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International journal of Advanced Biological and Biomedical Research

Volume 2, Issue 3, 2014: 840-846

Evaluation of leaf protein pattern in wheat genotypes under drought stress

K. Moradpour[1*], A. Najaphy[2], S. Mansoorifar[2], A. Mostafaie[3]

[1] Graduate MSc of Agricultural Biotechnology, Razi University, Kermanshah, Iran
[2] Assistant Professor, Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran
[3] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Drought is one of the most important factors limiting crop yields around the world. Drought stress in plants, the change (increase or decrease) in the production of plant proteins. This research was carried out using bread wheat genotypes. For evaluation of leaf protein pattern in wheat, 10 genotypes were assayed with 3 replications under irrigated (non-stress) and rain-fed (stress) conditions. At grain filling stage, 10 random plants were selected and flag leaf samples were harvested. SDS-PAGE Electrophoresis was used to evaluate protein pattern after applying water stress. Thirty five protein bands appeared. Most of the bands were similar in the entries and specific bands were rare. Under drought stress, high molecular weight proteins were intensified, while low molecular weight proteins were faint. Cluster analysis under non-stress conditions classified the genotypes into tree clusters but under stress conditions the entries were classified into four clusters.

Key words: drought stress, electrophoresis, protein pattern, wheat

Introduction

Drought is an important environmental factor that limit plant performance, growth and productivity (Chaves and Oliveira, 2004). where water stress often reduce crop yields and plant growth (Beltrano *et al.*, 2006). many Scientists studies on the mechanisms of drought tolerance of agriculturally important crops have been performed (Yamaguchi- Shinozaki *et al.*, 2002). With dwindling water resources, the Breeders breeding programs for varieties adapted to drought stress increased (Li *et al.*, 2011). Among the changes that occur due to drought stress in plants, the change (increase or decrease) in the production of plant proteins (Donnelly *et al.*, 2005 Jangpromma *et al.* 2007). Proteins play an important role in resistance to environmental stresses (Shen *et al.*, 2002). Qualitative and quantitative changes during stress proteins have been identified (Kottapalli *et al.* 2009). drought stress decreased soluble proteins have a molecular weight of more than 100 kDa in the leaves, while soluble proteins whit low molecular weight increase (Sujin and Ray wu. 2004). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is one of the most frequently employed techniques for separating macromolecules (DNA, RNA and proteins). This research aimed examined the changes of protein pattern in wheat leaves under drought stress and non-stress conditions.

Corresponding Author E-mail: Kamranmoradpour60@gmail.com

Materials and Methods

Experimental location and Plant material

This research was carried out using bread wheat genotypes (Table 1) during 2008-2009 at research farm and laboratories of Razi university and medical biology research center Kermanshah university of medical sciences. Ten wheat genotypes were assayed with 3 replications under irrigated (non-stress) and rain-fed (stress) conditions. Density was 400 plants per square meter. At grain filling stage, 10 random plants were selected and flag leaf samples were harvested. SDS-PAGE Electrophoresis was used to evaluate protein pattern after applying water stress. Thirty five protein bands appeared.

Protein Extraction and electrophoresis

This method, with change on the protein extraction method by Tsugita *et al*. 1996 proposed the modified method Damerval *et al*. 1986, respectively. In brief, One gram of fresh leaf tissue was powdered with liquid nitrogen then transferred into a microtube and extraction buffer were added. The mixture was placed at -20C ° for 1 h. Then at 12000 rpm for 20 min at 4 ° C. Then at 12,000 rpm for 20 min at 4 ° C was centrifuged. the supernatant discarded, the precipitate was washed. Then for 15 min at -20C ° was mixed. Then at 12,000 rpm for 20 min at 4 ° C. The mixture was centrifuged and the supernatant was discarded. the precipitate was repeated three times. The samples were incubated in acetone to evaporate it. then added 400 µl lysis buffer in samples and the refrigerator temperature (-4°C) were maintained.

Concentration of the protein in leaf extracts was performed using the Bradford (1976) method. In this method a standard curve of bovine serum albumin (BSA) was used. Proteins were electrophoresed by SDS-PAGE according to the Method of Laemmli (1970). Resolving gel 12.5 percent combination: 3.3 ml acrylamide stock, 2 ml Resolving gel buffer, 2.6 ml distilled water, 0.04 ml ammonium persulfate (10 %) and 0.04 ml TEMED, and then combining the Staking gel 5%: 0.49 ml acrylamide stock of 0.75 ml Staking gel buffer, 1.76 ml distilled water, 0.04 ml ammonium persulfate (10%) and 0.04 ml TEMED was prepared. After sample loading, electrophoresis was performed at 80 to 100 volts. Gels were stained with Coomassie brilliant blue and then destaining with methanol and acetic acid.

