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Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat

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Summary. The introgression of genetic material from alien species is assuming increased importance in wheat breeding programs. One example is the translocation of the short arm of rye chromosome 1 (1RS) onto homoeologous wheat chromosomes, which confers disease resistance and increased yield on wheat. However, this translocation is also associated with dough quality defects. To break the linkage between the desirable agronomic traits and poor dough quality, recombination has been induced between 1RS and the homoeologous wheat arm 1DS. Seven new recombinants were isolated, with five being similar to those reported earlier and two having a new type of structure. All available recombinants were characterized with DNA probes for the loci *Nor-R1*, *5SDna-R1*, and *Tel-R1*. Also, the amount of rye chromatin present was quantified with a dispersed rye-specific repetitive DNA sequence in quantitative dot blots. Furthermore, the wheat-rye recombinants were used as a mapping tool to assign two RFLP markers to specific regions on chromosome arms 1DS and 1RS of wheat and rye, respectively.

Key words: Wheat-rye recombinants – Homoeologous recombination – Repetitive DNA sequences – RFLP markers – mapping

Introduction

Wheat is one of the most important food crops in the world and attempts to expand the gene pool by introducing desirable genetic information from alien species were initiated more than a century ago (reviewed in Sharma and Gill 1983; Fedak 1985; Gale and Miller 1987). Since the early 1930s wheat breeders have successfully introduced important traits like disease resistance from related species (McFadden 1930), and many valuable cultivars have been released. Hexaploid wheat (*Triticum aestivum*) is included in one of the 25 genera in the tribe *Triticeae* (Miller 1987). Genetic information from many of the 300 or more species within the *Triticeae* can be transferred to wheat by simple crosses, while in other cases embryo rescue or chromosome pairing control mutants may be required to achieve the transfer.

Cereal rye (*Secale cereale*) is an important source of useful alien genes for wheat improvement. Whole genomes (Triticales), individual chromosomes (addition or substitution lines), or chromosome arms (translocation lines) have been introduced into wheat (reviewed in Gale and Miller 1987). Of special interest is the short arm of rye chromosome 1RS, which confers resistance to several foliar diseases (listed in Koebner and Shepherd 1986) along with wide adaptation and high yield performance (Rajaram et al. 1983). However, the flour obtained from 1BL.1RS and 1DL.1RS translocation lines leads to poor dough quality in bread making. To break the linkage between the desirable and nondesirable agronomic traits, recombination between 1RS and 1DS was induced using Sears' (1977) *ph1b* mutant or nullisomic 5B stocks, both of which allow rare recombination between wheat and rye chromatin (Koebner and Shepherd 1986). Two recombinants, 82–180 and I 93, were isolated initially and their agronomic performance was analyzed after backcrossing to cultivar Gabo, to reduce the content of undesirable alleles of Chinese Spring that had been introduced with the *ph* mutant or the nulli 5B line (Koebner and Shepherd 1988; Shepherd et al. 1990). Both recombinant lines were resistant to stem rust, but the dough quality

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was intermediate between the original 1DL.RS translocation and the Gabo recurrent parent. A secondary recombinant, DRA-1, which was derived from 82–180 (proximal rye) and I 93 (distal rye) by homologous recombination in their common rye segment, also possessed the rye source of stem rust resistance, but after only one cross to Gabo it still had poor dough quality (Shepherd et al. 1990). The failure to obtain rust-resistant recombinants having good dough quality in this initial work led to a search for additional recombinants, with the expectation that different crossover points between the wheat and the rye chromatin might lead to the desired break in linkage.

Besides their potential for wheat improvement, wheat-rye recombinants may prove useful as a mapping tool in wheat genetics. In human genetics, RFLP probes are routinely assigned to chromosome regions with the use of somatic rodent-human cell hybrids, which contain parts of human chromosomes (Murray et al. 1982; Van Keuren et al. 1986). Similarly, a series of wheat-rye recombinants with crossover points evenly distributed along a chromosome arm could be used for the localization of new genetic markers on that chromosome arm. This physical mapping is technically simpler than the painstaking determination of genetic distances. Depending on the density of crossover points, approximate localizations of new markers within chromosome regions or detailed genetic maps may be obtained. Thus, the wheat-rye recombinants can be used to assign markers of previously unknown location to specific chromosome regions.

