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Determination of Allelic Compositions of *Glu-1* and *Glu-3* Loci in Bread Wheat Genotypes

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ABSTRACT

Breeding high-quality wheat cultivars is one of the main targets of breeding programs. Identification of High Molecular Weight Glutenin Subunits (HMW-GS) and Low Molecular Weight Glutenin Subunits (LMW-GS) alleles that confer high quality may be one of the easy ways to determine promising wheat lines in early generations. HMW-GS and LMW-GS alleles of 64 bread wheat (*Triticum aestivum* L.) genotypes from a Turkish breeding program including advanced lines and cultivars were identified using Sodium-Dodecyl-Sulfate PolyAcrylamide Gel (SDS-PAGE) electrophoresis method. In total, 26 alleles were identified including three alleles at the *Glu-A1* locus, 6 alleles at the *Glu-B1* locus, 2 alleles at the *Glu-D* locus, 6 alleles at the *Glu-A3* locus, 7 at the *Glu-B3* locus, and 2 alleles at the *Glu-D3* locus. The most prevalent alleles among the 64 genotypes in the study were *2 (67.2%), *7+9* (42.2%), *5+10* (68.8%), *A3c* (28.1%), *B3b* (35.9%), and *D3c* (92.2%). The previously announced good flour quality conferring alleles *1, 17+18, 5+10* at *Glu-1* loci and *A3b, A3c, B3b, B3c, B3d, B3g* at *Glu-3* loci were also identified in the present study, indicating that these alleles can be used as markers for selection of higher-quality genotypes in wheat breeding programs. Introduction of the allele structure of the studied genotypes may enlighten the way for the wheat breeders in the crossing decisions.**

Keywords: Flour quality, Glutenin subunits, Plant breeding, SDS-PAGE.

INTRODUCTION

Due to its use in many food products, wheat is one of the most important products in the world. Breeding high-quality wheat cultivars has been basic aim of the wheat breeding programs since demand of wheat product having high quality flour has risen. Within this scope, breeders in the wheat breeding programs in Turkey have started to seek and develop ways to screen and identify high quality germplasm for development of high quality wheat cultivars. Protein content, hardness, test weight of the wheat grain and sedimentation volume of the flours, and dough strength and extensibility are some of the important quality parameters in wheat. Classical quality tests such as alveograph energy or baking tests are not suitable for early generations of breeding programs as these require more wheat grains and are also time consuming. Glutenin subunits (HMW and LMW) include more information about quality values of wheat genotypes, can be easily identified by SDS-PAGE electrophoresis, and require much less seeds.

The genes in *Glu-1* loci, which control HMW glutenin synthesis, are located on the long arm of chromosome 1 of wheat (Payne *et al*., 1980). The loci encoding subunits of HMW are designated as *Glu-A1*, *Glu-B1* and *Glu-D1* according to chromosome order in the wheat genome (Payne and Lawrence, 1983). Although HMW-GSs constitute 10% of the total seed proteins, their effect on bread-making quality is quite large (Payne *et al*., 1987; Gianibelli *et al*., 2001; Kaya and Akcura, 2014). Important protein bands

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observed by SDS-PAGE were grouped as A (HMW-GS), B (LMW-GS) and C (Lower MW than B subunits) (Payne, 1987). HMW-GSs are accepted as basic identifiers of bread-making quality as they affect visco-elastic values (dough strength) of wheat dough (Tatham *et al*., 1985). Payne and Lawrence (1983) reported the allelic forms of the *Glu-1* loci as 3 alleles at the *Glu-A1*, 11 alleles at the *Glu-B1*, and 6 alleles at the *Glu-D1*. Payne *et al*. (1981) investigated effect of different subunit compositions on SDS sedimentation volume and reported that some certain allelic subunits had different effects on gluten quality. According to Payne *et al*. (1981), subunit *5+10* is associated with good dough quality, but *2+12* is associated with weak dough quality. Similarly, Branland and Dardevet (1985) reported that associations of W (gluten strength) and P (resistance) and Zeleny sedimentation value were positive with subunits *7+9*, *5+10*, but negative with subunit *2+12*. On the other hand, subunit *1* correlated with G (swelling) and subunits *2** and *17+18* correlated with W. Payne *et al*. (1987) found that subunits *1* and *2**, at the *Glu-A1* locus, *17+18*, *7+8* and *9* at the *Glu-B1* locus, and *5+10* at the *Glu-1D* locus associated with strong dough and better cooking properties, but subunits null, *6+8* and *2+12* associated with poor baking quality.