Statistical analysis

Presence and absence of bands with numbers one and zero, respectively, were shown. Cluster analysis of molecular data was performed using the NTSYS software version 2.02 (Rohlf. 2000).

Table 1 - Names and genotypes coding

Genotype	Pedigree genotype
1	zarin
2	Bolani
3	HAMAM-4
4	Atila2/PBW65
5	M-79-7
6	KAR-1//RMNF12-71/JUP'S'
7	Marvdasht
8	M-81-13
9	TEVEE'S'//CROW/VEE'S'
10	Pishgam (Bkt/Zhong)

Results and discussion

Protein electrophoresis bands under drought stress and non-stress conditions the total number of 35 protein bands detected in leaves (Figure 1). In order to clarify the differences and similarities Between genotypes cluster analysis was performed. Since each protein is expressed in a characteristic difference Between the bands can be indicative of differences in various traits. Protein bands among genotypes were more common, while there were a few specific bands among the genotypes. cluster analysis was performed COMPLETE. The results of cluster analysis in non-stress conditions (Figure 2) genotypes were evaluated in three separate clusters. In the first cluster genotypes 2, 4 and 8 were grouped. As genotypes number 1, 5, 10 and 7 were grouped in cluster 3. While in the third cluster genotypes were 9 , 6 and 3. In drought stress conditions (Figure 3) genotypes were evaluated in four separate clusters. In the first cluster genotypes 2, 4, 1 and 9 were grouped. In Second cluster was single genotype number 7. While in the third cluster genotypes were 5, 6, 10 and 8. In the fourth cluster was genotype number 3. According to Figure 1 can be seen that in effect of drought stress in genotypes some bands are removed and in some other bands have emerged. From the above results it is concluded that the effects of drough stress , proteins whit low molecular weight which are located at the bottom of the gel have been intensified while proteins whit high molecular weight which are located at the above of the gel have been weakened.

Drought stress decreased soluble proteins whit high molecular weight, while soluble proteins whit low molecular weight increase (Farshadfar *et al* 2008, Zimmerman 1998, Ghasempour and Kianian 2007).

Acknowledgments

This study was supported by research farm and laboratories of Razi university and medical biology research center Kermanshah university of medical sciences. We are highly thankful to all colleagues who helped us in this research.

