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Identification of the 1RS rye chromosomal segment in wheat by RAPD analysis

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Abstract The introgression of rye DNA into the wheat genome was studied using random decamer and specific primers with the polymerase chain reaction (PCR). DNA from paired near-isolines in Chisholm and Arkan backgrounds differing with respect to the presence of a 1RS.1BL translocation was amplified with 120 arbitrary sequence primers. Two of the primers (OPR 19 and OPJ07) amplified rye-specific DNA fragments. The OPR19 primer amplified a 1.35-kb fragment that appeared to be specific to the 1RS.1BL translocation, based on its presence only in lines carrying the 1RS.1BL translocation. A fragment of the same size was also amplified in 1RS.1AL translocation lines. This 1RS.1BL marker locus was designated *Ximc 1*. The other primer, OPJ07, amplified a 1.2-kb DNA sequence, that was designated *Ximc 2*, specific to the wheat-rye translocation in various wheat backgrounds. The sequences of the two marker loci were found to be different from each other. The *Ximc 1* locus was a low-copy sequence which was also present in Balboa rye genomic DNA. Through the use of specific primers, the presence of the rye-specific marker was confirmed in hexaploid as well as in tetraploid wheat backgrounds. The use of RAPDs for the study of smaller alien introgressions into wheat is discussed.

Key words 1RS.1BL translocations · RAPD markers · Wheat · Near-isolines

Introduction

Rye (*Secale cereale* L.) contains genes that provide resistance to various wheat foliage diseases along with genes for many other useful characters. Several wheat-rye translocations, including 1RS.1BL, have been produced as a means for transferring desirable genes from rye to wheat (reviewed by Zeller and Hsam 1983). Biochemical (Dhaliwal et al. 1988; Howes et al. 1989; Lookhart et al. 1991) and cytological (Zeller 1973; Gill and Kimber 1977; Le et al. 1989; Friebe et al. 1991) strategies have been used to identify rye chromosome fragments in wheat backgrounds. The major limitation of these techniques is their failure to satisfy the need for markers for both advantageous as well as deleterious segments of rye.

Molecular markers based on the direct analysis of DNA sequence variation can satisfy the need for markers anywhere in the genome. Rogowsky et al. (1991) used various DNA probes to assign restriction fragment length polymorphism (RFLP) markers to specific regions on chromosome arms 1DS and 1RS of wheat and rye. Wheat-rye recombinants involving 1DL.1RS and 1BL.1RS have been characterized using various RFLP probes and the map locations of various genes have been established (Koebner et al. 1986; Rogowsky et al. 1993). While RFLPs have the potential to be useful in selection for various agronomic characteristics, RFLP analysis has not been easy to adapt to selection schemes in plant breeding due to its laborious and time-consuming nature, as well as the need for special supplies or equipment.

PCR (Polymerase chain reaction) with primers of specific amplicons such as gamma-gliadin genes (D'Ovidio et al. 1990) and on the alpha-amylase sequence (Weining and Langridge 1991) has been used to study genetic polymorphisms in wheat. Lee et al. (1993) used specific primers to amplify the gamma-secalin gene of rye and was able to identify the presence of 2RS in a wheat background. The PCR-based assays termed "randomly amplified polymorphic DNA" (RAPDs) of Williams et al. (1990) and the arbitrary primed PCR (AP-PCR) of Welsh and McClelland (1990) provide an unlimited number of markers and circumvent the need for specific primers. Since 1990, RAPD analysis has been

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extensively used to study polymorphisms among various crops (Devos and Gale 1992; He et al. 1992; Vierling and Nguyen 1992; Gonzalez and Ferrer 1993; Joshi and Nguyen 1993, Iqbal and Rayburn 1994). RAPD markers have been linked to various plant disease resistance genes (Uphoff and Wricke 1992; Haley et al. 1993. Miklas et al. 1993; Paran and Michelmore 1993; Penner et al. 1993). Devos and Gale (1992) linked a RAPD amplified fragment to chromosome 4 of barley (*Hordeum vulgare* cv Betzes) in a wheat (Chinese Spring) background. Similarly, King et al. (1993) identified various RAPD markers specific for chromosome 5E of *Thinopyrum bessarabicum* in a wheat/alien recombinant.

