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Glu-A3b allele has a significant effect on gluten quality of bread wheat in a recombinant inbred line population

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Glu-A3b allelinin rekombinant kendilenmiş hat popülasyonunda ekmeklik buğdayın gluten kalitesi üzerinde önemli bir etkisi vardır

Abstract: In this study, a total of 147 wheat lines with variying low molecular weight glutenin subunits (LMW-GS), obtained by crossing Tosunbey and Tahirova2000 bread wheats, were included. Milling, protein, dough-mixing properties of the genotypes were measured and their relations with LMW-GS were investigated in eight different environments. As the LMW-GS of the parents were quite different; milling, protein and dough-mixing properties of the lines were significantly influenced. In this regard, presence of rye translocation (*Glu-B3j*) reduced flour yield and increased damaged starch and protein contents. In terms of protein quality, *Glu-A3b+Glu-B3b* allellic combinations were better than *GluA3b+Glu-B3j* or *Glu-A3e +Glu-B3j* allellic combinations. It was observed that negative effects of rye translocation could be minimized by selecting proper *Glu-3* alleles, such as *Glu-A3b* instead of *Glu-A3e*. LMW-GS combinations of the lines influenced mixolab mixing and thermorheological properties. In this respect, the lines with *Glu-A3b* or *Glu-B3b* allelles showed increased mixing time and stability as compared to the lines with *Glu-A3e* or *Glu-B3j* allelles. The effect of LMW-GS alleles on gluten quality and dough strength was statistically bb > eb > bj > ej. In terms of myxolab stability value related to bread volume; 1 = 2*, 7 + 9 > 17 +18, b > e and b > j; in terms of mixing time; 1 > 2*, 7 + 9 < 17 +18, b > e and b > j. As a result, the *Glu-A3b* allele can be used to increase gluten quality, and the *Glu-B3j* allele can be used to increase protein content. Proper allellic combinations of LMW-GS in wheat can be developed for a given bakery product.

Key words: Bread wheat, technological quality, low molecular weight glutenin subunits, wheat-rye translocation

Özet: Bu çalışmaya, Tosunbey ve Tahirova2000 ekmeklik buğdaylarının melezlenmesiyle elde edilen, farklı düşük molekül ağırlıklı glutenin alt birimlerine (LMW-GS) sahip toplam 147 buğday hattı dahil edilmiştir. Genotiplerin öğütme, protein, hamur karıştırma özellikleri ölçülmüş ve LMW-GS ile ilişkileri sekiz farklı ortamda incelenmiştir. Ebeveynlerin LMW-GS'leri oldukça farklı olduğundan; hatların öğütme, protein ve hamur karıştırma özellikleri önemli ölçüde etkilenmiştir. Çavdar translokasyonunun (*Glu-B3j*) varlığı un verimini azaltmış, hasarlı nişasta ve protein içeriğini arttırmıştır. *Glu-A3b+Glu-B3b* allelik kombinasyonları protein kalitesi açısından *GluA3b+Glu-B3j* veya *Glu-A3e +Glu-B3j* allelik kombinasyonlarına göre daha iyi sonuç vermiştir. *Glu-A3e* yerine *Glu-A3b* gibi uygun *Glu-3* allellerinin seçilmesiyle çavdar translokasyonunun olumsuz etkilerinin en aza indirilebileceği gözlenmiştir. Hatların LMW-GS kombinasyonları, mixolab karışımını ve termoreolojik özellikleri etkilemiştir. Bu bakımdan *Glu-A3b* veya *Glu-B3b* allellerine sahip hatlar, *Glu-A3e* veya *Glu-B3j* allellerine sahip hatlara kıyasla daha yüksek karıştırma süresi ve stabilite göstermiştir. LMW-GS allellerinin gluten kalitesi ve hamur sertliği üzerine etkisi istatistiksel olarak bb > eb > bj > ej şeklindedir. Ekmek hacmine bağlı myxolab stabilite değeri açısından; 1 = 2*, 7 + 9 > 17 +18, b > e ve b > j; karıştırma süresi açısından; 1 > 2*, 7 + 9 < 17 +18, b > e ve b > j'dir. Sonuç olarak, *Glu-A3b* alleli gluten kalitesini arttırmak için, *Glu-B3j* alleli ise protein içeriğini arttırmak için kullanılabilir. Belirli bir unlu mamul için buğdaydaki LMW-GS'nin uygun allelik kombinasyonları geliştirilebilir.

Anahtar Kelimeler: Ekmeklik buğday, ekmeklik kalitesi, düşük moleküler ağırlıklı glutenin alt üniteleri, buğday-çavdar translokasyonu

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1. Introduction

The quality of wheat, which has a wide adaptability and rich biodiversity, varies depending on many criteria and has a very broad meaning depending on its intended use in industry. Wheat contains 60-70% starch, 10-15% protein, 5-10% non-starch carbohydrates, 1-2% lipids and 1-2% minerals (Lineback and Rasper, 1988; Pomeranz, 1988).

The amounts, properties, ratios and interactions of these components determine the suitability and intended use of wheat for different end products (Pomeranz, 1988; Hoseney, 1994).

