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Characterisation of wheat-rye recombinants with RFLP and PCR probes

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Summary. The introgression of genetic material from alien species into wheat has become an important tool in modern wheat breeding. Ideally, only the trait of interest and no flanking material should be transferred. Random recombination between the genetic material is therefore of paramount importance. In a model system, we examined 17 recombinants putatively between chromosome 1D of wheat and 1R of rye with 60 random RFLP and three PCR markers. The recombinants had been generated by removing the normal effect of the *Ph1* gene in the wheat background. Amongst the nine short-arm recombinants, three breakpoints were identified but no differentiation could be made between the five proximal recombinants. For the eight long-arm recombinants analysed only two breakpoints were identified with 36 markers. However, only a single RFLP marker was able to differentiate between the recombinants. Indeed the long-arm results are consistent with the possibility that only the rye telomeric region had been transferred. These results indicate either a strong clustering of the RFLP markers near the centromere or else imply that recombination induced between wheat and rye in the absence of the normal effect of the *Ph1* gene occurs at only restricted sites. The results allow new primary recombinants to be selected for intercrossing to generate secondary recombinants which are expected to have a smaller interstitial rye segment than that present in DR-A1.

Key words: Alien introgression – Wheat-rye recombinants – Homoeologous recombination – RFLP markers

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Introduction

The relatives of wheat offer a valuable source of genes for wheat improvement. However, their exploitation depends upon the development of techniques for the introgression into wheat of genetic material from alien species. Among other members of the tribe Triticeae, cereal rye (*Secale cereale*) has been successfully used as a source of disease-resistance genes and yield-benefit traits. Whole rye genomes, individual chromosomes or chromosome arms have been introduced into hexaploid wheat (for review see Sharma and Gill 1983; Gale and Miller 1987). Using wheat-rye translocation lines as the starting material, rare recombination between wheat and rye chromatin was achieved in chromosome pairing control mutants (like the *Ph1b* mutant) leading to rye segments smaller than the chromosome arms present in wheat. One example was the isolation of wheat-rye recombinants involving both the short (Koebner and Shepherd 1986) and long arms (Koebner and Shepherd 1985) of chromosomes 1R and 1D. Over several years a total of five recombinants with proximal, and four recombinants with distal, rye chromatin have been isolated for the short arm (Rogowsky et al. 1991a) while eight putative recombinants with proximal and eight with distal rye chromatin have been isolated for the long arm.

The classical scheme for the introgression of alien chromatin into wheat was devised by Sears (1977) and consists of two steps. First, homoeologous recombination is induced. Homologous recombination is then possible between the primary recombinants, leading to a derived recombinant in which the region of interest and varying amounts of flanking material are present. Following this scheme, the derived recombinant DRA-1 was recovered from the primary

wheat-rye recombinants 82-180 (proximal rye) and I93 (distal rye) in the short arm of chromosome group 1. Although the desired stem-rust resistance gene *SrR* was present in DRA-1, a certain yield and quality loss was also observed (Shepherd et al. 1990). Our strategy to reduce the amount of flanking sequences was to determine the cross-over points in other primary recombinants relative to 82-180 and I93, to select the pair of recombinants which showed a minimum overlap in the region of interest, and then to repeat the homologous recombination with this pair.

To address the fundamental question of recombination between wheat and rye chromatin on a broader basis, we also studied recombinants between the long arms of 1D and 1R. These recombinants were isolated by dissociation between the *Glu-R1* seed protein marker and *het*, a cytogenetic marker detecting a heterochromatic region in rye telomeres (Koebner and Shepherd 1985). These were less well characterised than the short-arm recombinants and no evidence had been found for an overlap between any of the distal and proximal recombinants. Furthermore, translocation to chromosomes other than 1D had not been excluded.

This paper describes the characterisation of a total of 17 recombinants with RFLP probes for chromosomes of homoeologous group 1 as well as with three PCR markers.

Materials and methods

Genetic material

The isolation of short- and long-arm recombinants (Rogowsky et al. 1991a) and of the initial long-arm recombinants (Koebner and Shepherd 1985) has already been described. All long-arm recombinants for which seed was available were included in the analysis. Out of a total of 16 putative long-arm recombinants (Koebner and Shepherd, 1985) only three (RL1–RL3) of the eight with proximal rye chromatin and five (R1–R4 and R7) of the eight with distal rye chromatin were analysed. Recombinants R5, R6 and R8 were included in the initial analyses (see Fig. 1) but were excluded when it was found that the particular plants used in the Southerns did not contain the recombinant chromosomes due to segregation from the heterozygous parent plant. The recombinant chromosomes were present in mixed wheat backgrounds with varying amounts of the wheat cvs Chinese Spring, Gabo and Warigal. All rye chromatin in the recombinants, as well as in the translocation lines used as controls, stemmed from rye chromosome 1R of Imperial rye, originally present as the disomic addition line (Driscoll and Sears 1971).

