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Analysis of Some Turkish Durum Wheat Cultivars and Landraces for HMW-Glutenin Subunits

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Abstract: Gluten is a complex mixture of proteins composed mainly of glutenins and the gliadins. A strong correlation exists between the presence of certain gliadin and glutenin proteins and the viscoelastic properties of the gluten. For instance, HMW-glutenin subunits, encoded by the Glu-A1, Glu-B1 and Glu-D1 loci that are located on the long arms of chromosome 1, are strongly related to quality. This study were made for screening for the presence of HMW glutenin alleles of the durum wheat varieties grown in different regions of Turkey in 2012. In search, 17 Turkish durum wheat cultivars and 15 durum landraces and seven bread wheat cultivars as standards were used. This study was performed to determine the composition of HMW-glutenin subunits in gathered from different regions of Turkey. Five seeds from each cultivar were cut into two halves and one half was used for protein electrophoresis. The SDS-PAGE was performed to determine the composition of HMW-glutenin subunits. As a result, HMW-Glutenin alleles obtained from the SDS-PAGE screenings were compared with known LMW-Glutenins and γ-gliadin alleles determined in previous studies and the quality-related situations of the durum genotypes were assessed. As a result of the study, it was observed that 1 and 7+8 HMW-G subunits, which had a negative effect on quality when they were found together with durum wheat seeds, also included LMW-1-Glutenin and γ-gliadin 42. It has been determined that the varieties containing LMW-2 glutenin and γ-gliadin 45 which have a positive effect on the quality of pasta also include 7+9 HMW-Glutenin and 14+15 HMW-Glutenin subunits separately. It was determined that a single conclusion could not be reached regarding the effect of 6+8 HMW-Glutenin and 17+18 HMW-Glutenin subunits on quality.

Keywords: Durum wheat, *Triticum durum*, HMW-Glutenin, LMW-Glutenin, γ-gliadin

Türkiye'de Yetiştirilen Bazı Tescilli ve Yerel Makarnalık Buğday Çeşitlerinin HMW-Glutenin Allelleri Bakımından Karakterizasyonu

Öz: Makarnalık buğdayda kalite, protein miktarı ve özelliği ile ilgilidir. Makarnalık buğdayda bulunan gluten, hamurun elastik özelliğinde etkili glutenin ve hamurun viskoz özelliğinde etkili gliadin proteinlerinden oluşmaktadır. Bu proteinlerden HMW-glutenin allelleri, 1. grup kromozomların uzun kolları üzerine haritalanmış *Glu-A1*, *Glu-B1*, *Glu-D1* lokusları tarafından kodlanmaktadır ve kalite ile ilişkili olduğu düşünülmektedir. Bu araştırma, Türkiye'nin farklı bölgelerinde yetiştirilen farklı makarnalık buğday çeşitlerinin HMW-Glutenin allellerinin varlığı bakımından taranması amacıyla 2012 yılında Karaman'da yürütülmüştür. Araştırmada 17 adet tescilli ve 15 adet yerel olarak yetiştirilen makarnalık buğday çeşidi ile standart çeşit olarak yedi adet ekmeklik buğday çeşidi kullanılmıştır. Makarnalık buğday çeşitlerinden beşer tohum alınarak embriyo ve endospermleri ayrılmıştır. Endospermlerden izole edilen gluteninler, 1. grup kromozomların uzun kolları üzerine haritalanmış HMW-Glutenin allelleri açısından, SDS-PAGE yöntemi kullanılarak taranmıştır. SDS-PAGE taramaları sonucunda elde edilen HMW-Glutenin allelleriyle daha önceki çalışmalarda belirlenmiş olan LMW-Glutenin ve γ-gliadin allelleri karşılaştırılarak, mevcut çeşitlerin durumları belirlenmiştir. Çalışma sonucunda, makarnalık buğday tohumunda birlikte bulunduğunda kalite üzerine olumsuz etki yapan 1 ve 7+8 HMW-G allellerinin aynı zamanda LMW-1-Glutenin ve γ-gliadin 42'e sahip