Protein quality is an important trait that determines the intended use. The effect of cultivation on protein quality is less and more genetically controlled (Graybosch et al., 1996). An important storage protein that influences the quality of wheat is gluten. Gluten proteins are composed of prolamin (gliadin) and glutelin (glutenin) fractions (MacRitchie, 2016). Gluten proteins, which have viscous, elastic and cohesive characteristics, are functional proteins unique to wheat due to their ability to form dough and retain gas, and have an irreplaceable role in the production of many bakery products. The viscous, elastic and cohesive stability of gluten proteins is due to the protein content of wheat and the structural properties of gluten proteins (Hoseney, 1994; Barak et al., 2015).

The gliadins that make up gluten proteins are monomeric proteins and are prominent in the viscous and cohesive properties of dough, while glutenines, which are polymeric proteins, are more determinant in dough elasticity (MacRitchie, 2016). The glutenin fraction is divided into high molecular weight (>80 kDa) glutenin (HMW-G) and low molecular weight (<80 kDa) glutenin (LMW-G) proteins according to electrophoretic mobility. (Rasheed et al., 1988). HMW-G on dough rheology and bread quality proteins have been studied in detail and largely clarified. However, the effects of LMW-G proteins on dough rheology and bread quality are still under investigation (Gianibelli et al., 2001, Dupont et al., 2007, Rasheed et al, 2014).

LMW-G protein alleles are encoded by the *Glu-A3*, *Glu-B3* and *GluD3* loci localized on the short arms of the group 1 chromosomes of wheat (Weegels et al., 1996). A total of 15 to 20 *Glu-3* alleles have been identified in bread wheat (Liu et al., 2001; Dangi et al., 2019; Cho et al., 2017). *Glu-A3* and *Glu-B3* alleles were reported to be more effective than *Glu-D3* alleles on dough and bread quality (Zhang et al., 2012). Studies have shown that the c or d allele on *Glu-A3*, the d, b, c or g allele on *Glu-B3* and the a or b allele on *Glu-D3* are associated with higher wheat quality, while the *Glu-A3e* allele is negatively associated with quality (Gupta et al., 1994; Bonafede et al., 2015; Yasmeen et al., 2015; Aktas and Baloch, 2017).

Glu-B3 alleles have a determining effect on dough stability (He et al., 2005; Ahn et al., 2014). In terms of sedimentation volume, the *Glu-A3b* allele, a simple but robust quality test for wheat, was found to be superior to other *Glu-B3* alleles (Ito et al., 2015), while the *Glu-A3e* allele was inversely related to wheat protein content (Gupta et al., 1989) and decreased the gluten index of wheat flour (Zhen et al., 2014). In particular, studies using mixographs have proven that the *Glu-A3e* null allele causes weakening of gluten (Flaete and Uhlen, 2003; Bonefede et al., 2015; Ito et al., 2015).

In addition to dough and bread quality, wheat has been reported to affect the milling characteristics of LMW-GS combinations. Ahn et al. (2014) and Bonafede et al. (2015) found that *Glu-A3* alleles affected flour yield, but *Glu-B3* alleles had no effect on flour yield and particle size distribution. Shin et al. (2012) compared the *Glu-B3h* allele with the *Glu-B3d* allele and concluded that the differences in flour yield and particle size distribution were due to puroindolines (*Pina-D1borPinb-D1b*). Similarly, Ahn et al. (2014) found that flour yield and grain physical properties are influenced by puroindolines rather than allelic combinations of gluten proteins.

Many wheat genotypes in the world carry the *1BL.1RS* wheat-rye translocation. The rye translocation, commonly referred to as the *Glu-B3j* allele, is known to increase grain yield and protein content but reduces the gluten quality of wheat (Gobaa et al., 2008; Moiraghi et al., 2013). Some studies (Fenn et al., 1994; Burnett et al., 1995; Kim et al., 2005) did not identify any effect of rye translocation on flour yield; however, Moiraghi et al (2013) found that rye translocation caused an increase in starch damage, pentosans and water absorption of flours.

Literature shows that LMW-GS or *Glu-3* allelic combinations affect milling characteristics, protein content and gluten quality of wheats, dough mixing characteristics and final product quality. Therefore, it is important to further expand our knowledge on the effects of various *Glu-3* allelic combinations on wheat quality. In this study, the effects of different *Glu-3* combinations (*Glu-A3b - Glu-A3e, Glu-B3b - Glu-B3j etc.)* in 147 recombinant selfed lines obtained by crossbreeding of Tosunbey and Tahirova2000 bread wheat varieties on milling, protein and dough mixing characteristics were investigated in eight different environments.

2. Materials and Method

A recombinant inbred line (RIL) population was developed by crossing Tosunbey and Tahirova2000 cv, was used in the study. Tahirova2000 variety carries the combination of 2*, 7+9, 5+10; *GluA3e* and *GluB3j*; Tosunbey variety carries the combination 1, 17+18, 5+10; *GluA3b* and *GluB3b* alleles. The RILs, composed of 147 genotypes including parents, were grown in eight environments to produce the data for flour yield, protein content, sedimentation volume, damaged starch content, 1000-kernel weight and test weight. The environments were Bafra/Samsun and Eskişehir locations for 2012-2013 growing season and Bafra/Samsun, Eskişehir and Pamukova/Sakarya locations for 2013-2014 and 2014-2015 growing seasons. Therefore, the study contained a grand total of 2352 wheat samples for these quality traits. For Mixolab dough-mixing studies, we used 524 samples from four environments that Bafra/Samsun and Eskişehir for 2012-2013 and 2013-2014 growing seasons.

