

STUDY OF POLYPEPTIDES INDUCED BY DROUGHT STRESS IN SOME LOCAL VARIETIES OF BARLEY FROM PAKISTAN

MADIHA SADIQ, REHANA ASGHAR, RAHMATULLAH QURESHI* AND AHMAD ALI

Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan

*Corresponding author's e-mail: rahmatullahq@yahoo.com

Abstract

The response against drought stress amongst cultivars of barley is explained on the basis of SDS-PAGE and western blot. In this study, four varieties of barley were collected from National Agriculture Research Center (NARC), Islamabad, Pakistan for the analysis of protein profile under drought stress. Morphological studies revealed that the most affected traits were germination percentage, leaf area, number of roots & leaves and fresh weight of plant at different stress levels. In all, the highest number of proteins was produced in response to stress in Frontier-87 at 5 and 10% PEG concentration compared with the rest of treatments. Although no significant difference was observed at protein level in Sanober-96, however number of proteins was higher at 10 and 15% PEG concentration. Immunoblot analysis of the total protein extracted at the seedling stage clearly showed that all the varieties were drought tolerant.

Introduction

Barley (*Hordeum vulgare* L.) is a fast growing, cool season crop that can be used as forage or as a cover crop to improve soil quality. It has very good heat and drought tolerance, making it is a valuable plant for arid and semiarid areas (Valenzuela & Smith, 2002). Numerous physiological and biochemical changes takes place in response to drought stress in various plant species. The alteration of protein synthesis is one of the most basic metabolically stimulated processes that may influence drought tolerance (Chandler & Robertson, 1994). Evidence is increasing in favors of a relationship between accumulation of drought induced proteins and Physiological adaptation to water limitations (Bray, 1993; Riccardi *et al.*, 1998). Close *et al.*, (1989) used the term DHN for these proteins for the first time and have since found widespread use. Although the term DHN was initially intended for only those protein which are induced against dehydration but now proteins are classified as DHNs based on sequence homology rather than after their expression characteristics in addition to being produced during the later stages of embryogenesis and in seeds. DHNs have been found to produce and accumulate in plants treated with ABA, salt, drought and low temperature.

A possible role of dehydrin is to bind with the ions accumulated under drought stress and also control solute concentration in the cytoplasm (Dure, 1993). Dehydrin also possess a cytoprotective role in macromolecule stabilization by binding water molecule to their hydrophilic surfaces, which reserves and prevents further denaturation of cellular protein (Close, 1996). To understand the contribution of dehydrins to stress tolerance, barley is an appropriate model plant since its genetic characterization is well advanced in particular for the localization of dehydrin genes (Choi *et al.*, 2000).

Previously Khan *et al.*, (2012) and Barozai *et al.*, (2012) carried out experiments on wheat and potato for seeking drought induced respectively. The present study was aimed to investigate morphological traits, such as root and shoot length of plant under drought stress, estimation of protein in plant tissue and the changes

caused by drought stress in the polypeptide composition of local varieties of barley by SDS-PAGE.

Materials and Methods

In order to carry out present study, seeds of four varieties viz., Sanober-96, Haider-93, Soorab-96 and Frontier-87 of Barley were collected from NARC, PGRI Islamabad during the month of May 2008. Seeds were surface sterilized in 20% sodium hypochlorite, 0.01% SDS for 10 minutes, washed three times with distilled water. Seeds were imbibed in Polyethylene glycol (PEG 6000) having concentrations 5%, 10%, 15%, 20%. The water potential of the solutions was measured by osmometer (Wescor). The seeds were germinated in glass trays of 12 X 6 X 2.5 inches on 2 layers of filter paper moistened with 7ml PEG solutions of variable osmolarities at 20°C for 10 hours light period and at 8°C for 14 hours dark period in an incubator. Germination percentage was determined. Dry weight of the seedlings was measured after drying at 72°C for 48 hrs. The length of root and shoot were measured. Growth of Root/shoot ratio was calculated. The leaf area was determined by using leaf area meter (CI-202 Area Meter). The tissue was ground in 1 ml of 50mM borate buffer, pH 9 containing 10µl of 100mM PMSF and kept for 1 hour at 4°C and then centrifuge at 14000rpm. After centrifugation supernatant was collected. The extracted crude proteins were recovered as clear supernatant.