This paper describes the isolation of wheat-rye recombinants, including five more of the 82–180 and I 93 types as well as two of a new type. Furthermore, the structure of all recombinants presently available has been tested using a variety of DNA probes, and two RFLP markers have been localized to specific regions of 1DS utilizing these recombinants.

Materials and methods

Genetic material

All translocation stocks and recombinant lines available are schematically presented in Fig. 1 A, B. The 1DL.1RS translocation line (Shepherd 1973) and the recombinant lines I 93 and 82–180 have been described earlier (Koebner et al. 1986). The derived recombinant lines DRA-1 and DRA-2 (Fig. 1 B) were obtained by homologous recombination after hybridizing I 93 with 82–180 (Shepherd et al. 1990). The isolation of the recombinant lines WR-1, WR-2, WR-3, WR-4, WR-5, WD-1, and WD-2 is described herein. Wheat cultivars Chinese Spring, Gabo, and Warigal and rye cv Imperial were used as controls.

Assays for biochemical markers

Dried seeds were cut in half. The embryo half was germinated, while an unreduced endosperm protein extract was prepared from the endosperm half according to Singh and Shepherd (1985). Protein bands corresponding to the biochemical markers *Tri-D1* and *Gli-D1* on chromosome arm 1DS and *Sec-R1* on chromosome arm 1RS were visualized by one-dimensional SDS-PAGE.

Origin of seed used to screen for wheat-rye recombinants

The starting material was a 1DL.1RS translocation line originally isolated in a Chinese Spring background (Shepherd 1973), but subsequently backcrossed first to cv Halberd and then to cv Warigal, as detailed in the following pedigree: [(1DL.1RS CS × Halberd^3) × Warigal^3]. This line was crossed to a stock of Chinese Spring heterozygous for Sears' (1977) *ph* mutant and, following the strategy and criteria of Koebner and Shepherd (1985), an F_2 plant (121/83) heterozygous for the 1DL.1RS translocation and homozygous for *ph1b* was obtained from this cross (Fig. 1). Homozygosity for *ph1b* was indicated by the occurrence of multivalent associations at metaphase I in pollen mother cells (pmcs) from this plant. The *ph1b* status was confirmed by analyzing meiotic pairing behavior in six progeny from testcrosses with *Aegilops variabilis*. However, plant 121/83 set only a few seed, and additional plants (F_3) heterozygous for 1D and 1DL.1RS and homozygous for *ph1b* were selected from among its progeny and allowed to self-fertilize. The F_4 progeny from one of these plants (448/84) were also screened for recombinants.

DNA probes

The 2.4-kbp *Taq*I fragment of plasmid pScR4 (Appels et al. 1986) was used as a probe for *Nor-R1*, plasmid pScT7 (Reddy and Appels 1989) as a probe for *5SDna-R1*, plasmid pTri25-11 (Dr. N.K. Singh, unpublished results) as a probe for *Tri-D1*, plasmid pAW161 (Guidet et al. 1990) as a probe for the rye-telomere-specific 350- to 480-bp family of repetitive DNA sequences (the locus on 1RS was designated *Tel-R1*), and plasmid pAW173 (Guidet et al. 1990) as a probe for the interspersed rye-specific R173 family of repetitive DNA sequences. Plasmid pL10a2 is an 800-bp *Eco*RI fragment subcloned from PSR161 (Sharp et al. 1988), which maps on the short arm of chromosome 1 in wheat and rye. 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

A segment of leaf approximately 10 cm long was placed in an Eppendorf tube, frozen in liquid nitrogen for 5 min, and crushed to a fine powder with a knitting needle. Six hundred microliters of DNA extraction buffer [100 m*M* TRIS-HCl (pH 8.5), 100 m*M* NaCl, 10 m*M* EDTA, 4% sarkosyl], and 600 µl phenol:chloroform:iso-amylalcohol (25:24:1) were added, and the material was extracted by frequent hand mixing for 5 min. The phases were separated by centrifugation in an Eppendorf centrifuge and the aqueous phase was re-extracted as described above. The DNA was precipitated by the addition of 50 µl 3 *M* Na-acetate (pH 4.8) and 1 ml 99% ethanol. It was collected by centrifugation, washed with 70% ethanol, dried, and resuspended in 50 µl TE buffer [10 m*M* TRIS-HCl (pH 8.0), 1 m*M* EDTA] containing 40 µg/ml heat-treated RNase A.

