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A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene, *CreR*

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Abstract A genetic map of the long arm of chromosome 6R of rye was constructed using eight homoeologous group-6 RFLP clones and five PCR markers derived from the rye-specific dispersed repetitive DNA family, R173. The map was developed using a novel test-cross F_1 (TC-F_1) population segregating for resistance to the cereal cyst nematode. Comparisons were made between the map generated with other rye and wheat group-6 chromosome maps by the inclusion of RFLP clones previously mapped in those species. Co-linearity was observed for common loci. This comparison confirmed a dramatic reduction in recombination for chromosome 6R in the TC-F_1 population. The *CreR* locus was included in the linkage map via progeny testing of informative TC-F_1 individuals. *CreR* mapped 3.7 cM distal from the RFLP locus, *XksuF37*. Comparative mapping should allow the identification of additional RFLP markers more closely linked to the *CreR* locus.

Key words CCN · RFLP · PCR · *Heterodera avenae* · Genetic mapping

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Introduction

The nematode *Heterodera avenae* Wollenweber, often referred to as the cereal cyst nematode (CCN), has been recorded in all of the major wheat-growing regions of Australia (Brown 1982). Yield losses in an intolerant cultivar may be up to 30% (Fisher 1982 a) and it has been estimated that economic losses to Australian wheat growers may be as high as $173 M (Stirling et al. 1992).

The available methods of control are crop rotation (Millikan 1938), nematicides (Gurner et al. 1980), cultural practice (Andersson 1982), host resistance (O'Brien and Fisher 1974) and host tolerance (Fisher et al. 1981). However, limitations in the current levels of understanding of nematode population dynamics, particularly multiplication rates and the relation between nematode densities and damage (Fisher and Hancock 1991), seasonal variation, particularly soil temperature and moisture (Banyer and Fisher 1971), and environmental concerns arising from the use of nematicides (Schumann 1991) emphasises the importance of the application of resistant and/or tolerant cultivars in the control of this organism.

To-date, one resistance locus, *Cre*, has been identified in hexaploid wheat (Nielsen 1966; O'Brien and Fisher 1974). This locus carries a monogenic dominant gene for resistance located on chromosome 2B in the lines "Loros" (Slootmaker et al. 1974) and "AUS10894" (O'Brien et al. 1980; Nielsen 1982). Unfortunately, *Cre* confers only a moderate degree of resistance to CCN. Consequently, there is a need to identify additional sources of resistance which may be utilised in wheat breeding programmes. It has been known for a long time that cereal rye, *Secale cereale*, also carries resistance to CCN (Brown and Meagher 1970; Fisher 1982 a).

While there have been no detailed reports on the inheritance of CCN resistance found in rye, Asiedu (1986) determined that the resistance of T701-4-6

(Fisher 1982 a) was controlled by a single dominant gene (*CreR*) located on chromosome 6R. Subsequent work further localised *CreR* to the long arm of 6R (Asiedu et al. 1990; Dundas et al. 1992). This gene confers very strong resistance to CCN and is now available to wheat breeders in the form of a Chinese Spring 6R(-6D) substitution line. It is hoped that *CreR* may be introgressed into wheat via homoeologous recombination. Until recently, the introgression of CCN resistance into commercial wheat cultivars relied upon a time-consuming and expensive bioassay to detect resistant progeny (Fisher 1982 b). However, the recent identification of molecular markers linked to CCN resistance in barley and wheat (Williams et al. 1994; Kretchmer et al. 1997) has rendered this bioassay almost obsolete. The identification of molecular markers linked to *CreR* would thus be useful for wheat breeders.

RFLP and PCR markers linked to nematode resistance loci have been identified in a number of plant species including potato (Gebhardt et al. 1993), sugarbeet (Jung et al. 1990), tomato (Messeguer et al. 1991), *Lycopersicon peruvianum* (Yaghoobi et al. 1995), *Triticum tauschii* (Eastwood et al. 1994), soybean (Weisemann et al. 1992), barley (Kretschmer et al. 1997) and hexaploid wheat (Williams et al. 1994). Although the *CreR* locus has been physically localised to the long arm of chromosome 6R, it has not been genetically mapped to this chromosome arm. Current genetic linkage maps of rye chromosome 6R are of low resolution and include few loci in common with those of wheat and barley group-6 chromosomes (Devos et al. 1993 a; Philipp et al. 1994; Loarce et al. 1996).

While chromosome 6R of rye is displays considerable homoeology to wheat and barley group-6 chromosomes, regions of 6R sharing homoeology to other chromosome groups have been identified. With respect to the long arm of rye 6R, early studies indicated homoeology to the long arm of group-3 and -7 chromosomes (Koller and Zeller 1976; Naranjo et al. 1987). The more recent study of Devos et al. (1993 a) has confirmed the presence of translocated segments of 3RL and 7RL and provided a much clearer picture of the structure of this chromosome arm.

The objectives of the present study were twofold; (1) to develop a genetic map for the long arm of chromosome 6R incorporating loci common to wheat and barley homoeologous group-6 chromosomes in order to further elucidate the degree of synteny shared between rye 6RL and wheat and barley group-6 chromosomes, and (2) to genetically map the CCN resistance locus, *CreR*.

Materials and methods

Plant material

A chromosome-6R test-cross F_1 (TC-F_1) population was generated for mapping studies. The CCN-resistant Chinese Spring-T701-4-6 6R(-6D) disomic substitution line was crossed as male to the CCN-susceptible Chinese Spring-Imperial 6R disomic addition line. This resulted in the generation of F_1 plants with an unbalanced chromosome configuration ($20W'' + 6R^{Imp\prime} + 6R^{T701\prime} + 6D'$). Since rye chromosomes rarely pair with wheat homoeologues in the presence of *Ph1* (Naranjo and Lacadena 1980), F_1 plants could be backcrossed to Chinese Spring wheat to generate a TC-F_1 population of progeny plants possessing recombinant 6R chromosomes. Two F_1 plants were selected and test-crossed to Chinese Spring wheat as female, generating 439 TC-F_1 seed. Due to the unbalanced chromosome constitution of the F_1 plants, and assuming some chromosome disjunction, six genomic configurations are possible within the TC-F_1 population [(1) $20W'' + 6R' + 6D'$; (2) $21W'' + 6R'$; (3) $20W'' + 6R'' + 6D'$; (4) $21W'' + 6R''$; (5) $20W'' + 6D'$; (6) $21W''$]. Consequently, it was necessary to screen the TC-F_1 population for individuals monosomic for chromosome 6R, [(1) $20W'' + 6R' + 6D'$ and (2) $21W'' + 6R'$].

