



Characterization of Wheat protein (Albumin) in different varieties of wheat cultivated in Sindh through SDS-PAGE Electrophoresis

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Abstract: 14 selected wheat varieties were analyzed for quantification and characterization using SDS-PAGE technique. Significant difference ($P < 0.05$) found in Albumin concentration among the selected wheat varieties of Sindh having ranged (17.03 - 22.36). The highest concentration of albumin found in variety Sindh-90 while lowest concentration seen in variety TJ-83. The electrophoretic patterns of molecular weight of Albumin ranged between 08- 72kDa. The highest banding pattern was seen in variety Kiran and Khirman with 14 bands whereas lowest banding pattern as seen in varieties Soghat with 13 bands while lowest banding pattern seen in varieties Sarsabz, Khirman, and Mehran having 09 bands. 12kDa proteins are common in all varieties except Sarsabz, Jouhar and GP-256 however 72 kDa proteins are common in all varieties. There is no any relation found in concentration and banding pattern in Albumin protein ($r = -0.08664$). Cluster analysis shows the similarity and dissimilarities of the albumin proteins pattern was carried out on the results of SDS-PAGE using the statistics software SPSS 19. Cluster analysis based on the linkage distance by the procedure of Unweighted Pair Group Method with Arithmetic average (UPGMA). Cluster analysis, sorting the wheat varieties into 02 major groups (lineages) at linkage distance 25. Albumin protein known to be the most important proteins causative of immediate hypersensitivity reactions to the Wheat. Bakers' asthma is a distinctive condition in which flour protein (mainly water soluble protein) reacts with serum IgE as a result of inhalation of particles of wheat flour.

Keywords: Albumin, wheat varieties, (UPGMA), Characterization

1. INTRODUCTION

Cereal protein is an important nutrient for human health and nutrition. Wheat is one of the most important crops and major source of energy and protein for human being and animals, while the quantity and character of protein are chief determinant of efficient characteristics of wheat flour (Wanget *al.*, 2005). The alternative classification of wheat protein rather than solubility described by Shewry and Halford (2002), described that wheat proteins consist of two classes on the basis of storage property seed, storage protein (gliadin/Glutenin) and non seed storage protein (Albumin/ Globulin). Albumin and globulin are non seed storage proteins, in cereal albumin/globulin are present the seed coat and aleurone cell and comprise about 25% of the total grain protein and the majority of these proteins are monomeric (Belderok *et al.*, 2000). Albumin is biological active proteins which are responsible for breakdown of starch and other enzymatic reactions e.g. Amylase and proteases. Albumin is known as metabolic proteins and is the first to hoard in significant amounts (Waga, 2004). The molecular weights of non prolamin (albumin & globulin) are usually blow 25,000 Dalton, even though a major proportion of these proteins have molecular weight in between 60,000 and 70,000 Da (Veraverbeke and Delcour, 2002). Many albumins known to be glycoprotein. The albumin is mainly enzymes, carbohydrates; as alpha- amylase, beta- amylase or glycosidase, or photolytic enzymes etc (Matz, 1991). Nutritionally albumin and globulin are rich in essential amino acid, contain tryptophan, methionin and lysine. The albumins which have high molecular weight subunit are generally β -amylases (Gupta *et al.*,

1996). Urminka and Basista, (2005) reported that albumin have a molecular weight ranged 17 to 28 kDa.

2. MATERIALS AND METHODS

Fourteen wheat varieties 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). were obtained from official organization, the Nuclear Institute of Agriculture Tandojam, while one known variety was collected from local market.

Purification of water soluble proteins (Albumin)

Took 1g of wheat powder and incubated the flour with 1ml of 0.5M NaCl over night, put in ultrasonic traction (100w for 30 min at room temperature, centrifuged for 15 min at 14000 rpm. Separated the supernatant, frozen and stored at -40°C .

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 were analyzed by using SDS-PAGE electrophoresis according to the method of Laemmli (1970) and modified by Shaista Khan *et al.* (2015).

For electrophoresis we used 30% acrylamide gel and for buffer system we used resolving gel buffer pH 8.8 and stacking buffer pH 6.8.

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. Staining 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 destining solution 30mL methanol and 10mL acetic acid were added in 60 mL distilled water.

Loading of the sample: Fixed the plates with the clamp, Filled the lower portion of the tank with reservoir buffer, than Load (20 μ l) sample 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, allowed to run till the tracking dye (bromophenol blue) reached to the lower part of the gel.

Interpretation of Bands

The protein bands were imaged through gel documentation system (Bio-Rad). At first well of each gel, the proteins engaged as the molecular weight markers (Kilo Daltons) ranging from 10-180kDa (SDS-PAGE MW standards, Fermentus pre staining protein ladder).

