Wageningen Academic Publishers

Molecular breeding of durum wheat cultivars for pasta quality

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Received: 22 November 2017 / Accepted: 30 September 2018

RESEARCH ARTICLE

Abstract

The quality of durum wheat (*Triticum durum*) is defined as its degree of suitability for pasta production. In order to meet the demand of pasta industry for high quality raw material, existing durum varieties should be first improved in terms of quality-associated genes or quantitative trait loci (QTLs). In this study, important QTLs (*Gli-B1* locus encoding γ-gliadin 45 protein and *Glu-B3* locus encoding LMW-2 type glutenins) that affect cooking quality of pasta were transferred to two durum wheat cultivars (Salihli-92 and Kızıltan-91) through marker-assisted selection (MAS) and backcross breeding. A Canadian durum wheat cultivar with a high quality, Kyle, was used as the donor parent. Each F1 and backcross (BC) plants were backcrossed four times to the recurrent parents, and backcrosses carrying the targeted QTLs were selected by linked molecular markers, i.e. DNA markers, acidic polyacrylamide gel electrophoresis (A-PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Quality analyses were performed on the kernels and semolinas of inbred backcross lines (BC4F4) and of parental cultivars to assess the effects of the transferred QTLs. Both advanced breeding lines (ABLs) had higher protein contents (14.9 and 15.8%) than their recurrent parents (14.0-14.8%). Also, SDS-sedimentation volumes (32.7 and 28.2 ml) of the ABLs were significantly higher ($P<0.05$) than their parents. It was found that the transfer of *Gli-B1* locus containing γ-gliadin 45 and *Glu-B3* locus containing LMW-2 type glutenins to the advanced breeding lines led to increases in their pasta-quality associated properties.

Keywords: durum wheat, γ-gliadin 45, LMW-2 type glutenins, pasta quality, SSR

1. Introduction

World production of durum wheat (*Triticum durum*) in 2016 was 39 million tons, approximately 6% of global wheat production. The leading countries in durum wheat production are the EU, Canada, Turkey, Mexico, USA, Algeria and Morocco (IGC, 2016). Although Turkey has a suitable ecology to produce high-quality durum wheat, the demand of the domestic pasta industry for high-quality wheat often cannot be supplied in sufficient quantities (Yıldırım *et al.,* 2013). Therefore, breeding programs in Turkey have recently focused on improving quality of major cultivars (Sakin *et al.,* 2011; Sayaslan *et al.,* 2012; Yıldırım *et al.,* 2013).

Quality characteristics of durum wheat products are strongly associated with physical and chemical properties, such as kernel size and homogeneity, hardness, vitreousness, milling properties, protein content and quality, yellow pigment content and activities of oxidative enzymes, including lipoxygenase (LOX), peroxidase (POD) and polyphenol oxidase (PPO) (Borrelli *et al.,* 1999; Clarke *et al.,* 1998; Sakin *et al.,* 2011; Sayaslan *et al.,* 2012; Troccoli *et al.,* 2000; Yıldırım *et al.,* 2013; Yüksel *et al.,* 2011). Of the physical properties, kernel size and homogeneity, hardness and vitreousness are particularly important in semolina yield and bright yellow appearance of semolina (Bushuk, 1998; Sayaslan *et al.,* 2012; Troccoli *et al.,* 2000; Yüksel *et al.,* 2011). As for the chemical properties, however, protein content together with gluten quality and yellow-coloured

pigment content along with the oxidative enzymes are of vital importance respectively on the so-called *al dente* cooking characteristics and bright yellow colour of pasta products (Borrelli *et al.,* 1999; Sakin *et al.,* 2011; Troccoli *et al.,* 2000; Yıldırım *et al.,* 2013).

