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Identification and Prediction of the Flour Quality of Bread Wheat by Gliadin Electrophoresis

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Abstract: Gliadin proteins were used to identify and characterize 30 wheat varieties. SDS-PAGE of gliadins revealed a total of 30 bands ranged from 74.5 to 6.5 kDa, where the number of total bands varied between varieties. The region of omega-gliadin had a wide range for a number of bands. Among gliadin protein bands, nine bands were monomorphic and 21 bands were polymorphic, which were used to characterize the wheat varieties. The similarity index and dendrogram demonstrated the genetic relationships among wheat varieties using the gliadin protein bands resulted from SDS-PAGE analysis. In the studied Egyptian wheat varieties, the gliadin band of γ-45 related to high gluten quality, while the ω-38 band related to moderate gluten quality. However, it was found that γ-42 band related to low gluten quality. The concentration of each gliadin protein subgroup was varied among the wheat varieties and some of gliadin subgroups were related partially to gluten quality in some varieties. The obtained results indicate that gliadin proteins analysis is useful as biochemical genetic markers for characterizing the wheat varieties and detecting flour quality.

Keywords: Wheat, Gliadin, Electrophoresis, Flour, Quality

INTRODUCTION

Gliadins are the major class of wheat grain proteins made in the endosperm and mainly monomeric, high in proline and glutamine, and contribute to dough physical characteristics[9]. The combination of gliadins and glutenins is important for the bread making quality of wheat. Gliadin proteins can be grouped into α-, β-, γ- and ω-gliadin families based on their mobility in protein gels[14,6]. Hou *et al*[3]. used A-PAGE coupled with densitometry using a known quantity of modified Osborne gliadins as a quantitative standard to identify and quantify the gliadin subgroups in 17 soft wheat patent flours from four wheat classes and seven straight-grade flours. They found that the quantity of alpha-gliadins was wheat class related. The percentages of individual gliadin subgroups in flour protein were significantly lower in the patent flours than in counterpart straight-grade flours. The quantities of certain gliadin subgroups and total gliadins have various associations with flour rheological properties, Japanese-type sponge cakes and sugar-snap cookies end-use quality for each class of wheat. Petrova *et al*.[8] analyzed and compared the gluten strength, mixing properties and pasta cooking quality of thirty samples of durum wheat variety Neptun 2, grown in Bulgaria at 6 locations for 5 years with other Bulgarian durum wheat varieties. They found that the gliadin proteins of Neptun 2 contain gamma-gliadin fraction 45 and possess strong gluten/protein characteristics and good pasta quality. The other Bulgarian durum wheats that belong to the gliadin type 42 and consequently have inferior pasta making potential. Tanaka *et al*[11]. used Acid PAGE to determine the electrophoretic patterns of gliadins in 107 common wheat lines derived from Japan. They found that the gliadin patterns of Japanese wheat cultivars and landraces greatly differed from the patterns of wheat lines from other countries, and the variation seen in wheat lines from Japan was limited to 46 patterns. Seven collection or breeding areas in Japan showed different frequencies in their gliadin patterns. Wegrzyn and Waga[13] evaluated the relationships of 45 cultivars and strains of winter wheat by polymorphism of gliadin and glutenin proteins. They found that the cluster analysis showed a considerable variation of the investigated genotypes and the genetic distances between the cultivars ranged from 1.00 to 0.12. Caglarrmak *et al*[2]. compared bread making and pasta making varieties of wheat and four breeding lines to determine which of the breeding lines most likely merits carrying forward for further refinement of the line. They examined the electrophoretic properties of

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the standard varieties and breeding lines to relate genetic characteristics to bread- or pasta-making potential, also glutenin profiles and gliadin subunits were among the factors studied. They found two of the breeding lines seemed to have merit for bread making purposes and one additional line for pasta making. Nizar[7] Used electrophortic pattern of gliadin groups to identify and classify 187 *Triticum durum* cultivars. He found that the region of omega-gliadins had a wider range for the number of bands than all other regions of gliadins. Cluster analyses put the cultivars of all groups in trees on the basis of the gliadin band distribution.

The objective of this study is to estimate the genetic diversity of 30 hexaploid wheat varieties and fingerprinting them by gliadin protein patterns and investigate the gliadin protein bands and the relative quantity of gliadin subgroups in relation to wheat flour quality as well.