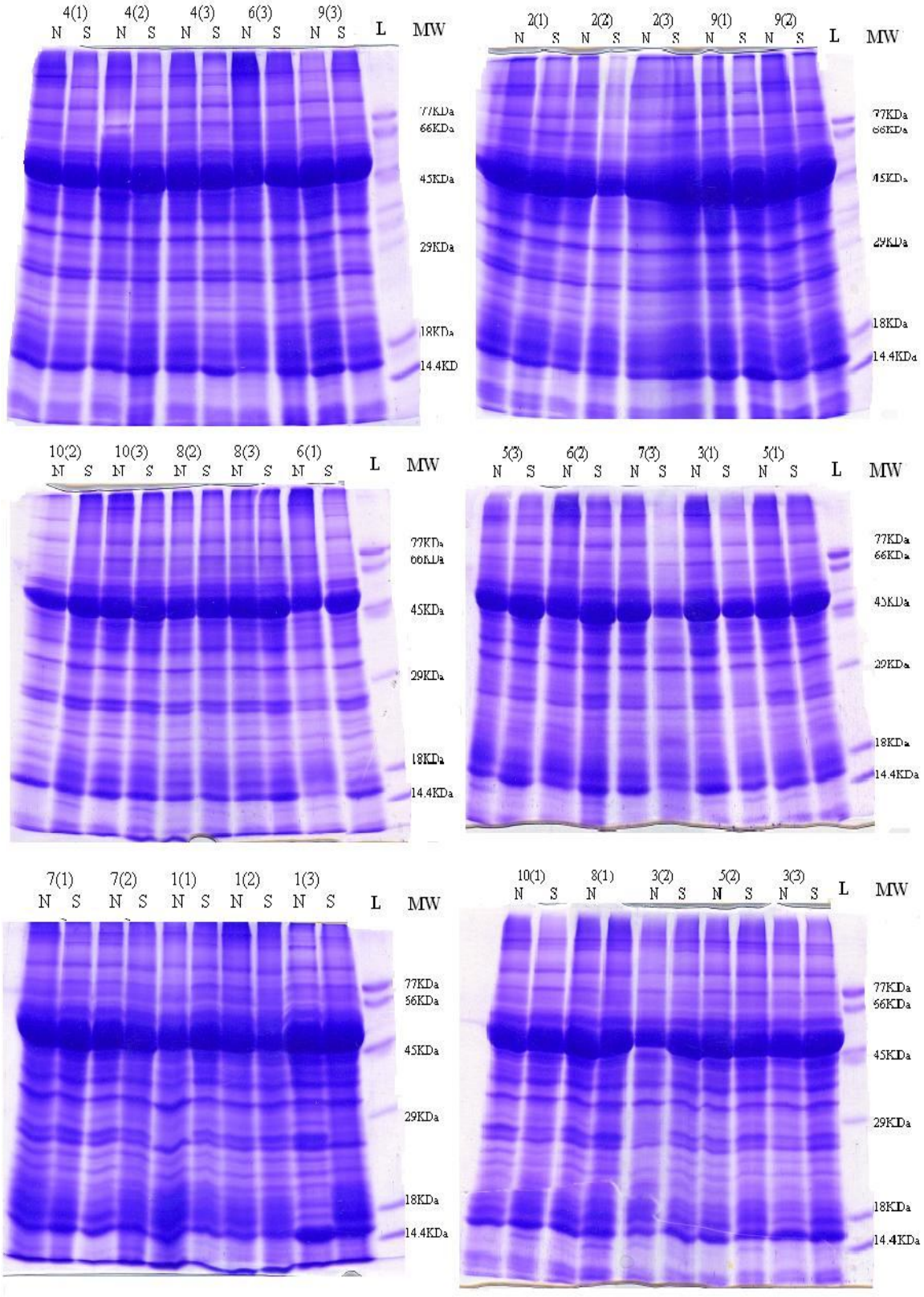

Figure 1 - protein Profiles of genotypes in non- stress (N) and stress (S) conditions

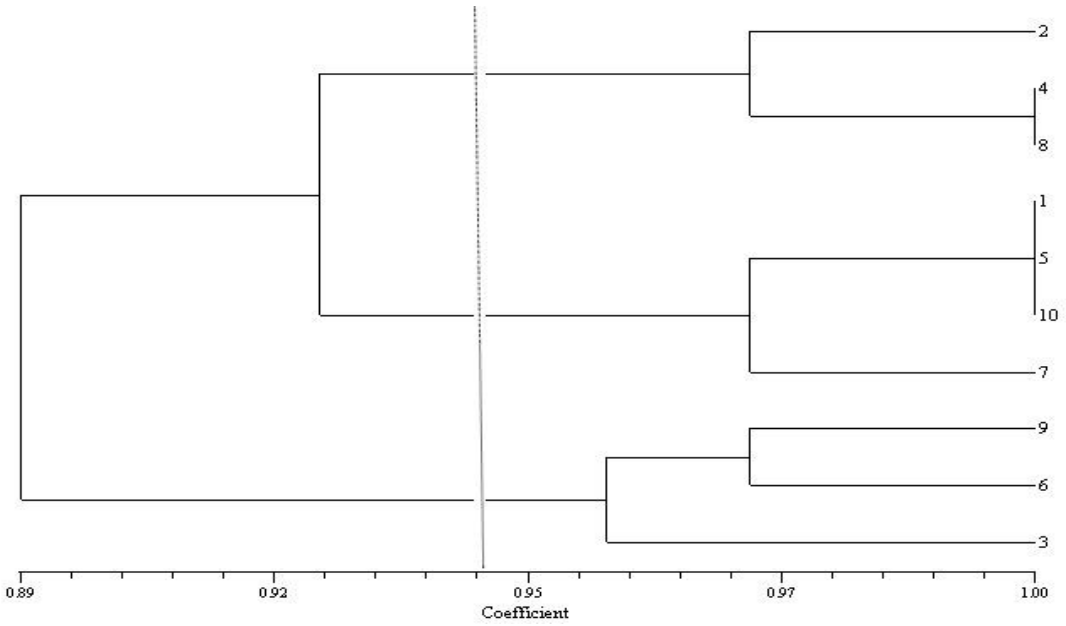

Figure 2 - Dendrogram resulting from cluster analysis based on protein bands in non-stress conditions

