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MOLECULAR AND BIOCHEMICAL SCREENING OF TURKISH DURUM WHEAT LANDRACES FOR γ-GLIADIN AND LMW-GLUTENIN PROTEINS ASSOCIATED WITH PASTA-COOKING QUALITY

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ABSTRACT

In recent years, pasta-cooking quality has become an important issue in durum wheat breeding. Pasta-cooking quality of durum wheat has been shown to depend mainly on protein content and gluten properties. Gluten is a complex mixture of proteins composed of gliadins and glutenins. A strong correlation exists between certain γ-gliadin and/or LMW-glutenin proteins and the viscoelastic properties of gluten affecting *al dente* cooking quality of pasta goods. Of those proteins, γ-gliadin 45 and LMW-2 glutenin alleles are correlated with proper gluten strength and superior pasta-cooking quality, whereas γ-gliadin 42 and LMW-1 glutenin tend to provide weak gluten with reduced cooking quality. In this study, DNA and protein markers have been jointly used for the analysis of γ-gliadin and LMW-glutenin QTLs of Turkish local durum wheat cultivars (landraces) affecting pasta-cooking quality. For that purposes, 13 SSR, one STS and two GAG primers linked to *Gli-B1* loci were used. Polymorphic relations of 28 Turkish durum wheat landraces with Canadian durum wheat cultivars of Kyle and Avonlea were determined through PCR reactions. Additionally, gliadin and LMW-glutenin proteins of the landraces were separated using A-PAGE and SDS-PAGE techniques, respectively. Of the 28 durum landraces, 17 were determined carrying γ-gliadin 45 and LMW-2 glutenin proteins associated with proper gluten strength and superior pasta-cooking quality.

Keywords: *Triticum durum*, Pasta-cooking quality, γ-Gliadin 45, SSR, A-PAGE, SDS-PAGE

INTRODUCTION

Durum wheat *(Triticum turgidum* L. *durum)* is an important food crop in the world because of its great importance in the human diet (Williams et al., 1984).Durum wheat is grown approximately in 25 million hectares worldwide and spreads over many countries, accounting for 8% of total world wheat production (Bozzini, 1988). The annual production of durum wheat, which constitutes about 19% of total wheat planting area in Turkey, is about 4 million tons (Anonymus, 2008). With this production amount, Turkey ranks second after Canada in the world. However, high quality durum wheat is not being produced in sufficient quantity required by the pasta industry.

Durum wheat has been traditionally used for pastamaking, where cooking quality is one of the most important quality criteria (Liu et al., 2006). Cooking quality of pasta, which is reflected by the viscoelastic nature of pasta dough and surface condition of cooked pasta (D'Egidio et al., 1993), is mostly influenced by protein content and composition, i.e. gluten quality. It has been well established that relationships exist between specific banding patterns of gliadin and/or glutenin proteins and gluten quality of durum wheats (Kosmolak et al., 1980). In particular, the presence of γ-gliadin 45 and LMW-2 glutenin proteins, as opposed to the absence of γ-gliadin 42 and LMW-1 glutenin proteins, has been shown to correlate strongly with gluten strength (Damidaux et al., 1978; Joppa et al., 1983; Autran et al., 1986; Pogna et al., 1990; Fares et al., 1997) It has been reported that concurrent transfer of LMW-2 glutenin and γ-gliadin 45 alleles in the same breeding program provide great increase in pasta-cooking quality (Fares et al., 1997).

Properties of gliadins and glutenins, the major components of gluten, determine the cohesive and viscoelastic traits of the dough and pasta-cooking characteristics (Miflin et al., 1983). Gliadins are monomeric proteins subdivided into four groups (alpha, beta, gamma and omega-gliadins) by their mobility when separated by acidic polyacrylamide gel electrophoresis (A-PAGE). The gene regions or QTLs encoding gliadins are located on the short arm of chromosomes l and 6 of A and B genomes (Impiglia et al., 2005). The γ-gliadin 45 protein, an indicator of proper pasta-cooking quality, is encoded by the *Gli-B1* locus on the short arm of chromosome 1B. As opposed to gliadins, glutenins are polymeric proteins that can be subdivided into

two main groups (HMW and LMW-glutenins) by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In durum wheats, two types of LMW-glutenin patterns (LMW-1 and LMW-2) correlating with pasta-cooking quality were recognized. The LMW-2 glutenins encoded by *Glu-B3* locus are tightly linked with *Gli-B1* locus (Impiglia et al., 2005).

