

Full Length Research Paper

Evaluation of wheat by polyacrylamide gel electrophoresis

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In this study 15 wheat varieties namely Nacozari-76, Ouqab, Tatar-96, Bakhtawar-92, Yecura-70, Raj, Bakkar, Sulilman-96, Pirsabak-05, Maria, Khyber-87, Fakhr-e-Sarhad, Pirsabak-04, Inqulab-91 and Rawal-87 were evaluated for analysis of variability in seed storage proteins by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophorogram for each variety were scored and presence or absence of each band noted and was entered in a binary data matrix. Based on the data of SDS-PAGE gels cluster analysis was performed to check the variations among varieties. The overall result shows low degree of heterogeneity however different varieties reveal differential protein banding pattern. It is concluded that SDS-PAGE analysis of wheat endosperm protein is useful for evaluation of genetic variability and cultivars identification that help in wheat breeding program.

Key words: SDS-PAGE, glutenin proteins, wheat varieties, genetic variation and cluster analysis (UPGMA).

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. Now the global wheat production is concentrated mainly in Australia, Canada, China, European Union, India, Pakistan, Russia, Turkey, Ukraine and United States accounting for over 80% of world wheat production. Pakistan is the 8th largest wheat producer, with 3.17% of the world wheat production from 3.72% of the wheat growing area. Wheat in Pakistan is a leading food grain and occupies a central position in agriculture and its economy (Khan et al., 2007)

The seed-storage proteins of wheat represent an important source of food and energy, being also involved in the determination of bread-making quality (Cooke and Law, 1998). The 2 groups of wheat grain proteins that is gliadins and glutenins have been extensively studied and their genetics and biochemistry are relatively well known. Wheat varieties are qualified to different classes, which exhibit different applications and differ in quantity and

quality of proteins, mainly gluten. Gluten, comprising roughly 78 to 85% of total wheat endosperm protein, is a very large complex composed mainly of polymeric (multiple polypeptide chains) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (MacRitchie, 1994). Glutenins confer elasticity to dough, whereas gliadins are viscous and give extensibility to dough (Payne et al., 1984).

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. Bietz and Wall (1972) reported that 2 types of glutenin subunits were present in the wheat grains, the low molecular weight (LMW) (10 –70 KDa) and the high molecular weight (HMW) glutenin subunits (80-130 KDa). The polyacrylamide gel electrophoresis has been used to show that large size variation exists between LMW and HMW glutenin subunits and it has been suggested that deletions and insertions within the repetitive region are responsible for these variations in length (Benmoussa et al., 2000). Allelic variations of high molecular weight (HMW) subunits of glutenin in 185 cultivars of bread wheat have been described, where about 20 different major subunits were distinguished by SDS-PAGE (Payne et al., 1981). The high molecular weight (HMW) and low molecular weight (LMW) glutenin

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subunits from 13 Pakistani wheat varieties were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), in order to characterize the plant material and test the variability by constructing the dendrogram using the bivariety data recorded from the gels (Shuaib et al., 2007).

The availability of different classes of biochemical (storage proteins, isozymes) and molecular markers (RFLP, RAPD, microsatellites, etc.) makes the assessment of a direct measure of genetic variation possible. Protein storage markers and RFLPs were more informative for their ability to classify 2 pre-definite germplasm classes (Figliuolo and Zeuli, 2006). Identification and registration of bread wheat cultivars is mainly based on morphologic and physiologic characteristics. Even though these descriptors are useful, they are limited in number and may be affected by environmental factors. Biochemical and molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivars identification early in plant development. Biochemical characterization of cultivars is also useful to evaluate potential genetic erosion, identification of a variety and genetic diversity. The present work was carried out to characterize 15 Pakistani wheat varieties by using SDS-PAGE.

MATERIALS AND METHODS

Plant sample

The wheat grains of 15 Pakistani varieties were collected from cereal crop research institute (CCRI) Noshehra, Pakistan. The grains were stored in labeled glass bottle with insecticide tablets to ensure safety. The analysis was carried out at the Department of Biotechnology University of Malakand.

