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Research article

TERMINAL DROUGHT INDUCED CHANGES IN LEAF PROTEIN PATTERN OF WHEAT

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ABSTRACT: For evaluation of changes in leaf protein pattern of wheat, 10 wheat genotypes were assayed under both terminal drought stress and non-stress conditions. SDS-PAGE electrophoresis was used to assess protein pattern of flag leave after applying the stress. As a whole, thirty five protein bands were detected. Most of the bands under the stress conditions were similar to those in non-stress environment and specific bands were rare. Under drought stress, some low molecular weight proteins were intensified, while high molecular weight proteins were faint. Cluster analysis under non-stress conditions classified the wheat genotypes into three groups but in stress environment, the entries were grouped into four clusters.
Key words: Drought stress, electrophoresis, protein pattern, wheat.

INTRODUCTION

Drought stress is the most adverse environmental condition that can seriously reduce plant yield, particularly when the stress occurs during reproductive stage. To cope with the stress, numerous morphological, physiological and biochemical changes occur in various plant species. These changes cause the retention of water and the maintenance of photosynthetic activity, while stomatal opening is reduced to counter water deficit [1]. The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought stress tolerance [2, 3]. Both quantitative and qualitative changes of proteins have been detected during the stress [4, 5, 6]. Sujin and Ray wu [7] reported that soluble proteins in rice leaf with molecular weight of more than 100 kDa were reduced as a result of drought stress, but low molecular weight proteins were increased. The aims of the present study were to evaluate the pattern of flag leaf proteins in wheat under terminal drought stress and non-stress conditions.

MATERIALS AND METHODS

Plant materials

This research was carried out using ten bread wheat genotypes (Table 1) during 2009-2010 at research farm and laboratories of Razi University and medical biology research center, Kermanshah University of medical sciences. Ten wheat genotypes were planted in a RCB design with three replications under irrigated (non-stress) and rain-fed (stress) conditions. Plant density was 400 plants per m^2. At grain filling stage, 10 random plants were selected and flag leaf samples were harvested.

Protein Extraction and electrophoresis

Flag leaf proteins were extracted according to Tsugita et al., [8] with some minor modifications. Initially, 1g of leaves were powdered with liquid nitrogen in a mortar and then transferred into microtubes and extraction buffer was added to them. The mixtures were placed at -20°C for an hour. Then they centrifuged at 12000 rpm for 20 min at 4° C. The supernatant in each tube was removed and centrifuged and the remaining sediment was mixed with washing buffer. The mixture was placed on a magnetic mixer for 15 minutes at -20°C and then centrifuged at 12000 rpm for 20 min at 4°C. The supernatant removed and the above stages were repeated three times. The samples were then incubated to evaporate acetone. 400 µl lysis buffer were added to samples and the tubes were kept at 4°C.
Concentration of protein in leaf extracts was performed using the method of Bradford [9]. Proteins were analyzed by SDS-PAGE electrophoresis. Separation of proteins was carried out at constant voltage (80 V for 30 min and 150 for 90 min). After electrophoresis, the gel was fixed in 20% trichloroacetic acid and stained in 0.1% Coomassi brilliant R-250, and then destained in 20% methanol and 7.5% (v/v) acetic acid.

Statical Analysis

Presence and absence of bands were determined by numbers one and zero, respectively. Cluster analysis of the molecular data was performed using the NTSYS software version 2.02 [10].

Table 1 - Names and code of wheat genotypes used in the study

Genotype code	Name
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

A total number of 35 protein bands were detected by electrophoresis of flag leaf proteins in stress and non-stress conditions (Fig. 1). Most of the bands under the stress conditions were similar to those in non-stress environment and specific bands were rare.

