Detection of terminal deletions in barley chromosomes by the PCR-based method

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Barley chromosomes have barley-specific repetitive sequences (HvT01) in the subtelomeric regions. The subtelomeric repeats were amplified by the polymerase chain reaction (PCR) in 11 wheat-barley telosome addition lines, but no or little amplification occurred in the euploid common wheat cultivar 'Chinese Spring'. A gametocidal chromosome 2C, derived from an *Aegilops cylindrica* chromosome, induces chromosome breaks in Chinese Spring. To explore the potential of using PCR as a screen for barley telosomes with terminal deletions, induced by chromosome 2C, the progeny of a barley ditelosomic (6HS) addition line of Chinese Spring carrying chromosome 2C was investigated. The progeny plants in which DNA amplification occurred by PCR always had a normal 6HS whereas those in which no DNA amplification was observed carried either no 6HS or a 6HS telosome with a deletion. In this work the PCR based assay described is shown to be a robust and reliable method of identifying terminal deletions in barley telosomes in a Chinese Spring background.

INTRODUCTION

Physical mapping of chromosomes reveals real distances between genetic markers. The translocation breakpoints of numerous barley reciprocal translocation lines produced by X-irradiation were studied by PCR analyses using the microisolated translocation chromosomes as templates and sequence-specific primers of genetically mapped DNA sequence (Künzel and Korzun, 1996; Korzun and Künzel, 1996). In barley, however, the genetic and physical maps have not yet been fully integrated.

Certain chromosomes of *Aegilops* species, which are related to wheat, are known to cause chromosome breakage in common wheat, *Triticum aestivum* and are designated as gametocidal chromosomes (Endo, 1988, 1990). Chromosome breaks occur in the wheat gametes lacking the *Aegilops* chromosome, and telomeres are added to the broken site during gametogenesis or early zygote development (Werner et al., 1992; Tsujimoto, 1993). Therefore, the deleted chromosomes become stable in the subsequent generations. An array of chromosome deletions induced by such gametocidal chromosomes have been identified cytologically to establish deletion stocks of common wheat (Endo and Gill, 1996). Using the deletion stocks, physical mapping studies have been conducted to map various genes (Gill et al., 1993; others) and RFLP markers (Gill et al., 1996; others).

With the object of inducing deletions in barley chromosomes Shi and Endo (1997) produced common wheat lines that are disomic for respective barley chromosomes and monosomic for a gametocidal chromosome (chromosome 2C derived from *Aegilops cylindrica*). Although they showed that barley chromosome deletions can be detected by C-banding, it was difficult to detect minor deletions and translocations by C-banding alone because the barley chromosomes do not have prominent terminal heterochromatic bands (Shi and Endo, 1997). The barley chromosomes carry the 118 bp tandemly repeated satellite DNA (Belostotsky and Ananiev, 1990) which is closely associated with the telomeres (Kilian and Kleinhofs, 1992). By in situ hybridization this subtelomeric sequences (HvT01) was localised mainly in the terminal regions of at least 12 barley chromosome arms (Röder et al., 1993; Brandes et al., 1995; Schubert et al., 1998). Schubert et al. (1998) demonstrated that deletions and translocations in barley chromosome 7H and chromosome arm 4HL can be detected reliably by in situ hybridization using the HvT01 probe.

In this study the polymerase chain reaction, using a primer set that amplifies HvT01 repeats, was tested as a means of detecting terminal deletions in barley chromosomes. The results of our research indicate that PCR analysis is a reliable means of detecting terminal deletions in barley telocentric chromosomes in the wheat genomic background.

