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ORIGINAL PAPER

Mapping genes Lr53 and Yr35 on the short arm of chromosome 6B of common wheat with microsatellite markers and studies of their association with Lr36

N. A. Dadkhodaie · H. Karaoglou · C. R. Wellings · R. F. Park

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Abstract The rust resistance genes *Lr53* and *Yr35*, transferred to common wheat from *Triticum dicoccoides*, were reported previously to be completely linked on chromosome 6B. Four F_3 families were produced from a cross between a line carrying *Lr53* and *Yr35* (98M71) and the leaf rust and stripe rust susceptible genotype Avocet "S" and were rust tested using *Puccinina triticina* pathotype 53-1,(6),(7),10,11 and *Puccinia striiformis* f. sp. *tritici* pathotype 110 E143 A+. The homozygous resistant lines produced infection types of ";1−" and ";N" to these pathotypes, respectively. The Chi-squared tests indicated goodness-of-fit of the data for one leaf rust gene and one stripe rust gene segregation. Linkage analysis using this population demonstrated recombination of 3% between the genes. Microsatellite markers located on the short arm of chromosome 6B were used to map the genes, with the markers *cfd1* and *gwm*508 being mapped approximately 1.1 and 4.5 cM, respectively, proximal to *Lr53*. Additional studies of the relationship between *Lr36*, also located on the short arm of chromosome 6B, and *Lr53* indicated that the two genes were independent.

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N. A. Dadkhodaie · H. Karaoglou · C. R. Wellings · R. F. Park (✉)
Plant Breeding Institute, The University of Sydney,
107 Cobbitty Road, Cobbitty, NSW 2570, Australia
e-mail: robert.park@sydney.edu.au

Present Address:
N. A. Dadkhodaie
Department of Agronomy and Plant Breeding,
College of Agriculture, Shiraz University,
Shiraz, Fars 71441-65186, Iran

Introduction

Rust diseases are major constraints to wheat production in many regions throughout the world. Rust resistant wheat cultivars may become susceptible as new rust pathotypes appear due to mutation within local pathogen populations or through the introduction of new pathotypes from outside a region. Maintaining sustained genetic control of rust diseases is, therefore, dependent upon discovering and deploying new resistance genes.

Alien gene transfer is a valuable means of increasing the amount of genetic diversity available to wheat breeders (Jiang et al. 1994). Rust resistance genes that have been transferred to hexaploid wheat from wild relatives include *Lr9* (Soliman et al. 1963), *Lr35* (Kerber and Dyck 1990), *Lr36* (Dvorak and Knott 1990), *Yr15* (Gerechter-Amitai et al. 1989), *Lr54* and *Yr37* (Marais et al. 2005a), *Lr56* and *Yr38* (Marais et al. 2006) and *Lr62* and *Yr42* (Marais et al. 2009).

Although a valuable source of new resistance, genes from alien species are not necessarily durable. For example, the deployment of *Lr9* was soon followed by the development of matching virulence in *Puccinia triticina* (*Pt*) (Friebe et al. 1996). Combining resistance genes has been proposed as a way of increasing the durability of individual genes by reducing the chance of a multiple mutation arising for matching virulence in rust pathogens. It is, however, difficult to select gene combinations without rust cultures with virulence to one or more of the genes being combined. Although there are some examples of morphological traits linked to resistance genes that aid in their selection (e.g. pseudo black chaff and *Sr2*; Hare and McIntosh 1979), expression of these traits may depend on growth stage and environmental conditions.

More recently, molecular markers have been developed that are independent of environmental and developmental factors, and these may facilitate the selection of traits such

as disease resistance genes. Microsatellites, or simple sequence repeats (SSRs), are a class of DNA sequences that are inherited as a Mendelian trait and are popular mainly due to their high variability, their abundance in genomes, and their co-dominance characteristics. Hiebert et al. (2005) used microsatellite markers to locate the rust resistance gene *Lr52* on chromosome 5B. Microsatellite markers linked with the leaf rust resistance genes *Lr10* (Schachermayr et al. 1997), *Lr16* (McCartney et al. 2005), *Lr34* (Bossolini et al. 2006; Lagudah et al. 2006; Suenaga et al. 2003), *Lr35* (Seyfarth et al. 1999), *Lr39* (Raupp et al. 2001), *Lr47* (Helguera et al. 2000), the stripe rust resistance genes *Yr34* (Bariana et al. 2006), *Yr36* (Uauy et al. 2005), and the stem rust resistance genes *Sr2* (Hayden et al. 2004; Spielmeyer et al. 2003), *Sr24* and *Sr26* (Mago et al. 2005) have been reported.