oldukları görülmüştür. Makarna kalitesi üzerinde olumlu etkisi olan LMW-2 glutenin ve γ-gliadin 45'i taşıyan çeşitlerin, ayrı ayrı 7+9 HMW-Glutenin ve 14+15 HMW-Glutenin allellerini taşıdığı belirlenmiştir. 6+8 HMW-Glutenin ve 17+18 HMW-Glutenin allellerinin ise tek başına kalite üzerinde etkisiyle ilgili bir sonuca varılamayacağı saptanmıştır.

Anahtar Kelimeler: Makarnalık buğday, *Triticum durum*, HMW-Glutenin, LMW glutenin, γ-gliadin

1.Introduction

Wheat is used in the production of breads, pasta, noodles, bulgur, couscous, biscuits, crackers, wafers, cakes, breakfast cereals and snack foods because of the unique viscoelastic and cohesive properties of wheat gluten. Gluten consists of glutenins and gliadins, of which the former is effective on the elastic properties of dough and the latter is effective on the viscose cohesive properties of the dough. The polymeric glutenins are divided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) according to their behavior in the SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) system (Troccoli et al., 2000). Gliadins are heterogeneous monomeric proteins that are separated into groups according to their electrophoresis behavior at low pH by A-PAGE (Acid Polyacrylamide Gel Electrophoresis) system. Gliadins (Gli-1 and Gli-2, respectively) are encoded by the six Gli loci mapped on the short arms of chromosomes 1 and 6 (Morris, 2004).

The LMW-GS are encoded by the Glu-A3, Glu-B3 and Glu-D3 loci mapped on the short arms of 1A, 1B and 1D chromosomes, respectively. The HMW-GS are encoded by Glu-A1, Glu-B1 and Glu-D1 loci mapped on long arms of group 1 chromosomes (Aalami et al., 2007). The most important of the specific gliadin proteins related to baking quality of macaroni produced from durum wheat are the γ-gliadin 42/45 proteins found in Gli-B1 locus (Troccoli et al.,2000). γ-Gliadin 45 is regarded as an indicator of optimum gluten strength and high cooking quality, while γ-gliadin 42 is a sign of poor gluten and low quality of cooking. Recent studies have revealed that the actual proteins that are the main determinants of gluten strength and macaroni cooking quality are LMW-1 and LMW-2 glutenin proteins encoded by the Glu-B3 locus that are closely related to the Gli-B1 locus and associated with the γ gliadin 42 and 45 proteins (Payne et al., 1982; Sönmezoğlu et al., 2010; Sayaslan et al., 2012).

HMW-GS are named according to the band differences in the SDS-PAGE system in bread and durum wheat (Branlard et al., 1989). Many studies on bread wheat have found that different HMW-GS are associated with some gliadin alleles and affect quality (Mohd et al., 2007). Similarly, it was determined that HMW-GS positively or negatively affect gluten strength in bread wheat (Gianibelli et al., 2002). There are 3 to 5 HMW-GS alleles in bread wheat, while 1 to 3 HMW-GS alleles are present in durum wheat (Waines and Payne, 1987). The reason for this is the D genome in bread wheat. It is reported that HMW-GS are directly related to gluten strength and loaf volume in bread wheat, while they have less effect on pasta cooking characteristics (Gianibelli et al., 2002). However, many studies have demonstrated that HMW-GS alleles have positive or negative effects on LMW-GS and gamma-gliadin alleles as well as pasta quality (Gianibelli et al., 2002).

In this study, HMW-GS and other alleles (LMW-1/LMW-2 glutenin and γ-gliadin 45/42) of registered durum wheat cultivars and landraces of Turkey were determined.

2. Materials and Method

In the study, 17 registered and 15 locally grown wheat varieties were characterized by the presence of HMW glutenin alleles as material (Table 1). Seven bread wheat varieties known to have different HMW-GS bands (Table 2) were used as standard. The tapes were numbered according to Payne and Lawrence (1983) (Figure 3, Figure 1).

