Marker-assisted breeding of a durum wheat cultivar for γ-gliadin and LMW-glutenin proteins affecting pasta quality

Ahmet YILDIRIM1,*, Özlem ATEŞ SÖNMEZOĞLU1, Abdulvahit SAYASLAN2, Mehmet KOYUNCU2, Tuğba GÜLEÇ1, Nejdet KANDEMİR3

1Department of Biology, Karamanoğlu Mehmetbey University, Karaman, Turkey
2Department of Food Engineering, Karamanoğlu Mehmetbey University, Karaman, Turkey
3Department of Field Crops, Gaziosmanpaşa University, Tokat, Turkey

* Correspondence: ahmet.yildirim@kmu.edu.tr

Abstract: The pasta quality of durum wheat is one of the most important properties for the industry and consumers. Therefore, breeding for improved grain quality without yield penalties, using modern breeding methods, has been a primary objective in durum wheat breeding programs in recent years. In this study, 2 important gene regions that encode pasta-quality associated proteins (γ-gliadin 45 and LMW-2 glutenin) were transferred to a registered Turkish durum wheat variety, Sarıçanak-98, from a high-quality Canadian durum wheat cultivar, Kyle, through a marker-assisted backcross breeding method. Each of the F1 and backcross (BC) plants were backcrossed 4 times to the recurrent parent, and the backcrossed plants carrying the targeted gene regions in all generations were selected by marker-assisted selection (MAS). DNA markers in combination with A-PAGE were used for tracking the introgression of the targeted gene regions. Transfer of Gli-B1 locus encoding γ-gliadin 45 and Glu-B3 locus encoding LMW-2 glutenin to the wheat variety Sarıçanak-98 led to a considerable increase in protein content and gluten quality.

Key words: Durum wheat, Triticum durum, γ-gliadin 45, LMW-2 glutenin, backcross breeding, MAS, SSR

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1. Introduction

Wheat quality is simply defined as its suitability for a given final product. For durum wheat, quality means its suitability for pasta processing. The quality of pasta products is strongly correlated with the physical and chemical properties of durum wheat. Kernel vitreousness, protein content and quality (proper gluten strength), milling properties (semolina yield and ash content), yellow pigment content, and activities of lipoygenase (LOX), peroxidase (POD), and polyphenol oxidase (PPO) enzymes are among the well-documented quality parameters (Clarke et al. 1998; Borrelli et al. 1999; Troccoli et al. 2000; Morris 2004). Of these quality parameters, protein content and quality, yellow pigment content, and the activities of the oxidative enzymes are of vital importance for pasta quality, as they overwhelmingly determine the so-called al dente cooking characteristics and bright yellow color of pasta products (Troccoli et al. 2000; Aalami et al. 2007).

However, protein content and gluten quality (including gliadin and glutenin proteins) are the 2 most important variables in determining pasta cooking properties (D’Edigio et al. 1990; Kovacs et al. 1995; Blanco et al. 1996; Troccoli et al. 2000). Moreover, there is an important correlation among specific gliadin and glutenin proteins and the gluten strength. The most important of those proteins are γ-gliadin 45 and LMW-2 glutenin, which are linked very tightly to each other. γ-gliadin 45 is strongly correlated with high gluten strength and pasta quality (Gupta et al. 1994; Kovacs et al. 1995; D’Ovidio and Porceddu 1996). It has been reported that transfer of a Gli-B1 locus encoding γ-gliadin 45 and a Glu-B3 locus encoding LMW-2 glutenin together into a variety leads to improvement in the pasta cooking quality of durum wheat (D’Ovidio et al. 1992; Kovacs et al. 1994; Clarke et al. 1998).

Turkey is blessed with agro-climatic regions suitable for the cultivation of high-quality durum wheat. It is among the top 3 durum wheat producing countries in the world with 3 Mt of production during 2009. However, only about 30%–40% of the total durum wheat is able to meet the quality criteria required by the pasta industry (IGC 2003), leading to a rise in importation of durum wheat with desirable pasta qualities each year. Therefore, the pasta processing quality of Turkish durum wheat cultivars must be improved using modern breeding methods, without adversely affecting grain yield. For this purpose, existing varieties may be improved through introgression of genes/QTLs associated
with pasta quality. Sarıçanak-98, a durum wheat cultivar, is widely grown in southeastern Turkey due to its high yield potential, resistance to common wheat diseases in the region, and tolerance of poor soil conditions, particularly in terms of microelement deficiencies (Özberk et al. 2010; CRIFC 2011). However, the cultivar Sarıçanak-98 suffers from poor end-use quality, as it has the γ-gliadin 42 and LMW-1 proteins, which are related to poor pasta quality. Sarıçanak-98 may be further improved by transfer of γ-gliadin 45 and LMW-2 glutenin proteins associated with superior pasta-cooking quality. The aim of this study was to transfer 2 important gene regions encoding γ-gliadin 45 and LMW-2 glutenin proteins from the high-quality Canadian durum wheat cultivar Kyle to Sarıçanak-98 through a marker-assisted backcross breeding method.

