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Full Length Research paper

Characterization of wheat varieties by seed storage-protein electrophoresis

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Wheat grains of thirteen varieties were collected from different ecological regions of Pakistan. The variability of seed storage-proteins was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophorogram for each variety were scored and Jaccard's similarity index (JSI) was calculated. Genetic diversity of wheat was evaluated by constructing the dendrogram for high molecular weight (HMW) and low molecular weight (LMW) gluten subunit bands. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development especially in a developing country like Pakistan.

Key words: Wheat varieties, SDS-PAGE, genetic diversity and cluster analysis.

INTRODUCTION

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 and Law, 1998). According to solubility properties, they are traditionally classified into four classes; albumins, globulins, prolamins and glutelins. Gliadins and glutenins have been extensively studied and the 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 rou-ghly 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, respecttively (MacRitchie, 1994). Glutenins confer elasticcity to dough, where as gliadins are viscous and give extensibility to dough (Payne et al., 1984).

The pioneer studies of Bietz and Wall (1972) showed that two types of subunits were present; the low molecular weight (10,000 – 70,000 Da) and the high molecular weight glutenin subunits (80, 000 – 130,000 Da). High Molecular Weight-Gluten Subunits (HMW-GS) are encoded at the *Glu-1* loci on the long arms of group 1 chromosomes (*Glu-A1*, *Glu-B1,* and *Glu-D1*) (Payne et al., 1980). Electrophoretic studies have revealed appreciable polymorphism in the number and mobility of HMW-GS in both bread wheat (Lawrence and Shepherd, 1980; Payne et al., 1980) and pasta wheat (Branlard et al., 1989). Bread wheat could, in theory, contain six different HMW-GS but due to the "silencing" of some of these genes, most common wheat cultivars possess three to five HMW-GS.

The Low Molecular Weight-Gluten Subunits (LMW-GS) represents about one-third of the total seed protein and 60% of total gluten (Bietz and Wall, 1973). Despite their abundance, they have received much less research attention than the HMW-GS. This has been mainly due to the difficulty in identifying them in one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels. The LMW-GS are controlled by genes at the *Glu-A3*, *Glu-B3,* and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS, and 1DS, respectively. Glia-

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dins are heterogeneous mixtures of single-chained polypeptides with molecular weight range is 30, 000 to 75,000 Da. Due to extensive polymorphism, these proteins have been widely used for cultivar identification in hexaploid and tetraploid wheats (Payne et al., 1984). Allelic variants differ in the number, mobility, and intensity of their components and can be characterized through A-PAGE or even SDS-PAGE.

The polyacrylamide-gel electrophoresis has been used to show that large size variation exists among 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 variation of high molecular weight (HMW) subunits of glutenin in 185 cultivars of bread wheat have been described by Payne et al. (1981), where about 20 different major subunits were distinguished by SDS-PAGE. The high molecular weight (HMW) glutenin subunits from seven Pakistani wheat genotypes were also fractionated SDSPAGE, in order to characterize the plant material and test the variability within species (Khan et al., 2002).

The use of registered crop varieties makes their expeditious identification important; its significance is increased by the diversity of varieties in many important traits. Each variety is characterized by a specific set of traits that determine its use. Gliadins and glutens are genetic markers allowing the expeditious and objective identification of a variety, determination of its genetic constitution, and determination of some important characteristics and traits. Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers (Fufa et al., 2005). The present study was undertaken to evaluate the genetic diversity in gluten-subunits in set of thirteen Pakistani wheat varieties using SDS-PAGE.

MATERIALS AND METHODS

Plant sample

Grains of thirteen wheat varieties were collected from different ecological regions of Pakistan. The samples were stored in labeled glass bottle to ensure safety, in the Department of Biotechnology, University of Malakand, for analysis.

SDS-PAGE electrophoresis

The variability of seed storage-proteins was analyzed by using SDS-PAGE (Damania et al., 1983). The grains were ground to fine powder and 10 mg was weighed in 1.5 ml microtube. 400 µl protein extraction buffer (Tris-HCl 0.05 M, 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. Gel preparation, running and staining were standard procedures.

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 a 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 for window 98.

