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EFFECT OF MANNITOL INDUCED DROUGHT STRESS ON SEEDLING TRAITS AND PROTEIN PROFILE OF TWO WHEAT CULTIVARS

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ABSTRACT

The experiment was carried out at the laboratory of Botany Department, Kohat University of Science and Technology, Kohat in December 2012. Two wheat cultivars (Janbaz and Atta Habib) were subjected to different mannitol levels (0, 125, 250 and 500 mM) under room temperature in petridishes. The objectives of the study were to investigate the effect of mannitol induced drought stress on seedling traits and protein profile of two wheat cultivars. Analyses of variance revealed significant differences for shoot and root length for mannitol levels as well as for interaction. Genotypes, however, showed significant variability for root length only. It was evident from the data that increased levels of mannitol decreased the shoot and root lengths linearly. The longest shoot (8.57 and 9.57 cm) and root (9.73 and 10.13 cm) were recorded from control treatments while the shortest shoot (3.03 and 2.67 cm) and roots (2.37 and 2.67 cm) were observed in 500 mM treatment for Janbaz and Atta Habib, respectively.96.1 and 88.6 % of variation in shoot length and 72.3 and 71.5 % variation in root length was due to the mannitol induced drought stress for Janbaz and Atta Habib, respectively. SDS-PAGE showed no band variation between genotypes as well as among mannitol levels.

Keywords: Drouht stress, mannitol, protein, root length, shoot length, SDS-PAGE.

INTRODUCTION

The global water crisis seriously influences crop productivity particularly in most of the Asian countries where irrigated agriculture accounts for 90% of total diverted fresh water (Huaqi *et al*., 2002). The intensity of the response to water stress depends on the stress severity and its duration, as well as the plant developmental stage. Wheat crop needs water for the entire period of growth, but some stages are more vulnerable to water shortage and moisture stress during those critical stages may result in significant yield losses, noteworthy in this regard are the phases of crown root initiation, booting and early grain fill period (Anonymous, 2007). But it is considered that water stress is usually less detrimental to grain yield when occurring early in the crop cycle (Blum, 1996). The productivity of crop plants could be increased through studying the nature of adaptation of cereal cropsto water stress and by identifying the possible physiological markers for evolving the best-adapted and high yielding wheat varieties for areas affected by water stress (Mujtaba and Alam, 2002).

Proteins are compounds of fundamental importance for all functions in the cell (Dose, 1998). It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions (Vierstra, 1993: Heing *et al*., 2004).Under conditions of water deficit (dehydration) numerous processes are modified or impaired (Bradford and Hsiao, 1982). Water stress affects the protein levels of plants but the results of different authors are contradictory. Some authors show decreased protein levels under water stress (Pierre and Savoure, 1990: Roy-Macauley *et al*., 1992). Others found an absence of deleterious effects of drought on protein levels (Todd and Basler 1965). Increases in protein levels have also been reported (Singh andRai 1982). One way of plants to tolerate abiotic stresses to some degree is by biosynthesis of so called stress proteins.

To survive under a biotic stresses plants have developed various adaptive strategies that manifest in them morphological, physiological, developmental and molecular changes (Bohnert *et al*., 1995; Bray, 1997). Drought tolerance is a multifaceted phenomenon in which several characteristics influence plant success during vegetative period (Ingram and Bartels, 1996; Khan and Khan, 2010) and has been reported in almost all plants but its extent varies from species to species (Chaitanya *et al*., 2003). Under water stress plants induce the accumulation of compatible solutes such as soluble sugars, proline and proteins. This phenomenon is called osmotic adjustment. Osmotic adjustment is accepted as an effective way of drought resistance in many crops

(Kramer and Boyer, 1995). Accumulation of sugars in response to drought stress is also well documented (Al-Hakimi, 2006). Mannitol induced drought stress is widely used in many crop species. Seong *et al*. (1988) reported that the moisture content and the seedling length decreased when the mannitol concentration increased. Polyethylene glycol (PEG) is also well known for drought induction as an agent of membrane injury (Ahmad *et al*., 2007) but it also reduces oxygen dissolution in the culture medium. The toxic symptoms of plants grown under PEG treatments are more evident than those induced by mannitol in terms of iso-osmotic potential (Slama *et al*., 2007).

