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Cultivar identification in *T. aestivum* using highly polymorphic RFLP probes

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Abstract. Two probes, specific for HMW-glutenins and γ-gliadins have been used to identify 50 common wheat Italian cultivars, most of which are closely related, and four common wheat cultivars originating outside Italy. The probes revealed complex polymorphic patterns; three probe/enzyme combinations had the necessary sensitivity for the identification of all 54 cultivars. As already shown for potato and barley, the use of four-cutter restriction enzymes and polyacrylamide gels proved particularly useful for detecting polymorphism.

Key words: *T. aestivum* – RFLP – Fingerprint

Introduction

Restriction fragment length polymorphisms (RFLPs) have been used for the purpose of varietal identification in potato (Görg et al. 1992), beets (Nagamine et al. 1989), maize (Smith and Smith 1991), and barley (Pecchioni et al. 1993). In the cases cited a sufficient amount of genetic variability was found by using cDNA or genomic random probes and DNA sequences from known genes. Dallas (1988) followed a different approach based on human minisatellite probes, highly polymorphic in man and in various animals, to detect polymorphism in rice. Beyermann et al. (1992) introduced the use of synthetic oligonucleotide probes such as $(GTG)_5$ and $(GATA)_4$, which reproduce the short tandem repeats of minisatellite families, to fingerprint various monocotyledonous and dicotyledonous species.

Clones equivalent to human minisatellites in their capacity to reveal a high degree of polymorphism have not yet been isolated from plant genomes. However, Liu et al. (1992) have discovered a moderately repeated, dispersed and highly variable genomic sequence of *T. aestivum* which allowed them to differentiate 56 common wheat cultivars. In common wheat the classification of species and cultivars has been mainly based on the electrophoresis of two particular classes of storage proteins: gliadins, which are monomeric, and glutenins, which are polymers of individual subunits associated by interchain disulphide bonds (Payne et al. 1982; Pogna et al. 1989; Metakovsky 1991; Shewry et al. 1992).

In the present paper we propose a simple method for varietal identification based on two probes, K9 and K32, specific for HMW-glutenins and γ-gliadins respectively. DNA restriction was based on the use of four-cutter restriction enzymes which allow the evaluation in wheat of the sensitivity of this method, already successfully used in potato (Gebhardt et al. 1989) and barley (Pecchioni et al. 1993). To assess the value of DNA probes revealing simple or complex Southern patterns, we also used two clones, 178 and 364, originating from a genomic library of wheat and compared the results obtained with those of probes K9 and K32.

Materials and methods

The following 54 common wheat cultivars were examined: Apulia, Rieti, Inallettabile 96, Frassineto, Mentana, Ardito, Impeto, S. Pastore, Mara, Produttore, Orso, Marzotto, Centauro, Mec, Manital, Loreto, Maestra, Oderzo, Irnerio, Costantino,

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Aquileia, Chiarano, Salmone, Etruria, Eridano, Farneto, Arquà, Bolero, Brasilia, Fiocco, Golia, Liocorno, Mirtos, Pandas, Pegaso, Santerno, Saul, Spada, Tiberio, Tullio, Nobel, Veronese, Gladio, N. Strampelli, Aurelio, Adria, Gemini, Leopardo, Saliente, Artù, MV 15, Thesee, FAP 74908, and Chinese Spring. The four cultivars (MV 15, Thesee, FAP 74809, and Chinese Spring) originating in countries outside Italy are not genetically related to the remaining 50, representing new and old cultivars bred in Italy. All the Italian cultivars except Fiocco and Tullio had as progenitor the local population Rieti. Moreover, at least one of the first 12 cultivars listed appears in the pedigrees of all others. The 54 cvs were classified into 20 groups according to their HMW-glutenin electrophoretic patterns and into 38 groups according to their γ-gliadin composition.

The K9 and K32 DNA probes were supplied by R. Thompson (Max Planck Institut, Köln). K9 is a cDNA clone hybridizing to all *Glu-1* DNA sequences, and K32 a cDNA clone recognizing most if not all γ-gliadin sequences at the *Gli-1* locus. WG 178 and WG364 are two wheat genomic clones provided by M. E. Sorrells (Cornell University, Ithaca).