The purpose of the present study was to identify marker loci by RAPD analysis which could be used to detect the 1 RS rye segment in a wheat background and to characterize their composition. This is a first step in the development to a saturation map of the 1 RS segment.

Materials and methods

Plant material

The paired near-isolines used in this study are described by Carver et al. (1993). Each pair of near iso-lines are genetically similar except for the presence or absence of the 1 RS.1 BL wheat-rye translocation. Six paired near-isolines, OK91G119 (1 RS.1 BL), OK91G120, OK91G123 (1 RS.1 BL), OK91G124, OK91G143 (1 RS.1 BL) and OK91G144, were produced involving a cross with Chisholm while the other six, OK91G147 (1 RS.1 BL), OK91G148, OK91G153 (1 RS.1 BL), OK91G154, OK91G161 (1 RS.1 BL) and OK91G162, were produced involving a cross with Arkan. Three 1 RS. 1 AL introgressions lines, OMC8811, OMC8814 and OMC8817, were studied. These lines contain the 1 RS.1 AL) segment from 'Amigo'. Tetraploid wheat containing the 1 RS.1 BL translocation was provided by Dr. B. S. Gill and Mr. J. Raupp of The Wheat Genetics Resource Center, Kansas State University. Balboa rye was obtained from Dr. D. G. Bullock, University of Illinois at Urbana-Champaign.

DNA isolation

DNA was isolated from 2 week-old plants. Leaves from three to six plants were washed in distilled water and quickly frozen in liquid nitrogen. The plants were ground into a very fine powder and DNA was isolated by the method of Rogers and Bendich (1988). DNA concentration was measured by a UV-VIS spectrophotometer and the DNA was diluted in distilled water.

PCR and primers

Decamer oligonucleotide primers (20 primers per kit) were obtained from Operon Technologies, Inc. (Alameda, Calif.). Operon Kits A, J, L, M, R, and primers OPG01, OPG02, OPG06, OPG07, OPG08, OPG09, OPG11, OPG14, OPG15, OPG16, OPG18, OPG19, OPG20, OPN02, OPN06, OPN08, OPN12, OPN14, OPN17, OPN20 were studied. Specific primers OPJ071F1 and OPJ071R1 were synthesized by the Genetic Engineering Facility, University of Illinois. $MgCl_2$, 10 × PCR buffer (100 mM Tris-HCL, 500 mM KCL, pH = 8.3) and dNTPs were obtained from Perkin Elmer Cetus, Alameda, Calif, *Taq* DNA polymerase from Bethesda Research Laboratories (BRL) and gelatin from Sigma Chemicals Inc. PCR was carried out in a 50-µl reaction volume containing 10 mM Tris-HCL (pH 8.3 at 25 °C), 50 mM KCl, 3 mM $MgCl_2$, 0.1 mM of each dATP, dGTP, dCTP and dTTP, 0.001% gelatin, 1 unit of *Taq* DNA polymerase, 6.25 ng of template DNA and 30 ng of primer. The reaction mixture was overlaid with two drops of mineral oil (Sigma, St. Louis, MO.) to avoid evaporation. The PCR reaction was run in a Hybaid Thermocycler for 40 cycles with the first step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min, with a ramp of 3 s/1 °C between annealing and extension steps. After 40 cycles, there was a final extension step of 4 min at 72 °C. The reaction mixture was cooled to 25 °C and maintained at this temperature until gel electrophoresis. For PCR with specific primers, the annealing temperature was raised to 55 °C, no ramp was used between annealing and extension and the amount of template DNA was increased to 75 ng in the case of hexaploid, and 25 ng per 50 µl reaction for tetraploid, wheat. The PCR products were separated on a 1.2% agarose gel in TBE (tris-borate EDTA) buffer. Forty nanograms of ethidium bromide (EtBr) were added to the gel and 400 ng of EtBr to the buffer. A 1 kb DNA ladder (BRL) was used as a size marker.

Screening of primers with paired near-isolines

One pair of near-isolines from the Chisholm and one from the Arkan background were selected. Twenty primers that appeared to amplify the 1 RS translocation in the wheat backgrounds during the screening were used to amplify DNA of the 12 isogenic lines.