RESULTS

Genetic diversity evaluation

In this study SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among different Pakistani wheat varieties. The electrophorogram showing proteins banding pattern of different wheat varieties are given in Figures 1 and 2. A total of 21 bands were obtained among which bands number 1, 2, 4, 7, 12, 13 and 20 were common in all varieties but the other bands show variation. The results from comparison with standard molecular weight marker reveal that wheat variety Tatara, Bakhtawar-92, Bhakkar-01 and Gaznawy contain 7 subunits in range of 50 -120 kDa while Fakhr-e-Sarhad and Zakht gave 11 and 9 subunits, respectively. At low molecular weight range of 15 - 50 kDa there are 6 -1 2 subunits reflecting less diversity but variety Chudry-97 and Wafaq-01 showed more variation than the rest of varieties. The varieties Watan, Gandam 711, Fakhre-Sarhad, Zakht and Chudry-97 appear to contain 85-kDa gluten.

Cluster analysis

Cluster analysis of wheat grain storage proteins was performed on the results of SDS-PAGE using the software STATISTICA to find out the diversity among the given wheat varieties. The results of cluster analysis are given in the dendrogram (Figure 3) on the bases of linkage distance (Euclidean distances) Table 1. The diagram revealed two main groups L1 and L2; the group L1 has only one variety Wafaq-01 and L2 comprised the remaining 12 wheat varieties. At Euclidean distance of 2.5, all the varieties show similarity with one another, and distributed into two categories; one containing the variety chudhry-97 while the second is further divided into sub-

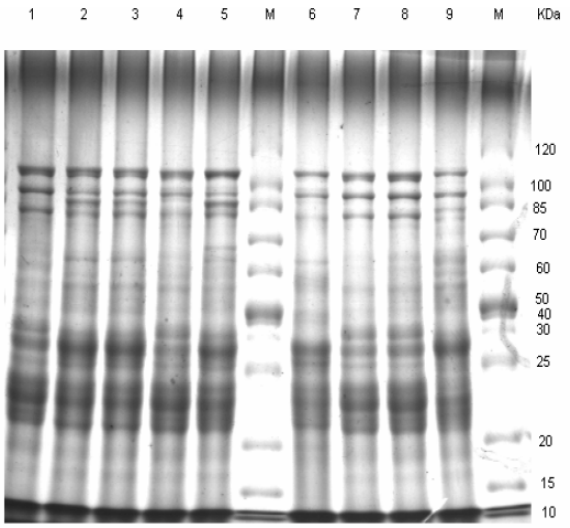

Figure 1. Electrophorogram showing banding pattern of wheat proteins and molecular weight marker. 1 = Tatara, 2 =Watan, 3 =Gandam 711, 4 =Bakhtawar-92, 5 =Fakhre-Sarhad, 6 =Bhakkar-01, 7 = Ghaznawy, 8 = Saleem-2000, and 9 = Zakht.

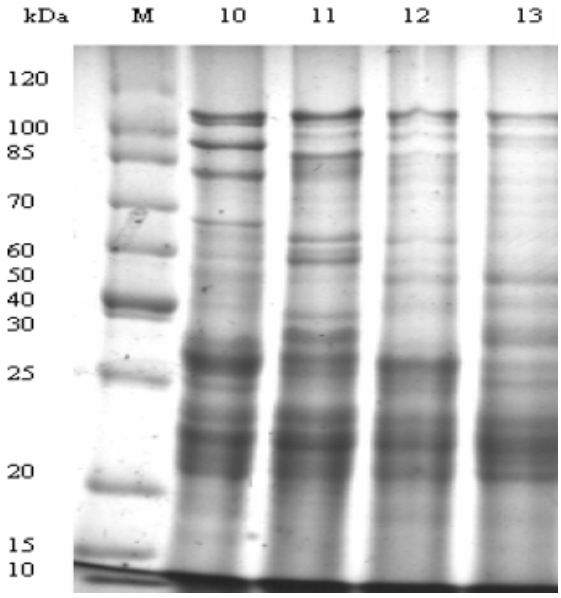

Figure 2. Electrophorogram showing banding pattern of wheat proteins and molecular weight marker. 10 = Gandam-2002, 11 = Chudry-97, 12 = Inqilab-91, and 13 =Wafaq-01.

groups in which one include only one variety Fakhre-Sarhad. At linkage distance 2 the Zakht variety show more than 50% distance with the rest of the varieties. Below the linkage distance of 1.5 only the variety Gandam-2002 and Watan are in one group and Saleem-2000, Bhakkar-01, Bakhtawar-92 and Tatara are in the other group showing less than 50% linkage distance. The

Table 1. Molecular weight analysis of wheat varieties.

