

Use of PCR amplification products of glutenin and gliadin as markers assisted selection for durum wheat improvement

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Abstract

To establish the linkage between biotechnology tools and breeding programs, PCR molecular markers were used as markers assisted selection in durum wheat at ICARDA to select the genotype with good (strong gluten) or bad (weak gluten) pasta quality instead protein markers. PCR specific primers for glutenin Low Molecular Weight (LMW) type 2 and 1 and for gamma-gliadin type 45 and 42, were used as markers assisted selection. A collection of 1580 durum wheat genotypes, were analysed. About 85 to 89,9% of the genotypes analysed possessed gamma-gliadin 45 and glutenin LMW type 2, respectively 10 to 8,7% of the genotype had gamma-gliadin 42 and glutenin LMW type 1 and 2,4 to 2% of the genotypes are heterozygous. Analysis of 92 lines from F6 durum wheat populations inbred lines Jenah Khattifa/ChamI show 47,82% of lines have glutenin LMW 2, 47,82% with LMW1 and 4,34% are heterozygous.

Key words : durum wheat, quality, marker-assisted selection, gliadin, glutenin, PCR

Résumé : Utilisation des produits d'amplification par PCR des glutenines et des gliadines comme marqueurs de sélection pour assister l'amélioration génétique du blé dur

Pour assister la sélection des variétés de blé dur pour leurs qualités technologiques, une collection de 1580 génotypes sélectionnés à l'ICARDA, a été testée par l'utilisation d'amorces spécifiques aux gliadines type 45 et type 42 et aux glutenines de faible poids moléculaire (LMW) type 2 et type 1. Les résultats obtenus ont montré que 85 à 89,9% des génotypes analysés, pos-

Material and methods

Plant materials

Durum advanced yield trial genotypes (ADYT) and durum ICARDA core collection were planted during 4 seasons (1994-95-96-97). A collection of 1388 genotypes from advanced yield trials ADYT (380 genotypes in 1994, 240 genotypes in 1995, and 264 genotypes in 1996 and 504 genotypes in 1997), and 192 genotypes from ICARDA core collection in 97 (DCC 97) were used. 92 lines from durum wheat populations F6 have been also tested.

DNA extraction

Seeds from different genotypes of ADYT and the core collection were planted in the field with two genotypes (ChamI and Jenah Khatifa) used as control. Leaves were collected, frozen in liquid nitrogen and dried in a freeze drier for at least three days. CTAB technique was used for DNA extraction (Saghai Marrof et al., 1984). Dry material was grounded by a mixer or with liquid nitrogen using pestle and mortar. About 50 mg from this material were mixed with DNA-CTAB buffer extraction (100mM Tris-HCl pH 7, 150 mM NaCl, 10 mM EDTA, 1.5% of CTAB and 1% of b-MercaptoEthanol) in 2 ml eppendorff tubes. After 1 h at 65°C under agitation, the tubes were incubated in ice for 5 minutes. One volume of chloroform/isoamyl alcohol (24 : 1) was added and mixed by agitation for 20 minutes. After centrifugation for 30 minutes at 12000g, the aqueous phase was transferred to new tubes. DNA was precipitated with 0.3-M sodium acetate and 2 volumes of Ethanol, then was washed by Ethanol 75%, dried and resuspended in 50 µl of distilled water or TE (10mM Tris-HCl PH7 & EDTA 1mM PH8).

PCR chain reaction

PCR analysis was carried out using specific primers for the complex coding regions of glutenin LMW type 1 and 2 and for gamma-gliadin 45 and 42. These PCR primers were developed in the University of Tuscia, Viterbo.

Screening for glutenin was done using three set of primers selected at different positions of coding regions of genome DNA sequences of glutenin LMW. For gliadin, the primer used represent the first 20 nucleotides of the 5' transcribed region and 19 nucleotides of the complementary strand in the 3' transcribed region between the position 986 and 1004 of a gamma-gliadin gene isolated from *Triticum aestivum* (D'Ovidio R., et al., 1991).