2.1 Molecular analysis

HMW-GS and LMW-GS of wheats were extracted by Singh et al. (1991) and separated on SDS-PAGE electrophoresis by Masci et al. (2000) and Gianibelli et al. (2001). HMW-GS and LMW-GS were identified following the nomenclature of Bekes et al. (2006). SDS-PAGE electrophoresis was used in the determination of *1BL.1RS* wheat-rye translocation and Glu-B3b allel, which were further confirmed by PCR technique (Wang et al., 2009). DNA was isolated from the leaves of plants that reached the 4-5 leaf stage. For this, the Plant/Seed DNA miniPrep™ Kit was used. Analysis of the *Glu-B3b* allele in the genotypes was also detected using the specified primers and as specified by Wang et al. (2009). PCR gels were imaged using the BioRad gel imaging system.

2.2 Quality analysis

Hectoliter weights and moisture contents of wheat kernels were measured on Dickey-John GAC Plus (Auburn, IL, USA) by the AACCI method 55-10 (AACCI, 2010). Thousand-kernel weights of RILs were determined using Numigral-I (Chopin, Villeneuve-la-Garenne, France) and converted to 14% moisture basis. Wheat samples (200 g) were milled on a Quadrumat Jr. type laboratory mill (Bastak, Ankara, Turkey) upon tempering at 15% moisture content for 24 hours, as elucidated by the AACCI method 26-50 (AACCI, 2000). Moisture contents of flours were measured using Ohaus MB-45 moisture tester (Ohaus, Greifensee, Swetzerland). Damaged starch contents of flours (14% moisture basis) were measured on SD-Matic device (Chopin, Villeneuve-la-Garenne, France) by the ICC method 172 (ICC, 2011). Protein contents of flours were quantified using Perten 9500 NIR spectroscopy (Perten, Hagersten, Sweden) and expressed as 14% moisture basis. SDS sedimentation volumes of flours (14% moisture basis) were measured according to the method of Maghirang et al. (2006), which was modified from the AACCI method 56-70 (AACCI, 2000). Dough-mixing properties of flours were measured on Mixolab system (Chopin, Villeneuve-la-Garenne, France) employing the "Chopin+" protocol by the ICC method 173 (ICC, 2011).

2.3 Statistical analysis

The experiments were conducted according to a balanced-lattice experimental design with two replications. The data collected within the scope of the study were analyzed by this experimental design using the JMP software (Patterson and Hunter, 1983) We investigated the effects of HMW-GS and LMW-GS and, their interactions on quality traits.

3. Results

3.1. Molecular analysis

DNA markers developed by Wang et al. (2009) were used to determine the *Glu-B3b* allele carrying status of the lines in the recombinant selfed line population and the lines carrying and not carrying the *Glu-B3b* allele were determined (Fig. 1). As a result of molecular screening using *Glu-B3b* primers, 1570 bc (base pair) long bands were obtained in lines carrying the relevant allele. Lines not carrying the relevant allele did not produce bands. In addition, lines carrying the *Glu-B3b* allele do not carry rye translocation. The results obtained are in agreement with the results determined by SDS-PAGE method (Fig. 2).

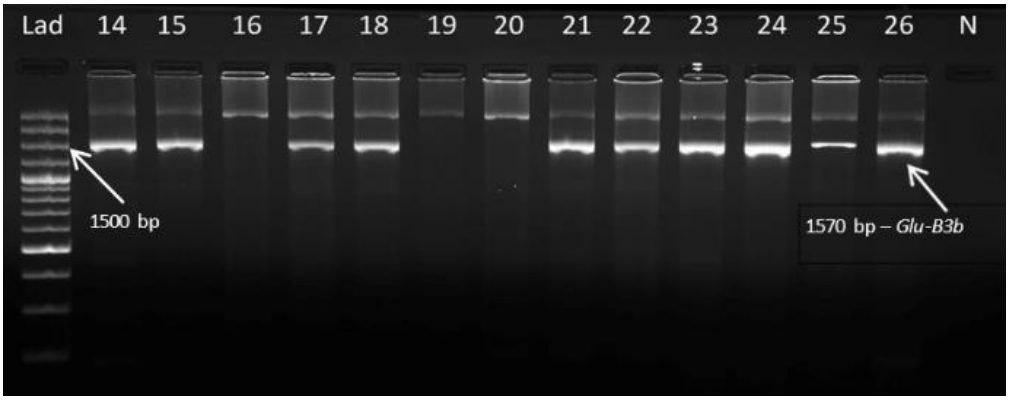

Figure 1. Gel photograph of some lines for *Glu-B3b* allele (Lad: Ladder, N: Control)