Glutamate oxaloacetate transaminase (GOT-2) screening

The dosage of chromosome 6R (nullisomy, monosomy or disomy) present in TC-F_1 seedlings was established by screening for the isozyme glutamate oxaloacetate transaminase (GOT-2) using the procedure of Hart (1975).

DNA clones

Wheat, barley and oat homoeologous group-6 clones were obtained from Dr. M. E. Sorrells (BCD-, CDO-, WG-series), Dr. B.S. Gill (KSU-series), Dr. G.E. Hart (TAM-series), Dr. R. Appels (CS) and Dr. P. Sharp (PSR). Clones from a barley root cDNA library (AW-series) were obtained from Dr. P. Murphy.

RFLPs

DNA extraction from reference plants was carried out as described by Guidet et al. (1991). Genomic DNA extraction from individual TC-F_1 plants was carried out using the mini-prep method described in detail by Rogowsky et al. (1991). Restriction digestion, Southern blotting and hybridisation was carried out as described by Guidet et al. (1991).

Selection of clones and mapping

Forty three homoeologous group-6 clones were selected and first hybridised to membranes containing genomic DNA from the wheat cultivars "Chinese Spring" and "Schomburgk", the rye cultivar "Imperial", the Chinese Spring-Imperial 6R disomic addition line (Driscoll and Sears 1971) and the Chinese Spring-T701 6R(-6D) disomic substitution line (Asiedu et al. 1990) digested with seven restriction enzymes (*Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I), for assignment to chromosome 6R. The RFLPs identified were then localised to the long arm of 6R via hybridisation to genomic DNA isolated from a $6RL^{T701}$ di-telosomic addition to Chinese Spring wheat (I. Dundas personal communication). Clones that detected one or more well-resolved RFLPs were then hybridised to membranes containing genomic DNA from the TC-F_1 mapping population that had been digested with the appropriate restriction enzyme.

PCR markers

The generation of PCR markers for the long arm of chromosome 6R followed the approach of Rogowsky et al. (1992 a). PCR markers

were based upon sequence data obtained for three rye-specific repeat elements from the R173 family (Rogowsky et al. 1992 b) and from the rye-specific sequence contained in the clone pSc119.1 (McIntyre et al. 1990). R173-derived PCR primers targeted the flanking regions of the three R173 elements for which sequence data was available (Rogowsky et al. 1992 b) while the pSc119.1-derived primers targeted the 5′ and 3′ ends of the sequence, reading out as determined from the sequence data of McIntrye et al. (1990). The primer PawC2 was designed from sequence information from the rye-specific dispersed sequence contained in the clone pAW173 (Guidet et al. 1991).

PCR reactions were carried out as described by Rogowsky et al. (1992 a). Primer combinations were initially assessed using genomic DNA from wheat, rye, and the Chinese Spring-Imperial 6R disomic addition and the Chinese Spring-T701 6R(-6D) disomic substitution lines. PCR products were assigned to the long arm of chromosome 6R if they could be amplified from genomic DNA isolated from a $6RL^{T701}$ di-telosomic addition to Chinese Spring wheat (I. Dundas personal communication). PCR products were analysed on 3% TBE gels as described by Rogowsky et al. (1992 a).

Nematode bioassay

The assay procedure reported by Fisher (1982 b) was used with minor modifications. Seedlings were planted in Palmer soil (sandy red loam) in polyvinyl chloride tubes (27 mm diameter × 125 mm long). Seedlings were inoculated with 100 larvae/ml of water at the time of planting and four times thereafter with alternating intervals of 3 and 4 days. Plants were grown at 15°C constant temperature with a 16 h day maintained by fluorescent light (12 × 500 Watt quartz halogen). Plants were grown for 9 weeks after the last inoculation before harvesting.

CreR progeny-test

Since it was impossible to undertake large-scale screening for *CreR* within the TC-F_1 population, a strategy was devised in order to genetically map this locus via the selection and screening of individuals demonstrating recombination around the *CreR* locus. *CreR* has been physically localised to an interstitial region of chromosome 6RL (Dundas et al. 1992). Loci *Xbcd758* and *AawS5/FG3-280* have been physically mapped proximal to and distal from *CreR* respectively (data not shown). Recombination events occurring within the interval flanked by these loci are likely to be informative for the *CreR* locus. Therefore, marker phenotypes for all 280 plants within the TC-F_1 population were examined and 12 plants demonstrating recombination between *Xbcd758* and *AawS5/FG3-280* were selected for progeny-testing.

The 12 selected plants were selfed in order to generate TC-F_2 seed. TC-F_2 seedlings were then screened for *Got-R2* to establish the presence or absence of chromosome 6R. For one plant, no chromosome 6R-positive TC-F_2 individuals were detected among the four seedlings tested. Transmission of chromosome 6R ranged from 0 to 50.00%. All *Got-R2*-positive progeny seedlings identified were selected along with five to six *Got-R2*-negative sib-lines. These plants were then assayed for their reaction to CCN inoculation.

Statistical analysis of cyst counts observed on TC-F_2 seedlings derived from TC-F_1 plants 99, 116 and 158 were carried out using the Students *t*-test (Sokal and Rohlf 1969).

Data analysis

Data were collected within the TC-F_1 mapping population for five PCR and nine RFLP markers. Linkage analysis and map construction was carried out with the computer programme CRIMAP (v. 2.4) installed on a Macintosh PowerBook 3400c. A two-point lod-score analysis using the *twopoint* function identified linkage between 14 of the 15 markers. All loci except *Xtam36B* demonstrated linkage to at least one other locus within the data set with a lod score > 3.0. Initial multipoint analysis using the *build* function was carried out with a mapping LOD of 3.0. This analysis allowed the generation of a framework map. Lowering the mapping LOD value allowed the inclusion of remaining loci. The most-likely order derived using the *build* function was then tested using the *flips* function under a mapping LOD of 3.0. *Flips5* allowed the comparison of $\log_{10}$ likelihoods resulting from the permution of five loci at a time. Centimorgan (cM) values were calculated using the Kosambi mapping function (Kosambi 1944).

