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Research Article

SALT STRESS TOLERANCE AND STRESS PROTEINS IN WHEAT (*Tritium Aestivum* L.)

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ABSTRACT
One of the unique properties of living organisms is growth. Growth 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. Salt stress influences all these metabolic activities and hence influences growth also. Salt stress is caused by concentrations greater than the required for optimum growth of a typical crop plant. 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. Because of the importance of wheat in Indian Agricultural Economy and in order to understand the processes that give rise to tolerance of salt and to identify the salt stress proteins into salt stress effect on plant growth was studied using different salt solutions like copper sulphate, cadmium chloride and zinc sulphate with different concentrations like 200µM, 150 µM and100 µM
Keywords: $CuSO_4.5H_2O$- Copper sulphate, $CdCl_2.H_2O$- Cadmium sulphate, $ZnSO_4.7H_2O$- Zinc sulphate, salt tolerance, Wheat.

INTRODUCTION

Plants have to exploit their immediate environment to maximum effect. Their inability to move swiftly means that the best way of dealing with many stresses is through physiological or morphological changes. Abiotic stresses and ways to adapt to them are numerous and interlocked. When exposed to salt stress, leaves from dark- grown wheat seedlings showed reduced accumulation of chlorophyll during irradiation. 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). Plants living in temperate climates require tolerance to the seasonal advent of cold and salt (Ghoulam *et al*., 2002). The ability of induced systems to tolerate severe levels of stress signifies the importance of stress proteins (Uma *et al*., 1995).

Salinity is the process of accumulation of soluble salts by which saline soils are produced (Chen et al., 2001). The composition of salts in large amounts mostly is Ca, Na, $MgCl_2$, and SO_4^{2-} ions and relatively small amounts are potassium carbonates, bicarbonates, borates 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). Survival these stressful conditions depends on the plants ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa *et al*., 2000) (Fedina *et al.,*2009).

Wheat, a plant of *Tritium aestivum* L. family, and most widely cultivated food crop in the world. It may be grown in a variety of soils and climates, and its excellent storing and shipping qualities make it available to people almost everywhere. Wheat takes second place as a grain food in eastern Asia, where rice is the loading cereal (Aykrod and Joyce., 1970). Wheat is most familiar in the form of breads and pastries made from wheat flour, and as a breakfast cereal. Wheat grain is a staple food used to make flour for leavened, meat and steamed breads, cookies, cakes, pasta and noodles and for fermentation to make beer, whiskey, bio-fuel, starches, industrial alcohols and some kinds of paper. Wheat is planted to a limited extent as forage crop for livestock and the straw can be used as fodder for livestock or as a construction material for roofing thatch (Inglelt.G.E.,1974).

The objective of the present study was to evaluate the growth rate on the metal stress and to identify and characterized the salt stress proteins synthesized on metal stress in wheat.

MATERIALS AND METHODS

Certified seeds of wheat (*Tritium aestivum* L.) were obtained Tamil Nadu Agricultural University, Coimbatore for the present investigators 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 hrs maintained at 28° C

Salt Stress

The effect of salt stress on plant growth was studied using different salt solution concentrations like 200µM, 150µM, and 50µM-Copper Sulphate, Cadmium chloride and Zinc sulphate.

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 (Girija *et al*., 2002) (Parida *et al*., 2004). 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) (Gorham., 1987). The growth of the seedlings

of wheat 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, 100µM, and 50µM showed a marked effect in growth (Khan et al., 2000) (Kaya *et al*., 2001). The effect of sudden verses progressive exposure to salt stress at the seedling stage was investigated in wheat differing in their mean level of salt and drought resistance (Kerepesi *et al*.,2000). The results were shown in table 1, 2 and 3.

Influence of salt stress on protein profile of wheat seedlings

$CuSO_4.5H_2O$

The protein extracted from the treatment groups of wheat 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 29 kDa in all the three concentrations. At 50 µM $CuSO_4.5H_2O$ (figure 4 Lane 2) there were a disappearance of number of polypeptides at 31, 35, 38, 44 and 47 kDa regions and appearance of new polypeptides at the region 50, 39, 36.5kDa whereas in 100 µM $CuSO_4.5H_2O$ (figure 4 Lane 3) there was an appearance of low molecular weight polypeptides at 27.3, 25.6, 24.6, 22.9 kDa regions. At 200µM $CuSO_4.5H_2O$ (figure 4 Lane 4) there was an appearance of a new polypeptide at 30 kDa and all other polypeptides were disappeared (Parvaiz et al., 2008).