Southern blots

Approximately 5 µg plant DNA was digested with restriction endonucleases for 4 h, separated on agarose gels overnight, and transferred to membrane Hybond C Extra (Amersham) following the recommendations of the manufacturer. Prehybridization was carried out at 65 °C in 20 m*M* PIPES (pH 6.8), 0.6 *M* NaCl,

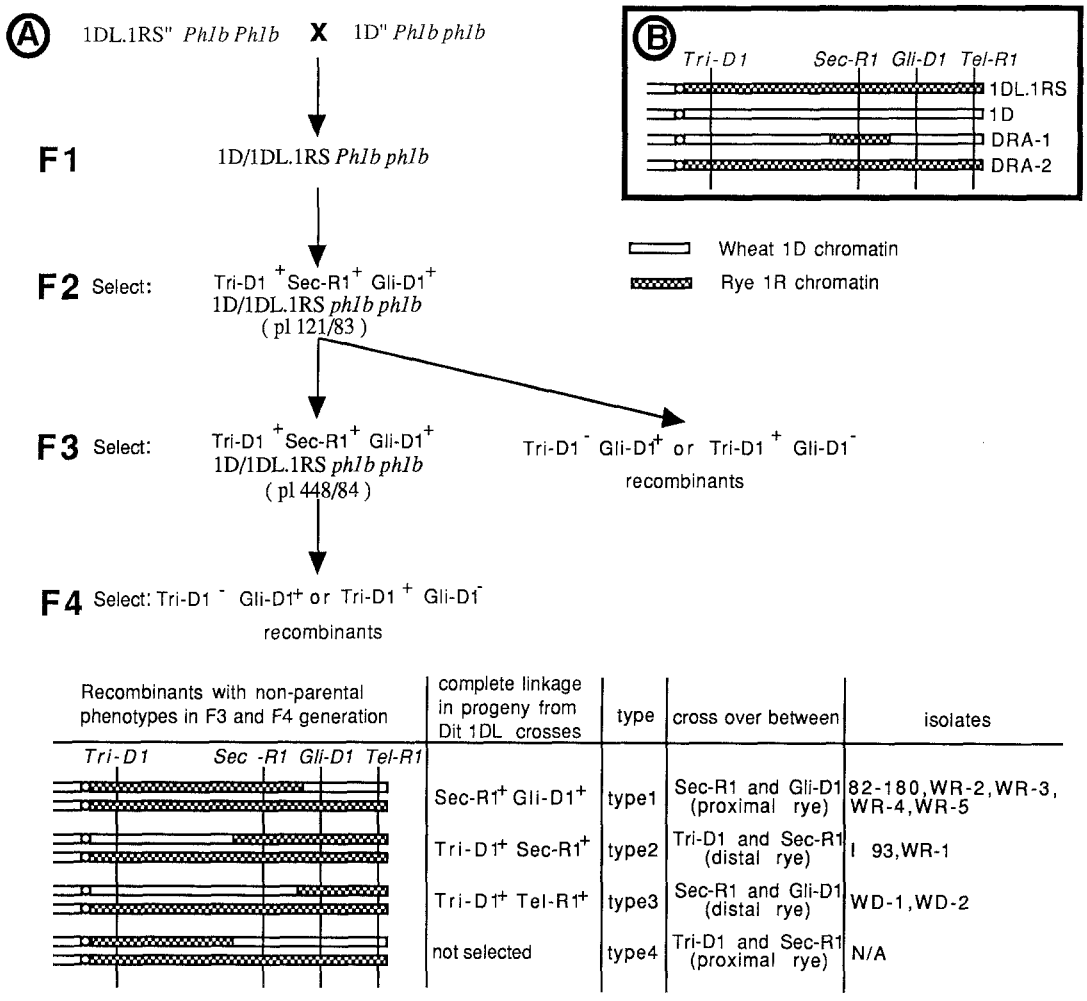

Fig. 1 A and B. Scheme for production, recovery, verification, and structure of wheat-rye recombinants in the short arm of chromosome 1 (**A**) and scheme for the structure of derived recombinants (**B**)

5 m*M* EDTA, 1% SDS, 0.2% gelatin, 0.2% ficoll, 0.2% polyvinyl-pyrollidone, 0.5% tetrasodium pyrophosphate, and 500 µg/ml carrier DNA, while the hybridization solution contained less carrier DNA (100 µg/ml) 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 (20 × SSC is 3 *M* NaCl, 0.3 *M* Na_3-citrate), 0.5 × SSC, and 0.1 × SSC, respectively.