LMW-GSs are controlled by the genes in the *Glu-3 loci* located on the short arm of chromosome 1 of wheat and the individual LMW-GSs are assigned to *Glu-A3*, *Glu-B3* and *Glu-D3* loci (Gupta and Shepherd, 1990). Gupta and Shepherd (1990) identified 20 different band patterns including 6 alleles for *Glu-A3* locus, 9 alleles for *Glu-B3* locus, and 5 alleles for *Glu-D3* locus. Recently, in a study, 7 alleles at the *Glu-A3* locus, 12 alleles at the *Glu-B3*, and 6 alleles at the *Glu-D3* were identified and a set of 30 known cultivars was suggested to identify LMW-GS alleles (Liu *et al*., 2010). Nine cultivars from this set were used in the present study as standards. LMW-GSs comprise nearly 30% of the total protein (Gupta *et al*., 1992). Many studies have indicated the associations of LMW-GS allelic variation with significant changes in bread wheat quality (Gupta *et al*., 1989, 1994; Payne *et al*., 1984; Cornish, 1995; Cornish *et al*., 1999). LMW-GSs are related to dough extensibility (Sissons *et al*., 1998, Branlard *et al*., 2003). LMW-GSs also found associated with gluten swelling index and mixing time (Sharma *et al*., 2012). In a study, positive effect of *Glu-A3a*, *Glu-A3d*, and *Glu-B3g* alleles *on* dough properties (strength and extensibility), and negative effects of *Glu-D3c* on dough properties have also been reported (Branlard *et al*., 2003).

Payne *et al*. (1987) developed a scoring system using HMW-GSs. The alleles were assigned quality scores from 1 (poor quality) to 4 (high quality). Although Payne's scoring system was useful for having an idea about the quality value of the wheat genotypes, some interactions coming from LMWs, gliadins, or abiotic factors may affect the score (Gianibelli *et al*., 2001).

The aim of this study was to determine the alleles encoding HMW and LMW glutenin subunits of 64 genotypes using SDS-PAGE electrophoresis method, to reveal higher quality genotypes for breeding programs as gene resources and to reveal applicability of SDS-PAGE electrophoresis method for MAS (marker-assisted selection).

MATERIALS AND METHODS

Plant Materials

Sixty-four genotypes having different quality traits were used as materials. Twenty- one of them were advanced lines from the MRI (Maize Agricultural Research Institute) wheat breeding program. Thirty of them were cultivars from other research institutions and different countries including CIMMYT (International Wheat and Maize Improvement Center-Mexico), and the other 13 genotypes were lines from crossing blocks of MRI breeding program and cultivars from MRI (Table-1).

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Table 1. HMW-GS and LMW-GS alleles at the *Glu-1* and *Glu-3* loci of the genotypes.

