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Investigation of Seed Storage Proteins (Glutenin) of Wheat on Sds-Page Electrophoresis

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Abstract: Wheat (*Triticum aestivum L*) varieties were analyzed for quantification and characterization using SDS-PAGE technique. ANOVA result show the significant difference ($P<0.05$) found in glutenin concentration among the selected wheat varieties having ranged (27.76±0.05 - 22.46±0.02). The highest concentration of glutenin found in variety Marvi although lowest concentration seen in variety Amber. The electrophoretic patterns of molecular weight of glutenin ranged between 17- 170 kDa, along with distribution of HMW-GS having molecular weight ranged 17 kDa-66 kDa and LMW-GS with molecular weight in between 66 kDa 170 kDa. The highest banding pattern was seen in variety Kiran and Khirman with 14 bands whereas lowest banding pattern seen in varieties Marvi having a 08 bands. 17 kDa proteins are common in almost all the varieties except Kiran, Amber, Sindh-90, Jauhar, Anmol, TJ-83 and GP-205 however 35 kDa proteins are common in all varieties. But there is no relation found in concentration and banding pattern of glutenin protein($r = -0.272$). Cluster analysis show the similarity and dissimilarities of albumin proteins pattern was carried out on the results of SDS-PAGE using the statistics software SPSS 19. Cluster analysis based on linkage distance by the procedure of Unweighted Pair Group Method with Arithmetic average (UPGMA). Cluster analysis categorize the wheat varieties into 2 main groups at linkage distance 25 and which are further scattered into 10 clusters at linkage distance 10. This study will be helpful for the future explored the wheat variety beneficial for baking products and for breeding of new cultivars with high nutritional benefits for consumers.

Keywords; Glutenin, SDS-PAGE, ,wheat varieties, Unweighted Pair Group Method with Arithmetic average (UPGMA).

1. INTRODUCTION

The word Protein derive from Greek word means *proteios* is measured as the most vital nutrient for humans and animals. Wheat proteins are classified into four classes on the basis of solubility into various solvent systems Albumins (soluble in water), Globulins (insoluble in pure water however soluble in dilute NaCl), Gliadins (soluble in 70% ethyl alcohol), Glutenins (soluble in dilute acid). Prolamin of wheat are the major component of gluten, the properties of which determine the quality of wheat flour for various technological processes including bread making (Peter, 1990) the 75% of the total protein content covered by the storage proteins glutenins and gliadins and these proteins are mainly located in endosperm. Gliadins and glutenins are technologically active protein involve in dough making. Glutenins and Gliadins do not possess any enzyme activity but have an important role in dough formation because these proteins retain gas and provide the spongy character of baking products. Glutenin is the class of wheat flour proteins having high molecular weight (mol wt) and formed by inter chain disulfide cross linking of several distinct polypeptides. Glutenin seems to be the most important determinant of structure and elasticity in bread dough and also of wheat flour quality, Differences in performance between wheat varieties may be due largely to variations in glutenin (Bietz *et al*., 1973). Glutenin polymers are made up of single polypeptides linked through intermolecular disulfide bonds that contain about 45% of total protein in grain end sperm Bietz *et al*., (1992). Glutenin and gliadin are complex proteins separated in to monomeric gliadin having molecular weight between 30-80 kDa and heterogeneous mixture glutenin polymers with molecular weights vary from 80 kDa to several thousand (Kasarda 1989). The glutenin fraction is formed of a concoction of polymers, high-molecular weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) on the basis of their SDS-PAGE electrophoresis mobility. The seed storage protein Glutenin is divided into two assembly high-molecular weight (100-140 kDa) and low molecular weight (30-55 kDa) based on electrophoretic mobility Bietz *et al*., (1992).

2. MATERIALS AND METHODS

Fourteen wheat varieties were collected from the Nuclear Institute of Agriculture Tandojam, namely Kiran-95, Amber, Sindh-90, Sarsabz, Khirman, Jauhar-18, Mehran-89, Anmol-91, TJ-83, GP-256, GP-205, Marvi-2000, Soghat-90, Local (Unknown).

Purification of Insoluble proteins (Glutenin)

Took residue from globulin extract , precipitated and washed with distilled water (3X) then incubated over night with 1 mL with 50% isopropanol, then put in sonicator (100 w for 30 min) after that centrifuged for 20 min at 14000 rpm, separated the glutenin extract frozen and stored at -40 ^{0}C

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Electrophoresis:

The electrophoresis is a technique in which migration of charged particles under the influence of an electric current, most of biological molecules such as protein and amino acid can be forced to migrate under electrical influence at any given pH.

