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Evaluation of seed storage protein patterns of ten wheat varieties using SDS-PAGE

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Abstract. SDS-PAGE was used to evaluate and characterize the patterns of seed storage proteins in ten bread wheat (*Triticum aestivum* L.) genotypes. Electrophorogram for each variety were scored for presence/absence in each sample, which were transformed into a binary data matrix. Jaccard's similarity coefficient, generated to determine the genetic similarity among genotypes, revealed that most of the genotypes were genetically distant from each other. The cluster analysis with UPGMA method separated the genotypes into 2 clusters. Based on Jaccard's similarity matrix, we represented a protein marker and it is clear that genotype 14GB & Hama-4, 14GB & un11 and Hama-4 & un11 have the highest similarity and lowest genetic distance. Genotypes Boomaz and Saji have the least similarity and maximum distance.

Key Words: *Triticum aestivum*, protein patterns, SDS-PAGE, Cluster analysis.

Introduction

Wheat (*Triticum aestivum* L.) is considered as one of the most primitive domesticated crop. Bread wheat plays a major role among the few crop species being widely grown as food sources and was likely a central point to the beginning of agriculture (Shuaib et al. 2007). One practical application of knowledge of genetic diversity is in the design of populations for genome mapping experiments (Kaga et al. 1996). Seem concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of genotypes. The morphological markers were not quite enough to expose the genetic diversity between the morphological overlap cultivars and the morphological identical accessions. Therefore, the need for a new tool was disparate. The advent of the electrophoretical as an analytical tool provides an indirect method for genome probing by exposing structural variations in enzymes or other protein genome (Cook 1984, Gilliand 1989). Wheat (*Triticum aestivum* L.) seed-storage proteins represent an important source of food and energy, being also involved in the determination of bread-making quality (Cooke & Law 1998). Plant storage proteins in general and wheat storage proteins in particular contribute to the major source of the required nutritional protein and carbohydrates to nearly all nations around the globe (Izadi–Darbandi et al. 2010). The most common cultivated wheat nowadays is hexaploid *T. aestivum*, AABBDD, (2n= 6X= 42) (Zohary & Hopf 1993). Acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has become one of the most widely used techniques to separate and characterize wheat storage proteins (Shuaib et al. 2010). SDS-PAGE could be conveniently used for identification of wheat varieties suitable for chapatti making (Prabhasankar & Rao 2001). Seed storage proteins have been used as genetic markers in four major areas: (1) analysis of genetic diversity within and between accessions, (2) plant domestication in relation to genetic resource conservation and breeding, (3) establishing genome relationships, and (4) as a tool in crop improvement (Ghafoor & Ahmad 2005). Studying of storage proteins has been reported in many researches such as phylogenetic relationships, genomic homologies and genetic diversity (Lufiudra & Benedetelli 1985). These are considered as practical and reliable methods because seed storage proteins and nucleotide sequences are largely independent of environmental fluctuations (Iqbal et al. 2005). The aim of the present paper was to study the genetic diversity of wheat on the basis that SDS-PAGE may provide useful information for the use of genotypes in different breeding experiments.

Materials and Methods

The present study used 10 wheat genotypes (Table 1) that prepared from Dryland Agricultural Research Institute, Kermanshah, Iran. To extract total proteins, single grain powder was added with protein extraction buffer (Tris-Hcl, pH=8.5; NP-40, 0.2%; PMSF, 1mM and EDTA, 1mM) vortex and gently shaken overnight, centrifuged at 12,500 rpm for 15 minutes, following the method described by Xi and collaborators (2006) with some modifications (Kakaei et al. 2010). SDS-PAGE method in resolving gel with 12.5% acrylamid and stacking gel 5% acrylamid was applied for extraction and resolving of these genotypes. Then, after gel electrophoresis, protein bands were revealed by Coomassie Brilliant Blue R-250 staining and destained by methanol and acetic acid. Dissociating polyacrylamide gel electrophoresis (SDS-PAGE) was adopted after Laemmli (1970) with some modifications. Protein assay was made according to Bradford (1976). The molecular weight of each band was identified by the standard curve obtained from the standard proteins. Standard proteins were used and their molecular weights were: ovotransferrin (78 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), actinidin (29 kDa), β lactoglobulin (18 kDa) and lysozyme (14 kDa).

Table 1. The wheat genotypes that have been used in study of seed proteins pattern are divided in two groups.

No.	Genotypes name	Genome	Cluster No.
1	Azar-2	AABBDD	1
2	ww33G	AABBDD	2
3	14GB (Awhadi)	AABBDD	2
4	Pato	AABBDD	2
5	Boomaz	AABBDD	2
6	Shahi	AABBDD	2
7	Hama-4	AABBDD	2
8	Un11	AABBDD	2
9	Cross Alborz	AABBDD	2
10	Saji	AABB	1

Cluster analysis

Cluster analysis of wheat grain storage proteins was performed on the results of SDS-PAGE using the software NTSYS to find out the diversity among the given wheat varieties.

Data analysis

Electrophoregrams for each cultivar were scored and the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's similarity index.

