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Molecular Breeding of Selçuklu-97 Durum Wheat Cultivar for Some Genes Affecting Pasta Quality

Özlem ATEŞ SÖNMEZOĞLU[1*] Ahmet YILDIRIM[2] Tuğba ESERKAYA GÜLEÇ[1]
Nejdet KANDEMİR[1] Abdulvahit SAYASLAN[3] Mehmet KOYUNCU[3]

[1]Gaziosmanpasa University, Agricultural Faculty, Department of Field Crops, Molecular Biotechnology Lab., Tokat, TURKEY
[2]Karamanoglu Mehmetbey University, Science Faculty, Department of Biology, Karaman, TURKEY
[3]Karamanoglu Mehmetbey University, Vocational School, Food Technology Program, Karaman, TURKEY

[*]**Corresponding Author**
e-mail: ozlemsonmezoglu@gmail.com

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Abstract

The most efficient way of producing and supplying proper raw material desired by the pasta industry is the development of high quality durum wheat varieties. Therefore, quality of Turkish durum wheat varieties should be improved using modern breeding methods without adversely affecting their yields.

In this study, important genes (y-gliadin 45 and LMW-2 glutenin) affecting the quality of pasta products were transferred to a Turkish durum wheat variety, Selçuklu-97, in a backcross breeding method in combination with marker assisted selection (MAS). A Canadian durum wheat cultivar with high quality, Kyle, was used as the donor parent. Each of F1 and backcross (BC) plants was backcrossed four times to the recurrent parent and in all of the generations, backcrossed plants carrying the targeted QTLs were selected by the MAS. The MAS method was employed in combination with embryo culture and rapid plant growth in a controlled greenhouse conditions. In identifying and transferring processes of the gene regions, molecular DNA markers (SSR, STS and GAG) were employed with A-PAGE and SDS-PAGE methods. A-PAGE was used for selection of y-gliadin 45, SDS-PAGE for selection of LMW-2 glutenins, and four SSR primers (Xgwm550, Xgwm608, Stm553actc, Stm542acag), one STS primer and one PCR primer (GAG5-6) linked to Gli-B1 and Glu-B3 loci for selection of y-gliadin 45 and LMW-2 glutenins either all together or alternatively. As a result, the study was completed in three years instead of six years required in a classical backcross breeding study, meaning about 50 % time saving, and obtained a high quality candidate variety.

Key words: Durum Wheat, Triticum Durum, y-gliadin 45, LMW-2 glutenin, Backcross Breeding, SSR, A-PAGE, SDS-PAGE.

INTRODUCTION

World production of durum wheat in 2009 was 38,5 million tonnes (t), approximately 6% of total wheat production. The top six durum wheat producing countries are Canada (5.5 million t), Italy (5.2 million t), Turkey (3.0 million t), Kazakhistan (2.6 million t), USA (2.3 million t) and France (2.1 million t) [2].

Turkey is the diversity origin of durum wheat like many other plants such as barley. Although Turkey has suitable ecology for producing high quality durum wheat, she has imported durum wheat in recent years. The most important reason is that the demand of pasta industry for high quality wheat is not supplied in sufficient quantities. Therefore, pasta quality of Turkish durum wheat varieties must be increased using the modern breeding methods without adversely affecting their yields. First of all, the existing varieties must be improved in terms of the quality genes.

A basic criterion that determines the quality of durum wheat is the degree of suitability for pasta production. There is a strong relationship between the quality of pasta and gliadin and glutenin proteins [16, 26, 36]. Moreover, there is an important correlation between specific gliadin and glutenin proteins with gluten strength and viscoelastic properties, respectively. The most important of these proteins are y-gliadin 45, localized in Gli-B1 locus, and LMW-2 glutenins, encoded by Glu-B3 locus linked very tightly to each other. The y-gliadin 45 is strongly correlated with high gluten strength and pasta quality, whereas the y-gliadin 42 is associated with weak gluten and poor pasta quality [20]. Similarly, LMW-2 glutenin subunits positively affect gluten strength and quality of durum wheat.