MATERIALS AND METHODS

Eighteen Egyptian and twelve foreign hexaploid wheat varieties (*Triticum aestivum* L.) from different genetic background that shown in Fig. (1) were used in this study.

For gliadin grain storage protein electrophoresis; sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to identify 30 wheat varieties by their gliadin protein patterns. Gliadin proteins were performed on vertical slab using gel electrophoresis apparatus according to method of Laemmli[5] modified by Studier[10]. Gel was photographed using a 35 mm color film (100 ASA) and scanned by phoretix 1D Quantifier, Image Analysis Software, England Imaging System, Epson GT 9500 Scanner.

Regarding gluten quality; wet, dry gluten and gluten–index were determined by Glutomatic 2200 apparatus in the "Food Technology Research Institute, Agriculture Research Center".

RESULTS AND DISCUSSIONS

Gliadin Protein Electrophoresis: The results of electrophoretic analysis of gliadins for 30 wheat varieties are illustrated in Fig. (1) and Table (1). The SDS-PAGE revealed a total of 30 bands ranged from 74.5 to 6.5 kDa that were unnecessarily present in all varieties. The number of total bands varied between varieties whereas the lowest number was 11 bands appeared in five varieties Giza 164, Sakha 69, Sakha 93, Sids 8 and Golan while the highest number was 20 bands appeared in variety Sakha 202. The region of omega-gliadin had a wider range for the number of bands (16 bands) than all other regions of gliadins, this result agreed with that found by Nizar[7]. Among such gliadin protein bands, nine bands were commonly detected in all wheat varieties with different molecular weights (monomorphic bands). Two common bands at mobility 0.09 and 0.26 with molecular weights 70.9 and 41.3 kDa in omega zone, two at mobility 0.47 and 0.49 with molecular weights 22.5 and 20.4 kDa in gamma zone, three at mobility 0.62, 0.65 and 0.69 with molecular weights 15.4, 13.9 and 12.5 kDa in beta zone and two at mobility 0.73 and 0.89 with molecular weights 11.3 and 7.7 kDa in alpha zone.

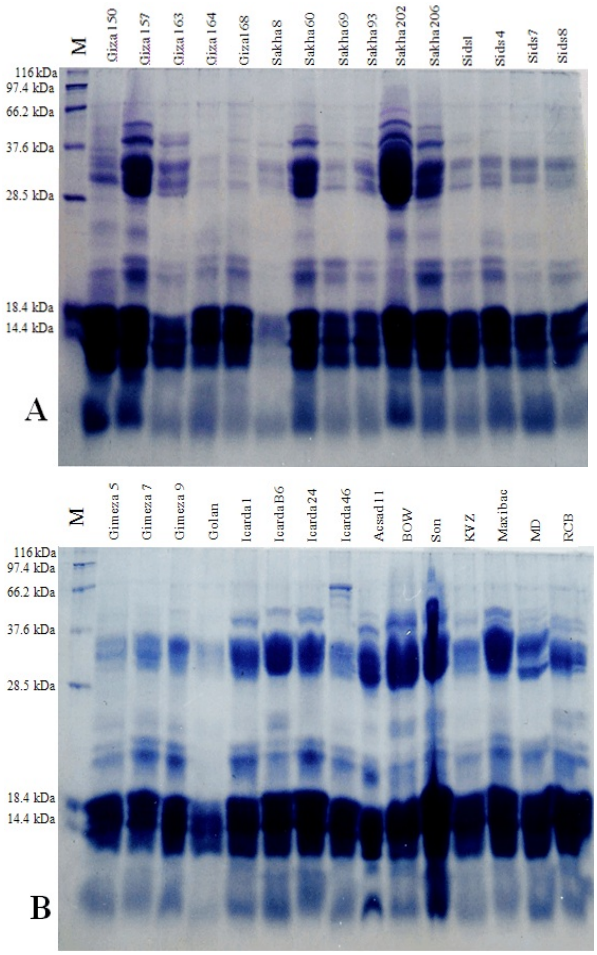

Fig. 1(A and B): SDS-PAGE profile of gliadin proteins extracted from thirty hexaploid wheat cultivars, M is standard proteins.

