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EFFECT OF HMW AND LMW GLUTENIN ALLELES ON QUALITY TRAITS OF BREAD WHEAT

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This study was performed to investigate impact of HMW and LMW glutenin alleles on quality traits of bread wheat cultivars. Fifteen bread wheat varieties were used for field trails during 2012-13 and 2013-14 under irrigated conditions of Diyarbakır and Mardin locations, Turkey. We investigated the quality of varieties that have same HMW-GS (High molecular weight subunits) and different LMW-GS (Low molecular weight subunits) or vice versa. Results indicated that *GluA3-c > d > e, GluB3-g > b' = i,* and *GluD3-b ≥ c > a for LMW-GS, and GluB1-7+8 > GluB1-17+18; GluD1-5+10 > GluD1-2+12 for HMW-GS* have higher extensograph dough energy, extensibility, resistance, also higher farinograph stability time, gluten index and grain hardness values. The overall evaluation of the results obtained from this study demonstrated that *GluA1-1 or 2*, GluB1-7+8 and GluD1-5+10* for HMW glutenin alleles and *GluA3-d, GluB3-g and b, GluD3-c* for LMW glutenin alleles are associated with a strong gluten structure and accordingly selection of lines harboring these alleles can contribute to the development of genotypes with high quality in wheat breeding programs.

Keywords: HMW, LMW, glutenin, quality, rheology, dough

INTRODUCTION

Wheat, having one of the highest production and consumption rates in the world, has great importance in terms of both economy and nutrition (AKTAŞ and BALOCH, 2017). In an attempt to develop yielding varieties to meet the wheat needs of the growing world population, wheat breeders also need to take into account the quality values of these varieties. In this regard, the presence of a negative correlation between grain yield and quality parameters of wheat constitutes challenges for wheat breeders.

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Gluten proteins (HMW and LMW) determine the quality characteristics of wheat flour. *HMW* glutenin subunits (GS) are synthesized at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arms of *1A*, *1B* and *1D* chromosomes (HORVAT *et al.*, 2002; ALSALEH et al., 2019). The genes encoding *LMW-GS* are localized at *Glu-A3*, *Glu-B3* and *Glu-D3*, which are linked to *Gli-1* on the short arm of *1A*, *1B* and *1D* chromosomes (JOHANSSON *et al.*, 2002). Although it is generally accepted that *HMW-GS* and *LMW-GS* have an effect on the dough characteristics of wheat flour, it is also acknowledged that the *HMW-GS* are more effective on dough traits. Thus, the use of *LMW-GS* for selection in wheat breeding programs is not as common compare to HMW-GS. This is because there is only little information about the actual effect of *LMW-GS* on the dough properties and quality process. In addition, the exact effect of *LMW-GS* on quality is yet to be confirmed compare to HMW-GS. Therefore, further research and effort is required to elucidate the impact of *LMW-GS* on the dough and bread quality characteristics. Different combinations of glutenin alleles of wheat genotypes have positive or negative effects on bread quality. Therefore, *HMW* and *LMW* glutenin alleles are also used as biochemical markers in parental selection in the early and advanced stages of breeding programs. Of the *HMW* alleles, *Glu-A1 (1 and 2*)*, *Glu-B1 (7+8 and 17+18)*, and *Glu-D1 (5+10)* have a positive effect on gluten quality and dough characteristics, while *Glu-A1 (Null)* and *Glu-D1 (2+12)* have a negative effect on gluten quality, compared to the other alleles in the same group (TOSI *et al.* 2011). The concept of quality varies depending on the product to which wheat flour will be processed in the final stage; e.g., varieties possessing the *Glu-D1-2+12* allele and weak or moderately strong gluten are more appropriate for flat bread, whereas those with a strong gluten structure and *Glu-D1-5+10* alleles are more desirable for a bread loaf (NAZCO *et al.*, 2014; HE *et al.*, 2005). In this sense, glutenin alleles are useful markers in terms of suitability for processing to the final product.

Despite the considerable amount of research concerning the agronomic and traditional quality parameters of varieties grown in Turkey, limited studies have been undertaken to investigate the actual factors affecting gluten quality in wheat; i.e., *HMW* and *LMW* glutenin alleles and their relationship with quality parameters (YILDIRIM *et al.* 2011; TEMIZGÜL *et al.*, 2018).

The current study aimed to: a-) Investigate the quality characteristics of genotypes with the same *HMW* but different *LMW* alleles to determine effet of LMW alleles on quality traits. b-) Determine the *HMW* and *LMW* glutenin alleles of wheat varieties that are intensively grown in Turkey, and the relationship between these alleles and quality parameters. c-) Determine individual effect of HMW and LMW glutenin subunits and also their combinations on quality traits of wheat. d-) Provide useful information regards to HMW and LMW glutenin alleles which can be used as marker to select high quality genotypes in breeding programs.

MATERIAL AND METHOD

Fifteen bread wheat varieties intensively cultivated in Turkey were used as plant material in this study. The field trials were conducted in the provinces of Diyarbakır and Mardin located in the Southeastern Anatolia Region of Turkey, under irrigated conditions in the 2012-13 and 2013-14 growing seasons. The experiments were carried out in a randomized block design with three replications, and the seeds were sown in six rows in each plot with 20 cm distance within the row. Irrigation was performed once during the spike emergence period. In all experiments, 80 kg ha^{-1} P_2O_5 and 160 kg ha^{-1} nitrogen (N) were used. In the harvest period, four middle rows were harvested for quality analyses. After cleaning the harvested seed samples, two replications were selected from each location to conduct quality analysis and statistical analyses made based on two replications.

