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Identification of durum wheat cultivars with good and poor quality by PCR-based markers

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SUMMARY - Polymerase chain reaction was used to amplify γ-gliadin and low molecular weight glutenin (LMW) sequences from genomic DNA of durum wheat genotypes. Amplification reactions, carried out by using several oligonucleotide primers, produced specific amplification products. PCR analyses carried out using a pair of primers specific for γ-gliadin and a different pair specific for LMW glutenin sequences gave amplification patterns characteristic of durum wheat cultivars with good and poor technological properties. The usefulness of PCR-based markers for selecting durum wheat genotypes with desirable traits are discussed.

Key words: *Triticum durum*, PCR, wheat storage proteins, molecular markers, quality, γ-gliadin, LMW glutenins.

RESUME *- "Identification des cultivars de blé dur présentant une qualité bonne ou médiocre au moyen de marqueurs à PCR". La réaction PCR a été utilisée pour amplifier les séquances de λ-gliadine et de gluténine à faible poids moléculaire (LMW) provenant de l'ADN génomique des génotypes de blé dur. Les réaction d'amplification, réalisées en utilisant plusieurs amorces d'oligonucléotides, ont donné des produits spécifiques d'amplification. Les analyses PCR effectuées en utilisant deux amorces spécifiques à la λ-gliadine et deux autres différentes spécifiques aux séquences de gluténine LMW ont donné des modèles d'amplification caractéristiques des cultivars de blé dur présentant des propriétés technologiques bonnes ou médiocres. Dans cet article est discutée l'utilité des marqueurs à PCR pour la sélection de génotypes de blé dur ayant des caractères souhaitables.*

Mots-clés : *Triticum durum, PCR, protéines de réserve du blé, marqueurs moléculaires, qualité, λ-gliadine, gluténines à faible poids moléculaire.*

Introduction

The presence of specific components of gliadins and glutenins, the most abundant storage proteins of wheat endosperm, has been associated with dough technological properties. In durum wheat a highly significant correlation has been detected between specific durum wheat γ-gliadin components and gluten strength: cultivars containing γ-45 gliadin component possess superior qualitative characteristics as compared with those containing its allelic variant γ-42 (Damidaux *et al.*, 1978). The genes coding for these proteins are located at the *Gli-B1* locus and are linked to LMW glutenin genes at the *Glu-B3* locus, and to ω-gliadin genes. In particular, the gene encoding γ-45 gliadin is linked to genes coding for LMW-2 glutenin subunits, and to a gene coding for ω-35 gliadin, whereas the gene encoding γ-42 gliadin is linked to genes coding for LMW-1 glutenin subunits, and to ω-gliadin genes encoding components designated 33, 35 and 38. On the basis of these observations, Payne *et al.* (1984) hypothesized that the LMW-2 and LMW-1 glutenin subunits are responsible for qualitative differences in durum wheat, and that γ-gliadins 45 and 42 are only genetic markers. Recent studies carried out using the Italian cultivar Berillo, which presents a genetic recombination within the *Gli-B1* locus (Margiotta *et al.*, 1987), demonstrated that allelic variation among LMW glutenin subunits is responsible for differences in gluten strength of durum wheat cultivars (Pogna *et al.*, 1990).

Distinction between durum wheat cultivars possessing γ-gliadin 42 or 45, and LMW-1 or LMW-2 glutenin subunits is currently done by analyzing the protein pattern on polyacrylamide gels, a fast and

reliable method that, however, requires the use of toxic compounds, such as acrylamide and reducing agents. In order to overcome these problems, the possibility of developing a polymerase chain reaction (PCR) method for identifying γ-gliadin and LMW glutenin genes present in a genotype was investigated (Saiki *et al.*, 1985, 1988).

In the present paper it is reported the possibility of distinguishing between durum wheat cultivars with good or poor technological properties by analyzing the PCR amplification products of γ-gliadin and LMW glutenin gene sequences.