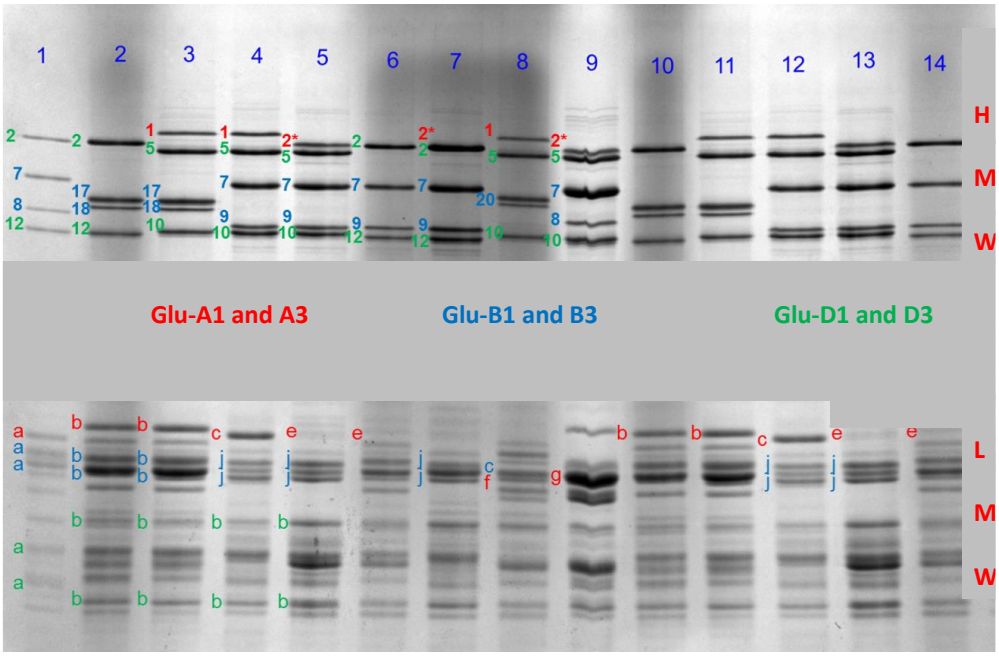

Figure 2. SDS-PAGE screening results of some genotypes (11: Tosunbey, 13: Tahirova2000)

SDS-PAGE analysis was performed for HMW and LMW subunits in all lines and alleles carried by some lines are shown in Figure 2. As can be seen in the Figure 1, *GluA3e* and *GluB3j* alleles are null alleles for wheat, while the presence of *GluB3j* allele indicates the presence of rye translocation.

3.2. Quality analysis

The mean values of some technological traits of *Glu-A1* alleles 1 and 2*, *Glu-B1* alleles 17+18 and 7+9 are given in Table 1. Variance analysis results of some technological traits of the genotypes in the study are given in Table 2. Year, location, *Glu-A1, Glu-B1, Glu-B1, Glu-B3* and some interactions were found significant at 1% or 5% level. In other words, besides the environment, both HMW-G and LMW-G alleles seem to have an effect on technological quality traits.

There was no statistical difference between *Glu-A1* alleles (1 and 2*) in terms of damaged starch content, hectoliter weight and protein ratio. In *Glu-A1* 1 allele, hectoliter weight and flour yield of 7+9 were higher than 17+18, while kernel weight and damaged starch content were lower. It was noteworthy that the SDS sedimentation values indicating gluten quality were higher (28.2 ml) in the 17+18 carriers of these two subunits with the same protein ratio average (11.2%) in 1 of the *Glu-A1* alleles. When the same two alleles were evaluated in 2* *Glu-A1* allele, 7+9 again had slightly higher flour yield, but lower hectoliter weight and kernel weight and higher damaged starch content. Although the protein ratio was slightly lower (11.0%), the SDS sedimentation values of the two subunits were close. The 17+18 subunit had a significantly higher SDS sedimentation value and damaged starch value at a protein ratio close to the 1 allele compared to the *Glu-A1* 2* allele. In addition, grain physical properties and flour yield were lower. The 7+9 subunit also gave higher gluten quality and protein content with the 1 allele. Although kernel weight and damaged starch content were lower, hectoliter weight and flour yield were similar in both alleles.

In the *Glu-B1* 17+18 subunit, the effects of *Glu-A3/B3* LMW-GS alleles on gluten quality (sedimentation value) were statistically *bb* > *eb* > *bj* > *ej*. Statistically, *eb* = *bj* for the *Glu-A1* 1 allele and *bj* = *ej* for the *Glu-A1* 2 allele. Clearly, the presence of the *-bb* allele was associated with high and the presence of the rye translocation *(j)* with low gluten quality. For protein ratio there is an inverse effect *bj* = *ej* > *bb* = *eb*. The effects of *Glu-A3/B3* alleles on gluten quality are similar in the 7+9 allele. *Glu-A3/B3 -bb* was highly effective and *-ej* was low while the other two alleles were close to each other. In general, in terms of protein ratio, *-ej* and *-bj* gave high values in both *Glu-A1* alleles, while the other two alleles had low values.

In the study, it was noteworthy that in the *Glu-A1* 1 allele, the *-bj* and *-ej* alleles had higher amounts of damaged starch than the other two alleles. Kernel weight in *Glu-A1* 1 allele of *-ej* allele is lower than the other alleles, while it is good in 2* allele. Among alleles, *-bj* allele did not decrease the kernel weight in general. In 2*, 7+9 alleles, high kernel weight of *-eb* allele (43.4 g) and high hectoliter weight of *-bb* and *-eb* were remarkable. Apart from these alleles, *-bj* allele had low hectoliter weight. Flour yield was found to be high in all combinations of *-bb* allele and *-eb* allele which are effective in terms of protein quality. The presence of wheat rye translocation was therefore found to be unfavorable in terms of both bread making and milling quality. Especially in combination 1, 17+18, low kernel weight in *-bj* allele is remarkable (Table 1).