The thousand grain weight was determined as described by ELGÜN *et al.* (2001), and the grain protein content was obtained using the Dumas method with an NDA 701 Dumas Nitrogen Analyzer according to the AACC (2000) method number 46-12. The samples of bread wheat cultivars stored for 48 hours with 14.5 % moisture according to the AACC (2000) method 44-16, and ground in a Brabender Junior mill following the AACC (1995) method 26-50. Zeleny sedimentation was determined according to AACC (2000) method 56-60, and the wet gluten content was calculated based on the average of two repetitions using the method described in the ICC (1994), standard 137/1 with a glutomatic instrument. The farinograph analysis (Farinograph-AT-Brabender Germany) was performed according to AACC (2000) method 54-21, and the extensograph analysis (Extensograph-E Brabender, Germany) was conducted in accordance with AACC (2000) method 54-10.

Protein extraction was undertaken using four seeds of each variety as described by PAYNE *et al.* (1987). The SDS-PAGE analysis was performed using 11.5% polyacrylamide according to the method developed by PAYNE *et al.* (1987). The *HMW* alleles at the *Glu-1* locus were identified by null the bands at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. The *HMW* alleles at the *Glu-3* locus and the *LMW* alleles were determined by recording the bands at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci. The quality score of *HMW* alleles was obtained according to the description in the study of PAYNE *et al.* (1987). HMW and LMW glutenin alleles of cultivars obtained from SDS-PAGE analysis are showed Fig 1 and Fig 2.