## 2. Materials and methods

### 2.1. Plant materials

Sarıçanak-98, widely grown in Turkey and lacking the γ-gliadin 45 and LMW-2 glutenin encoding genes, was used as the recurrent parent. The donor parent was a high-quality Canadian durum wheat cultivar, Kyle. In each generation, F1 and BC plants were backcrossed 4 times (BC4) to the recurrent parent, and progenies carrying the targeted gene regions were selected by MAS. In each generation, an average of 50–100 BC plants were screened by molecular DNA markers, and all screening results were verified and tested with A-PAGE. Advanced breeding lines (ABLs) were obtained by selfing BC4F2 plants twice. Seeds of the lines were multiplied by growing in field conditions during the 2009–2010 growing seasons.

The cultivars Marquis and Kyle were used as check genotypes for the identification of γ-gliadin 42/45 bands on A-PAGE.

### 2.2. DNA screening

Seeds of F1 and backcross plants were cut into halves. One half was used for A-PAGE and the other half, including the embryo, was germinated and used for screening with DNA markers. DNA was extracted from the leaf of each plant using a genomic DNA purification kit (Fermentas Life Sciences).

DNA samples were screened. For this purpose, the gene-based marker GAG5-6 (Von Büren et al. 2000), linked to both Gli-B1 and Glu-B3 loci, was used for screening of the F1 and backcross population for the presence of γ-gliadin 45 and for LMW-2 glutenin alleles.

### 2.3. PCR amplification

Polymerase chain reactions (PCRs) were performed under the conditions given elsewhere (Von Büren et al. 2000) with some modifications. PCR reaction volume was 40 μL. PCR reactions contained 50–100 ng of genomic DNA, 0.25 μM of each primer, 0.2 μM dNTP mix, 2.5 μM MgCl₂, 10X PCR buffer, and 0.5 units of Taq DNA polymerase. The PCR was run on a Thermo (Px2) thermal cycler as follows: an initial denaturation step of 3 min at 94 °C was followed by 32 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, extension for 1 min at 72 °C, and conclusion with a final extension step for 5 min at 72 °C. PCR products were analyzed on 3% metaphere agarose gels or 1% agarose gels. Electrophoresis was conducted at 90 W of constant power for 3–4 h.

### 2.4. A-PAGE

The gliadins (γ-gliadin 42/45) of wheats were identified using the A-PAGE method that was originally described by Bushuk and Zillman (1978) and modified by Khan et al. (1985). In each generation, an average of 50–100 BC plants were screened by A-PAGE.

### 2.5. Embryo culture

To speed up BC generations, some BC plants were generated by immature embryo cultures following the process described by Arzani and Mirodjagh (1999).

### 2.6. Quality measurements on grain

Selected grain properties associated with quality were measured using the following methods in 3 replications: kernel vitreousness by Köksel et al. (2000), thousand kernel weight and kernel size distribution by Elgün et al. (2002), and test weight, kernel color, yellow pigment content, SDS sedimentation volume, moisture content, protein content, and ash content by the AACC methods of 55-10, 14-22, 14-50, 56-70, 44-15A, 46-10, and 08-01, respectively (AACC 2000).

Lipoxygenase (LOX) activity was determined by spectroscopic measurement of a conjugated diene formation upon the reaction of wheat extracts with a linoleic acid substrate, as described by Rani et al. (2001) and Aalami et al. (2007). One unit of LOX activity (EU) was described as 1.0 unit min⁻¹ change in the absorbance under the assay conditions and reported as EU per g wheat. Polyphenol oxidase (PPO) activity was determined following the method of Coseteng and Lee (1987), as modified by Aalami et al. (2007), where color changes in the mixture of wheat extract–catechol substrate were monitored at 420 nm. One unit of PPO activity was described as 0.1 unit min⁻¹ change in the absorbance under the assay conditions and reported as EU per g wheat. Peroxidase (POD) activities were measured according to the method of Aparicio-Cuesta et al. (1992), modified by Aalami et al. (2007), where colored product formation upon the reaction of wheat extracts with an o-dianisidin substrate in the presence of hydrogen peroxide, was monitored at 460 nm. One unit of POD activity was described as 1.0 unit min⁻¹ change in the absorbance under the assay conditions and reported as EU/g wheat.

### 2.7. Quality measurements of semolina

Selected quality characteristics of semolina were measured in 3 replications as follows: a dark-colored speck count
based on Elgün et al. (2002) and milling yield, color, pigment content, gluten content/index, moisture content, protein content, and ash content according to the AACC methods of 26-41/42, 14-22, 14-50, 38-12A, 44-15A, 46-10, and 08-01, respectively (AACC 2000).