DNA probes

Plasmids pScR4 (*Nor-R1*), pScT7 (*5SDna-R1*), pNTR-Adh (*XAdh-R1*), pSec-1.5 (*Sec-R1*), pSec-3 (*Sec-3*), pEM (early methionine, *XEm-1R*) and pIH69 (multiple loci on 1R) were from R. Appels (Baum and Appels 1991), plasmids pTa-Tam2, pTa-Tam22 and pTa-Tam52 from G. Hart (Texas A and M), plasmids pPSR161 and pPSR162 from M. Gale (Sharp et al. 1988). Plasmids pTri25-11 (*Tri*) and pAW161 (*Tel-1RS* and *Tel-1RL*) have been described in Rogowsky et al. (1991a), the KSU plasmids in Gill et al. (1991), and the BCD, CDO and WG plasmids in Anderson et al. (1992). Plasmid DNA was isolated by alkali lysis according to Maniatis et al. (1982). Plasmid inserts were isolated from preparative agarose gels and radioactively labelled with the 'Oligo Priming Labelling Kit' from Amersham.

Plant DNA isolation

The miniprep method used for the isolation of total DNA from wheat leaves has been described in detail by Rogowsky et al. (1991a). Briefly, a 10 cm-long leaf segment was frozen in liquid nitrogen, crushed to a fine powder and resuspended in DNA-extraction buffer. Total DNA was prepared by phenol: chloroform extraction, ethanol precipitation and RNase treatment. The yield was approximately 25 µg.

Southern blots

Approximately 5 µg plant DNA were digested with restriction endonucleases for 4 h, separated on agarose gels overnight and transferred to Hybond C Extra (Amersham) membrane following the recommendations of the manufacturer. Prehybridisation was carried out at 65 °C in 20 mM PIPES pH 6.8, 0.6 M NaCl, 5 mM EDTA, 1% SDS, 0.2% gelatin, 0.2% ficoll, 0.2% polyvinylpyrollidone, 0.5% tetrasodium pyrophosphate and 500 µg/ml carrier DNA, while the hybridisation solution contained only 100 µg/ml carrier DNA and 10% dextran sulphate. The membranes were washed at 65 °C for 20 min in each of three washing solutions containing 0.1% SDS and 2 × SSC, 0.5 × SSC and 0.1 × SSC, respectively.

PCR reactions

The PCR markers were based upon sequence data obtained for three rye-specific repeat elements, R173 (Guidet et al. 1991). Although the R173 repeat elements analysed are flanked by other repetitive sequences, the spacing of the R173 element with respect to these other repeats proved to be unique. The PCR primers targetted the flanking regions of the three R173 elements for which sequence data was available (Rogowsky et al. 1992a). These primers generated several PCR bands of which one could be assigned to the 1RS recombinants. The techniques used to generate and map the PCR markers are described elsewhere (Rogowsky et al. 1992b).

Results

Characterisation of wheat-rye recombinants with RFLP probes and PCR

Although isozyme and cytogenetic markers were used to isolate the wheat-rye recombinants, RFLP markers are far more suited for their characterisation. They are abundant, theoretically they are evenly distributed along chromosomes, and they can be tested with the same assay. The order of cross-over points between wheat and rye chromation in our collection of 17 primary recombinants was assayed with a total of 60 RFLP and three PCR markers. Restriction enzymes