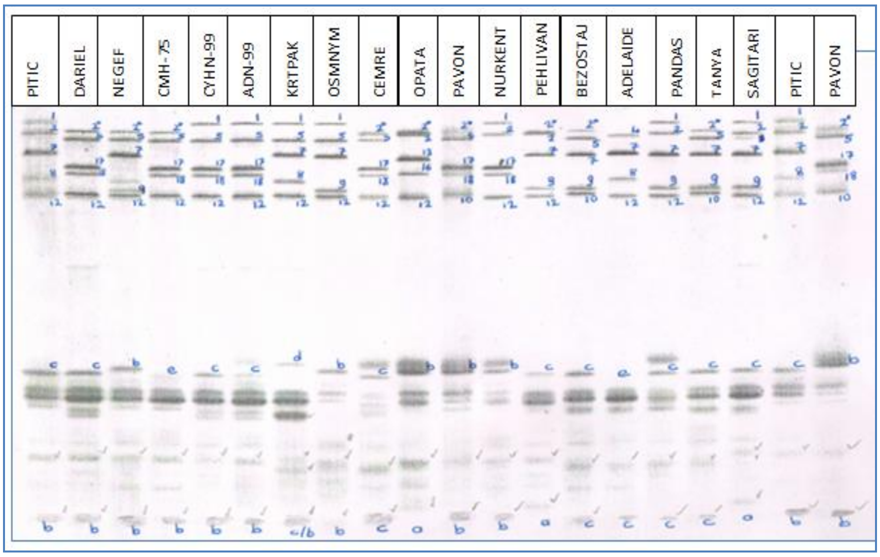

Fig. 1. HMW and LMW Glutenin Alleles of Variety for Glu-A1-B1-D1 and Glu-A3-D3

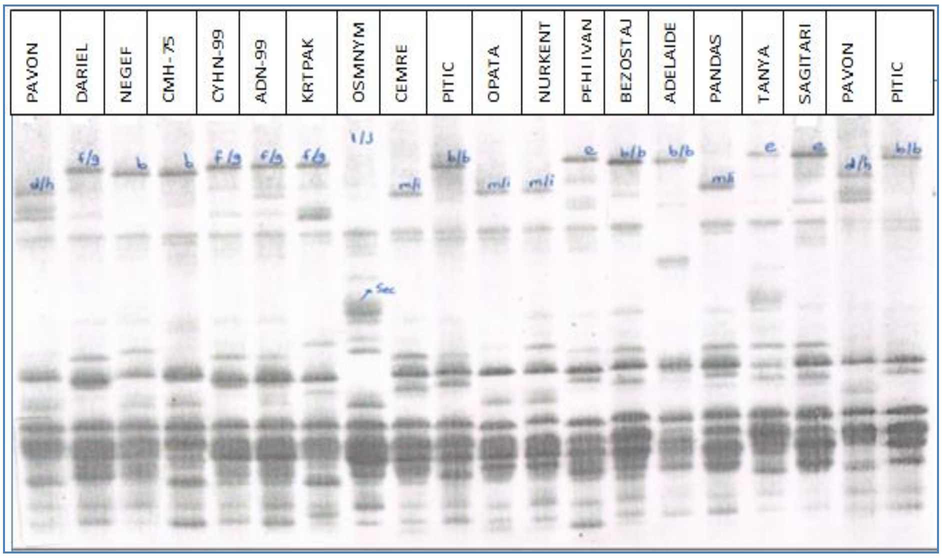

Fig. 2. LMW Glutenin Alleles of Variety for Glu-B3

RESULTS AND DISCUSSION

Anova Analysis and Average of Locations for Investigated Traits

According to Combine ANOVA analysis significant statistical differences ($P < 0.01$ or 0.05) was determined between test environments and genotypes for examined quality traits (Table 1). The highest values for dough extensibility (215 mm), dough resistance (398 BU), dough energy value (115 cm^2), gluten index (87 %) were obtained in KZT2 environment (Table 1). While, DYB1 had higher values for wet gluten (37.1%), sedimentation (32.4 ml) and protein content (14.1 %). Also, high moderate values for extensibility (396 mm), protein content (14.1%) were obtained from KZT1 location. In current study, we applied irrigation in all test environments, because of variations of quality traits of the bread showed high variation under drought or limited water conditions. Four groups were obtained in GGE biplot analysis based on environment and examined traits (Fig. 3), and total variations for examined traits across test environments was 88.52%,. Fırst group consist of dough traits, such as dough extensibility, dough resistance, dough energy and gluten index located polygon corner that presented by KZT2 location, second group located corner that represented by DYB1 location and consist of protein contents and thousand grain weight, and only sedimentation include in group of sector KZT1 location and wet gluten in DYB2 location. GGE biplot methodology indicates that there is high correlation or relation between traits in the same group (TEKDAL *et al.* 2018). Also, environments or genotype that located in corner of polygon has highest or suitable for related traits. (KENDAL *et al.* 2015). Results of GGE biplot also indicated that test environment KZT2 was suitable for higher dough extensibility, dough resistance, dough energy and gluten index; DYB1 had higher values for protein contents and thousand grain weigh. While the lowest values for investigated traits were determined in DYB2 location except wet gluten content. Test environments located in different sector of GGE biplot polygon indicated that there is high variations between environments for investigated traits. We also tried to show relation between individual of HMW - LMW glutenin alleles and some quality traits by GGE biplot analysis (Fig. 4). According to results *GluA3-d*, *GluB3-b*, *GluB1-7+8*, *GluD1-5+10* and dough energy value, sedimentation, farinograph stability time located in same sector and near each other which means that, there are relationship between these glutenin subunits and qualiy traits. On the other hand *GluA3-e* and TGW and PSI traits located in same group, this results show that *GluA3-e* is related to TGW and PSI traits. HE *et al.* (2005) investigated glutenin subunits of Chines bread wheat cultivars reported similar results of our study.

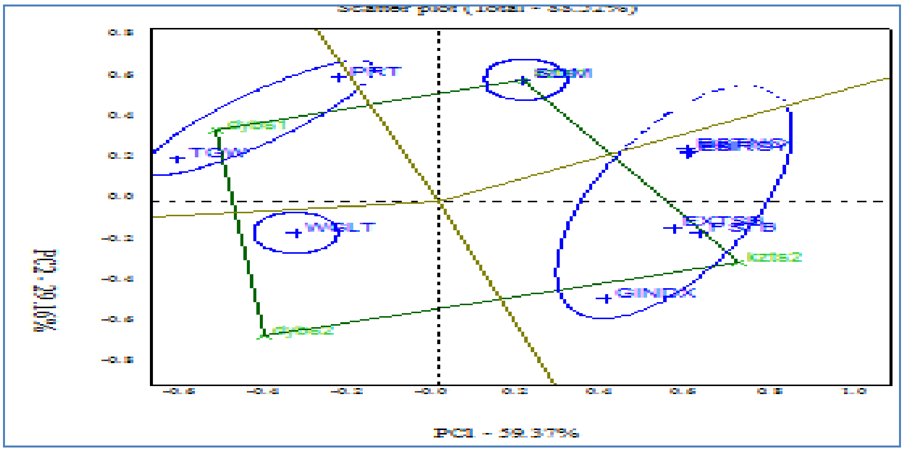

Fig. 3. Biplot graph shows relation of environment and traits.