Several biochemical methods, mostly electrophoresis, have been used to estimate the genetic diversity among different plant species (Hamesand Richwood, 1990). Kakaei, (2009) compared seven genotypes of *Brassica napus*in gel SDS-PAGE which showed that protein pattern of the genotypes are significantly similar except the intensity of protein bands with molecular mass of 13 and 50 kDa. Among biochemical techniques, Sodium Dodecyl Sulphate Poly acrylamide Gel Electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm (Ahmad and Slinkard, 1992).Thus the objectives of the study were to check the response of studied wheat cultivars to mannitol induced drought stress and to determine the changes in protein profiles of both the cultivars in response to drought stress.

MATERIALS AND METHODS

Two wheat cultivars namely Janbaz and Atta Habib were procured from the molecular biology lab of the departmentof Plant Breeding and Genetics, The University of Agriculture, Peshawar. The samples were graded accordingly and were sterilized in 70 % alcohol. The petidishes were also autoclaved. The stock solution of mannitol was prepared by dissolving 18.217 gm of mannitol in 100 ml water for preparation of 1 M mannitol solution. The solution was further diluted to 125, 250 and 500 mM by adding 12.5, 25 and 50 ml of stock solution to 50 ml Hoagland's solution. In 125 and 250 mM dilutions, distilled water of 37.5 and 25 ml were also added. Thus, 100 ml of each dilution were prepared and were kept in flasks for watering the experimental material.

Protein Extraction and Separation through SDS-PAGE: Protein was extracted by grinding 100 mg lyophilized plant material under liquid nitrogen to fine powder in a mortar and pestle. The powder was homogenized with buffer containing 100 mMTrisHCl (pH 7.5), 1 % SDS and 0.1 % ß mercepto ethanol, centrifuged at 15,000 rpm for 10 minutes at 4ºC and supernatant was collected. Protein was quantified using Bradford method. 15µg protein from each variety was separated through 15% SDS-PAGE. Protein molecular weight marker was also loaded in the similar manner and electro poresed on a power supply of 50 mA at 500Vuntilthedyefront reached the bottom of the gel. Gels were removed from the plates and were shaken in staining solution (440 ml methanol, 60 ml acetic acid, 500 ml distilled water, 2.25 g Coomassie Brilliant Blue) for 2 h, then transferred to a distaining solution (200 ml methanol 50 ml acetic acid and 750 ml distilled water) and left for shaking for several hours until the protein bands appeared. The gels were observed under gel documentation system and photographed.

Layout and experimental design: Statistical design used was Completely Randomized Design (CRD) with two factors. Three replicates of each treatment were used to minimize the experimental error. Five seed of each variety were sown in each petri dish. The petri dishes had been watered with 2 ml of solutions on the alternate days for three weeks. The factors used were genotypes and mannitol with two (Janbaz and Atta Habib) and four (0, 125, 250 and 500 mM) levels, respectively.

Parameters and statistical analysis: Data were recorded on root and shoot length after 21 days of sowing date. One gram of shoot sample was taken for each level from both of the varieties and were subjected to SDS-PAGE for protein profile. The data were subjected to MSTATC for analysis of variance. Regression analysis was carried out using statistical package Prism while graphs were developed through MS Excel 2007.