The pasta quality of durum wheat containing the g-gliadin 45 and LMW-2 glutenin proteins is usually higher than durum wheat containing the g-gliadin 42 and LMW-1 glutenin proteins, which have been confirmed by different researchers [7, 11, 14, 26, 28, 30, 31].

Transferring of Gli-B1 locus containing the y-gliadin 45 and Glu-B3 locus containing LMW-2 glutenin to a variety together in the same breeding program provides a great raise in pasta quality [9, 10, 15, 25, 32].

In wheat quality breeding, marker assisted selection (MAS) and backcross breeding method are used together. The use of molecular markers in backcross breeding provides time and economic advantages and accelerates the development of new varieties [39, 40]. With the help of MAS, the wheat breeding studies, which take many

years, were completed in a short time. For instance, resistance genes to important diseases such as yellow rust [38], leaf rust [24], and powdery mildew [22] were quickly transferred to new varieties.

Use of biochemical methods as well as molecular markers increases the reliability of breeding studies. It was reported that when protein and molecular markers were used together, the reliability and efficiency of study was increased [1].

In this study, two QTLs (y-gliadin 45 and LMW-2 glutenin) affecting end-use quality were transferred to a registered Turkish durum wheat cultivar, Selçuklu-97, in a backcross breeding study. In identifying and transferring of these gene regions, molecular (SSR, STS and GAG) markers were used in combination with A-PAGE and SDS-PAGE methods.

MATERIALS AND METHODS

Plant Materials

In this study, Selçuklu-97 durum wheat variety, which is widely grown in Turkey but does not carry the y-gliadin 45 and LMW-2 glutenin genes, was used as a recurrent parent. The donor parent was the high quality Canadian durum wheat cultivar Kyle. In each generation, F1 and BC plants were backcrossed four times with recurrent parent and progenies carrying the targeted QTLs were selected by MAS.

Lira-1, Lira-2, Marquis and Kyle are used in the determination of LMW-1, LMW-2 glutenin and y-42, y-45 gliadin bands.

DNA Isolation and PCRs

Seeds of F1 and backcross plants were cut into half, one of which was used for protein electrophoresis and the other including embryo was germinated and used for molecular screening. DNA was extracted from leaf material of each plant using genomic DNA purification kit (Fermentas Life Sciences Genomic DNA Purification Kit).

The obtained DNA was screened with in the presence of y-gliadin 45 encoded by the Gli-B1 locus and LMW-2 glutenin alleles encoded by the Glu-B3 locus. For this purpose, Stm553actc [21], Stm542acag, Xgwm 550, Xgwm 608 [34] microsatellite (SSR) markers, and a STS marker [8] and GAG5-6 [37] primers linked to Gli-B1 and Glu-B3 loci and mapped at end of the short arm of chromosome 1B were used.

PCR Amplification

Polymerase Chain Reactions (PCR) were performed under conditions shown in the source article for each primer with some modifications. PCR reactions were carried out in 40 µl volume. PCR mixes contained 50-100 ng of genomic DNA, 0.25 µM of each primer, 0.2 µM dNTP mix, 2.5 µM MgCl2, 10 X PCR Buffer and 0.5 units of Taq DNA polymerase. PCR was run on Thermo (Px2) thermocycler 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 50-60 oC (different annealing temperature of primers), extension for 1 min 72 oC, and concluded with a final extension step for 5 min at 72°C. PCR products were analyzed on 3% metaphore agarose gels or 1% agarose gels. Electrophoresis was applied at 90 watt constant power for 3-4 hours.

A-PAGE

The gliadins (y-gliadin 42 and 45) in the durum wheat cultivar were analyzed and identified by using Acidic Polyacrylamide Gel Electrophoresis (A-PAGE) that was described by Bushuk and Zillman [6] and modified by Khan et al. [23].

SDS-PAGE

Low molecular weight (LMW) glutenin subunits (LMW-1 / LMW-2) were prepared according to Singh et al. [33] and analyzed using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method of Masci et al. [27] and Gianibelli et al. [19].

Embryo Culture

Immature embryo culture was prepared according to Arzani and Mirodjagh [3]. BC seeds were increased by immature embryo culture.