Squash blots

In a modification of the procedure of Hutchinson et al. (1985), green leaf tissue was directly squashed onto a nylon membrane. Three sheets of Whatman 3 MM paper were soaked in 0.4 *M* NaOH, placed in the lid of a lunch box, and overlaid with a sheet of Hybond-N^+ nylon membrane (Amersham, also soaked in 0.4 *M* NaOH) and a plastic screen with holes 0.5 cm in diameter. A piece of leaf tissue was held just above one of the holes and squashed onto the membrane with a metal plunger. Subsequently, the membrane was rinsed in 5 × SSC for 2 min and hybridized as described above.

Copy number determination

Serial dilutions of plant DNA (1–1,000 ng) and of the plasmids pAW173 and pUC19 (both 1–1,000 pg) were denatured in 0.4 *M* NaOH for 10 min, spotted on a Hybond-N^+ membrane (Amersham) in a Minifold dot-blot apparatus (Schleicher and Schuell) washed with 0.4 *M* NaOH, and hybridized with the 450-bp *Bam*HI insert of pAW173. After autoradiography the dots were cut from the membrane and counted in a scintillation counter. Following correction with the pUC19 values, the pAW 173 values were used to establish a standard curve and to calculate the copy number, assuming that all the recombinant genomes (6n) are the same size as wheat (36.2 pg).

Results

Isolation of additional 1DS-1RS wheat-rye recombinants

Electrophoretic screening to detect new recombinants was carried out on 110 F_3 seeds and 645 F_4 seeds from plants of genotype 1D/1DL.1RS *ph1bph1b*. In both cases, the large majority of progeny exhibited parental phenotype combinations segregating in the 1:2:1 ratio expected if there was no selection against gametes carrying the rye arm 1RS (Table 1). However, 1 and 25 putative recombinants were identified among the F_3 and F_4 prog-

Table 1. Endosperm protein phenotypes and their frequency in progeny from F_2 and F_3 plants with genotpye 1D/1DL.1RS *ph1bph1b*

Endosperm protein phenotype			Frequency in		Classification
Tri-D1	*Gli-D1*	*Sec-R1*	F_3 progeny	F_4 progeny	
+	+	+	56	303	parental
+	+	−	27	165	parental
−	−	+	26	152	parental
+	−	+		11	potential recombinant
+	−	−		2	potential recombinant
−	+	+	1	11	potential recombinant
−	+	−		1	potential recombinant

+ = present
− = absent

eny, respectively. These 26 plants were retained for progeny testing to confirm their recombinant status. Two plants were shown to be misclassified and 8 plants died as seedlings . The other 16 plants were grown to maturity and crossed with a stock of ditelocentric 1DL (Dit 1DL) in a Gabo background, in order to confirm their phenotype and to study their segregation of markers in progeny of this first cross and also in the next generation after self-fertilization. Dissociation of the *Tri-Gli* markers could only be selected in approximately half of the F_3 or F_4 progeny since, in order to express its phenotype, it was necessary for the gamete carrying this dissociation to combine with a gamete carrying the 1RS arm. Furthermore, gametes showing *Tri-Gli* dissociation were expected to include the desired 1DS-1RS wheat-rye recombinants, as well as wheat-wheat recombinants resulting from homoeologous recombination between 1DS and related 1AS and 1BS arms. As shown in Fig. 1, four types of wheat-rye recombinants are expected with respect to the wheat markers *Tri-D1* and *Gli-D1* and the rye markers *Sec-R1* and *Tel-R1*. Three of these four types could be distinguished by the complete linkage between *Sec-R1*$^+$ and *Gli-D1*$^+$ (type 1), *Tri-D1*$^+$ and *Sec-R1*$^+$ (type 2), and *Tri-D1*$^+$ and *Tel-R1*$^+$ (type 3) in progeny from crosses between the putative recombinants and Dit1DL, and also in the next generation from selfing those plants that were heterozygous for Dit1DL and the recombinant chromosome. The other type of wheat-rye recombinant (type 4) could not be distinguished from wheat-wheat recombinants with this method of analysis. After carrying out these tests, 4 of the putative recombinants were classified as type 1 wheat-rye recombinants (WR-2, WR-3, WR-4, WR-5) and 1 as type 2 (WR-1), similar to 82–180 and I 93, respectively, as isolated earlier (Koebner and Shepherd 1986). Also, two type 3 recombinants (WD-1, WD-2) were detected with the rye-telomere-specific probe pAW161; this type of recombinant had not been detected in the earlier study. These tests indicated that the remaining 9 putative recombinants were either wheat-wheat or type 4 wheat-rye recombinants and they have not been analyzed further.