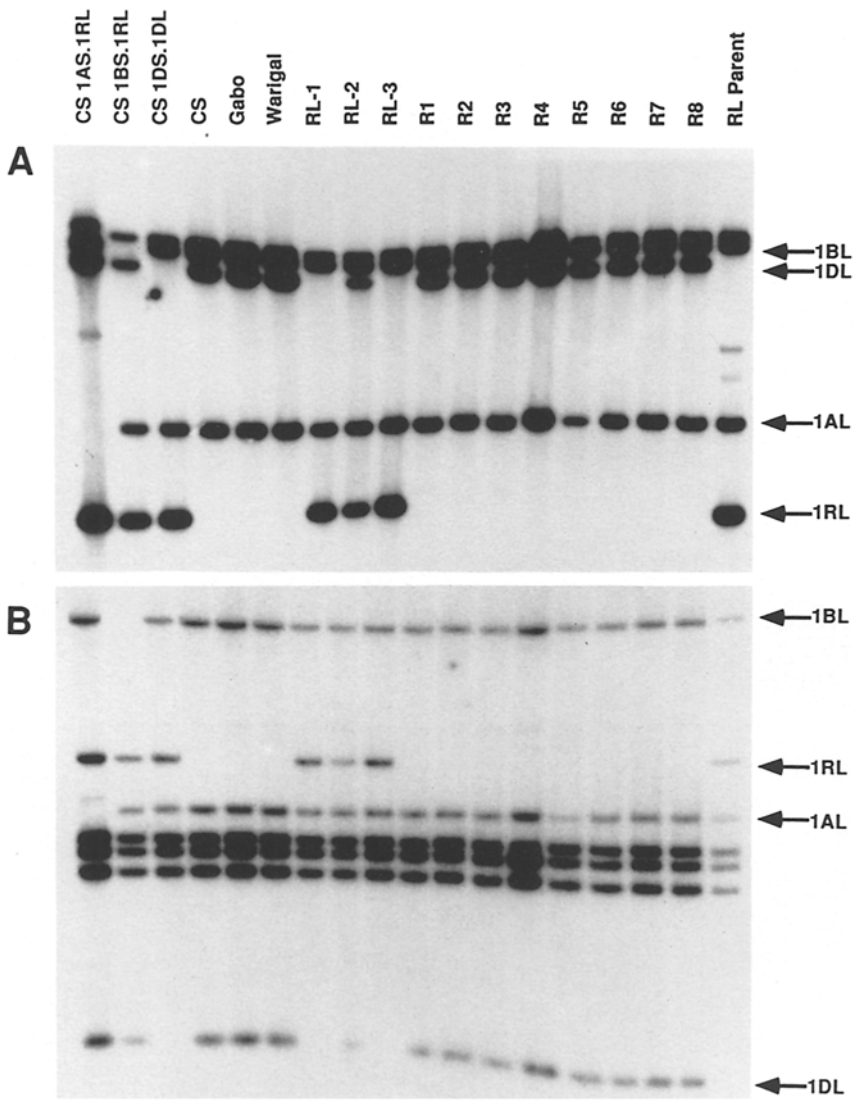

Fig. 1 A, B. Analysis of long-arm recombinants with RFLP probes. Southern blots of the wheat lines shown were probed with BCD454 (**A**) or CDO1396 (**B**). Bands mapping to the long arms of chromosomes 1A (1AL), 1B (1BL), 1D (1DL) or 1R (1RL) are indicated

were chosen so that the probe detected polymorphisms between the A, B and D genomes of wheat.

As an example, Fig. 1 shows the analysis of the long-arm recombinants with probes BCD454 (A) and CDO1396 (B). The recombinants RL-1 to RL-3 (proximal rye chromatin) and R1 to R8 (distal rye chromatin) were run in parallel with six control lines. The three wheat-rye translocation lines, 1AS.1RL, 1BS.1RL and 1DS.1RL, allowed the assignment of chromosome group 1 long-arm bands to the A, B, D and R genomes. The three wheat cultivars, Chinese Spring (C.S.), Gabo and Warigal, which constitute the mixed wheat background of the recombinants, were also used to distinguish between wheat-rye polymorphisms caused by recombination and wheat-wheat polymorphisms caused by the mixed wheat background. In both panels, a band, identified as a 1DL band due to its absence in the 1DS.1RL translocation line, was present in recombinants R1 to R8 as well as in RL-2 but absent from RL-1 and RL-3. Unfortunately the parent plant of recombinants R5, R6 and R8 proved to be heterozygous for the recombinant chromosome and the progeny plants used for the Southerns did not contain this chromosome. The 1RL band, which replaced the 1DL band in the 1DS.1RL translocation line, was present in RL-1, RL-2 and RL-3 but absent in the remaining recombinants. Therefore, the RFLP markers corresponding to the two probes, mapped proximally to the breakpoints of all eight recombinants. Only one probe was found that could differentiate between the distal recombinants. This was CDO393 which divided the five recombinants tested into two groups. No differences were found within the group of three recombinants with proximal rye