Results

RFLP markers

Table 1 lists all cDNA and genomic DNA (gDNA) RFLP clones used in this study. A total of 43 RFLP clones were analysed, of which 26 had been previously mapped to the long arm of wheat and/or barley group-6 chromosomes. The chromosomal arm location of the remaining 17 clones was unknown. Of the 43 clones used, 26 were cDNA and 17 were gDNA. For all probe/enzyme combinations employed, cDNA clones demonstrated stronger hybridisation with rye and resulted in less complex banding patterns to those observed using genomic DNA clones.

Twenty (76.9%) cDNA clones and seven (41.2%) genomic DNA clones were mapped to chromosome 6R (Table 1). A greater proportion of cDNA clones detected polymorphism between $6R^{Imp}$ and $6R^{T701}$ than did the genomic DNA clones (85.0% and 71.4% respectively).

Five cDNA clones (AW9, AW13, BCD221, BCD339 and CDO419) revealed more than six bands for most enzymes used. Of these, only CDO419 could be mapped to chromosome 6R of rye. Three clones (CDO1400, AW23 and CDO676) showed RFLP bands in one rye line relative to the other for all probe/enzyme combinations, suggesting possible structural differences between these two chromosomes. AW9 and AW13 could not be mapped to chromosome 6R and BCD339 failed to hybridise to rye under the conditions employed.

Dominant expression of marker phenotype was observed for a number of probe/enzyme combinations. The majority of dominant polymorphisms were identified in the $6R^{T701}$ substitution line (30.3% of all polymorphisms scored) compared to 7.9% identified in the $6R^{Imp}$ addition line. Dominant clone phenotypes accounted for 38.2% of all polymorphisms observed between chromosome $6R^{Imp}$ and chromosome $6R^{T701}$.

The arm location of five clones previously mapped only to homoeologous group-6 chromosomes was established. The clones AW15, AW23, BCD269, CDO419 and CDO1400 were all localised to the long arm of chromosome 6R.

Table 1 A list of cDNA and gDNA clones, their chromosomal arm location in either hexaploid wheat or barley, the restriction enzyme used, and source/reference from which clones were obtained. Clones mapped to chromosome 6R of rye are indicated by an asterix

Clone	cDNA or gDNA	Restriction enzyme	Chromosomal location	Additional chromosomal locations	Source and Reference[a]
AW1	C	Unknown	6H		1
AW9	C	Unknown	6H		1
AW13	C	Unknown	6H		1
AW15*	C	Unknown	6H		1
AW19	C	Unknown	6H		1
AW23*	C	Unknown	6H		1
BCD1*	C	*Eco*RV	6AL, BL, DL		2
BCD102*	C	*Dra*I	6AL, BL, DL	3BS, 3DS	2
BCD221	C	*Eco*RV	6H	2HS	2
BCD269*	C	*Xba*I	6H		2
BCD276*	C	*Dra*I	6AL, BL, DL		2
BCD339	C	*Eco*RV	6H	2HL, 3HL	2
BCD340*	C	*Eco*RI	6AL, BL, DL		2
BCD758*	C	*Eco*RV	6AL, BL, DL		2
BCD1426*	C	*Eco*RV	6AL, BL, DL	3BL	2
CDO419*	C	*Eco*RI	6H	3HL	2
CDO497*	C	*Eco*RV	6AL, BL, DL		2
CDO676*	C	*Dra*I	6DL	7AS, 7BS, 7D	2
CDO772*	C	*Eco*RV	6AL, BL, DL		2
CDO1091*	C	*Eco*RV	6AL, BL, DL		2
CDO1380*	C	*Eco*RV	6AL, BL, DL		2
CDO1400*	C	*Dra*I	6D	4AS, 5D, 7AS, 7D	2
WG222	G	Unknown	6H	2HS	2
WG282	G	*Eco*RV	6H		2
WG286*	G	*Eco*RI	6AL, BL, DL		2
WG341	G	*Eco*RI	6BL	5AS, 5DS, 7BL	2
WG405	G	*Dra*I	6DL	5BL, 2H, 3HL	2
WG522*	G	*Eco*RI	6AL, DL	7AS, 7BS, 7D	2
WG933*	G	*Eco*RI	6AL, BL, DL		2
KSUF37*	G	Unknown	6DL		3
KSUD1*	G	Unknown	6DL		3
TAM3	G	*Hin*dIII	6 A, B, D		4
TAM9	G	*Bam*HI	6AL, BL		4
TAM17*	G	*Bam*HI	6AL, BL, DL		4
TAM21*	C	*Hin*dIII	6AL, BL, DL		4
TAM25*	G	*Hin*dIII	6AL, BL, DL		4
TAM26	G	*Hin*dIII	6 A, B, D		4
TAM28	G	*Sac*I	6BL, DL		4
TAM30*	C	*Hin*dIII	6AL, BL, DL		4
TAM36*	C	*Hin*dIII	6AL, BL, DL		4
TAM57	G	*Bam*HI	6 A, B, D		4
CSIH90	G	Unknown	6D		5
PSR154*	C	*Hin*dIII	6AL, BL, DL	6HL, 6RL	6

[a] 1, P. Murphy, University of Adelaide, Australia (Murphy et al. 1995); 2, M. E. Sorrells, Cornell University, USA (Heun et al. 1991); 3, B. S. Gill, Kansas State University, USA (Gill et al. 1991); 4, G. E. Hart, Texas A&M University, USA; 5, R. Appels, CSIRO, Australia (Lagudah et al. 1991); 6, P. Sharp, University of Sydney, Australia (Sharp et al. 1988)

PCR markers

Table 2 lists the origin and sequence information for primers used in this study. The primer combinations and the annealing temperatures employed are shown in Table 3.

