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Fractionation of globulin protein in different varieties of Wheat cultivated in Sindh

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Abstract: Wheat is the principal cereal and major source of energy worldwide, Different varieties of Wheat were analyzed for quantification and fractionation on Sodium Dodecyl Sulphate poly acrylamide gel electrophoresis, the percentage of salt soluble proteins (globulin) content among the selected wheat varieties of Sindh having ranged 4.74% to 7.81%. The Globulin content was found significantly high in variety TJ-83 (7.816±0.01) while the lowest content of globulin in a Sarsabz variety about 4.746±0.06 and Mehran (4.833 ±0.02). The banding patterns of molecular weight of globulin protein ranged between 07kDa - 60kDa, the 7 kDa band was common in all varieties except Kiran-95 and Anmol-90 although variation seen in banding pattern of different varieties . The correlation of banding pattern and concentration of globulin (r= 0.25). The result of cluster analysis based on the linkage distance by the procedure of UPGMA using the statistical software SPSS 19. Cluster analysis arrangement the wheat varieties into 2 major groups at linkage distance 25 and which are further scattered into 09 clusters at linkage distance 10.

Keywords; Globulin, Wheat proteins, molecular weight, SDS-PAGE, fractionation

1. INTRODUCTION

Wheat is most important cereal and major source of energy worldwide. It supply one-fifth of the entire caloric requirement of the individuals of the world. The characteristics/quality of wheat flour is mainly determined by its protein, the fully grown wheat grain contain approximately 8.2-15.4% of proteins (Khan *et al*., 2013). Wheat proteins are classified into 02 different classes on the bases of their solubility; albumin and globulin are soluble proteins while glutenin and gliadins are insoluble proteins. Albumins and globulins are non prolamin proteins comprise 15-20% of total wheat proteins. Globulins are salt soluble proteins (Singh and Skerritt, 2001). Albumins and globulins are generally biologically active proteins perform the catalytic and regulating functions. Albumins and globulins formulate approximately 90% of the total grain protein in the first ten days of grain growth. Even though they are deposited in the grain for most of the grain-filling period, the percentage of albumin and globulin in total grain protein decline for the period of grain growth, and make up 20-30% at development, This turn down occurs due to the synthesis of storage protein (70-80% of the mature protein mass) occurs fairly later in grain filling (Lasztity, 1996). The molecular weights of albumin and globulin in between 60,000 and 70,000 Da (Veraverbeke and Delcour, 2002). Singh *et al.,(* 2001) reported that the molecular weight of globulin lower than 25,000 Dalton while a considerable amount has a molecular ranged 60,000 and 70,000 Dalton. Dupont *et al.,* (2006*)* studied the 19 bread wheat and 23 durum wheat varieties and found the molecular weight ranged 76.4-12.4 kDa. (Zilic *et al*., 2011 and Dvoracke and Curn, 2003) reported that the banding pattern of albumin and globulin was alienated into two relatively wide area, 69-23 kDa found in bread and 16-2 kDa proteins in drum Genotypes. (Graveland *et al*., 1982) investigated that the globulin proteins showed the heterogeneous fraction on electrophoresis with molecular weight of ranged 98000 to 10000 while albumins showed the molecular weight less than 15 000 dalton.

2. MATERIALS AND METHODS

Different 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 Nuclear (NIA) Tandojam, whereas one unknown variety was collected from local market.

Purification of water soluble proteins (Globulin)

Took residue from albumin extraction, Precipitate (residue) washed three times (3X) with water, and incubated the residue with 1 ml of PBS over night at room temperature , Put in ultrasonic extraction (For 30 min at room temperature), Centrifuge for 20 min at 14000 rpm, Separated the supernatant, Frozen and stored at -40 ^{0}C.

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Gel Electrophoresis (SDS-PAGE)

The fractionation of globulin protein was investigated by using SDSPAGE electrophoresis according to the method of (Laemmili 1970), modified by Shaista Khan *et al*: (2015).