SDS-PAGE analysis

The variability of seed storage-proteins was analyzed by using SDS-PAGE (Damania et al., 1983) to investigate genetic diversity and characterized wheat varieties. The present study is the extension of previous experiment using different wheat Pakistani varieties (Shuaib et al., 2007). The grains were ground to fine powder and 10 mg was weighed in 1.5 ml microtube, 400 µl protein extraction buffer (Tris-HCl 0.05M (pH 8), 0.02% SDS, 30.3% urea, 1% 2-mercaptoethanol) was added to each micro-tube, kept overnight at 40°C and centrifuged at 13000 rpm for 10 min. The supernatant contain dissolved extracted protein ready for experiment purposes, which could be kept for longer time at 4°C.

Preparation of resolving gel (10% acrylamide gel)

The separating gel was prepared by mixing 3 ml (1.875M Tris-HCl Ph 8.80), 6.9 ml distilled water, 5 ml (5% acrylamide), 140 µl (SDS 10%), 90 µl (APS 5%) and 14 µl TEMED.

Preparation of stacking gel

Mixed 1 ml (0.6 M Tris-HCl pH 6.8), 7.2 ml distilled water, 1.66 ml

(30% acrylamide), 100 µl (SDS 10%), 80 µl (APS 5%) and 9 µl (TEMED) at the last.

Gel preparation

Glass plates were cleaned with 70% ethanol and fixed by using seal gasket and clips, separating gel was poured to the cell and layered with water. After 30 min distilled water was removed, stacking gel was added and comb was inserted into the stacking gel.

Sample loading and electrophoresis

Glass cabinet was fixed with electrophoresis apparatus, fill the electrophoretic trays with electrode buffer (25 Mm Tris, 0.1%SDS, 192 mM glycine). The wells was clean with running buffer and the sample was load (12 ul) and molecular weight marker 10 - 200 kDa (Fermentas protein ladder) (5 µl) at the bottom of each well using micropipette and connect the power supply at 80 volts.

Staining

After electrophoresis the gel was transferred to tray containing staining solution shake gently for 40 min, followed by destaining until the background of gel disappeared. The picture was taken by gel documentation with white light illuminator.

Data analysis

Electrophoregrams for each variety 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 (JSI) was calculated by the formula (Sneath and Sokal, 1973).

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

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. The similarity matrix generated was converted to a dissimilarity matrix (Dissimilarity = 1 - similarity) and used to construct dendrogram by the unweighed pair group method with arithmetic means (Sneath and Sokal, 1973). All analysis was carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993) and STATISTICA.

RESULTS

SDS-PAGE analysis

In this study high and low molecular weight glutenin subunits of different Pakistani wheat varieties were separated by SDS-PAGE electrophoresis for characterization and evaluation of genetic diversity among the given set of varieties. The molecular weight marker of 10 to 200 kDa was used for this purpose. Electrophorogram showing proteins banding pattern of different wheat varieties are presented in Figures 1 and 2. The result recorded from the figures show that the total number of bands varies from 10 to 22 in different varieties. The bands on the gel were divided into 2 categories, category first comprises of major bands, second involve minor bands based on the