Material and methods

Genomic DNA was extracted from fresh material of single plant from several durum wheat genotypes as previously reported (D'Ovidio *et al.*, 1992a). Total DNA was extracted with rapid procedures either from part of the endosperm of a single dry seed (Benito *et al.*, 1993), from roots or from young hypocotyl of a single germinating seed (Edwards *et al.*, 1991). PCR analyses of γ-gliadin and LMW glutenin sequences were carried out following the conditions reported by D'Ovidio *et al.* (1990) and D'Ovidio (1993), respectively.

Results and discussion

Protein electrophoretic patterns of cultivars possessing γ-42 or γ-45, and LMW-1 or LMW-2 glutenin subunits are shown in Figs 1 and 2.

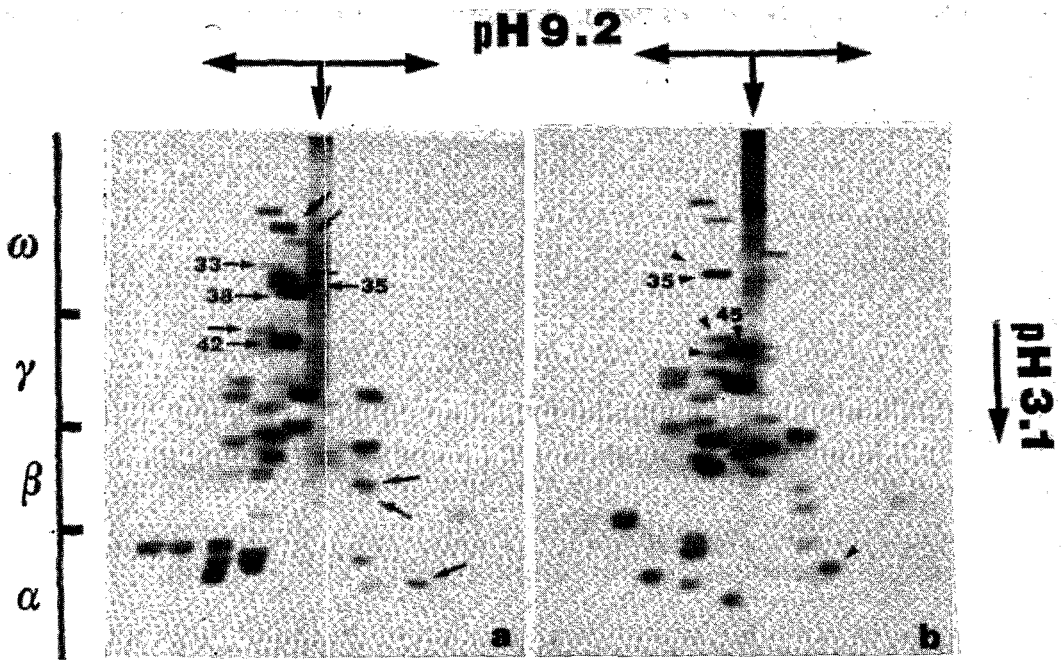

Fig. 1. Two-dimensional analysis of gliadin components of durum wheat cultivars types γ-42 (a) and γ-45 (b).

On the basis of the nucleotide sequence of a γ-gliadin gene from *Triticum aestivum* (Scheets and Hedgcoth, 1988), it was possible to design a series of primers specific for this class of genes. Interesting results were obtained with a pair of primers spanning almost the entire coding region of the γ-gliadin gene (Fig. 3). Electrophoretic analysis of the PCR reactions carried out on genomic DNA of several durum wheat cultivars showed, in fact, the presence of a band which allowed the identification of the durum wheat cultivars belonging to types γ-42 or γ-45 (Fig. 4). In particular, the pattern of

amplification products is represented by two bands of 750 bp and 850 bp, which have the same size in both groups, and a third band, which is 950 bp in cultivars type γ-45 and 900 bp in cultivars type γ-42.