Primer combination PawS5/PawFG3

Figure 1 shows results obtained using the primer combination PawS5/PawFG3. This combination produced a profile which included bands identified when each primer was used independently as well as a number of bands in both wheat and rye which were clearly the result of amplification with both primers. Specifically, two bands of 170 bp and 280 bp were observed in both the $6R^{T701}$ addition line and the $6R^{T701}$ di-telosomic addition line (lanes 18 and 19; arrows). While neither band was observed in the $6R^{Imp}$ addition line, both were observed in rye cv Imperial (lane 9). A band of about 240 bp was amplified from all three rye cultivars tested. This band was mapped to rye chromosome 4R using the Chinese Spring-Imperial disomic addition

Table 2 Origin and sequence of oligonucleotide primers used in this study

Primer	Length (bp)	5′ → 3′ sequence	Origin
PawC2	18	GGCCCAATGTTCTTCTCT	pAW173, nucleotides 35–52
PawFG3	20	ATGCTGGAGCCCGAGGCTCA	pSc119.1, nucleotides 30–50
PawFG4	20	CTGTTTCGCTTCTGTTGCTC	pSc119.1, nucleotides 690–710
PawS5	18	AACGAGGGGTTCGAGGCC	Border of R173 (R173–2 and R173–3)
PawS6	18	GAGTGTCAAACCCAACGA	Flanking R173 (R173–3)
PawS11	18	GAATTCTTGGAAAATGTA	Flanking R173 (R173–2)
PawS13	18	GATCATATTTGGACTAAC	Border of R173 (R173–1)
PawS14	18	AATCCAATACATAGAGGA	Border of LTR (R173–1)
PawS15	18	CCGGGTCCGCACTGGATC	Border of R173 (R173–3)

Table 3 Summary of the results obtained using the primer combinations and conditions employed in this study

Primer combination	Annealing temperature	Number of PCR cycles	Size of PCR products mapped to chromosome 6R (bp)		PCR products mapped to other rye chromosomes
			$6R^{Imp}$	$6R^{T701}$	
PawC2	50	35	–	710 bp	2R
PawS5/PawS6	55	40	125 bp	125 bp	1R, 2R, 4R, 5R, 7R
PawS5/PawS11	50	40	–	–	–
PawS5/PawS14	55	32	630 bp	630 bp	–
PawS5/PawS15	55	32	–	–	1R, 2R
PawS5/PawFG3	55	32	–	170 bp and 280 bp	4R
PawS5/PawFG4	55	32	400 bp	450 bp	1R, 2R, 3R, 4R, 7R
PawS6/PawS14	55	32	–	–	–
PawS6/PawS15	55	32	–	–	–
PawS6/PawFG3	50	40	–	–	1R, 4R, 5R, 7R
PawS6/PawFG4	50	40	–	–	–
PawS11/PawFG3	55	32	–	–	–
PawS11/PawFG4	55	32	–	–	–
PawS13/PawS14	50	32	–	–	–
PawS14/PawS15	55	32	310 bp	310 bp	5R
PawS14/PawFG3	55	32	–	–	–
PawS14/PawFG4	55	32	–	–	–
PawS15/PawFG3	55	32	–	–	7R
PawS15/PawFG4	55	32	–	–	–

– PCR products not mapped

lines (lane 13). Two additional bands were identified, one in the rye cv Vila Pouca and the other in rye cv Petkus-R5 (lanes 7 and 8). Neither band was observed in rye cv Imperial nor could they be mapped to individual rye chromosomes.

Other primer combinations

Results using the primer combination PawS5 and PawFG3 are indicative of those observed using other primer combinations. Typically, amplification products were a mixture of bands produced by both primers *individually* as well as in *combination*. A total of 26 amplification products were mapped to individual rye chromosomes. Primer combinations involving PawFG3 and PawFG4 generated eight (30.8%) and seven (26.6%) of all the markers observed. The primer combinations PawS5/PawFG3 and PawS5/PawFG4 accounted for ten (38.5%) of all the amplification products mapped. Five primer combinations generated seven new PCR-based markers for chromosome 6R of rye. However, only primer combinations PawS5/PawFG3 and PawS5/PawFG4 yielded products which were polymorphic between $6R^{Imp}$ and $6R^{T701}$.

Generation of a chromosome-6R TC-F_1 mapping population

The approach described for the generation of the TC-F_1 mapping population resulted in six possible genomic configurations necessitating selection of TC-F_1 plants monosomic for chromosome 6R.

GOT-2 screening

Table 4 shows the results obtained from screening the entire TC-F_1 population for GOT-2. Individual plants

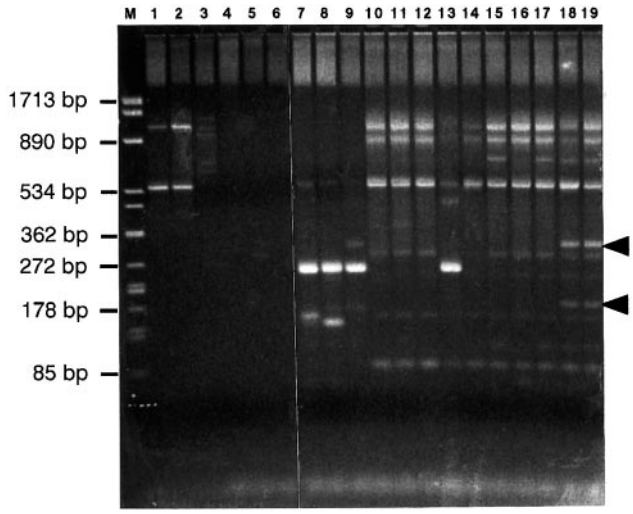

Fig. 1 Mapping of the PCR amplification products generated using the primer combination PawS5/PawFG3. *Lanes* 1–3 are Chinese-Spring (C.S.) wheat, Schomburgk wheat and Imperial rye amplified using only the primer PawS5, while *lanes* 4–6 contain the same DNA samples amplified using only the primer FG3. *Lanes* 7–19 were amplified using both PawS5 and FG4 and contained the following DNA samples: rye cv Vila Pouca, rye cv Petkus-R5, rye cv Imperial, C.S.-Imperial disomic addition lines (1R-7R), C.S.-Imperial 6R disomic addition line, C.S.-T701 6R(-6D) disomic substitution line, C.S.-T701 6RL di-telosomic addition line. The 280-bp and 170-bp bands mapped to chromosome $6RL^{T701}$ are indicated (*lanes 18 and 19*; *filled arrows*, right)

were classified based on their likely chromosome constitution as deduced from GOT-2 zymogram phenotypes (data not shown). About 68% (288/423) of the TC-F_1 population appeared to be monosomic for chromosome 6R, indicating that chromosomes $6R^{Imp}$ and $6R^{T701}$ pair in the F_1 hybrid at meiosis. However, a significant proportion of plants were found to be nullisomic (21.75%) or disomic (10.16%) for chromosome 6R, indicating relatively high levels of asynapsis and/or desynapsis.