3. Results
3.1. Molecular and biochemical marker-assisted selection
The genes encoding γ-gliadin 45 and LMW-2 glutenin proteins, which affect end-use quality of durum wheat, were transferred to the Turkish durum wheat cultivar Sarıçanak-98 following a marker-assisted backcross breeding program. The F1 and BC plants were backcrossed 4 times to the recurrent parent, and the backcross plants (from BC1F1 to BC4F1) were screened for the targeted alleles through molecular markers (Figure 1) and A-PAGE (Figure 2) in each generation.

A 1-to-1 segregation ratio was clearly seen in the BC1F1 generation. Fifty percent of the backcross plants carried the recurrent parent allele (Sarıçanak-98), while the rest were heterozygous, as expected.

In each backcross generation, screening was carried out in combination with molecular DNA markers and A-PAGE. As a result of the screening, heterozygous backcross plants having the targeted alleles were determined and the BC4F1 seeds were obtained through hybridization of those plants. The BC4F2 seeds were obtained by selfing of BC4F1 plants. In the BC4F2 plants, homozygous plants (25%) were identified by molecular (Figure 3a) and A-PAGE screening (Figure 3b), and homozygous BC4F2 seeds were increased by selfing to use for the yield trials later.

![Figure 1. Representative PCR profiles of parents and BC1F1 plants due to GAG 5-6 markers linked to Gli-B1 locus. (H: plants heterozygous for targeted gene regions.)](image1)

![Figure 2. Representative profiles of parents and BC1F1 plants screened with A-PAGE. (A: BC1F1 plants homozygous for the recurrent parent alleles, H: BC1F1 plants heterozygous for the targeted gene regions, K: cv. Kyle, S: cv. Sarıçanak-98.)](image2)
Immature embryo cultures were used to reduce the time required for seed generation after hybridization and acceleration of the backcross processes. Half of the BC seeds in each generation were germinated through immature embryo cultures and the rest were left on BC spikes for normal maturation. Thus, each generation was produced approximately 40 days earlier. Consequently, more than 2 generations were obtained in a year with the help of the embryo culture approach.

3.2. Quality improvement

Selected physical, chemical, and technological properties of the advanced breeding line (BC4F4) and its parents (cultivars Kyle and Sarıçanak-98) were investigated and the findings summarized in Tables 1–3. The breeding line and its parents were fairly comparable in terms of kernel physical properties, although the differences were statistically significant (P < 0.05) (Table 1). However, the breeding line significantly differed from its parents with respect to gluten quality and protein content (Table 2). Gluten quality of the breeding line was significantly improved, as indicated by its higher SDS sedimentation and specific sedimentation volumes, which was the aim of this work. Additionally, protein content of the breeding line was significantly higher (P < 0.05) than that of its recurrent parent, Sarıçanak-98. Indeed, simultaneous improvement of gluten quality and protein content is a significant achievement for the advanced breeding line. Initial observations indicated that yield of the ABL did not

![Figure 3. Representative profiles of parents and BC4F2 plants screened with GAG 5-6 markers (a) and A-PAGE (b). (A: Backcross plants homozygous for the recurrent parent alleles, B: Backcross plants homozygous for the donor parent alleles, H: Heterozygous backcross plants.)](image)

<table>
<thead>
<tr>
<th>Parents / Advanced breeding line</th>
<th>Thousand kernel weight (g)</th>
<th>Test weight (kg hL⁻¹)</th>
<th>Kernel vitreousness (%)</th>
<th>Kernel size distribution (%)</th>
<th>Homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyle</td>
<td>36.7 a</td>
<td>79.2 c</td>
<td>99.8 a</td>
<td>23.6</td>
<td>37.3 29.1 10.2</td>
</tr>
<tr>
<td>Sarıçanak-98</td>
<td>36.8 a</td>
<td>82.3 a</td>
<td>99.1 b</td>
<td>27.4</td>
<td>47.6 18.6 6.5</td>
</tr>
<tr>
<td>ABL (BC4F4)</td>
<td>35.5 b</td>
<td>80.6 b</td>
<td>99.5 ab</td>
<td>47.3</td>
<td>34.0 14.6 4.1</td>
</tr>
</tbody>
</table>

*14% moisture basis  bDifferent letters in a column indicate significant difference (P < 0.05)
ABL: Advanced breeding line, BC: Backcross