3. RESULTS

Globulin protein considered as a minor group of total protein comprises 5% of total protein (Yadav and Singh, 2011), albumin and globulin have nutritionally good protein because these proteins contain high amount of amino acid and biological function which may influence that these soluble proteins have the baking quality of wheat (Lasztity 1984). Apart from the gluten protein, water soluble albumin and salt soluble globulins constitute 10-2 2% of total flour protein (Singh and Mactchie, 2001). Albumins and globulins are non gluten proteins of wheat, these proteins contribute about 20 - 25% of whole grain protein and majority of these proteins are monomeric in nature (Merlino *et al.,* 2009).

Globulin protein (salt soluble protein) has been quantified, illustrated in **(Table 1),** ranged 4.74% to 7.81% of total wheat protein. Globulin content was found significantly highest in variety TJ 83(7.816±0.01) followed by Kiran-95(7.523±0.01), Khirman (6.540 ±0.01), GP-256(6.416±0.05), GP-205 (6.033±0.02), Soghat -90 (6.016±0.01), Marvi (5.456±0.02), Amber (5.323±0.09), Jauhar (5.256± 0.01), Local variety (5.166±0.06), Sindh-90 (4.856±0.06), Anmol (4.823 ±0.03) however the lowest globulin content found in variety Sarsabz(4.746±0.06) and Mehran (4.833 ±0.02).

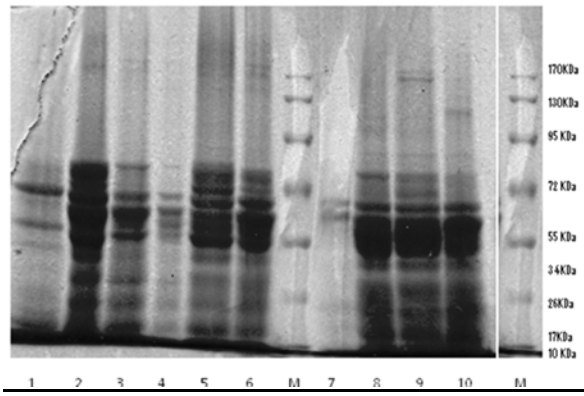
Similarly finding of different scientist and researchers has also been reported the globulin content of wheat varieties, Gafurova (2002) reported that the concentration of globulin protein was approximately 4.73-6.0% in wheat grain grown in Uzbekistan. While, Stone and Savin (1999) reported that the concentration globulin in grain was about 15%. Wieser *et al*., (1980) reported that globulin concentration in wheat grain during the different stage of development was 7%.

The results of globulin content of wheat grain in present study are in accordance with the findings of Gafurova (2002) and Wieser *et al*., (1980). Albumin and globulin are enzymatic, synthetic, metabolic proteins which regulate the protective function in plant, but in some sort these proteins also have allergenic effect for some peoples (Horvath-Szanics *et al*., 2006).

SDS-PAGE pattern of Wheat varieties of present study shown in Electrogram **(Fig. 1, 2 and Table 2).** The characterization of wheat globulin shows the number of bands varied from 10-12 having a molecular weight ranged 7kDa- 60kDa. The highest banding pattern seen in variety GP-205 and Soghat while lowest banding pattern seen in varieties Mehran-89, Sindh-90 and local varieties. The electric mobility of different varieties have molecular weight ranged 7 kDa- 55 kDa, In which 7 kDa and 55 kDa band was common in almost all the varieties while variation in banding pattern seen in different varieties.

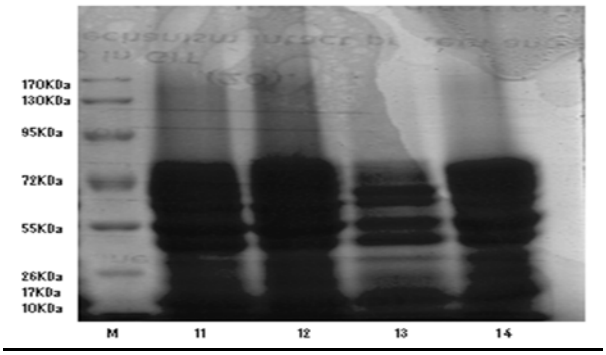
Kiran-91 and Anmol variety contains 11 bands with molecular weight 9kDa- 55kDa. The fractionation of variety Amber, Sindh-90, Jauhar, GP-205, GP-256 Marvi and local wheat variety have molecular weight ranged 7kDa- 55kDa. Sarsabaz wheat variety contains 11 bands, sequence of band with molecular weight 7kDa-52kDa. The molecular weight of Mehran-89 variety ranged 7kDa- 60kDa. TJ-83 wheat variety has 11 bands with molecular weight ranged 7kDa- 50kDa. Soghat wheat variety has 12 fractions with molecular weight 7kDa- 50kDa. Local variety contains 10 bands with banding pattern 7kDa- 55kDa. Similarly finding of different scientist and researchers has also been characterized the globulin (banding pattern and molecular weight), (Urminska and Basista 2005) reported that globulin have relatively low molecular weight of about 25-300kDa. (Dvoracke and Curn, 2003) reported that the range of globulin in bread and drum wheat was 16-2KDa. Present result of globulin is nearly agreement with the finding of Urminska and Basista (2005).