The wild tetraploid wheat species *Triticum dicoccoides* is considered a member of the primary gene pool for wheat breeding at the hexaploid level (Jiang et al. 1994), from which traits such as resistance genes can be transferred by direct hybridization, homologous chromosome recombination, backcrossing, and selection. The leaf rust resistance gene *Lr36* (Dvorak and Knott 1990) and the stripe resistance gene *Yr36* (Uauy et al. 2005) were transferred from *T. dicoccoides* to wheat and are located on 6BS. The two linked seedling resistance genes *Lr53* and *Yr35* were also transferred from *T. dicoccoides* to hexaploid wheat by Marais et al. (2005b) and mapped to the short arm of chromosome 6B using the Chinese Spring monosomic series and telosomic stocks for 6B. Virulence has not been reported for either gene, making them potentially valuable in developing rust resistant wheat cultivars. Although tight linkage was initially reported between *Lr53* and *Yr35* (Marais et al. 2003), preliminary studies indicated recombination between the two loci (C. R. Wellings unpublished data). A study was, therefore, conducted to confirm and assess the recombination between leaf rust resistance gene *Lr53* and stripe rust resistance gene *Yr35*, to identify SSR markers linked to these genes, and to determine linkage relationships between *Lr53* and *Lr36*.

Materials and methods

Plant and pathogen materials

Four families of an F_3 population derived from a cross between the resistant line 98M71 and the susceptible genotype Avocet "S" (a selection from cultivar Avocet, lacking seedling resistance gene *YrA*; Wellings 2007) were inoculated as seedlings in the greenhouse with *Pt* pathotype (pt.) 53-1,(6),(7),10,11 (accession number 810043; avirulent for *Lr1*, *Lr2a*, *Lr3a*, *Lr3bg*, *Lr3 ka*, *Lr14a*, *Lr15*, *Lr17b*, *Lr19*, *Lr23*, *Lr24*, *Lr26*, *Lr28*, and *Lr37*; partially virulent for *Lr17a* and *Lr27 + Lr31*; and virulent for *Lr10*, *Lr13*, *Lr16*, and *Lr20*) and *Puccinia striiformis* f. sp. *tritici* (*Pst*) pt. 110 E143 A+ (accession number 861725; avirulent for *Yr1*, *Yr5*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr27*, *Yr32*, and *YrSpaldings Prolific*; virulent for *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *YrA*, *YrNord Desprez*, *YrStrubes Dikkopf*, and *YrSuwon Omar*). From 15 to 20 seeds of each line were planted in clumps in 9 cm diameter pots and inoculated 12 days later with urediniospores of each pathotype suspended in light mineral oil (Shellsol T®, Shell Chemicals, Australia). The inoculated material was incubated at 100% RH for 24 h at 20°C and 11°C, and then kept in greenhouse microclimates at temperatures of 23–25°C and 17–20°C, for leaf rust and stripe rust, respectively. Lines were scored using the 1–4 infection type (IT) scale (McIntosh et al. 1995). The goodness-of-fit of the observed data to expected models and the homogeneity of the overall data were tested using the Chi-squared statistic. Lines selected at random were examined for chromosome behavior. Seeds were germinated and root tips were collected and fixed in 3:1 ethanol to acetic acid. Root tip cells were squashed in acetocarmine 1% and mitotic chromosomes were counted.

DNA extraction, primers and polymerase chain reaction (PCR)

Fresh leaves were collected from parents and all F_3 lines, with a minimum of ten plants from each line. DNA was extracted using a SDS small scale DNA extraction method (Bansal et al. 2010) with some modifications in the time and speed of centrifugation. Leaves were dried in 2 ml flat bottom tubes in silica gel for 3 days. The dried leaves were then crushed into a fine powder using two ball bearings in a mixer mill (Retsch® MM300, Germany) and 700 µl of preheated extraction buffer (5 ml of 1 M Tris, 2 ml of 0.5 M EDTA, 2 ml of 5 M NaCl, 5 ml of 20% SDS, 70 µl of 10 mM β mercaptoethanol) was added to each tube and incubated for approximately 10–15 min in a water bath at 65°C with occasional mixing by gentle inversion. Following incubation, 150 µl of 3 M potassium acetate (pH = 5.2) was added to each tube, the tubes were kept in a −20°C freezer for 15–20 min, and were then centrifuged at 12,000 revolutions per minute (rpm) for 10–15 min. The supernatant was transferred to new 1.5 ml Eppendorf tubes and an equal volume of cold isopropanol was added to each, followed by centrifugation at 12,000 rpm for 15 min. At this stage, the supernatant was discarded and the pellets were floated in 200 µl of distilled water and kept overnight at 4°C. The next day, tubes were centrifuged at 12,000 rpm for 10 min and 180 µl of supernatant was transferred to new 1.5 ml tubes and 25 µl of 3 M Na-acetate (sodium acetate, $3H_2O$ 40.82 g, ddH_2O 50 ml, pH = 5.2–5.5 adjusted with