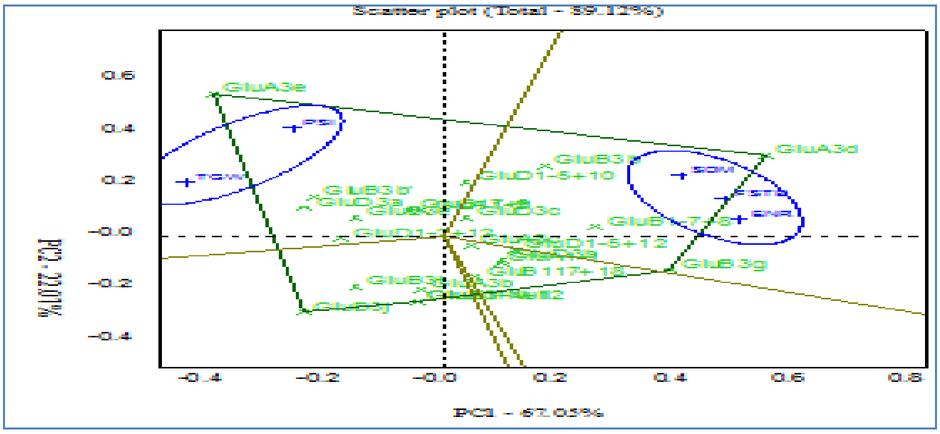

Fig. 4. Biplot graph shows relation of HMW-LMW-GS and some quality traits

Table 1. Anova Analyses and Average of Locations of Examined Traits

		Mean squares of Examined Traits									
Source	DF Num	Ext.	Resis.	Energy	W.Glt	G.Index	F. Stb	Sdm	Prt	TGW	PSI
Rep[Loc]&Rndm	4	30 ns	225 ns	5,63 ns	1,05 ns	2,4 ns	0,02 ns	1,6 ns	0,36 ns	0,82 ns	12,3 ns
Genotype	14	2591**	101579**	7191**	97**	624**	49**	10**	2,8**	65**	164**
Location	3	3539**	283858**	17008**	15*	476**	111**	15*	15,5**	49**	132*
Genoype*Loc	42	1037**	18222**	1100**	15**	67**	8,8**	4,2**	0,63**	5,1**	31,5**
Error	56	91 ns	867 ns	61 ns	1,4 ns	14 ns	0,007 ns	5 ns	0,22 ns	1,6 ns	0,03 ns
C. Total	119	804**	25954**	1692**	18**	116**	12**	19**	1,05**	11,5**	34**
		Average of Examined Traits in Locations									
DYB1 (2012-13)		194 b	247 b	79 c	37,1 a	78 d	3,9 d	32,4 a	14,1 a	38,5 a	35,0 b
DYB2 (2013-14)		191 b	213 c	63 d	36,3 b	86 b	4,5 c	28,5 b	12,9 b	37,9 ab	34,6 b
KZT1 (2012-13)		195 b	396 a	106 b	35,4 c	83 c	5,5 b	33,0 a	14,1 a	37,6 b	38,4 a
KZT2(2013-14)		215 a	398 a	115 a	36,3 b	87 a	8,3 a	31,7 a	12,8 b	35,6 c	38,4 a
Lsd (loc)		3.92**	10.72**	1.72**	0.74**	1.11**	0.09**	2.03*	0.43**	0.64**	2.5**
Lsd (Loc*gen)		19.1**	60**	15.7**	2.34**	7.46**	0.17**	4.5**	0.93**	2.54**	0.36**

Means followed by the same letter in the column are not statistically different*: Statistically significant at 0.05, **: Statistically significant at 0.01,

Ext: Extensograph dough extensibility, DF: Degree of freedom Resis: Extensograph dough maximum resistance, Energy: Extensograph dough energy value, Glt:Wet gluten content, F.Stability: Farinograph dough stability time, Sdm: Zeleny sedimentation value, Prt: Protein content, TGW: Thousand grain weight,PSI: particle size index, DYB1: Location of Diyarbakır in 2012-13 season, DYB2: Location of Diyarbakır in 2013-14 season, KZT1: Location of Kızıltepe in 2012-13 season, KZT2: Location of Kızıltepe in 2013-14 season

Frequences of HMW and LMW Glutenin Alleles

The *HMW* and *LMW* glutenin alleles belonging to the investigated varieties and their distribution were showed in Table 2, Fig. 1., and Fig. 2. Ten alleles (with ten combinations) were found for *HMW-GS* and 14 alleles (with 11 combinations) for *LMW-GS*. At *Glu-A1*, there was a variation of three alleles, and the distribution among the varieties was noted as *2** (46.6%), *1* (46.6%) and *Null* (6.6%). *17+18* (40%), *7+9* (46.6%) and *7+8* (13.3%) alleles were detected at *GluB1* and *5+12* (53.3%), *2+12* (26.6%) and *4+12* (6.6%) alleles at *GluD1*. Concerning *LMW-GS*, *b* (20%), *c* (60%), d (6.6%), *e* (6.6%), and *f* (6.6%) alleles were identified at *GluA3; b* (13.3%), *b'* (13.3%), *e* (20%), *g* (26.6%), *i* (20%), and *j* (6.6%) alleles at *GluB3*; and *a* (13.3%), *b* (46.6%) and *c* (40%) alleles at *GluD3*. Only one variety, Osmaniyem had secale translocation which had negative effect on gluten structure. Table 3., also presents the quality scores of the HMW alleles of the varieties according to PAYNE *et al.* (1987). The quality score of the varieties used in the study varied between 5 and 9. Some earlier researchers suggested that the quality scores of *HMW* glutenin components of a genotype are not in agreement with the results of actual conventional dough rheological analysis, *HMW-GS* and *LMW-GS* should be evaluated together, secale translocation of the genotypes should be taken into consideration, and genotypes with secale translocation have lower gluten qualities despite their high protein, sedimentation and wet gluten ratio values (ANTES and WIESER, 2001; KIM *et al.*, 2003).

Table 2. Frequences and Mean of Some Quality Traits of each HMW - LMW Glutenin Allelles

Glu Locus	Alleles	Numbers	Frequence (%)	Energy	F. Stability	Sdm	PSI	TGW
	2*	n=7	46,6	91	4,98	31,5	38,4	38,3
GluA1	1	n=7	46,6	91	6,27	31,7	34,9	36,7
	Null	n=1	6,6	87	4,61	29,1	36,2	36,2
	17+18	n=6	40	91	5,35	31,2	35,0	36,6
GluB1	7+8	n=2	13,3	121	7,64	32,1	37,0	35,2
	7+9	n=7	46,6	83	5,14	31,7	37,9	38,8
	5+10	n=2	13,3	95	6,26	32,4	38,7	38,4
GluD1	2+12	n=3	26,6	68	4,17	30,3	38,3	38,6
	5+12	n=9	53,3	101	6,19	32,0	35,4	36,7
	4+12	n=1	6,6	87	4,61	29,1	36,2	36,2
	b	n=3	20	80	4,34	30,3	36,1	36,3
	c	n=9	60	92	5,69	31,8	35,5	37,8
GluA3	d	n=1	6,6	148	10,67	35,0	37,8	34,2
	e	n=1	6,6	59	3,81	30,1	47,5	41,2
	f	n=1	6,6	87	4,61	29,1	36,2	36,2
	b	n=2	13,3	107	6,06	35	37,9	37,5
	b'	n=2	13,3	73	4,21	29,6	41,9	38,7
GluB3	e	n=3	20	78	5,58	29,5	39	39,3
	g	n=4	26,6	126	8,28	33,1	32,6	34,8
	i	n=3	20	63	3,38	30,5	36,1	37,4
	j	n=1	6,6	60	2,82	29,6	33,8	39,4
	a	n=2	13,3	61	4,63	30,4	38,7	40,5
GluD3	b	n=7	46,6	93	6,66	31,2	35,5	36,5
	c	n=6	40	91	5,8	32	37,6	37,5