DNA was extracted from 7-day-old seedlings according to a modified CTAB procedure (Murray and Thompson 1980), and digested with 4 units/μg DNA of the restriction enzymes *Alu*I, *Hae*III, *Rsa*I and *Taq*I for 5 h according to the supplier's instructions (Promega). DNA fragments were separated on denaturing 4% polyacrylamide gel and electrophoretically transferred to a nylon membrane (Hybond N, Amersham) according to Gebhardt et al. (1989).

DNA probes were labelled using α^{32}P-dCTP (Amersham) with the Prime-a-Gene kit (Promega). Prehybridization and hybridization of the membranes were as described by Gebhardt et al. (1989). Kodak X-OMAT AR films were exposed to the membranes, using an intensifying screen, at −70 °C for 3–15 days. Membranes were re-used up to ten times.

For each probe/enzyme combination, the hybrid fragments were identified, numbered, and their presence or absence recorded as 1 or 0. Data were analyzed using the NTSYS-pc package (Rohlf 1989).

Results and discussion

In Table 1, all single probe/enzyme combinations and some double or triple combinations are listed. The restriction patterns of probes 178 and 364 were simple, as expected, and showed low polymorphism: they classified the 54 varieties into 17 and 15 groups, respectively.

Probes specific for wheat storage proteins revealed complex polymorphic patterns (Fig. 1). None of the single probe/enzyme combinations tested was able to differentiate all the cultivars: the best result was obtained with the K9/*Alu*I combination which discriminated 38 cultivars out of 54. Using this combination, the doublets Salmone/Pandas, Brasilia/Mirtos, Rieti/Frassineto, S. Pastore/Aurelio, Costantino/ Aquileia, Eridano/Saul, Ardito/Gemini, Thesee/FAP, Veronese/Chinese Spring, Centauro/Chiarano, Tiberio/Artù, Oderzo/Irnerio, and the groups of three cultivars Mara/Produttore/Adria and Liocorno/Saliente/MV15, all had similar RFLP patterns. All doublets and triplets were resolved into unique component patterns by the combination K32/*Alu*I, except for Ardito/Gemini which was resolved by the K32/*Hae*III combination. The three cultivars Maestra, Fiocco and MV15, all carrying the wheat-rye translocation 1B/1R, were recognized by the K32 probe as a single group.

The best double combination was K9/*Alu*I + K32/*Alu*I, which was able to distinguish 53 groups out of 54. A complete fingerprinting of all 54 cvs was obtained with the combination K9/*Alu*I + K9/*Rsa*I + K32/*Alu*I.

Table 1. Number of different RFLP patterns obtained with several probe/enzyme combinations. The total number of genotypes considered was 54

Probe-enzyme combination	No. fragments	No. different RFLP patterns observed
Genomic probes with simple RFLP pattern		
178/*Hae*III	15	17
178/*Taq*I	5	6
364/*Alu*I	7	15
Genomic probes with complex RFLP pattern		
K9/*Alu*I	36	38
K9/*Hae*III	23	13
K9/*Rsa*I	37	35
K32/*Alu*I	11	25
K32/*Hae*III	15	22
K32/*Taq*I	31	24
K9/*Alu*I + K9/*Hae*III	59	44
K9/*Alu*I + K9/*Rsa*I	73	49
K32/*Alu*I + K32/*Hae*III	26	45
K32/*Alu*I + K32/*Taq*I	42	45
K9/*Alu*I + K32/*Alu*I	47	53
K9/*Alu*I + K9/*Hae*III + K9/*Rsa*I	96	49
K32/*Alu*I + K32/*Hae*III + K32/*Taq*I	57	49
K9/*Alu*I + K9/*Rsa*I + K32/*Alu*I	84	54