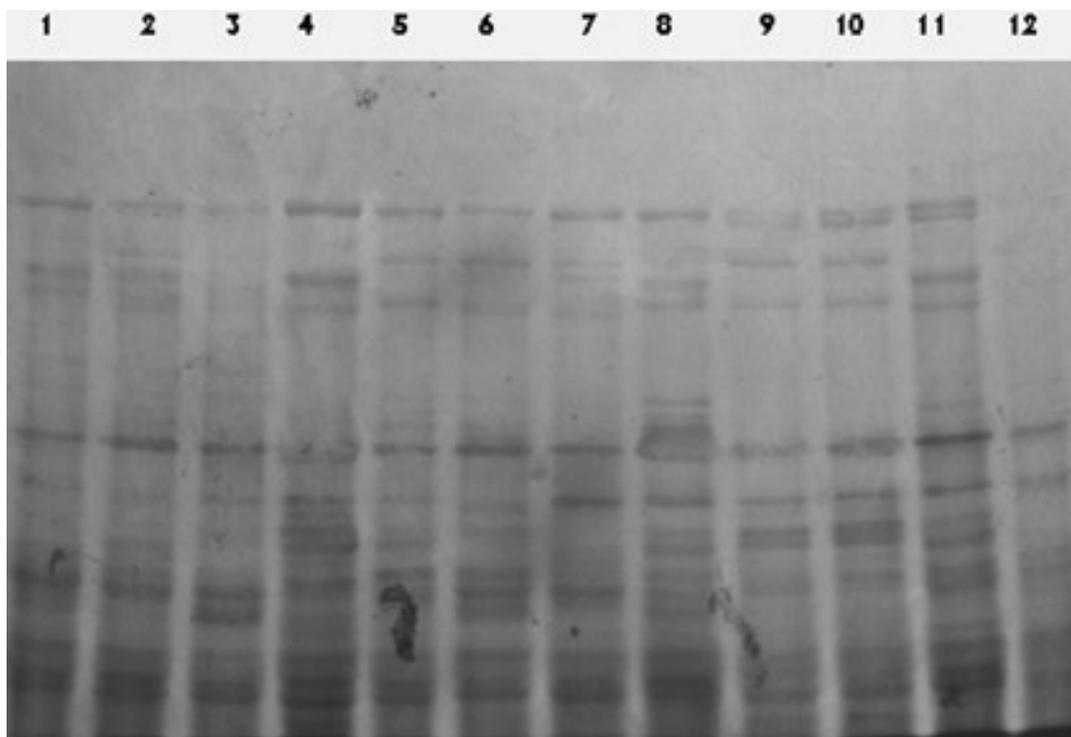


Figure 1. Electrophorogram showing banding pattern of wheat proteins of different varieties. 1= Nacozari-76, 2= Ouqab, 3= Tatar-96, 4= Bakhtawar-92, 5= Yecura-70, 6= Raj, 7= Bakkar, 8= Sulilman-96, 9= Pirsabak-05, 10= Maria, 11= Khyber-87, 12= Fakhr-e-Sarhad.

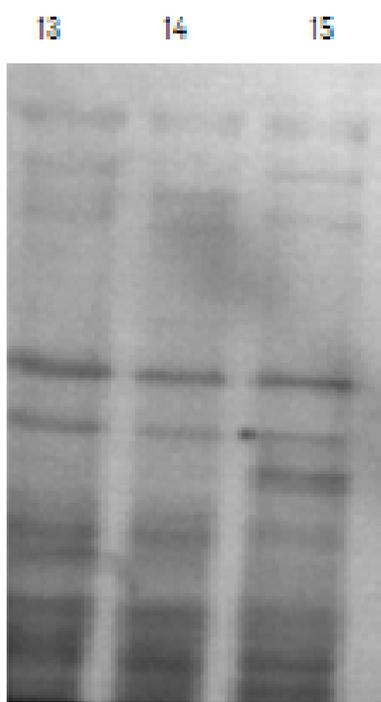


Figure 2. Electrophorogram showing banding pattern of wheat proteins of different varieties. 13 = Pirsabak-04, 14 = Inqilab-91, and 15 = Rawal-87.

glance (sharpness) of the bands. The major bands particularly band number 1, 2, 4, 6, 7 and 8 were common among most of the varieties but the other bands shows variation. The lane number 1, 12, 14, 15 and 16 representing varieties Nacozari-76, Fakhr-e-Sarhad, Inqilab-91, Rawal-87 and Ghaznavi-98, respectively, showing less number of protein bands as compared to the other varieties while there is no clear band in Pirsabak-04 represented by lane no 13.