The other twenty one variable bands were used to characterize the thirty wheat varieties (polymorphic bands). The band at mobility 0.08 with MW 74.5 kDa identified two varieties Sakha 202 and Bow. Band at mobility 0.10 with MW 67.2 kDa identified only one variety Icarda 46. Band at mobility 0.12 with MW 62.7

Table 1: Densitometer analysis of gliadin proteins (SDS–PAGE) representing band number (BN), molecular weight (MW), relative front (RF) and intensity as percentage of total concentration for thirty wheat varieties.

	BN	MW	RF	Varieties															*
				Giza 150%	Giza 157%	Giza 163%	Giza 164%	Giza 168%	Sakha 8%	Sakha 60%	Sakha 69%	Sakha 93%	Sakha 202%	Sakha 206%	Sids 1%	Sids 4%	Sids 7%	Sids 8%	
ωZone	1	74.5	0.08										0.31						
	2	70.9	0.09	0.48	0.42	0.43	1.22	1.63	1.42	0.34	0.13	0.28	0.45	0.39	0.4	0.28	0.44	0.3	
	3	67.2	0.10																
	4	62.7	0.12		2.22					0.9			1.87	0.79					
	5	61.3	0.13																
	6	58.3	0.15	0.28		0.73				0.75			4.78	0.49	0.42	0.24	0.28		
	7	56.1	0.16		5.54	3.06		0.99	1.66	4.98	0.56	1.14		3.66	0.88			0.37	
	8	54.3	0.17	1.34															
	9	49.1	0.20	1.19			0.77						9.1						
	10	47.0	0.22	3.32	1.88			2.45			4.16	12.7			3.82	3.42			
	11	45.0	0.24			8.62			10.1	2.33			10.6	6.05		0.91	6.58	4.86	
	12	41.3	0.26	7.41	2.28	8.54	0.47	2.78	6.87	19.5	3.22	3.94	2.78	1.02	2.78	2.32	3.67	1.29	
	13	40.0	0.28		14.3	2.69			0.92				16.6	21.5					
	14	35.2	0.32		1.14	0.1				0.91			1.33	0.94					
	15	29.3	0.36	2.99															
	16	28.0	0.38		3.57			0.25		0.92			2.13	1.13					
γZone	17	26.5	0.42				0.47									1.28			
	18	25.3	0.45																
	19	22.5	0.47	0.24	3.73	0.34	1.45	3.96	1.45	3.14	5.54	2.38	0.12	2.38	2.27	1.65	0.21	1.05	
	20	21.9	0.48										0.21						
	21	20.4	0.49	1.71	10.9	4.45	3.5	10.4	8.52	8.68	6.21	3.54	0.07	8.48	2.83	4.18	0.83	2.67	
	22	18.4	0.56		7														
βZone	23	15.4	0.62	2.45	22.8	6.87	21.7	19.3	22.8	22.9	19.2	21.3	5.24	5.45	14.7	50.8	37.3	15.2	
	24	13.9	0.65	34.2	2.32	25.3	6.36	6.54	2.5	2.15	27.3	23.8	26.3	27.5	40.7	6.08	3.02	31.8	
	25	12.5	0.69	13.1	0.16	16.0	46.4	17.0	17.0	15.0	2.01	2.32	3.05	1.64	5.65	8.89	17.3	33.1	
αZone	26	11.3	0.73	9.05	7.55	14	14.7	21.6	0.97	8.3	20.9	13.9	5.09	9.31	15.4	11.9	7.86	8.57	
	27	10.5	0.75	0.25	1.99					2.1			0.21	1.13			3.13		
	28	8.5	0.82							2.16			0.86				3.36		
	29	7.7	0.89	22.1	12.3	8.78	2.81	13.1	25.2	5.03	10.6	14.9	9.56	9.08	10.0	7.98	15.9	0.64	
	30	6.5	0.91																
Total number of bands				15	17	14	11	12	12	17	11	11	20	17	12	13	13	11	
	BN	MW	RF	Gimeza 5%	Gimeza 7%	Gimeza 9%	Golan %	Icarda 1%	Icarda B6%	Icarda 24%	Icarda 46%	Acsad 11%	Bow %	Son %	KVZ %	Maxibac %	MD %	RCB %	*
ωZone	1	74.5	0.08										0.94						
	2	70.9	0.09	o.41	0.57	0.41	0.38	0.54	0.38	0.46	4.15	0.25	0.44	0.38	0.79	1	2.22	2.51	30
	3	67.2	0.10								1.86								1
	4	62.7	0.12								0.54								5
	5	61.3	0.13									0.54		2.26			0.32		3
	6	58.3	0.15	0.49	0.59	0.62			2.17	4.98						3.46			14
	7	56.1	0.16					3.56				2.16	7.49	6.82	2.8		1.47	1.34	17
	8	54.3	0.17			0.2												2.44	3
	9	49.1	0.20																3
	10	47.0	0.22	2.96				2.52	4.61							13.7	20.6	3.13	13
	11	45.0	0.24		5.74	12.3	16.2		8.76		14.0	16.4		8.83	14.6	20.7			17
	12	41.3	0.26	6.58	8.17	1.67	0.52	22.7	14.5	22.1	3.64	8.67	37.7	22.6	4.91	11.5	8.67	21.9	30
	13	40.0	0.28	0.88	5.92	1.5		3.25		4.61	5.92	15.4	4		3.67		12.0	2.85	16