glacial acetic acid) was added. After adding, 500 µl of absolute ethanol to each tube, they were kept at −20°C for 15 min and then centrifuged at 13,200 rpm for 15 min and the supernatant was discarded. This process was then repeated with 70% ethanol. The supernatant was discarded, pellets were dried completely and 200 µl of autoclaved ddH_20 was added to each and left overnight to dissolve. RNA was degraded by adding 10 µl of RNase to the DNA stock solution and the tubes were incubated for 1–2 h in a 37°C water bath or oven.

Thirty-one microsatellite markers were selected from those mapped previously on the short arm of chromosome 6B (Gupta et al. 2002; Guyomarćh et al. 2002; Röder et al. 1998; Somers et al. 2004). The *gwm* markers were mapped previously in the population Opata 85 × w7984 (Röder et al. 1998). All primer sequences were as described by Röder et al. (1998) or as listed at http://wheat.pw.usda.gov.

PCR was performed in a 25 µl volume that included 3 µl of 25 ng/µl DNA template, 6.75 µl of distilled water, 2.5 µl of 10× buffer (Applied Biosystems, U.S.), 2.5 µl of 2 mM dNTP (Roche Diagnostic, Australia), 5 µl of 10 ng/µl each forward and reverse primers (Sigma-Genosys, Australia), and 0.25 µl (1.25 U) of Taq polymerase enzyme (Applied Biosystems, U.S.). Neither $MgCl_2$ nor $Mg(C_2H_3O_2)_2$ was used unless mentioned. PCR was performed according to the conditions mentioned in Table 1. Initially, PCR products were separated on 2% agarose gels with 1× TBE in a horizontal submarine gel tank at 100 volts for 1.5 h. PCR products that were non-polymorphic on agarose were detected on polyacrylamide gel electrophoresis (PAGE) using silver-staining. The glass plates were washed thoroughly and then cleaned with 70% alcohol. About 1 ml of bind-saline solution (comprising of 1 ml Bind saline, 49 ml ethanol, 10 ml glacial acetic acid and 40 ml dH_2O) and 1 ml of Repel (1 ml of DMCS and 99 ml of ethanol) were applied on uncut and cut plates once and twice, respectively. Once the plates were assembled, 60 ml of gel solution (each 50 ml consisted of 24 g urea, 7.5 ml 40% acrylamide, 10 ml of 5× TBE and 15 ml of dH_2O) was mixed thoroughly with 600 µl of 10% APS (1.0 g of ammonium persulfate in 10 ml of dH_2O) and 60 µl of TEMED. The gel was poured and left for 1.5 h to set. Once the gel had set, the wells were cleaned thoroughly and a pre-run in an electrophoresis apparatus in 1× TBE buffer for 30 min was carried out to bring the gel temperature to 50°C. Four µl of PCR product was then mixed with 2 µl of loading buffer, denatured in a thermocycler at 95°C for 5 min, and then kept on ice, while 3 µl of it was loaded onto the gel. A 25 bp DNA ladder (Invitrogen, Australia) was used as the reference marker. The gel was run for 1.5 h and after separating the plates, was fixed for 40 min in fixing solution (10% acetic acid), washed twice in dH_2O (each time for 5 min) and stained in silver nitrate (0.2%) for 45 min. After staining, the gel was washed in dH_2O for 10–20 s and developed in 1× developer (diluted from 5× developer, comprising of 625 g sodium carbonate, 20 ml formaldehyde and 1 ml sodium thiosulphate) for 5–10 min and then stopped in stopper solution (5% acetic acid and 2% glycerol) for 3–5 min. It was finally rinsed with dH_2O for 3–5 min and left upright overnight to dry and then scored manually.

