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Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines

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Abstract The short arm of rye (*Secale cereale*) chromosome 1 has been widely used in breeding programs to incorporate new disease resistance genes into wheat. Using wheat-rye translocation and recombinant lines, molecular markers were isolated and mapped within chromosomal regions of 1RS carrying rust resistance genes *Lr26*, *Sr31*, *Yr9* from 'Petkus' and *SrR* from 'Imperial' rye. RFLP markers previously mapped to chromosome 1HS of barley – flanking the complex *Mla* powdery mildew resistance gene locus – and chromosome 1DS of *Aegilops tauschii* – flanking the *Sr33* stem rust resistance gene – were shown to map on either side of rust resistance genes on 1RS. Three non cross-hybridising Resistance Gene Analog markers, one of them being derived from the *Mla* gene family, were mapped within same region of 1RS. PCR-based markers were developed which were tightly linked to the rust resistance genes in 'Imperial' and 'Petkus' rye and which have potential for use in marker-assisted breeding.

Keywords 1RS · AFLP · Gabo 1BL·1RS · Gabo 1DL·1RS · Imperial rye · Petkus rye · Resistance gene analogs (RGA) · Sequence-tagged site (STS) · Wheat-rye recombinants

Introduction

The short arm of chromosome 1 (1RS) of rye (*Secale cereale*) carries several important disease resistance genes that confer resistance to rusts (*Puccinia* spp.) and powdery mildew. Many successful wheat cultivars containing 1RS translocations have been released including the 'Veery' lines developed at CIMMYT (Rajaram et al. 1983). These varieties were derived from three-way crosses between the Mexican spring semi-dwarf and winter wheat variety 'Kavkaz', which is presumed to carry 1B/1R translocation from 'Petkus' rye (Zeller 1973; Schlegel and Korzun 1997). This rye chromosome arm carries genes *Lr26*, *Sr31*, *Yr9* and *Pm8* conferring race-specific resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*), respectively. Another translocation line involving 1RS of 'Imperial' rye was developed as 1DL·1RS translocations in the 'Gabo' wheat background (Koebner and Shepherd 1986; Koebner et al. 1986). The 'Imperial' 1RS translocation carries the stem rust resistance gene *SrR*, but no known leaf, stripe rust or powdery mildew resistance genes. The group of rust resistance genes *Lr26*, *Sr31* and *Yr9* from 'Petkus' rye and *SrR* from 'Imperial' rye were mapped in separate crosses and located near the end of chromosome 1RS, approximately 5 cM distal to the seed-storage protein genes (*Sec-1*) (Singh et al. 1990). In a separate study the map location of *Lr26* was confirmed to be distal to the *Sec-1* locus of rye (Hsam et al. 2000). No recombination has been detected between rust resistance genes from 'Petkus' rye (*Lr26*, *Sr31* and *Yr9*). The allelic relationship of the stem rust resistance genes *Sr31* and *SrR* remains unresolved. Furthermore, no rust strains have been reported that can distinguish *Sr31* and *SrR* resistance specificities.

Although 1RS has contributed important disease resistance genes, wheat cultivars carrying this chromosome arm were shown to produce grain with quality defects, in particular, sticky dough and a reduction in dough strength (Martin and Stewart 1990; Graybosch et al. 1993). The reduction in dough strength was thought to be due to the presence of monomeric secalins from rye (*Sec-1*) and the substitution of glutenins and gliadins (*Glu-3* and *Gli-1*) (Dhaliwal and MacRitchie 1990). In an attempt to eliminate or reduce these deleterious effects on grain quality, studies were undertaken to induce homoeologous recombination between chromo-

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some arms 1RS of 'Imperial' rye and 1DS of wheat (Koebner and Shepherd 1986; Koebner et al. 1986; Rogowsky et al. 1991). Later, a series of 1DS·1RS wheat-rye recombinants were isolated that carried different interstitial rye segments separating the *Sec-1* locus from *SrR* (Shepherd 2001, personal communication) (see Fig. 1a). In a similar approach, several cycles of homoeologous recombination between the chromosome arms 1RS of 'Petkus' rye and 1BS of wheat variety 'Pavon' produced recombinant lines that incorporated a number of breakpoints between the *Sec-1* locus, disease resistance genes and the *Gli-1*/*Glu-3* loci within the distal end of the chromosome (Lukaszewski 2000).