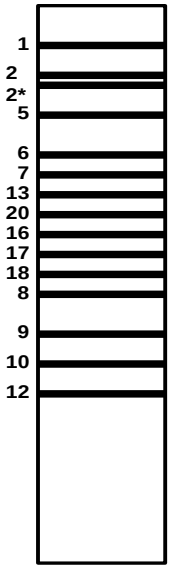

Figure 1. Possible positions of HMW-GS bands in A, B and D genomes
Şekil 1*. A, B ve D genomlarındaki HMW-GS bantlarının muhtemel pozisyonları*

HMW-GS, which are effective in gluten strength of the wheat varieties, have been determined using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method described by Gianibelli et al., (2002) and Singh et al., (1991) on the vertical electrophoresis system.

3. Results and Discussion

Based on the SDS-PAGE screenings, HMW-GS patterns of all varieties were determined (Figure 2, 3). HMW-GS band patterns of all varieties and presence of LMW-1/LMW-2 glutenin and γ-gliadin 42/γ-gliadin 45 determined in previous studies are given in Table 4.

Table 4 shows that varieties with 7+8 HMW-GS alleles carry (Salihli, Selcuklu-97, Sarıcanak-98, Çeşit-1252, Aday-3, Bağacak, Sarı Başak, Havrani) LMW-1 glutenin and γ-gliadin 42 band patterns that adversely affect pasta quality at the same time. This result shows that pasta qualities of varieties with 7 + 8 alleles may be lower than others. Similary results have been determined in some other studies (Oak et al., 2004; Fan et al., 2009). However, it was reported that the same situation was indicative of the quality of bread wheat varieties as well (Payne and Lawrence, 1983). In addition, it was shown that the varieties having both 1 and 7+8 alleles had lower qualities (Zarki et al., 2010). This was the case in Selçuklu-97 and Çeşit-1252 varieties in our study. Both groups had both 1 and 7+8 alleles as well as LMW-1-glutenin and □-gliadin 42, which had adverse effects on pasta quality.

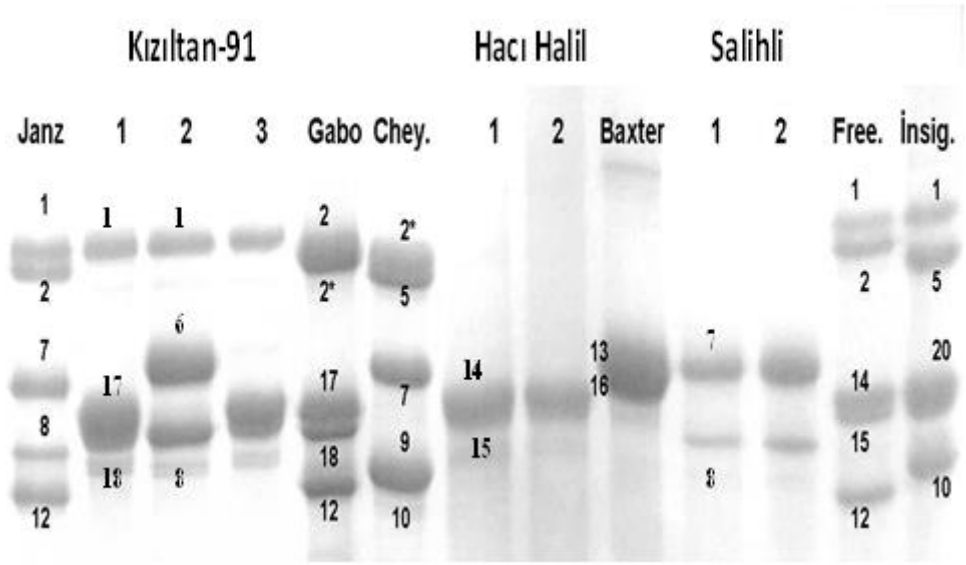

Figure 2. SDS-PAGE photograph of Kiziltan-91, Hacı Halil and Salihli varieties
Şekil 2. *Kızılltan-91, Hacı Halil ve Salihli çeşitlerinin SDS-PAGE fotoğrafları*