Probe synthesis

The $OPJ07_{1200}$ fragment was excised from the low-melting-point agarose gel. The fragment was purified by the addition of 3 vol of TE buffer followed by sequential melting of the agarose (70 °C for 5 min) and rapid freezing in dry ice (5 min). After brief stirring, the agarose was separated from the DNA containing the supernatant by centrifugation. The DNA fragment was precipitated with ethanol. This purified fragment was then used as a template in the PCR labeling of the probe. Twenty-five percent of the normal dATP was replaced in the PCR reaction by Biotin 7-dATP (BRL) and the reaction was run as described earlier for RAPD analysis. Again the DNA fragment was precipitated with ethanol.

Southern hybridization of PCR products

The PCR products were separated on a 1.2% agarose gel with ethidium bromide in the gel and buffer. The gels were pretreated and blotted to nitrocellulose membranes according to Maniatis et al. (1982). For hybridization and detection, the BRL Blue Gene nonradioactive detection system was used according to the manufacturer's instructions.

Genomic Southern hybridization

Five µg of genomic DNA was digested separately by 25 U of *Eco*R1, *Bam*H1 and *Hin*dIII for 3 h. The whole sample was loaded on a 0.8% agarose gel and electrophoresed at 47V (15 mA) in 0.5 × TBE (tris borate EDTA buffer) for 7 h. The gel was depurinated (0.25 M HCl) for 30 min, denatured (0.5 M NaOH, 1.5 M NaCl) for 30 min, and neutralized [1 M Tris-HCl (pH 8.0), 1.5 M NaCl] for 30 min. The gels were blotted and hybridized.

Cloning and sequencing of the rye-specific fragment

The rye-specific $OPJ07_{1200}$ fragment was purified from a gel and cloned into the pGEM-T vector. After blue/white screening, the recombinant plasmid was isolated (Magic™ Minipreps DNA Purification Systems, Promega). Restriction analysis with *Nco*1 and *Not*1 was carried out and Southern hybridization was done using a biotin-labeled PCR-amplified probe (with the OPJ07 primer). $OPJ07_{1200}$ was sequenced with pUC/M13 forward and reverse sequencing primers using a Silver SequenceTM DNA Sequencing System (Promega Corporation, Madison, Wis.).

Results and discussion

Markers for 1RS.1BL

Two primers out of the 120 studied produced rye-specific amplification fragments. With OPR19 (5′CCTCCTCATC3′), the three 1RS.1BL introgressions in the Chisholm background (OK91G119, OK91G123 and OK91G143) exhibited a fragment (1.35 kb, labeled as $OPR19_{1350}$) that was not present in the non-translocation lines (OK91G120, OK91G124 and OK91G144) (Fig. 1). The amplification profiles with OPR19 of the translocation lines in the Arkan background (OK91G147, OK91G155 and OK91G161) also contained the 1.35-kb DNA fragment, while in non-translocation lines (OK91G148, OK91G156 and OK91G162) it was missing. The presence of $OPR19_{1350}$ in the introgression line amplification profiles and its absence in the non-introgression amplification profiles suggest that $OPR19_{1350}$ is a marker for the 1 RS.1 BL chromosome. We have assigned it the designation *Ximc1*. This 1.35-kb fragment was also amplified from the three 1 A. 1 R introgressions (Fig. 2). The amplification of $OPR19_{1350}$ from the 1 RS.1 BL and 1 RS.1 AL introgression lines indicates that this fragment is associated with 1 RS introgression.

The other primer that amplified a 1 RS. 1 BL-specific product was OPJ07 (5′CCTCTCGACA3′), which amplified a 1.2-kb rye-specific marker (labeled as $OPJ07_{1200}$). Figure 3 a shows the presence of $OPJ07_{1200}$ in the amplification profiles of 1 RS.1 BL introgressions in Chisholm backgrounds (lanes 1, 3 and 5) and its absence in the non-introgression line amplifications (lanes 2, 4 and 6). The locus identified by this band is designated *Ximc2*. This fragment was also amplified from 1 RS.1 BL introgressions in the Arkan background and was present in the amplification profiles of OMC8817, a 1 RS.1 AL translocation line. The rye-specific band $OPJ07_{1200}$ was amplified from tetraploid wheat KS91WGRC14 containing the 1 RS. 1 BL translocation by reducing the template DNA to half (3.1 ng/50 µl reaction) (Fig. 3 b) that of the hexaploid wheat. This is apparently due to the higher ratio of rye DNA to wheat DNA in the tetraploid versus the hexaploid wheat-rye translocation lines.