The aim of the present work was to identify DNA markers linked to rust resistance genes on 1RS. Because suitable rust strains or rye genetic stocks for mapping the resistance genes directly in rye were not available, we have used a set of wheat translocation lines containing 1RS and a set of recombinants between 1RS and homoeologous wheat chromosomes (Koebner and Shepherd 1986; Koebner et al. 1986; Rogowsky et al. 1991; Lukaszweski 2000; Shepherd 2001, personal communication). Using these recombinant lines, previously mapped markers from barley, *Aegilops tauschii* and wheat were used to delineate the resistance gene region within rye. In addition, markers detected by resistance gene analogs (RGAs), which share significant sequence homology with the major class of plant disease resistance genes that are predicted to encode nucleotide-binding site/leucine-rich repeat proteins, were isolated and mapped (Ellis and Jones 1998; Meyers et al. 1999). We have also developed a comparative map of a chromosomal region on 1S by combining the previous Triticeae mapping efforts with current results from rye and *Ae. tauschii*. This study is a preliminary work leading to the cloning of the rust resistance genes on 1RS.

Materials and methods

Plant material

The 1DL·1RS translocation line and wheat-rye recombinants involving exchange between 1RS and 1DS were kindly provided by Dr. K.W. Shepherd, Waite Agricultural Research Institute, University of Adelaide, and have been described in detail by Rogowsky et al. (1991) and Shepherd (2001, personal communication) (see Fig. 1a).

Similar recombinants involving 1RS of 'Petkus' rye in a 1BL·1RS translocation in a 'Pavon' wheat background (introduced from cv Kavkaz) were kindly provided by A.J. Lukaszewski, University of California, Riverside, USA, and have been described in detail in Lukaszewski (2000) (see Fig. 2a).

An F2 family of 116 individuals was derived from a cross between *Ae. tauschii* var. *meyeri* accession CPI 110799, carrying the stem rust resistance gene *Sr33*, and accession AUS 18911, carrying the leaf rust resistance gene *Lr21* and stem rust resistance gene *Sr45* (Spielmeyer et al. 2000).

Isolation of AFLP markers linked to *SrR*

To isolate AFLP markers from the region carrying the stem rust gene *SrR*, the 1DL·1RS translocation line, the secondary recombinant DR-A1 and tertiary recombinants $Sr^{+}Sec^{-}$ and $Sr^{-}Sec^{+}$ were used as templates. AFLP analysis was performed using the standard protocol (Vos et al. 1995). For selective amplification, *Pst*I and *Mse*I primers with three additional nucleotides were used.

Cloning and analysis of AFLP fragments

X-ray films were aligned with the dried acrylamide gels and AFLP products of interest excised. DNA was eluted into 100 µl of water and boiled for 15 min. After a brief spin the supernatant was transferred to a fresh tube and DNA precipitated overnight at –20 °C by adding 10 µl of 3 M sodium acetate (pH 5.2), 5 µl of 10 mg/ml glycogen and 2.5 vol of ethanol. After centrifugation for 10 min, the pellet was washed with 70% ethanol, dried and resuspended in 10 µl of sterile water. Two microliters of this eluate were used for re-amplification employing the same primers and PCR conditions used for selective PCR. The PCR products were separated on an agarose gel, excised and purified using the QIAquick gel extraction kit (Qiagen, Germany). The PCR products were cloned into the pGEM-T easy vector (Promega, USA) according to the manufacturer's instructions.

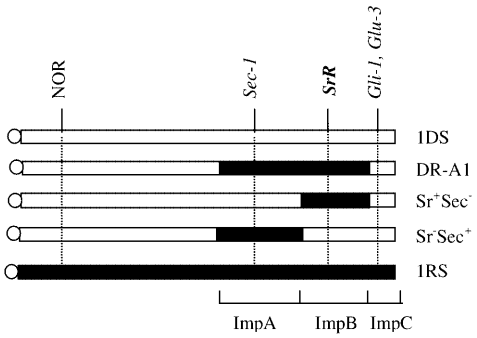
In all cases, the cloned fragments contained a mixture of different fragments of the same size. To identify the correct fragment, 20 clones per amplification were analysed. The insert from each of these clones was amplified using the T7 and SP6 primers, which flank the cloning sites, and digested with the restriction endonuclease *Alu*I. The digested samples were run on a 2% agarose gel. The clone which appeared most frequently amongst these clones was further used as a DNA probe to detect the restriction fragment length polymorphism between the recombinant lines. This RFLP analysis confirmed the association of the AFLP products and the *SrR* rust resistance locus.