Protein content from the samples was determined by a Protein-Dye Binding assay (Bradford) by using BSA as standard. Electrophoresis was carried out using discontinuous Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) having 12% (w/v) separating gel and 4.5% stacking gel.

While gel was running, transfer buffer (1L per transfer unit) was placed in the freezer to chill. Equilibrated complete gel is transfer buffer for 15 min. For immunoblot analysis, proteins were transferred electrophoretically from gels to nitrocellulose membrane in 25mM tris-HCl (pH 8.3), 192mM Glycine and 20% methanol using Mini transfer unit (Cleaver Scientific).

For analysis of antibody recognition of proteins, nitrocellulose sheets were blocked with 3% gelatin (w/v) in 1X tris buffered saline (TBS) incubated with goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma). Secondary antibody was detected using 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate.

Statistical Analysis: All the experimental studies carried out using CRD design (Steel *et al.*, 1997). All the statistical analysis carried out using software MSTAT-C. LSD analysis of each variety is performed individually to check the response of that variety to stress.

Result and Discussion

In the present study, plants when treated with control, 5% and 10% PEG treatment showed relatively better and fast growth than at 15 and 20% PEG treatments. When the plants were germinated at higher levels of PEG concentrations viz., 15 and 20% PEG, they showed slow growth and took relatively more time as compared to the other levels of stress. Germination of seeds in PEG

solutions during a period of 5 days caused a growth reduction of shoots of barley seedling, its root dry matter; however, the growth was increased with increasing concentration of the stressor (Leinhos *et al.*, 1996). Soorab-96 showed maximum shoot and root length at 5 and 10% PEG conc. than rest of treatments. It also showed increase number of roots as the level of PEG concentration raised. Soorab-96 and Frontier-87 showed the maximum number of roots at 15% PEG concentration (Table 1).

There was a significant reduction in the number of leaves at 15 and 20% PEG (Table 2). Water stress of 9 days caused a drastic decrease in leaf area and shoot length of both the cultivars. This was probably due to a decrease in cell enlargement (Hsiao, 1973). All the four varieties showed decrease in leaf area on increasing the PEG concentration. All the varieties showed maximum increase in fresh and dry weight at 10% PEG concentration. Water stress dry weight of both the plant parts was reduced to half of the control plants. This was in response to the internal water deficits, which impair the normal metabolic and physiological processes of plant (Anjum *et al.*, 2003).

Table 1. Effect of drought on root length (cm) in seedlings of different local cultivars of *Hordeum vulgare* L.

Treatment	Frontier-87	Sanober-96	Haider-93	Soorab-96
Control	11.833 A	15.667 BC	16.633 AB	16 A
5% PEG	10.167 A	10.167 D	20.833 A	18 A
10%PEG	11.283 A	20.667 A	12.333 BC	18 A
15%PEG	10.5 A	20 AB	11 C	6.833 B
20%PEG	11.333 A	12 CD	5.1 D	6.333 B

LSD value = 4.474, at alpha = 0.050: Coefficient of variation: 19.70%

Table 2. Effect of drought on Leaf area in seedlings of different local cultivars of *Hordeum vulgare* L.

Treatment	Frontier-87	Sanober-96	Haider-93	Soorab-96
Control	4.613 B	6.420 A	5.26 A	4.823 A
5% PEG	5.277 A	4.853 B	4.157 B	4.517 AB
10%PEG	5.170 AB	4.050 C	4.05 B	4.103 BC
15%PEG	4.933 AB	3.700 CD	3.7 BC	3.830 C
20%PEG	3.467 C	3.290 D	3.29 C	3.203 D

LSD value = 0.5950 at alpha = 0.050: Coefficient of variation: 8.47%

Protein concentration was estimated by using BSA standard curve. The 10% PEG concentration showed the maximum protein concentration than all other treatment levels. During the drought process, total soluble proteins of plant were separated by SDS-PAGE (Figs.1-3) and immunoblotted (Figs. 4-6). It was revealed that changes in protein profile occurred as a result of stress. Certain kinds of protein induced due to drought stress, while the others decrease their concentration or totally disappeared. Fig. 1 showed the protein profile of a variety of barley i.e. Haider-93, it was observed, that 130, 95, 71, 43,41 and 40 kDa are that kind of proteins which became disappear in other treatments with the progressive increase of PEG concentration. At 5% PEG concentration 55, 22, 28 and 30 kDa protein bands were induced and which were absent at 10% PEG concentration. At 15% PEG concentration, 34, 32 and 30 kDa protein was induced, whereas at 15% PEG concentration, this variety showed the maximum production of proteins i.e., 26, 28, 30 and 21 kDa proteins. But the 20% protein concentration

showed the lesser amount of protein as compared to the other which was not different from other stress levels. Close and Chandler (1990) detected a 25 kDa dehydrins in stressed wheat and barley seedlings along with faint bands between 18 and 21 kDa.