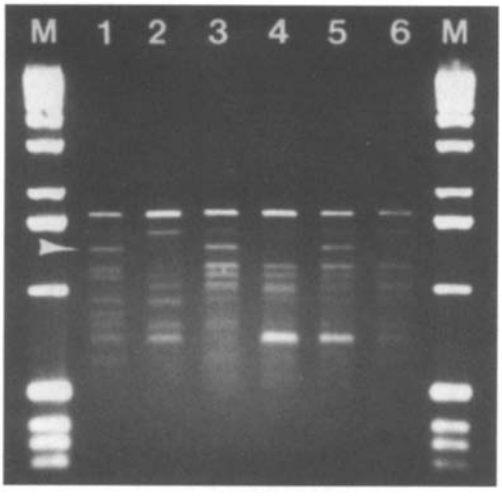

Fig. 1 Amplification profiles of 1 RS.1 BL translocations in a Chisholm background using the OPR19 primer. The *arrow* indicates the 1 RS-specific $OPR19_{1350}$ marker. *Lane 1* = OK91G119 (1 RS. 1BL), *2* = OK91G120, *3* = OK91G123 (1 RS. 1 BL), *4* = OK91G124, *5* = OK91G143 (1RS.1BL), *6* = OK91G144; *M* = 1-kb DNA ladder (BRL)

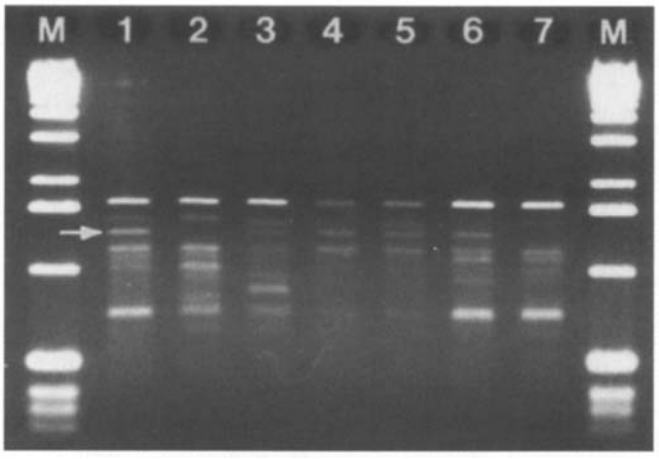

Fig. 2 Amplification profiles of 1 RS.1 AL with the OPR19 primer. The *arrow* indicates the 1 RS-specific $OPR19_{1350}$ marker. *Lane 1* = OK91G143 (1RS.1BL), *2* = OK91G144, *3* = OMC8811 (1 RS. 1 AL), *4* = OMC8814 (1 RS.1 AL), *5* = OMC8817 (1 RS.1 AL), *6* = OK91G155 (1 RS.1 BL), *7* = OK91G156; *M* = size marker (1-kb DNA ladder from BRL)

Sequence similarity of two rye-specific markers

In order to determine whether $OPJ07_{1200}$ is a length polymorphism of $OPR19_{1350}$ or a unique sequence, an $OPJ07_{1200}$ biotin-labeled probe was hybridized to OPJ07 amplification products of various translocation and non-translocation lines. The results obtained (Fig. 4) show that hybridization took place primarily with the $OPJ07_{1200}$ fragment in the introgression lines. When purified $OPJ07_{1200}$ was reamplified by the OPJ07 primer, it produced only the same-sized fragment, indicating that there is no internal priming site for OPJ07 in this 1.2-kb amplification product. These results confirm that $OPJ07_{1200}$ has limited sequence homology to other amplification products of the same primer and represents a unique sequence which is amplified from the 1RS introgression. In order to determine the level of sequence homology between the two rye-specific loci (*Ximc1* and *Ximc2*), the $OPJ07_{1200}$ biotin-labeled probe was hybridized to the OPR19 amplification products in 1RS.1BL introgressions and non-introgression lines. No homology was observed between the $OPJ07_{1200}$ probe and any of the OPR19 amplification products of the translocation and non-translocation lines. The two se-