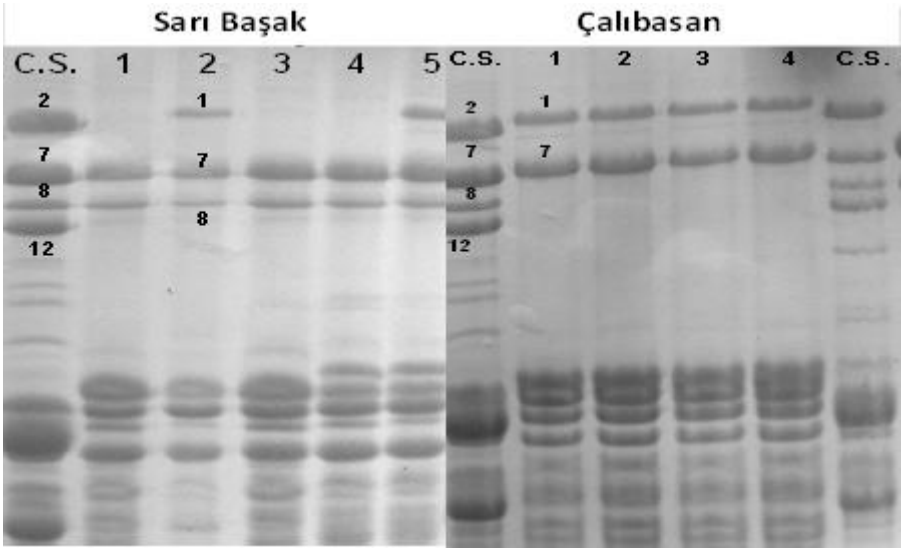

Figure 3. SDS-PAGE photograph of Sarı Başak and Çalıbasan varieties
Şekil 3. *Sarı Başak ve Çalıbasan çeşitlerinin SDS-PAGE fotoğrafları*

Table 1. Registered and local durum wheat varieties used in the research
Çizelge 1*. Araştırmada kullanılan tescilli ve yerel makarnalık buğday çeşitleri*

Number	Variety Name	Regist. Year	Pedigree	Number	Variety Name	Regist. Year	Pedigree
1	Gediz-75	1976	LD 57E/TC2//Jori"S"	**17**	Aday-18	……	……
2	Kızıltan-91	1991	---- ----------	**18**	Beyaziye	---	Local variety
3	Aydın-93	1993	OMRABIA "S"	**19**	İskenderiye	---	Local variety
4	Salihli	1995	B.BAL//BYE*2/ TC60	**20**	Menceki	---	Local variety
5	Harran-95	1995	Korifla//D.S-15/Geiger	**21**	Sorgül	---	Local variety
6	Selcuklu-97	1997	073-44*2/Ovi/3/DF21-72//61-130/Uvy162	**22**	Karakılçık	---	Local variety
7	Yılmaz-98	1998	---------- -	**23**	Bağacak	----	Local variety
8	Ankara-98	1998	---------- ---	**24**	Akbaşak	----	Local variety
9	Altıntoprak-98	1998	ACONCH189 =ALTAR84/AOS	**25**	Beyaz Buğday	----	Local variety
10	Saricanak-98	1998	DACK/GEDIZ//USPA575	**26**	Sarı Başak	----	Local variety
11	Kümbet-2000	2000	----------	**27**	Bintepe	……	Local variety
12	Çeşit-1252	----	----------	**28**	Havrani	……	Local variety
13	Quashar	----	---------- ---	**29**	Kozmidor	……	Local variety
14	Aday-1	……	……..	**30**	Çalıbasan	……	Local variety
15	Aday-3	……	……	**31**	Hacı Halil	……	Local variety
16	Aday-6	……	……	**32**	Ağ Buğ.	……	Local variety