RESULTS AND DISCUSSION

Shoot Length: Analysis of variance showed significant differences for mannitol stress and interaction between genotypes and mannitol stress (Table 4.1). Genotypes, however, showed no statistical variation for shoot length. No variation between genotypes for shoot length in this experiment is in line with the result of Simova-Stoilova *et al*. (2008) who concluded from their study that physiological response to drought was similar among the varieties

Mannitol effect on Shoot length is presented in figure 4.1. Shoot length decreased gradually with increasing stress. Highest values (8.57 and 9.57 cm) were recorded at control while lowest values (3.03 and 2.67 cm) were noted at 500 mMMannitol concentration for Janbaz and Atta Habib, respectively. Decrease in vigor of wheat genotypes due to drought stress have been reported previously by many researchers (Bohnert*et al*., 1995; Bray, 1997). Regression analysis revealed a variation of 96.1 and 88.6 % in the performance of Janbaz and Atta Habib, respectively due to mannitol induced stress. The rest of variation was the combined effect of genotypes and interaction.

Table 4.1. Mean squares for shoot and root length of two wheat cultivars

Sources	Degrees of freedom	Shoot Length	Root Length
Genotypes (G)	1	0.002^{NS}	0.304^{*}
Mannitol(S)	3	40.214^{**}	64.489^{**}
G x S	3	0.949^{**}	0.194^{*}
Error	16	0.144	0.047
CV (%)	----	6.59	4.18

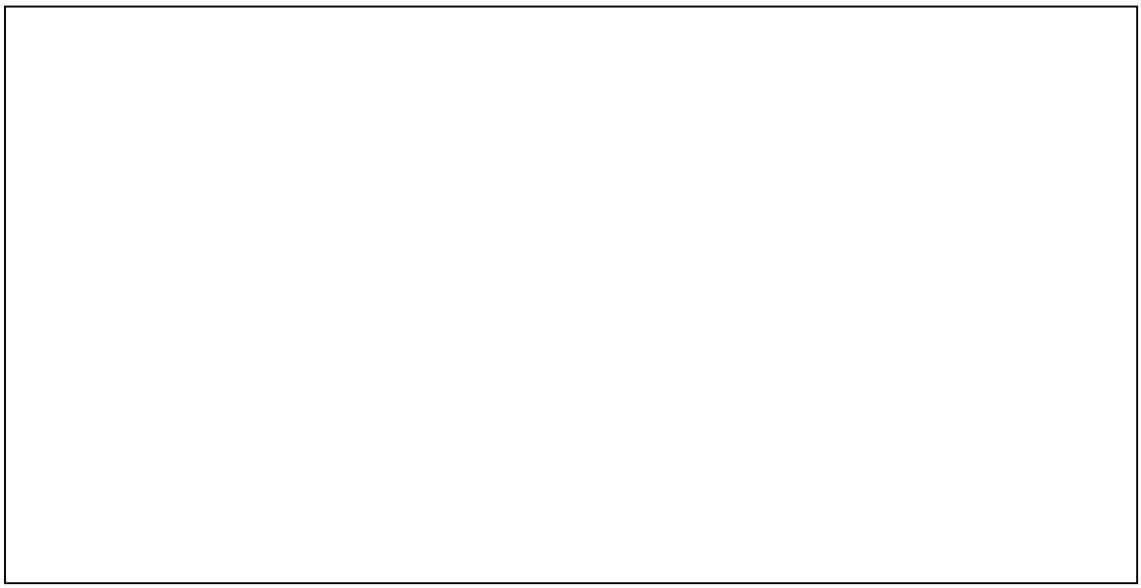

Figure 4.1. Effect of mannitol induced drought stress on shoot length of two wheat cultivars.