Molecular markers are important tools in marker assisted selection (MAS) and classification of germplasm (Edwards and McCounch, 2003). Different kinds of molecular markers exist; including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs) markers, amplified fragment length polymorphisms (AFLPs), microsatellites (SSRs) and single nucleotide polymorphisms (SNPs). They may differ in a variety of ways, such as the requirements of time, money and labor, and amount of genetic variation found for each marker in a given population (Ruane and Sonnino, 2003). Of those molecular markers, SSRs have prevailed with several advantages. SSR markers are co-dominant; the heterozygous state can be discerned from the homozygous state. They are easily automated using florescent primers on an automated sequencer. Additionally, the rate of polymorphism is quite high in SSRs (Edwards and McCounch, 2003).

The purpose of this study was to investigate Turkish durum wheat landraces with respect to γ-gliadin 45 and LMW-2 glutenin QTLs that were associated with pasta-cooking quality by means of DNA and protein markers.

MATERIALS AND METHODS

Materials

This study was performed to determine the presence or absence of γ-gliadin 45 and LMW-2 glutenin proteins in 28 Turkish durum wheat landraces obtained from different regions of Turkey (Table 1). Kyle and Avonlea, which are the most commonly grown cultivars of Canada carrying γ-gliadin 45 and LMW-2 glutenin proteins, were used as the control wheats.

Table 1. Turkish durum wheat landrace

Number	Landrace	Number	Landrace
1	Sofu	16	Üveyik
2	Akbaşak	17	Sarı Buğday
3	Sarıçam	18	Vatan
4	Bağacak	19	Meram
5	Beyaziye	20	Altın
6	İskenderiye	21	Durnadili
7	Menceki	22	Ağ Buğdayı
8	Sorgül	23	Bintepe
9	Karakılçık	24	Havrani
10	Hevidi	25	Devedişi
11	Mısıri	26	Çalıbasan
12	Beyaz Buğday	27	Haci Halil
13	Yerli Sarı	28	Akçakale
14	Karabaşak	Control 1	Kyle
15	Sarıbaşak	Control 2	Avonlea

Plant growth and DNA extraction

Five seeds from each cultivar were divided into two halves; one half was used for protein electrophoresis and the other half including embryo was germinated for DNA extraction. DNA was extracted from leaf material of each genotype using genomic DNA purification kit (Fermentas Life Sciences, Genomic DNA Purification Kit).

PCR analysis

Thirteen SSR, one STS and two GAG primers, linked to *Gli-B1* and *Glu-B3* locus, were used for PCR amplification (Carrillo et al., 1990; Gale, 1995; Von Büren et al., 2000; Gupta et al., 2002; Somers et al., 2004; Hayden et al., 2006). Polymorphic relationships of 28 landraces and two control wheats (Kyle and Avonlea) were determined through the PCR reactions. Possible positions on the map of DNA markers is given in Figure 1.

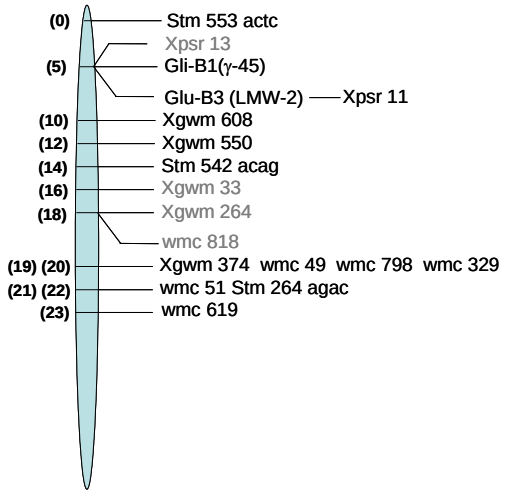

Figure 1. Positions on the map of DNA markers. (The values shown distances in cM)