Protein content and gluten quality overwhelmingly determine the strength and cooking quality of pasta. For the processing of *al dente* cooking pasta products, durum wheats are expected to contain elevated levels of protein (>13%), which is largely dictated by growing conditions (Yüksel *et al.,* 2011). On the other hand, the viscoelastic and cohesive nature of gluten proteins, i.e. gluten quality, is heavily dependent on genotype (Bushuk, 1998). Many analytical approaches have been developed to assess durum wheat gluten quality, including SDS-sedimentation volume, rheological measurements and electrophoretic screening for specific gliadin and glutenin proteins that are linked to gluten viscoelasticity (Bushuk, 1998; Cubadda *et al.,* 2007; Marchylo *et al.,* 2001; Sakin *et al.,* 2011; Sayaslan *et al.,* 2012; Yüksel *et al.,* 2011). It is known that pasta-cooking quality is strongly influenced by allelic compositions of endosperm storage proteins, namely gliadins and glutenins (Porceddu *et al.,* 1998; Vita *et al.,* 2007). The most recognised proteins in this respect are γ-gliadin 45 and LMW-2 type glutenins, which were found to be tightly linked (Kovacs *et al.,* 1995). It is generally accepted that γ-gliadin 45 and LMW-2 type glutenins are highly correlated with superior gluten strength and pasta-cooking quality. Adversely, γ-gliadin 42 and LMW-1 type glutenins are associated with low gluten strength and poor pasta-cooking quality (Kovacs *et al.,* 1995). The simultaneous transfer of *Gli-B1* locus (encoding γ-gliadin 45) and *Glu-B3* locus (encoding LMW-2 type glutenins) in the same breeding program has been shown a substantial improvement in pasta-cooking quality (Clarke *et al.,* 1998; D'Ovidio and Porceddu, 1996; D'Ovidio *et al.,* 1992).

Pigment contents of durum wheats were reported to vary from 4 to 8 mg/kg (Sakin *et al.,* 2011), where carotenoids constitute the major pigments (Clarke *et al.,* 1998; Troccoli *et al.,* 2000). However, those pigments are easily oxidised, resulting in detrimental effects on colour of pasta products (Aalami *et al.,* 2007). It is known that LOX causes oxidative bleaching of yellow-coloured carotenoids, whereas PPO and POD cause oxidative darkening of wheat phenolics (Aalami *et al.,* 2007; Borrelli *et al.,* 1999). Pigment contents and LOX activities were shown to be influenced by both genotype and environmental conditions, thus leading to its large variation in durum wheats (Aalami *et al.* 2007; Sakin *et al.,* 2011). Therefore, wheat cultivars that are high in yellow pigments but low in oxidative enzyme activities are preferred in the processing of bright yellow-coloured products.

In recent years, introgression of quantitative trait loci (QTLs) or genes into elite varieties has been achieved with the use of marker-assisted selection (MAS) technique. The MAS approach improved the efficiency and reliability of conventional backcrossing (Sönmezoğlu *et al.,* 2012; Tyagi *et al.,* 2014). It also enabled effective selection of target loci, minimal linkage drags and maximum recovery of recurrent parent. Additionally, molecular and biochemical markers were successfully used in combination with MAS in wheat breeding (Sönmezoğlu and Balkan, 2014; Sönmezoğlu *et al.,* 2010). Furthermore, the MAS was employed in combination with immature embryo culture in order to shorten generations in backcross breeding (Baenziger and DePauw, 2009). Subsequently, the time required for the development of high-quality candidate wheat varieties in a backcross breeding was reduced almost by half through the MAS.

The aim of this study was to transfer two important QTL regions encoding pasta-quality associated proteins (g-gliadin 45 encoded by *Gli-B1* locus and LMW-2 type glutenins encoded by *Glu-B3* locus) from the high-quality Kyle cultivar to two registered Turkish durum wheat varieties, namely Salihli-92 and Kızıltan-91, through marker assisted backcross breeding method and to assess their effects on durum wheat quality characteristics.