Table 2. Bread wheat varieties used as standards in HMW-GS of wheats (Bekes et al., 2011)
Çizelge 2. *HMW-GS allelleri için standart olarak kullanılan ekmeklik buğday çeşitleri*

Variety Name	Bands Found in A Genome	Bands Found in B Genome	Bands Found in C Genome
Chinese Spring	Null	7+8	2+12
Janz	1	7+8	2+12
Gabo	2*/ Null	17+18	2+12
Baxter	….	13+16	…….
Cheyenne	2*	7+9	5+10
Free Gallipoli	1	14+15	5+10
Insignia	1	20	5+10

Table 3. HMW-GS alleles in genomes of bread wheat by Payne and Lawrence (1983)
Çizelge 3. *Payne ve Lawrence (1983) tarafından bulunan ekmeklik buğday genomlarındaki HMW-GS allelleri*

Bands Found in A Genome	Bands Found in B Genome	Bands Found in D Genome
1	6+8	5+10
2*	7	2+12
Null	7+8	
	7+9	
	13+16	
	14+15	
	17+18	
	20	

Among the varieties having 6+8 HMW-GS alleles, Beyaz Buğday and Yilmaz-98 varieties were carrying LMW-1 glutenin and γ-gliadin 42, while others (Kozmidor, Gediz-75, Quashar, Ankara-98) were carrying LMW-2 glutenin and γ-gliadin 45. Earlier studies have reported that 6+8 alleles are not a single indicator of quality. (Oak et al., 2004; Sissons et al., 2005).

Lukow (1991) and Naghavi et al. (2009) reported that the varieties carrying the 7+9 HMW-GS allele were higher than those of other varieties. It was determined that only Kümbet-2000 varieties had 7+9 alleles in our study, and it was determined that LMW-2 glutenin and γ-gliadin 45 were also carried in this variety at the same time.

Some of the varieties and lines which had 17+18 HMW-GS alleles (Ağ Buğdayı, Bintepe, Akbaşak, Aday-1 Aday-6 Aday-18) had also LMW-2 gluten and γ-gliadin 45 at the same time however İskenderiye, Beyaziye, Menceki varieties carried LMW-1 glutenin and γ-gliadin 42 instead. In this case, it can be concluded that 17 + 18 alleles are not effective on pasta quality alone. (Branlard and Dardevet, 1985; Butow et al., 2003).

When Table 4 was examined, it was seen that some varieties had different alleles in some seeds (Kızıltan-91, Harran-95, Sorgül ve Karakılçık). The same was true for the detection of γ-gliadin 42, 45 and LMW-1, LMW-2 glutenin. The reason for this could be the lack of seed purity or mixing seeds of different varieties.

As a result of the screenings, Aydın-93, Altıntoprak-98 and Hacı Halil varieties with 14+15 HMW-GS alleles were found to have LMW-2 gluten and γ-gliadin 45 at the same time. Similar results were obtained in other studies, so it can be said that 14+15 alleles have positive effect on pasta quality (Butow et al., 2003; Oak et al., 2004).

Previous studies have shown that 20 HMW-GS alleles have an adverse effect on pasta and bread wheat quality when compared to other HMW-GS alleles, reducing gluten power (Butow et al., 2003; Sissons et al., 2005). There are no 20 alleles in the durum wheat varieties used in our study.

The study by Butow et al. in 2003 on bread wheat showed that 17+18 and 7 HMW-GS alleles gave stronger pulp power than 20 HMW-GS alleles. In the same year, Brites and Carrillo determined that 14+15 HMW-GS alleles in durum wheat varieties gave higher gluten power than 20 HMW-GS alleles. In recent years, quality concepts in wheat trade have been evaluated according to the shape of final consumption.