Energy:Extensograph dough energy value, F.Stability: Farinograph dough stability time,
Sdm: Zeleny sedimentation value, TGW: Thousand grain weight

Average of Examined Traits for Each HMW and LMW Alleles (Effect of Particular Alleles on Quality Traits)

The average values of each *HMW-GS* and *LMW-GS* for dough and some other quality properties are given in Table 2. The mean farinogram stability and sedimentation values of *GluA1-2** and *GluA1-1* alleles were higher compared to the *GluA1-Null* alleles, while the lowest grain hardness (PSI 38.4) and highest grain weight (38.3 g) were obtained from the *GluA1-2** alleles. Among the *GluB1* alleles, the highest energy value (121 cm^2), farinogram stability value (7.64 min) and sedimentation (32.1 ml), and the lowest thousand grain weight were observed in the *GluB1-7+8* allele. Some researchers reported that the *GluB1-7+8* allele had a positive effect on the rheological properties of dough and technological quality parameters (RAM *et al.*, 2003). The mean energy values of the *GluD1-5+10* and *GluD1-5+12* alleles were higher compared to the *GluD1-2+12* (68 cm^2) and *GluD1-4+12* (87 cm^2) alleles. Similar results were obtained in relation to the farinogram stability and sedimentation values. In the literature, it is accepted that

GluD1-2+12 is associated with a weak gluten structure and *5+10* with a strong gluten structure (MAUCHER *et al.*, 2009; KILIÇ *et al.*, 2017).

The highest energy (148 cm^2), farinogram stability (10.7 min) and sedimentation (35 ml) values were obtained from the *GluA3-d* alleles. In contrast, the PSI value representing the softest grain structure (47.5 %) and the highest thousand grain weight was observed in the *GluA3-e* allele. For *GluB3*, the highest energy, farinogram stability and sedimentation values were obtained from the *GluB3-g* and *b* alleles, respectively. However, *GluB3-b', i* and *j* alleles had low energy, farinogram stability and sedimentation values, softer grain structure, and higher thousand grain weight. The *GluD3-b* allele had higher energy, farinogram stability, sedimentation, and grain hardness (PSI) values than the remaining alleles in the same group.

When the data obtained from the individual evaluation of *HMW* and *LMW* alleles in this study were collectively evaluated, it was seen that development of genotypes with combinations of *GluA1-2** or *1*, *GluB1-7+8*, *GluD1-5+10, GluA3- d, GluB3- g* and *b, GluD3-b* alleles would indirectly contribute to obtaining products with an intact gluten structure. Many researchers previously showed that the *GluA1- 1* and *2** alleles and the *GluD1 5+10* allele had a more positive effect on bread quality characteristics compared to the *Null* allele and the *2+12* allele, respectively (CUNSOLO *et al.*, 2004). However, there is limited or no research in the literature on the effect of the *4+12* and *5+12* alleles of *GluD1* on quality characteristics. It was reported that among the *GluB1* alleles, *17+18* and *7+8* had higher quality scores than *7+9* (NAZCO *et al.*, 2014). Although there are only few studies on the effect of *LMW* alleles on bread-making quality characteristics, the positive effect of the *GluA3-b* allele on sedimentation and that of the *GluB3-g* allele on gluten strength are known (EAGLES *et al.*, 2002). We obtained similar results in the current study.

Evaluation of HMW and LMW-GS Combinations for Examined Traits (Effect of HMW and LMW-GS Combinations on Quality Traits)

Mean of quality traits of varieties based on four locations and HMW (Glu1) – LMW (Glu3) glutenin alleles of varieties are given Table 3. When the mean energy values of the Dariel, Cumhuriyet-75 and Cemre varieties with the same HMW-GS (*GluA1- 2**, *Glub1- 17+18* and *GluD1-5+12*) but different *LMW* alleles were compared, the highest mean energy value (143 cm^2) was obtained from Dariel possessing the *GluA3-c*, *GluB3-g* and *GluD3-b* LMW alleles. Cumhuriyet-75 with the *GluA3-e*, *GluB3-b'* and *GluD3-b* alleles and Cemre with the *GluA3-c*, *GluB3-i* and *GluD3-c* alleles had a mean energy value of 59 cm^2 and 65 cm^2, respectively. Similar results were obtained for dough resistance and extensibility, gluten index, and farinograph stability time. The mean resistance value of Dariel was calculated as 475 BU, while that of Cumhuriyet-75 and Cemre was 188 BU. Concerning extensibility, the highest mean value was recorded for Dariel at 220 mm, followed by Cemre and Cumhuriyet-75 (161 mm and 189 mm, respectively). Similarly, Dariel was found to be superior in terms of hardness (32.2 %), and also had a slightly higher mean sedimentation value (32.5 ml) compared to Cumhuriyet-75 (47.5 % and 30.1 ml, respectively) and Cemre (33.2 % and 31 ml, respectively). However, the thousand grain weight, protein ratio and wet gluten values were higher in Cumhuriyet-75 and Cemre varieties. According to these results, for energy, strength, extensibility, gluten index, farinograph stability, sedimentation and grain hardness properties, the following hypothesis can