No	Genotype [a]	Feature	Glu-1 alleles			Glu-3 alleles		
			A1	B1	D1	A3	B3	D3
1	Pamukova-97/Sönmez	Advanced line	2*	7+9	2+12	c	f	c
2	Tnmu/3/HD2206/Hork//Buc/Bul	Advanced line	1	7+9	5+10	b	f	c
3	Ocoroni 86/ Pewit3	Advanced line	1	7+8	2+12	d	g	c
4	Tahirova2000/Zornitcha	Advanced line	2*	7+9	5+10	a	f	c
5	Tahirova2000/Zornitcha	Advanced line	2*	7+9	5+10	a	f	c
6	Ağrı/Bjy"S"//Vee"S"/Mmtc /4/LL/3/Orso/Akv/Ska	Advanced line	1	17+18	2+12	c	b	c
7	Pamukova-97/Arostor	Advanced line	2*	17+18	5+10	e	i	c
8	Pamukova-97/Arostor	Advanced line	2*	17+18	5+10	e	i	c
9	Momtc/4/LL/3/Orso//Akv/ Ska/Prostor	Advanced line	1	7+9	2+12	e	i	c
10	Stozher/3/Kal/Mus//Har	Advanced line	2*	7+9	5+10	e	i	c
11	Sunvale/Sultan95	Advanced line	1	7	5+10	e	i	c
12	Stozher//Sibia/Milan	Advanced line	2*	7+9	5+10	d	g	c
13	Stozher//Sibia/Milan	Advanced line	2*	7+9	5+10	d	g	c
14	Sunco/Pastor	Advanced line	2*	7+9	5+10	d	g	c
15	Doğu-88/Ziyabey98	Advanced line	2*	17+18	5+10	c	b	c
16	Adana-99/Sultan95	Advanced line	1	7	5+10	b	b	a
17	Adana-99/Sultan95	Advanced line	1	7	5+10	b	b	c
18	Aköz/Galil	Advanced line	2*	17+18	5+10	e	h	c
19	Aköz/Dariel	Advanced line	2*	17+18	5+10	e	h	a
20	Bau/Kauz// Tahirova2000	Advanced line	2*	7+9	5+10	a	f	c
21	Tahirova-2000/Yakar	Advanced line	2*	7+9	5+10	a	f	c
22	Adana-99	Cultivar-TR	1	17+18	5+10	f	g	c
23	Ağrı/Bjy"S"//Vee"S"	MRI-Line	2*	17+18	5+10	c	g	c
24	Aköz	Cultivar-TR	2*	7+8	2+12	c	g	c
25	Arostor	Cultivar-BG	2*	7+8	2+12	c	b	c
26	Bau/Kauz	MRI-Line	2*	7+9	5+10	c	f	c
27	Dariel	Cultivar-TR-IL	2*	17+18	5+10	c	g	c
28	Lancer	Cultivar-TR	N	14+15	5+10	d	c	c
29	Galil	Cultivar-TR-IL	2*	7+9	5+10	f	f	c
30	HD2206/Hork//Buc/Bul	MRI-Line	2*	17+18	2+12	d	a	c
31	Kal/Mus//Har	MRI-Line	1	17+18	2+12	b	b	c
32	Momtc/4/LL/3/Orso/Akv/Ska	MRI-Line	2*	7+8	2+12	b	b	a
33	Ocoroni 86	CIMMYT	2*	7+8	2+12	d	a	c
34	Pastor	CIMMYT	2*	7+9	5+10	c	f	c
35	Pewit3	CIMMYT	1	7+9	2+12	d	g	c
36	Pamukova-97	MRI-Cultivar	2*	17+18	5+10	b	a	c
37	Prostor	Cultivar TR	2*	7+8	5+10	b	b	c
38	Sibia/Milan	MRI-Line	1	7+9	5+10	d	b	c
39	Sönmez	Cultivar TR	1	7	2+12	b	g	c
40	Stozher	Cultivar BG	2*	7+9	5+10	e	g	a
41	Sultan-95	Cultivar TR	2*	7	5+10	b	b	c
42	Sunco	Cultivar AU	1	7+8	2+12	b	b	c
43	Sunvale	Cultivar AU	1	7+8	2+12	b	b	c
44	Tahirova-2000	MRI-Cultivar	2*	7+9	5+10	a	f	c
45	Tinamou	CIMMYT	2*	17+18	5+10	b	f	c
46	Yakar-99	Cultivar TR	2*	13+16	5+10	e	h	c
47	Ziyabey-98	Cultivar TR	2*	7	5+10	e	i	c
48	Zornitcha	Cultivar BG	2*	7+9	2+12	c	f	c

[a] The genotypes 4-5, 7-8, 12-13, 16-17 are sisters.

Table 1 continued …

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Continued of Table 1.

No	Genotype [a]	Feature	Glu-1 alleles			Glu-3 alleles		
			A1	B1	D1	A3	B3	D3
49	Basribey-95	Cultivar TR	2*	7+9	5+10	c	b	c
50	Osmaniyem	Cultivar TR	1	7+9	5+10	b	b	c
51	Gönen-98	Cultivar TR	2*	17+18	2+12	c	g	c
52	Pehlivan	Cultivar TR	2*	7+9	2+12	c	g	a
53	Aldane	Cultivar TR	1	7+9	5+10	c	b	c
54	Flamura 85	Cultivar TR	2*	7+8	5+10	c	b	c
55	Tosunbey	Cultivar TR	1	17+18	5+10	b	b	c
56	Konya-2002	Cultivar TR	2*	7+8	2+12	e	b	c
57	Harmankaya-99	Cultivar TR	N	7+8	5+10	e	b	c
58	Çetinel-2000	Cultivar TR	2*	7	2+12	e	h	c
59	Yıldız 98	Cultivar TR	1	7	5+10	c	b	c
60	Bezostaja-1	MRI-RU	2*	7+9	5+10	c	b	c
61	Momtchil	MRI-BG	2*	7+9	5+10	c	b	c
62	Bandırma-97	MRI-Cultivar	2*	7+9	2+12	f	h	c
63	Beşköprü	MRI-Cultivar	1	7+9	5+10	e	i	c
64	Hanlı	MRI-Cultivar	2*	7+8	5+10	e	b	c

[a] The genotypes 4-5, 7-8, 12-13, 16-17 are sisters.