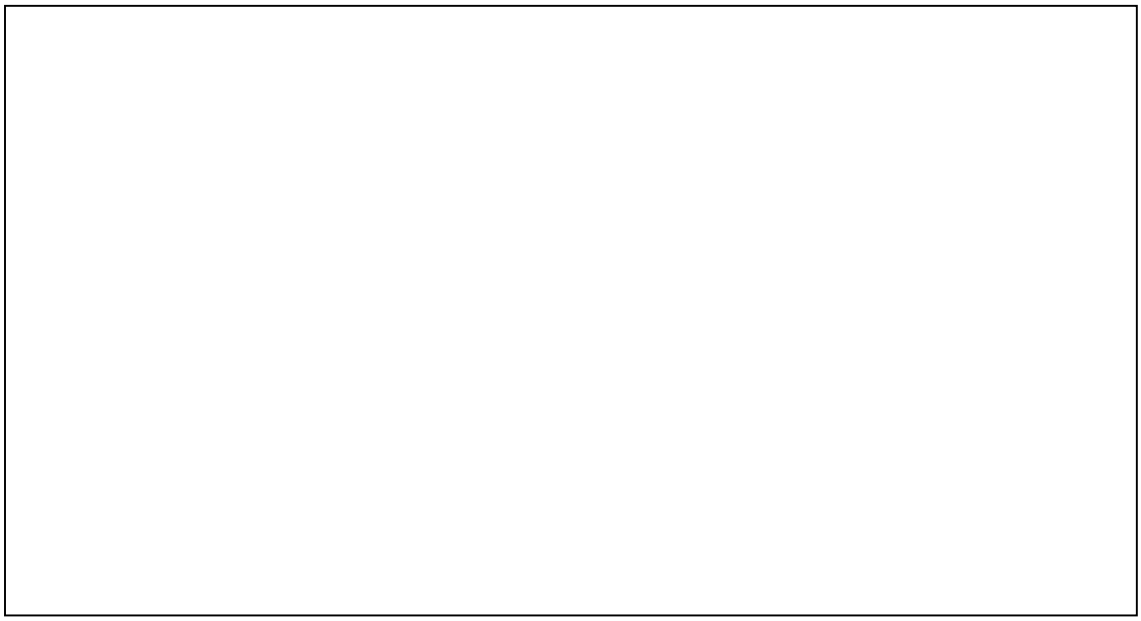

Figure 4.2.Regression of mannitolconcentration on shoot length of two wheat cultivars.

Root Length: Root systems have an important role to play in contributing to crop performance. Wheat roots extend more than 1 m in depth (Thorup-Kristensen *et al*. 2009), although root density is highest in the top few centimeters and optimal for interception of applied nutrients and rainfall. Improvement in yield require efficient uptake of water and nutrients that must be captured from the soil via the roots.

Analysis of variance revealed significant differences for root length between genotypes, for mannitol levels and their interaction as well (Table 4.1). The significant interaction revealed that the genotypes responded differently to stress condition. The effect of mannitol concentration on root length is shown in Figure 4.3. It is clear from the Figure that mannitol reduced the root lengths to a greater extent. Minimum root lengths (2.37 and 2.67 cm) were noted at 500 mM mannitol concentration as compared to maximum in control (9.73 and 10.13 cm) for Janbaz and Atta Habib, respectively. It is evident from the Figure that the root length dropped rapidly at low stress but as the stress increased the effect of the stress lowered down. This can be explained by the findings of different researchers (Kerepesi and Galiba, 2000; Alfoecea and Larher, 1994; Jones and Turner, 1980) who reported increase accumulation of carbohydrates in roots under drought stress condition. Thus, because of high carbohydrates accumulation at higher stress may have compensated the severe adverse effect of stress.

Regression analysis showed that 72.3 and 71.5 % of total variation in Janbaz and Atta Habib, respectively, were because of the mannitol induced drought stress. The rest of the variation was the cumulative effect of genotypes and interaction.