All except two (WR-4, WR-5) of the new wheat-rye recombinants were tested for reaction to stem rust, and all of those carrying the *Sec-R1* locus (WR-1, WR-2, WR-3) were resistant, while the two not carrying it (WD-1, WD-2) were susceptible.

Analysis with the rye-telomere-specific probe pAW161

After their isolation, the four existing and the seven new recombinants were all backcrossed to wheat cv Gabo, in order to assess their agronomic performance later in a common genetic background. Homozygotes for the recombinant chromosomes, which were selected from the F_2 of the backcross and were mainly in a mixed Chinese Spring/Gabo background, were used for the analysis with DNA probes. The parental chromosomes (1DL.1RS and 1D) in wheat cv Chinese Spring, the cultivar used for the backcrosses (Gabo), and another translocation line (1AL.1RS in wheat cv Chinese Spring) were included as controls.

The recombinants were probed with pAW161 in squash blots (Fig. 2a). In addition to WD-1 and WD-2, which had been isolated with this probe, the other two recombinants with distal rye chromatin (WR-1 and I 93) as well as DRA-2 were positive for the corresponding locus *Tel-R1*. Recombinants with proximal rye chromatin and DRA-1 were negative, confirming the structure of the recombinant chromosomes deduced from the biochemical markers.

Analysis of the ribosomal DNA with pScR4 and pScT7

The rye *Nor-R1* locus is located proximal to the *Sec-R1* marker on 1RS (Lawrence and Appels 1986). To test for its presence in the recombinants, the 2.4-kbp *Taq*I fragment of plasmid pScR4 was used as a probe in Southern blots (Fig. 2b). This subfragment of the insert stems from the rDNA spacer region of rye and hybridizes strongly to the main locus of the 18S-5.8S-26S rDNA operon on chromosome arm 1RS. Only weak cross-hybridization happens to the main wheat loci on chromosomes 1B and 6B (Appels et al. 1986). As indicated by a strongly hybridizing band of 2.4 kbp in *Taq*I digests, *Nor-R1* was present in all type 1 recombinants (82–180, WR-2, WR-3, WR-4, WR-5) as well as in the derived recombinant DRA-2, and absent from all recombinants with distal rye as well as DRA-1. These results narrowed down the crossover point of the type 2 recombinants, I 93 and WR-1, to the region between *Nor-R1* and *Sec-R1*. Furthermore, they are consistent with a crossover in type 1 and type 3 recombinants distal to *Sec-R1*.