Gel Electrophoresis (SDS-PAGE)

The variability of protein was analyzed by using SDSPAGE electrophoresis according to the method of Laemmili (1970).

Sample preparation

Took 40 µl of extracting 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 4oC. The supernatant contain dissolved extracted protein readily for experimental purposes (Prepare each time fresh sample).

Acrylamide solution

30g acrylamide monomer, 0.8g bisacrylamide were dissolved in distilled water and final volume was made 100mL with distilled water in a volumetric flask. The solution was filtered and stored at 4ºC in dark bottles.

Resolving gel buffer:

36.6g Tris base and 40mL of 0.1M HCl were mixed, adjusted the pH to 8.8 and made the volume 100mL with distilled water and store at room temperature.

Stacking gel buffer:

6.06g Tris base was dissolved in 40mL of distilled water adjusted the pH to 6.8 with 0.01M HCl and made the volume 100mL with distilled water and store at room temperature.

Sodium Dodecyl Sulphate (SDS) Solution

10g SDS was dissolved in distilled water and made the volume upto100ml with water.

Ammonium per Sulphate (APS) Solution (1.5%)

0.15g APS was dissolved in 10mL of distilled water (prepare fresh before use)

Reservoir Buffer

3.03 g Tris, 14.4g glycine and 1g SDS were dissolved in distilled water, and made final volume up to 500 mL (use for two or three run only).

Staining Solution and Destining Solution

Dissolved 1.1g Coomassie brilliant Blue R 250 in 40mL acetic acid than add 40ml of 2-propanol dissolved in distilled water. The final volume (100 ml) was made with distilled water.

For distaining solution 30mL methanol and 10mL acetic acid were added in 60 mL distilled water, the final volume (100 mL) was made with distilled water.

Gel preparation

Glass plate, silicon tube and comb were 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 stacking gel solution, the comb was inserted into the stacking gel.

Loading of the sample:

Remove the glass plates from the stand and smoothly remove spacer and transferred the plates to electrophoretic tank (Atto Company), Fixed the plates with the clamp. Filled the lower portion of the tank with reservoir buffer, than Load (20 µl) sample (10ml tracking dye, 50µl sample and 50µl of sample buffer were mixed and the sample protein was denatured by heating at 100ºc for 2 min before being applied to the gel) in sample well by using micro syringe, than filled the upper reservoir with buffer till it comes in contact with the sample wells Connect the electrophoresis apparatus to the power supply ,Switch on power pack and adjust current 25mA (300V constant current), allowed to run till the tracking dye (bromophenol blue) reached to the lower part of the gel.

Interpretation of Bands

Gel documentary system (Bio-Rad) were used for visualizing and counting of the protein bands. The first well of each gel, the proteins engaged as the molecular weight markers (Kilo Daltons) ranging from 10-180 kDa (SDS-PAGE MW standards, Fermentus pre staining protein ladder).

Data analysis

Electrograme of each variety was measured in the presence (1) or absence (0) of band in each wheat variety of Sindh. Presence and absence of the band were based on the binary data matrix and was calculated by Jaccard`s similarity index (Sneath and Sokal, 1973) by using the following formula: S = W (A+B-W)

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

3. RESULTS ANS DISCUSSION

Glutenin is component of seed storage protein and main part of gluten provides the elasticity in dough formation (Belderok *et al*., 2000). Glutenins proteins

have an important role in intensification wheat dough by conferring elasticity; whereas gliadins give to the viscous properties of dough by confer extensibility (Shewry and Halford, 2003). Glutenins are heterogeneous mixtures of proteins comprise subunits connected by disulphide bonds even though all wheat seed storage proteins are a fraction of the gluten, the glutenin polymers are deliberate as the most important factor of viscoelastic property (Gupta *et al*., 1992).

The glutenin contents of different wheat varieties of Sindh presented in Table 1**,** which ranged (21.62 - 27.76%). The wheat variety possessed significantly high glutenin content in Marvi (27.76±0.05) followed by Sarsabz (26.81±0.05), TJ 83 (26.46±0.01), Sindh-90 (26.26±0.01), Anmol (26.03±0.05), GP-256 (25.43±0.03), Local variety (25.39±0.09), GP-205 (24.52±0.02), Khirman (24.32±0.03), Jauhar (23.35± 0.09), Mehran (23.22±0.02), Kiran-95 (22.53± 0.05) However the lowest glutenin content found in variety Soghat-90 (21.62±0.01) and Amber (22.46± 0.02).