RESULTS

Two QTLs (y-gliadin 45 and LMW-2 glutenin) affecting end-use quality were transferred to a registered Turkish durum wheat cultivar (Selçuklu-97) in a MAS supported backcross breeding program. F1 and BC plants were backcrossed four times with the recurrent parent and backcross plants were selected for targeted QTL regions by screening with molecular markers (Figure 1-A), A-PAGE (Figure 1-B) and (when necessary) SDS-PAGE (Figure 1-C) in each generation.

In each backcross generation, screening processes were carried out in combination. As a result of screening, heterozygous backcross lines having the target genes were determined and BC4F1 seeds were obtained by hybridization of these plants. BC4F2 seeds were obtained by selfing of BC4F1 plants. In BC4F2 plants, homozygous ones were identified by molecular (Figure 2) and A-PAGE screening and homozygous BC4F2 seeds were increased by selfing.

In the study, immature embryo cultures were employed for speeding up backcross processes and decreasing the time required for seed generation after hybridizations (Figure 3). New seedlings were obtained by taking immature embryos in culture only 20 days after hybridization, and thus, each generation could be produced approximately 30 to 40 days earlier. It is provided that, in a year, more than two generations were

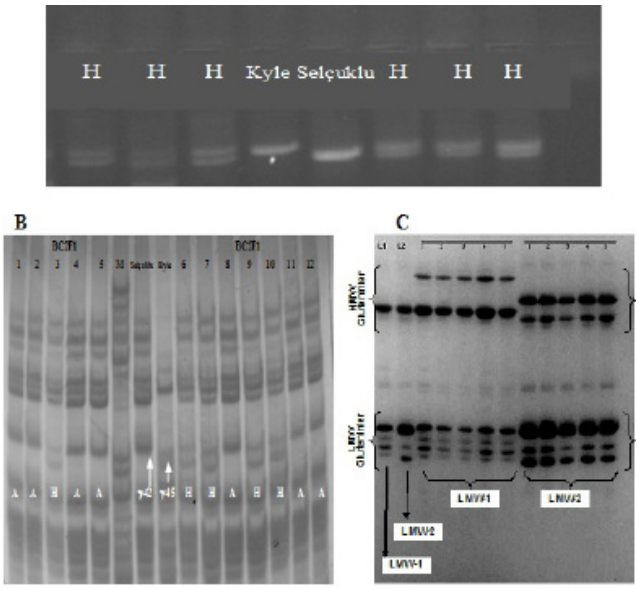

Figure 1. Examples of parents and BC2F1 plants screened with markers. A: screening with GAG 5-6 markers B: screening with A-PAGE C: screening with SDS-PAGE

(H: The heterozygous backcross plants which carry the target gene regions.)

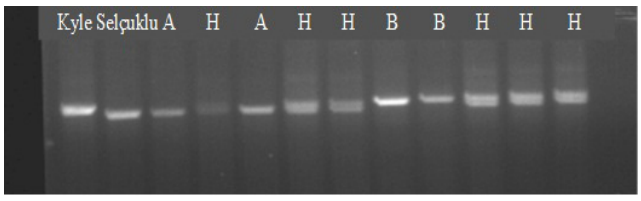

Figure 2. Sample results of parents and BC4F2 plants were screened with molecular marker.

(A: The backcross plants are homozygous for the recurrent parent alleles, B: The homozygous backcross plants which carry the target gene regions, H: The heterozygous backcross plants which carry the target gene regions)

taken with the help of immature embryo culture.

DISCUSSION

In this study, the important quality genes affecting the quality of pasta were transferred to Turkish durum wheat variety, Selçuklu-97. Thus, the present variety was improved for pasta quality. A-PAGE, SDS-PAGE methods and molecular markers were used in MAS applications either all together or alternatively.

Marker assisted selection can support plant breeding by saving time and labour in breeding programs. As a result, the study was completed in three years instead of six years commonly required in a classical backcross breeding study, meaning about 50 % time saving. In other words, a breeding work, which usually requires many years, were completed much more quickly by taking two or more generations in a year. Similarly, 350 marker assisted backcross programs, initiated by the International Triticeae Mapping Initiative, have been completed in shorter time than conventional breeding methods by taking average two generations in a year [13].