Methods

This research was carried out in the fields of MRI, Sakarya, and in the Biotechnology Laboratory of Transitional Zone Agricultural Research Institute, Eskişehir, under Republic of Turkey Ministry of Food, Agriculture, and Livestock.

The Field Study

The ear planting area was located at Sakarya, Turkey, with an altitude of 31 m, clay loam soil having medium organic matter content with pH 7.4 and with a mean annual rainfall of 672 mm. During the growing season, monthly minimum and maximum temperatures were 4.3 and 27.0°C, respectively. The rows were fertilized with 80 kg N ha^{-1} and 80 kg P_2O_5 ha^{-1} at the planting and 70 kg N ha^{-1} in spring at tillering.

The study materials were planted in 1 m long 30-ear rows in November 2011. To maintain seed purity, 25 ears from each genotype were isolated with paper bags in order to prevent fertilization of the foreign pollens. Non-homogeneous or mixed rows were discarded. All of the 25 ears of each genotype, grown isolated, were harvested and threshed separately in July 2012. Ten healthy ears having homogeneous seeds from 25 ears were selected for SDS-PAGE electrophoresis of HMW and LMW glutenin subunits.

Glutenin SDS-PAGE Electrophoresis

Gluten Electrophoresis Method and Sample Preparation

For the glutenin extraction and SDS-PAGE electrophoresis, we employed a standard method described by UPOV (1994) (http://www.upov.int/edocs/tgdocs/en/tg003.pdf, 22.10.2013) with some modifications and optimizations. Studies of Glutenin SDS-PAGE electrophoresis were completed in October 2013. Nine varieties, Chinese Spring, Gabo, Courtot, Norman, Opata, Buck Pingo, Halberd, Ruso, and Neepawa, which were provided by CIMMYT were used as standards in the glutenin analyses. These varieties were suggested as standards for determination of HMW-GS and LMW-GS alleles (Liu *et al*., 2009, 2010).

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For the extraction of glutenin, the endosperm of a single wheat kernel representing each genotype was crushed into fine powder and 0.05 g sample of the powdered endosperm was put into an eppendorf tube and labeled. In a beaker, 1 mL H_2O (distilled water), 0.40 mL extraction solution [20 mL glycerol, 24.1 mL H_2O, 4 g SDS and 20 mg pyronin Y (0.02 g) were added into 12.5 mL separating gel buffer (12.11g Tris, added 80 mL H_2O, made up to 100 mL adjusted with HCl to pH 8.8) and the solution mixed and adjusted to pH6.8.] and 0.07 mL merkapto-ethanol (ME) were put and mixed. During all sample preparation 5% ME was used. One mL from this final extraction solution [1 mL H_2O (distilled water), 0.40 mL extraction solution, 0.07 mL merkapto-ethanol (ME)] was added for each sample. Later, each sample was vortexed for 30 seconds several times at interval of 15 min over 2 hours. The samples were then incubated for 3-4 minutes at 85°C and were taken out of the incubator and waited to reach room temperature. Finally, the samples were centrifuged for 7 minutes at 12,000 rpm before gel electrophoresis.

Gel Preparation and Electrophoresis Application

We used a gel system consisting of two layers, stacking and separating layers for the sake of clarity of HMW and LMW glutenin subunits. Stacking and separating gel layers contained, respectively, 14 and 3% acrylamide. The main separating gel layer consisted of buffer (12.11 g Tris, added 80 mL H_2O, made up to 100 mL adjusted with HCl to pH 8.8), acrylamide solution, 34.85 g acrylamide, 0.154 g bisacrylamide, and 0.25 g SDS, added 94 mL separating gel buffer, made up to 250 mL). For polymerization of separating gel, 90 µL APS (10%) and 20 µL TEMED were added into 30 mL separating gel solution. The stacking gel layer consisted of buffer (12.11 g Tris, added 78 mL H_2O made up to 100 mL), adjusted with HCl to pH 6.8, acrylamide solution, 2.99 g acrylamide, 0.043 g bisacrylamide and 0.1 g SDS, added 12.5 mL stacking gel buffer, made up to 100 mL. For polymerization of stacking gel, 180 µL APS (10%) and 60 µL TEMED were added to 45 mL stacking gel solution. The electrode buffer contained 15.15 g Tris, 72.075 g glycine, 5 g SDS, dissolved in 5 L H_2O and adjusted to pH 8.3 with 6N HCl. The prepared glutenin protein samples as described earlier were loaded into each well using a 20 well comb as 15 µL and electrophoresis was run at a constant current of 60 mA using 500 volt power and 15°C for the two gels for 5 hours. After electrophoresis, the gels were rinsed slowly on a shaker for 5 hours in a solution containing 100 mL TCA (100%), 330 mL methanol and 570 mL H_2O and left overnight in the solution. Following rinsing, the gels were stained for at least 24 hours in a solution containing 35 mL 10N KOH and 50 mL TCA (100%) in 300 mL staining solution (from the combination of 1,000 mL H_2O containing 2.25 g Coomassie Brilliant Blue G-250 and 1,000 mL 2N H_2SO_4). After staining, the gels were left in H_2O for 1 hour for imaging process.