Data analysis

Electrogram 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: $S = \frac{W}{A+B-W}$ 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 AND DISCUSSION

Albumins are biological active protein and perform the metabolic function. Albumin is water soluble protein (Pence *et al.*, 1954 *et al.*, 2001). The albumins are mainly enzymes, carbohydrates as alpha and beta amylase, glycosidase or proteolytic enzymes etc. (Matz, 1991). Albumins and globulin in wheat have rich banding pattern the number of band varied 19-2 (Zilic *et al.*, 2011).

Albumin protein which is water soluble protein has been quantified, as depicted in Table 1; ranged 17.03 to 22.36%, it is found that Sindh-90 variety contain the highest Albumin content (22.36 \pm 0.05) followed by Jauhar (21.46 \pm 0.09), Mehran (21.33 \pm 0.05), Sarsabz (21.31 \pm 0.05), Soghat-90 (20.56 \pm 0.05), GP-256 (20.51 \pm 0.03), Local variety (19.55 \pm 0.03), Marvi (19.37 \pm 0.01), Khirman (19.32 \pm 0.01), GP-205 (18.62 \pm 0.05), Amber (18.45 \pm 0.01), Anmol (18.41 \pm 0.02) while lowest albumin level was found in TJ 83 (17.03 \pm 0.03) and Kiran-95 (18.23 \pm 0.05) variety.

Different researchers reported that the concentration of albumin in wheat; Merlino *et al.*, (2009) reported that albumin plus globulin content ranged from 20-25% of total proteins in different wheat varieties. Orth *et al.*, (1972) reported that albumin in wheat grain ranged 8-9%, Wieser *et al.*, (1980) reported that an albumin concentration in wheat grain during the different stage of development was 15%. Gafurova *et al.*, (2002) reported the albumin concentration in wheat grain grown in Uzbekistan ranged 18.0-21.26%. Present result of albumin is in full agreement with that finding of Gafurova *et al.*, (2002).

The result of characterization of albumin showed that the number of band varied from 9 to 13 with molecular weight ranged 08 to 72 kDa shown in electrogram (**Fig. 1 and 2 and Table 1**). The highest banding pattern is seen in variety Soghat with 13 bands and lowest banding number seen in variety Sarsabz followed by Khirman and Mehran having a 09 bands. Similarly Kiran, Sind-90, TJ-83 and GP-256 wheat variety contain 12 bands having molecular weight ranged 10kDa-72 kDa while Jauhar, Anmol-91, Marvi and local wheat varieties contain 11 bands. Jauhar, Anmol-91 has molecular weight ranged 08kDa-72 kDa whereas Marvi, and Local have banding ranged from 10kDa -72 kDa. Amber and GP-205 varieties have 10 numbers of bands having electric mobility with molecular weight 08kDa -72 kDa. 12kDa proteins are common in all the varieties except Sarsabz, Jauhar and GP-256 while 72 kDa proteins are common in all investigated varieties.

Different scientist reported the banding pattern and molecular weight of Albumin fraction. Urminska and Basista, (2005) reported that Albumins proteins are water-soluble proteins with a relatively low molecular weight of about 17 to 28 kDa.

Dvoracke and Curn, (2003) reported that the pattern of albumin within relatively wide was area with molecular weight ranged 69-23kDa in bread and drum genotypes. Hassan *et al.*, (2010) reported that the molecular weight distribution of protein isolates and their fractions was found in the range 19-67 kDa.

Results of present study are almost consistent with the finding of Zilic S. *et al.*, (2011) who reported the molecular weight of Albumin varied from 12.4kDa-76.4 kDa.

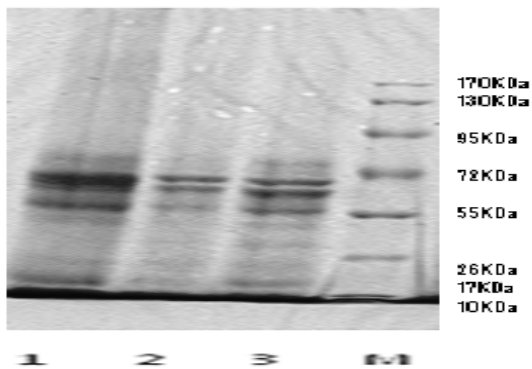
The water soluble protein (albumin) belong to the class of alpha amylase inhibitor and trypsin family, these proteins are disturbed with defense mechanism of plant, but also well know allergens in baker's asthma (Tatham and Shewry, 2008 and Shewry and Lucas, 1997). The result regarding characterization of albumin fractions of different varieties of Wheat cultivated in Sindh depicted in (**Table 1**). Result shows that 12 kDa, 15 kDa, 17 kDa, 36 kDa are possible wheat allergens present in almost all varieties of Sindh.