Table 3. Mean of Quality Traits of Varieties Based on 4 Locations and HMW (Glu1) – LMW (Glu3) Glutenin Alleles of Varietis

Varieties	Ext	Resis.	Enrgy	W. Glt	G.Indx	F. Stb	Sdm	Prt	TGW	PSI	GluA1	GluB1	GluD1	Score	GluA3	GluB3	GluD3	1B/1R
1-Dariel	220^{b}	475^{b}	143^{a}	34^{g}	92^{ab}	5.79^{g}	32.5^{cd}	13.4^{df}	33^{j}	32.2^{m}	2*	17+18	5+12	9	c	g	b	1B/1B
2-Negev	222^{ab}	411^{c}	117^{b}	36^{ef}	88^{bc}	7.07^{d}	32.7^{cd}	13.6^{cd}	35^{hi}	38.1^{e}	2*	7+9	5+12	8	b	b	b	1B/1B
3-Cumhuriyet	161^{g}	188^{g}	59^{f}	39^{c}	73^{f}	3.81^{k}	30.1^{ei}	13.8^{bd}	41.2^{b}	47.5^{a}	2*	17+18	5+12	9	e	b'	b	1B/1B
4-Ceyhan	209^{c}	407^{cd}	112^{b}	33^{h}	93^{a}	9.73^{b}	33.5^{bc}	13.1^{eh}	36^{gh}	27.4^{n}	1	17+18	5+12	9	c	g	b	1B/1B
5-Adana	195^{de}	377^{de}	102^{c}	33^{hi}	91^{ac}	6.91^{e}	31.4^{cg}	12.9^{gi}	36.1^{gh}	33^{l}	1	17+18	5+12	9	c	g	b	1B/1B
6-Karatopak	231^{a}	506^{a}	148^{a}	35^{fg}	91^{ac}	10.67^{a}	$35a^{b}$	14^{bc}	34.2^{ij}	37.8^{f}	1	7+8	5+12	10	d	g	c	1B/1B
7-Osmaniyem	180^{f}	202^{g}	60^{f}	39^{c}	70^{fg}	2.82^{n}	29.6^{fj}	14.1^{b}	39.4^{cd}	33.8^{j}	1	7+9	5+12	8	b	j	b	1B/1R
8-Cemre	189^{ef}	213^{g}	65^{f}	39^{c}	73^{f}	2.74^{n}	31^{dh}	13.3^{dg}	38.6^{de}	33.2^{k}	2*	17+18	5+12	9	c	i	c	1B/1B
9-Nurkent	207^{c}	188^{g}	62^{f}	36^{ef}	83^{d}	3.13^{l}	28.4^{ij}	12.9^{fi}	34.4^{i}	36.5^{h}	1	17+18	2+12	6	b	i	b	1B/1B
10-Pehlivan	200^{cd}	190^{g}	61^{f}	41^{b}	67^{g}	2.93^{m}	29.2^{gj}	13.2^{eh}	43.4^{a}	40.2^{b}	2*	7+9	2+12	6	c	e	a	1B/1B
11-Bezostaja	189^{ef}	375^{e}	96^{cd}	42^{a}	78^{e}	5.06^{h}	37^{a}	14.8^{a}	39.9^{c}	37.8^{f}	2*	7+9	5+10	8	c	b	c	1B/1B
12-Adelaide	189^{ef}	303^{f}	87^{e}	31^{j}	90^{ac}	4.61^{i}	29.1^{gj}	12.7^{hi}	36.2^{gh}	36.2^{i}	Null	7+8	4+12	7	f	b'	c	1B/1B
13-Pandas	192^{de}	191^{g}	61^{f}	37^{d}	84^{d}	4.29^{j}	32.2^{ce}	13.5^{ce}	39.2^{cd}	38.6^{d}	1	7+9	2+12	6	c	i	c	1B/1B
14-Tanya	187^{ef}	376^{e}	95^{cd}	32^{ij}	88^{c}	7.47^{c}	27.7^{j}	12.6^{i}	36.8^{fg}	39.6^{c}	2*	7+9	5+10	8	c	e	c	1B/1B
15-Sagitario	208^{c}	294^{f}	91^{de}	37^{de}	88^{c}	6.33^{f}	31.6^{cf}	13.6^{ce}	37.6^{ef}	37.2^{g}	1	7+9	2+12	6	c	e	a	1B/1B
ORT	199	313	90	36	83	5.56	31.4	13.4	37.4	36.6								
CV(%)	4.8	9	9	3	5	1.6	7.1	3.5	3.4	0.5								
Lsd (gen)	9.5**	29.4**	7.85**	1.2**	3.74**	0.09**	2.4**	0.46**	1.26**	0.18**								

Means followed by the same letter in the column are not statistically different, *: Statistically significant at 0.05, **: Statistically significant at 0.01,

Ext: Extensograph dough extensibility, Resis: Extensograph dough maximum resistance, Energy: Extensograph dough energy value, W. Glt: Wet gluten content,