Dendrogram study show the similarity and dissimilarities of globulin proteins fractions in Sindh wheat varieties was conceded based on the results of SDS-PAGE using the software SPSS 19, examined the genetic variation in globulin protein fractions among the selected wheat varieties of Sindh shown in **(Table 3) (Fig. 3).** The result of cluster analysis based on linkage distance by the procedure of UPGMA. Cluster analysis arrangement the wheat varieties into 2 major groups at linkage distance 25 and which are further scattered into 09 clusters at linkage distance 10. The first group is composed of 02 clusters and there are 07 clusters in the second group. Among the lineage first, clusters 2 contain single variety TJ 83 while cluster 1 further distributed into 3 cluster at linkage distance 5, in which cluster 01 have two varieties Marvi and Local variety and cluster 2 and 3 have single variety Soghat and GP-205. Similarly among the second cluster lineage cluster 4, 5,6,7,8 and 9 has single wheat variety namely Amber, Sindh-90, Anmol, Sarsabz, Jauhar, GP-256 and Kiran-95 while cluster 3 at linkage distance 5 divided into 2 out of 2 clusters in first cluster have 2 varieties Khirman and Mehran and second cluster have single variety Amber.



1-Kiran,2- Amber, 3- Sindh, 4- Sarsabz, 5- Khirman, 6-Jauher, 7-Mehran, 8-Anmol, 9- TJ.83, 10-GP.256, M-Marker

Fig. 1: Banding pattern of Salt soluble protein (Globulin) fractions in selected wheat varieties of Sindh.



11-GP.205, 12- Marvi, 13-Soghat, 14- Local, M-Marker

Fig.2: Banding pattern of Salt soluble protein (Globulin) fractions in selected wheat varieties of Sindh.

Table 1: Globulin protein concentration (%) and their relation with banding pattern in selected wheat varieties.

Wheat Varieties	Salt Soluble protein (Globulin) content (%)	Band numbers in Globulin protein in Sindh Wheat varieties	Correlation
Kiran-95	7.523±0.01	11	0.2550
Amber	5.323±0.09	11	
Sindh-90	4.856±0.06	10	
Sarsabz	4.746 ±0.06	11	
Khirman	6.540 ±0.01	10	
Jauher-18	5.256± 0.01	11	
Mehran-89	4.833 ±0.02	10	
Anmol-91	4.823 ±0.03	11	
TJ-83	7.816±0.01	11	
GP-256	6.416±0.05	11	
GP-205	6.033±0.02	12	
Marvi	5.456±0.02	11	
Soghat	6.016±0.01	12	
Local(unknown)	5.166±0.06	10	

Table 2: Banding pattern of Salt soluble protein (Globulin) fractions in selected wheat varieties of Sindh (Pakistan)