A plant breeder, making phenotypic selections, has to obtain approximately 16.7 times as much generation in comparison to a plant breeder using MAS method [17]. As a result of another study, used phenotypic and molecular selections together, Davies et al. [12] has determined that selection with molecular markers in backcross breeding program is more advantageous and the result was reached a much shorter time. Ohm et al. [29] transferred specific traits into a selected recurrent parent soft winter wheat lines with marker assisted backcrossing. They transferred new useful traits into the wheat advanced lines by two backcross cycles per year and MAS.

Both breeding time is reduced and efficiency of backcross breeding is increased with the help of MAS method. Babu et al. [4] reported that percentage of recurrent parent genome in BC2F1 generation is 83.7-

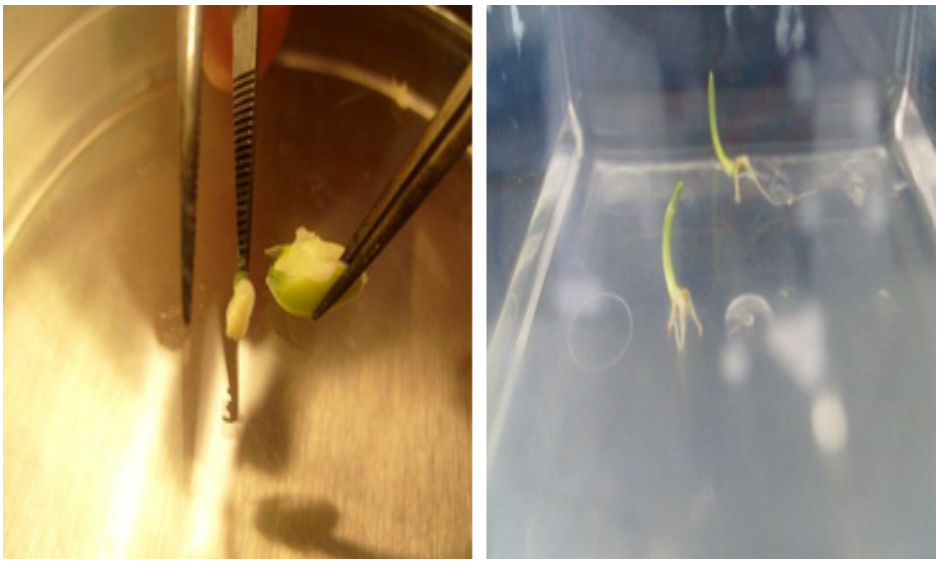

Figure 3. Application of immature embryo culture

94.8 percent using MAS method.

The major problem encountered in MAS, is the lack of close linkage between markers and the gene of interest. Therefore, identification of tightly linked (preferable <2 cM) and/or flanking co-dominant markers (<5 cM on either side of gene locus) is necessary [5]. The GAG5-6 primers, used in our study, were developed directly from the γ-gliadin 45 gene. They are special markers for the gene [37]. Additionally, the Stm553actc marker is in the most end region of the 1BS chromosome and only 5 cM away from the QTL region [21]. The other SSR markers [34] used in this study was selected to flank the QTL region. Molecular selection with flanking markers in the desired region is much more reliable. Also linkage disequilibrium was interrupted and the only transfer of a desired particular region could be increased. The rate of error is theoretically 5% when using a single marker that is 5 cM away from the desired gene or locus, but the rate of this error is decreased to 0.04% (0.08 x 0.05) by using together the flanking two markers which are 5 and 8 cM away from gene. Biradar et al. [5] indicated that flanking SSR markers for Gm1, a major gene for resistance to rice gall midge, helped accurately predict the presence of Gm1 with an error of less than 1%.

In their study, Frisch et al. [18] determined the optimum distances between the usable markers and the interested gene(s) in a MAS supported backcross breeding with a minimum proportion of donor genome. Their approach can increase the efficiency of marker-assisted backcrossing by reducing the required number of individuals and marker data points.

When different marker types, such as protein and molecular markers, were used in MAS, it was determined that the reliability and efficiency of the study increased[1].

Results of our study are shown that marker assisted selection and backcross breeding were successfully employed with combination. Similarly, Stuber [35] successfully used combined marker assisted selection with corn backcross breeding in transfer of alleles providing strength against drought.

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