African Journal of Biotechnology Vol. 8 (9), pp. 1855-1859, 4 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Protein landmarks for diversity assessment in wheat genotypes

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Accepted 6 March, 2009

Grain proteins from 10 Pakistani registered wheat genotypes were evaluated for diversity assessment based on sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Genetic diversity was evaluated via UPGMA cluster analysis by constructing dendrogram of fractions of proteins, which were used for the calculation of similarity coefficients between these varieties. The greatest similarity (95%) was observed between Shahkar-95 and Mehran-89, while the lowest similarity (16%) was observed between Parwaz-94 and Abadgar-93. Adoption of this technology would be useful to plant protection regulatory systems, especially for plant variety identification and registration of new plant varieties, breeding programs and protection purposes.

Key words: SDS-PAGE, genetic diversity, coefficient of similarity.

INTRODUCTION

Wheat is the primary staple food of Pakistan. Total area under wheat cultivation is 8,358 thousand hectares with a total production of 21,612.3 thousand tones and an average yield of 2,586 kg/ha (MINFAL, 2004 - 2005). Varieties have been a landmark in the genetic improvement of wheat, as it resulted in increase in its potential for grain yield. Information about genetic diversity and genetic relatedness among elite material is a fundamental element in plant breeding (Zhu et al., 2000). Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up DUS (distinctness-uniformity-stability) test for candidate cultivar (Chan and Sun, 1997). For acquiring plant breeder's rights (PBR), varieties of agricultural importance have to be tested for distinctness (D), uniformity (U) and stability (S) (DUS testing) (Ardley and Hoptroff, 1996). Evaluation of genetic diversity in wheat has been on differences in morphological and agronomic traits or pedigree information (Bernard et al., 1998). A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These methods have relied on pedigree, morphological, agronomic performance, biochemical and molecular (DNA-based) data (Mohammadi and Prasanna, 2003). Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. The use of biochemical/molecular markers for the evaluation of genetic diversity has received much attention in recent years. A large number of germplasm lines can be characterized for biochemical markers in a short period of time. In addition the data reflects more truly the genetic variability as biochemical markers are direct product of genes and the environment does not influence their expression (Perry and McIntosh, 1991; Masood et al., 2000).

Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm (Murphy et al., 1990; Javaid et al., 2004; Anwar et al., 2003.). The analysis of storage protein variation in wheat has proved to be a useful tool not only for diversity studies but also to optimize variation in germplasm collections (Ciaffi et al., 1993; Masood et al., 2000). SDS-PAGE can be used as a promising tool for distinguishing cultivars of particular crop species (Jha and Ohri, 1996). The main objective of our research was to evaluate the potential of SDS-PAGE technique to assess the genetic diversity and relatedness among 10 Pakistani wheat genotypes based on protein profiles and to develop an optimized and efficient opera-

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Table 1. Pakistani Wheat varieties studied.

S.No	Variety Name	Breeding Centre/ Institute	Parentage	Province	Yr. of Registr.
1	Pirsabak 2004	CCRI, Pisabak	KAUZ/STAR	NWFP	2004
2	Anmol-91	WRI, Sakrand	Lira'S'	Sindh	1996
3	Chakwal-86	WRI, AARI, Faisalabad	FLN-ACC x ANA	Punjab	1990
4	Mehran-89	ARI, Tandojam	Veery 5'S' CM33027-F-15M-500Y-OM-57B-OY	Sindh	1990
5	Punjab-96	WRI, AARI, Faisalabad	SA 42*2/4/CC/INIA//BB/3/INIA/HD 832	Punjab	1997
6	Abadgar-93	WRI, Sakrand	Yaktanan54 x NORIN10-Brevor x Son 64	Sindh	1996
7	Parwaz-94	WRI, AARI, Faisalabad	Pb.20089-7A-4A-0A	Punjab	1996
8	TJ 83	ARI, Tandojam	Tzpp-PL-7C	Sindh	1984
9	Shahkar-95	WRI, AARI, Faisalabad	WL 711//F3.71/TRN	Punjab	1997
10	Kiran -95	AEARC, Tandojam	WL711NaN3/CROW'S'	Sindh	1997

tional system for their use.