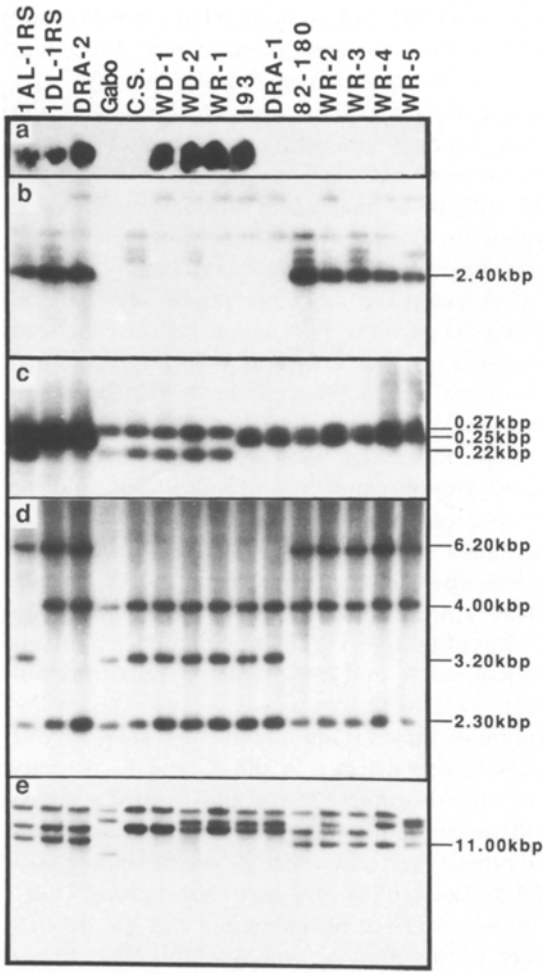

Fig. 2a–e. Analysis of wheat-rye recombinants with DNA probes. Squash blots (**a**) and Southern blots (**b–e**) of the lines shown were probed with pAW161 (**a**), pScR4 (**b**), pScT7 (**c**), PSR161 (**d**), and Tri25-11 (**e**). The restriction endonuclease used, the concentration of the agarose gel, and the autoradiography time were: **b** *Taq*I, 1%, 3 h; **c** *Taq*I, 3%, 12 h; **d** *Bam*HI, 0.8%, 24 h; and **e** *Bam*HI, 0.8%, 48 h

One major locus of the rye 5S RNA, *5SDna-R1,* is located distal to *Nor-R1* on 1RS (Reddy and Appels 1989), while its position relative to *Sec-R1* is not known. It can be detected as a strong 0.25-kbp band in *Taq*I digests of rye DNA probed with pScT7, which contains one 5SDNA unit. The probe cross-hybridizes to 5SDNA loci on wheat chromosome arms 1BS and 1DS, represented by 0.27- and 0.22-kbp bands, respectively. Similar to the *Nor-R1* locus, all type 1 recombinants had the rye allele and all type 3 recombinants, the wheat allele (Fig. 2c). An interesting difference could be seen between the two type 2 recombinants. While WR-1 possessed the 0.22-kbp band typical of 1DS (Fig. 2c), I 93 exhibited the 0.25-kbp band, indicating the presence of the rye allele (Fig. 2c). This different pattern showed clearly that the crossover points of WR-1 and I 93 were not in the same

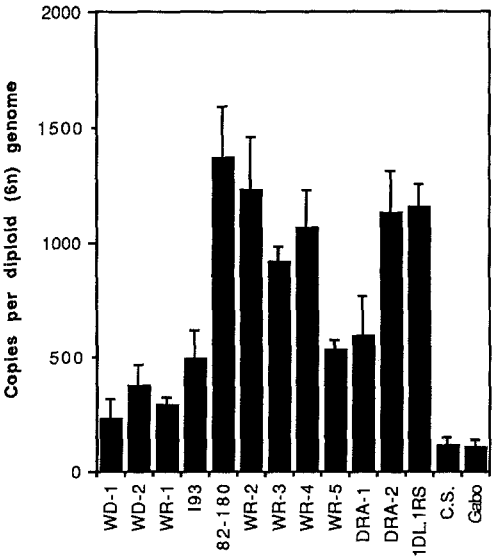

Fig. 3 Copy number of the R173 family in recombinants between 1RS and 1DS. *Error bars* indicate the standard error above the mean based on at least three independent measurements

position and that the *5SDNA-R1* locus was located between them. Furthermore, the *5SDNA-R1* locus was located proximal to *Sec-R1* because both recombinants were *Sec-R1* positive.

Quantitation of rye chromatin with pAW173

Even though the crossover points of individual isolates from one given type of recombinant (Fig. 1) all lie in a region defined by seed storage protein markers, they are probably not in identical positions. Due to the lack of additional well-characterized genetic markers on the short arms of group 1 homoeologous chromosomes (1S) in the *Triticeae,* we attempted to quantify the amount of rye chromatin present in each recombinant by utilizing probe pAW173. This probe hybridizes to the R173 family of rye-specific repetitive DNA sequences, which is dispersed throughout all rye chromosomes (Guidet et al. 1990). Assuming a reasonably even distribution along chromosome arm 1S, it was theoretically possible to estimate the copy number of the R173 family and to use it as an indication of the amount of rye chromatin present in individual recombinants.

The estimate of copy number in the recombinants ranged between the extreme values obtained for the wheat control lines (Chinese Spring and Gabo) and the 1DL.1RS translocation line (Fig. 3). The significantly lower copy number in WR-5 compared to the other type 1 recombinants was the first indication for different crossover points within this group. Among the type 2 recombinants, the copy number of WR-1 was significantly lower than that of I 93, consistent with the result obtained with probe pScT7 showing that the crossover

point of WR-1 is distal to that of I 93. No significant differences were observed between WD-1 and WD-2, DRA-1 and I 93, or DRA-2 and 1DL.1RS.

