



## Evaluation of leaf protein pattern in wheat genotypes under drought stress

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### 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.

## 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)

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### 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 drought 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).

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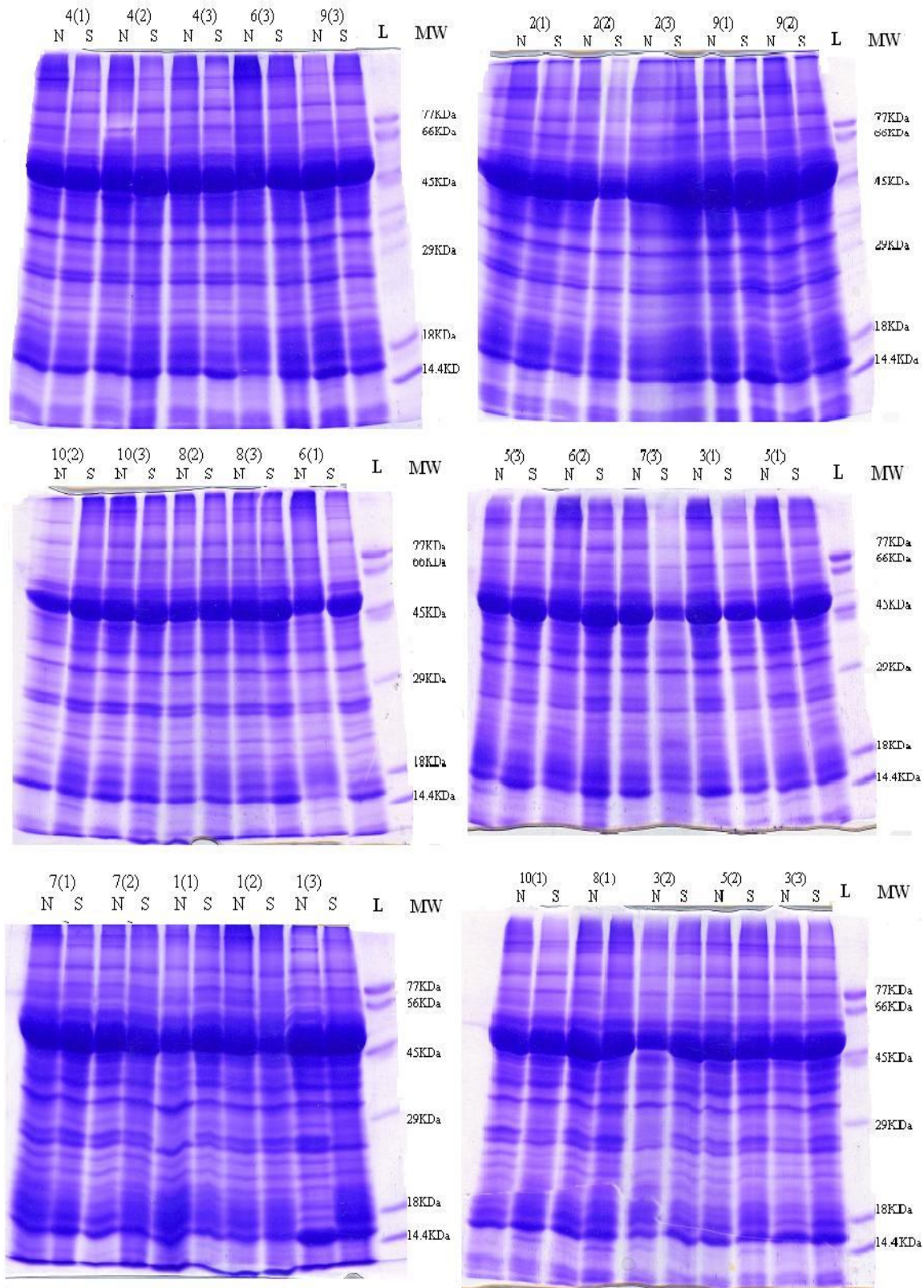


Figure 1 - protein Profiles of genotypes in non- stress (N) and stress (S) conditions