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MATERIALS AND METHODS

Plant materials. The eleven wheat-barley telosome addition lines used in this study were constructed by Islam (1983). In each of these lines one of the eleven telocentric chromosomes of barley (*Hordeum vulgare* cv. Betzes, $2n = 2x = 14$, genome formula HH), except for both arms of barley chromosome 1H and the short arm of barley chromosome 2H, is added to common wheat (*T. aestivum* cv. Chinese Spring, $2n = 6x = 42$, AABBDD). They were kindly provided by H. Tsujimoto, Yokohama City University, Japan. Their chromosome constitutions were examined by C-banding to confirm that all the lines but three had a pair of the critical telosomes. The lines for 3HS, 4HS, and 7HS appeared to have been monosomic for the respective barley telosomes and therefore monotelosomic addition plants were cytologically selected in this study. Euploid Chinese Spring wheat and Betzes barley were used as controls. We follow the designations of barley chromosomes proposed by Linde-Laursen et al. (1997)

The ditelosomic 6HS addition line was crossed to a Chinese Spring line with a pair of gametocidal chromosomes 2C derived from *Ae. cylindrica* ($2n = 4x = 28$, CCDD). The hybrid was backcrossed to the ditelosomic 6HS addition line, and from the backcrossed progeny a 45-chromosome plant with a pair of telosomes 6HS and a single 2C chromosome was selected (Fig. 1). This plant was cross-pollinated with euploid Chinese Spring, and chromosomal aberrations were expected to occur in the progeny.

Total DNA extraction. Young leaves of ca. 5 cm long from individual plants were ground to fine powder in liquid nitrogen with a small plastic pestle, fitted to a 1.5 ml

Fig. 1. A C-banded mitotic metaphase of a root-tip cell of the 45-chromosome plant ($21'' + 1''6HS + 1''2C$). Arrowheads indicate the barley 6HS telosomes and an arrow indicates chromosome 2C. Bar = 10 μ m.

centrifuge tube. To the leaf powder were added 400 μ l glycine buffer (100 mM glycine, 100 mM NaCl, 100 mM EDTA, pH 8.5), 40 μ l 10% SDS, and 4 μ l 1% bentonite. Equal volumes of TE-equilibrated phenol and chloroform/isoamyl alcohol (24/1) were added to the well-mixed solution. The solution was centrifuged for 10 min at 15,000 rpm. Isopropanol was added to the supernatant to precipitate DNA, which was rinsed with 70% ethanol and resuspended in 100 μ l of TE buffer. Thus prepared DNA solutions contained ca. 100 ng DNA/ μ l.

PCR analysis of HvT01 repeats. Primers for PCR were designed based on the HvT01 repeat sequences reported by Belostotsky and Ananiev (1990). They were HvT-1, 5' CGAAACTCGCATTTTTGGCC 3', and HvT-R1, 5' AGAGT-TCCCGTAACCGGCC 3'. Each PCR was conducted in a 25 μ l solution containing ca. 100 ng total DNA, 125 μ M each of dATP, dTTP, dGTP, and dCTP, 5 pmol each of the primers, 2.5 unit Taq polymerase, and 1 $\times$ amplification buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.8). PCRs were conducted with TAKARA PCR Thermal Cycler MP; 1 min at 94°C, 2 min at 65°C, and 3 min at 72°C. The amplified DNAs were separated in 1.5% agarose gels by electrophoresis. The DNA amplification was also examined by placing 1 μ l of the PCR product mixed with 5 μ l ethidium bromide solution (2 μ g/ml) on a sheet of Parafilm which was then illuminated by UV light (This is described as 'dot analysis' in this paper).