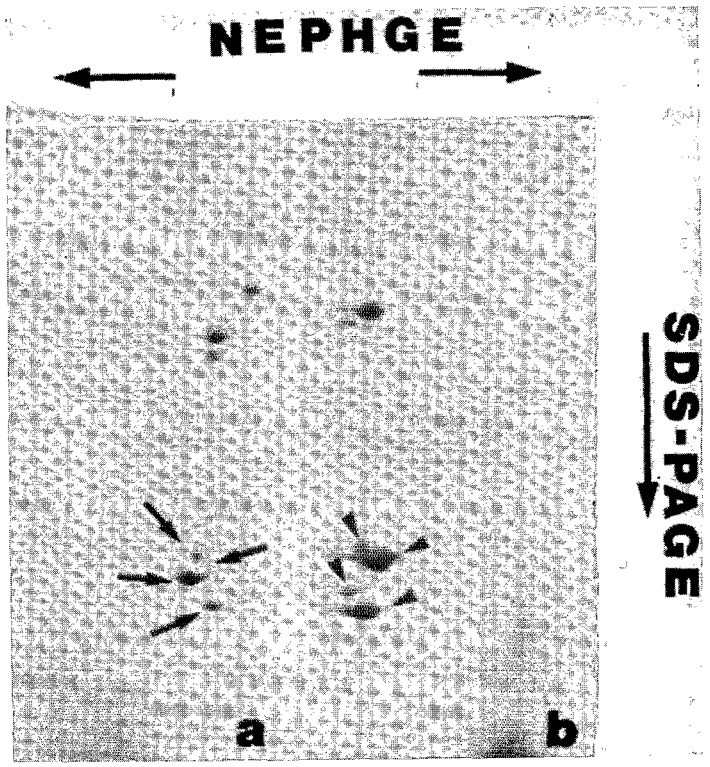

Fig. 2. Two-dimensional analysis of LMW glutenin subunits of durum wheat cultivars possessing LMW-1 (a) or LMW-2 (b).

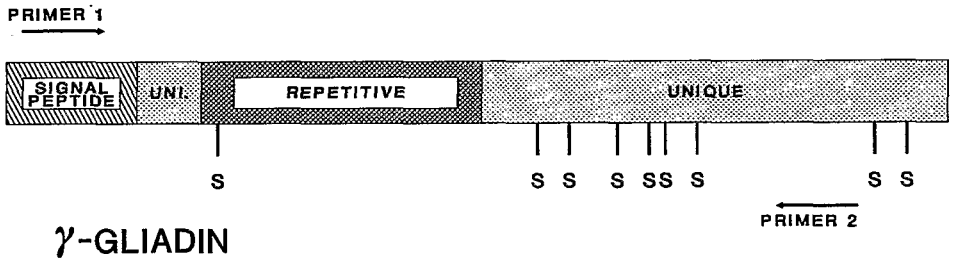

Fig. 3. Diagram of a γ-gliadin gene. Arrows indicate the position of primers used for PCR analysis. S indicate the position of codons coding for cysteine residues.

Nevertheless, since the use of γ-45 and γ-42 as molecular markers are not effective in genotypes which either do not express such polypeptides, like in the genotype MG 41392 (D'Ovidio *et al.*, 1992b), or have a recombination within the *Gli B1* locus, such as the cultivar Berillo (Margiotta *et al.*, 1987), the possibility of developing PCR markers specific for LMW glutenin genes, which are directly responsible for qualitative characteristics of durum wheat, was investigated.

On the basis of nucleotide sequences of LMW glutenin genes (Colot *et al.*, 1987; Cassidy and Dvorak, 1991; D'Ovidio *et al.*, 1992c) we constructed several primers and a pair of them could distinguish between durum wheat cultivars possessing LMW-1 or LMW-2 glutenin subunits (Fig. 5).