Table 1 Continued:

	14	35.2	0.32							0.05		0.58				0.04			8
	15	29.3	0.36																1
	16	28.0	0.38											2.15					6
γZone	17	26.5	0.42									1.09	3.71					2.91	5
	18	25.3	0.45	1.7	1.98	1.19			2.88		0.53								5
	19	22.5	0.47	1.31	2.12	2.66	1.65	2	0.52	0.41	1.9	1.65	2.27	0.26	1.02	0.99	1.54	0.99	30
	20	21.9	0.48																1
	21	20.4	0.49	6.32	15.5	11.6	3.54	11.4	3.16	16.1	8.1	11.4	3.49	0.97	6.53	4.98	11.5	5.62	30
	22	18.4	0.56				4.54						0.27	0.03				0.73	5
βZone	23	15.4	0.62	15.2	13.1	12.7	19.1	9.25	14.6	8.14	9.03	11.4	9.95	14.3	14.3	2.42	15.4	5.02	30
	24	13.9	0.65	27.6	5.01	27.3	9.6	3.12	5.09	15.4	14.2	16.1	7.37	0.91	12.7	10.2	10.2	28.4	30
	25	12.5	0.69	14.6	15.5	6.64	26.3	19.6	18.8	18.0	25.2	3.17	5.05	6.21	5.09	6.21	6.21	5.05	30
αZone	26	11.3	0.73	10.2	14.8	11.9	12.6	6.05	5.09	5.31	6.05	6.85	6.18	10.8	19.1	17.3	2.94	10.6	30
	27	10.5	0.75			0.27		8.9	15.2	3.1	2.37	0.64	6.79		7.74	3.46			15
	28	8.53	0.82		0.21	0.45		0.27	0.77	0.76				0.74					9
	29	7.07	0.89	11.5	10.8	8.54	5.8	6.88	2.7	1.05	8.61	14.0	6.23	22.8	7.41	4.28	15.6	6.49	30
	30	6.55	0.91						1.86										1
Total number of bands				13	14	16	11	14	16	14	15	16	15	15	13	14	13	15	