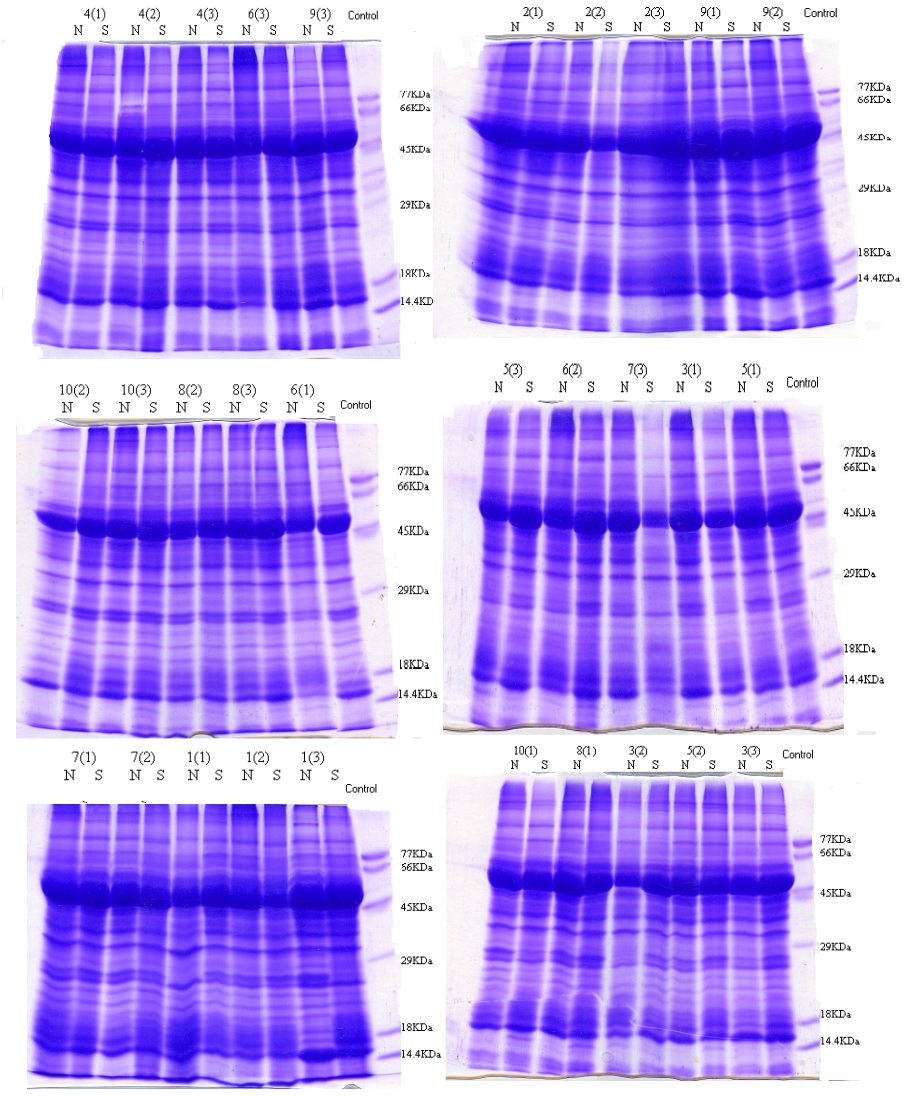

Figure 1 - Protein profile of wheat leaves under drought stress (S) and non-stress (N)
The numbers in and out of paranthesis refer to replication and genotype number, respectively.

Under drought stress, some low molecular weight proteins were intensified, while high molecular weight proteins were faint. These findings are in accordance with Farshadfar et al [11] and Ghasempour and Kianian [12]. Jiang and huang [3] reported that two polypeptides were intensified in drought-stressed tall fescue plants than well watered conditions. Water deficit stress increased concentration of soluble proteins in chickpea leaves up to 43% in comparison with normal watering treatment, but didn't significantly affect electrophoretic pattern of protein profiles [6].

Cluster analyses based on protein banding in the both stress and non-stress conditions were performed to clarify differences and similarities among the wheat genotypes. In non-stress conditions (Fig. 2), genotypes were grouped in three separate clusters. The first cluster included genotypes 2, 4 and 8. Genotypes 1, 5, 10 and 7 were placed together in the second cluster. The third group comprised genotypes were 9, 6 and 3. In drought stress conditions, the wheat accessions were classified in four clusters (Fig. 3). The first cluster comprised genotypes 2, 4, 1 and 9. Genotype number 7 was placed alone in the second cluster. The third group included accessions 5, 6, 10, 8. Finally, genotype No. 7 formed the forth cluster. Intensified and weakened protein bands in chickpea under drought stress and different grouping of cultivars in stress and non- stress conditions were reported by Kakaei *et al*. [13] Figures 2 and 3 showing that the clustering pattern and responses of the wheat genotypes based on leaf proteins were different in stress and non-stress environments.