<table>
<thead>
<tr>
<th>Parents / Advanced breeding line</th>
<th>Moisture content (%)</th>
<th>Ash content (%)</th>
<th>Protein content (%)</th>
<th>Sedimentation volume (mL)</th>
<th>Specific sedimentation volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyle</td>
<td>10.4 ns</td>
<td>1.54 b</td>
<td>14.8 a</td>
<td>22.1 a</td>
<td>1.49 a</td>
</tr>
<tr>
<td>Sarıçanak-98</td>
<td>9.8 ns</td>
<td>1.48 b</td>
<td>13.8 b</td>
<td>17.9 b</td>
<td>1.30 b</td>
</tr>
<tr>
<td>ABL (BC4F4)</td>
<td>10.3 ns</td>
<td>1.65 a</td>
<td>14.8 a</td>
<td>22.5 a</td>
<td>1.52 a</td>
</tr>
</tbody>
</table>

*14% moisture basis  bDifferent letters in a column indicate significant difference (P < 0.05)  ns: Not significant
ABL: Advanced breeding line, BC: Backcross
show any difference from Sarıçanak-98. However, reliable results can be obtained from replicated field trials in the wheat’s natural environment.

As seen in Table 3, yellow pigment content and color properties (L*, a*, and b*) of the breeding line were significantly higher (P < 0.05) than those of the recurrent parent. On the other hand, the breeding line and its recurrent parent were statistically comparable in terms of their oxidative enzymes (LOX, PPO, and POD) that contribute to the bright yellow color of pasta products.

Semolina yields of the breeding line and its parents were quite similar; however, the breeding line had significantly higher levels of pigment and protein with slightly improved gluten quality (Table 4). It is obvious that quality measurements recorded on the grain (Tables 2 and 3) were in close agreement with those recorded on the semolina (Table 4).

4. Discussion

Pasta products are appreciated worldwide as an end-product of durum wheat. Considering increased competition in the global pasta market, improving the end-use quality of durum wheat is essential. It has been reported that there is a positive relationship between γ-gliadin 45 protein (LMW-2 glutenin) and the optimum gluten strength associated with the al dente cooking quality of pasta (Kovacs et al. 1995; Troccoli et al. 2000). It has been widely reported that the gluten quality of durum wheat containing γ-gliadin 45 and LMW-2 glutenin is better than that of γ-gliadin 42 and LMW-1 glutenin (Damidiau et al. 1978; Chihab 1990; Kaan et al. 1995; Nachit et al. 1995; Pena 2000; Edwards et al. 2007; Yüksel 2009). It is also well known that gluten quality and the protein content of the durum are the main parameters that determine pasta quality (Bushuk 1998; Troccoli et al. 2000).

During this study, important gene regions encoding pasta-quality associated proteins (γ-gliadin 45 and LMW-2 glutenin) were transferred from the cultivar Kyle to the cultivar Sarıçanak-98, leading to the development of an advanced breeding line with improved quality. As expected, this line has increased gluten quality, as judged by the sedimentation and gluten index tests. Additionally, the protein content of the breeding line also increased.

Besides the protein content and gluten quality, a yellow pigment color of the grain also contributes to improved pasta quality. Pigment contents ranging from 4 to 8 mg kg⁻¹ in durum wheat have been reported by different researchers. The pigment content (7.55 mg kg⁻¹) of the breeding line developed during the present study was higher than the pigment content (4 to 8 mg kg⁻¹) reported in earlier durum wheat studies (Kaan et al. 1995; Köksel et al. 2000; Troccoli et al. 2000; Sakin et al. 2011). Thus, the breeding lines developed should produce yellow-colored pasta products, preferred by consumers.

The results of this study confirm that marker-assisted selection and backcross breeding can be successfully

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<tr>
<th>Table 3. Color properties and oxidative enzyme activities of the advanced breeding line and its parents.a,b</th>
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<tr>
<td>Parents / Advanced breeding line</td>
</tr>
<tr>
<td>Kyle</td>
</tr>
<tr>
<td>Sarıçanak-98</td>
</tr>
<tr>
<td>ABL (BC4F4)</td>
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*a14% moisture basis  bDifferent letters in a column indicate significant difference (P < 0.05)  ns: not significant

ABL: Advanced breeding line, BC: Backcross

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<tr>
<th>Table 4. Chemical and technological properties of semolina milled from the advanced breeding line and its parents.a,b</th>
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<td>Parents / Advanced breeding line</td>
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ABL: Advanced breeding line, BC: Backcross
employed in wheat breeding programs and that molecular markers can be used alone or in combination with the A-PAGE technique in each backcross generation. Quality-targeted breeding through marker-assisted backcross breeding in this work was completed in about 3 years by raising 3 generations per year. With the help of marker-assisted selection, breeding time was reduced and the efficiency of backcross breeding was increased, leading to the development of a durum wheat candidate with elevated protein content and gluten quality.

Acknowledgments

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References


Yüksel F (2009) Quality characteristics and stability traits of advanced durum wheat lines (MSc Thesis). Gaziosmanpaşa University, Department of Food Engineering, Tokat, Turkey (in Turkish).