Screening the TC-F_1 mapping population

On the basis of GOT analysis, 288 seedlings were classified as being monosomic for chromosome 6R and DNA samples were prepared from 282 *Got-R2*-positive individuals. Eight cDNA, two genomic DNA clones and five PCR markers were suitable for linkage mapping studies. The total population was screened with PCR markers but only 149 individuals were screened using RFLP markers.

Of the RFLP clones used, difficulties were encountered with PSR154. Scoring of this marker was not reliable due to reduced intensity of RFLP bands mapping to chromosome 6R and the presence of a faint band in wheat cultivars which was of similar size to the band mapping to chromosome $6R^{Imp}$. This marker was excluded from linkage analysis. The clone KSUF37

Table 4 GOT-2 screening. Chromosome constitution of TC-F_1 individuals. Nomenclature for genomic configurations are as described in Materials and methods. Plants were scored for the intensity of bands for the isozymes GOT-2a, GOT-2d and GOT-2e

Chromosome constitution	Number of plants	% TC-F_1 population
1 (20W″ + 6R′ + 6D′)	179	42.32
2 (21W″ + 6R′)	109	25.77
1 + 2	288	68.09
3 (20W″ + 6R″ + 6D′)	22	5.20
4 (21W″ + 6R″)	21	4.96
3 + 4	43	10.16
5 (20W″ + 6D′)	67	15.84
6 (21W″)	25	5.91
5 + 6	92	21.75
1 + 3 + 5	268	63.36
2 + 4 + 6	155	36.64
Total	423[a]	100.00

[a] Sixteen of the 439 TC-F_1 seeds failed to germinate

also presented problems due to the weakness of signal intensity. Phenotypes were recorded conservatively for 45 individuals. One TC-F_1 individual was found to possess both the $6R^{T701}$ allele and the $6R^{Imp}$ allele for all RFLP loci, indicating that this plant was disomic for chromosome 6R. A second plant was also found to be disomic for chromosome 6R using two RFLP clones. Data derived from these plants were omitted from linkage analysis.

Screening the TC-F_1 population with the PCR primer combination PawS5/PawFG4 revealed five plants which possessed both the $6R^{Imp}$ band and the $6R^{T701}$ band while six plants possessed neither band. It was unknown at this stage whether these products were allelic or non-allelic. Using the primer combination PawS5/PawFG3, seven plants were found to possess only the 280 bp band while eight plants possessed only the 170-bp band, indicating that the bands amplified with this primer combination are non-allelic.

Progeny testing for *CreR*

CCN status could be determined for 8 of the 11 families screened (Table 5). TC-F_2 progeny derived from four TC-F_1 individuals were clearly resistant while TC-F_2 progeny derived from two plants were clearly susceptible. A single TC-F_2 plant possessing chromosome 6R was isolated for CCN screening of two TC-F_1 individuals. No cysts were found on one plant while a single cyst was observed on the other. These two plants were scored as resistant. The bioassay failed for two lines, while for one line the bioassay was inconclusive.

Additional *CreR* phenotypes were included via the visual inspection of the data set. Individuals within the data set possessing parental phenotypes for markers were identified. This allowed 48 individuals to be classified as CCN-resistant and 38 individuals CCN-susceptible. Individuals which could not be assigned to a

Table 5 Number of cysts on the roots of TC-F_2 plants ± chromosome 6R derived from selfed TC-F_1 individuals selected based upon putative recombination events. Number of cysts on the roots of control susceptible and resistant lines and the rye cultivar Imperial are also shown

Line	No. of seedlings tested	Number of cysts per plant		CCN reactions[d]
		Mean ± SD[a]	Range	
Control lines:				
Schomburgk	6	20.00 ± 6.81	13–28	S
Chinese Spring	5	4.80 ± 2.59	1–8	S
Imperial Rye	6	0.83 ± 1.33	0–3	R
Triticale cv T701-4-6	2	0.00 ± 0.00	0	R
Chinese Spring-Imp. 6R	6	3.33 ± 2.58	1–7	S
Chinese Spring-T701 6R	1	1.00 ± 0.00	1	R
Sib-line ± 6R chromatin:				
TC-F_2-99 + 6R	4	0.25 ± 0.50	0–1	F
− 6R	6	1.50 ± 0.84[b]*	1–3	
TC-F_2-116 + 6R	4	1.25 + 2.50[c]	0–5	I
− 6R	6	3.00 ± 2.97[b]	0–7	
TC-F_2-124 + 6R	4	0.50 ± 0.57	0–1	R
− 6R	6	9.83 ± 4.71	4–15	
TC-F_2-158 + 6R	10	0.10 ± 0.32	0–1	F
− 6R	8	1.38 ± 2.13[b]*	0–6	
TC-F_2-186 + 6R	2	0.00 ± 0.00	0	R
− 6R	5	4.60 ± 1.95	2–6	
TC-F_2-209 + 6R	5	3.00 ± 2.83	1–7	S
− 6R	6	6.33 ± 3.56	1–11	
TC-F_2-210 + 6R	1	0.00 ± 0.00	0	R
− 6R	5	3.60 ± 3.21	0–8	
TC-F_2-254 + 6R	4	0.00 ± 0.00	0	R
− 6R	6	3.33 ± 2.88	0–8	
TC-F_2-274 + 6R	5	0.00 ± 0.00	0	R
− 6R	6	7.33 ± 6.31	1–16	
TC-F_2-284 + 6R	1	1.00 ± 0.00	1	R
− 6R	6	7.17 ± 6.31	3–10	
TC-F_2-295 + 6R	5	6.00 ± 4.53	3–14	S
− 6R	6	9.17 ± 6.19	3–17	

* Significant at $P \leqslant 0.05$
[a] SD = standard deviation
[b] Mean number of cysts observed on chromosome 6R-deficient sib-lines was compared with the mean number of cysts on Chinese Spring using the students *t*-test
[c] Mean number of cysts observed on chromosome 6R-proficient TC-F_2-116 sib-lines compared with the mean number of cysts observed on chromosome 6R-deficient TC-F_2-116 sib-lines using the students *t*-test
[d] S, susceptible; R, resistant; F, failed bioassay; I, inconclusive bioassay

particular parental type due to insufficient marker information (i.e. no phenotype information for markers within the interval flanked by loci *Xbcd758* and *AawS5/FG3-280*) were scored as unknown.

