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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Fractionation and Characterization of Seed Storage Proteins from Different Wheat Varieties Cultivated in Sindh on SDS-PAGE Electrophoresis

Shaista Khan, A.N. Memon, Allah Bux Ghanghro and Ibtessam Tahir
Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan

Abstract: Wheat is most widely cultivated cereal and important source of food and protein worldwide. Wheat is a staple diet containing most of the ingredient, for wide range of food. The unique property of wheat flour is to form dough and this property is due to gluten required for making preparation of chapatti and bread. Fractionation and characterization of seed storage proteins of fourteen wheat varieties cultivated in sindh was carried out by Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Wheat grains of fourteen (14) varieties were collected from different region of sindh (Pakistan). Electrogram of each variety were calculated by Jaccard's Similarity Index (JSI). Genetic diversity of different sindh wheat varieties was measured by constructing dendogram of High Molecular Weight (HMW) and Low Molecular Weight (LMW) gluten sub unit bands. The dendrogram profile data showed the variation in the number and position of bands from one variety to the other, while some bands are common.

Key words: SDS-PAGE, genetic diversity, UPGMA

INTRODUCTION

Wheat is most widely cultivated cereal and important source of food and protein worldwide contain most of ingredients required for calories and health.

Wheat is the basic raw material for preparation of chapatti and bread (Haridas Rao *et al*., 1986).

Wheat endosperm protein were among the first protein to be studied in 1745 reported the isolation of gluten (Beccari, 1745).

According to solubility, wheat proteins are classified into four classes: albumin, globulin, prolamin and glutens, gluten comprising 78-85% of total protein and the large complex mainly polymeric and monomeric protein are known as glutenin and gliadins (MacRitchie, 1994).

Wheat contains two subunits, one is Low Molecular Weight (LMW-GS) (10,000-70,000 Da) and the High Molecular Weight (HMW-GS) glutenin subunits (80,000-130,000 Da) (Payne *et al*., 1980).

Gluteins are soluble in alcohol under reducing condition and in diluted alkali.

Gliadins form a large protein family in which α/β-γ ω gliadins can be distinguished (Woychik *et al*., 1961).

Both high and low molecular weight glutenin subunit play a major role in determining the viscoelastic properties of wheat flour.

Glutenin comprises large molecules formed from polypeptide chains linked by disulphide bond, which are through out to have different molecular weight ranging from few hundred thousand to many million (MacRitchie, 1994).

Glutein protein with High Molecular Weight (HMW) (10-140k) and (LMW) (30-50k) protein subunit determined by Sodium Dodecyl Sulfate (SDS-PAGE) (Bietz and Simpson, 1992).

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.

Allelic variation of High Molecular Weight (HMW) subunits of glutenin in 185 cultivars of bread wheat have been described by (Payne *et al*., 1981), The High Molecular Weight (HMW) glutenin subunits from seven Pakistani wheat genotypes were also fractionated on SDSPAGE, in order to characterize the plant material and test the variability within species (Khan *et al*., 2002).

MATERIALS AND METHODS

The variability of protein were analyzed by using SDS-PAGE electrophoresis according to the method of Laemmili (Laemmli, 1970).

Sample preparation: Took 40 µl of extracted protein in sample buffer (0.5M tris HCl, pH 8), 5% SDS, 30% glycerol, 5% 2-Mercaptoethanol and 0.06% BPB) was added to each tube, boil the sample for 5 min than ice cold for 5 min and centrifuge at 14000 rpm for 5 min at 4^{0}C. The supernatant contain dissolved extracted protein readily for experimental purposes (Prepare each time fresh sample).

Corresponding Author: Shaista Khan, Institute of Biochemistry, University of Sindh Jamshoro, Pakistan

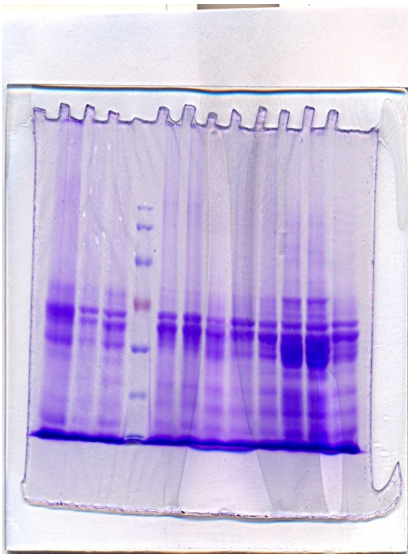

Fig. 1: Electrogram showing banding patterns of different molecular weight. 1- Kiran, 2- Amber, 3- Sindh, 4- Sindh, 5- Sarsabz, 6- Khirman, 7- Jauhar, 8- TJ78, 9- Anmol, 10- Local