The protein profile of Frontier-87 under drought stress is shown in Fig. 2. The proteins of molecular wt. 55 and 53 kDa were induced at stress levels of 10, 15 and 20 % PEG concentration. 32 kDa was present only at 15% and 20% PEG concentration. Drought induced polypeptides have been observed in many studies (Riccardi *et al.*, 1998) and they were considered to play an important role in water stress tolerance.

The protein profile of Sanober-96 and Soorab-96 is shown in Fig. 3. The maximum number of proteins was induced at 10 and 15% PEG concentration in Soorab-96 with 130, 95 and 80kDa molecular weights. 26kDa protein was induced at all levels of treatment including control. The protein of molecular weight 40 and 22 kDa is present only at control level and absent at other treatment levels.

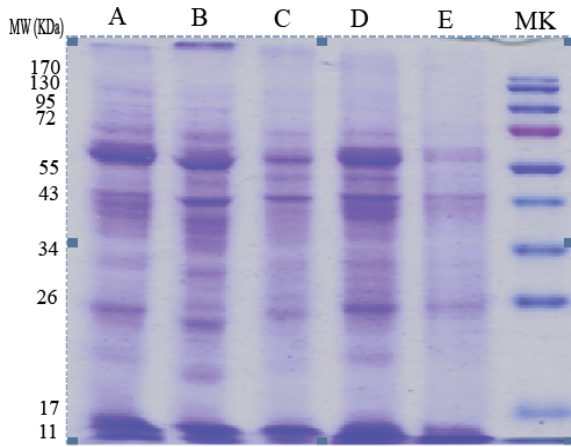


Fig. 1. The SDS-PAGE profile of proteins under drought stress for Barley Var. Haider-93: A: control, B: PEG 5%, C: PEG 10%, D: PEG 15%, E: PEG 20%.

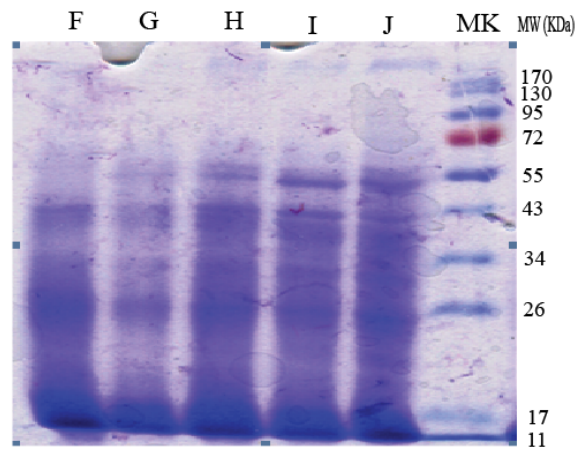


Fig. 2. F: Representing the var. of Barley, Frontier-87, control, G: 5% PEG concentration, H: 10% PEG conc. I: 15% PEG concentration, J: Frontier-87 with 20% PEG conc., Mk: marker with molecular weights given on left side.

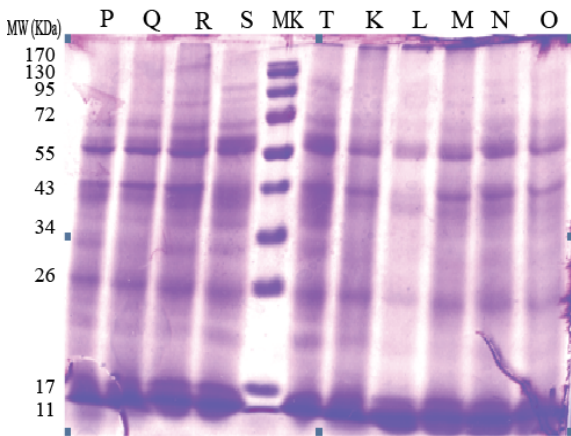


Fig. 3. The SDS-PAGE profile of proteins under drought stress; P Q, R, S and T represent the Barley Var.-Soorab-96 control, with PEG 5%, 10%, 15%, and 20% respectively. While K, L, M, N and O represent the Barley Var.-Sanober-96 control, with PEG 5%, 10%, 15% PEG and 20%.