Banding patterns of HMW-GS and LMW-GS of the studied cultivars were identified according to standard cultivars (Liu *et al*., 2009, 2010) provided by CIMMYT. The cultivars were scored according to Payne *et al*. (1987) and the alleles were assigned quality scores from 1 (poor quality) to 4 (high quality).

RESULTS AND DISCUSSION

The alleles of HMW-GS and LMW-GS identified at the *Glu-1* and *Glu-3* loci of the 64 genotypes are given in Table 1. Unknown Glu-1 and Glu-3 allele combinations of 21 advanced lines of the MRI wheat breeding program were identified for the first time. Also, Glu-1 and Glu-3 allele combinations of 43 cultivars from different origins were identified, although glutenin allele compositions of some of the cultivars have

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been previously revealed (Yıldız, 2011, Kilic *et al*., 2017).

Twenty-six of glutenin alleles were identified in total. Eleven of the identified glutenin alleles were at the *Glu-1* loci and 15 were at the *Glu-3* loci. Three alleles were identified at the locus *Glu-A1*, among which the subunit *2** was the most frequent with the frequency of 68.8% (Table 2). Six alleles were identified at the locus *Glu-B1* and subunits *7+9*, *17+18* and *7+8* had high frequencies of 42.1, 23.4, and 18.8%, respectively. Two alleles were identified at the locus *Glu-D1*, and subunit *5+10* had higher frequency than *2+12*, respectively, 68.8 and 31.2%.

Six alleles were identified at the locus *Glu-A3*. Among these six alleles, the subunit *c* was most frequent with the frequency of 28.1%. Seven alleles were identified at the locus *Glu-B3*, and subunit *b* had the highest frequency of 35.9%. Two alleles were identified at the locus *Glu-D3* and subunit *c* had higher frequency than subunit *a*, (92.2 and 7.8%, respectively).

The results of the frequency of the HMW and LMW-GS alleles in this study were similar to the results of the previous studies. According to Tabasum *et al*. (2011), the most frequent subunits were *2** (50%), *17+18* (42.11%) and *5+10* and *2+12* (48.68% for both subunits) at the *Glu-1* loci in 76 Pakistani wheat genotypes including land races. Nosrati *et al*. (2013) identified subunits *2** (60%), *7+9* (64%) and *5+10* (66.7%) at the *Glu-1* loci as the most common alleles in 16 Iranian advanced wheat lines. Keser and Pena (2004) reported that the most frequent subunits were *2** (57.8%), *7+9* (42.6%), *5+10* (60.1%) at the

Table 2. Identified HMW-GS and LMW-GS alleles, their distribution, and proportion in 64 bread wheat genotypes.

Locus	Total number of the alleles	Identified alleles	Distribution of the alleles in 64 genotypes	Proportion of the alleles (%)
Glu-A1	3	N	2	3.1
		1	18	28.1
		2*	44	68.8
Glu-B1	6	7	8	12.5
		7+8	12	18.8
		7+9	27	42.2
		13+16	1	1.6
		14+15	1	1.6
		17+18	15	23.4
Glu-D1	2	2+12	20	31.3
		5+10	44	68.8
Glu-A3	6	Aa	5	7.8
		Ab	14	21.9
		Ac	18	28.1
		Ad	9	14.1
		Ae	15	23.4
		Af	3	4.7
Glu-B3	6	Ba	3	4.7
		Bb	23	35.9
		Bc	1	1.6
		Bf	12	18.8
		Bg	13	20.3
		Bh	5	7.8
		Bi	7	10.9
Glu-D3	2	Da	5	7.8
		Dc	59	92.2