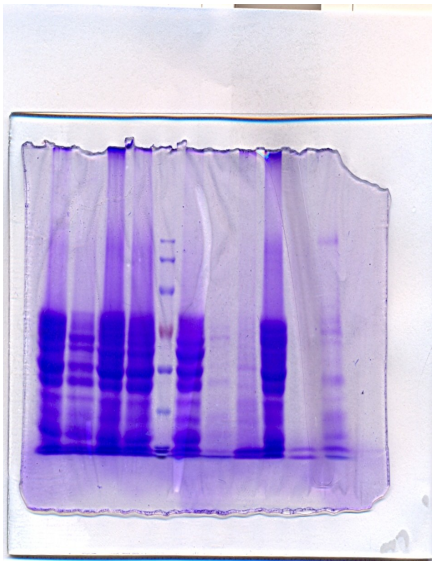

Fig. 2: Electrogram showing banding patterns of different molecular weight 11- GP526, 12- GP256, 13- Marvi, 14- Soghat, M = Molecular weight marker

Preparation of resolving gel (15%): The separating gel was prepared by mixing 2.5 ml (1.5M tris HCl pH 8.8), 1.4 ml distilled water, 5.0 ml of (30% Acrylamid bis), 1.0 ml of (10% APS) and 40 µl TEMED.

Preparation of stacking gel (4.5%): The staking gel was prepared by mixing 0.5M tris HCl pH 6.8, 1.8 ml distilled water, 5.0 ml of 30% Acrylamid bis, 10% APS, 1% SDS and 40 µl TEMED.

Gel preparation: Glass plate, silicon tube and comb was cleaned with ethanol and set a silicone tube and clip. Resolving gel was poured up to 6 cm and layered with water, for intercepting the air bubbles, water was removed after a clear intercept appear between two layers then pour staking gel solution, comb was inserted into the staking gel.

Electrophoresis: Glass plate was fixed in electrophoresis apparatus and fill the upper tank with SDS running buffer then load the sample (20 µl) and molecular weight marker (10-180KDa Fermentus pre staining protein ladder) Connected the power supply at 30mA (300V constant current) Stop when PBS line reach to lower part of the gel.

Staining and decolorization: After electrophoresis gel was put into staining solution (0.25% CBB-R 250/2) for 30-40 min with continuous shaking, wash the gel with water and put into decolorizing solution (7% acetic acid, 5% Methanol) until the back ground of gel disappear Gel was put into the gel documentary system to find out the molecular weight of different varieties.

Data analysis: Electrophoretic electrogram of each variety was measured in the presence (1) or absence (0) of band in each wheat varieties of sindh. Presence and absence of band were based on the binary data matrix and was calculated by Jaccard`s similarity index (Sokal, 1973) by using following formula:

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

Where, W is the common bands present having a same mobility, A is the number of band in type A, B is the number of band in type B (A and B show the 0 and 1).

RESULTS

Genetic diversity: This study was performed in order to determine the molecular subunits and investigate the genetic diversity among the different varieties of wheat cultivated in Sindh. Electrogram Fig. 2 shows the banding pattern of different wheat varieties.

Electrogram show the variation in number of bands in which 35KDa and 50 KDa were common, in each variety while 30kDa, 45KDa, 58KDa and 66KDa show the slight variation in the molecular weight, 95KDa is only present in GP-526 and 85 KDa protein is only present in khirman Variety of sindh.