PCR amplifications were performed using the procedure described by Röder et al. (1995) with some modifications. PCR reactions were carried out in a thermal cycler with a final volume of 40 µl (Thermo Px2). PCR mixture contained 50 ng of genomic DNA, 0.25 µM of each primer, 0.2 µM dNTP mix, 2.5 µM $MgCl_2$, 10x PCR Buffer and 0.5 U of Taq DNA polymerase per reaction volume. PCR cycles were started at 95°C for 5 min. Thirty cycles were performed as follows; 1 min at 94°C, 1 min at 50-60°C (depending upon the annealing temperature of the primers), 1 min at 72°C and a final extension of 5 min at 72°C. PCR products were separated on 3% metaphore agarose and 1% agarose gel.

A-PAGE screening

One half of the divided seeds from each landrace was used for the sequential extraction of gliadin and glutenin proteins. Gliadin proteins were separated using the A-PAGE method that was originally described by Bushuk and Zillman (1978) and later modified by Khan et al. (1985).

SDS-PAGE screening

LMW-glutenins of each landrace prepared by Singh et al. (1991) were separated using the SDS-PAGE procedure of Masci et al. (2000) and Gianibelli et al. (2001).

RESULTS AND DISCUSSION

In this study, DNAs from 28 durum wheat landraces (28x5=140 genotype) were amplified with 13 SSR and GAG5-6 markers. Most of the markers surrounding *Gli-B1* and *Glu-B3* locus showed polymorphism. Polymorphism status of four of those markers is given in Figure 2A and Table 2.