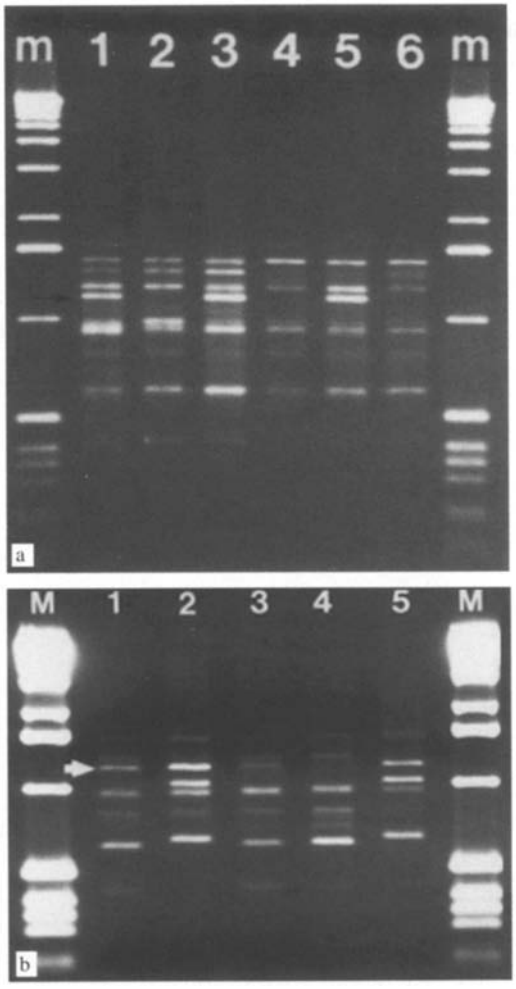

Fig. 3 Amplification profiles of translocation and non-translocation lines in a Chisholm (**a**), and tetraploid (**b**) wheat background with the OPJ07 primer. The *arrow* indicates the rye-specific $OPJ07_{1200}$ marker. **a** *Lane 1* = OK91G119 (1 RS.1 BL), *2* = OK91G120, *3* = OK91G123 (1 RS.1BL), *4* = OK91G124, *5* = OK91G143 (1 RS.1 BL), *6* = OK91G144; *m* Equal size marker (1-kb DNA ladder) **b** *1* = OK91G119 (1 RS.1 BL), *2* = KS91WGRC14 (1 RS. 1 BL, tetraploid), *3* = OK91G119 (1 RS.1 BL), *4* = OK91G120, *5* = KS91WGRC14 (1 RS.1 BL, tetraploid); *M* = size marker (1-kb DNA ladder, BRL)

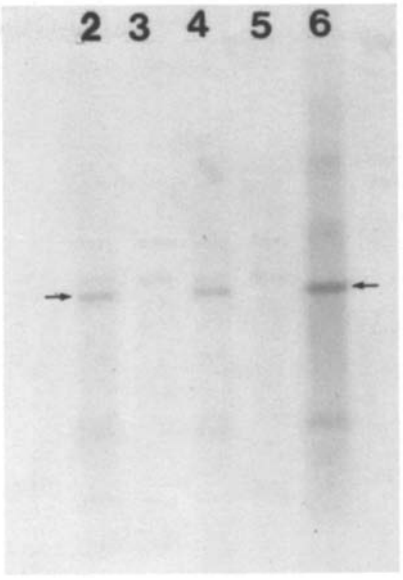

Fig. 4 Southern hybridization of OPJ07 amplification products of OK91G119 (1 RS. 1 BL) (*lane 2*), OK91G120 (*lane 3*), OK91G161 (1 RS. 1 BL) (*lane 4*) and OK91G162 (*lane 5*) with the 1 RS-specific $OPJ07_{1200}$ probe. *Lane 6* represents amplification products of the purified 1 RS-specific fragment ($OPJ07_{1200}$) with the OPJ07 primer

quences are different sequences that are amplified from 1 R introgressed into wheat and they represent different loci.