$CdCl_2.H_2O$

The polypeptides resolved at 12.5% SDS-PAGE six polypeptides were clearly visualized in all the untreated control of the wheat seedlings (figure 5, Lane 1). These polypeptides had apparent molecular weights of 29, 31, 35, 38, 44 and 47 kDa. The protein profile of the treatment groups revealed the disappearance of the above polypeptides and an appearance of a new polypeptide at 21kDa (figure 5, Lane 2). Treatment at 100 µM (figure 5, Lane 3) there were appearance of number of polypeptides at 27, 30 and 33 kDa region. Non occurrence of all the polypeptides and occurrence of high molecular weight poly peptides were observed at 62-72.3 kDa and some low molecular weight poly peptides at 25 and 23 kDa regions.

$ZnSO_4.7H_2O$

Figure 6 Lane 1 shows the various polypeptides of control (untreated 28^oC) seedlings. The polypeptide at 29 kDa was of high intensity. The polypeptides at other regions showed comparatively low intensities. Figure 6-Lane 2 represents the protein profile of the seedlings exposed to salt stress at 50µM $ZnSO_4.7H_2O$ for 3 hours. There was appearance of new polypeptide at 21 kDa. It clearly indicates the disappearance of high molecular polypeptide and appearance of low molecular polypeptide at 21 kDa. In figure 6-Lane 3, there is an appearance of new polypeptide at 46 kDa region.

At 200 µM there was an appearance of a new polypeptide at 40 kDa and 60 kDa regions (figure 6-Lane 4).

CONCLUSION

Salt stress is caused by concentrations greater than required for optimum growth of typical crop plant. Salt stress is an important factor affecting crop productivity. Its productivity decreases when crops are subjected to salt stress. 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 wheat beyond traditional areas where these varieties are being grown.

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Table-1: Effect of salt stress on wheat by $CuSO_4.5H_2O$

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	7.32±0.8643
$CuSO_4.5H_2O$	50µM	3 days	4.02±0.1920
	100µM	3 days	3.10±0.3670
	200µM	3 days	2.34±0.7050

Table-2: Effect of salt stress on wheat by $CdCl_2.H_2O$

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	7.32±0.8643
$CdCl_2.H_2O$	50µM	3 days	4.6±0.4359
	100µM	3 days	4.14±0.4827
	200µM	3 days	3.02±0.4764

Table-3: Effect of salt stress on wheat by $ZnSO_4.7H_2O$

Type	Concentration	Duration	Length (cm)
Standard in water	-	3 days	7.32±0.8643
$ZnSO_4.7H_2O$	50µM	3 days	4.6±0.5958
	100µM	3 days	3.9±0.8330
	200µM	3 days	3.6±0.1410