Table 1 PCR conditions used for microsatellite primers

Microsatellite locus	PCR conditions
CFD1	Initial denaturation (94°C): 5 min
	Denaturation (94°C): 30 s
	Annealing Tm: 30 s
	Extension (72°C): 30 s
	30 cycles
	Final extension (72°C): 10 min
All *GWM*s, *WMC*s, *BARC*s, and *GDM108*	Initial denaturation (94°C): 3 min
	Denaturation (94°C): 1 min
	Annealing Tm: 1 min
	Extension (72°C): 2 min
	35 cycles
	Final extension (72°C): 10 min
BARC134, *BARC198*, *GWM508*, *GWM626*, *WMC95*	Touchdown PCR
	Initial denaturation (94°C): 3 min
	Denaturation (94°C): 1 min
	Annealing Tm: 1 min with 1°C decrement per cycle
	Extension (72°C): 2 min
	7 cycles
	Denaturation (94°C): 1 min
	Annealing (51°C): 1 min
	Extension (72°C): 2 min
	28 cycles
	Final extension (72°C): 10 min

Approximate sizes of the polymorphic markers (bp) *cfd1* (220, 275), *gwm508* (135), *barc198* (145), *gwm191* (128–134), and *wmc487* (160)

Data analysis

Images of agarose gels were captured using a gel Doc-It imaging system (UVO®), while polyacrylamide gels were digitally scanned. The gels were scored manually for the whole population and data were analysed and linkage groups constructed by Map manager QTX version b20 (Manly et al. 2001) using the Kosambi mapping function.

Allelism tests

To test linkage between *Lr36* and *Lr53*, line ER84018 (carrying *Lr36*) was crossed to line 98M71 (carrying *Lr53*), and the F_1 plants were grown to flowering and allowed to self.

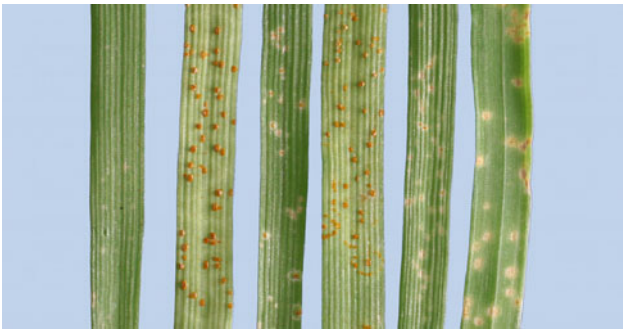

Fig. 1 Responses of Line 98M71 (*Lr53*), Avocet "S" (Susceptible), homozygous resistant line (line 71), homozygous susceptible (line 97), Agent (*Lr24*) and Harrier (*Lr17b*) to *Puccinia triticina* pathotype 53-1,(6),(7),10,11

Four F_2 families were inoculated with *Pt* pt. 53-1, (6),(7),10,11 as described previously. The inoculated plants were kept in a greenhouse microclimate at 20°C and notes were taken 12 days later.

Results

Seedling tests

Resistant F_3 lines showed ITs of ";1−" (Fig. 1) and ";N" to the selected *Pt* and *Pst* rust pathotypes (Table 2). Results from leaf rust tests showed that each family segregated in the ratio of 1:2:1, supporting the hypothesis of a single gene conferring the resistance ($P \leq 0.01$; Table 3). Chi-squared analysis of results obtained with stripe rust also indicated a goodness-of-fit of the data for one gene segregation (Table 4). Examination of mitotic root tip cells showed normal chromosome numbers in all lines tested (data not shown).

Table 3 Frequencies of lines in four F_3 families from a cross between line 98M71 and Avocet S, classified as either non-segregating resistant (resistant), segregating, or non-segregating susceptible (susceptible) to *Puccinia triticina* pathotype 53-1,(6),(7),10,11

Family	Number of lines			χ^2 value	*P*
	Resistant	Segregating	Susceptible		
1	6	23	13	2.71	0.2573
2	9	29	12	1.64	0.4404
3	9	30	11	2.16	0.3395
4	8	23	13	1.22	0.5413
Pooled data	32	105	49	6.20	0.0540

A Totaled Chi sq. (2.71 + 1.64 + 2.16 + 1.22) = 7.73, 3 *df*

B Pooled Chi sq. (32:105:49) = 6.20, 2 *df*

Homogeneity Chi sq. (A−B) = 1.53, 1 *df* ($P > 0.10$)

The homogeneity Chi-squared value for data from both leaf rust (1.22) and stripe rust (5.33) tests (Tables 3, 4) was less than the tabulated Chi-squared value ($P \leq 0.01$ and $P \leq 0.05$, 16.81 and 12.59, respectively, $df = 6$), indicating consistency of the overall pattern of inheritance for a single gene to both pathogens. On this basis, data for all families were pooled to enable inspection of the joint segregation of both traits (Table 5). The data indicated that the population segregated for one leaf rust resistance gene and one stripe rust resistance gene. As shown in Table 5, 11 lines (underlined) out of a total 186 involved recombination events between the two rust resistance loci.