PCR amplification was performed on PCR thermocycler Perkin Elmer 9600 using conditions reported by D'Ovidio et al. (1990) with minor modification. PCR analyses were performed in a final reaction volume of 50µl by using 50-200ng of genomic DNA, 1 unit of Taq DNA polymerase (Boehringer), 1xTaq PCR buffer (Boehringer), 250µM of each deoxyribonucleotide, 250ng of each primer. Amplification conditions for glutenins LMW were for 30

cycles at 94°C for 1 min, 60°C for 2 min and 72°C for 2 min. A final step at 72°C for 7 min was also used. Amplification conditions for gliadins were performed in the same conditions only annealing temperature were 55°C instead of 60°C. Oligonucleotides used as primers were synthesised on the basis of the glutenin LMW genes or gliadin genes previously published (D'Ovidio R. et al., 1992 ; 1993 ; 1996 ; 1997). The amplification PCR products were fractionated on 1.5% agarose gel in 1XTAE buffer following standard procedures (Sambrook et al. 1989).

Nucleic sequences of the primers set 1 used in PCR amplification of LMW-glutenin

5' ATg AAg ACC TTC CTC gTC TT 3'

5' CAA CgC CgA ATg gCA CAC TA 3'

Nucleic sequences of the primers set 2 used in PCR amplification of LMW-glutenin

5' CgT TgC ggC gAC AAg TgC AA 3'

5' gTA ggC ACC AAC TCC ggT gC 3'

Nucleic sequences of the primers set 3 used in PCR amplification of LMW-glutenin

5' -CgT TgC ggC gAC AAg TgC AA- 3'

5' -Cgg ATT gCA TCA TA gCgg-3'

Nucleic sequences of the primers set used in PCR amplification of Gamma-gliadin 45 and 42

5' ATg AAg ACC TTA CTC ATC CT 3'

5' ACA TAC ACg TTg CAC ATg g

Results

PCR amplification of glutenins low molecular weight (LMW) type 2 and 1

A number of 1388 genotypes of durum wheat advanced yield trials ADYT94-95-96-97 and 192 genotypes of durum wheat core collection DCC and 92 lines from F6 Jenah Khatifa/ChamI durum wheat population were analysed by PCR technique. ADYT 94 were screened for glutenin low molecular weight using primer set 1 (Pagnotta et al., 1993), ADYT 95 and 96 were screened using primer set 2 (D'Ovidio R. et al., 1993). ADYT 97 and DCC97 and F6 Jenah Khatifa/ChamI durum population were screened using primer set 3 (D'Ovidio R. et al., 1996). The PCR amplification using primer set 1 shows three bands with molecular weights ranging between 900 and 1160 bp (fig. 1). The two lower bands were similar in all genotypes. The hi-

ghest band varied between genotypes, its molecular weight was 1110bp or 1160bp. The band with 1160bp was specific for genotypes possessing glutenin LMW type 2, and the band with 1110bp was specific for genotypes possessing glutenin LMW type 1.