Cluster analysis show the similarity and dissimilarities of albumin proteins pattern in selected wheat varieties of Sindh was carried out on the results of SDS-PAGE using the statistics software SPSS 19 in order to examine the genetic variation in albumin protein fractions among the Sindh wheat varieties shown in (Fig. 3 and table 2).

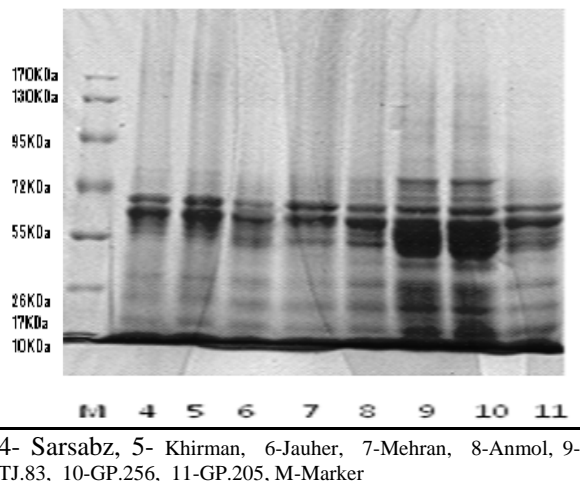
The result of cluster analysis based on linkage distance by the procedure of UPGMA. Cluster analysis sorting the wheat varieties into 2 major groups (lineages) at linkage distance 25 and which are further distributed into 09 clusters at linkage distance 15. The first group is composed of 06 clusters and there are 03 clusters in the second group. Among the first heredity, clusters 2, 4, 5 and 6 each containing single variety GP-256, Anmol, Kiran-95 and Sarsabz respectively and in cluster 3 contain 2 varieties GP-205 and Soghat while in cluster 1 at linkage distance 5 contain 04 clusters have 2 varieties Khirman and Local variety and cluster 2, 3 and 4 involves variety TJ 83, Mehran, Marvi. In second group have 3 clusters having the variety names Amber, Jauhar and Sindh-90.

Table 1: Albumin concentration (%) with bands numbers of selected varieties of Wheat cultivated in sindh.

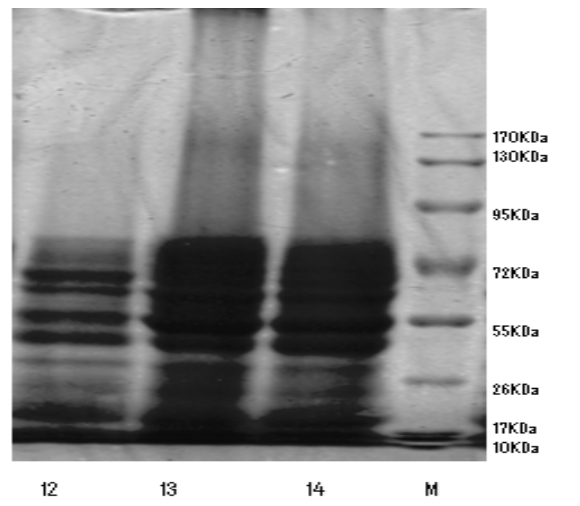
Wheat Varieties	Albumin content (%)	Band numbers in Albumin protein in Sindh Wheat varieties	noitalerroc
Kiran-95	18.23 ±0.05	12	-0.08664
Amber	18.45 ±0.01	10	
Sindh-90	22.36 ±0.05	12	
Sarsabz	21.31 ±0.05	09	
Khirman	19.32 ±0.01	09	
Jauher-18	21.46 ±0.09	11	
Mehran-89	21.33 ±0.05	09	
Anmol-91	18.41±0.02	11	
TJ-83	17.03±0.03	12	
GP-256	20.51±0.03	12	
GP-205	18.62±0.05	10	
Marvi	19.37±0.01	11	
Soghat	20.56±0.05	13	
Local(unknown)	19.55±0.03	11	



1-Kiran,2- Amber, 3- Sindh, M-marker
Fig. 1: Banding pattern of Water soluble protein fractions in selected wheat varieties of Sindh, Pakistan.



4- Sarsabz, 5- Khirman, 6-Jauher, 7-Mehran, 8-Anmol, 9-TJ.83, 10-GP.256, 11-GP.205, M-Marker
Fig.2: Banding pattern of Water soluble protein fractions in selected wheat varieties of Sindh, Pakistan.



12- Marvi, 13-Soghat, 14- Local, M-Marker
Fig. 3: Banding pattern of Water soluble protein fractions in selected wheat varieties of Sindh, Pakistan.