Microsatellite polymorphism

Of the 31 microsatellite loci tested, only five, *gwm508*, *gwm191*, *barc198*, *cfd1*, and *wmc487*, were polymorphic between the parents. Only one band was detected for the

Table 2 Infection types of parents and HR lines from a cross 98M71 and Avocet S, and selected differential genotypes to *Puccinia triticina* pathotype 53-1,(6),(7),10,11 and *Puccinia striiformis* f. sp. *tritici* pathotype 110 E143 A+

P. triticina pathotype 53-1,(6),(7),10,11			*P. striiformis* f. sp. *tritici* pathotype 110 E143 A+		
Line/differential	IT	Gene(s)	Line/differential	IT	Gene(s)
98M71	;1−	*Lr53*	98M71	;N	*Yr35*
Avocet "S"	3+	–	Avocet "S"	3^+	–
F_3 HR line	;1−	*Lr53*	F_3 HR line	;N	*Yr35*
Webster[a]	0;	*Lr2a*	Clement[b]	0;;	*Yr2*, *Yr9*
Thew[a]	3+	*Lr20*	Selkirk[b]	1NB	*Yr27*
Gaza[a]	;1−	*Lr23*	Fed/4* Kav[b]	0;	*Yr9*
Egret[a]	3+	*Lr13*	Avocet R[b]	3+	*YrA*
Exchange[a]	33	*Lr16*	Lee[b]	0;	*Yr7*
Mildress[a]	0;	*Lr26*	Vilmorin 27[b]	33+	*Yr3*
Agent[a]	;	*Lr24*	Heines VII[b]	33+	*Yr2*

[a] Differential genotypes used to characterize isolates of *P. triticina*

[b] Differential genotypes used to characterize isolates of *P. striiformis* f. sp. *tritici*

Table 4 Frequencies of lines in four F_3 families from a cross between 98M71 and Avocet S classified as either non-segregating resistant (resistant), segregating, or non-segregating susceptible (susceptible) to *Puccinia striiformis* f. sp. *tritici* pathotype 110 E 143 A+

Family	Number of lines			χ^2 value	*P*
	Resistant	Segregating	Susceptible		
1	5	24	13	3.91	0.1419
2	9	33	8	5.16	0.0757
3	10	31	9	2.92	0.2322
4	9	22	13	0.73	0.6951
Pooled data	33	110	43	7.29	0.0261

A Totaled Chi sq. (3.91 + 5.16 + 2.92 + 0.73) = 12.72, 3 *df*

B Pooled Chi sq. (33:110:43) = 7.29, 2 *df*

Homogeneity Chi sq. (A−B) 12.72−7.29 = 5.43, 1 *df* (*P* = 0.022)

Table 5 Joint segregation data from four F_3 families from a cross between 98 M71 and Avocet S in response to *Puccinia triticina* and *Puccinia striiformis* f. sp. *tritici* pathotypes 53-1,(6),(7),10,11 and 110 E143 A+, respectively

		Leaf rust phenotype			Total
		Resistant	Segregating	Susceptible	
Stripe rust phenotype	Resistant	30	3	–	33
	Segregating	2	102	6	110
	Susceptible	–	–	43	43
Total		32	105	49	186

Underlined numbers indicate recombinant lines

loci *wmc487* and *barc198*, while *gwm191* produced multiple bands in which the polymorphic band showed a presence/absence pattern. Loci *cfd1* and *gwm508* were monomorphic on agarose gels but polymorphic on polyacrylamide gels. The *cfd1* marker was codominant while *gwm508* showed absence/presence pattern for segregating and susceptible lines. Two different alleles with approximate sizes of 220 and 275 bp (Fig. 2) were amplified from the *cfd1* locus. The 225 bp band was present in resistant the parent, contrasting with 275 bp band, which was present in the susceptible parent. Both of these alleles were present in segregating lines. A band with an approximate size of 135 bp was amplified from the *gwm508* locus. This marker gave a distinct pattern for homozygous resistant lines, however, segregating and non-segregating genotypes were also distinguishable because a band was present in segregating lines and absent in susceptible ones. Based on this information, the number of segregating lines was determined.