Linkage analysis

Distorted segregation ratios

Distorted segregation of markers was determined using the chi-square test (Table 6). Due to the nature of the crosses used in the generation of the TC-F_1 population, the segregation of all markers should be restricted to 1 : 1 (female). Three loci (*AawC2*, *Xtam36B* and *Xbcd1426*) demonstrated distorted ratios. The segregation of all other markers fitted the expected 1 : 1 ratio.

Construction of a genetic linkage map of chromosome 6RL

Figure 2 shows the most likely order of loci included in the linkage map. Fourteen loci including *CreR* were placed into a single linkage group. The locus *Xtam36B* was not linked to any other loci within the group. This map spans almost 80 cM and comprises eight RFLP loci, five PCR loci and *CreR*.

PCR loci

The PCR locus *AawC2* mapped about 32 cM from *Xbcd758*. Initial analysis using a mapping LOD of 3.0 failed to include *AawC2* in the linkage group. Using a mapping LOD of 1.0, *AawC2* was placed 60-cM proximal to *Xbcd758*. Examination of the data set using

Table 6 Phenotypic frequencies for RFLP and PCR markers mapped in the TC-F_1 population

Locus	Expected	Observed		χ^2-Values
		$6R^{T701}$	$6R^{Imp}$	
AawS5/FG4-1	1:1	76	69	1.036
AawS5/FG4-2	1:1	77	68	1.896
AawC2	1:1	53	77	20.643*
AawS5/FG3-170	1:1	72	68	0.443
AawS5/FG3-280	1:1	74	66	0.824
Xbcd758	1:1	60	41	3.176
Xaw15	1:1	46	34	1.800
XksuF37	1:1	25	20	0.556
Xwg933	1:1	48	35	2.036
Xcdo1380	1:1	48	32	3.200
Xtam36B	1:1	9	76	47.472**
Xbcd1426	1:1	44	26	4.696*
Xbcd1	1:1	56	44	1.198
Xbcd340	1:1	44	30	2.649
CreR	1:1	54	40	2.085

** Significant at $P \leqslant 0.01$
* Significant at $P \leqslant 0.05$

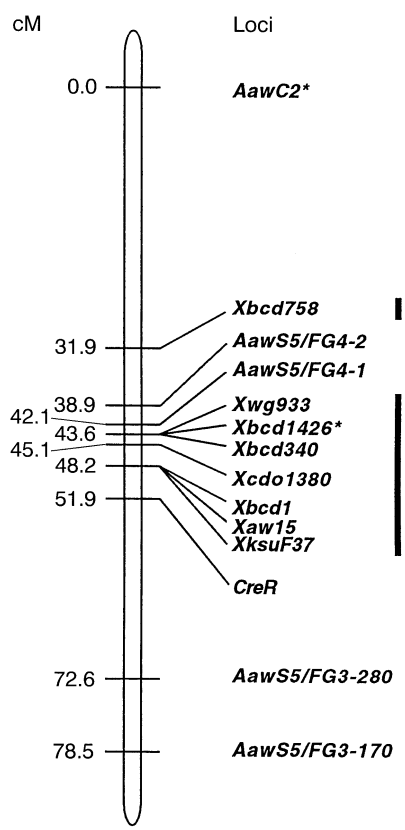

Fig. 2 Genetic linkage map of the long arm of chromosome 6R. Markers exhibiting distorted segregation ratios are indicated by an *. *Vertical bars* to the right indicate markers which were linked at LOD < 3.0

the *chrompic* function in CRIMAP indicated a number of likely typing errors. Genotype information for this marker was checked and data were deleted where inconsistencies were identified. Upon re-analysis, *AawC2* was included in the linkage group under a mapping LOD of 3.0. PCR loci *AawS5/FG4-2* and *AawS5/FG4-1* mapped 3.2 cM apart, indicating that these loci are not allelic.

CreR

The *CreR* locus mapped 3.7 cM distal from *XksuF37* (Fig. 2). Loci *Xbcd1*, *Xaw15*, *XksuF37* and *CreR* were unseparated by cross-overs. As a consequence, a number of orders with equal likelihoods were identified in which loci within this group were inverted with respect to each other. However, *CreR* was always the most-distally placed locus within the group.

Comparison with wheat and rye group-6 maps

Comparison of the map presented in Figure 2 with those of Devos et al. (1993 a), Philipp et al. (1994), Wanous et al. (1995) and Loarce et al. (1996) highlights a number of points. Clearly there is an obvious clustering of 9 loci (*AawS5/FG4-2-XksuF37*) spanning only 9.3 cM (two-point analysis for loci *AawS5/FG4-2* and *XksuF37* reveals a direct map distance of 4.5 cM). Two clusters or blocks of loci were observed; *Xwg933*, *Xbcd1426* and *Xbcd340*, and *Xbcd1*, *Xaw15*, *XksuF37* and *CreR* (Figure 2). The two groups of loci were separated by a number of recombination events and could be ordered relative to each other. However, recombination between loci *within* either group was not observed.

There is a gap of about 21 cM between *XksuF37* and *AawS5/FG3-280*. The lack of RFLP clones mapping to this region is likely to be a combination of the selection of group-6 homoeologous clones and the known breakdown in homoeology in the distal region of 6RL. This region is likely to demonstrate homoeology to group 3RL and group 7RL. In the chromosome-6RL map of Loarce et al. (1996), the group-3 clone BCD147 was mapped to the same position as the group-6 clone BCD1 indicating that the locus *Xbcd1* (and *XksuF37*) lies immediately proximal to the 6RL/3RL translocation breakpoint. This raises the possibility that the *CreR* locus was transferred to chromosome-6RL from rye chromosome 3RL or 7RL.

Of the four chromosome-6R maps currently available, only that of Loarce et al. (1996) has loci in common with the map presented in Fig. 2. In the map of Loarce et al. (1996), *Xbcd758* and *Xbcd1* map at least 11.8 cM apart. In the present study, these loci mapped 16.3-cM apart (Fig. 2). However, a two-point analysis indicates a direct map distance of 7.5 cM for these loci.