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Glu-1 loci in 218 genotypes consisting of cultivars and lines in a Turkish wheat breeding program. Aktaş *et al*. (2014) reported subunits *2** (68%), *7+8* (32%) and *5+10* (56%) at the *Glu-1* loci as the subunits having the highest frequency in five Turkish cultivars and 20 IWWIP (International Winter Wheat Improvement Program) based wheat genotypes. Shan *et al*. (2007) reported that the most frequent subunits were *2** (76.1%), *7+9* (33.3%), *5+10* (81%) at the *Glu-1* loci, and *A3c* (46.8%), *B3g* (41%), and *D3a* (30.2%) at the *Glu-3* loci in U.S. hard white winter wheat cultivars. According to Lerner *et al*. (2009), the most frequent subunits were *2** (63.9%) at the *Glu-A1* locus, *7+9* (41.2%) at the *Glu-B1* locus, *5+10* (94.1%) at the *Glu-D1* locus, and the most frequent alleles were *c* (34.5%) at the *Glu-A3* locus, *j* (36.1%) at the *Glu-B3* locus, *b* (49.6%) at the *Glu-D3* locus in Argentinean wheat cultivars. Tsenov *et al*. (2009) reported subunits *2** (42.5%), *7+9* (68.5%) and *5+10* (76.7%) at the *Glu-1* loci and alleles *A3c* (65.8%), *B3b* (56.2%) and *D3c* (82.2%) at the *Glu-3* loci as the most common alleles in 73 Bulgarian winter wheat cultivars. Similarly, Atanasova *et al*. (2012) reported subunits *2** (41.5%), *7+9* (65.4%) and *5+10* (69.2%) at the *Glu-1* loci and alleles *A3c* (69.2%), *B3b* (47.7%) and *D3c* (80%) at the *Glu-3* loci as the most common alleles in 98 Bulgarian genotypes. Yıldız (2011) reported that the most frequent subunits were *2** (74.6%), *7+8* (49.2%) and *5+10* (54.2%) at the *Glu-1* loci, and the most frequent alleles in Turkish winter wheat cultivars were *A3b* (35.9%), *B3a* (25.4%), *D3c* (47.5%) at the *Glu-3* loci.

The alleles of HMW-GS affect bread making quality differently. The quality scores of the genotypes used in the study were determined and the overall score of each genotype was calculated (Table 3). Individual scores for each allele present in a genotype may be pooled to provide the overall quality score for that genotype. Thus, by looking at overall quality scores, breeders may have an idea about their breeding materials.

The genotypes having the highest *Glu-1* quality score 10 were the sister lines of Pamukova-97/Arostor (No. 7 and 8), the lines Doğu-88/Ziyabey-98 (No.15), Aköz/Galil (No.18), Aköz/Dariel (No.19), Ağrı/Bjy"S"//Vee"S" (No.23), and the cultivars Adana-99 (No.22), Dariel (No.27), Pamukova-97 (No.36), Prostor (No.37), Tinamou (No.45), Flamura-85 (No.54), Tosunbey (No.55), and Hanlı (No.64). On the other hand, cultivars Lancer (No.28) with the quality score 5, Sönmez (No.39) and Çetinel-2000 (No.58) with the quality score 6 were the genotypes having the lowest *Glu-1* quality scores.

The range of the quality scores of the genotypes of this study was 5-10. Fourteen genotypes were scored as 10, 21 genotypes were scored as 9, 19 genotypes were scored as 8, 6 genotypes were scored as 7, 2 genotypes were scored as 6, and only one genotype was scored as 5. Fifty-four genotypes out of 64 were scored from 8 to 10, suggesting that the efforts of generating high quality genotypes deserve appreciation. Some other studies in the literature also report similar ranges of the quality scores. Both Tabasum *et al*. (2011) and Dvoracek *et al*. (2013) reported a range of 4-10 total quality scores for their genotypes. According to Aktaş *et al*. (2014), the quality scores of their genotypes ranged 6-10 in total.

The alleles *1, 17+18, 5+10* at *Glu-1* loci were suggested as markers of good bread making (Kocourková *et al*., 2008; Kaya and Akcura, 2014). Since the alleles associated with good dough quality such as subunits, *1, 17+18, 5+10* combined in a cultivar, better bread making quality may be obtained. In all cultivars, only Adana-99 and Tosunbey which are known having technological quality in the market had good subunits combination (1, 17+18, 5+10) in this study at the *Glu-1* loci. The quality scores of these two genotypes were 10, confirming their high bread making quality.

The alleles *A3b*, *A3c* at *Glu-A3* and *B3b*, *B3c*, *B3d*, *B3g* at *Glu-B3* loci were reported as good quality promoting alleles in bread

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Table 3. HMW-GS alleles at the *Glu-1* and quality scores of the genotypes.