Electrophoretic analysis of PCR products showed, in fact, an amplification pattern represented by two bands with molecular sizes between 1.0 and 1.2 Kb. The band with higher mobility has a uniform size in all genotypes, whereas the band of lower mobility was about 50 bp larger in genotypes possessing LMW-2 glutenin subunits (Fig. 6, lanes No. 1, 2 and 6) than in genotypes possessing LMW-1 subunits.

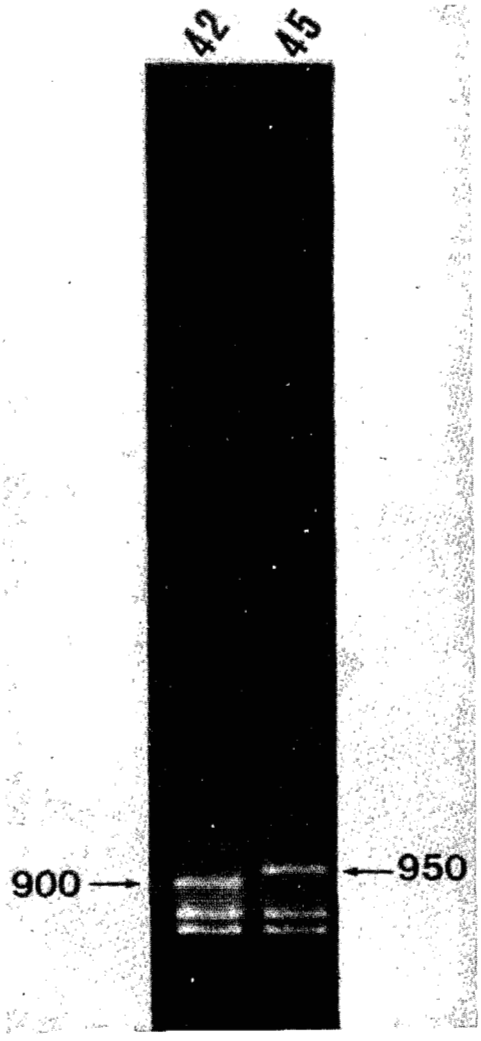

Fig. 4. PCR products of γ-gliadin sequences fractionated on 1.2% agarose gel. 1) Durum wheat cultivar type γ-42; 2) Durum wheat cultivar type γ-45. Arrows indicate the PCR band which identify cultivars type γ-42 and type γ-45.

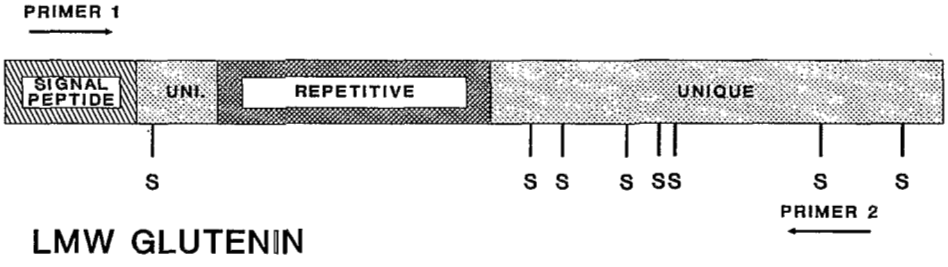

Fig. 5. Diagram of a LMW glutenin gene. Arrows indicate the position of primers used for PCR analysis. S indicate the position of codons coding for cysteine residues.