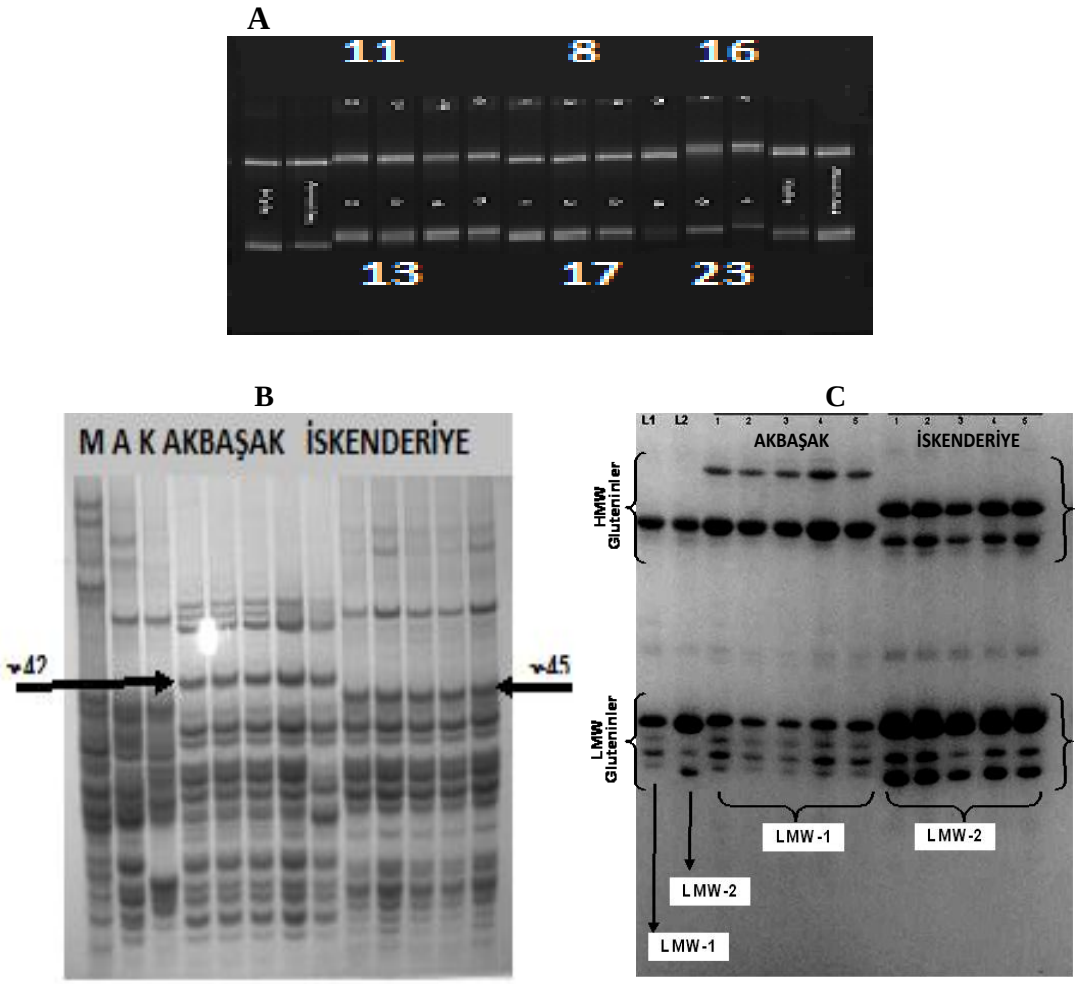

Figure 2. **A:** 3% metaphore agarose gel electrophoresis of Stm 542acag marker (8: Sorgül, 11: Mısıri, 13: Yerli sarı, 16: Üveyik, 17: Sarı Buğday, 23: Bintepe) **B:** Screening with A-PAGE (M: Marquis standart, K: Kyle, A: Avonlea) **C:** Screening with SDS-PAGE (L1: Lira 1 standart, L2: Lira-2 standart, Akbaşak and İskenderiye).

Based on the STS marker, A-PAGE and SDS-PAGE screenings, 17 out of 28 durum landraces had γ-gliadin 45 and LMW-2 glutenin proteins, whereas eight of the landraces had γ-gliadin 42 and LMW-1 glutenin proteins (Table 2, Figure 2B and 2C). In other words, the great majority of Turkish local durum wheats were determined to carry γ-gliadin 45 and LMW-2 glutenin proteins that are strongly associated with superior pasta-cooking quality.

This study revealed that the majority of Turkish durum wheat landraces had γ-gliadin 45 and LMW-2 glutenin proteins. However, certain commonly grown landraces, namely Akbaşak and Ağ Buğdayı (Zencirci et al., 1994), had γ-gliadin 42 and LMW-1 glutenin alleles that were known to have poor pasta-cooking characteristics (Damidaux et al., 1978). Some landraces were determined to differ in γ-gliadin 45 and LMW-2 glutenin alleles even within their own accessions. For instance, three out of five seeds of Beyaziye carried γ-gliadin 45 and LMW-2 glutenins whereas two of the seeds had the γ-gliadin 42 and LMW-1 glutenins (*data not shown*). This could be due to the genetic variations within the landraces and/or due to the heterogenic traits of landraces even though they are homozygous (Allard et al., 1964; Tanksley and Mccouch, 1997; Dreisigacker et al., 2005)

On the other hand, Sofu, which is a preferably grown landrace in some localities, contained both γ-gliadin 42 / LMW-1 glutenins and γ-gliadin 45 / LMW-2 glutenins (Table 2). Oak et al. (2004) reported similar results, where two out of Indian durum wheat landraces contained both γ-gliadin 45 and γ-gliadin 42 proteins. These findings emphasize that local durum wheats with remarkable genetic variations are invaluable breeding materials.