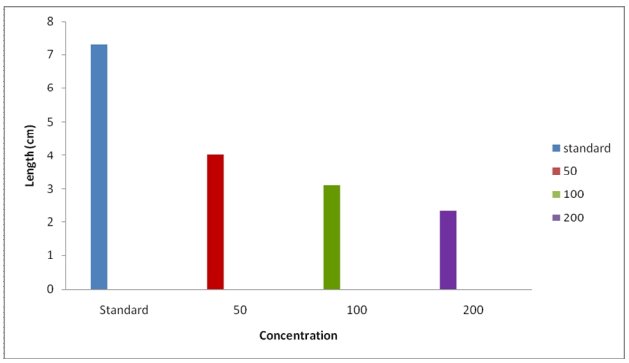


Fig.1

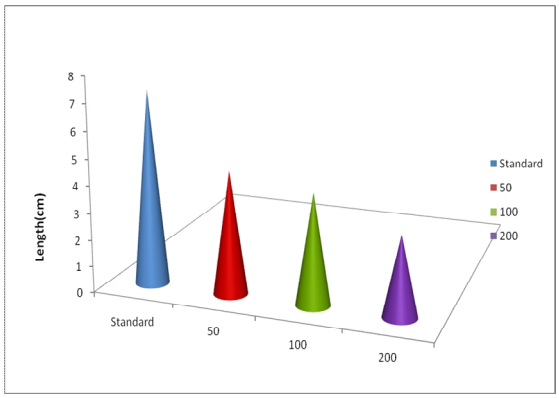


Fig.2

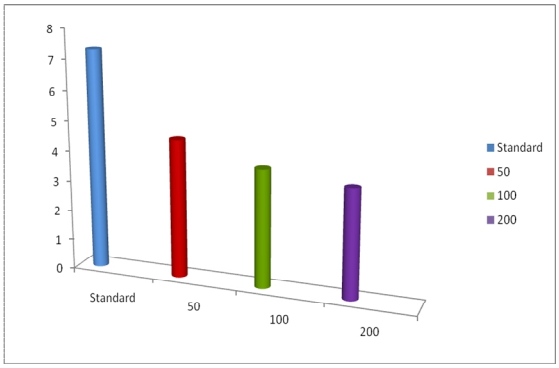


Fig.3

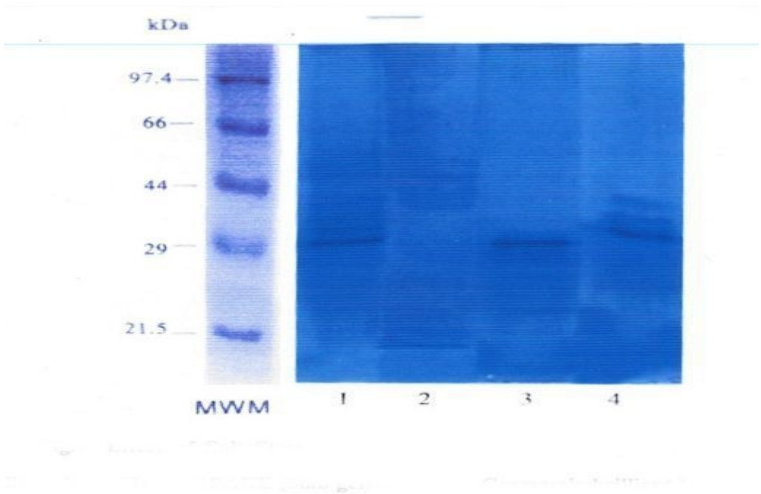
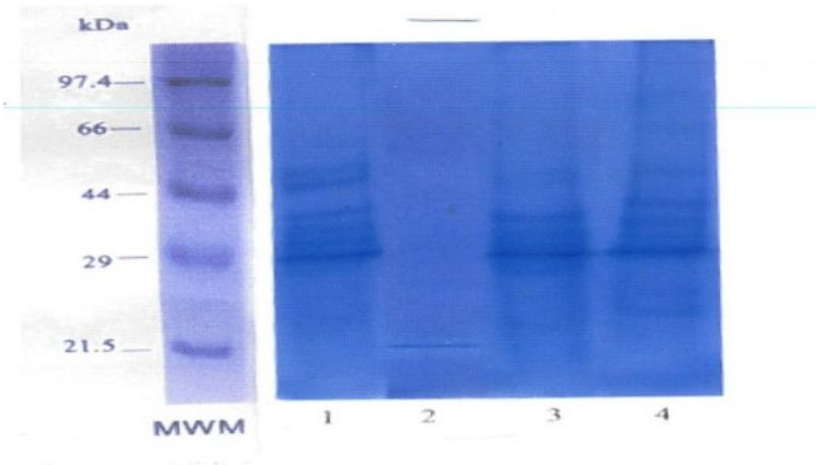


Fig.4: Effect of Salt stress ($CuSO_4.5H_2O$) on protein profile of Wheat seedlings in 12.5% SDS-PAGE (Slab gel) Stained in Coomassic brilliant blue. Each line was loaded with 100µg of protein.
MWM- Molecular weight marker proteins (phosphorlyase-97.4kDa, bovine serum albumin-66.0kDa, ovalbumin-44.0kDa, carbonic anhydrase-29.0kDa and soybean trypsin inhibitor-21.5kDa.
Lane1- Untreated control seedlings (28°C)
Lane2- treated 50µM $CuSO_4.5H_2O$ seedlings (28°C-3 hours)
Lane3- treated 100µM $CuSO_4.5H_2O$ seedlings (28°C-3 hours)
Lane4- treated 200µM $CuSO_4.5H_2O$ seedlings (28°C-3 hours)



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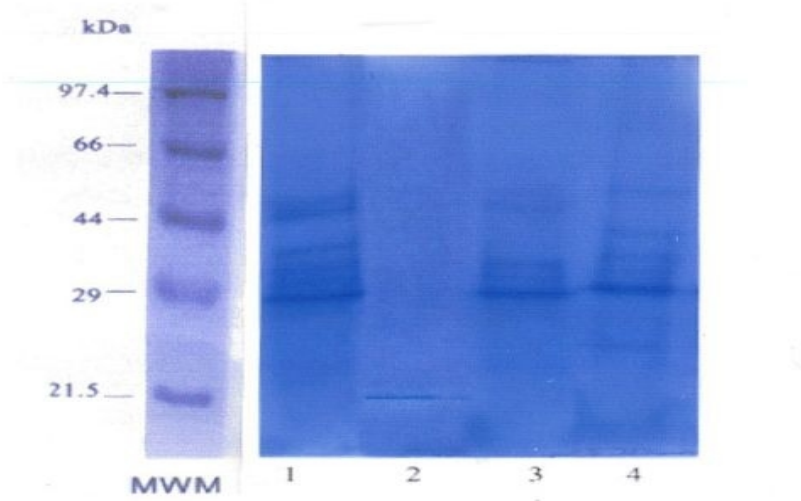

Fig.6: Effect of Salt stress ($ZnSO_4.7H_2O$) on protein profile of Wheat seedlings in 12.5% SDS-PAGE (Slab gel) Stained in Coomassic brilliant blue.
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Lane4- treated 200µM $ZnSO_4.7H_2O$ seedlings (28°C-3 hours)

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