2. Materials and methods

Plant materials

In this study, registered Turkish durum wheat cultivars of Salihli-92 and Kızıltan-91, which lack the encoding genes of γ-gliadin 45 and LMW-2 type glutenins, were used as the recurrent parents. A high-quality Canadian durum wheat cultivar, Kyle, was used as the donor parent. In each generation, F1 and (backcross) BC plants were backcrossed four times to the recurrent parents and MAS was used to determine the progenies carrying the targeted gene regions. In each generation, about 50-100 BC plants were screened by the GAG5-6 marker and results were verified with the acidic polyacrylamide gel electrophoresis (A-PAGE). Marquis and Kyle cultivars were used for the identification of γ-gliadin 42/45 bands in the A-PAGE, and Lira-1 and Lira-2 cultivars for the identification of LMW-1/LMW-2 glutenin patterns by in the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Yıldırım *et al.,* 2013). Marker assisted backcrossing method was employed in combination with immature embryo culture (Arzani and Mirodjagh, 1999) to accelerate BC generations. Seeds of the lines were multiplied by growing in field conditions during the 2009-2010 growing seasons.

Molecular and biochemical marker-assisted selection

The γ-gliadins (γ-gliadin 42/45) of durum wheat genotypes were determined using the A-PAGE method (Khan *et al.,* 1985). The LMW glutenin types (LMW-1/LMW-2) were extracted and prepared according to Singh *et al.* (1991) and identified following the SDS-PAGE method (Gianibelli *et al.,* 2002).

DNA isolation was performed as described by Yıldırım *et al.* (2013). The obtained DNA was screened for the presence of γ-gliadin 45 and for LMW-2 type glutenin alleles. For this purpose, Stm553actc, Stm542acag (Hayden *et al.,* 2006), Xgwm 550, Xgwm 608 (Somers *et al.,* 2004) microsatellite (SSR) markers, mapped at the end of the short arm of chromosome 1B, were used. In addition, GAG5-6 (Von Büren *et al.,* 2000) primers linked to *Gli-B1* and *Glu-B3* loci were used.

PCR was performed under the conditions given in the source articles (Hayden *et al.*, 2006; Somers *et al.*, 2004; Von Büren *et al.*, 2000) for each primer with some modifications. The PCR amplifications were carried out in reaction mixture of 40 µl containing 50 ng of wheat template DNA, 0.2 µM dNTPs, 2.5 µM $MgCl_2$, 0.25 µM of each primer, 10× PCR buffer and 0.5 U *Taq* DNA polymerase. The PCR started with an initial denaturation step for 3 min at 94 °C. The details of 32 PCR cycling reactions were as follows: 1 min at 94 °C, 1 min at 50-60 °C (different annealing temperature of primers), 1 min at 72 °C with a final extension step of 5 min at 72 °C and storage at 4 °C. PCR products were separated on 1% agarose or 3% metaphor agarose gels. Electrophoresis was applied at 90-watt constant power for 3-4 h, and 0.5× TBE buffer was used as the running buffer during electrophoresis.

Pasta-quality associated measurements on wheat kernel and semolina

Kernel vitreousness, thousand-kernel weight and kernel size distributions were measured by Elgün *et al.* (2002). Test (hectolitre) weight, kernel colour, yellow pigment content, SDS-sedimentation volume, moisture, protein and ash contents of wheat kernels were determined by the American Association of Cereal Chemists International (AACCI) methods, respectively 55-10, 14-22, 14-50, 56-70, 44-15A, 46-10 and 08-01 (AACCI, 2000). Specific sedimentation volumes of wheats were calculated by dividing SDS-sedimentation volumes by the corresponding protein contents (Sakin *et al.,* 2011). The LOX, PPO and POD enzyme activities were determined by Yıldırım *et al.* (2013), which was a slight modification from Aalami *et al.* (2007). Wheats were tempered at 16% moisture content and milled into semolina using CD2 semolina mill (Chopin technologies, Villeneuve-la-Garenne, France). Dark-coloured speck count in semolina was carried out by Elgün *et al.* (2002). Milling yield, colour, pigment, moisture, protein and ash contents of semolina samples were determined by the AACCI methods, respectively 26-41/42, 14-22, 14-50, 44-15A, 46-10 and 08-01 (AACCI, 2000).

Statistical analysis

The data collected with at least two replications were subjected to one-way analysis of variance and the means were compared with Duncan's multiple comparison test using SPSS statistical software (2010 release, 19th version, IBM, Armonk, NY, USA).