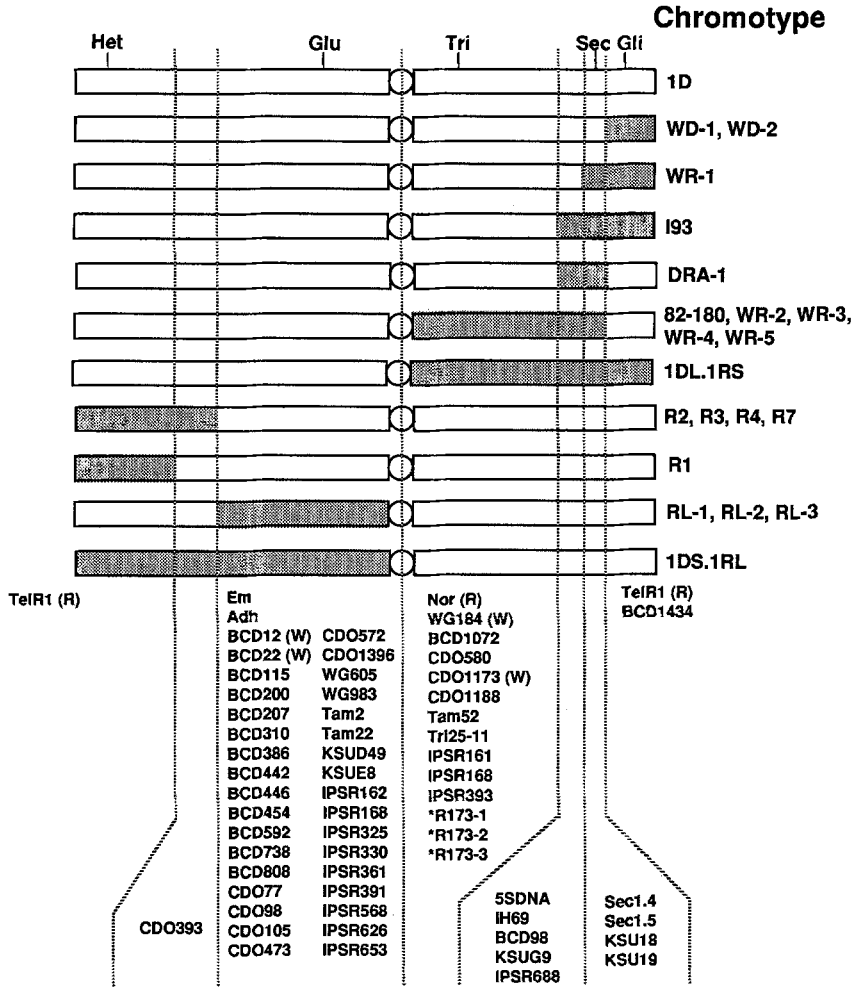

Fig. 2. Mapping of RFLP and PCR markers to chromosome regions defined by chromosome breakpoints of wheat-rye recombinants (chromotypes). The structure of the wheat-rye recombinants is shown schematically with *empty* and *dotted boxes* representing wheat and rye chromatin, respectively. The seed protein and cytogenetic markers used for the isolation of the recombinants are shown on top, the RFLP and PCR (*) markers used for their characterisation at the bottom, of the chromosome segments they mapped to. Rye (*R*) or wheat (*W*) specificity of RFLP probes is indicated. The positions of markers or breakpoints do not reflect genetic or physical distances

chromatin. This includes recombinant RL-2, where the analysis was complicated by the heterozygosity of the individual plant used for the DNA preparation. The results obtained with another 36 RFLP probes on the long-arm recombinants and a total of 22 RFLP probes and three PCR markers on the eight primary and one derived short-arm recombinants, are summarized in Fig. 2.

Rye and wheat specific probes

Using wheat cv Chinese Spring as a reference, the lack of one or more 1D bands usually was accompanied by the appearance of one or more 1R bands. This was not the case for the two probes pScR4 and pAW161 (marked with an 'R' in Fig. 2), which were known to detect rye-specific sequences in the spacer of the rDNA and near the rye telomeres, respectively (Appels et al. 1986; Guidet et al. 1991). Furthermore four RFLP probes (marked with a 'W' in Fig. 2) appeared to be wheat specific, that is, the recombinants lacking 1D bands do not possess extra 1R bands or show the increased signal strength of the A or B genome bands. Possible explanations include true wheat specificity or experimental artefacts such as excessive stringency in the Southern washes (some 1R bands were considerably fainter than their 1D counterparts) or the small size of the rye fragments (fragments smaller than 0.7 kb were not retained in the gels). Despite their apparent specificity these probes were useful for the analysis of breakpoints in the recombinants and the results are included in Fig. 2.