Cluster analysis on the bases of SDS-PAGE

Cluster analysis was carried out on the results of SDS-PAGE using the computer software STATISTICA in order to investigate genetic variation among the given wheat varieties. The result of cluster analysis is given in the dendrogram (Figure 3) on the bases of linkage distance by the procedure of “unweighted pair group method with arithmetic means” (UPGMA). Cluster analysis sorting the wheat varieties into 2 major groups (lineages) at linkage distance 7 and which are further distributed into 11 clusters at linkage distance 4. The first group is composed of 4 clusters and there are 7 clusters in the second group. Among the lineage first, clusters 2, 3 and 4 each containing single variety Fakh-r-sarhad, Pirsabak-04 and Khyber-87 respectively while cluster 1 involves 2 varieties Rawal-87 and Inqilab-91. Similarly among the second

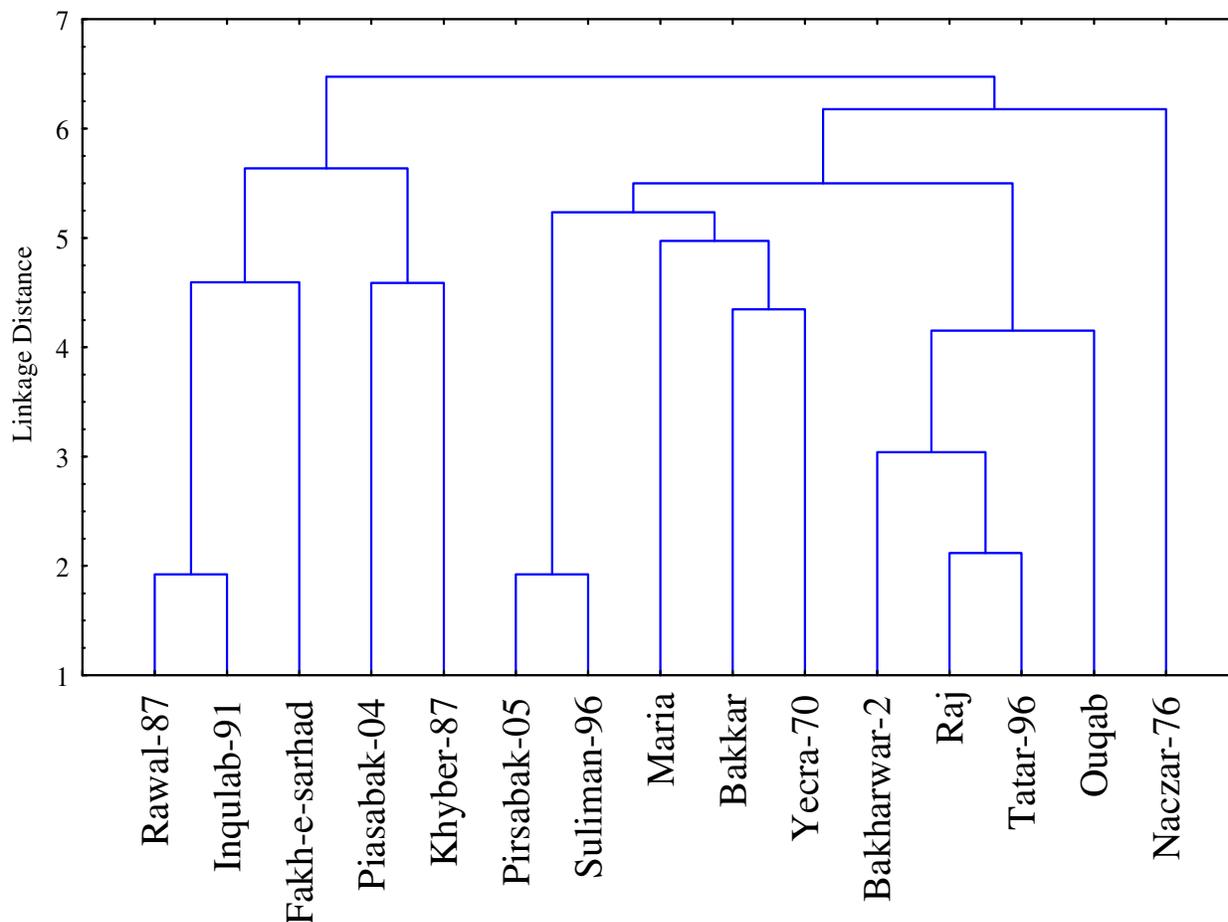


Figure 3. Dendrogram of fifteen different wheat varieties based on SDS-PAGE (UPGMA).

lineage, clusters 6, 7, 8, 10 and 11 each has single wheat variety namely Maria, Bakkar, Yecora-70, Ouqab and Naczar-76. Below 50% linkage distance cluster 5 consist of 2 varieties Pirsabak-05 and Suliman-96 while cluster 9 represent 3 varieties Bakhtawar-2, Raj and Tatar-96.

DISCUSSION

The results from SDS-PAGE analysis of wheat endosperm protein indicate differential banding pattern for different wheat varieties but the overall degree of variation is relatively low, similar result was reported in the previous study for 13 varieties of wheat (Shuaib et al., 2007). Electrophorogram showing proteins banding pattern of different wheat varieties are presented in Figures 1 and 2. The result recorded from the figures show that the total number of bands varies from 10 to 22 in different varieties. The diversity in high molecular weight protein subunits is the result of gene silencing in some varieties encoding these proteins (Lawrence and shepherd, 1980). The major bands having similar banding pattern in some varieties but minor bands show variations. There is also

difference in the density of common major bands.