Quality and standard product can only be produced from a quality raw material For this reason, in order to increase our quality durum wheat production, it is necessary to evaluate and develop suitable varieties for regions, quality genes and alleles. In the breeding studies carried out in recent years, it has also become clear that it is also necessary to increase quality along with yield. It has become a necessity now that the variety candidates are at a certain quality level considering the quality criteria that the pasta industry wants before they are presented to the testers.

Table 4. HMW-Glutenin, LMW-1/LMW-2 Glutenin and γ-gliadin 42/45 results of all varieties
Çizelge 4. *Tüm çeşitlerin HMW-Glutenin, LMW-1/LMW-2 Glutenin ve γ-gliadin 42/45 sonuçları*

Number	Variety Name	HMW-GS (A Genome)	HMW-Glutenin (B Genome)	LMW-1 Glutenin / LMW-2 Glutenin	γ-gliadin 42 /γ-gliadin 45
1	Gediz-75	Null	6+8	LMW-2	γ-gliadin 45
2	Kızıltan 91	1	6+8 / 17+18	LMW-1/ LMW-2	γ-gli. 42 / γ-gli. 45
3	Aydın -93	Null	14+15	LMW-2	γ-gliadin 45
4	Salihli	Null	7+8	LMW-1	γ-gliadin 42
5	Harran-95	Null	7+8 / 17+18	LMW-1/ LMW-2	γ-gli. 42 / γ-gli. 45
6	Selcuklu-97	1	7+8	LMW-1	γ-gliadin 42
7	Yılmaz -98	Null	6+8	LMW-1	γ-gliadin 42
8	Ankara -98	Null	6+8	LMW-2	γ-gliadin 45
9	Altıntoprak -98	Null	14+15	LMW-2	γ-gliadin 45
10	Saricanak-98	Null	7+8	LMW-1	γ-gliadin 42
11	Kümbet-2000	1	7+9	LMW-2	γ-gliadin 45
12	Çeşit-1252	1	7+8	LMW-1	γ-gliadin 42
13	Quashar	Null	6+8	LMW-2	γ-gliadin 45
14	Aday-1	Null	17+18	LMW-2	γ-gliadin 45
15	Aday-3	Null	7+8	LMW-1	γ-gliadin 42
16	Aday-6	Null	17+18	LMW-2	γ-gliadin 45
18	Beyaziye	Null	17+18	LMW-1	γ-gliadin 42
17	Aday-18	Null	17+18	LMW-2	γ-gliadin 45
19	İskenderiye	1	17+18	LMW-1	γ-gliadin 42
20	Menceki	1	17+18	LMW-1	γ-gliadin 42
21	Sorgül	Null	6+8 / 17+18	LMW-1/ LMW-2	γ-gli. 42 / γ-gli. 45
22	Karakılçık	Null	7+8 / 17+18	LMW-1/ LMW-2	γ-gli. 42 / γ-gli. 45
23	Bağacak	Null	7+8	LMW-1	γ-gliadin 42
24	Akbaşak	1	17+18	LMW-2	γ-gliadin 45
25	Beyaz Buğday	Null	6+8	LMW-1	γ-gliadin 42
26	Sarı Başak	Null / 1	7+8	LMW-1	γ-gliadin 42
27	Bintepe	1	17+18	LMW-2	γ-gliadin 45
28	Havrani	Null	7+8	LMW-1	γ-gliadin 42
29	Kozmidor	Null	6+8	LMW-2	γ-gliadin 45
30	Çalıbasan	1	7	LMW-1	γ-gliadin 42
31	Hacı Halil	Null	14+15	LMW-2	γ-gliadin 45
32	Ağ Buğdayı	1	17+18	LMW-2	γ-gliadin 45

In the majority of durum wheat varieties used as material in the study, it was concluded that, even though HMW-Glutenin, LMW-Glutenin and γ-gliadin alleles which have a positive effect on the quality of pasta were found together, HMW-Glutenin subunits did not give enough information about quality alone. However, it is important that this work and its consequences lead to further breeding work and determine the circumstances of our existing varieties.

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