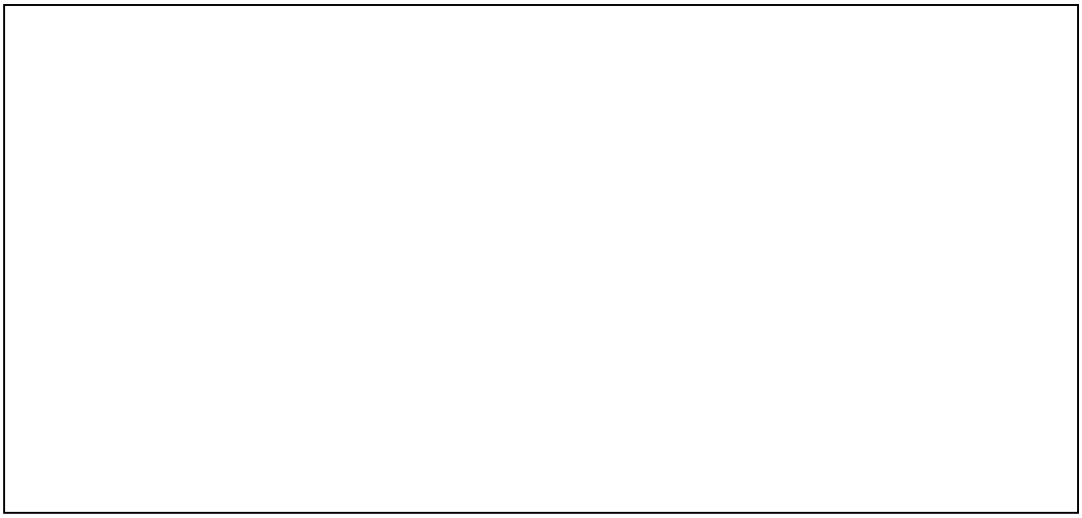

Figure 4.3. Effect of mannitol induced drought stress on root length of two wheatcultivars.

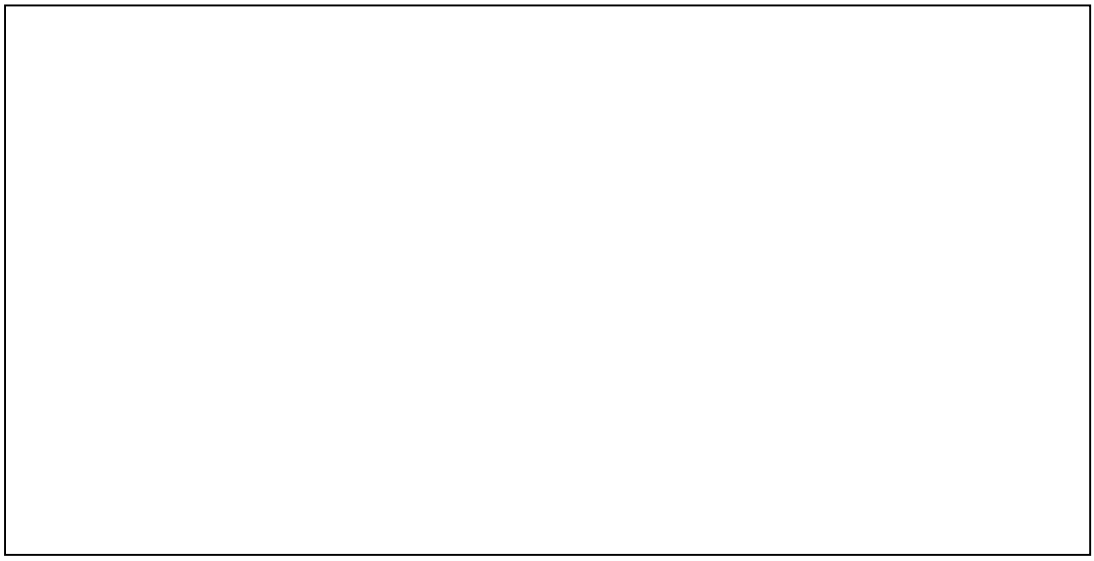

Figure 4.4.Regression of mannitolconcentration on root length of two wheat cultivars.