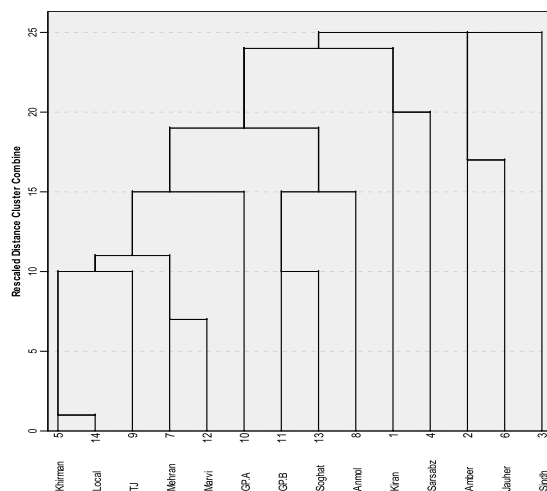


Fig. 4: Dendrogram of water soluble proteins showing the similarity index.

Table 2: Banding pattern of Water soluble protein fractions in selected wheat varieties of Sindh, Pakistan.

Wheat varieties	Banding pattern of water soluble proteins(kDa)												
	I	II	III	VI	V	IV	IIV	IIIV	XI	X	IX	IIX	IIIX
sdnaB													
Kiran-95	10	12	16	20	26	31	36	45	57	60	70	72	dn
Amber	8	12	15	17	26	42	47	55	70	72	dn	dn	dn
Sindh-90	8	10	12	17	23	27	36	39	45	55	70	72	dn
Sarsabz	8	10	17	20	26	31	57	62	72	dn	dn	dn	dn
Khirman	10	12	17	26	31	50	55	70	72	dn	dn	dn	dn
Jauher-18	8	10	15	17	20	26	45	55	67	70	72	dn	dn
Mehran-89	10	12	17	20	26	36	55	70	72	dn	dn	dn	dn
Anmol-91	8	10	12	15	20	26	36	40	50	70	72	dn	dn
TJ-83	10	12	15	17	21	26	36	39	50	55	70	72	dn
GP-256	10	16	17	21	23	26	31	36	50	55	70	72	dn
GP-205	10	12	20	26	36	40	50	55	67	72	dn	dn	dn
Marvi	10	12	16	20	23	26	36	50	55	70	72	dn	dn
Soghat	10	12	17	20	26	36	40	45	50	55	57	67	72
Local	10	12	17	26	31	36	50	55	67	70	72	dn	dn

nd : not detected

Table2:Cluster pattern of Water soluble protein fractions in selected wheat varieties of Sindh (Pakistan).

Mw KDa	Kiran-95	Amber	Sindh-90	Sarsabz	Khirman	Jauher-18	Mehran-89	Anmol-91	TJ-83	GP-256	GP-205	Marvi	Soghat	Local
72	1	1	1	1	1	1	1	1	1	1	1	1	1	1
70	1	1	1	0	1	1	1	1	1	1	0	1	0	1
67	0	0	0	0	0	1	0	0	0	0	1	0	1	1
62	0	0	0	1	0	0	0	0	0	0	0	0	0	0
57	1	0	0	1	0	0	0	0	0	0	0	0	1	0
55	0	1	1	0	1	1	1	0	1	1	1	1	0	1
50	0	0	0	0	1	0	0	1	1	1	1	1	1	1
47	0	1	0	0	0	0	0	0	0	0	0	0	0	0
45	1	0	1	0	0	1	0	0	0	0	0	0	1	0
42	0	1	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	1	0	0	1	0	1	0
39	0	0	1	0	0	0	0	0	1	0	0	0	0	0
36	1	0	1	0	0	0	1	1	1	1	1	1	1	1
31	1	0	0	1	1	0	0	0	0	1	0	0	0	1
27	0	0	1	0	0	0	0	0	0	0	0	0	0	0
26	1	1	0	1	1	1	1	1	1	1	1	1	1	1
23	0	0	1	0	0	0	0	0	0	1	0	1	0	0
21	0	0	0	0	0	0	0	0	1	1	0	0	0	0
20	1	0	0	1	0	1	1	1	0	0	0	1	1	0
18	0	0	0	0	0	0	0	0	0	1	0	0	0	0
17	0	1	1	1	1	1	1	0	1	1	0	0	1	1
16	1	0	0	0	0	0	0	0	0	1	0	1	0	0
15	0	1	0	0	0	1	0	1	1	0	0	0	0	0
12	1	1	1	0	1	0	1	1	1	0	1	1	1	1
10	1	0	1	1	1	1	1	1	1	1	1	1	1	1
8	0	1	1	1	0	1	0	1	0	0	0	0	0	0

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