Similarly finding of different scientist and researchers has also been reported the glutenin content, Halford *et al*., (1992) reported that Glutenins make a contribution up to 12% of the total protein in the wheat endosperm, Zilic S. *et al*., (2011) reported that glutenin concentration ranged 56.25-64.48% of total protein and Bietz J.A *et al*., (1992) described that glutenin polymers contain about 45% of total protein in grain endosperm, Fu *et al*, (1996) reported glutenin content as high as 48.0-52.1% of total protein.

The present study results of the glutenin content are in agreement with the finding of Gafuroua (2002) and Stone and Savin (1999). Who reported the glutenin content ranged 23.7-27% while Stone and Savin (1999) reported the glutenin account 23% of total protein.

Fractionation and banding pattern depicted in (**Fig. 1,2 and 3)** showed the banding pattern in different wheat varieties of Sindh. **(Table 1)** show the bands number of selected wheat varieties. The highest banding pattern found in wheat variety kiran-95 and khirman varieties whereas lower banding pattern seen in Marvi and GP-205.

The molecular weight of glutenin polypeptide is divided into two classes, the Low molecular weight glutenin subunits (LMW-GS) and High molecular weight glutenin subunits (HMW-GS). The glutenin polypeptide fractionation detected in present study ranged 17kDa to 170 kDa, and divided into two regions, LMW-GS of present study ranged 17 kDa-66 kDa, while HMW-GS ranged varying 67 kDa -170kDa.

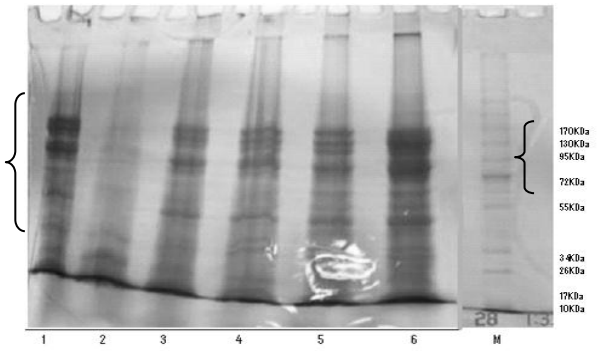
Kiran-95 and Khirman variety contain 14 bands, the band ranged in Kiran-95 have two region the LMW-GS region are 26kDa - 66 kDa while HMW-GS region are 70 kDa- 170 kDa. And Khirman wheat variety have LMW region ranged 17kDa- 66 kDa while LMW region 72kDa- 170kDa. Sarsabz and Soghat wheat varieties contain 13 band numbers, Sarsabz have LMW region are 17kDa- 55kDa and HMW region ranged are 72kDa- 170kDa, however Soghat variety have LMW ranged 17kDa- 65kDa even as HMW are 70kDa - 130kDa. TJ-83 is the only variety which contains 12 bands having LMW 20kDa- 55kDa while HMW glutenin molecular weight is 70kDa- 160kDa. Mehran, Anmol, GP-256 Local wheat variety contain 11 bands. The bands ranged in Mehran wheat variety have LMW region ranged 17kDa- 55kDa and HMW region 70kDa-160kDa. Anmol wheat variety have LMW region ranged 30kDa - 60 kDa although HMW have 70kDa-160kDa. GP-256 wheat variety has LMW region are 17kDa - 60kDa and HMW are 70kDa- 95kDa. Local wheat variety bands with molecular masses ranged 17kDa- 170kDa. Sindh and Jauhar wheat variety have 10 bands. Banding pattern in Sindh variety have LMW region ranged 26kDa- 66kDa whereas HMW are 75kDa - 170kDa. Jauhar variety have LMW region ranged 26kDa- 66kDa and HMW region are 70kDa- 130kDa. Amber variety contain 10 bands in which LMW-GS region are 22kDa- 58 kDa whereas HMW-GS region are 90kDa- 130kDa Similarly GP-205 wheat variety contain 09 bands, LMW region have 20kDa-55kDa while HMW region have molecular weight ranged 70kDa- 90kDa. Marvi wheat variety contain lowest band number having 08 bands have LMW region 17kDa- 55kDa while HMW region 70kDa- 85kDa.