MATERIALS AND METHODS

Plant sample

Wheat varieties were collected from different ecological regions of Pakistan. The samples were stored in labeled glass bottles to ensure safety in the National Agricultural Research Centre (NARC), Islamabad, Pakistan (Table 1).

SDS-PAGE analysis

The variability of seed storage-proteins was analyzed by using SDS-PAGE (Damania et al., 1983). For extraction of protein, a single seed was ground to fine powder with mortar and pestle. Total 400 ul sample buffer was added to a 0.01 g (10 mg) seed powder and mixed thoroughly by vortex in an Eppendorf tube (1.5ml) with a small glass rod. The extraction buffer contained the following final concentration: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10 % glycerol and 5% 2-mercaptoethanol, kept overnight at 40℃ and centrifuged at 13000 rpm for 10 min. To monitor the movement of the protein in the gel, bromophenol blue (BPB) was used as a tracking dye. Seed protein was analyzed through slab-type SDS-PAGE using 7% polyacrylamide gel. The molecular weights of the dissociated polypeptides were determined using protein standards (MW-SDS-70) of Sigma, USA. SDS-PAGE of total seed protein was carried out in a discontinuous buffer system following the method of Laemmli (1970). The gels were stained with Coomassie brilliant blue (CBB) and destained till the background became transparent.

Data analysis

For each variety, electrophoregram were scored and the presence (1) or absence (0) of each band was noted. Presence and absence of data were entered in a binary data matrix. Based on electrophoretic band spectra, Jaccard's similarity index (JSI) was calculated by the formula (Sneath and Sokal, 1973):

Table 2. Molecular weight analysis of wheat varieties.

Protein Type	Molecular weight (KDa)	Wheat varieties									
		1	2	3	4	5	6	7	8	9	10
HMW-GS	120	1	1	1	1	1	1	1	1	1	1
	100	0	0	0	0	0	0	0	0	0	0
	85	1	1	1	1	1	1	1	1	1	1
	70	1	1	1	1	1	1	1	1	1	1
	60	1	1	1	1	0	1	1	0	1	0
LMW-GS	50	1	1	1	1	1	1	1	1	1	1
	40	1	1	1	0	0	1	1	0	1	0
	30	1	1	1	0	1	0	1	1	0	1
	20	1	1	1	1	1	1	1	1	1	1
	10	1	1	1	1	1	1	1	1	1	1