Linkage analysis

The loci showing co-dominant and dominant alleles were analyzed separately. The null alleles were considered as either paternal or maternal dominant. In this way, three different linkage groups with LOD scores higher than 3.0 were formed and then merged to construct a consensus map. The final map of chromosome 6BS is shown in Fig. 3. The genetic linkage positioned *Lr53* and *Yr35* approximately 3 cM apart. The closest marker to *Lr53* was *cfd1*, which was proximal at an approximate distance of 1.1 cM. The markers spanned a segment of 50 cM. Marker *gwm508* was mapped at a distance of 3.4 cM from *cfd1*. The only marker mapped distal to the resistance genes was *gwm191*, which was 18.9 cM from *Yr35*. Markers *wmc487* and *barc198* were also mapped to the proximal region with distances of 18 and 27.6 cM from *Lr53*, respectively.

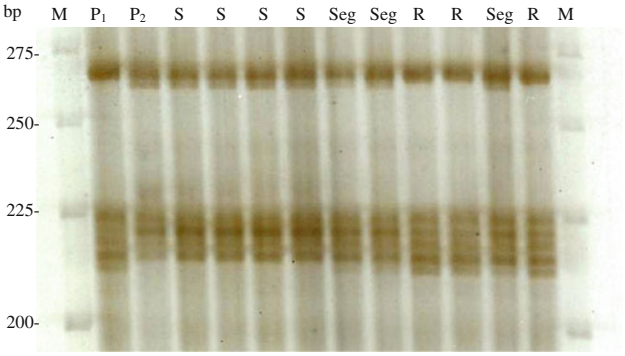

Fig. 2 The *cfd1* locus polymorphic on 6% polyacrylamide gel (P_1 non-segregating resistant parent, P_2 non-segregating susceptible parent, *R* resistant line, *S* susceptible line and *Seg* segregating lines for *Puccinia triticina* pathotype 53-1,(6),(7),10,11). *M* 25 bp DNA ladder (Invitrogen, Australia)

Allelism tests

The lines ER84018 (*Lr36*) and 98M71 (*Lr53*) showed ITs of "1+2CN" and ";1=", respectively, and the resistant F_2 plants produced ITs ";1−" and "1++2N", while the susceptible line had higher ITs of "33−" (Fig. 4). A Chi-squared analysis, based on the three different phenotypic groups indicated that *Lr36* and *Lr53* segregated independently (Table 6). The data were interpreted on a theoretical F_2 segregation ratio of 12:3:1, where *Lr53* (IT ";1−") was epistatic to *Lr36* (IT "1++2N"). The homogeneity Chi-squared test also indicated that pooling the data was justified (4.43, *df* = 1, *P* = 0.037).

Discussion

While previous studies had indicated that *Lr53* and *Yr35* were completely linked (Marais et al. 2005b), the present study demonstrated approximately 3% recombination between these two genes. No F_3 line non-segregating resistant for *Lr53* and lacking *Yr35*, or vice versa, was observed

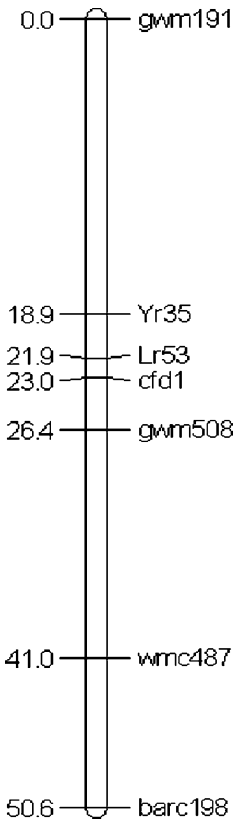

Fig. 3 Genetic map for *Lr53* relative to *Yr35* and polymorphic microsatellite markers on chromosome 6BS constructed using a population of 186 individuals (distances are shown in centi Morgans (*cM*)