Mapping of PSR161 and Tri25-11 with wheat-rye recombinants

The introgression of human chromatin into foreign cell lines has been used as a routine mapping method in human genetics for many years. To demonstrate the use of the wheat-rye recombinants in 1S as a mapping tool, PSR161, an RFLP marker of unknown location on 1S (Sharp et al. 1988), was mapped to a chromosome region in a model system. According to Sharp et al. (1988), the probe gives rise to characteristic *Bam*HI bands for the A-genome (13.5 and 4.0 kbp), the B-genome (7.8 and 2.3 kbp), the D-genome (4.0 kbp), and the R-genome (6.2 kbp). Since the corresponding plasmid actually contained four insert fragments, which could possibly stem from noncontiguous regions on chromosome arm 1S, we subcloned the largest fragment, an 800-bp *Eco*RI fragment, resulting in plasmid pL10a2. The pattern obtained with this fragment (Fig. 2d) was slightly different from that published for PSR161. The control lanes with DNA from the 1AL.1RS and 1DL.1RS translocation lines and the wheat cultivars Gabo and Chinese Spring revealed hybridizing bands of 6.2, 4.0, 3.2, and 2.3 kbp, which were characteristic for the R-, A-, D-, and probably the B-genome, respectively. While the absence of the 7.8 and 13.5-kbp bands could be easily explained by the subcloning of only one insert from PSR161, the presence of a 3.2-kbp band was surprising. However, this band must correspond to the D-genome, since it is replaced by the 6.2-kbp rye band in the 1DL.1RS translocation line (Fig. 2d, lane 2).

While the patterns of all recombinants with distal rye chromatin (WD-1, WD-2, WR-1, I 93), as well as DRA-1 were identical to the wheat control lines, the patterns of DRA-2 and all type 1 recombinants (82–180, WR-2, WR-3, WR-4, and WR-5) were identical to that of the 1DL.1RS translocation line. These results placed this RFLP marker between the centromere and the wheat-rye breakpoint in I 93.

In a similar manner, the RFLP marker detected with probe Tri25-11 was also mapped to the chromosome segment between the centromere and the wheat-rye crossover point in I 93. An 11.0-kbp band characteristic for the rye allele was present only in type 1 recombinants (Fig. 2e). This result was consistent with the isolation of this probe from a cDNA clone for the *Tri-D1* locus (N. Singh, personal communication, 1991).

Discussion

We present here the isolation and characterization of seven new wheat-rye recombinants between chromosomes arms 1DS and 1RS. Among them are WD-1 and WD-2, the first two representatives of type 3 recombinants (Fig. 1), which are characterized by distal rye chromatin and a crossover point between the *Sec-R1* and *Gli-D1* loci. Recombination between 1DS and 1RS occurred in a plant heterozygous for the translocation 1DL.1RS and the normal wheat chromosome 1D and homozygous for the *ph1b* mutation, which allows the otherwise restricted, allosyndetic recombination. The selection of potential recombinants among self-fertilized progeny was based on a dissociation between the seed storage protein markers *Tri-D1* and *Gli-D1*. These two markers originally had been chosen by Koebner and Shepherd (1986) because they were easy to score and only loosely linked; thus, large numbers of individuals could be analyzed without major technical problems, and the frequency of recombination was maximized by the large genetic distance. Using the presence of the *Sec-R1* marker as an indication for the involvement of 1RS and not 1AS or 1BS in the recombination with 1DS, recombinant WR-1, with distal rye chromatin, and recombinants WR-2, WR-3, WR-4, and WR-5, with proximal rye chromatin, were isolated.