Breakpoint differences detected only in short-arm recombinants with distal rye chromatin

The RFLP probes clearly demonstrated at least three different breakpoints for the four short-arm recombinants with distal rye chromatin. The recombinant I93 showed the 1RS pattern with the probes BCD98, IH69, KSUG9, IPSR688 and ScT7 (*5SDNA-R1*), while recombinants WR-1, WD-1 and WD-2 showed the 1DS pattern. Therefore, the I93 breakpoint is located proximal to these markers and to the three other breakpoints. Probes, Sec 1.5, 1.4, KSU18 and KSU19,

on the other hand, showed a rye band with I93 and WR-1, but not with WD-1 or WD-2. This places the WD-1 and WD-2 breakpoint(s) distal to these markers. Since no probe detected a polymorphism between WD-1 and WD-2, it could not be decided whether or not these two recombinants had different breakpoints.

In contrast, only one other breakpoint difference was found within the other three groups of recombinants. All five short-arm recombinants with proximal rye chromatin, and all three long-arm recombinants with proximal rye chromatin, showed identical patterns within the respective groups with all probes used. The long-arm recombinants with distal rye could only be differentiated by one probe out of 37 tested.

Confirmation of the DRA-1 genotype

The recombinant DRA-1 had been derived by crossing the primary recombinants 82-180 and I93 (Shepherd et al. 1990). Its genotype had been verified for the *Tri*, *Sec*, *SrR* and *Gli* markers. To confirm the structure of the group 1 chromosome as a true recombination event DRA-1 was assayed with all RFLP and PCR probes in the same manner as all the primary short-arm recombinants. As summarized in Fig. 2, DRA-1 behaved as expected for an homologous recombination event between 82-180 and I93. Five markers for the rye chromatin present in DRA-1 were identified. They may be of considerable value in fine mapping and possibly for isolating the disease resistance genes.

Discussion

A total of 17 recombinants, putatively between wheat chromosome 1D and rye chromosome 1R, were screened with RFLP and PCR probes in an attempt to map the chromosome breakpoints. While three different breakpoints were established for the group of four short-arm recombinants with distal rye chromatin, and two breakpoints were found between the five distal long-arm recombinants, no differences were found within the group of five short-arm and three long-arm recombinants with proximal rye chromatin. Theoretically, the failure to discover breakpoint differences within these groups might be caused by a clustering of breakpoints in certain chromosome regions or by a non-random distribution of the markers assayed. Neither possibility can be excluded. Breakpoint clustering reflects variable homoeologous recombination frequencies along a chromosome. An increase in the frequency of homologous recombination from the centromere to the telomeres has been demonstrated by comparisons of genetic and physical maps of 1R (Gustafson et al. 1990) and 1D (B. Gill, personal communication). For example *Sec-R1*, the most distal marker common to the group of proximal short-arm recombinants, has been mapped in the proximal third of 1RS on a genetic consensus map (Baum and Appels 1991) but in the distal sixth of the arm on a physical map (Gustafson et al. 1990). The genetic consensus map of 1R also gives clear evidence of a non-random distribution of supposedly random RFLP markers. With the exception of the multi-locus probe IH69, all probes map to the central third of the chromosome. Similar clustering, which is not always located close to the centromere, has been found in genetic maps of other cereals based on RFLPs, such as *T. tauschii* (Gill et al. 1991) and barley (Heun et al. 1991).

In the case of the proximal short-arm recombinants, consideration must be given to the scheme used for the detection of the wheat-rye recombinants. Only recombinants with a rye segment distal to *Sec-R1* could be recognised (Rogowsky et al. 1991a). The clear clustering of the 11 RFLP and three PCR markers proximal to *Sec-R1* (i.e., in the proximal third of the genetic map), made it impossible to detect possible breakpoint differences. It remains unclear whether the five recombinants are structurally identical or whether they have variable breakpoints as with the distal short-arm recombinants. They are certainly not simple deletions, because they have lost the subtelomeric repeat detected by pAW161 (*Tel-1RS*) but gained the *Gli-D1* seed protein marker of wheat.

The most distal marker which has been mapped on the long arm of wheat group 1 chromosomes is *XAdh* (Mitchell et al. 1989), at about a third of the arm length from the centromere. The results presented in Fig. 2 clearly show that the chromosome breakpoints of all long-arm recombinants are located distal to this marker. Since there was no selection for certain recombination events during the isolation of the long-arm recombinants, the results imply that homoeologous recombination occurs more frequently at greater distances from the centromere. This conclusion is made under the assumption that the recombinants are true meiotic recombinants and did not arise from 'special events' such as deletions or translocations of telomers. It also remains unclear whether the distal recombinants involve 1D or another wheat chromosome and whether there is an overlap in the rye segments of the distal and proximal recombinants.

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