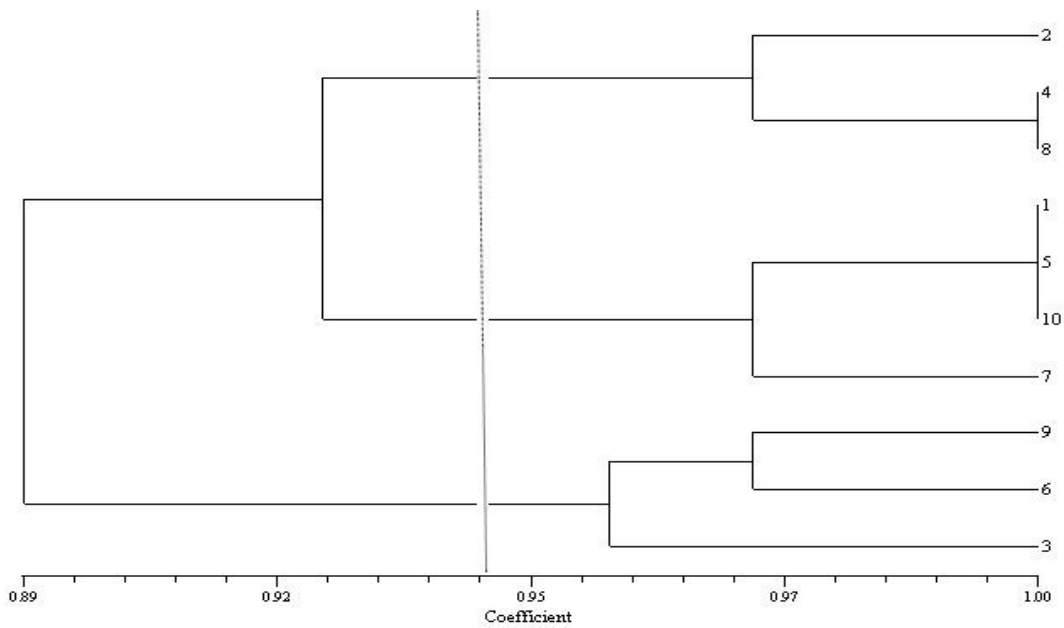


Figure 2 - Dendrogram resulting from cluster analysis based on protein bands in non-stress conditions

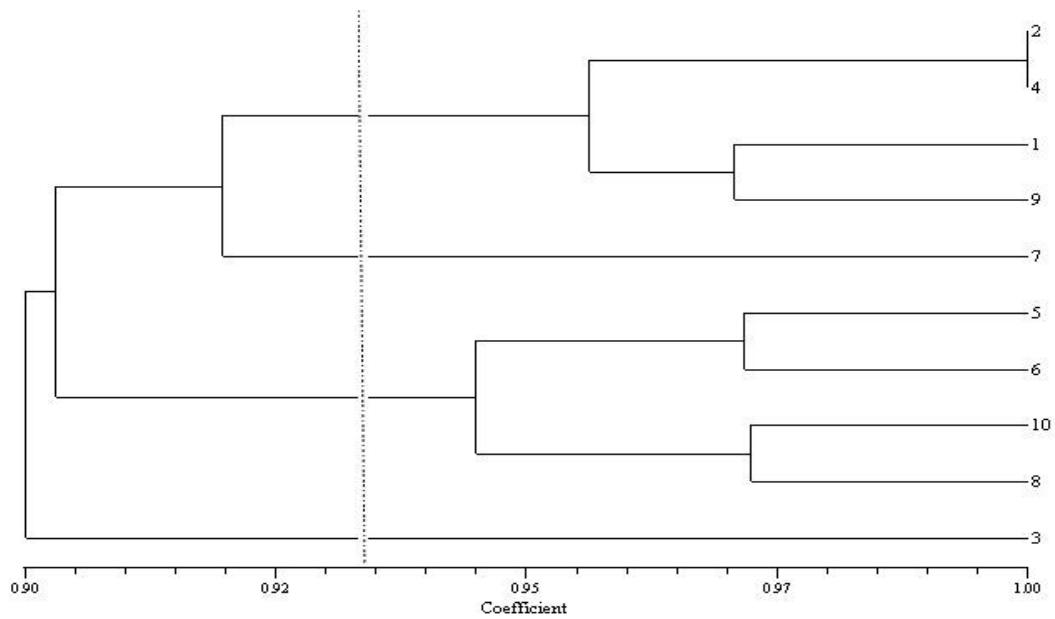


Figure 3 - Dendrogram resulting from cluster analysis based on protein bands in stress conditions

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