A feature of these results was the high frequency of *Tri-Gli* dissociation detected in the F_3 and F_4 progeny groups tested. Altogether, 26 out of 755 progeny seed showed dissociation, but only 16 of these could be confirmed in progeny tests; two were shown to be misclassified and 8 died as seedlings. Of these dissociations, 7 were shown to be wheat-rye recombinants and the other 9 were either wheat-wheat or type 4 wheat-rye recombinants. These results may be contrasted with the earlier experiments aimed at recombining 1RS and 1DS (Koebner and Shepherd 1986). In testcross progeny from a *ph1b* homozygote, they only detected four dissociations between *Tri-Gli* in 394 progeny tested, whereas they observed 14 (12 confirmed in progeny tests) dissociations in 531 progeny, equivalent to an F_2 generation, derived from plants nullisomic for chromosome 5B. However, only 2 of these 18 selected plants were confirmed to be wheat-rye recombinants, with one (82–180) coming from the testcross progeny and the other (I 93) from the F_2 progeny. The reason for the much higher frequency of *Tri-Gli* dissociations and wheat-rye recombination observed in the present study is not clear, since it also involved the *ph1b* mutant and F_2-equivalent progeny, just as in the previously described experiments. The different genetic backgrounds, the growing conditions at meiosis or different recombination frequencies in male and female gametes may have influenced the degree of homoeologous pairing and recombination.

The four new type 1 isolates (crossover between *Sec-R1* and *Gli-D1*, proximal rye chromatin) could not be distinguished from each other or from the previous isolate 82–180 with probes for *Nor-R1*, *5SDNA-R1*, and

Tel-R1. Two probes with unknown location on 1 S also gave identical patterns for the five recombinants. However, the quantitative copy number assay for the dispersed rye-specific R173 family suggested that less rye chromatin is present in WR-5 and that the crossover point of this isolate is proximal to those of the other four type 1 recombinants. Considering the low density of markers on chromosome arm 1S and the rather large error in the copy number determination, no conclusions could be drawn on the relative positions of crossover points for 82–180, WR-2, WR-3, and WR-4.

The new type 2 isolate, WR-1, shared with the previous isolate, I 93, a crossover point between *Tri-D1* and *Sec-R1*, distal rye chromatin, the absence of *Nor-R1* and the presence of *Tel-R1*. However, differences were observed with the probe for *5SDNA-R1* and in the quantitative assay with pAW173. These results demonstrated that the crossovers between 1DS and 1RS occurred at different positions for WR-1 and I 93, and that the WR-1 breakpoint is closer to the telomere. They indicate that the *5SDNA-R1* locus is situated between the two breakpoints and, therefore, also between *Nor-R1* and *Sec-R1*. The proposed gene order on 1RS is centromere/*Nor-R1*/I 93 breakpoint/*5SDNA-R1*/WR-1 breakpoint/*Sec-R1*/telomere.

The novel type 3 recombinants WD-1 and WD-2 were isolated by screening for *Tel-R1* instead of *Sec-R1*. *Tel-R1* is the locus of the 350- to 480-bp family of rye-telomere-specific repetitive DNA sequences on chromosome arm 1RS. Using this outside marker, wheat-rye cross overs between *Sec-R1* and *Gli-D1*, rather than *Tri-D1* and *Sec-R1*, were detected. Recent results suggest that this type 3 recombination may provide the desired break in linkage between the disease resistance and the poor dough quality factor. The stem rust resistance marker *Sr-R* has been mapped distal to *Sec-R1* (Singh et al. 1990), and it has been suggested that the *Sec-R1* marker itself may be responsible for poor dough quality. Although the two isolates WD-1 and WD-2 were susceptible to stem rust (data not shown), further attempts for the isolation of recombinants will focus on type 3 recombinants.

The value of wheat-rye recombinants as mapping tools was exemplified by the mapping of probes PSR161 and Tri25-11 in the region proximal to the wheat-rye breakpoint in I 93. The crucial question, whether the crossovers occur at random along the length of the chromosome arm or whether they are clustered in certain hot spots, was only partially answered. Differences in the crossover points were demonstrated for one of five type 1 isolates, for the two type 2 isolates, but not for the two type 3 isolates. The current activity in several laboratories to isolate additional RFLP markers for chromosome 1 may provide an answer to this question. When sufficient molecular markers have been isolated to densely map chromosome arm 1RS, it will be possible to accurately define the crossover points involved in wheat-rye recombination. This will allow much more precision in these 'chromosome engineering' experiments, since it will then be possible to choose two recombinants, one with a proximal and the other a distal rye segment, with a small overlapping segment of rye carrying the target gene. By intercrossing these two primary recombinants it will be possible to select secondary recombinants, similar to DRA-1 (Shepherd et al. 1990), which will carry only the small overlapping segment of rye chromatin bearing the desired rye gene.

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