The mean values of alleles at *Glu-A1, B1, Glu-A3* and *Glu-B3* loci are statistically compared in Table 3. While sedimentation value (gluten quality) was higher in *Glu-A1* 1 allele, flour yield and kernel weight were higher in 2* allele. No significant difference was observed among other parameters. *In Glu-B1*, 7+9 gave higher values in terms of flour yield and 17+18 gave higher values in terms of damaged starch content, while no statistically significant difference was observed between the other parameters. At the *Glu-A3* and *B3* loci, the b allele showed superiority over

Table 1. The mean results of grain yield and based on the averaged data from eight environments

HMW-GS		LMW-GS		Line number	Flour yield (%)	Protein content (14% mb)	SDS (ml)	Damaged starch content (%)	1000-kernel weight (g)	Test weight (kg)
Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*							
1	17+18	b	b	18	55.5 cd	10.9 ef	33.3 b	3.72	40.8	77.1
1	17+18	e	b	16	55.6 bc	10.8 fg	27.4 ef	3.71	40.6	77.2
1	17+18	b	j	1	50.9 j	11.9 a	28.9 de	3.91	42.6	75.7
1	17+18	e	j	7	53.4 hi	11.0 d-f	23.0 j	3.86	39.0	76.6
					53.9	**11.2**	**28.2**	**3.80**	**40.8**	**76.7**
2*	17+18	b	b	21	55,3 cd	10.9 ef	30.9 c	3.63	40.3	77.4
2*	17+18	e	b	17	55.3 cd	11.0 de	26.1 h	3.74	41.3	77.2
2*	17+18	b	j	3	55.0 c-f	11.6 ab	24.2 i	3.49	42.9	76.9
2*	17+18	e	j	5	54.5 d-g	11.5 a-c	20.5 i	3.67	43.1	77.2
					55,0	**11.3**	**25.4**	**3.63**	**41.9**	**77.2**
1	7+9	b	b	1	56.2 a-e	11.4 a-e	36.8 a	3.27	41.8	77.3
1	7+9	e	b	4	57.2 a	10.5 g	23.6 i	3.59	39.4	77.6
1	7+9	b	j	15	53.4 i	11.2 cd	26.8 fg	3.73	41.7	76.7
1	7+9	e	j	6	53.5 g-i	11.6 a	20.2 j	3.67	38.4	76.6
					55.1	**11.2**	**26.9**	**3,57**	**40.3**	**77.1**
2*	7+9	b	b	5	55.7 bc	10.6 fg	28.4 d	3.65	41.0	76.6
2*	7+9	e	b	5	56.2 ab	10.8 fg	25.6 h	3.66	43.4	76.8
2*	7+9	b	j	9	54.1 f-h	11.3 bc	26.5 gh	3.68	40.7	77.2
2*	7+9	e	j	14	54.7 ef	11.3 bc	20.6 j	3.71	41.0	77.1
					55.2	**11.0**	**25.3**	**3.68**	**41.5**	**76.9**

Table 2. ANOVA of grain yield properties by *Glu-1* and *Glu-3* alleles based on averaged data from four environments

		Flour yield (%)	Protein content (14% mb)	SDS (ml)	Damaged starch content AACC-7631	1000-kernel weight (g)	Test weight (kg)
Locus	*DF*			MS			
Lokasyon (Yıl)	2	433.49	44.47	1299,27	2.76	87.61	10.3
Tekekkür (Yıl, Lokasyon)	4	13.25	19.82	89.08	0.36	23.72	4.8
Glu-A1	1	113,9**	0,65	1194,7**	0,19	336,7**	8,88
Glu-B1	1	129,9**	2,87	94,7	2,39**	39,7	1,97
Glu-A1* Glu-B1	1	62,8*	5,60*	20,1	4,89**	0,18	28,2
Glu-A3	1	86,6**	7,80*	9482,9**	1,13**	122,2	8,20
Glu-A1*Glu-A3	1	32,8	12,8**	777,5**	0,05	726,2**	3,86
Glu-B1*Glu-A3	1	0,21	1,53	256,1**	0,01	0,49	2,53
Glu-A1* Glu-B1*Glu-A3	1	37,9	0,66	290,5**	1,27**	44,5	1,73
Glu-B3	1	1260,3**	74,0**	6847,7**	2,29**	3,80	35,6
Glu-A1*Glu-B3	1	321,6**	0,41	70,2	4,05**	31,5	68,8
Glu-B1*Glu-B3	1	17,3	0,48	0,01	0,99**	273,9**	15,9
Glu-A1*Glu-B1*Glu-B3	1	24,5	0,66	355,2**	0,04	137,6	2,55
Glu-A3* Glu-B3	1	1,78	0,08	107,7*	0,40	205,7*	1,95
Glu-A1*Glu-A3* Glu-B3	1	17,3	2,14	383,3**	1,02**	9,37	0,00
Glu-B1*Glu-A3* Glu-B3	1	33,7	22,4**	29,4	0,61*	5,07	17,6
Glu-A1* Glu-B1* Glu-A3* Glu-B3	1	50,4*	18,4**	492,5**	0,33	57,1	0,65
Error	2336	12,3**	1,40**	27,0**	0,12**	38,1**	24,9

* P <0.05, ** P <0.01 level of significance, DF: degrees of freedom, MS: mean square

the *e* and *j* alleles in both. This is the opposite for the amount of starch damaged. In terms of protein ratio, *b* > *e* in *Glu-A3* and *j* > *b* in *Glu-B3*. In terms of flour yield, *e* > *b* and *b* > *j*.