RESULTS AND DISCUSSION

PCR analysis of barley subtelomeric repeats. Thirty-cycle PCRs were conducted for euploid Chinese Spring, Betzes, and the wheat-barley telosome addition lines. As shown in Figure 2A, the HvT01 repeat sequence was amplified by PCR when the total DNAs of Betzes and all the wheat-barley telosome addition lines were used as templates, but no or scarce amplification occurred when the total DNA of Chinese Spring was used. This difference in amplification was also seen by the dot analysis, which is easier and faster than gel electrophoresis (Fig. 2B). Occasionally the amplification was not sufficient for the dot analysis in telosomics 7HS, 7HL, 2HL, 3HL, and 5HL: The amount of amplification was between those in Chinese Spring and Betzes. The amounts of DNAs amplified by PCR using 35 cycles were, however, sufficient for the dot analysis for telosomics 7HS, 7HL, and 5HL, but was not always so for telosomics 2HL and 3HL. PCR with 40 cycles did not produce an increase in the amount of DNA and amplified the same amount of DNA in euploid Chinese Spring as in the telosomic lines (data not shown). This result is consistent with the previous in situ hybridization studies on barley mitotic cells (Röder et al., 1993; Brandes et al., 1995; Schubert et al., 1998). By the in situ hybridization no or faint signals of HvT01 were observed on 2HL

Fig. 2. Gel electrophoresis (A) and dot analysis (B), both negative images, of DNAs amplified by 30-cycle PCR using HvT01 primers in the wheat-barley telosomic addition lines (2HL to 7HL), Betzes barley (Bt), Chinese Spring wheat (CS), and no template (bl). SM indicates the size marker, indicated in kilobases.

and 3HL, indicating the numbers of the subtelomeric repeats are smaller in 2HL and 3HL than in the other barley chromosome arms. Thus, when the barley telosomes, except 2HL and 3HL, are present singly in the genomic background of common wheat, the presence or absence of their subtelomeric HvT01 repeats could be revealed reliably by gel electrophoresis or the dot analysis of PCR products.

Screening for terminal deletions in barley telosome 6HS. The backcrossed progeny of the 45-chromosome plant ($21'' + 1''6HS + 1''2C$) was expected to include plants bearing 6HS telosomes with terminal deletions, as well as intact 6HS telosomes. Total DNAs extracted from 107 plants of the backcrossed progeny were used as templates for the 30-cycle PCR to amplify the barley HvT01 repeat. These plants were also examined cytologically for the presence of 6HS. All 90 plants in which the DNA amplification of the HvT01 repeat occurred by PCR carried the 6HS telosome. The DNA amplification was not observed in the remaining 17 plants both in the gel electrophoreses and on the dotted sheets (Fig. 3A, B). No 6HS was present in 15 of the 17 plants, presumably a result of meiotic pairing failure, and deficient 6HS telosomes were observed in two plants (Fig. 4). The frequency (two out of 107, 1.9%) of

Fig. 3. Gel electrophoresis (A) and dot analysis (B), both negative images, of DNAs amplified by 30-cycle PCR using HvT01 primers in part of the backcrossed progeny of the 45-chromosome plant ($21'' + 1''6HS + 1''2C$). Plants a to g were judged to have lost the barley subtelomeric repeats: C-banding revealed that plant d had a deleted 6HS that is shown in Fig. 4 (left), and that the others had no 6HS. Note plants c, d, and e are included in both gel electrophoresis and dot analysis.

Fig. 4. Two C-banded deletions of barley telosome 6HS (indicated with arrows): The breakpoints are in the short arm (left) and in the satellite. Normal 6HS is in a rectangular box.

detection of the terminal deletions of 6HS is comparable to that of wheat deletions detected by C-banding (Endo and Gill, 1996). Thus the PCR analysis described has been

shown to be a valuable means of identifying the loss of barley subtelomeric repeats and therefore can be used for the initial screening of terminal deletions in the single barley telosomes in Chinese Spring wheat. Once individuals which putatively carry terminal deletions have been identified, in situ hybridization using the HvT01 repeat as a probe, and genomic in situ hybridization can be applied to confirm the presence of structural aberrations in the barley telosome. PCR analysis using the barley telosomic lines does have some drawbacks: A high frequency of non-transmission of the telosome to the progeny would lower the efficiency of detecting terminal deletions of the barley telosome; only one chromosome arm can be surveyed at a time. Also, this analysis can not detect translocations between wheat chromosomes and barley telosome fragments with the subtelomeric repeats. Irrespective of these drawbacks the PCR analysis is useful in detecting barley terminal deletions because it needs no cytological expertise.

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