F.Stability: Farinograph dough stability time, Sdm: Zeleny sedimentation value, Prt: Protein content, TGW: Thousand grain weight, PSI: particle size index be established: *GluA3-c >e*, *GluB3-g > b' = i,* and *GluD3-b ≥ c*. These results indicate that *GluA3-c*, *GluD3-b*, *GluB3-b* and especially *GluB3-g* alleles have a positive effect on energy, extensibility and strength values, and thus they can be used as markers in the development of varieties that produce dough with high strength and extensibility traits. Many researchers have reported that the *GluA3-d, GluB3-g* and *GluB3-b* alleles have a positive effect on bread-making quality parameters (BRANLARD *et al.,* 2003; HE *et al.*, 2005). For example, LUO *et al.* (2000) showed the positive effect of the *GluA3-d*, *GluB3-b* and *GluD3-b* alleles on dough strength and extensibility. ZHANG *et al.* (2012) found that the *GluB3-g, GluB3-b* and *GluB3-i* alleles were associated with high quality bread-making properties, while *GluA3-e* and *GluB3-c* were related to lower bread quality. In the current study, in general very similar results were obtained, and the only difference was observed in relation to *GluB3-i*. Our results indicated that adding *GluB3-g* to the allelic combination for the development of varieties in breeding programs can provide benefits. For the remaining properties investigated; i.e., thousand grain weight, protein ratio, and wet gluten, the hypothesis of *GluA3-e > c, GluB3- b' > g = i,* and *GluD3-c ≥ b* appeared to be valid.

Varieties, Ceyhan-99 and Adana-99, which have same HMW and LMW-GS (*GluA1-1; GluB1-17+18 and GluD1-5+12; GluA3-d; GluB3-g and GluD3-b*) had lower values for examined traits compare to Karatopak variety (*GluA1-1; GluB1-7+8 and GluD1-5+12; Glu3-d; GluB3-g and GluD3-c*) that has different alleles (*GluB1-7+8* and *GluD3-c)* from both Ceyhan-99 and Adana-99. Variety, Karatopak with the *GluB1-7+8*, *GluA3-d* and GluD3-c alleles had higher extensograph energy (148 cm^2), extensibility (231 mm), resistance (506 BU), wet gluten (35.2 %), farinograph stability (10.67 min), sedimentation (35 mm) and protein ratio (14 %) values. While, Ceyhan-99 which had higher energy (112 cm^2), extensibility (209 mm), resistance (407 BU), wet gluten (32.8 %), farinograph stability (9.73 min), sedimentation (33.5 mm) and protein ratio (13.1 %) values than Adana-99, but had a lower values for these traits compare to Karatopak variety. However, Ceyhan-99 and Adana-99 had higher thousand grain weight and PSI values than the Karatopak variety. This shows that the *HMW* and *LMW* alleles of Ceyhan-99 and Adana-99 varieties were more effective or superior for TGW and grain hardness (PSI). Based on these results, it is possible to hypothesize *GluB3-7+8 > GluB3-17+18; GluA3-d > c* and *GluD3-b = GluD3-c* for the energy, extensibility, strength, sedimentation, wet gluten, protein ratio and farinograph stability properties and *GluB3-17+18 > GluB3-7+8; GluA3-c > d and GluD3-c = GluD3-b* for thousand grain weight and grain hardness. It is suggested that for some quality parameters, *GluA1-1 and GluB1-2** alleles have superiority over the *GluA1-Null* allele in the presence of the *GluD1-5+10* allele, and therefore these alleles have an epistatic effect on quality properties. Similarly, it has been reported that the *GluB1-7+8 and 7+9* alleles are superior to the remaining alleles in the same group in the presence of *GluD1-5+10*. OBREHT *et al.* (2007) ordered the *GluB1* alleles as *'7+8 > 7+9 > 7=6+12* and pointed to the superior characteristics of *GluB1-7+8* combined with *GluD1-2+12* compared to the other *GluB1* alleles combined with the same allele. COSTA *et al.* (2013) investigated the effect of *HMW-GS* and *LMW-GS* on the physicochemical characters of 16 different Brazilian varieties and noted that in the presence of *GluD1-5+10*, the effect of *Glu3* and *Glu1* alleles on quality parameters was dominated by *5+10* and made it difficult to determine the effect of other alleles, whereas in combinations without the *5+10* allele, the *GluB1-7+8* and *GluA3-d* alleles had observable effects on quality. In the current study, it was found that the combination of *GluB1-7+8* and *GluA3-d* alleles could provide great benefits in the development of varieties with a strong gluten structure in breeding programs.

For the extensograph energy and resistance values, farinograph stability, and gluten index, Bezostaja (*GluA1-2*; GluB1-7+9 and GluD1-5+10; GluA3-c; GluB3-b and GluD3-c*) and Tanya (*GluA1-2*; GluB1-7+9 and GluD1-5+10; GluA3-c; GluB3-e and GluD3-c*) with the *GluD1-5+10* and *GluD3-c* alleles had superior characteristics to Pehlivan (*GluA1-2*; GluB1-7+9 ve GluD1-12+12; GluA3-c; GluB3-e ve GluD3-a*), whereas Pehlivan and Sagitario with the same LMW alleles (*GluA1-1; GluB1-7+9 and GluD1-12+12; GluA3-c; GluB3-e and GluD3-a*) performed better in dough extensibility compared to Bezostaja and Tanya. These results support the hypotheses of *GluD1-5+10 > GluD1-2+12 and Glu-D3-c > GluD3-a* for dough energy, resistance values and farinograph stability and gluten index, and *GluD1-2+12 > GluD1-5+10 and GluD3-a > GluD3-c* for dough extensibility. Many studies reported that the varieties carrying the *2+12* allele generally had a weak gluten structure, but there were also varieties possessing this allele but a stronger gluten structure. Indeed, the Sagitario variety of Italian origin is one of the high-quality wheat varieties that easily finds buyers in the wheat market in Turkey. However, it is also possible to evaluate dough extensibility independently from strength and energy traits since varieties without a strong gluten structure may also exhibit high extensibility. In this sense, dough extensibility and strength properties should be evaluated as different characteristics. The combination of *HMW* and *LMW* alleles may also provide unusual results considering the combined effect of *HMW-GS* and *LMW-GS* on dough characteristics (ZHU *et al.*, 2015).

