms of acclamatory stress tolerance and signaling. Plant Physiology. 1997;100:241-254.

Greenway H. and Munns. Mechanism of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. 1980;31:149-190.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol. 2000;51: 463-499.

Hussain K, Ashraf M, Ashraf MY. Relationship between growth and ion relation in pearl millet (*Pennisetum glaucum* (L.) R. Br.) at different growth stages under salt stress. Afr. J. Plant Sci. 2008;2(3): 23-27.

Hussain K, Majeed A, Nawaz K, Nisar FK, Khan F, Afghan S and Ali K. Comparative study for salt stress among seed, root stock and direct regenerated violet (*Viola odorata* L.) seedlings in relation to growth, ion contents and enzyme activities. Afr. J. Biotechnol. 2010;9(14): 2108-2117.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of T4 bacteriophage. Nature. 1970;227: 680-685.

Noctor G, Foyer C. Ascorbate and glutathione: keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1998;49: 249–279.

Parimita Agarwal, Pradeep K. Agarwal, Suresh Nair, S.K. Sopory and M.K. Reddy. Stress inducible transcription factor from pennisetum glaucum is a phosphoprotein. Molecular genetics and genomics. 2007;277:189-198.

Sairam R K, and Tyagi A. Physiological and molecular biology of salinity stress tolerance in plants. *Curr, Sci*. 2004 ;(86): 407–420.

Uma S., Prasad T.G. and Udayakumar M. Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in finger millet. Annals of Botany. 1995;76:43-49.

Zhu JK. Plant salt tolerance trends. Plant Sci. 2001;6: 66–72.