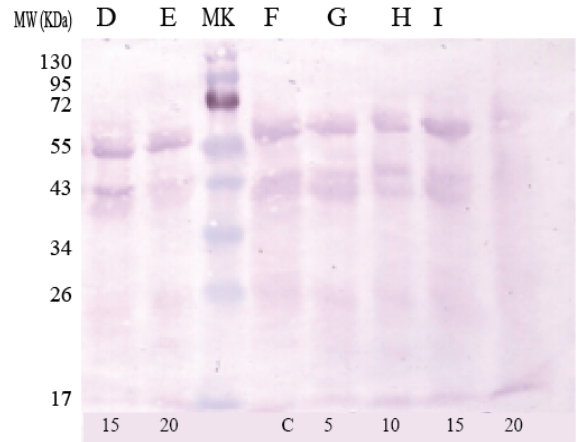


Fig. 4. Western Blot, D: Haider-93, 15% PEG concentration, E: 20% PEG concentration, F: Frontier-87 control, G: Frontier-87 with 5% PEG concentration, H: Frontier-87 with 10% PEG conc., I: Frontier-87 with 15% PEG concentration Mk: marker with molecular weights.

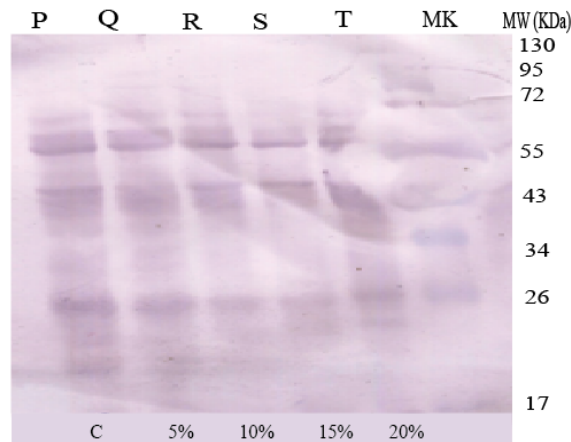


Fig. 5. western Blot, P, Q, R, S and T representing Soorab-96, control, PEG 5%, 10%, 15%, and 20% respectively.

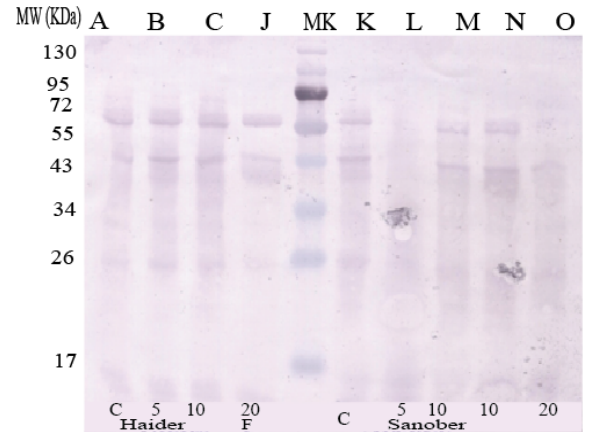


Fig. 6. A, B and C represent Barley Var.-Haider-93 control, PEG 5%, and 10% conc. respectively; J represent Frontier-87 with 20% PEG conc. K, L, M, N and O represent Sanober-96 control, with PEG 5, 10, 15 and 20% conc.

The presence of dehydrin in response to different levels PEG treatment was studied using anti dehydrin-antiserum. The western blot analysis of Haider-93 is shown in Fig. 4 and Fig. 6. The dehydrin protein of molecular weight 58 was present at all level of treatment, whereas the 43 and 26 kDa dehydrin proteins were present at 5 and 10 % PEG treatment. The dehydrin of molecular weight 43 and 38 kDa proteins were found to be induced at 15% PEG treatment and the intensity of the dehydrin was increase at 15 % PEG treatment. The presence of 58 kDa in all treatment is not surprising because all these stresses produce cell dehydration and osmotic adjustment (Zhu, 2001).