Sequence-tagged site (STS) analysis

Four cloned AFLP fragments isolated from the region carrying the stem rust resistance gene *SrR* on Imperial rye chromosome 1S, ACC/GTT-159 (IB-159), ACA/GTC-262 (IB-262), ACC/GTG-267 (IB-267) and ACC/GAT-544 (IB-544), were sequenced using the dye terminator sequencing system and analysed on an ABI prism system. Specific primers were designed for amplification of each of these fragments. The DNA probe that detected the RFLP marker iag95 (Philipp et al. 1994) was sequenced from both ends and specific primers for PCR amplifications were designed.

Resistance gene analogue (RGA) clones

The clone PIC31 was isolated from the 1DL·1RS tertiary recombinant line $Sr^{+}Sec^{-}$ using the primers specific to the conserved P-loop and GLPLAL motifs in the nucleotide-binding site (NBS)-domain region of rice *Xa-1* (Acc. No. AB002266) and rice blast resistance gene *Pib* (Acc. No. AB013451), respectively. The PCR was carried out in a volume of 20 µl and contained 200 ng of genomic DNA, 0.2 mM of dNTPs, 10 pmol of each primer, 2.0 mM of $MgCl_2$, 50 mM of KCl, 10 mM of Tris-HCl (pH 9.0 at 25 °C), 0.1% TritonX-100 and 0.5 U of *Taq* DNA Polymerase. PCR was carried out in a PC-960C thermal cycler (Corbett Research, Sydney, Australia) with the following program: 95 °C for 3 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 50 s; 10 min at 72 °C. RGA39 was amplified from barley cultivar 'Morex' using the primers 39F236 and 39B318 as described in Wei et al. (1999). RGA39 has since been demonstrated to belong to a small gene family in barley that includes the *Mla1* and *Mla6* powdery mildew resistance genes (Halterman et al. 2001; Zhou et al. 2001). The wheat RGA probe cslrgh3 was amplified from wheat variety 'Chinese Spring' using the primer sequences designed from the wheat EST (Acc. No. BE 518223) and was closely related to the barley RGA b6 (96% identical) (Leister et al. 1998).



b

Position of markers on 1RS of 'Imperial' rye

Markers	ImpA	ImpB	ImpC
abc156	+		
mwg68	+		
ksuE19	+		
ksuG9	+		
IA-215	+		
IA-294	+		
IA-299	+		
IB-159		+	
IB-262		+	
IB-267		+	
IB-544		+	
RGA39		+	
mwg60		+	
PIC31		+	
cslrgh3		+	+
iag95			+

Fig. 1 **a** Schematic representation of the short arm of the 1DL·1RS translocation line and secondary and tertiary recombinants of rye chromosome arm 1RS with wheat chromosome arm 1DS (adapted from Rogowsky et al. 1991, 1993). Rye segments are *solid*; wheat segments are *blank*; the centromeres are on the left, represented by *blank circles*. Designations of recombinant lines are shown on the right. The start and end points of blocks of rye chromatin are not known precisely and may lie anywhere between two marker loci where one is present and the other is absent. The recombination events delineate three regions on the short arm of chromosome 1 in 1DS·1RS. Region 'ImpA' contains the rye seed storage protein locus, *Sec-1*. Region 'ImpB' represents the segment carrying the stem rust resistance gene *SrR* and region 'ImpC' corresponds to the distal region of wheat that contains seed storage protein genes, gliadins (*Gli-1*) and glutenins (*Glu-3*). **b** The position of various markers on 1RS are shown in a tabular form, based on hybridisation with the 'Gabo' wheat, the 1DL.1RS translocation line and the secondary and tertiary recombinants

Table 1 RFLP markers and probes used

Probe	Source/reference
abc156	A. Kleinhofs, Washington State University, USA
b6	Leister et al. 1998
cslrgh3	This study
IA-215	This study
IA-294	This study
IA-299	This study
iag95	G. Wrickle, University of Hannover, Germany
IB-159	This study
IB-262	This study
IB-267	This study
IB-544	This study
ksuE19	B.S. Gill, Kansas State University, USA
ksuG9	B.S. Gill, Kansas State University, USA
mwg36	A. Graner, IPK, Gaterslaben, Germany
mwg60	A. Graner, IPK, Gaterslaben, Germany
mwg68	A. Graner, IPK, Gaterslaben, Germany
mwg2083	A. Graner, IPK, Gaterslaben, Germany
mwg2197	A. Graner, IPK, Gaterslaben, Germany
PIC31	This study
RGA39	Wei et al. 1999

DNA isolation and RFLP mapping of probes

Genomic DNA isolation from leaves and DNA-blot analysis was carried out according to Lagudah et al. (1991a, b). DNA was restricted with endonucleases under conditions recommended by the manufacturer (New England Biolabs, USA).