To confirm the presence of the rye-specific OPJ07 amplified sequence in rye, genomic Southern hybridization was performed using $OPJ07_{1200}$ as a probe. With *Hin*dIII-digested Balboa rye DNA, this probe hybridized to an approximately 1.6-kb fragment. It did not show a smear characteristic of repetitive DNA sequences. This suggests that the 1R locus *Ximc2* is a low-copy sequence which is present in Balboa rye. When Balboa rye DNA was amplified with the OPJ07 primer and hybridized with the $OPJ07_{1200}$ probe, no hybridization to any of the Balboa rye amplification products was observed. The 1 RS segment in wheat is not from Balboa rye and DNA polymorphisms exist between various rye cultivars (Iqbal and Rayburn 1994). That the 1 RS-specific $OPJ07_{1200}$ was not amplified in Balboa rye DNA was not surprising. Genomic Southern analysis does confirm that a sequence complementary to $OPJ07_{1200}$ is present in the Balboa rye genome.

1 RS markers with specific primers

Two specific primers, J07IF1 and J07IR1, were designed. The 22 nucleotide-long J07IF1 primer (5′TAA GCC GTA AAG CAT GGT GCA C3′) starts from the 87th base (sequence determined with the pUC/M13 forward primer of the cloned $OPJ07_{1200}$ fragment). The 24 nucleotide-long J07IR1 primer (5′CTT CAA CGA AAT GTT TTC CTC TTC3′) starts from the 92nd base of the alternate strand (sequence of the $OPJ07_{1200}$ cloned fragment determined with the pUC/M13 reverse primer). These two primers were used to amplify the 1.RS.1BL translocation lines (both tetraploid and hexaploid) and normal wheat. A rye-specific fragment (1 kb in size) was amplified in all of translocation lines (Fig. 5). The specificity of this marker was confirmed in both Chisholm and Arkan backgrounds. There was no amplification with these specific primers in wheat lines not containing the 1RS.1BL translocation. When the two primers were used to amplify Balboa rye genomic DNA, the rye-specific 1-kb fragment was amplified indicating that the sequence found in *Ximc2* locus is present in rye. These results further confirm that *Ximc2* is a marker locus for the 1 RS segment introgressed into wheat.

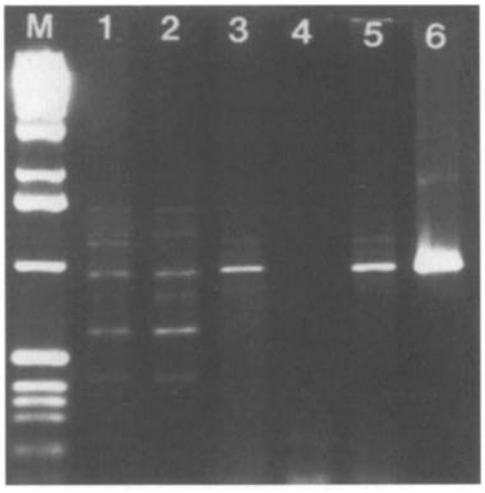

Fig. 5 Amplification profiles with the OPJ07 (*lanes 1–2*) J07IF1 and J07IR1 (*lanes 3–6*) primers. *1* = OK91G119 (1RS.1BL), *2* = OK91G120, *3* = OK91G119 (1 RS. 1 BL), *4* = OK91G120, *5* = KS91WGRC14 (1 RS. 1 BL, tetraploid) *6* = Plasmid containing the $OPJ07_{1200}$ fragment; *M* = size marker (1-kb DNA ladder)

Conclusions

The results presented in this study demonstrate that random primers can be used to target rye translocations in wheat backgrounds. RAPD markers have been used earlier to identify barley and *T. bessarabicum* chromosomes in wheat (Devos and Gale 1992; King et al. 1993). In addition to their use in genome identification and genetic relatedness, polymorphic RAPD markers from segregating populations have been used to generate linkage maps (Rowland and Levi 1994). In many cases RAPDs have been used in combination with RFLPs and isozymes to produce saturation maps and identify various linkage groups (Torres et al. 1993; Chaparro et al. 1994; Kesseli et al. 1994). Since a large number of random primers are available, more 1 RS marker loci can be identified. These loci can then be used to produce a RAPD-based saturation map of 1 RS. Moreover, specific primers can be designed by cloning and sequencing the RAPD markers. RAPD loci act as dominant markers, but when longer primers are designed from the sequence of DNA fragments obtained by RAPD amplification, they could act as co-dominant markers [sequence-characterized amplified regions (SCARs) of Paran and Michelmore (1993)]. The specific primers designed from the sequence of rye-specific $OPJ07_{1200}$ now provide the potential to reveal co-dominant markers for recombination analysis.

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