Currently, the only wheat group-6 map which shares more than one marker in common with the map of chromosome 6RL presented in Fig. 2 is the genetic map of chromosome 6D of *T. tauschii* (Gill et al. 1993). Two loci are in common, *Xwg933* and *XksuF37*. In rye, these loci map 4.6 M apart, while in wheat the distance is 151.3 cM. Two-point analysis indicates a direct map distance of 2.2 cM.

Discussion

RFLP markers

With the exception of two clones, polymorphism was detected with at least five restriction enzymes indicating that deletion and/or insertion events account for the majority of polymorphisms observed in rye. This result is consistent with the study of Loarce et al. (1996) in rye, as well as for barley (Graner et al. 1990) and wheat (Chao et al. 1989).

Not surprisingly, the proportion of cDNA clones localised to chromosome 6R of rye was significantly higher than that observed for genomic DNA clones (77% and 41%, respectively). Other workers have attributed similar observations to the reduced level of sequence conservation and the non-homoeologous behaviour of genomic DNA clones when used to screen across species (Gale 1990).

The level of polymorphism observed in the present study is higher than reported by Loarce et al. (1996) for rye, but comparable to that reported by Hart (1990) between *Triticum turgidum* var. *durum* cv Langdon and *T. turgidum* var. *dicoccoides* and by Gill et al. (1991) between two divergent accessions of *T. tauschii*. Such high levels of polymorphism are likely to reflect the diverse nature of the materials under investigation rather than any intrinsic feature of the clones or restriction enzymes employed.

Hemizygous loci have been observed in *T. tauschii* (Gill et al. 1991), hexaploid wheat (Liu and Tsunewaki 1991) and rye (Loarce et al. 1996; Wanous et al. 1997). In *T. tauschii*, the proportion of such loci was 16%, while in rye figures of 66% (Wanous et al. 1997) and 24.13% (Loarce et al. 1996) have been reported. In the present study, 3 of 27 clones (11.1%) demonstrated dominant phenotypes for all restriction enzymes used. While the true nature of these loci is unknown, it is tempting to speculate that they may indicate small rearrangements in one chromosome relative to the other. Such rearrangements could take the form of small deletions, which is consistent with the observation regarding the importance of deletion/insertion events for the generation of polymorphism in rye.

Sixteen homoeologous group-6 clones could not be mapped to chromosome 6R. The most-likely explanation is that these clones reside on those segments of chromosome 6RL known to have been translocated to chromosomes 3RL or 2RS during the evolution of rye (Devos et al. 1993 a). Polymorphism for RFLP and PCR markers was observed between rye cv Imperial and the Chinese Spring-Imperial 6R disomic addition line. In a number of instances, bands observed in Imperial rye were not observed in the addition line. These observations presumably reflect heterozygosity in the "Imperial" original stock or may indicate deletions in chromosome 6R in the Chinese Spring addition line.

PCR markers

On average, each PCR primer revealed 3.25 new markers for the rye genome, while each primer combination revealed 1.44 new markers for the rye genome. Seven (26.9%) markers were generated for chromosome 6R of rye. However, amplification products were mapped to all seven rye chromosomes, highlighting the generality of the approach used. These results compare favourably to other PCR-based approaches such as RAPD analysis (Williams et al. 1990).

The initial study of Rogowsky et al. (1992 a) has been extended via the inclusion of primers derived from the dispersed repetitive sequence contained within the clone pSc119.1. The results presented confirm that primers derived from R173 elements are an efficient and essentially limitless source of molecular markers for the rye genome. This clearly extends to primers derived from other dispersed repetitive DNA sequences, such as pSc119.1. Primer combinations involving PawFG3 and PawFG4 account for some 57.7% of all markers scored. Since these two primers were derived from a dispersed repetitive family of DNA sequences found within the rye genome, it seems reasonable to conclude that sequences defined by these primers occur in the vicinity of R173 elements in slightly different configurations. This rationale also applies to the results observed with other external primers derived from multicopy sequences such as PawS6. Therefore, it is likely that many of the PCR products observed are the result of specific amplification events at the border of individual R173 elements.

Screening the TC-F_1 population

Screening the TC-F_1 population with RFLP and PCR markers identified two plants disomic for chromosome 6R, while using PCR data alone three additional putative disomic plants and six putative nullisomic plants were identified. Misclassification of TC-F_1 plants based upon GOT-2 isozyme phenotypes therefore ranged between 0.71% (2/282) and 3.90% (11/282). The relative age of the leaf tissue, the amount of tissue extracted and the amount of extract loaded in each lane are factors

likely to influence the accuracy of this method. However, classification of the dosage of chromosome 6R based on GOT-2 isozymes appears to be a relatively robust and efficient technique.

The dosage of chromosome 6D for TC-F_1 progeny was also ascertained via GOT-2 screening. Female transmission of wheat monosomes is about 25% (Sears 1952). Therefore, the proportion of TC-F_1 plants *disomic* for chromosome 6D should be about 25%. The figure of 36.64% derived from GOT-2 screening is higher than expected, even if one assumes an error rate similar to that observed for determining the dosage of chromosome 6R. These observations may indicate differential selection for chromosome 6D and, hence, selection against chromosome 6R in female gametes.

Linkage map of 6RL

The linkage map of 6RL presented is characterised by an interstitial clustering of markers. Clustering of loci has been observed for other Triticeae chromosome maps, particularly around centromeres (Devos et al. 1993 a, b; Kleinhofs et al. 1993). In rye, Loarce et al. (1996) observed clustering of loci mapping to chromosomes 4R, 5R and 6R. In most instances, clustering of loci is the result of marker paucity and reduced levels of recombination in proximal chromosomal regions (Lukaszewski 1992; Langridge et al. 1995). In contrast, the clustering of loci observed in the present study is likely to reflect bias due to the selection of only homoeologous group-6 clones coupled with suppression of recombination in the TC-F_1 population used for mapping.