Results and Discussion

Electrophorogram showing proteins banding pattern of different wheat varieties was presented in Fig.1. Based on the results, it is concluded that evaluation of genetic diversity and identification of wheat varieties by SDS-PAGE is easy, if early approached and useful for molecular weight analysis of wheat seed storage proteins. The study could help wheat breeding programs. The wheat genotypes that have been used in the study of seed proteins pattern are shown in Table 1. The diagram from Fig. 2 revealed two main groups L1 and L2; the group L1 has Azar-2 and Saji and the group L2 has cultivars ww33G, 14GB, Pato, Boomaz, Shani, Hama-4, un11, Cross Alborz. More differences related to the pattern of protein bands were expressed as protein bands with molecular weight ranging from 50 to 78 kDa and the greatest differences are seen in the expression intensity and weak bands in the range of less than 18 kDa. SDS-PAGE techniques are used for studying the genetic diversity in several researches (Sofalian & Valizadeh 2009, Kakaei 2009, Kakaei & Kahrizi 2011, Gantait et al. 2009, Yuzbasioglu 2008, Solouki & Emamjomeh 2007, Zarkti et al. 2010). Differences in expression protein pattern include the following: In genotypes 1(Azar-2) and 10 (Saji) the protein bands above 66 kDa were removed in a group. Genotypes 2(ww33G), 3(14GB), 6(Shahi) and 8(un11) were near the 19 kDa protein band and they are more poorly than other experimental materials. Fig. 2 shows the resulting dendrogram based on protein Jaccard's coefficient that is of r= 0.966, and shows the data are fitted dendrogram. In Table 2, based on Jaccard's similarity matrix, it is represented a protein marker as is clear genotype 3(14GB), 7(Hama-4), 3(14GB) & 8(un11) such as genotypes 7(Hama-4) and 8(un11) with the highest similarity and the distance is minimal. Genotypes 5 (Boomaz) and 10 (Saji) have the least similarity and the highest distance.

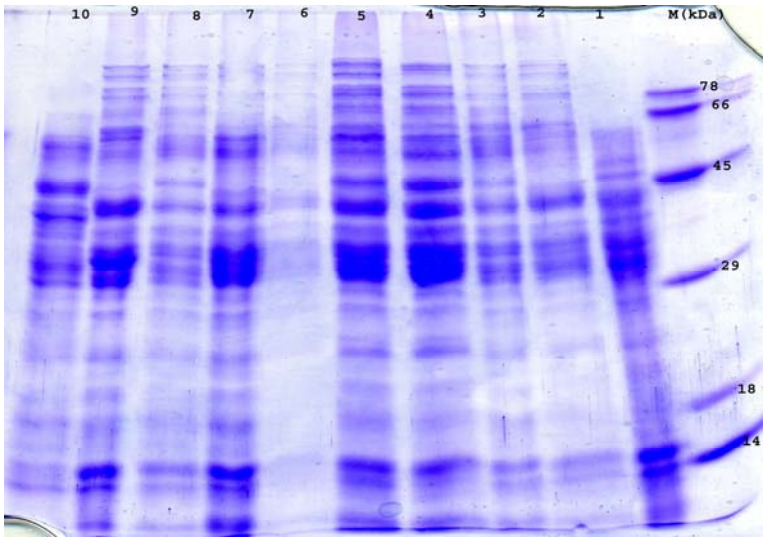

Figure 1. Seed protein banding pattern of *T. aestivum*
[lane M: Molecular marker others lanes: wheat cultivars].

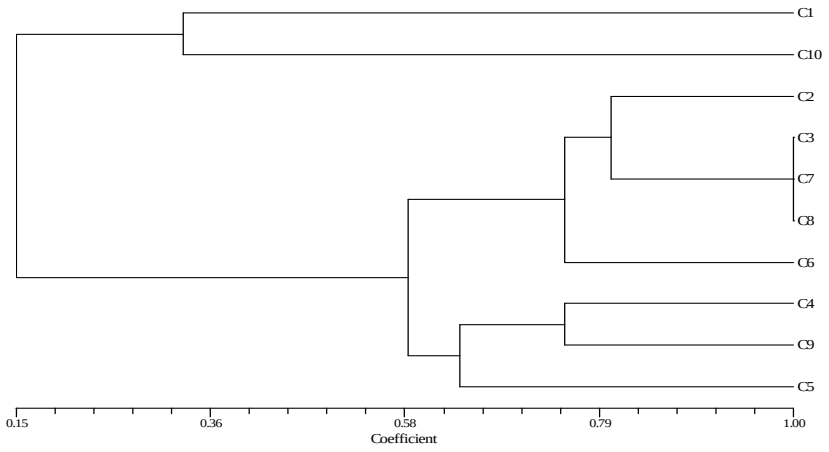

Figure 2. Dendrogram based on seed protein banding pattern in wheat.

Table 2. Jaccard's similarity matrix based on seed protein banding pattern.

	1	2	3	4	5	6	7	8	9	10
1	1									
2	0.2	1								
3	0.2	0.8	1							
4	0.28	0.5	0.66	1						
5	0.12	0.36	0.5	0.71	1					
6	0.15	0.75	0.75	0.5	0.5	1				
7	0.2	0.8	1	0.66	0.5	0.75	1			
8	0.2	0.8	1	0.66	0.5	0.75	1	1		
9	0.22	0.54	0.7	0.75	0.55	0.66	0.7	0.7	1	
10	0.33	0.11	0.11	0.16	0	0.08	0.11	0.11	0.12	1

Experimental results showed that there was no significant correlation between them. Based on the SDS-PAGE patterns all genotypes were classified into three clusters. According to cultivars distance of various groups, parents in two different extremes may be identified and used in the crossing projects for generating further variability in order to select and realize the plant improvement. So, cultivars specific band(s) that were identified may be exploited for hybrid identification in breeding program and also which could be used for the breeder's needs. Further, we suggest that seed protein SDS-PAGE be used as a rapid, easy, inexpensive and reliable method for routine identification of *Triticum aestivum* genotypes in breeding programs.

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