Wieser (2007) reported that the HMW glutenin ranged 83-88kDa and LMW ranged 67-74kDa. Gianibelli *et al*., (2001) reported that LMW of glutenin ranged 55-70kDa and HMW ranged 80-130kDa. MacRitchie, (1992) reported that glutenins made up of LMW and HMW, the range of LMW 31- 45kDa while HMW was 100kDa. Zhang *et al*., (2004) found that the low molecular weight glutenin subunits (LMW-GS) molecular weight ranged from 32.3 kDa to 67.4 kDa of different wheat varieties.

Our results are consistent with the findings of Gianibelli *et al*., (2001) and Bietz *et al*., (1992) who found that the LMW glutenin ranged 30-55kDa and HMW gultenin ranged 100-140kDa.

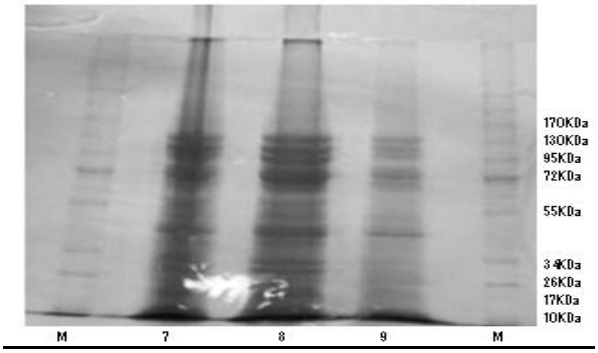
Cluster analysis shown in (**Table-2) (Fig.4)** described the similarity and dissimilarities of glutenin proteins in selected wheat varieties of Sindh on SDS-PAGE using the software SPSS 19 .The result of cluster analysis based on linkage distance by the procedure of

UPGMA. Cluster analysis categorize the wheat varieties into 2 main groups at linkage distance 25 and which are further scattered into 10 clusters at linkage distance 10. The first group is consisting of 05 clusters and second group also contain 05 clusters. Among the lineage first cluster contain 3, 4 and 5contain single variety TJ 83, Marvi and Soghat while cluster 2 contain two varieties Mehran and Anmol whereas cluster 1 contain three varieties GP-256, GP-205 and Local variety. Similarly among the second group lineage cluster 6, 7, 9 and 10 contain single variety Amber, Jauhar, Kiran-95 and Khirman while cluster 8 contain two varieties Sindh-90 and Sarsabz.



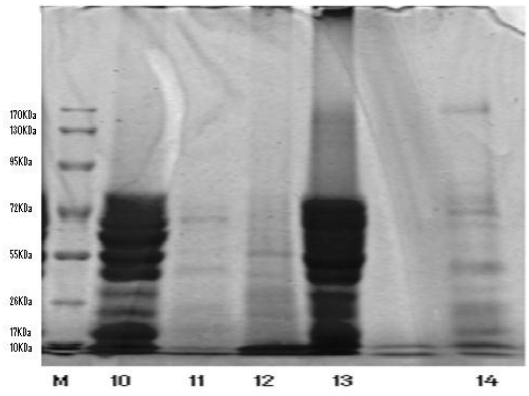
1-Kiran,2- Amber, 3- Sindh, 4- Sarsabz, 5- Khirman, 6-Jauher

Fig. 1. Banding pattern of Insoluble protein (Glutenin) fractions in selected wheat varieties of Sindh.



7-Mehran, 8-Anmol, 9- TJ.83,

Fig.. 2 : Banding pattern of Insoluble protein (Glutenin) fractions in selected wheat varieties of Sindh.



10-GP.256, 11-GP.205, 12- Marvi, 13-Soghat, 14- Local

Fig. 3 : Banding pattern of Insoluble protein (Glutenin) fractions in selected wheat varieties of Sindh.

Table 1. Concentration (%) of glutenin with bands numbers of selected varieties of Wheat cultivated in sindh.