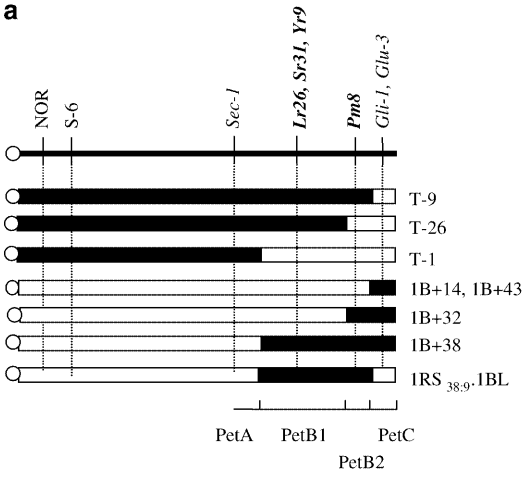
RFLP clones previously mapped to 1S in several species within the Triticeae have been listed in Table 1. DNA probes used for hybridisation to the DNA digests were labelled with [P^{32}]-dCTP using the Megaprime DNA labelling system (Amersham Pharmacia).

Results

Identification of markers linked to stem rust resistance gene *SrR* on 1RS from 'Imperial' rye

To characterise the region carrying the stem rust resistance gene, *SrR*, RFLP markers previously assigned to the short arms of homoeologous group-1 chromosomes of wheat, barley and rye were used for DNA hybridisation with 'Gabo' wheat, the 1DL·1RS translocation line and derived wheat-rye recombinant lines. These lines incorporated recombination events between the short arm of chromosome 1D of wheat and 1R of 'Imperial' rye consisting of the secondary recombinant, DR-A1 and the two tertiary recombinant lines, $Sr^{+}Sec^{-}$ and $Sr^{-}Sec^{+}$ (Fig. 1). Comparison of the RFLP patterns was the basis of the assignment of markers to regions delineated by the recombination breakpoints. RFLP probes abc156, mwg68, ksuG9 and ksuE19 detected markers within a region proximal to the rust resistance gene (region ImpA; Fig. 1a, b), whereas the RFLP marker iag95 mapped distal to the stem rust resistance gene (region ImpC). RFLP probe mwg60 identified markers within the rye segment carrying *SrR* (region ImpB).

RFLP markers linked to *SrR* (mwg68, mwg60) were previously mapped to the corresponding region on 1HS of barley flanking the complex *Mla* powdery mildew resistance locus (Wei et al. 1999). There were at least three non cross-hybridising classes of NBS-LRR sequences at the *Mla* locus (RGH1, RGH2 and RGH3). Using specific primers (39F236 and 39B318) for the P-loop region of one of the NBS-LRR sequence classes(RGH1), a member of this gene family (RGA39) was amplified from the barley cv Morex. RGA39 hybridised to multiple restriction fragments on genomic DNA of the wheat-rye recombinant lines. Depending on the restriction enzyme



b

Position of markers on 1RS of 'Petkus' rye

Markers	PetA	PetB1	PetB2	PetC
abc156	+			
mwg68	+			
ksuE19	+			
ksuG9	+			
IA-215	+			
IA-294	+			
IB-159		+		
RGA39		+	+	
mwg36			+	
iag95			+	
cslrgh3			+	+
mwg60				+

Fig. 2 **a** Schematic representation of the primary and tertiary recombinants of rye chromosome arm 1RS with wheat chromosome arm 1BS. Rye segments are *solid*; wheat segments are *blank*; the centromeres are on the left, represented by *blank circles*. The map positions of NOR, S6, *Sec-1* and disease resistance genes and the rust testing have been described by Lukaszewski (2000). Designations of the recombinant lines are shown on the right. The short arm of chromosome 1 in the recombinant lines is divided into three regions (A–C). Region 'PetA' contains the rye seed storage protein locus, *Sec-1*. Region 'PetB' represents the segment carrying the disease resistance genes *Lr26*, *Sr31* and *Yr9* of Petkus. Region 'PetC' corresponds to the distal region of wheat that contains seed storage protein genes, gliadins (*Gli-1*) and glutenins (*Glu-3*). Region 'PetB' is further subdivided into sections PetB1 and PetB2 to account for 1BL·1RS lines that incorporate a recombination event between rust resistance genes and *Pm8*. The start and end points of blocks of rye chromatin are not known precisely and may lie anywhere between two marker loci where one is present and the other is absent. **b** The position of various markers on 1RS are shown in a tabular form, based on hybridisation with 'Pavon' wheat, the 1BL·1RS translocation line and the primary and tertiary recombinants