Wheat varieties	Banding pattern of Salt soluble proteins(Globulin) in kDa												
Bands	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Kiran-95	9	15	18	20	31	34	35	42	45	47	55	nd	nd
Amber	7	15	17	20	26	34	37	45	47	50	55	nd	nd
Sindh-90	7	15	20	24	29	32	37	45	50	55	nd	nd	nd
Sarsabz	7	9	15	20	26	29	37	45	47	48	52	nd	nd
Khirman	7	13	15	20	26	35	37	45	50	55	nd	nd	nd
Jauher-18	7	15	18	20	26	35	38	42	47	50	55	nd	nd
Mehran-89	7	9	15	20	26	37	45	50	55	60	nd	nd	nd
Anmol-91	9	15	18	20	26	35	37	38	45	47	55	nd	nd
TJ-83	7	9	15	18	20	25	30	38	40	45	50	nd	nd
GP-256	7	9	12	15	20	24	31	35	42	47	55	nd	nd
GP-205	7	9	12	15	20	21	30	32	38	45	50	57	nd
Marvi	7	12	15	20	25	30	32	38	42	50	55	nd	nd
Soghat	7	9	12	15	20	25	30	32	42	45	47	50	nd
Local	7	12	15	25	30	32	38	45	50	55	nd	nd	nd

nd : not detected

Table 3: Cluster pattern of Salt soluble protein (Globulin) fractions.

Mw kDa	Kiran-95	Amber	Sindh-90	Sarsabz	Khirman	Jauher-18	Mehan-89	Anmol-91	TJ-83	GP-256	GP-205	Marvi	Soghat	Local
60	0	0	0	0	0	0	1	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	1	0	0	0
55	1	1	1	0	1	1	1	1	0	1	0	1	0	1
52	0	0	0	1	0	0	0	0	0	0	0	0	0	0
50	1	1	1	0	1	1	1	0	1	0	1	1	1	1
48	0	0	0	1	0	0	0	0	0	0	0	0	0	0
47	1	1	0	1	0	1	0	1	0	1	0	0	1	0
45	1	1	1	1	1	0	1	1	1	0	1	0	1	1
42	0	0	0	0	0	1	0	0	0	1	0	1	1	0
41	1	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	1	0	0	0	0	0
38	0	0	0	0	0	1	0	1	1	0	1	1	0	1
37	0	1	1	1	1	0	1	1	0	0	0	0	0	0
35	1	0	0	0	1	1	1	1	0	1	0	0	0	0
34	1	1	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	1	0	0	0	0	0	0	0	1	1	1	1
31	1	0	0	0	0	1	0	0	0	1	0	0	0	0
30	0	0	0	0	0	0	0	0	1	0	1	1	1	1
29	0	0	1	1	0	0	0	0	0	0	0	0	0	0
26	0	1	0	1	1	1	1	1	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	1	0	0	1	1	1
24	0	0	1	0	0	0	0	0	0	1	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	1	0	0	0
20	1	1	1	1	1	1	1	1	1	1	1	1	1	0
18	1	0	0	0	0	1	0	1	1	0	0	0	0	0
17	0	1	0	0	0	0	0	0	0	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	1	1	1	1	1
9	1	0	0	1	0	0	1	1	1	1	1	0	1	0
7	0	1	1	1	1	1	1	0	1	1	1	1	1	1

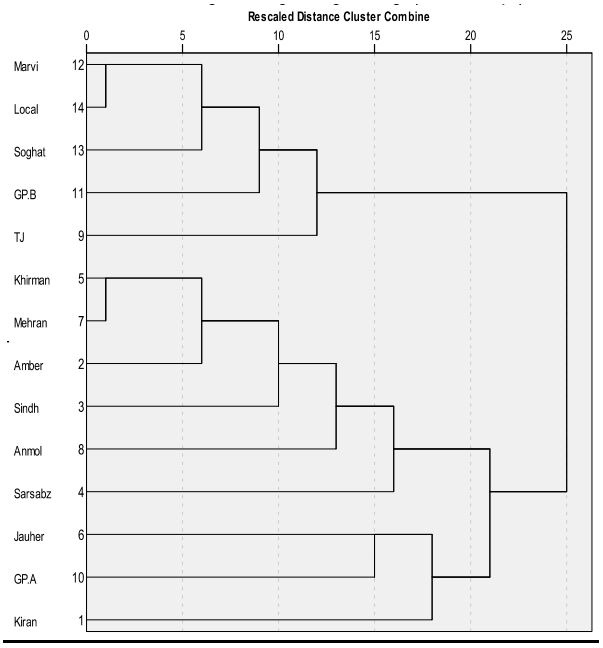


Fig. 3: Dendrogram of salt soluble proteins

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