3. Results

The QTLs (γ-gliadin 45 and LMW-2 type glutenins) affecting end use quality of durum wheat were transferred to Salihli-92 and Kızıltan-91 durum wheat cultivars in a marker-assisted backcross breeding program. The BC plants were backcrossed four times to the recurrent parents, and the progenies carrying the targeted gene regions were selected through the combination of biochemical markers (Figure 1), A-PAGE (Figure 2) and SDS-PAGE in each generation. The heterozygous backcross plants (H) carrying the targeted gene regions were selected in F1 and BC generations.

Heterozygous and homozygous backcross lines carrying the transferred genes were successfully selected by the MAS. The homozygous BC4F2 plants were selected by the

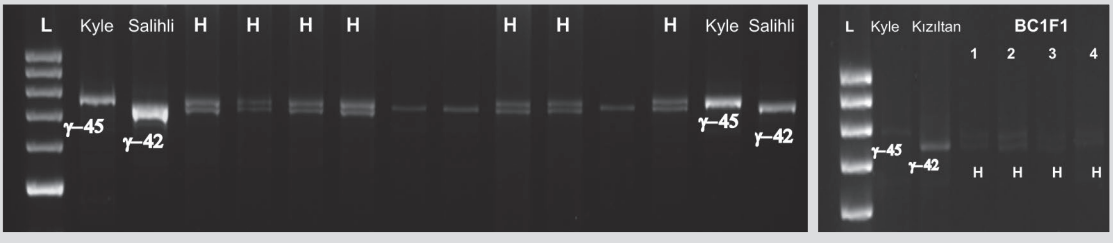

Figure 1. Molecular selection of backcross (BC) plants screened with GAG 5-6 markers linked to *Gli-B1* locus. H = heterozygous backcross plants. L = DNA length marker.

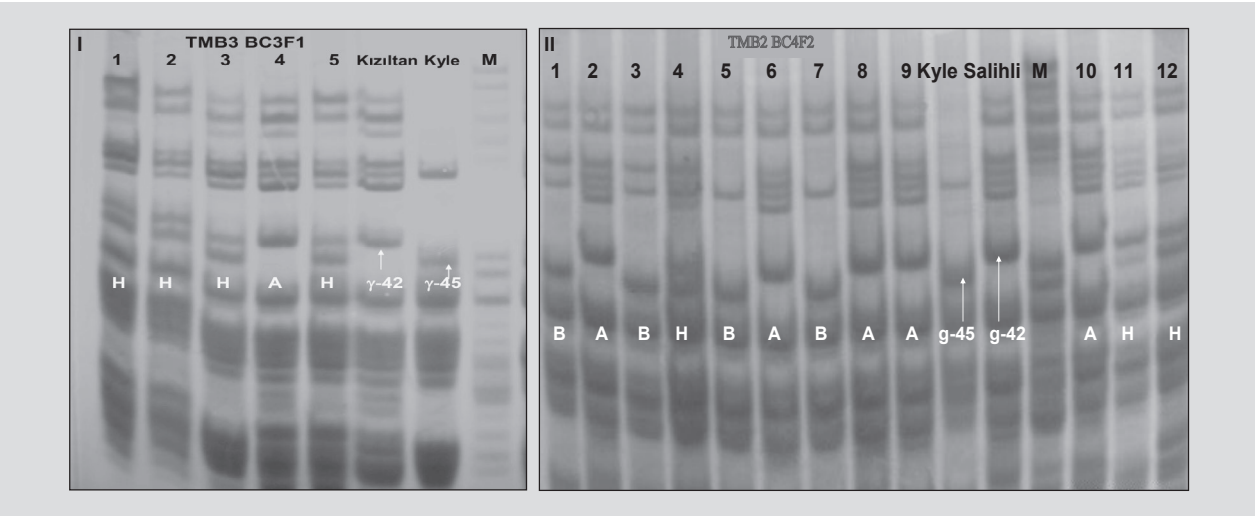

Figure 2. Examples of BC3F1 (I) and BC4F2 plants (II) screened with acidic polyacrylamide gel electrophoresis (A-PAGE). A = Backcross plants homozygous for the recurrent parent alleles; B = Backcross plants homozygous for the donor parent alleles; H = heterozygous backcross plants; M = DNA length marker.