used in the assay, one or more RFLPs were mapped to the region containing *SrR* on 1RS of 'Imperial' rye (ImpB, Fig. 1a, b). Another RGA marker, PIC31, was amplified from the region containing *SrR* with primers specific to the P-loop region of the rice *Xa1* gene (Yoshimura et al. 1998) and the GLPL motif of the rice blast gene *Pi-b* (Wang et al. 1999). PIC31 was amplified from DR-A1 and $Sr^{+}Sec^{-}$, placing this marker in the ImpB interval. This map location was confirmed using PIC31 DNA as an RFLP probe. A third RGA marker (cslrgh3) which was closely related to the barley RGA probe b6 (Leister et al. 1998), hybridised to multiple restriction fragments of genomic DNA of the wheat-rye recombinant lines, and several polymorphic fragments amongst these mapped to either the ImpB or ImpC regions (Fig. 1a, b). Because RGA39, PIC31 and cslrgh3 do not cross hybridise, three distinct RGA loci have been identified in the ImpB region.

To isolate additional markers from the region carrying *SrR*, we employed the AFLP technique using the secondary recombinant line DR-A1 and the tertiary recombinant lines $Sr^{+}Sec^{-}$ and $Sr^{-}Sec^{+}$ as templates (Fig. 1). We used 24 primer combinations with two *Pst*I+3 and 12 *Mse*I+3 selective primer sets. An average number of 85 fragments were amplified per primer combination. Three polymorphic fragments were amplified from the *Sec-1* region in the tertiary recombinant $Sr^{-}Sec^{+}$ and were designated as IA-215, IA-294 and IA-299, respectively. Four fragments designated as IB-159, IB-262, IB-267 and IB-544 were amplified from the region carrying the stem rust gene *SrR* in the tertiary recombinant $Sr^{+}Sec^{-}$ (Fig. 1a). All AFLP fragments were cloned and used as RFLP probes on recombinant lines to confirm their location on 1RS (Fig. 1b).

Identification of markers linked to disease resistance genes (*Lr26*, *Sr31*, *Yr9* and *Pm8*) on 1RS from 'Petkus' rye

To map DNA markers on 1RS of 'Petkus' rye, we compared the RFLP patterns of 1BL·1RS, 'Pavon' wheat and seven wheat-rye recombinants shown in Fig. 2a (Luskaszewski 2000). This allowed the assignment of markers to at least four regions of the 1RS chromosome defined by various recombination breakpoints. All RFLP markers that mapped on the proximal side of *SrR* on 1RS of 'Imperial' rye were also located to the corresponding region on 1RS of 'Petkus' rye (Fig. 2a, b). Two AFLP-derived markers (IA-215, IA-294) isolated from the *Sec-1* region of 'Imperial' (region ImpA) mapped to the corresponding region proximal to the rust resistance genes in 'Petkus' rye (region PetA). One of the AFLP-derived markers isolated from the *SrR* region of 'Imperial' (IB-159) was located to the region containing rust resistance genes in 'Petkus' (PetB1). Other AFLP-derived markers isolated from the *SrR* region could not be mapped in 1BS·1RS recombinant lines due to a lack of polymorphism between wheat and rye and/or lack of hybridisation to 'Petkus' rye.

The RGA39 probe detected multiple hybridising restriction fragments in genomic DNA of 1BS·1RS recombinant lines. Polymorphic bands were mapped to regions containing rust resistance genes (PetB1) and *Pm8* resistance (PetB2). RGA probe cslrgh3, a closely related sequence of the barley probe b6 (but not

of RGA39), hybridised to multiple bands on 'Petkus' genomic DNA, some of which were mapped to a region located distal to the rust resistance genes (PetB2, PetC). PIC31, a RGA clone isolated from the *SrR* region of 'Imperial', failed to hybridise to genomic DNA of 'Petkus' rye.

RFLP probe iag95 hybridised to at least two fragments in the genomic DNA of 'Petkus' isolated from the recombinant lines (Fig. 3a). Iag95 detected two bands (bands A and B) in the 1BL·1RS translocation line (lane 3) and in the recombinant line T9 (lane 4), but both bands were absent in lines T26 and T1 (lanes 5, 6) thereby positioning iag95 distal to the rust resistance genes and *Pm8*. The recombinant lines 1B+14 and 1B+43, both lacking the gene for *Pm8* resistance, contained one and two iag95 gene members, respectively (lanes 7, 8), suggesting that 1B+14 contained a shorter segment of rye chromatin as compared to 1B+43 (Fig. 3b). The presence of both iag95 bands in the tertiary recombinant $1RS_{38:9}$·1BL (lane11) indicated that rye segments were overlapping between lines 1B+14, 1B+43 and $1RS_{38:9}$·1BL and that iag95 mapped to the two closely linked loci on 1RS of 'Petkus' rye.