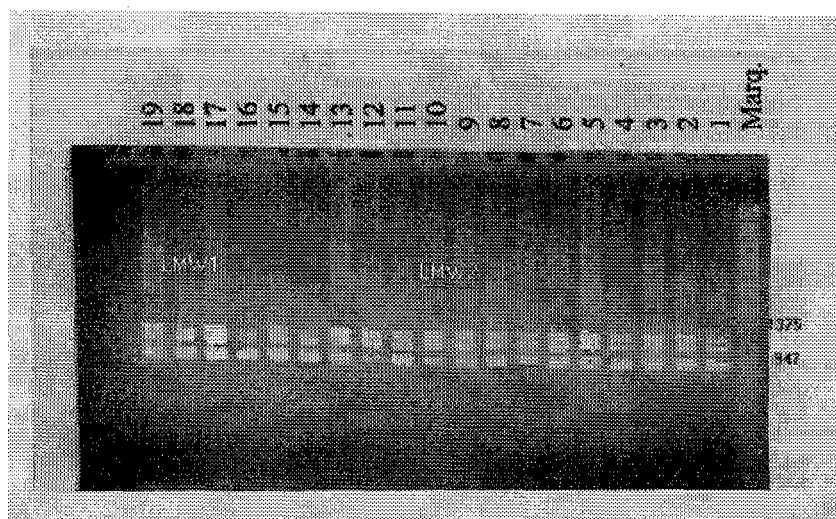


Figure 1. PCR amplification of LMW glutenin type 2 and 1 using primer Set 1 (Pagnotta et al., 1993). Line designed by: Marq correspond to molecular weight marker III (Roche) ; Lines with number 1 to 19 correspond to ADYT 94 Durum collection ; lines with number 1 to 15 and 19 are genotypes with LMW glutenin type 2 (LMW2) ; lines with number 16 and 18 are genotypes with LMW glutenin type 1 (LMW1).

By using glutenin primer set 2, PCR amplification produced only two bands. The lowest one had 1010bp and was similar in all genotypes, while the highest one had either 1160bp and was characteristic for genotypes possessing glutenin LMW type 2 or 1110bp and was characteristic for genotypes with glutenins LMW type 1 (fig. 2). For these two sets of primers the only difference was the generation of a small fragment (900bp) obtained by using primer set 1 but not by primer set 2.

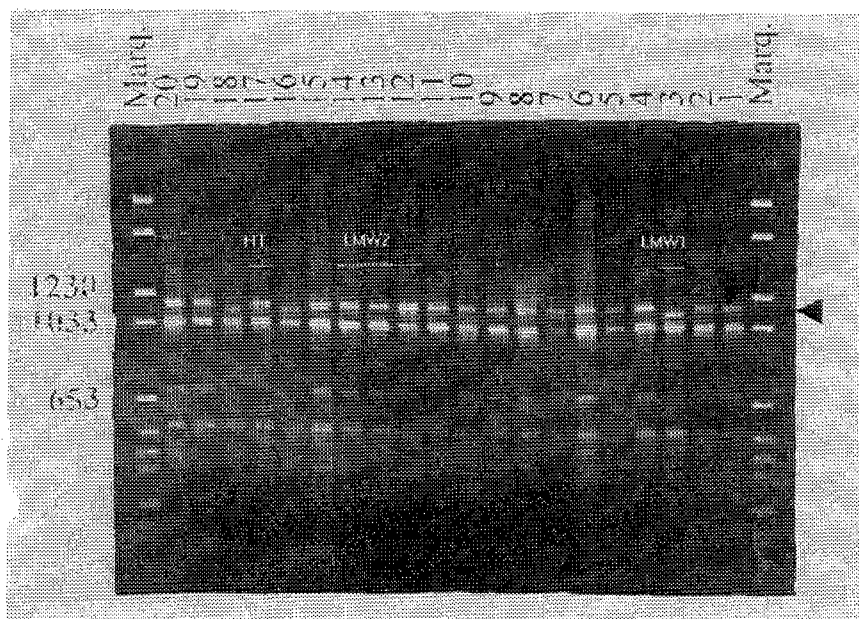


Figure 2. PCR amplification of LMW glutenin type 2 and 1 using primer Set 2 (D'Ovidio et al., 1993). Line Marq is molecular weight marker VI (Roche); Lines with number 1 to 20 are ADYT 95 and ADYT 96 Durum collection; lines numbered 2, 16 and 18 are genotypes with LMW glutenin type 1 (LMW1); line numbered 17 is heterozygote genotype with LMW glutenin 1 and 2 (HT); others lines are genotypes with LMW glutenin type 2 (LMW2).

The PCR amplification using primer set 3, showed only one specific band for glutenin LMW type 2 (band with 840bp) or glutenin LMW glutenin type 1 (band with 790bp) (fig. 3A, 3B). The molecular weight of the specific band characteristic of genotypes possessing glutenin LMW type 2 obtained by using the three primers set 1, 2 and 3 was larger by 50bp than the band obtained in genotypes with glutenin LMW type 1. In the heterozygous genotypes, the bands corresponding to glutenin LMW type 1 and 2 were detected using the three sets of primers.