Protein Type	Molecular Weight (kDa)	*Wheat Variety*												
		1	2	3	4	5	6	7	8	9	10	11	12	13
HMW-GS	120	0	0	0	0	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0	0	0	0	0	0
	85	0	1	1	0	1	0	0	0	1	0	1	0	0
	70	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	1	1	1	1	1	1	1	1	1	1	1	1	0
LMW-GS	50	1	1	1	1	1	1	1	1	1	1	1	1	1
	40	0	0	0	0	0	0	0	0	0	0	1	0	0
	30	1	1	1	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	1	1	1	1	1	1	1	1	1	1	1	1	1
	15	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0

V1 = Tatara, V2 =Watan, V3 = Gandam 711, V4 = Bakhtawar-92, V5 = Fakhre-Sarhad, V6 = Bhakkar-01, V7 = Ghaznawy, V8 = Saleem-2000, V9 = Zakht, V10 = Gandam-2002, V11 = Chudry-97, V12 = Inqilab-91, and V13 = Wafaq-01.

varieties Saleem-2000 and Bakhtawar-92 show 100% similarity.

DISCUSSION

According to the results of the SDS-PAGE, the overall pattern of seed storage-proteins shows low degree of heterogeneity. The diversity 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 seven wheat varieties (including Inqilab-91) 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 (Khan et al., 2007; Zeb et al., 2006) this study present 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 3. 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 proteins level 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

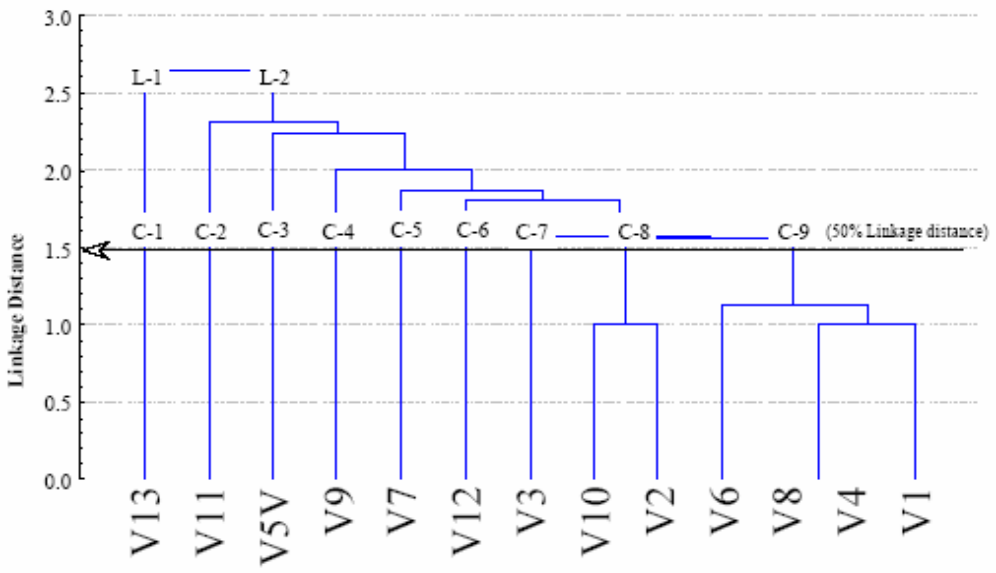

Figure 3. Dendrogram of Thirteen Wheat Varieties Based on SDS-PAGE (UPGMA). V1 = Tatara, V2 =Watan, V3 = Gandam 711, V4 = Bakhtawar-92, V5 = Fakhre-Sarhad, V6 = Bhakkar-01, V7 = Ghaznawy, V8 = Saleem-2000, V9 = Zakht, V10 = Gandam-2002, V11 = Chudry-97, V12 = Inqilab-91, and V13 = Wafaq-01.

a highly selected trait. The variety Saleem-2000 and Bakhtawar-92 show 100% similarity with one another representing a possible duplication of the variety with different names in different resource laboratories.

It is therefore concluded that seed storage protein profiles could be useful markers in cultivar 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.

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