Marker mwg60, which mapped to the *SrR* region of 'Imperial' rye and located proximal to iag95 (region ImpB), showed hybridisation to the recombinant lines 1B+14, 1B+32 and 1B+38, but not to the tertiary recombinant ($1RS_{38:9}$·1BL) placing it distal to iag95. This discrepancy of marker order between mwg60 and iag95 in the two sets of wheat-rye recombinant lines may represent differences between 'Imperial' vs 'Petkus' rye chromatin. Alternatively, some rearrangement of 'Petkus' rye chromatin may have occurred during the development of wheat-rye recombinant lines. Marker mwg36 showed a rye specific band in the 1BL·1RS translocation line and the primary recombinant T9, but did not hybridise to the recombinant lines T26 and T1, thereby placing it distal to the rust resistance genes. However, there was no hybridisation with the primary recombinant, 1B+32, implying that primary recombinants T-26 and 1B+32 do not overlap and that mwg36 is located in the gap between the two segments of rye chromatin in T26 and 1B+32 (data not shown).

In an attempt to order the DNA markers in the 1RS region, we have developed a mapping family in wheat derived from a 1BL·1RS (Petkus) and 1BL·1RS (Imperial) heterozygote. However, it appears that recombination in the 1RS region is strongly suppressed in this family, possibly due to heterogeneity in the heterochromatic knob regions at the ends of the 'Petkus' and 'Imperial' 1RS arms (Spielmeyer, unpublished). Therefore we have used an *Ae. tauschii* mapping family which is highly recombinogenic in this region (Spielmeyer et al. 2000), to map and order the *SrR* and *Sr31*-linked markers identified in the present study. This *Ae. tauschii* family also segregates for stem rust resistance gene, *Sr33*, which has been mapped to the short arm of chromosome 1DS. Markers distal (iag95, mwg60) and proximal (ksuG9) to rust resistance genes on 1RS, also

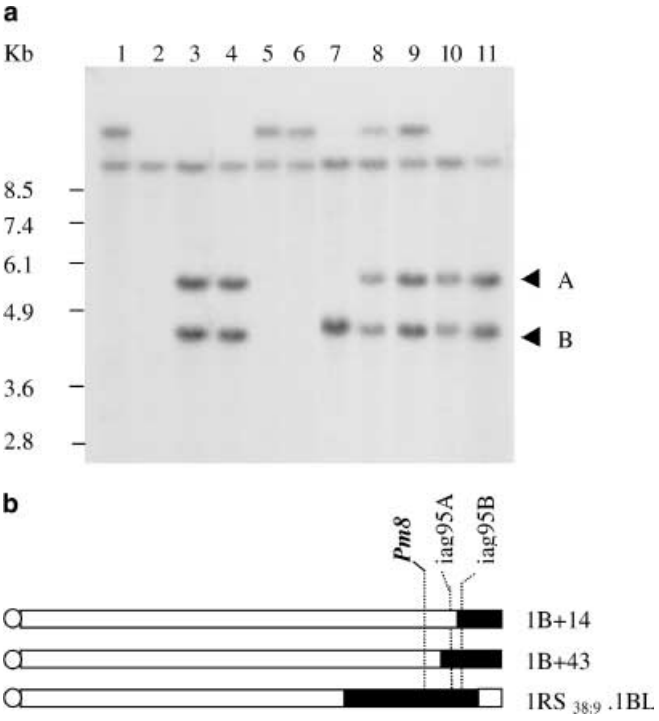


Fig. 3 **a** DNA hybridisation of iag95 probe to *Dra*I-digested genomic DNA from recombinant lines between *Triticum aestivum* cv Pavon76 and the short arm of rye (*S. cereale* L.) chromosome 1R. *Lanes: 1, T. aestivum* cv Pavon76; *2*, Pavon Dt·1BL; *3*, translocation line Pavon 1BL·1RS; *lanes 4–10*, primary recombinants: *4*, T-9; *5*, T-26; *6*, T-1; *7*, 1B+14; *8*, 1B+43; *9*, 1B+32; *10*, 1B+38. *Lane11*: tertiary recombinant $1RS_{38:9}$·1BL obtained by crossing T-9 and 1B+38. *Arrows* indicate the position of the two bands (A and B) on 1RS of 'Petkus' rye. **b** Detailed analysis of the primary recombinant lines 1B+14 and 1B+43 in comparison to the tertiary recombinant $1RS_{38:9}$·1BL based on hybridisation with iag95. Although 1B+14 and 1B+43 belong to the same recombinant class, 1B+43 has a larger piece of rye chromatin and thus shows both the alleles of iag95

mapped distal and proximal to the stem rust resistance gene *Sr33*. Two DNA probes, IB-159 and RGA39, were mapped to three loci on 1DS (Fig. 4), none of which cosegregated with *Sr33*. The other probes did not detect any RFLPs, or in the case of PIC31 did not hybridise to *Ae. tauschii*.