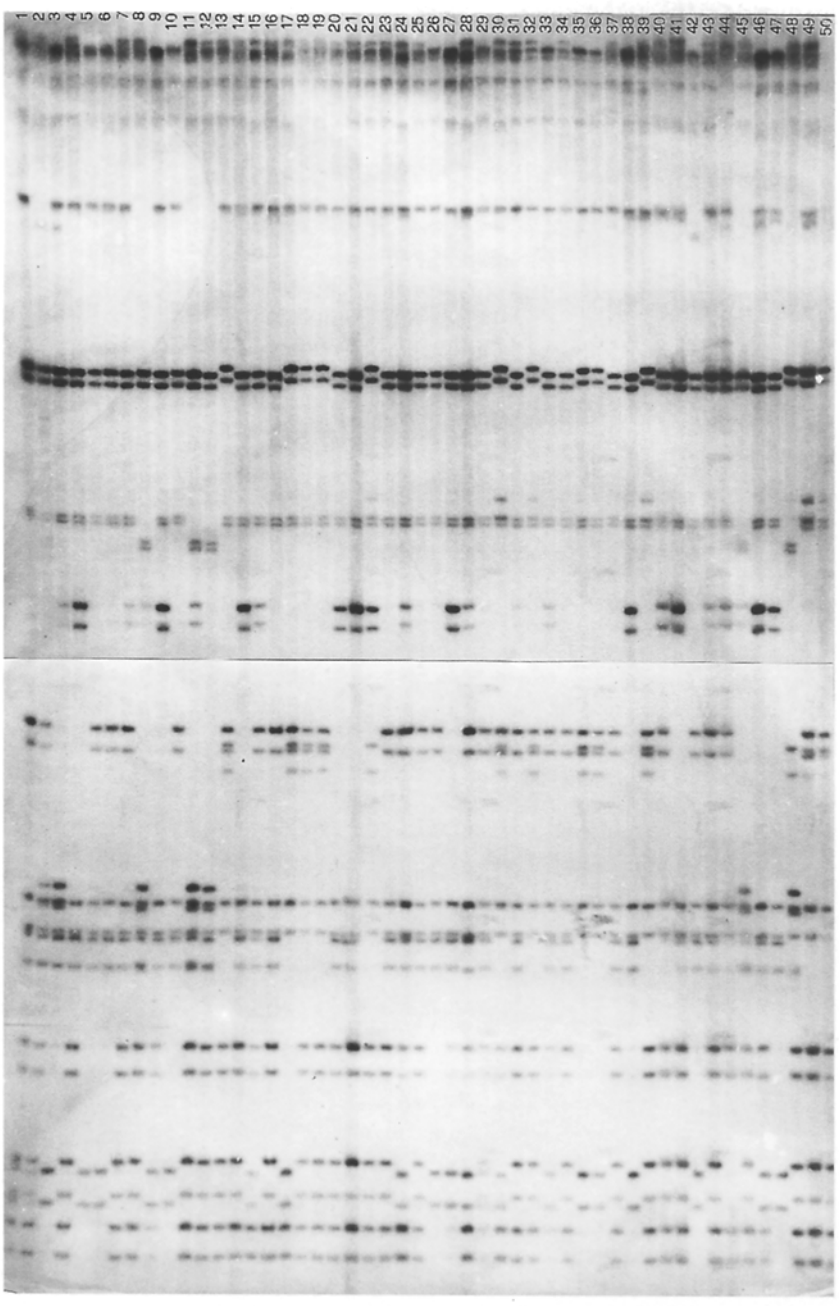

Fig. 1. Example of a complex RFLP pattern in 50 common wheat cultivars (ordered as in Materials and methods) obtained with the probe/enzyme combination K9/*Rsa*I

This study has demonstrated that, in wheat varietal fingerprinting, the RFLP technique based on the use of protein probes can provide more information than electrophoretic analysis.

As pointed out by Gebhardt et al. (1989) in potato and Pecchioni et al. (1993) in barley, the use of the four-cutter restriction enzymes combined with denaturing polyacrylamide gels was particularly useful for detecting polymorphism. In most cases, in spite of pattern complexity, autoradiographical films were easily read and three probe/enzyme combinations had the necessary sensitivity for identification of all 54 cultivars, most of them closely related.

Of particular interest is the fact that the cultivars Maestra, Fiocco and MV15 carry a translocation consisting of the long arm of wheat chromosome 1B, including its centromere, and the short arm of rye chromosome 1R. The K32 probe was able to identify RFLP fragments specific for this translocation, supporting a close homology between gliadins and secalins.

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