Combinations of *HMW* and *LMW* alleles had an effect on gluten quality, confirmed by the finding that when these alleles were not considered, the Cemre, Nurkent and Pandas varieties with the *GluB3-i* allele were found to have lower extensograph energy, resistance and gluten index values compared to varieties possessing the *GluB3-g, GluB3-b,* and *GluB3-e* alleles. In some studies, the *GluB3-i* allele was associated with a strong gluten structure, whereas in our study, the opposite results were obtained; i.e., the dough extensibility values for the *GluB3-i* allele were close to the average, which suggests that this allele is related to a softer gluten structure, and thus probably has a positive effect on dough extensibility.

The results lead to the hypothesis of *GluB3- g ≥ GluB3-b ≥ GluB3-e > GluB3-b' ≥ GluB3-i > GluB3-j* and indicate that the selection of genotypes combined with *GluB3-b* and *GluB3-e*, and especially *GluB3-g* in breeding programs would be beneficial in developing varieties with favorable dough properties. In this study, the only variety with secale translocation was Osmaniyem, which had a near-average or above-average protein ratio, sedimentation and thousand grain weight values but the lowest gluten index, energy, strength and extensibility values. It is reported that varieties with secale translocation generally result in a soft, sticky and weak gluten structure (EAGLES *et al.*, 2002). However, some researchers suggested that the varieties with secale translocation were resistant particularly to diseases, and when combined with the *HMW* alleles associated with a strong gluten structure (*2*, 7+8* and *5+10*), their dough characteristics were observed to be within normal value ranges.

CONCLUSION

The results of our study indicated that ten alleles (with ten combinations) were found for HMW-GS and 14 alleles (with 11 combinations) for LMW-GS in the tested varieties. Different widely grown varieties were tested under different environments to check the effect of various HMW and LMW alleles on quality traits of bread wheat. There was plentiful variation among tested environments for all studies varieties for different quality traits. Test environments located in different sector of GGE biplot polygon indicated that there is high variations between environments for investigated traits. The results clearly indicated that, there is relationship between these glutenin subunits and quality traits in bread wheat. The overall evaluation of the results obtained from this study demonstrates that *GluA1-1 or 2**, *GluB1-7+8 and GluD1-5+10* for HMW glutenin alleles and *GluA3-d*, *GluB3-g and b*, *GluD3-c* for LMW glutenin alleles are associated with a strong gluten structure and integrating these subunits/alleles can contribute to the development of genotypes with strong gluten characteristics when used in combination in breeding programs of Turkey. In this study also we concluded that combinations of *Glu-B1-2+12* and *GluB3- i* subunits of HMW and LMW respectively are indicator of softer gluten structure while *GluD1-5+10 and GluD3-c* have positive effect on dough energy value compare to *GluD1-2+12 and Glu-D3-a.* Results indicates that combinations of *GluD1-2+12 and GluD3-a* are more effective for dought extensibility; *GluA3-e*, *GluB3-b', i* and *j* alleles had low energy, farinogram stability and sedimentation values, softer grain structure, but higher thousand grain weight. According to our study results, we suggest that reducing the presence of the *1B/1R* translocation and alleles/subunits that has negative effects on examined quality traits in our study will contribute to improving varieties with gluten strength and extensibility and breadmaking quality. The results showed that Karatopak with *GluB1-7+8*, *GluA3-d*, *GluB3-g and GluD3-c* alleles has potential to improve high quality genotypes in breeding programs, because of it's highest performance for most quality traits.

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UTICAJ ALELA HMV i LMV GLUTENINA NA OSOBINE KVALITETA HLEBNE PŠENICE

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Izvod

Ovo istraživanje je urađeno da se ispita uticaj alela HMV i LMV glutenina na osobine kvaliteta kod sorata hlebne pšenice. Petnaest sorti hleba pšenice korišćeno je za poljske oglede tokom 2012-13 i 2013-14 u navodnjavanim uslovima na lokacijama Diiarbakır i Mardin, Turska. Ispitivali smo kvalitet sorti koje imaju iste HMV-GS (visoko-molekularne podjedinice) i različite LMV-GS (niskomolekularne podjedinice) ili obrnuto. Rezultati su pokazali da *GluA3-c > d > e, GluB3-g > b' = i,* i *GluD3-b ≥ c > a for LMW-GS, and GluB1-7+8 > GluB1-17+18; GluD1-5+10 > GluD1-2+12 for HMW-GS*, imaju veću energiju testa ekstenzorgrafa, istegljivost, otpornost, takođe veće vreme stabilnosti farinografa, indeks glutena i tvrdoću zrna. Ukupna ocena dobijenih rezultata pokazala je da su *GluA1-1* ili *2**, *GluB1-7 + 8* i *GluD1-5 + 10* za HMV gluteninske alele i *GluA3-d*, *GluB3-g* i *b*, *GluD3-c* za LMV alele glutenina povezani sa jakom glutenskom strukturom, pa prema tome izbor linija koje sadrže ove alele može doprineti razvoju genotipova visokog kvaliteta u programima oplemenjivanja pšenice.

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