A-PAGE in addition to the DNA markers (Figure 2), and they were increased by selfing for quality analysis.

In the study, immature embryo cultures were used to reduce the time required for seed generation after hybridisations. New seedlings were obtained by taking immature embryos in culture 20 days after hybridisation, and thus, each generation was produced approximately 40 days earlier. Consequently, about three generations were obtained in a year with the help of immature embryo culture and rapid plant growth under controlled greenhouse conditions. Background selections of the ABL were also done by 24 SSR markers, which were polymorphic between parents and represented all chromosomes. Based on the screening results T2 ABL has gained back 98.5% of its recurrent parent (Salihli-92), whereas T3 ABL has recovered back 97.5% of its recurrent parent (Kızıltan-91).

Physical and chemical analyses were performed on wheat kernels and semolinas to assess the effects of the transferred gene regions to the advanced backcross lines (BC4F4). As listed in Table 1, kernel physical properties of the ABLs (T2 and T3) were somewhat comparable to those of their parents (Kyle, Salihli-92 and Kızıltan-91), though some statistically significant differences were observed. The ABLs gave homogeneous kernel size distributions in contrast to their parents. The thousand-kernel weights, test weights and vitreousness of the T2 and T3 lines were respectively 42.6-34.8 g, 79.0-75.8 kg and 99.9-99.8% (Table 1), which were within the acceptable ranges for durum wheats (Sakin *et al.,* 2011; Yüksel *et al.,* 2011).

In terms of kernel chemical compositions, the ABL and their parents showed significant differences ($P<0.05$), especially in protein contents and qualities (Table 2). Both ABLs had

Table 1. Kernel physical properties of durum wheat breeding lines and their parents.[1]

Parent or advanced breeding line (ABL)	Thousand-kernel weight[2] (g)	Test weight (kg/hl)	Kernel vitreousness (%)	Kernel size distribution (%)				
				>2.8 mm	>2.5 mm	>2.2 mm	<2.2 mm	homogeneity
Kyle	36.7 b	79.2 b	99.8 a	23.6	37.3	29.1	10.2	heterogeneous
Salihli-92	37.1 b	82.3 a	99.0 b	24.5	37.4	27.2	11.5	heterogeneous
T2 ABL (BC4F4)	42.6 a	79.0 b	99.9 a	42.6	36.0	17.0	4.3	homogeneous
Kyle	36.7 a	79.2 a	99.8 ns	23.6	37.3	29.1	10.2	heterogeneous
Kızıltan-91	37.6 a	78.2 b	99.8	33.7	34.2	26.1	6.1	heterogeneous
T3 ABL (BC4F4)	34.8 b	75.8 c	99.8	40.7	38.1	17.4	4.2	homogeneous

[1] Different letters in a column within the same group indicate significant difference ($P<0.05$). ns = not significant ($P>0.05$).
[2] 14% moisture basis.

Table 2. Kernel chemical properties of durum wheat breeding lines and their parents.[1,2]

Parent or Advanced Breeding Line (ABL)	Moisture content (%)	Ash content (%)	Protein content (%)	Sedimentation volume (ml)	Specific sedimentation volume (ml)
Kyle	10.4 a	1.54 b	14.8 a	22.1 b	1.49 b
Salihli-92	9.4 b	1.62 ab	14.0 b	18.2 c	1.30 b
T2 ABL (BC4F4)	11.0 a	1.65 a	14.9 a	32.7 a	2.19 a
Kyle	10.4 ns	1.54 b	14.8 b	22.1 c	1.49 b
Kızıltan-91	10.3	1.45 b	14.2 c	25.9 b	1.82 a
T3 ABL (BC4F4)	10.0	1.73 a	15.8 a	28.2 a	1.78 a

[1] Different letters in a column within the same group indicate significant difference (P<0.05). ns = not significant (P>0.05).
[2] 14% moisture basis.

