J. Genet. & Breed. 48: 207-210 (1994)

Identification of polymorphisms in reference barley genotypes based on the polymerase chain reaction

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Received April 28, 1993

ABSTRACT

The polymerase chain reaction (PCR) can be used to detect DNA polymorphism. Four reference barley genotypes were evaluated for variability using primers designed to amplify specific segments of genomic DNA. Segregation of such PCR-based markers in F_5 SSD progeny was also analysed. Suitability of PCR-based markers for use as genetic marker in barley germplasm is discussed.

Key words: Characterization, DNA marker, Germplasm, *Hordeum vulgare.*

INTRODUCTION

Molecular markers reflecting DNA sequence differences between individuals can constitute useful genetic markers for a variety of genetic and plant breeding applications (TANKSLEY *et al.*, 1989; MELCHINGER, 1990). Several linkage maps based mainly on RFLP have been or are being constructed in barley (SHIN *et al.*, 1990; HEUN *et al.*, 1991; GRANER *et al.*, 1991). The general application of RFLP techniques to barley breeding however, is limited by several factors such as the very significant costs, time required for RFLP assays, and the low level of polymorphism found in barley when compared to maize and other outbreeding species (GRANER *et al.*, 1990; HEUN *et al.*, 1991). With the development of polymerase chain reaction (PCR) technology (SAIKI *et al.*, 1985), some alternative strategies for generating molecular markers have emerged (ERLICH *et al.*, 1989).

The purpose of the present study was to assess the use of PCR-based markers to identify polymorphisms in a barley germplasm of importance in ICARDA's barley breeding program.

MATERIALS AND METHODS

Plant material

Four barley lines (Tadmor, *Hordeum spontaneum* 41.1, Er/Apm and Athenaïs) were selected for their wide genetic variability for adaptation to the dry and cold areas of West Asia and North Africa. Tadmor is a pure-line selection from a Syrian 2-row landrace. *Hordeum spontaneum* 41.1 is a pure line from a population of *Hordeum spontaneum* collected in Israel. Er/Apm is a 2-row cultivar susceptible to cold and drought. Athenaïs is a 6-row cultivar well adapted to dry areas. In addition, a population of 26 F_5 inbred lines derived from the cross Tadmor/Er/Apm through SSD was used.

DNA isolation and PCR markers analysis

Total DNA was isolated from young leaves according to SAGHAI-MOROOF *et al.* (1984). All primer sets used for PCR are shown in Table 1 and were provided by T. Blake (M.S.U., MT, USA). Each primer set is composed of two oligonucleotide primers binding opposite DNA strand and flanking the target sequence. Primers are designed based on sequence information of either barley clones or known genes, obtained after sequencing or directly from genebank database.

Polymerase chain reaction sequence amplification was set up in volume of 35 µl as described by SHIN *et al.* (1990) using approximately 100 ng of genomic DNA and 20 ng of each primer. The PCR amplification profile consisted of initial denaturation (5 min at 95°C) followed by 32 cycles (1 min at 94°C, 1 min at 52°C, 2 min at 72°C). The amplifications finished with an incubation at 72°C for 7 min. For several primer sets and before analysis, parts of the amplified product were digested with different restriction endonucleases (Table 1). Reaction products were resolved by electrophoresis in 1% agarose or 6% polyacrylamide gels for 2 h. Gels were strained with ethidium bromide and analysed on a UV screen.

TABLE 1

PCR-based markers assayed

Primer set	Gene/Clone (Location)	Enzymes used for restriction fragment analysis of PCR products
TB 13/14	Thionins	/
TB 15/16	Ubiquitin	/
TB 17/18	Hordothionin	Taq I
KV 1/9	B Hordein (chr. 5)	/
KV 5/6	Lectin	/
KV 7/8	Dehydrin	/
M 17/19	Adh 2	Taq I
316 63/64	CSU 316	**Msp I, Hae III**
319 63/64	CSU 319	/
327 63/64	CSU 327	**Msp I, Hae III**, Hinf I
337 63/64	CSU 337	**Msp I**
340 63/64	CSU 340	**Msp I, Hae III**
464 63/64	CNL 464 (chr. 4)	/
686 63/64	CNL 686 (chr. 1)	Hae III

CSU: Colorado State University.
CNL: Cornell University.

Goodness-of-fit to the 1:1 pattern of segregation for marker DNA bands, as predicted for Mendelian characters in a SSD progeny, was determined using the chi-square test.

RESULTS AND DISCUSSION

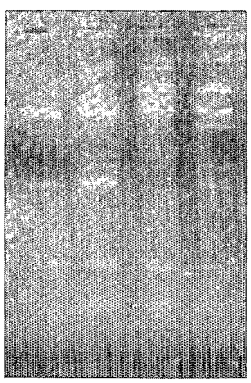
A total of 14 primer sets were used to detect PCR-based polymorphisms between barley genotypes. Only the primer sets KV1/9 and TB 13/14 revealed length polymorphism between the genotypes analysed (Figure 1). For several primer sets, the possibility of polymorphism after restriction of the amplified product was assayed. Different four-bases recognition restriction enzymes were used (Table 1). The amplified product of two additional primer sets, TB17/18 and 327 63/64, were found polymorphic using acrylamide gel electrophoresis. Combinations of the four informative PCR-based markers allow unambiguous identification of barley genotypes (Table 2).