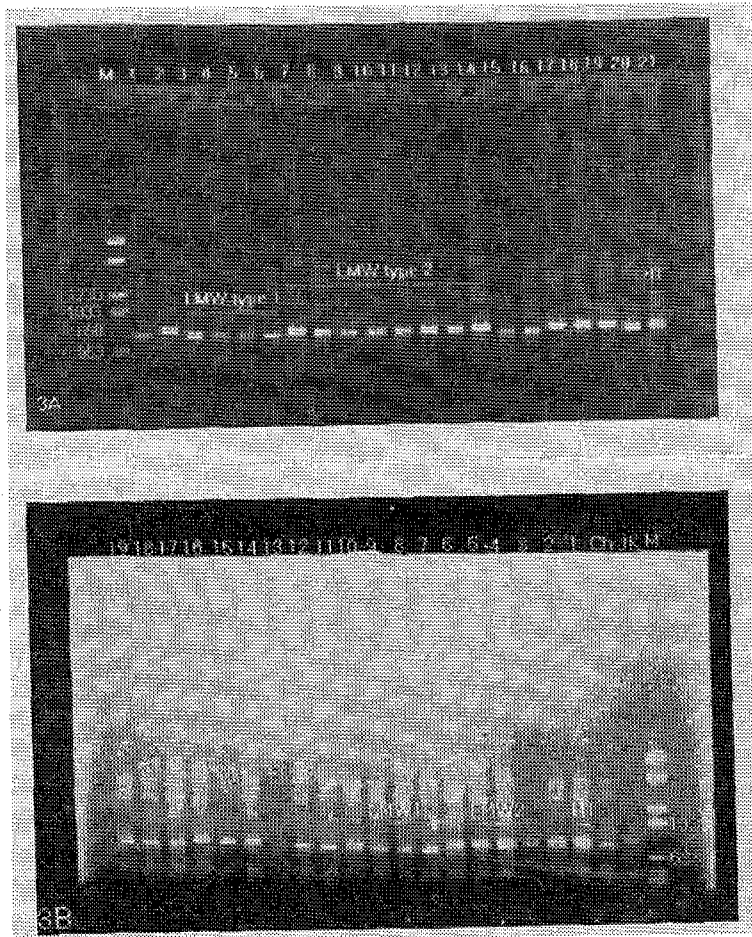


Figure 3. PCR amplification of LMW glutenin type 2 and 1 using primer Set 3 (D'Ovidio et al., 1996). (A): Fig (A) are ADYT 95 and ADYT 96 Durum collection. Line M is molecular weight marker VI (Roche) ; Line numbered 1 and 2 is respectively durum wheat varieties ChamI and Jenah Khatifa -used as control ; lines numbered 3 to 21 are ADYT95 and ADYT 96 Durum collection ; varieties ChamI (lines- numbered 1) has glutenin LMW type 1 ; varieties Jenah Khatifa (lines- numbered 2) has glutenin LMW type 2 ; lines numbered 15, 16 and 18 are genotypes with LMW-glutenin type 1 (LMW1) ; line numbered 17 is heterozygote genotype with LMW glutenin 1 and 2 (HT) ; others lines are genotypes with LMW glutenin type 2 (LMW2). Fig (B) is durum wheat Jenah Khatifa (JK)/ChamI (Ch) inbred lines population. Line M is molecular weight marker VI ; JK is variety Jenah Khatifa ; Ch is variety ChamI ; lines numbered 1 to 19 are inbred lines F6.

PCR amplification of gamma gliadin type 45 and 42

Durum advanced yield trials ADYT94 (380 genotypes) and ADYT95 (240 genotypes) were analysed using gliadin primer (D'Ovidio R. et al., 1992). The PCR product obtained using DNA genomic has been assigned to a specific locus Gli-B1 (D'Ovidio R. et al., 1991). PCR amplification of the complete coding regions of the genes at Gli-B1 locus of durum genotypes regenerated four fragments with different sizes ranging from 750bp to 1050bp (fig. 4). The two fragments with greater mobility (lower bands at 740 and 790bp) and the fragment with lower mobility (highest band at 1055bp) were similar in all genotypes. The fragments within medium mobility at 950bp or at 900bp were characterised in durum genotypes. The fragment at 950bp was specific to the genotype possessing gamma gliadin 45, whereas the fragment at 900bp was specific to the genotype possessing gamma-gliadin 42. In the heterozygous genotypes, the two fragments at 950bp and 900bp present in genotypes with gamma gliadin 45 and in genotypes with gamma gliadin 42 were detected (fig. 4).