No.	Genotype [a]	Glu-1 alleles			Glu-1 score			Total score
		A1	B1	D1	A1	B1	D1	
1	Pamukova-97/Sönmez	2*	7+9	2+12	3	2	2	7
2	Tnmu/3/HD2206/Hork// Buc/Bul	1	7+9	5+10	3	2	4	9
3	Ocoroni 86/ Pewit3	1	7+8	2+12	3	3	2	8
4	Tahirova2000/Zornitcha	2*	7+9	5+10	3	2	4	9
5	Tahirova2000/Zornitcha	2*	7+9	5+10	3	2	4	9
6	Ağrı/Bjy"S"//Vee"S"/Mmtc /4/LL/3/ Orso/Akv/Ska	1	17+18	2+12	3	3	2	8
7	Pamukova-97/Arostor	2*	17+18	5+10	3	3	4	10
8	Pamukova-97/Arostor	2*	17+18	5+10	3	3	4	10
9	Momtc/4/LL/3/Orso//Akv/ Ska/Prostor	1	7+9	2+12	3	2	2	7
10	Stozher/3/Kal/Mus//Har	2*	7+9	5+10	3	2	4	9
11	Sunvale/Sultan95	1	7	5+10	3	1	4	8
12	Stozher//Sibia/Milan	2*	7+9	5+10	3	2	4	9
13	Stozher//Sibia/Milan	2*	7+9	5+10	3	2	4	9
14	Sunco/Pastor	2*	7+9	5+10	3	2	4	9
15	Doğu-88/Ziyabey98	2*	17+18	5+10	3	3	4	10
16	Adana-99/Sultan95	1	7	5+10	3	1	4	8
17	Adana-99/Sultan95	1	7	5+10	3	1	4	8
18	Aköz/Galil	2*	17+18	5+10	3	3	4	10
19	Aköz/Dariel	2*	17+18	5+10	3	3	4	10
20	Bau/Kauz// Tahirova2000	2*	7+9	5+10	3	2	4	9
21	Tahirova-2000/Yakar	2*	7+9	5+10	3	2	4	9
22	Adana-99	1	17+18	5+10	3	3	4	10
23	Ağrı/Bjy"S"//Vee"S"	2*	17+18	5+10	3	3	4	10
24	Aköz	2*	7+8	2+12	3	3	2	8
25	Arostor	2*	7+8	2+12	3	3	2	8
26	Bau/Kauz	2*	7+9	5+10	3	2	4	9
27	Dariel	2*	17+18	5+10	3	3	4	10
28	Lancer	N	14+15	5+10	1	-	4	5
29	Galil	2*	7+9	5+10	3	2	4	9
30	HD2206/Hork//Buc/Bul	2*	17+18	2+12	3	3	2	8
31	Kal/Mus//Har	1	17+18	2+12	3	3	2	8
32	Momtc/4/LL/3/Orso/Akv/Ska	2*	7+8	2+12	3	3	2	8
33	Ocoroni 86	2*	7+8	2+12	3	3	2	8
34	Pastor	2*	7+9	5+10	3	2	4	9
35	Pewit3	1	7+9	2+12	3	2	2	7
36	Pamukova-97	2*	17+18	5+10	3	3	4	10
37	Prostor	2*	7+8	5+10	3	3	4	10
38	Sibia/Milan	1	7+9	5+10	3	2	4	9
39	Sönmez	1	7	2+12	3	1	2	6
40	Stozher	2*	7+9	5+10	3	2	4	9
41	Sultan-95	2*	7	5+10	3	1	4	8
42	Sunco	1	7+8	2+12	3	3	2	8
43	Sunvale	1	7+8	2+12	3	3	2	8
44	Tahirova-2000	2*	7+9	5+10	3	2	4	9
45	Tinamou	2*	17+18	5+10	3	3	4	10
46	Yakar-99	2*	13+16	5+10	3	-	4	7
47	Ziyabey-98	2*	7	5+10	3	1	4	8
48	Zornitcha	2*	7+9	2+12	3	2	2	7
49	Basribey-95	2*	7+9	5+10	3	2	4	9
50	Osmaniyem	1	7+9	5+10	3	2	4	9

[a] The genotypes 4-5, 7-8, 12-13, 16-17 are sisters.

Table 3 continued …

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Table 3. HMW-GS alleles at the *Glu-1* and quality scores of the genotypes.