Protein Profile: Drought tolerance trait is related to protein expression. Some proteins are produced by plant only under drought stress condition and are called drought induced proteins. Others protein that are always present in tissues and do not affect due to drought are called constitutive proteins. The major of research on drought tolerance related proteins is focused on induced protein.

Figure 4.5 represent the protein profile of two wheat genotypes obtained from their leaf extract. There is no apparent variation in the protein profile of the two cultivars at any mannitol stress level. No variation in the protein profiles of both cultivars at any mannitol concentration level maybe because of the use of simple SDS-PAGE which categorize all seedling proteins into 20 bands only. The results are in line with those obtained by (Todd and Basler 1965) who concluded that drought impose no adverse effect on the protein profile of wheat genotypes. Kamal *et al*. 2010, however, identified 9 drought stress responsive proteins in genotype China-108, 15 in Yeonnon-78, 20 in Norin-61, and 26 in Kantou-107 during their study. The controversial results can be explained by the use of the techniques as they used 2D gel electrophoresis to investigate the protein profile, a more advanced technique or it may be because of the genetic makeup of the genotypes. The former of which looks to be a stronger explanation according to our view, as most of the genotypes respond to the drought stress but the extent is different in different genotypes. Another reason in support of the statement is given Table 4.1 which illustrates that response of the genotypes was different to the drought stress condition. This, obviously, shows that different genes were induced in the genotypes in response to drought stress but simple SDS-PAGE was unable to separate them.

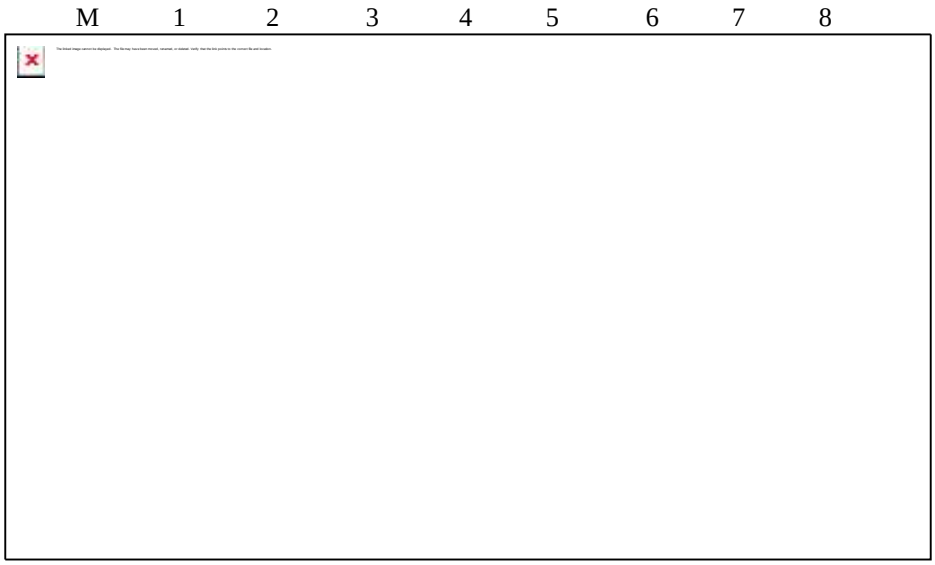

Figure 4.5. Protein profile from leaf extract of two wheat genotypes obtained through SDS-PAGE.
M= Markers
Janbaz (1= control, 2=125 mM, 3=250 mM and 4=500 mM)
Atta Habib(5= control, 6=125 mM, 7=250 mM and 8=500 mM)

Conclusions: It is concluded from the present studies that shoot and root length of both the genotypes was decreased as the level of the stress increased. Further, the effect of drought on shoot and root length was linear but on roots the effect was sever at first stress and later on the effect of stress was decreased which maybe because of the greater carbohydrates accumulation in the roots as compare to the shoots. Lastly, no variation in protein profile of the genotypes was observed by simple SDS-PAGE.

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