Development of PCR-based markers linked to rust resistance genes for marker-assisted selection in breeding

Sequence information derived from markers linked to *SrR* of 'Imperial' and *Lr26*, *Sr31*, *Yr9* of 'Petkus' was used to develop PCR primers for marker-assisted breeding. Specific primers were developed from the AFLP-derived marker IB-267 that was isolated from the *SrR* region of 'Imperial' and tested in a range of wheat-rye translocation lines carrying the *SrR* rye segment. The primers amplified a marker band associated with the presence of the rye segment containing *SrR* (Fig. 5a).

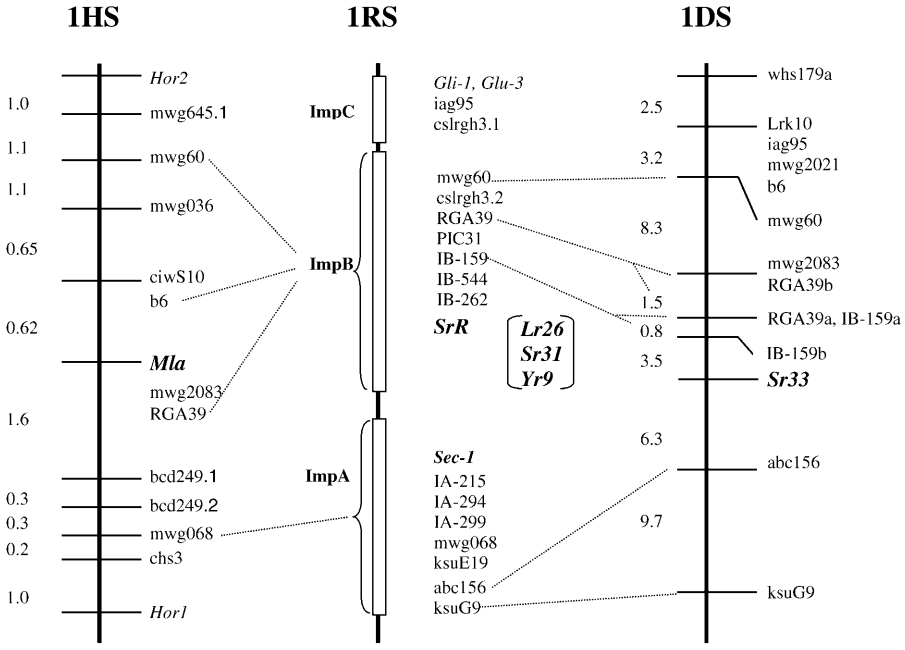


Fig. 4 Comparative map of the group-1 chromosomes 1HS of barley (Wei et al. 1999), 1DS of *Ae. tauschii* (Spielmeyer et al. 2000) and 1RS of Imperial rye. The mapping on 1RS is based on the position of the markers in 1DL·1RS recombinant lines (Rogowsky et al. 1993). Map distances for 1HS and 1DS are shown in centi Morgans (cM)

Table 2 Primer sequences and PCR conditions for amplification of SCAR markers

Marker	Primers	PCR conditions	
IB-267	5′ GCAAGTAAGCAGCTTGATTTAGC 3′ 5′ AATGGATGTCCCGGTGAGTGG 3′	94 °C, 3 min 94 °C, 30 s 55 °C, 60 s 72 °C, 60 s 25 °C, 60 s	1 cycle 30 cycles 1 cycle
iag95	5′ CTCTGTGGATAGTTACTTGATCGA 3′ 5′ CCTAGAACATGCATGGCTGTTACA 3′	94 °C, 3 min 94 °C, 30 s 55 °C, 60 s 72 °C, 70 s 25 °C, 60 s	1 cycle 30 cycles 1 cycle