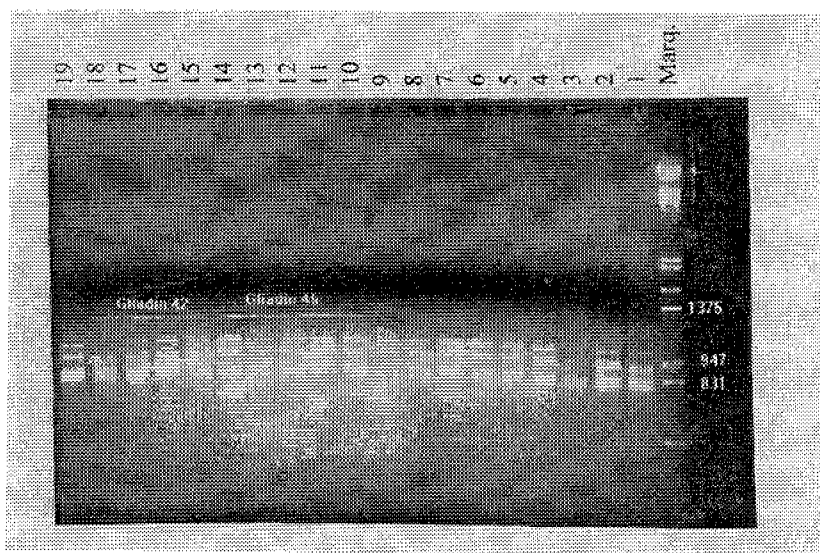


Figure 4. PCR amplification of gamma-gliadin type 45 and 42 using gamma-gliadin primer set 4 (D'Ovidio et al., 1994) ; Marq is molecular weight marker III (Roche) ; line numbered 1 and 2 are durum wheat varieties ChamI and Jenah Khatifa used as control ; lines numbered 3 to 19 are ADYT 94 and ADYT95 genotypes durum collection ; varieties ChamI has gamma gliadin 42 and varieties Jenah Khatifa has gamma gliadin 45 ; lines numbered 15, 16, 17 and 18 are genotypes with gamma-gliadin type 42 ; others lines are genotypes with gamma-gliadin type 45.

Durum genotypes screening

ICARDA durum collection ADYT94 (380 genotypes) and ADYT 95 (240 genotypes) were analysed using glutenin primers set 1 and 2 and gliadin primers set. Durum ADYT96 (264 genotypes) was analysed using glutenin primers set 2, ADYT97 (504 genotypes) and DCC97 (192 genotypes) were analysed by glutenin primers set 3.

In the case of glutenin, using glutenins primers set 1, 2, similar results were obtained in the analysis of the durum collection during four seasons. About 89.9% of the genotypes analysed showed the pattern for glutenin LMW type 2 (genotypes with good pasta quality), 8.7% of the genotypes had glutenins LMW type 1 (genotypes with bad pasta quality) and 2.4% of the genotypes were heterozygous (fig. 5).

PCR primer set 2 and 3 were used to evaluate durum ADYT 96 (264 lines) ADYT 97 (504) and durum ICARDA core collection, DCC (192 lines). The screening shows that more than 85% of the genotypes have glutenin low molecular weight 2, about 10% has glutenin low molecular weight 1 and about 2% of genotypes are heterozygous (fig 5).

The analysis of 92 lines from F6 Jenah Khatifa/ChamI durum population, using primer set 3 show that 47,82% (44 lines) of lines have glutenin LMW type 2, 47,82% (44 lines) of lines have glutenin LMW type 1 and 4,34% (4 lines) were heterozygous (fig. 5).