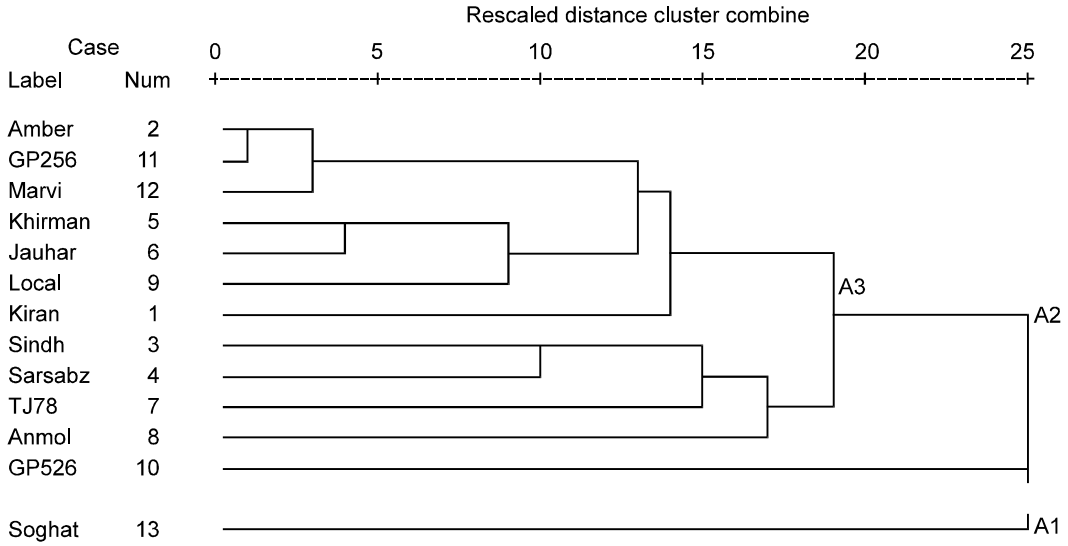

Fig. 3: Dendrogram using average linkage (Between groups). 1- Kiran, 2- Amber, 3- Sindh, 4- Sindh, 5- Sarsabz, 6- Khirman, 7- Jauhar, 8- TJ78, 9- Anmol, 10- Local, 11- GP526, 12- GP256, 13- Marvi, 14- Soghat

Table 1: Molecular weight analysis of different wheat varieties cultivated in sindh Pakistan

		Wheat varieties													
	M	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HMW-GS	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	97	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	95	0	0		0	0	0	0	0	0	0	1	0	0	0
	85	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	82	0	0	1	1	0	0	1	1	0	0	0	0	0	0
	80	1	1	0	0	1	1	1	1	0	0	1	1	1	1
	75	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	66	1	1	1	1	1	1	1	0	1	1	1	1	1	1
	64	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	60	1	0	0	0	0	0	1	0	0	0	0	0	0	0
	58	0	1	0	1	1	1	1	1	1	1	1	0	1	1
	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	54	0	1	0	0	0	0	0	0	0	0	0	1	1	1
	50	1	1	1	1	1	1	1	1	1	1	1	0	1	1
LMW-GS	47	1	0	1	0	0	1	1	0	1	1	1	1	0	1
	45	1	1	1	1	1	1	0	1	0	1	0	1	1	1
	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	38	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	35	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	30	1	0	1	1	1	1	1	1	1	1	1	0	1	0
	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	17	0	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	0

1 -Kiran, 2- Amber, 3- Sindh, 4- Sindh, 5- Sarsabz, 6- Khirman, 7-Jauhar, 8-TJ78, 9-Anmol, 10-Local, 11-GP526, 12-GP256, 13- Marvi, 14-Soghat

Cluster analysis: Cluster analysis of different varieties of wheat proteins was preformed on the basis of results of SDS-PAGE by using Hierarchical clustering on the soft ware SPSS 16.0.

The goal of cluster analysis to form similar and dissimilar group present in wheat varieties based on (UPGMA). The diversity of different wheat varieties shown in dendrogram in Fig. 3 on the basis of linkage distance.

The dendrogram show that there are three main groups A1, A2, A3 in A1 only one variety Soghat and A2 also contain only one variety GP-526 while A3 comprised remaining 10 varieties. A3 is further divided into two sub group P1 and P2, P2 contains Anmol, TJ-78, Sarsabz and Sindh while P1 comprised two subgroups T1 and T2, T1 contain only one Variety Kiran while T2 contain Jauher, Khirman, Marvi, GP-256 and Amber.

The linkage distance of all varieties is 25. Variety of GP-526, Anmol, TJ-78 and Sarsabz show the 100% similarities.

DISCUSSION

For characterization of wheat proteins, on SDS-PAGE electrophoresis of different varieties was done in order to screen out the molecular weight of gluten subunit and genetic diversity of varieties of sindh Pakistan.

Documentation of gel on gel doc system provided the information about banding pattern of different wheat varieties Fig. 1 and 2 show the variation in number of bands in which 35KDa and 50 KDa are common in all varieties but other bands are different.

The ranging of gluten proteins according to the molecular weight found 20KDa-97KDa of standard molecular weight marker (Farmantus page ruler ranging 10KDa-170KDa pre stained marker).

Gluten protein of sindh wheat varieties categorized the molecular weight into HMW-GS and LMW-GS, in the LMW-GS have bands 30 KDa, 32 KDa, 53 KDa, 45 KDa and 47 KDa while HMW-GS have bands 50 KDa, 54 KDa, 58 KDa, 66 KDa, 80 KDa, 97 KDa and 98 KDa.

According to the result of SDS-PAGE on different varieties of wheat cultivates in Sindh show the low degree of heterogeneity, there are variation in number of bands and position of bands (Shuaib and Alam Zeb, 2007).

The diversity in high molecular weight proteins subunit is the result of gene slicing in some variation encoding these proteins (Lawrence and Shepherd, 1980).

SDS-PAGE of wheat varieties has been previously investigated, however their Variety is different but result is correlated.

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