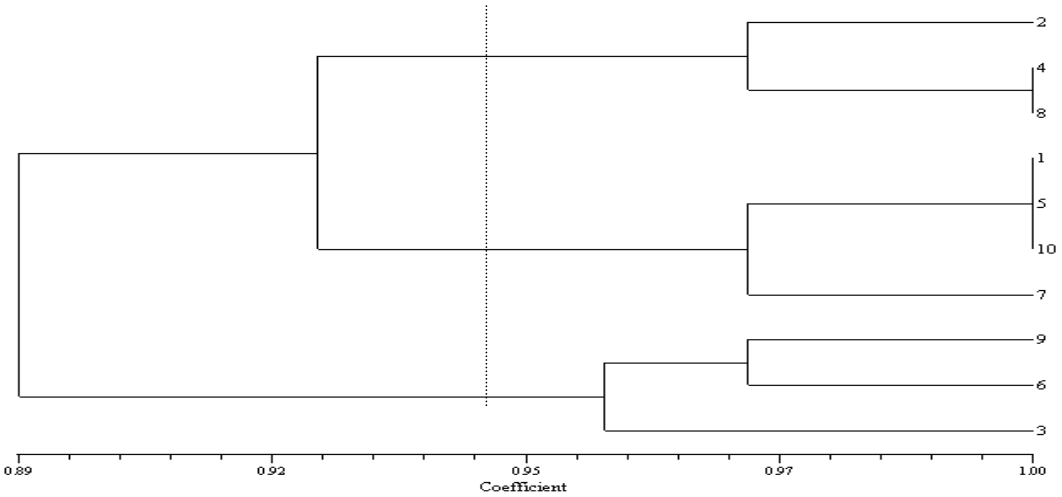

Figure 2 – Dendrogram of cluster analysis based on leaf protein bands in irrigated conditions (non-stress)

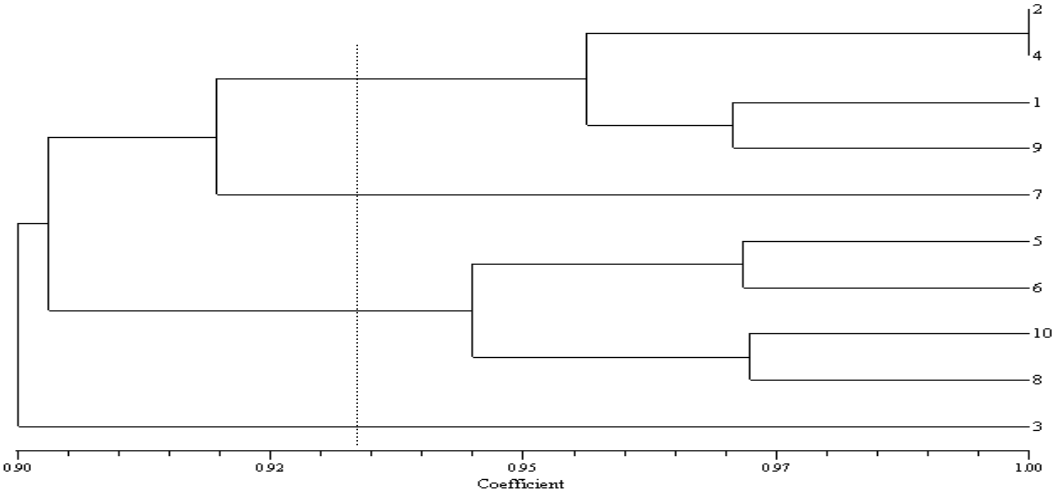

Figure 3 - Dendrogram of cluster analysis based on leaf protein bands in drought conditions (stress)

CONCLUSION

Terminal drought stress led to quantitative and minor qualitative changes of leaf proteins in wheat. Under drought stress, some low molecular weight proteins were intensified, while high molecular weight proteins were faint. Cluster analysis under non-stress conditions classified the wheat genotypes into three groups but in stress environment, the entries were grouped into four clusters. Clustering pattern and responses of the wheat genotypes based on leaf proteins were different in stress and non-stress conditions.

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