* Presence of each band in all varieties

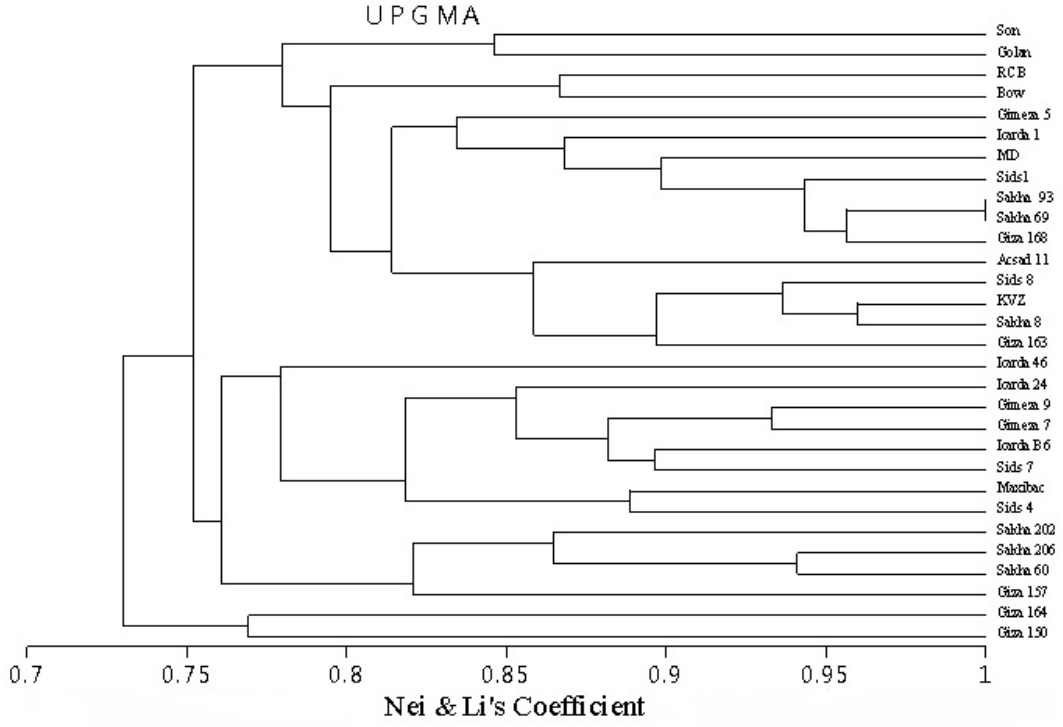

Fig. 2: Dendrogram representing the genetic relationships among the thirty wheat varieties using UPGMA cluster analysis of Jaccard genetic similarity coefficients generated from gliadin protein patterns.

kDa was detected among five varieties Giza 157, Sakha 60, Sakha 202, Sakha 206 and Icarda 46. Band at mobility 0.13 with MW 61.3 kDa was presented among three varieties Acsad 11, Son and MD. Band at mobility 0.17 with MW 54.3 kDa was found in three varieties Giza 150, Gimeza 9 and RCB. Band at mobility 0.20 with MW 49.1 kDa was observed in three varieties Giza 150, Giza 164 and Sakha 202. Band at mobility 0.36 with MW 29.3 kDa identified only one variety Giza150. Band at mobility 0.42 with

MW 26.5 kDa was found in five varieties Giza 164, Sids 4, Acsad 11, Bow and RCB. Band at mobility 0.45 with MW 25.3 kDa was detected among five varieties Gimeza 5, Gimeza 7, Gimeza 9, Icarda B6 and Icarda 46. Band at mobility 0.48 with MW 21.9 kDa identified only one variety Sakha 202. Band at mobility 0.56 with MW 18.4 kDa was detected in five varieties Giza 157, Golan, Bow, Son and RCB. Band at mobility 0.91 with MW 6.5 kDa identified only one variety Icarda B6. The other bands appeared in large number of varieties.

Our results are in agreement with those of Jones *et al.*[4] and Teng *et al.*[12] who used polyacrylamide gel electrophoresis (PAGE) to identify some wheat cultivars according to gliadin electrophoregrams banding patterns. They indicated that most of cultivars were readily differentiated, some very closely related cultivars gave identical patterns and gliadin patterns could be used as genetic markers.

The dendrogram tree (Fig. 2) demonstrated the relationships among the thirty wheat varieties according to the similarity index detected by gliadin protein patterns, using the UPGMA cluster analysis. The dendrogram divided the varieties into two main clusters. The first contained two Egyptian varieties, Giza 150 and Giza 164 with similarity value 77 %, while the second cluster was divided into two subclusters contained the rest of varieties. The first subcluster contained 16 varieties, among such varieties; two varieties Son and Golan were grouped together with similarity value 85 %. The two Mexican varieties Bow and RCB were grouped together with similarity value 87 %. The two Egyptian varieties Sakha 69 and Sakha 93 with similarity value 100 % were grouped with G168 with similarity value 96 % followed by Sids1, MD, Icarda 1 and Gimeza 5. The tow varieties KVZ and Sakha 8 with similarity value 96 % were grouped with Sids 8, Giza 163 and Acsad 11.

Table 3: The relationship between gluten quality and gliadin bands in ten Egyptian wheat varieties.

varieties	ω-38	γ-42	γ-45	Gluten-Index (%)
Gimeza 7			+	96.39
Gimeza 5			+	94.37
Gimeza 9			+	92.11
Sids 8				78.43
Sids 1				77.56
Sakha 202	+			77.48
Giza 168	+			73.83
Sakha 69				67.96
Giza 164		+		52.71
Sakha 93				52.66

Table 4: Concentration (%) of gliadin subgroups and gluten quality index (%) as indicated to the flour quality for baking performance in the ten Egyptian wheat varieties.