No.	Genotype [a]	Glu-1 alleles			Glu-1 score			Total score
		A1	B1	D1	A1	B1	D1	
51	Gönen-98	2*	17+18	2+12	3	3	2	8
52	Pehlivan	2*	7+9	2+12	3	2	2	7
53	Aldane	1	7+9	5+10	3	2	4	9
54	Flamura 85	2*	7+8	5+10	3	3	4	10
55	Tosunbey	1	17+18	5+10	3	3	4	10
56	Konya-2002	2*	7+8	2+12	3	3	2	8
57	Harmankaya-99	N	7+8	5+10	1	3	4	8
58	Çetinel-2000	2*	7	2+12	3	1	2	6
59	Yıldız 98	1	7	5+10	3	1	4	8
60	Bezostaja-1	2*	7+9	5+10	3	2	4	9
61	Momtchil	2*	7+9	5+10	3	2	4	9
62	Bandırma-97	2*	7+9	2+12	3	2	2	7
63	Beşköprü	1	7+9	5+10	3	2	4	9
64	Hanlı	2*	7+8	5+10	3	3	4	10

[a] The genotypes 4-5, 7-8, 12-13, 16-17 are sisters.

wheat (Kaya and Akcura, 2014; Aktaş and Baloch, 2017). These alleles were identified in the present study for the distinguished genotypes such as Adana-99, Aldane, Bezostaja, Pamukova-97, and Tosunbey suggesting that these alleles may be used as markers of good quality in bread wheat.

Identification of HMW-GS and LMW-GS glutenin alleles expressing high quality for MAS (marker-assisted selection) by SDS-PAGE electrophoresis were found applicable in breeding programs. It is suggested especially for early generation selection. In case MAS methods are integrated into breeding programs, the results will be more satisfying and higher-yielding cultivars with high quality may be released.

In conclusion, by managing HMW-GS and LMW-GS glutenin alleles, improving advanced lines or releasing cultivars having better bread making quality seem possible. Since molecular studies in the quality field in Turkey are quite limited, this study will provide insight quality compositions of common gene pools of bread wheat. In this study, 11 allelic forms encoding HMW-GS at the *Glu-1* loci and 15 allelic forms encoding LMW-GS at the *Glu-3* loci were found. Glu-1 and Glu-3 allele combinations of all advanced lines and most of the cultivars were unknown. The cultivars in the studied material included high and low bread quality, but they were mostly better yielding genotypes. Consequently, HMW-GS and LMW-GS glutenin allele compositions of all advanced lines and many important cultivars were revealed. Also, quality values of the material were exposed. This information will also be helpful to other breeding programs in the world that concentrate on improving high yielding bread wheat cultivars having high bread making quality.

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تعیین ترکیب آللی مکان های ژنی *Glu-1*و *Glu-3* در ژنوتیپ های گندم نان

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چکیده

بهنژادی کولتیوارهای گندم با کیفیت بالا یکی از اهداف اصلی برنامه های اصلاح نژاد است. در این ارتباط، شناسایی آلل واحد های گلوتنین با وزن مولکولی بالا (HMW-GS) و واحد های گلوتنین با وزن مولکولی کم (LMW-GS)که کیفیت بالا می دهد شاید یکی از راههای آسان برای تعیین رگه های امیدبخش گندم در نسل های ابتدایی باشد. در این پژوهش، با استفاده از روش الکتروفورز ژل پلی آکریل آمید سدیم-داودسیل سولفات (SDS-PAGE)، در ۶۴ ژنوتیپ گندم نان (*Triticum aestivum* L.) شامل رگه های پیشرفته و کولتیوارها ی مربوط به یک برنامه اصلاح نژاد در ترکیه، آللهای (HMW-GS) و (LMW-GS)شناسایی شد. در کل، ۲۶ آلل شناسایی شد شامل ۳ آلل در مکان *Glu-A1*، ۶ آلل در مکان *Glu-B1*، دو آلل در مکان *Glu-D*، ۶ آلل در مکان *Glu-A3*، ۷ آلل در مکان *Glu-B3*، و ۲ آلل در مکان *Glu-D3*. فراوان ترین آلل در میان ۶۴ ژنوتیپ در این پژوهش از این قرار بود: *(67.2%) 2*، (42.2%) 7+9* ، *(68.8%) 5+10*، *(28.1%) A3c* ، *(35.9%) B3b*، و *(92.2%) D3c*. همچنین، آللی های که قبلا به عنوان دهنده کیفیت بالا اعلام شده بود و شامل *1, 17+18, 5+10*در مکان *Glu-1*و *A3b, A3c, B3b, B3c, B3d, B3g* در مکان *Glu-3* بود نیز در این مطالعه شناسایی شد و حاکی از این بود که این آلل ها را می توان به عنوان نشانگرهایی برای گزینش و انتخاب ژنوتیپ های کیفیت بالا در برنامه های اصلاح نژاد به کار برد. معرفی ساختمان آلل ژنوتیپ های بررسی شده می تواند روشنگر راه بهنژادگران گندم در تصمیم گیری برای تلاقی دادن باشد.

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