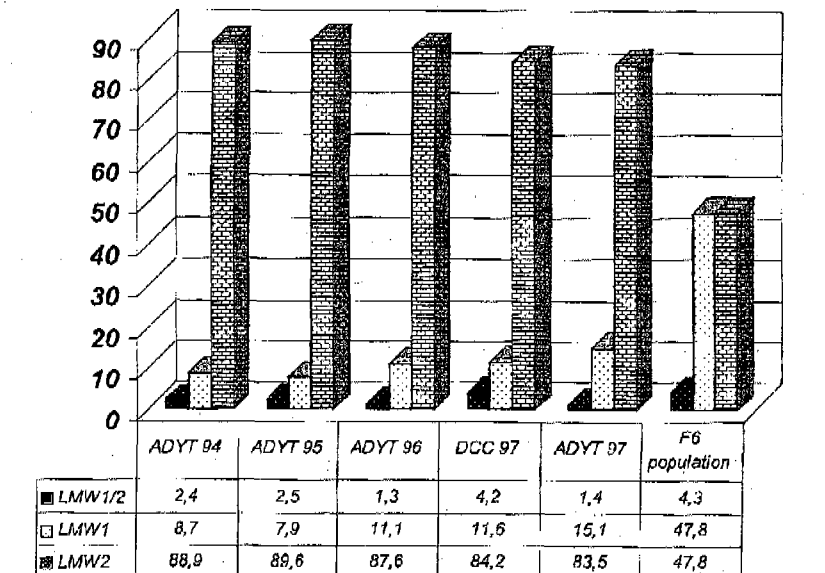


Figure 5. Evaluation of glutenin LMW type 1 and 2 in advanced yield trials (ADYT) for the 1994, 1995, 1996 and 1997 seasons and F6 Jenah Khatifa/Cham I durum population.

In case of gliadin similar results as glutenins were obtained in the analysis of the collection of durum ADYT 94 and ADYT95 with gliadin primers set 4. More than 88.9% of the same genotypes with glutenin LMW type 2 have gliadin 45 ; 7,9% of the genotypes with glutenin LMW type 1 have gliadin 42 and about 2.4% were heterozygous (fig. 6).

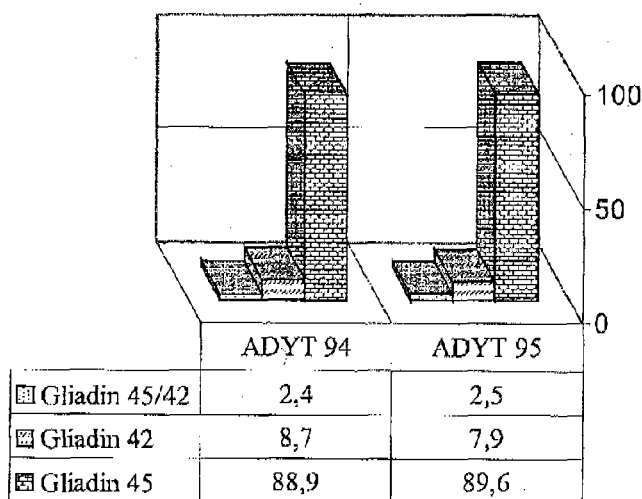


Figure 6. Evaluation of gamma gliadin type 45 and type 42 in durum advanced yield trials (ADYT) for the 1994 and 1995 seasons.

Discussion

DNA molecular markers were used to screen 1580 durum wheat genotypes cultivated in the WANA (West Asia and North Africa) region, for their performance in pasta making and to assist durum-breeding programs. The screening of the different genotypes, using the specific primers for glutenins and gliadins, showed about 85 to 89% of ADYT possessed glutenin LMW type 2 and gliadin 45. This high value is the result of intensive selections conducted for many years. Almost all the new released varieties are carrying glutenin LMW type 2 which is important component to meet the demand of the expanding pasta industry.

This screening of durum ADYT 94 -95 for glutenins and gliadins has so far not identified true recombinant genotypes. The absence of recombinant genotypes within the genotypes analysed by glutenin and gliadin primers could be explained by the high association between glu-

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