In the study, the averages of some myxolab traits of HMW-Gs *Glu-A1* and *Glu-B1*, LMW-Gs *Glu-A3* and *Glu-B3* alleles were statistically evaluated and given in Table 4. The analysis of variance results of allele combinations on myxolab traits are also given in Table 6. Year, location, *Glu-A1, Glu-B1, Glu-B1, Glu-B3* and some interactions were found significant at 1% or 5% level. Besides environment, both HMW-G and LMW-G alleles seem to have an impact on myxolab traits. In *Glu-A1* 1, when looking at 17+18 and 7+9, the water absorption of 17+18 was slightly higher, while the stability and kneading time of 7+9 were found to be higher. In *Glu-A1* 2*, the water absorption and kneading time of 17+18 were higher, while the stability value was slightly lower. The 17+18 subunit gave similar values in *Glu-A1* 1 and 2* alleles The water absorption and kneading time of 17+18 were slightly higher in 1 and the stability value was slightly lower. When the 7+9 subunit was compared in *Glu-A1* 1 and 2* alleles, better results were obtained with 1 in terms of all parameters.

In *Glu-B1* 17+18 subunit, the effects of myxolab stability value, which is the most important parameter related to dough strength, are statistically *bb* > *eb* > *bj* > *bj* > *ej*. Water absorption is the opposite. Kneading time similarly indicates dough strength. In both *Glu-A1* alleles, *-bb* stood out in dough kneading time. While *-ej* gave the lowest value; *-eb* and *-bj* gave close values. In the *Glu-B1* 7+9 subunit, the effect on stability at 1 and 2* is *bb* > *eb* > *bj* > *ej*. 1, this effect is more pronounced. *-ej* gave the lowest stability value in both alleles. In the 2* allele *-ej* did not differ from the other alleles in terms of stability value. In terms of water absorption, *-ej* gives the highest value in both alleles (1 and 2*) and *-bb* gives the lowest value, while the other two alleles have similar values. In terms of kneading time, *-bb* is clearly separated in *Glu-A1* 1, while *-bj* is high in the allele. Interestingly, *-bb* and *-ej* had the same value in the 2* allele.

The mean values of alleles at *Glu-A1, Glu-B1, Glu-A3* and *Glu-B3* loci are statistically compared in Table 5. In terms of myxolab stability value, which is related to dough durability and bread volume, 1 = 2*; 7+9 > 17+18 and *b* > *e* and *b* > *j*; in terms of kneading time, 1 > 2*; 7+9 < 17+18 and *b* > *e and b* > *j*. Especially the *b* allele was found to be

Table 3. Mean values of alleles at *Glu-A1, B1, Glu-A3* and *Glu-B3* loci

Locus	Subunit	Line number	Flour yield (%)	Protein content (14%)	SDS (ml)	Damaged starch content AACC-7631	1000-kernel weight (g)	Test weight (kg)
Glu-A1	1	68	54.5 b	11.2	27.6 a	3.68	40.6 b	76.9
	2*	79	55.1 a	11.1	25.4 b	3.65	41.7 a	77.0
Glu-B1	7+9	60	55.2 a	11.1	26.2	3.62 b	40.9	77.0
	17+18	87	54.4 b	11.2	26.8	3.72 a	41.3	77.0
Glu-A3	b	73	54.5 b	11.2 a	29.5 a	3.63 b	41.5	76.9
	e	74	55.1 a	11.1 b	23.4 b	3.70 a	40.8	77.0
Glu-B3	b	87	55.9 a	10.9 b	29.1 a	3.62 b	41.1	77.1
	j	60	53.7 b	11.4 a	23.9 b	3.71 a	41.2	76.8

Table 4. Mixolab dough mixing properties of wheat lines by *Glu-1* and *Glu-3* allelic combinations based on averaged data from four environments

HMW-GS	LMW-GS			Line number	Optimum water absorption(%)	Optimum mixing time (min)	Stability (min)
Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*				
1	17+18	b	b	7	57.0 cd	5.04 abc	9.66 a
1	17+18	e	b	4	56.3 def	4.48 c-f	8.50 c
1	17+18	b	j	1	57.9 a-d	4.95 a-f	7.14 efg
1	17+18	e	j	5	58.2 a	4.25 c-f	6.05 gh
					57.4	**4.68**	**7.84**
2*	17+18	b	b	5	56.8 cde	5.36 ab	9.54 ab
2*	17+18	e	b	5	56.9 cde	4.21 def	8.98 c
2*	17+18	b	j	3	57.0 b-e	4.56 a-f	6.91 f
2*	17+18	e	j	4	57.6 abc	3.90 def	6.13 fgh
					57.1	**4.51**	**7.89**
1	7+9	b	b	1	56.3 c-f	5.83 a	10.01 a
1	7+9	e	b	4	57.0 ef	4.17 def	8.63 c
1	7+9	b	j	6	57.1 bcd	4.87 a-d	7.84 de
1	7+9	e	j	5	57.9 ab	4.15 def	5.69 h
					57.1	**4.76**	**8,04**
2*	7+9	b	b	4	55.7 f	3.71 ef	8.95 bc
2*	7+9	e	b	3	56.7 cde	4.57 b-e	8.78 c
2*	7+9	b	j	5	56.1 ef	4.70 a-e	8.46 cd
2*	7+9	e	j	4	58.1 a	3.71 f	5.62 h
					56.7	**4.17**	**7.95**

associated with high dough strength. In terms of water absorption, *Glu-A3* and *Glu-B3 b* allele gave lower values than other alleles. Again, in terms of water absorption, 1 = 2* and 17+18 > 7+9. As a result, lines with the *Glu-A3b* or *Glu-B3b* allele showed longer kneading time and stability compared to lines with the *Glu-A3e* or *Glu-B3j* allele.