varieties	Gliadin subgroups (%)				Gluten analysis		
	ω	γ	β	α	Wet-gluten (%)	Dry- gluten (%)	Gluten-index (%)
Gimeza 7	20.97	19.61	33.64	25.84	31.38	10.83	96.39
Gimeza 5	11.29	9.33	57.49	21.86	24.35	8.78	94.37
Gimeza 9	16.25	15.45	46.66	21.17	28.52	7.16	92.11
Sids 8	6.82	3.72	80.26	9.21	25.51	7.66	78.43
Sids 1	8.30	5.10	61.11	25.48	30.61	9.97	77.56
Sakha 202	49.13	0.40	34.68	15.72	37.3	12.1	77.48
Giza 168	7.85	14.3	42.9	34.7	28.42	9.75	73.83
Sakha 69	8.07	11.75	48.68	31.54	32.43	10.60	67.96
Giza 164	2.46	5.42	74.4	17.6	32.14	10.76	52.71
Sakha 93	18.08	5.92	47.12	28.91	33.06	10.23	52.66

The second subcluster contained 12 varieties, among such varieties; two varieties Maxibac and Sids 4 were grouped together with similarity value 89 %. Two Egyptian varieties Gimeza 7 and Gimeza 9 with similarity value 93 % were grouped with Sids 7 and Icarda B6 with similarity value 90 % followed by Icarda 24 and Icarda 46. Two varieties Sakha 60 and Sakha 206 with similarity value 94 % were grouped with Sakha 202 followed by Giza 157.

The dendrogram tree showed that some foreign varieties were found in one group with the local varieties, due to the use of varieties in breeding programs to improve local varieties.

These results are on the same line with those of Nizar[7] who reported that the tree clustering based on gliadin electrophoregrams may be used as an additional tool in revealing genetic relationship among cultivars.

The Relationship Between Gluten Quality and Gliadin Bands: The relationship between gluten quality and gliadin bands in ten Egyptian wheat varieties is shown in Table (3). The gliadin band γ-45 (RF-45) related to high gluten quality because it was presented in the varieties Gimeza 7, Gimeza 5 and Gimeza 9 that have high gluten quality index (96.39, 94.37 and 92.11), respectively. On the other hand, gliadin band ω-38 (RF-38) may be related to moderate gluten quality, where it was found in the varieties Sakha 202 and Giza 168 that have slightly high gluten quality index (77.48 and 73.83). The gliadin band γ-42 (RF-42) may be related to low gluten quality due to its presence in the variety Giza 164 that gave low gluten quality index (52.71). The other varieties of Sids 8, Sids 1, Sakha 69 and Sakha 93 with gluten quality index (78.43, 77.56, 67.96 and 52.66) did not show any of these bands.

These results agreed with those of Autran and Galterio[1] who found that the band of γ-45 was high positively correlated with gluten quality, while γ- 42 gliadin band was negatively correlated with these criteria. Therefore, the varieties of Icarda 1 and Icarda 46 that have γ-45 gliadin band may be also having good flour quality. In contrast, the varieties Sids 4, Acsad 11 and Bow may be have low flour quality due to presence of γ-42 gliadin band in their gliadin patterns.

Association Between Wet, Dry Gluten, Gluten Quality, and Gliadin Subgroup Concentrations in Ten Egyptian Wheat Varieties: The concentration of each gliadin protein subgroup and gluten quality determined for ten Egyptian wheat varieties indicated differences in gliadin subgroups between different genotypes as shown in Table (4). The results illustrated that; the variety Sakha 202 showed the highest quantity of ω-gliadin subgroup (49.13), Gimeza 7 revealed the highest quantity of γ-gliadin subgroup (19.61), Sids 8 exhibited the highest quantity of β-gliadin subgroup (80.26) and Giza 168 manifested the highest quantity of α-gliadin subgroup (34.7).

The varieties Gimeza 7 and Gimeza 9 had high gluten quality index (96.39 and 92.11, respectively) and contained the highest quantity of γ- gliadin subgroup (19.61 and 15.45, respectively) comparing to the other varieties. The varieties Sakha 202 had the highest quantity of ω- gliadin subgroup (49.13) comparing to the other varieties and also the highest percentage of wet- gluten (37.3) and dry- gluten (12.1) and slightly high gluten index (77.48). This indicated partially positive correlations between concentrations of γ- and ω- gliadin subgroups and gluten quality.

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