TABLE 2

Analysis of reference barley genotypes for PCR-based markers

Informative PCR-based markers		Marker patterns of genotypes			
Primer set	Enzyme	*H. spontaneum* 41.1	Tadmor	Er/Apm	Athenaïs
TB 17/18	Taq I	A	A	B	B
KV 1/9	/	A	A	B	A
327 63/64	Hae III	A	B	B	A
TB 13/14	/	A	A	A	B

Segregation of three PCR-based markers in F_5 SSD progeny from an individual cross between Tadmor and Er/Apm was analysed (Table 3). All the F_5 inbred lines appeared homozygous for the regions assayed by the primers and showed either alleles from Tadmor or Er/Apm (Figure 2). The distributions observed at each locus were tested for conformance to Mendelian expectation. PCR-based polymorphism appeared as valuable genetic marker, although one segregation ratio was significantly different from 1:1. The small progeny size as well as differential survival or competitiveness of gamete or plant may be implicated as a possible explanation for such deviation. Similar phenomenon seemed to be of a common occurence in both



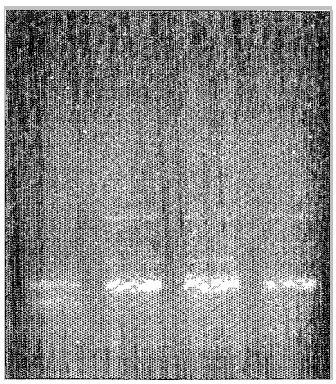


FIGURE 1 - Examples of PCR-based markers: agarose gel electrophoresis of amplified DNA samples from different barley genotypes (A:Athenaïs, E:Er/Apm, H: Hor. spon. 41.1, T:Tadmor) using primer sets TB 17/18 (left) and TB 13/14 (right).

TABLE 3

Chi-square analysis for goodness of fit to a 1:1 ratio of PCR-based markers in a population of inbred lines derived from the cross Tadmor Er/Apm

PCR-based marker		Lines with allele from Tadmor	Lines with allele from Er/Apm	χ^2
Primer set	Enzyme			
TB 17/18	Taq I	18	7	4.84*
KV 1/9	/	4	13	3.24
327 63/64	Hae III	9	15	1.50

* Significant at 5% level.

isozyme and RFLP analysis in barley (SCHÖN *et al.*, 1990; HEUN *et al.*,, 1991).

The results obtained in this study demonstrate the value of the PCR technique using specific primer sets of target known sequences in detecting polymorphisms in barley. Sufficient polymorphisms were observed to discriminate between all four barley genotypes. The method is rapid, simple, technically less demanding to use than RFLP's, requires low amount and crude quality of DNA and does not involve radioactivity.

Regarding the construction of genetic maps, limitations may appeared. The level of polymorphism detected was relatively low when compared with RFLP's revealed by genomic DNA clones for the same set of barley lines (LASHERMES *et al.*, 1991). Although many plant sequences have been published, there are too few available to allow the generation of large number of markers. Alternative PCR procedures as the RAPD-technology (WILLIAMS *et al.* 1990) or the use of primers that target a range of genes such as the consensus sequence for intron splice junctions (WEINING and LANDGRIDGE, 1991) have been proposed, but they may present limitations as well. DEVOS *et al.* (1992) using RAPD-marker technology (random amplified polymorphic DNA) in wheat have shown that amplified products originate mostly from repeated DNA sequences. RAPD markers behave as dominant markers. Moreover, results of amplification using RAPD-primer (short oligonucleotide) are often difficult to transfer from one laboratory to the next. In comparaison, the DNA sequences amplified with primers of known sequences are more specific, reliable and repeatable, probably due to the longer primer and the more stringent conditions during PCR cycling. With a given primer set, alleles of the same gene or DNA sequence will be amplified in different barley genotypes.

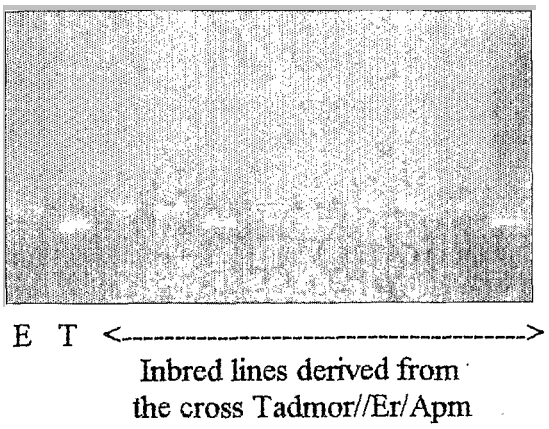


FIGURE 2 - Segregation of a PCR-based marker produced with primer set KV 1/9. The first lines contain amplified DNA from the parental lines Er/Apm (E) and Tadmor (T).

Evidence from this study indicates that the PCR technique using specific primer sets of target known sequences provide a method of choice for genotype identification.

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