4. Discussions

Within the scope of the study, milling, protein and dough kneading characteristics of the genotypes were measured and the relationships between these quality parameters and HMW-GS and especially LMW-GS allele combinations were investigated. Recent studies have shown that the effects of small regions formed by translocations or a locus should be investigated in the investigation of protein quality and that determining the variation to be generated for this purpose is more important for genomic studies on quality (Lukaszewski et al., 1987; Wang et al., 2016).

Among the parents used in the study, Tosunbey cultivar 1, 17+18, 5+10 and Tahirova2000 cultivar 2*, 7+9, 5+10 carry HMW-GS alleles and their quality scores were 10 and 9, respectively (Payne et al., 1987). In terms of LMW-GS alleles, Tosunbey has *Glu-A3b*, *Glu-B3b* and *Glu-D3b* alleles and Tahirova2000 has *Glu-A3e* (null/empty), *Glu-B3j* (null/empty, rye translocation) and *Glu-D3b* alleles. Since the Tahirova2000 variety carries a rye translocation (*Glu-B3j*), the *Glu-1* score corrected for rye decreases from 9 to 6. Among the parents, Tosunbey variety is considered as first class bread wheat in terms of protein quality, while Tahirova2000 variety has low protein quality (Aydın et al., 2016). Differences in the LMW-GS alleles of the cultivars, especially the fact that Tahirova2000 carries *Glu-A3e* (null/empty) and *Glu-B3j* alleles are effective in this difference, since the cultivars have similar quality score in terms of HMW-GS. As a result of this, the variation resulting from the crossing of Tosunbey variety, which has high protein content and quality, and Tahirova2000 variety, which has relatively high protein content but poor protein quality, is important and constitutes the essence of this study.

Flour yield and damaged starch content are important milling quality parameters of bread wheats. Commercial flour yields of bread wheats are desired to be high (>75%) and damaged starch contents within a certain range (5-8%) (Hoseney, 1994; Bushuk, 1998; Elgün et al., 2002). Since a laboratory type mill was used to grind the wheat in this study, it is expected that the flour yields and damaged starch contents of the genotypes (Table 1,3) would be lower than the values in commercial flour production.

Tables 1 and 3 show that *Glu-A1*, *Glu-B1* and *Glu-B3* alleles have an effect on flour yield, while *Glu-A3* allele has no effect. Ahn et al. (2014) reported that *Glu-A3* allele had a significant effect on flour yield. The reason for this difference may be the difference in *Glu-1* and *Glu-3* allele combinations of the material used in the studies. When the tables are analysed, the average flour yields of the combinations carrying rye translocation (*Glu-B3j*) are lower than the combinations not carrying rye translocation (*Glu-B3b*). While *Glu-A3* allelic difference (*Glu-A3b* and *Glu-A3e*) had no effect on flour yield, *Glu-B3* allelic difference (*Glu-B3b* and *Glu-B3j*) caused significant difference in flour yield. However, in previous studies (Fenn et al., 1994; Burnett et al., 1995; Kim et al., 2005), the effect of rye translocation on flour yield was found statistically insignificant.

It is known that rye translocation not only improves the breeding characteristics of wheat but also increases its protein content, but decreases its quality (Dhaliwal et al., 1987; Fenn et al., 1994; Burnett et al., 1995; Graybosch, 2001; Lelley et al., 2004; Kim et al., 2005; Liu et al., 2005; Gobaa et al., 2008; Moiraghi et al., 2013). However, the data in this study indicate that protein quality may vary depending on the *Glu-A3* allele despite carrying the rye translocation. Indeed, it was observed that the protein quality of the combinations carrying the *Glu-A3b* allele was

Table 5. Mixolab dough mixing properties of wheat lines by specific *Glu-1* and *Glu-3* alleles based on averaged data from four environments

Locus	Subunit	Line number	Optimum water absorption(%)	Optimum mixing time (min)	Stability (min)
Glu-A1	1	33	57.1	4.72	7.94
	2*	33	56.9	4.34	7.92
Glu-B1	7+9	32	56.7 b	4.46	8.00
	17+18	34	57.2 a	4.59	7.86
Glu-A3	b	32	56.7 b	4.88 a	8.54 a
	e	34	57.2 a	4.18 b	7.30 b
Glu-B3	b	33	56.5 b	4.67	9.13 a
	j	33	57.5 a	4.39	6.73 b

higher than those carrying the *Glu-A3e* (null/null) allele, although they carried the rye translocation (Table 3).