Wheat Varieties	**Glutenin content (%)**	**Band numbers in Glutenin protein in Sindh Wheat varieties**	Correlation
Kiran-95	22.53±0.05	14	
Amber	22.46±0.02	09	
Sindh-90	26.26±0.01	10	
Sarsabz	26.81±0.05	13	
Khirman	24.32±0.03	14	
Jauher-18	23.35±0.09	10	
Mehran-89	23.22±0.02	11	-0.27252
Anmol-91	26.03±0.05	11	
TJ-83	26.46±0.01	12	
GP-256	25.43±0.03	11	
GP-205	24.52±0.02	09	
Marvi	27.76±0.05	08	
Soghat	21.62±0.01	13	
Local(unknown)	25.39±0.09	11	

Table 2. Cluster pattern of glutenin protein fractions in selected wheat varieties of Sindh.

Mw KDa	Kiran-95	Amber	Sindh-90	Sarsabz	Khirman	Jauher-18	Mehan-89	Anmol-91	TJ-83	GP-256	GP-205	Marvi	Soghat	Local
170	1	0	1	1	1	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	1	1	1	0	0	0	0	0
130	1	1	1	1	1	1	1	1	1	0	0	0	0	1
95	0	0	1	1	1	1	1	1	1	1	1	0	0	0
90	1	1	1	1	0	1	0	0	0	0	0	1	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	1	0
80	1	0	0	0	0	0	0	0	1	0	0	0	1	1
78	0	0	0	1	0	0	0	0	0	0	0	0	0	0
75	1	0	1	1	1	0	0	0	0	0	0	0	0	0
72	0	0	0	0	1	0	1	1	1	1	1	1	0	1
70	1	0	0	0	0	1	1	1	1	1	1	1	1	1
66	1	0	1	1	1	1	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	0	1
60	0	0	0	0	0	0	0	1	0	1	1	0	0	1
58	0	1	0	0	0	0	0	0	0	0	0	0	0	0
57	1	1	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	1	0	1	1	1	1	1	1	1	1
54	0	1	0	0	0	1	0	0	0	0	0	0	0	0
52	1	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	1	0	1	0	0	1	0	0	1	0	0
47	1	0	1	1	0	0	0	0	0	0	0	0	0	0
45	1	0	1	1	1	0	1	1	0	1	1	0	0	1
40	0	0	0	0	1	0	0	1	0	0	0	0	0	0
39	0	0	0	0	1	1	0	0	0	0	0	0	0	0
35	1	1	1	1	1	1	1	1	1	1	1	1	0	1
30	1	1	0	0	1	0	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	0	1	1	1	1	1	1
22	0	1	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	1	1	1	1	1	1
17	0	0	0	1	1	0	1	0	0	0	1	0	1	1

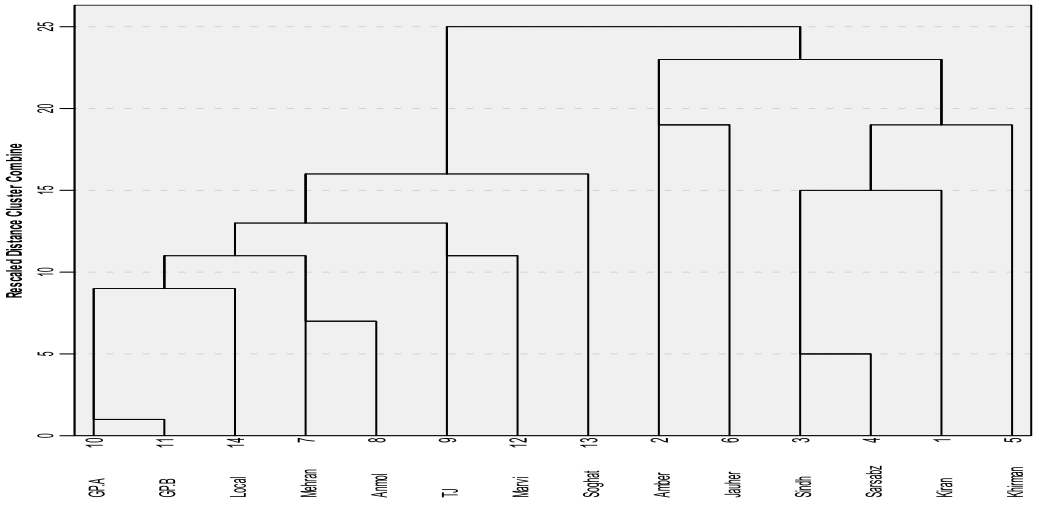


Fig. 4. Dendrogram of Insoluble proteins showing the similarity index of Sindh wheat Varieties.

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