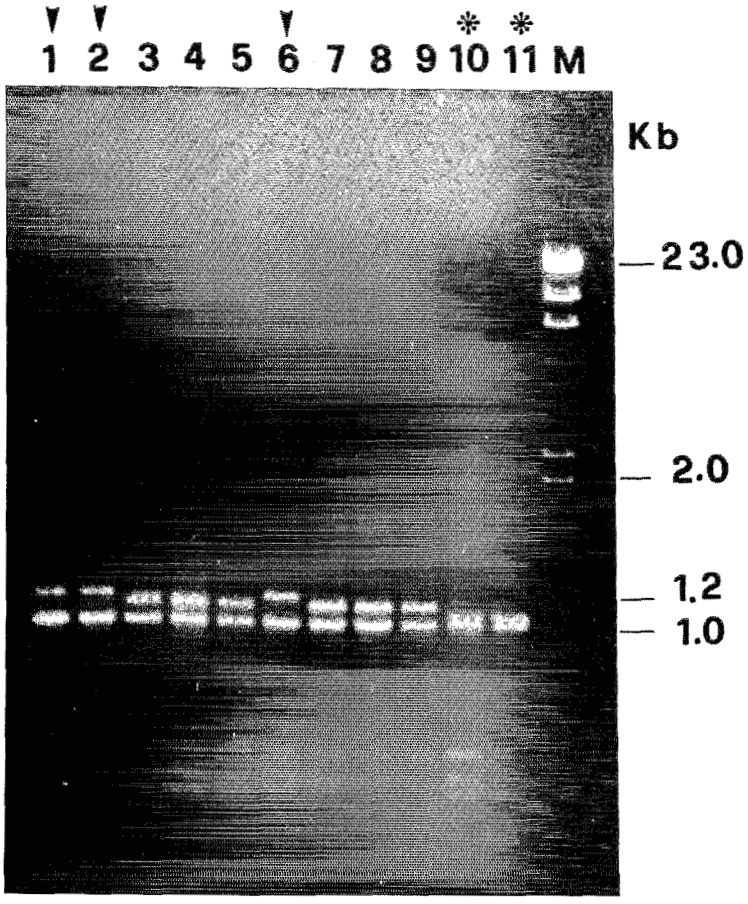

Fig. 6. PCR products of LMW glutenin sequences fractionated on 1.2% agarose gel. 1) Valforte; 2) Valnova; 3) Tito; 4) Quadruro; 5) Castel del Monte; 6) Creso; 7) Aldura; 8) Augusto; 9) Produra; 10) Doro; 11) Drago; M) Molecular weight marker. Arrow-heads indicate cultivars possessing LMW-2 glutenin subunits. Asterisks indicate cultivars which do not possess either LMW-1 or LMW-2 glutenin subunits.

The efficiency of this approach was further verified in genotypes lacking either LMW-1 or LMW-2 glutenin subunits and by using total DNA extracted with rapid procedures. The pattern of the amplification products from genotypes lacking either LMW-1 or LMW-2 was different from those obtained from cultivars possessing LMW-1 and LMW-2 glutenin subunits (Fig. 6, lanes no. 10 and 11). Similar results to those reported from genomic DNA were obtained when the PCR analyses were carried out on total DNA extracted with rapid procedures from small amounts (10 mg) of either leaf, root or endosperm tissues. Finally, the amplification band characteristic of durum wheat cultivars possessing LMW-1 glutenin subunits was located on chromosome 1B (D'Ovidio, 1993) using the substitution lines of the durum wheat cultivar Langdon (Joppa and Williams, 1988).

Conclusions

The simplicity, rapidity and the avoidance of toxic substances, such as acrylamide and reducing agents, makes the proposed PCR-based approach a valid alternative to protein electrophoretic techniques for selecting genotypes possessing either γ-gliadin 42 or 45 and LMW-1 or LMW-2 glutenin subunits. The possibility of performing the analysis on small amount of material makes the approach non destructive. The selected plant or the remaining embryo of the selected seeds can be grown and subsequently evaluated for other characteristics. In addition, the possibility to automatize the PCR analysis by using a computer-robot, which can extract DNA, prepare the PCR reactions and analyze the results, makes the PCR-based method the approach of choice for effective selection in breeding programs.

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