The lane numbers 1, 12, 14, 15 and 16 representing varieties Nacozari-76, Fakhr-e-Sarhad, Inqilab-91, Rawal-87 and Ghaznavi-98, respectively, shows less number of protein bands as compared to the other varieties while there is no clear band in Pirsabak-04 represented by lane no 13. SDS-PAGE electrophoresis of 7 wheat varieties have been investigated including Inqilab-91 for HMW gliadin, however their varieties were different but the final result is correlated (Khan et al., 2002).

In this study dendrogram was calculated from the Jaccard similarity coefficient and un-weighted pair group method with averages constructed by high molecular weight (HMW) and low molecular weight (LMW) glutenin subunit bands. Genetic diversity of European spelts wheat was evaluated by constructing the dendrogram for HMW and LMW glutenin subunit bands (Xueli et al., 2005). The result of cluster analysis is given in the dendrogram (Figure 3) on the bases of linkage distance. The dendrogram revealed 2 major groups at linkage distance 7 and which are further distributed into 11 clusters at linkage distance 4. The first group is composed of 4 clusters and there are 7 clusters in the second group. Among the

lineage first, clusters 2, 3, and 4 each containing single variety Fakh-r-sarhad, Pirsabak-04 and Khyber-87, respectively, while cluster 1 involves 2 varieties Rawal-87 and Inqilab-91. Similarly among the second lineage, clusters 6, 7, 8, 10 and 11 each has single wheat variety namely Maria, Bakkar, Yecora-70, Ouqab and Naczar-76. Below 50% linkage distance cluster 5 consists of 2 varieties Pirsabak-05 and Suliman-96 while cluster 9 represents 3 varieties Bakhtawar-2, Raj and Tatara-96. The dendrogram as a whole represent low heterogeneity because most of the varieties were 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. However this help to classify varieties in different groups.

From the result of this study it is concluded that evaluation of genetic diversity and identification of wheat varieties by SDS-PAGE is easy and early approach and it is also useful for molecular weight analysis of wheat seed-storage proteins. The study could help in improving the efficiency of wheat breeding programs in cultivars development especially in developing world like Pakistan.

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