Gupta et al. (1989) found that *Glu-A3e* (null/empty) allele was negatively correlated with protein content in wheat, while Zhen et al. (2014) found that it decreased gluten index. Wang et al. (2016) reported that deficiency of the *Glu-B3* allele decreased protein content and dough kneading properties. Ito et al. (2015) found that *Glu-B3b* allele was better than other *Glu-B3* alleles in terms of sedimentation volume. The effects of wheat HMW-GS alleles on dough and bread quality have been largely clarified and are listed as follows: 1>2*>null/empty for *Glu-A1* locus, 7+8≥13+16>17+18=7+9 for *Glu-B1* locus and 5+10>2+12>4+12 for *Glu-D1* locus (He et al., 2005). The effects of wheat LMW-GS alleles on dough and bread quality are still being intensively studied. The ranking of wheat in terms of dough strength and bread quality according to LMW-GS alleles was *Glu-A3d>Glu-A3b>Glu-A3c>Glu-A3f>Glu-A3a>Glu-A3e* for *Glu-A3* locus, *Glu-B-3b=Glu-B3d=Glu-B3g>Glu-B3h>Glu-B3a>Glu-B3c>Glu-B3j* for the *Glu-B3* locus and *Glu-D3d=Glu-D3f>Glu-D3e>Glu-D3a=Glu-A3c=Glu-D3b* for the *Glu-D3* locus (Zhang et al., 2012). Jin et al. (2013) and Bonafede et al. (2015) also reported that *Glu-A3e* allele weakened the dough. The results of these studies are compatible with the results found in this study. HMW-GS alleles of the lines were similar in terms of bread quality, but LMW-GS alleles were different. These differences were significantly reflected on the protein contents and sedimentation values of the wheats (Tables 1-3).

As shown in Tables 4 and 5, the optimum water holding capacities of the lines were higher in combinations carrying *Glu-A3e* and *Glu-B3j* (rye translocation) alleles. This result is closely related and compatible with the higher protein and damaged starch contents of the same combinations (Tables 1 and 3). This is because the water holding capacity of flours increases depending on their protein and damaged starch contents (Hoseney, 1994). In addition, since the pentosan content of the lines carrying rye translocation may also be high (Moiraghi et al., 2013), it may have contributed to the water retention capacity of the flours.

Table 6. ANOVA of Mixolab dough mixing properties by *Glu-1* and *Glu-3* alleles based on averaged data from four environments

Locus	DF	Optimum water absorption (%)	Optimum mixing time (min)	Stability (min)
			MS	
Yıl	1	117.41	30.93	66.95
Lokasyon (Yıl)	2	110.12	11.35	50.03
Tekekkür (Yıl, Lokasyon)	4	4.94	2.01	1.18
Glu-A1	1	4.12	8.81	0.02
Glu-B1	1	14.7*	1.04	1.10
Glu-A1* Glu-B1	1	0.09	2.55	0.31
Glu-A3	1	15.5*	29.89**	98.60**
Glu-A1*Glu-A3	1	12.11*	2.77	1.89
Glu-B1*Glu-A3	1	10.13*	0.31	8.21**
Glu-A1* Glu-B1*Glu-A3	1	1.97	7.29	0.13
Glu-B3	1	57.13**	4.64	329.34**
Glu-A1*Glu-B3	1	8.71	0.11	0.85
Glu-B1*Glu-B3	1	0.41	0.33	2.68
Glu-A1*Glu-B1*Glu-B3	1	1.10	3.46	3.63
Glu-A3* Glu-B3	1	11.38*	0.30	12.08**
Glu-A1*Glu-A3* Glu-B3	1	0.07	4.40	4.61*
Glu-B1*Glu-A3* Glu-B3	1	0.25	1.52	10.37**
Glu-A1* Glu-B1* Glu-A3* Glu-B3	1	0.31	11.22	2.48
Error	2336	2.43	2.04	1,12

When the optimum kneading times and stability of the genotypes (Tables 4 and 5) are analysed, the effect of LMW-GS alleles is clearly seen. In general, the kneading times and stability of the genotypes carrying *Glu-A3b* and *Glu-B3b* alleles were higher than the other combinations (*Glu-A3e* and especially *Glu-B3j*). Long kneading times and stability of bread flours indicate strong dough and quality bread production. This result coincides with the previously discussed high sedimentation values of the same combinations (Table 6). In previous studies using a mixograph (Flaete and Uhlen, 2003; Bonafede et al., 2015; Ito et al., 2015), the *Glu-A3e* (null/empty) allele was found to cause gluten weakening. Both *Glu-A3* (Zhen et al., 2014) and *Glu-B3* alleles were found to be significant in dough stability measured using Farinograph (He et al., 2005; Ahn et al., 2014). Ito et al. (2015) associated *Glu-A3b* and *Glu-A3d* alleles with long dough stability and good bread quality.

Within the scope of the study, milling, protein and dough kneading properties of the genotypes were measured and the relationships between these quality parameters and HMW-GS and especially LMW-GS allele combinations were investigated in eight different environments. The effects of LMW-GS alleles on gluten quality and dough strength were statistically as *bb* > *eb* > *bj* > *ej*. In terms of myxolab stability value, which is related to dough durability and bread volume, 1 = 2*; 7 + 9 > 17 + 18 and *b* > *e* and *b* > *j*; in terms of kneading time, 1 > 2*; 7 + 9 < 17 + 18 and *b* > *e* and *b* > *j*. The milling, protein and dough kneading data of the RIL lines, especially those obtained due to the LMW-GS allele combination, are internally compatible and the related ones support each other. Rye translocation *(Glu-B3j*) reduced flour yield although it increased damaged starch and protein content in genotypes. It was also observed that the negative effects of rye translocation could be minimized by selecting appropriate alleles such as *Glu-A3b* instead of *Glu-A3e.* In conclusion, this study shows that it is important to work on mapping populations containing different HMW-GS and LMW-GS alleles; with this approach, the most suitable HMW-GS and LMW-GS allele combinations can be developed for any bakery product.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' Contribution

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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