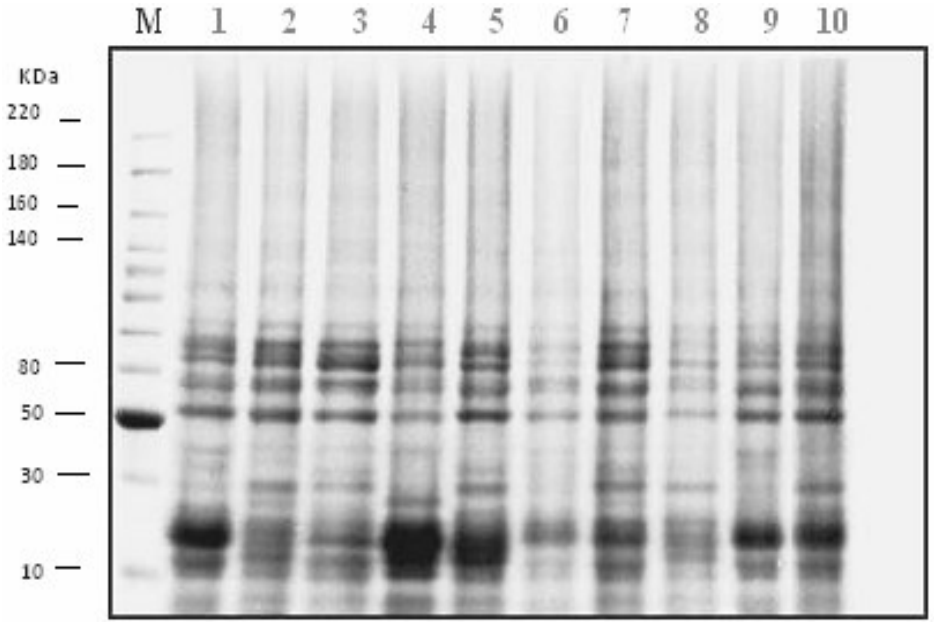

Figure 1. Electrophorogram showing banding pattern of wheat proteins and molecular weight marker. M: marker; L1 = Pirsabak; 2 = Anmol 91; 3 = Chakwal 86; 4 =Mehran 89; 5 = Punjab 96; 6 = Abadgar 93; 7 = Parwaz 94; 8 = TJ 83; 9 = Shahkar 95; and 10 = Kiran 95.

$$S = W/(A+B-W)$$

where W is the number of bands of common mobility, A the number of bands in type A and B is the number of bands in type B.

All analyses were carried out using statistical software NTSYS-PC, version 2 (Rohlf, 1993).

RESULTS

Genetic diversity evaluation

The 10 wheat varieties used in the present study showed various banding pattern using SDS-PAGE technique. In this study SDS-PAGE of grain proteins was performed in order to investigate genetic diversity among wheat varieties. Electrophoregram showing protein banding pattern of different wheat varieties are given in Figure 1. A total of 17 bands were obtained among which band number 1, 4, 12 and 13 were common in all varieties but other bands show variation (Table 2).

Cluster analysis on the basis of SDS-PAGE

The genetic similarity coefficient matrix of 10 wheat varieties based on SDS-PAGE using UPGMA method (Nei and Lie, 1979) was used to construct a dendrogram using a computer program NTSYS-PC, version 2.0, to

Table 3. Similarity coefficients among 10 wheat varieties from Pakistan.

Variety	1	2	3	4	5	6	7	8	9	10
Pirsabak	1.00									
Anmol-91	0.91	1.00								
Chakwal- 86	0.91	0.90	1.00							
Mehran-89	0.91	0.81	0.81	1.00						
Punjab-96	0.81	0.80	0.90	0.80	1.00					
Abadgar-93	0.81	0.80	0.80	0.90	0.77	1.00				
Parwaz-94	0.86	0.85	0.85	0.76	0.73	0.63	1.00			
TJ 83	0.81	0.70	0.70	0.90	0.66	0.77	0.73	1.00		
Shahkar-95	0.86	0.76	0.76	0.95	0.73	0.84	0.80	0.94	1.00	
Kiran -95	0.86	0.85	0.85	0.85	0.84	0.73	0.90	0.84	0.90	1.00