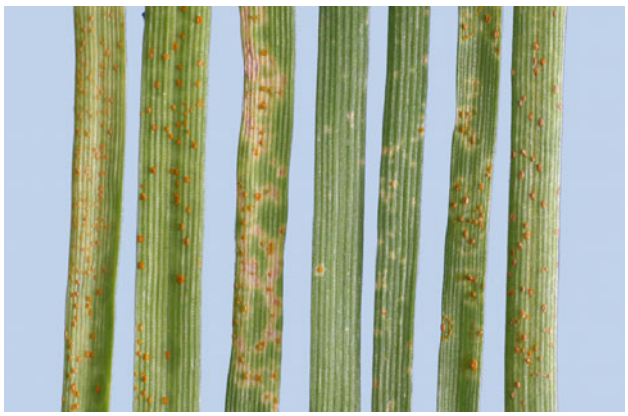

Fig. 4 Responses of genotypes Manitou (*Lr13*), Neepawa (*Lr13*), ER84018 (*Lr36*), 98M71 (*Lr53*), resistant F_2 plants (*Lr53* and *Lr36*, respectively), susceptible F_2 plant to *Puccinia triticina* pathotype 53-1,(6),(7),10,11

in this study. However, the expected recombinant lines were recovered in F_4 lines from a separate population derived from a cross between 98M71 and Avocet "S" (data not presented), and so it can be hypothesized that their absence in the population examined here was due to chance. The lack of recombination between these two genes observed previously (Marais et al. 2005b), and the 3% recombination observed in the present study, suggests that recombination rates between the two genes may differ between crosses. This could relate to differences in homology between the susceptible parents, as previous studies by Marais et al. (2005b) used cultivar W84-17 as the susceptible parent, while Avocet "S" was the susceptible parent used here.

In the present study, there was an observed excess of susceptible lines in tests for both leaf rust and stripe rust, but this was not a statistically significant departure from a single gene segregation (Tables 3, 4). It is possible that the transmission of the genes was low because the homology between the 6B chromosome of 98M71, which carries the alien segment from *T. dicoccoides*, with that of Avocet "S", may be low. If this is the case then poor homology would result in the chromosome carrying the resistance gene being lost at meiosis, and this would expect to result in monosomy. Although some seedlings in the greenhouse tests appeared weak, indicative of monosomy, random mitotic chromosome counts did not reveal any evidence of aneuploidy. Previous studies by Marais et al. (2005b) established that the transmission rate of the introgressed region differs between male and female parents, with the preferential transmission of the genes *Lr53* and *Yr35* through pollen being 96–98% in contrast to their transmission through egg cells, which was 41–66%.

Most polymorphic primers produced a band pattern of absence and presence that could be attributed to changes in sequence giving rise to null alleles. Null alleles, where locus specific primers give no PCR product, have been reported in different organisms including wheat (Plaschke et al. 1995; Prasad et al. 2000). As the primers used have shown polymorphism in previous studies, it can be assumed that mutations most likely caused the results obtained in the present study. Null alleles were common in the population examined (most of the polymorphic loci produced a pattern of present/absent alleles), and this may be due to the genetic divergence between the wild *T. dicoccoides* donor and Avocet "S". During the evolutionary process, some nucleotides may have been deleted or added. Gupta and Varshney (2000) suggested that if a mutation occurs within the binding site of a DNA primer or a primer site that is too close to the microsatellite, a null allele may occur. In either case, faint bands may be produced or primer binding may be prevented completely. More than one band was produced for the two loci examined in this study (*cfd1* and *gwm191*). Multiple banding patterns like this suggest the existence of inter and intra-chromosomal duplications within the wheat genome (Guyomarćh et al. 2002).

One problem with null alleles is that segregating lines cannot be recognized from non-segregating lines; thus when the resistant parent carries the null allele, both segregating and non-segregating susceptible lines will have the band present; when the resistant parent carries the null allele, the segregating lines are indistinguishable from the

Table 6 Frequencies of lines in four F_2 families from a cross between the wheat genotypes 98M71 (carrying *Lr53*) and ER84018 (carrying *Lr36*), classified as either resistant (two groups) or susceptible to *Puccinia triticina* pathotype 53-1,(6),(7),10,11

Family	Number of plants in three IT classes[a]			χ^2 value (12:3:1)	*P*
	Resistant (;1−)	Resistant (1+2N)	Susceptible (33−)		
1	71	11	5	2.27	0.3210
2	53	10	4	0.68	0.7112
3	63	21	6	1.33	0.5134
4	45	10	2	0.85	0.6544
Pooled data	232	52	17	0.69	0.71

A Totaled Chi sq. (2.27 +0.68+ 1.33 + 0.85) = 5.13, 3 *df*

B Pooled Chi sq. (232:52:17) = 0.6971, 2 *df*

Homogeneity Chi sq. (A−B) 5.13−0.69 = 4.43, 1 *df* (*P* = 0.037)

[a] Numbers in parenthesis show infection types of different groups

non-segregating resistant ones. In the former case, a breeder can select for homozygous resistant lines, while in the latter case, the focus should be on eliminating susceptible progeny.