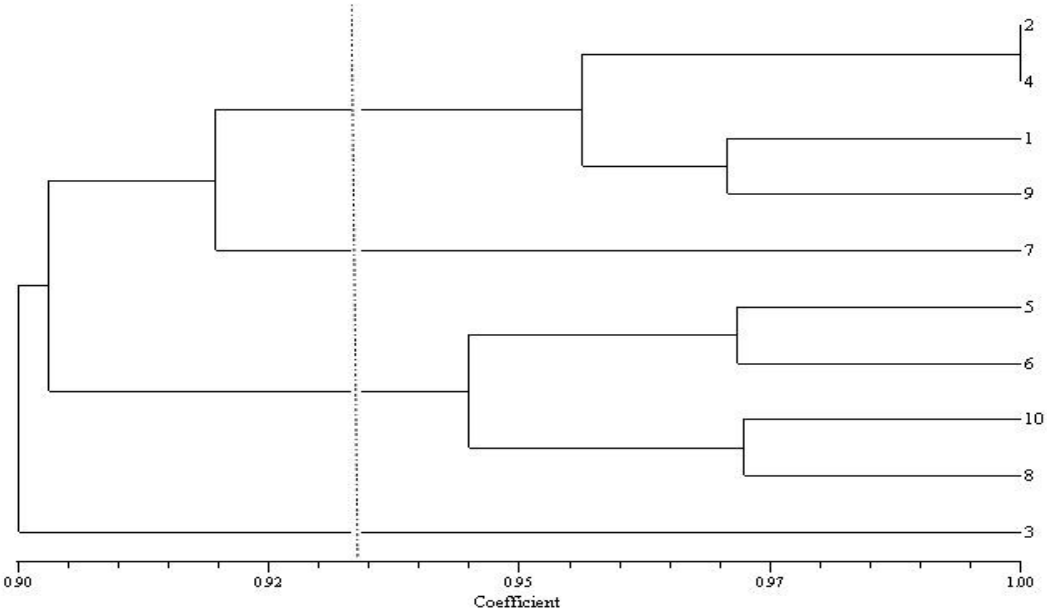

Figure 3 - Dendrogram resulting from cluster analysis based on protein bands in stress conditions

REFERENCES

Beltrano J, Ronco M, Arango MC (2006) Soil drying and rewatering applied at three grain developmental stages affect differentially growth and grain protein deposition in wheat (*Triticum aestivum* L.). Braz. J. Plant Physiol. 18:341-350.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteinutilizing the principle of protein- dye binding. Ann. Biochem. 72:248- 254.

Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture, J. Exp. Bot., 55, N 407, 2365-2384.

Damerval C, deVienne D, Zivy M, Thiellement H (1986). Technical improvements in two- dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 7:52-54.

Donnelly B, Madden R, Ayoubi P, Porter D, Dillwith J (2005) The wheat*(Triticum aestivum L.)* leaf proteome. J. Proteomics. 5: 1624–1633.

Farshadfar E, Ghasempour H, Vaezi H (2008) Molecular aspects of drought tolerance in bread wheat (*T. aestivum*). Pak. J. Biol. Sci. 11(1): 118-112.

Ghasempour HR, Kianian J (2007) The study of desiccation-tolerance in draying leaves of the desiccation-tolerance grass *sporobolus elongates* and the desiccation-sensititive grass *sporobolus pyramidalis.* Pak. J. Biol. Sci. 10(5): 797-801.

Jangpromma N, Kitthaisong S, Daduang S, Jaisil P, Thammasirirak S (2007) 18kDa protein accumulation in sugarcane leaves under drought stress conditions. KMITL Sci. Tech. J.Voi. 7No. S1 Nov. pp. 44-54.

Kottapalli KR, Rakwal R, Shibato J, Burow G, Tissue D, Burke J, Puppala N, Burow M, Payton A (2009) Physiology and proteomics of the water-deficit stress response in three contrasting peanut genotypes, Plant Cell Environ, 32: 380- 407.

Laemmli U K (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680- 685.

Li Q, Huang J, Liu Sh, Li1 J, Yang X, Liu Y, Liu Zh (2011) Proteomic analysis of young leaves at three developmental stages in an albino tea cultivar. Proteome Science, 9-44.

Rohlf F J (2000) NTYSYS-pc ver. 2.02 Numerical taxonomy and multivariate analysis system. Exeter software, Setauket, NY.

Shen S, Matsubae M, Toshifuma T, Komatsu S (2002) A proteomic analysis of leaf sheaths from rice. J. Biochem 132: 613-620.

Sujin, Ray wu (2004) Stress-inducible synthesis of proline in transgenic rice confers growth under stress conditions than that with constitutive synthesis. Plant Sci., 166(4): 941-948.

Tsugita T, Kamatsu S, Kajiwara H (1996) Two- Dimensional Electrophoresis of plant proteins standardization of gel patterns. Electrophoresis. 17: 855-866.

Yamaguchi-Shinozaki K, Kasuga M, Liu Q, Nakashima K, Sakuma Y, Abe H, Shinwary ZK, Seki M, Shinozaki K (2002) Biological mechanisms of drought stress response. JIRCAS Working Report, 1-8.

Zimmerman MH (1998) Hydraulic architecture of some diffuse propous trees. Can. J. Bor. 56: 2286-2295.