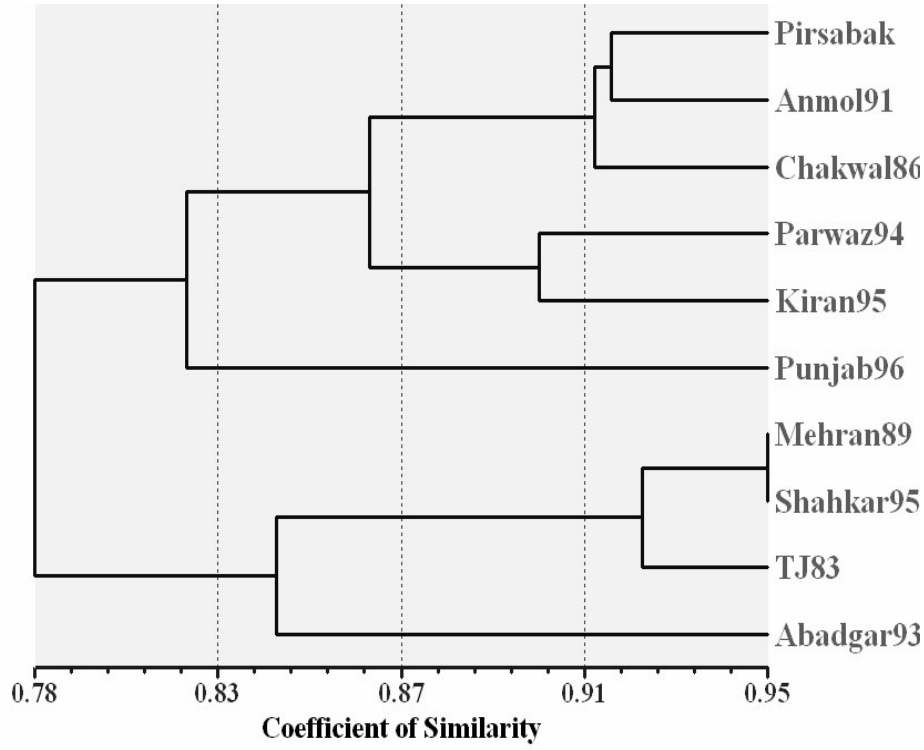

Figure 2. UPGMA cluster analysis showing the diversity and relationship among 10 wheat varieties based on SDS-PAGE.

find the diversity among given wheat varieties. The result of the cluster analysis are given in a dendrogram (Figure 2) on the basis of similarity coefficient (Table 3). The diagram revealed two main groups G1 and G2; the group G2 is further divided into two sub clusters I and II, subcluster I contains only one variety Abadgar 93 and II contains 3 varieties; Mehran 89, Shahkar 95 and TJ 83 and G2 contain 6 varieties. At a coefficient of similarity of 78%, all varieties show similarity with one another. The varieties Shahkar 95 and Mehran 89 were found to be 95% similar. Similarly Parwaz 94 and Abadgar 93 showed only 63% similarity.

DISCUSSION

Although variation in storage protein banding pattern was revealed by SDS-PAGE however, its magnitude was low. Based on SDS-PAGE, 13 bands were used for analysis and genetic diversity was estimated based on the number of different protein peptides between the 2 compared. A low level of genetic diversity may be attributed to narrow genetic base of a wheat crop.

According to the results of the SDS-PAGE, the overall blueprint of seed storage-proteins shows low degree of heterogeneity. A low level of genetic diversity may be

attributed to narrow genetic base of a wheat crop. The variation in high molecular weight protein subunits is the result of gene silencing in some varieties encoding these proteins (Lawrence and Shephred, 1980). SDS-PAGE electrophoresis of 7 wheat varieties has been previously investigated; however their varieties were different but the final result is correlated (Khan et al., 2002). Together with physiochemical and molecular characteristics already reported (Zeb et al., 2006), this study presents a good tool to characterize seed storage protein.

The dendrogram calculated from the Jaccard similarity coefficient and unweighted pair group method with averages constructed by HMW and LMW glutenin subunit bands cluster analysis is presented in Figure 2. Genetic diversity of European spelts wheat was evaluated by constructing the dendrogram for HMW and LMW glutenin subunit bands (Xueli et al., 2005). The dendrogram as a whole revealed low genetic diversity at protein levels because most varieties are in the same cluster. Fufa et al. (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait. The variety Shahkar-95 and Mehran-89 show 95% similarity with one another.

Conclusion

It is therefore concluded that seed storage protein profiles could be useful markers in genotype identification, registration of new varieties, pedigree analysis and in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development.

ACKNOWLEDGMENTS

The authors express their genuine thanks to the Federal Seed Certification and Registration Department (FSC and RD), Islamabad, Pakistan, for providing the seed material of wheat genotypes and the National Agriculture Research Centre (NARC), Islamabad, Pakistan for providing research facilities.

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