The western blot analysis of Frontier-87 is represented in Fig. 4 and Fig. 6. The bands of 58 and 43, 26 and 17 kDa dehydrin proteins were present in all levels of treatment however its concentration varied. At 20 % PEG treatment showed the more dense and concentrated bands than the all others. The protein of 41 kDa molecular weight was observed only at 10% PEG concentration.

The western analysis of Sanober-98 was shown in Fig. 6. The dehydrin protein of mol. weight 58, 43 and 26 kDa were present clearly at control, 10 and 15% PEG treatment; however these bands are denser at 15% PEG treatment. Fig. 5 described the western blot analysis of Soorab-96. It showed the protein of 58, 43 kDa is present at all levels of treatment. The proteins are more concentrated at 5, 10 and 20% PEG treatment and a protein of 22 kDa molecular weight is also present in addition. Plants have some physiological responses to defend themselves against drought stress.

Among these responses, dehydrins is one of the expressions, which is also known as group II late-embryogenesis abundant (LEA) proteins, with the size range from 9 to 200kDa (Ingram & Bartels, 1996). Evidence is increasing in the favour of a relationship between the accumulation of drought-induced proteins and physiological adaptation to water limitation (Bray, 1993; Riccardi *et al.*, 1998). As a result, it was concluded that all the selected varieties has potential to produced dehydrin but their response varied due to treatment or progressive effect of PEG concentration.

References

- Anjum, F., M. Yaseen, E. Rasool, A. Wahid and S. Anjum. 2003. Water stress in barley (*Hordeum vulgare* L.). I. Effect on morphological characters. *Pak. J. Agri. Sci.*, 40(1-2): 43-44.
- Barozai, M.Y.K. and H.A. Wahid. 2012. In silico identification and characterization of cumulative abiotic stress responding genes in potato (*Solanum tuberosum* L.). *Pak. J. Bot.*, 44 (SI): 57-69.
- Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principles of protein binding. *Anal. Biochem.*, 72: 248-254.
- Bray, E.A. 1993. Molecular responses to water deficit. *Plant Physiol.*, 103: 1035-1040.
- Chandler, P.M. and M. Robertson. 1994. Gene expression regulated by Abscisic acid and its relation to stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, 45: 113-141.
- Choi, D.W., M.C. Koag and T.J. Close. 2000. Map locations of barley Dhn genes determined by gene-specific PCR. *Theoretical and Applied Genetics*, 101: 350-354.
- Close, T.J. 1996. Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiol Plant.*, 97: 795-803.
- Close, T.J. and P.J. Chandler. 1990. Cereal Dehydrins. Serology. Gene mapping and potential functional roles. *Aust. J. Plant Physiol.*, 17: 333-334.
- Close, T.J., A.A. Kortt and P.M. Chandler. 1989. A cDNA based comparison of Dehydration induced proteins (Dehydrins) in barley and corn. *Plant Mol. Biol.*, 13(1): 95-108.
- Dure, L. 1993. The Lea proteins of higher plants. In: *Control of plant gene expression*. (Ed.): D.P. Verma. CRC Press, Inc, Boca Raton, 325-335.
- Hsiao, T.C. 1973. Plant response to water stress. *Ann. Rev. Plant Physiol.*, 24: 519-570.
- Ingram, J and D. Bartels. 1996. The molecular basis of dehydration tolerance in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 47: 377-403.
- Khan, S.U., A. Bano, J.U. Din and A.R. Gurmani. 2012. Abscisic acid and salicylic acid seed treatment as potent inducer of drought tolerance in wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 44 (SI): 43-49.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Leinhos, V., S. Tiroke and H. Bergmann. 1996. Influence of osmotic stress and amino alcohol treatment on protein content, protein patterns and growth of germinating barley. *J. Applied Bot.*, 70: 199-204.
- Riccardi, F., P. Gazeau, D. de Vienne and M. Zivy. 1998. Protein changes in response to progressive water deficit in maize, quantitative variation and polypeptide identification. *J. Plant Physiol.*, 117: 1253-1263.
- Steel, R.G.D. J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics: a biometrical approach. 3rd ed. New York: McGraw Hill Book, 666p.
- Valenzuela, H. and J. Smith. 2002. Barley. Sustainable Agriculture Green Manure Crops.
- Zhu, J.K. 2001. Plant salt tolerance. *Trends Plant Science*, 6: 66-71.

(Received for publication 15 December 2011)