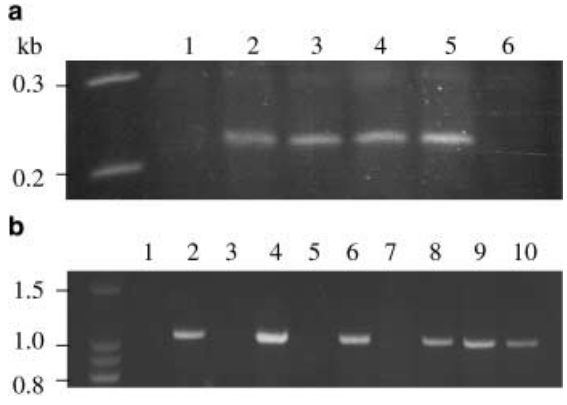


Fig. 5 a PCR amplification of the STS marker IB-267 from different wheat varieties carrying stem rust gene, *SrR*, from 'Imperial' rye. *Lanes: 1*, *T. aestivum* cv Gabo; *2*, Gabo 1BL·1RS; *3*, Gabo 1DL·1RS; *4*, secondary recombinant line DR-A1; *5*, tertiary recombinant $Sr^{+}Sec^{-}$; and *6*, reciprocal tertiary recombinant line $Sr^{-}Sec^{+}$. **b** PCR amplification of the STS marker iag95 from various wheat varieties containing 1RS of Petkus rye. *Lanes: 1*, *T. aestivum* cv Pavon76; *2*, Pavon 1BL·1RS; *3*, Thatcher; *4*, Thatcher + Lr26; *5*, Avocet; *6*, Avocet + Yr9; *7*, Federation; *8*, Federation/Kavkaz; *9*, Veery3 and *10*, Bobwhite

Similarly, specific primers were developed from the iag95 probe, that amplified a diagnostic marker associated with the presence of the rye segment carrying *Lr26*, *Sr31*, *Yr9* from a range of germplasms (Fig. 5b). Table 2 lists the primer sequences and PCR conditions for the amplifications.

Discussion

We have identified AFLP, RFLP and RGA markers located on the short arm of chromosome 1 of rye which carries leaf (*Lr26*), stem (*Sr31*/*SrR*) and stripe (*Yr9*) rust resistance genes. The markers were identified initially using wheat lines with either a 1BL·1RS (Pavon wheat/Petkus rye) or 1DL·1RS (Gabo wheat/Imperial rye) translocation chromosome. Subsequently the markers were assigned to particular segments of 1RS using a series of wheat-rye recombinant lines that contained only part of the 1RS arm. Some of the RFLP markers were identified using probes that originated from the short arms of chromosomes 1 of barley, wheat and

Ae. tauschii. In general, as shown in Fig. 4, the locus order on 1RS is in agreement with published maps of that region of rye, wheat and barley (Rowgowsky et al. 1993; Van Deynze et al. 1995; Börner and Korzun 1998; Wei et al. 1999; Lukaszewski 2000; Spielmeyer et al. 2000).

The probes that identified several RFLP markers flanking the barley *Mla* locus on chromosome 1HS detected orthologous markers flanking rust resistance genes on chromosome arms 1RS and 1DS. RGA probe, RGA39, derived from a member of the *Mla* gene family, identified markers within the rye segments of 'Imperial' carrying *SrR* and 'Petkus' carrying *Lr26*, *Sr31* and *Yr9*. RGA39 also mapped to two loci linked to the stem rust resistance gene *Sr33* located on chromosome 1DS of *Ae. tauschii*. Whether *Sr33* is orthologous to either *SrR* or *Sr31*, cannot be determined.

The stem rust resistance genes (*SrR* and *Sr31*) located on the short arms of chromosome 1R of 'Imperial' and 'Petkus' rye, respectively, are effective against all current Australian stem rust pathotypes. Recently developed wheat-rye recombinant lines containing interstitial regions of 1RS without the *Sec-1* locus (Lukaszewski 2000; Shepherd 2001, personal communication) may allow breeders to transfer stem rust resistance from rye into adapted germplasm without possible quality defects. PCR-based markers developed for the interstitial rye segments carrying the stem rust resistance genes will permit the transfer of these genes through marker-assisted selection, without depending on rust testing. This will be important for adding the rye genes to lines which already contain other stem rust resistance genes. However, testing the specificity of these PCR markers in a wider range of wheat germplasm carrying the 1RS of 'Imperial' or 'Petkus' rye is essential.

Although several markers linked to rust resistance genes on 1RS were identified, the exact map positions of these genes need to be determined. A mapping family segregating for the rust resistance genes *Lr26*, *Sr31* and *Yr9*, but without the problem of recombination suppression, is currently being generated. The present study prepares the groundwork for the isolation of candidate genes for rust resistance from 1RS of rye.

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