Table 3. Kernel colour properties and oxidative enzyme activities of durum wheat breeding lines and their parents.[1,2]

Parent or Advanced Breeding Line (ABL)	Pigment content (mg/kg)	Colour			LOX[3] activity (EU/g)	POD[3] activity (EU/g)	PPO[3] activity (EU/g)
		L*	a*	b*			
Kyle	7.58 a	77.0 a	5.05 b	18.59 a	44.9 c	29.3 b	6.8 b
Salihli-92	6.67 b	58.1 b	5.64 a	16.72 b	46.9 b	100.6 a	4.0 c
T2 ABL (BC4F4)	6.60 b	76.3 a	5.44 a	15.66 c	49.0 a	76.9 a	15.9 a
Kyle	7.58 a	77.0 ns	5.05 b	18.59 a	44.9 b	29.3 b	6.8 b
Kızıltan-91	6.80 c	76.6	5.42 a	16.22 b	48.8 a	65.6 a	13.9 a
T3 ABL (BC4F4)	7.14 b	74.9	5.48 a	18.04 a	50.6 a	60.1 a	4.3 b

[1] Different letters in a column within the same group indicate significant difference (P<0.05). ns = not significant (P>0.05). LOX = lipoxygenase; POD = peroxidase; PPO = polyphenol oxidase.
[2] 14% moisture basis.

much higher protein contents (14.9 and 15.8%) than their parents (14.0-14.8%). As expected, SDS-sedimentation volumes (32.7 and 28.2 ml) of the ABLs were significantly higher (P<0.05) than those of their parents (18.2-25.9 ml), indicating their superior gluten qualities. It is clear that the transfer of gluten-quality associated QTLs, namely γ-gliadin 45 and LMW-2 type glutenins encoded respectively by *Gli-B1* and *Glu-B3* loci, remarkably improved gluten characteristics of the durum wheat lines. The results of this study agreed well with the previous studies (Clarke *et al.,* 1998; D'Ovidio and Porceddu, 1996; D'Ovidio *et al.,* 1992; Yıldırım *et al.,* 2013) that the simultaneous transfer of *Gli-B1* and *Glu-B3* loci in the same breeding program improved pasta-cooking quality. As for the ash contents (Table 2), however, ABLs had slightly yet significantly higher ash contents (1.65 and 1.73%) than the parental varieties (1.45-1.62%), which might be due to increase in protein contents of the ABLs (Table 2).

As seen in Table 3, colour and oxidative enzyme activities of wheats varied with no specific trend. In general, the ABLs had colour properties lower than their donor parent (Kyle) but similar to or higher than their recurrent parents. It is also evident that the T3 ABL had better colour properties than the T2 ABL. Pigment contents of the ABLs were similar to or lower than the parental varieties. In terms of oxidative enzyme activities, however, slight increase in LOX and somewhat fluctuation in POD and PPO activities were observed. The reasons for these changes could not be elucidated; however, it is known that pigment contents and LOX activities of durum wheats were influenced by both genotype and environmental conditions (Aalami *et al.,* 2007; Sakin *et al.,* 2011). In this study, the variations in the oxidative enzymes of the ABL might have thus stemmed from their parents.

The ABLs and their parents were comparable in terms of semolina milling yield (Table 4). Likewise, the ABLs and their parents had semolina with similar dark-coloured speck counts and ash contents. However, the semolina milled from the ABLs had significantly higher (P<0.05) protein contents than those of their recurrent parents (Table 4). It

Table 4. Properties of semolina milled from durum wheat breeding lines and their parents.[1,2]