Yerli Sarı and Üveyik landraces were determined to carry neither γ-gliadin 45 / LMW-2 glutenins nor γ-gliadin 42 /

Table 2. Screening of Turkish durum wheat landraces by DNA and protein markers

Landrace	Stm542acag	Stm553acat	Xgwm550	Xgwm798	STS	A-PAGE	SDS-PAGE
Sofu	+*	+	-	+	+	**γ-gliadin 42 γ-gliadin 45**	-
Akbaşak	-**	-	+	+	-	**γ-gliadin 42**	**LMW-1**
Sarıçam	-	-	-	+	-	**γ-gliadin 42**	**LMW-1**
Bağacak	+	-	-	+	-	**γ-gliadin 42**	**LMW-1**
Beyaziye	+	+	+	+	+	γ-gliadin 45	LMW-2
İskenderiye	+	-	+	+	+	γ-gliadin 45	LMW-2
Menceki	+	-	+	+	+	γ-gliadin 45	LMW-2
Sorgül	+	+	+	+	+	γ-gliadin 45	LMW-2
Karakılçık	+	+	-	+	+	γ-gliadin 45	LMW-2
Hevidi	-	-	-	+	+	γ-gliadin 45	LMW-2
Mısıri	+	-	+	+	-	**γ-gliadin 42**	**LMW-1**
Beyaz Buğday	-	-	-	-	+	γ-gliadin 45	LMW-2
Yerli Sarı	+	+	+	+	-	**γ-gliadin 43**	-
Karabaşak	+	-	+	-	+	γ-gliadin 45	LMW-2
Sarıbaşak	-	-	-	+	+	γ-gliadin 45	LMW-2
Üveyik	-	+	+	+	+	**γ-gliadin 43**	-
Sarı Buğday	-	-	+	+	+	γ-gliadin 45	LMW-2
Vatan	-	+	+	-	+	γ-gliadin 45	LMW-2
Meram	-	-	-	+	+	γ-gliadin 45	LMW-2
Altın	-	-	-	+	+	γ-gliadin 45	LMW-2
Durnadili	-	-	-	+	-	**γ-gliadin 42**	**LMW-1**
Ağ Buğdayı	-	+	+	+	-	**γ-gliadin 42**	**LMW-1**
Bintepe	-	+	+	+	-	**γ-gliadin 42**	**LMW-1**
Havrani	-	+	+	-	+	γ-gliadin 45	LMW-2
Devedişi	-	-	+	+	+	γ-gliadin 45	LMW-2
Çalıbasan	-	-	-	+	+	γ-gliadin 45	LMW-2
Hacı Halil	-	+	+	-	-	**γ-gliadin 42**	**LMW-1**
Akçakale	-	+	-	-	+	γ-gliadin 45	LMW-2

*(+)Similar to Kyle and Avonlea
**(-)Different from Kyle and Avonlea

LMW-1 glutenins; instead, they carried γ-gliadin 43 or 44 alleles. According to Oak et al. (2004), gluten strength of durum varieties having γ-gliadin 43, γ-gliadin 44 and γ-gliadin 45 alleles were as follows: γ-gliadin 43 > γ-gliadin 45 > γ-gliadin 44.

In this study, 28 Turkish durum wheat landraces were effectively screened for the QTLs linked to pasta-cooking quality through DNA and protein markers. In a comparable study, Kudryavtsev et al. (1996) reported that protein markers (γ-gliadin 45 or 42) were invaluable tools for the identification of gliadin polymorphism in durum wheats and their genetic differences**.** Similarly, Siddiqui and Naz (2009) demonstrated the genetic diversity of 10 wheat genotypes by protein markers. Moreover, Sofalian and Valizadeh (2009) used A-PAGE and SDS-PAGE screenings to assess protein characteristics of some wild wheat genotypes.

CONCLUSIONS

In this study, SSR, STS and GAG markers together with A-PAGE and SDS-PAGE screenings were successfully used to assess the pasta-cooking quality associated traits of Turkish durum wheat landraces. The results of the study confirmed that DNA markers together with protein markers could be effectively used for the identification of QTLs or gene regions affecting durum wheat quality. Of the 28 durum landraces included in the study, 17 were determined to carry γ-gliadin 45 and LMW-2 glutenin proteins associated with proper gluten strength and superior pasta-cooking quality. Additionally, some notable genetic variations were observed among the Turkish local durum wheats.

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