Five of the 31 microsatellite primers tested were polymorphic and linkage analysis positioned them all on the short arm of chromosome 6B, consistent with the chromosomal location of the two rust resistance genes on 6BS (Marais et al. 2003; Marais et al. 2005b). The genes were mapped at 3 cM apart, distal to the markers *cfd1* and *gwm508*. Because the null alleles were considered as dominant markers, the distance on the map is not precise and the position of the loci *gwm191*, *barc198*, and *wmc487* must be considered tentative. The position of *gwm508* and *cfd1* is more accurate because the segregating lines were distinguishable from non-segregating ones. Both *cfd1* and *gwm508* may be suitable for marker-assisted selection breeding because all three possible genotypes can be recognized.

Uauy et al. (2005) observed that two substitution lines carrying the high temperature adult plant resistance gene *Yr36* in DIC (a chromosome segment from *T. turgidum* ssp. *dicoccoides* located between the loci *Xgwm193* and *Xgwm508*) could be selected using the marker *gwm508* (Khan et al. 2000). However, this gene is neither effective at seedling growth stages nor at low temperatures. Although the gene *Yr35* is a seedling resistance gene and *Yr36* is an adult plant resistance gene, *gwm508* is common for the segment carrying both. Although they may have originated from a common ancestor, the apparent chromosomal location and common microsatellite marker does not necessarily suggest that they are the same gene. In another study, wild populations of tetraploid *Triticum turgidum* ssp. *dicoccoides* showed promise as a source of high grain protein content (GPC). The lines produced from a cross between Langdon (LDN) and a Langdon 6B substitution line with 6B from *T. dicoccoides* (Joppa et al. 1997) were used to map a QTL for GPC on the proximal region of the short arm of chromosome 6B that accounted for 66% of the variation in GPC present in that particular cross. The marker *gwm508* gave 136 and 138 bp fragments for LDN (DIC 6B) and LDN, respectively (Khan et al. 2000). The distal marker *Xgwm508* can be used in combination with any of the three tightly linked proximal microsatellite markers (*Xgwm193*, *Xgwm361*, and *Xgwm644*) to monitor the transfer of a segment of *dicoccoides* chromatin.

The linkage analysis of genes *Lr53* and *Lr36* showed that they segregate independently, even though they are located on the same chromosome. When two genes act independently, four phenotypes are expected in an F_2 population resulting from selfing a dihybrid plant (A-B−, A-bb, aaB− and aabb, in the ratio 9:3:3:1). In the case of rust resistance genes, the gene conferring the lowest IT is expressed (Dyck and Kerber 1985) when two effective genes act independently. Therefore, in the F_2 population derived from intercrossing 98M71 and ER84018, the genes *Lr53* and *Lr36* acted independently with genotypes A-B− and A-bb producing an IT of;1− (*Lr53*), lines carrying *Lr36* producing an IT of 1++2N (aaB−), and those lacking both genes producing a susceptible response of 3+. This resulted in a ratio of 12:3:1 in all four F_2 families. The two genes should be at least 50 cM apart to account for the independent segregation observed, despite reports of the length of the short arm of chromosome 6B being less that 40 cM (Somers et al. 2004). Two reasons could account for this: firstly, one of the genes has been wrongly assigned to 6BS; secondly, and most probably, one of these genes does not pair with the other gene. The latter possibility is in agreement with the results of this study, which indicated differential transmission rates of *Lr53*.

Lr53 and *Yr35* originated from a wild relative of wheat, and provided they are not associated with negative traits,

they are of potential importance in resistance breeding because virulence has not been reported for either to date. However, their use singly should be avoided. The markers *gwm508* and *cfd1* could facilitate the early selection of these genes in combination with other resistance genes. Marker enrichment for this region would assist in resolving the map locations and distances, and thus improve the possibilities for marker-assisted selection. Possibilities for new markers include sequenced tagged microsatellites (Hayden and Sharp 2001a; 2001b) and the sequencing and conversion of the null allele markers for *gwm508* to produce a co-dominant marker.

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