Parent or Advanced Breeding Line (ABL)	Semolina yield (%)	Dark-speck count (no/100 cm^2)	Pigment content (mg/kg)	Ash content (%)	Protein content (%)
Kyle	54.7	110 ns	7.31 a	0.59 ns	13.8 a
Salihli-92	55.3	105	6.52 b	0.60	12.9 b
T2 ABL (BC4F4)	56.6	120	4.43 c	0.58	14.0 a
Kyle	54.7	110 ns	7.31 a	0.59 ns	13.8 b
Kızıltan-91	55.2	95	5.68 c	0.65	13.6 b
T3 ABL (BC4F4)	53.3	125	6.23 b	0.66	14.7 a

[1] Different letters in a column within the same group indicate significant difference (P<0.05). ns = not significant (P>0.05).
[2] 14% moisture basis.

is obvious that quality measurements taken on the semolina (Table 4) were in agreement with those conducted on the kernels (Table 2). Pigment content of the T2 ABL was much lower than the parents; however, the T3 ABL had pigment content between the parents.

4. Discussion

Wheat quality means the suitability of a cultivar for a targeted final product. In durum wheat, it is mostly defined as its suitability for pasta processing (Bushuk, 1998). Protein content and gluten properties of durum wheats are the main determinants of pasta-cooking quality (Troccoli *et al.*, 2000). It has been well documented that g-gliadin 45 and LMW-2 type glutenins have a positive relationship with optimum gluten strength associated with proper pasta-cooking quality (Kovacs *et al.*, 1995; Troccoli *et al.*, 2000). Therefore, many researchers suggested that high-quality durum wheats carry g-gliadin 45 and LMW-2 type glutenins rather than g-gliadin 42 and LMW-1 type glutenins (Gregova *et al.*, 2012; Yıldırım *et al.*, 2013).

In this study, *Gli-B1* and *Glu-B3* loci, encoding, respectively, g-gliadin 45 and LMW-2 type glutenins and associated with pasta-cooking quality, were transferred to two Turkish durum wheat varieties, namely Salihli-92 and Kızıltan-91, from the cultivar Kyle. The advanced breeding lines carrying the targeted QTLs had improved gluten quality as evidenced by their higher SDS-sedimentation and specific SDS-sedimentation values. Additionally yet unexpectedly, protein contents of the ABLs also increased. Thus, the ABLs with increased protein content and gluten quality were developed.

In addition to proper protein characteristics that are relevant to pasta-cooking quality (Bushuk, 1998; Troccoli *et al.*, 2000), higher levels of yellow-coloured pigments and lower activities of oxidative enzymes are preferred in durum wheats in order to process pasta products with a bright yellow colour (Aalami *et al.,* 2007; Borrelli *et al.,* 1999; Clarke *et al.,* 1998; Troccoli *et al.,* 2000). Pigment contents of durum wheats vary from 4 to 8 mg/kg (Sakin *et al.,* 2011). In this respect, both ABLs, especially the T3 ABL with quite high (7.14 mg/kg) pigment content, was promising. Since the enzyme activity is influenced by both genetics and environmental conditions in wheat (Aalami *et al.,* 2007), variations in the LOX and PPO enzyme activities of the ABLs could be attributed to their parents.

In general, the synchronised transfer of *Gli-B1* and *Glu-B3* loci, encoding respectively g-gliadin 45 and LMW-2 type glutenins, from Kyle to Salihli-92 and Kızıltan-91 durum wheat varieties resulted in advanced breeding lines with improved protein contents and qualities. However, the T3 ABL had inferior kernel physical properties. In line with the objective of the study, gluten quality of the ABLs, especially of ABL T3, was improved. The results of this study also confirmed that the MAS in combination with background screening is an effective approach in transfer of specific QTLs in durum wheat breeding. Combination of molecular markers with the A-PAGE and the SDS-PAGE in each backcross generation reduced the time required for the gene transfer almost by half. In addition, the rate of gaining back of the recurrent parents, which was revealed by backcross screening with SSR markers, indicated the usefulness of molecular markers. Obviously, the values are relative and use of more markers could have given better results. In conclusion, the utilisation of the MAS-supported backcross breeding and backcross selection resulted in durum wheat candidates with improved quality characteristics for pasta industry.

Acknowledgements

This study was partially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK; Project no. 107O004) within the framework of COST-FA-0604 TritiGen Action.

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