PCR loci

PCR loci *AawS5/FG3–170* and *AawS5/FG3-280* mapped 5.9 cM apart, while *AawS5/FG4-2* and *AawS5/FG4-1* mapped 3.2 cM apart. These results indicate that PCR markers derived from R173 elements mapping to chromosome 6R are non-allelic, in agreement with observations for similarly derived PCR markers for the short arm of chromosome 1R (Rogowsky et al. 1992 a).

Loarce et al. (1996) concluded that the only advantage to the inclusion of RAPD markers was that they enabled the generation of markers for regions poorly covered by RFLP markers. PCR markers *AawC2*, *AawS5/FG3-280* and *AawS5/FG3-170* were observed to map proximal to and distal from the main cluster of loci, allowing greater map coverage. However, while some of the PCR markers generated in this study by-passed potential problems arising from a breakdown in homoeology (i.e. *AawS5/FG3-280* and *AawS5/FG3-170*), they were inherited in a dominant fashion thereby providing less information for linkage analysis.

CreR

CCN progeny testing of putative informative families and the assignment of *CreR* status to additional TC-F_1 plants upon visual inspection of the data set allowed linkage mapping of *CreR*.

The accuracy of assigning *CreR* phenotypes to non-progeny tested individuals will affect the ability to map *CreR* and may result in a shrinkage of the overall map as a result of double-recombination events within the interval *Xbcd758-AawS5/FG3-280*. The probability of a double recombination event occurring within this interval was estimated to be 0.0031 using the recombination fractions derived by CRIMAP for the intervals *Xbcd758-CreR* (0.21) and *CreR-AawS5/FG3-280* (0.20). The probability of accurately assigning *CreR* phenotypes was estimated to be 93.9%. This figure verifies the approach taken in this study to map *CreR* and indicates that the effect upon the overall map generated is negligible.

CreR was placed 3.7 cM distal from the RFLP locus, *XksuF37*. However, these loci were not separated by cross-overs within the TC-F_1 population. Consequently, the order *CreR-XksuF37* is possible. In either instance, the RFLP clone KSUF37 represents a useful marker for future mapping and introgression studies involving the *CreR* locus.

Factors affecting locus order

Both distortion of phenotypic ratios and the mode of inheritance influence the derivation of LOD values via maximum-likelihood methods and, as a consequence, locus order (Lorieux et al. 1995). Loci *AawC2* and *Xbcd1426* demonstrated distorted ratios. *AawC2* also demonstrated dominant inheritance. Dominant RAPD markers have been observed to demonstrate non-conformity with expected Mendelian segregation ratios (Reiter et al. 1992). Factors such as the combined probabilities associated with the competitive nature of annealing and primer extension (Heun and Helentjaris 1993) and the temperature profiles and annealing temperatures of the PCR reaction have been implicated (Ellsworth et al. 1993). In the case of *AawC2*, the majority of individuals were observed to possess the null ($6R^{Imp}$) allele. Distortion from the expected ratios observed for *AawC2* is probably the result of failed PCR reactions.

Distorted ratios have been reported previously for RFLP markers mapping to chromosome 6R (Philipp et al. 1994; Wanous et al. 1995; Loarce et al. 1996). The locus *Xpsr915* demonstrated distorted segregation ratios in the study of Loarce et al. (1996). Loci *Xbcd758*

and *Xbcd1* are common to the map presented in Fig. 2 and the linkage map of Loarce et al. (1996) and flank *Xbcd1426* in this study and *Xpsr915* in the study of Loarce et al. (1996). That both maps indicate distorted segregation ratios in the same chromosomal region may be indicative of linkage to lethal or sublethal gene(s).

Recombination suppression in chromosome 6R

About 68% of the TC-F_1 plants isolated were monosomic for chromosome 6R and, hence, had apparently undergone normal assortment for that chromosome. However, the linkage map generated via screening of the TC-F_1 population indicates dramatically reduced levels of recombination; no recombination was observed between loci *Xwg933*, *Xbcd1426* and *Xbcd340* or loci *Xbcd1*, *Xaw15*, *XksuF37* and *CreR*. In chromosome 6A of hexaploid wheat, loci *Xwg933* and *XksuF37* map 151.3 cM apart, while in this study they are separated by only 4.6 cM – a 30-fold reduction. In contrast, similar map distances are observed between loci common to the study of Loarce et al. (1996) in rye and Marino et al. (1996) in wheat.

Pairing and the formation of chiasmata in rye chromosomes is greatly reduced in wheat-rye derivatives (Benavente and Orellana 1989). In addition, heterozygosity for interstitial and terminal C-heterochromatin has been shown to reduce the frequency of chiasmata, possibly due to differences in chiasma localisation patterns (Naranjo and Lacadena 1980). Moreover, major structural differences between homologues, such as translocations, have also been shown to reduce levels of meiotic pairing (Sybenga et al. 1990). Structural differences were observed in this study between chromosomes $6R^{Imp}$ and $6R^{T701}$ (data not shown). Chromosome $6R^{T701}$ was characterised by a satellite region present on the short arm while the long arm of this chromosome was apparently longer than that of $6R^{Imp}$. An obvious secondary constriction was also present on $6RL^{T701}$ but absent in $6RL^{Imp}$. It is therefore not surprising that a significant reduction in chiasma frequency, and possibly chiasma distribution, was observed within the TC-F_1 population for chromosome 6RL.

Concluding remarks

The present study has utilised homoeologous group-6 RFLP markers common to a number of wheat, barley and rye maps. Co-linearity was observed for loci common to the rye and wheat group-6 chromosome maps. Consequently, the results presented should facilitate a better integration of the rye map into those of related species such as wheat, barley, rice or maize. The *CreR* locus which confers resistance to CCN was mapped 3.7 cM distal from the RFLP marker KSUF37. This marker may be useful in any future attempt to introgress *CreR* into wheat. Significantly, comparison with the chromosome 6R map of Loarce et al. (1996) suggests that *CreR* may have been transferred to 6RL from 3RL or 7RL, indicating that selection and screening of homoeologous group-3 and -7 RFLP markers may yield a more-closely linked marker for the *CreR* resistance gene.

It is possible that the order of loci as presented, particularly those loci which were not separated by recombination events, may not be the true order. Locus order and map distances are a function of the quality of the data (Lorieux et al. 1995). Moreover, dramatically reduced recombination levels were observed in the TC-F_1 data set. Therefore, both the locus order and map distances presented should be viewed as preliminary.

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