Detection of quantitative trait loci associated with leaf rust resistance in bread wheat

H.M. William, D. Hoisington, R.P. Singh, and D. González-de-León

Abstract: Leaf rust, caused by *Puccinia recondita* Rob. ex Desm., is a common disease in wheat. The objective of this study was to develop molecular markers associated with the quantitative trait loci (QTLs) putatively conferring durable leaf rust resistance in *Triticum aestivum* L. em. Thell. A population of 77 recombinant inbred lines (RILs) developed from 'Parula' (resistant) and 'Siete Cerros' (moderately susceptible) was used. Bulked segregant analysis was done using random amplified polymorphic DNAs (RAPDs) with DNA enriched for low-copy sequences using hydroxyapatite chromatography. Out of 400 decamer primers screened, 3 RAPD markers were identified between the bulk of the most resistant and the bulk of the most susceptible lines. These were cloned and used as probes on the RILs in Southern hybridizations. Two probes revealed two tightly linked loci. One-way analysis of variance showed that these two loci, and another revealed by the third probe, were linked to QTLs controlling leaf rust resistance based on data taken from 2 years of replicated field trials. Cytogenetic analysis placed the two tightly linked loci on the long arm of chromosome 7B. The third probe detected loci located on the short arms of chromosomes 1B and 1D. It is suggested that the QTL detected on 7BL may well be homoeoallelic to *Lr34*.

Key words: bulked segregant analysis, RAPDs, RFLPs, DNA enrichment, leaf rust, QTL.

Résumé : La rouille brune, causée par le *Puccinia recondita* Rob. ex Desm., est une maladie commune chez le blé. L'objectif de ce travail était de mettre au point des marqueurs moléculaires associés avec les loci à caractère quantitatif (QTLs) qui confèrent apparemment une résistance durable à la rouille brune chez le *Triticum aestivum* L. em. Thell. Une population de 77 lignées recombinantes fixées (RILs) dérivées du croisement entre 'Parula' (résistant) et 'Siete Cerros' (modérément sensible) a été employée. Une analyse de ségrégants regroupés (BSA) a été réalisée à l'aide de marqueurs RAPD (ADN polymorphe amplifié au hasard) sur de l'ADN enrichi en séquences à simple copie par chromatographie sur colonnes d'hydroxyapatite. Des 400 amorces décanucléotidiques employées, trois présentaient un polymorphisme entre l'ADN des plantes les plus résistantes et celui des plantes les plus sensibles. Ces fragments polymorphes ont été clonés et employés comme sondes pour réaliser des hybridations Southern sur les RILs. Deux sondes ont révélé des loci très fortement liés. Une analyse de variance à un critère de classification a montré que ces deux loci et un troisième détecté par la troisième sonde étaient liés à des QTLs contrôlant la résistance à la rouille brune telle que mesurée en essais au champ avec réplication et sur 2 années. Une analyse cytogénétique a permis de placer les deux loci fortement liés sur le bras long du chromosome 7B. La troisième sonde a détecté des loci situés sur les bras courts des chromosome 1B et 1D. Il est suggéré que le QTL situé sur le chromosome 7BL pourrait bien être homéoallélique au locus *Lr34*.

Mots clés : analyse de ségrégants regroupés, RAPD, RFLP, enrichissement de l'ADN, rouille brune, QTL.

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Introduction

The primary objective of plant breeding is to develop cultivars that are high yielding and stable and that can withstand biotic and abiotic stresses. Rusts are the most common diseases of cultivated wheat and the use of resistant cultivars offers the most effective and economic form of disease control. The use of single gene resistance to leaf rust (*Puccinia recondita* Roberg ex Dezmaz f.sp. *tritici*) in cultivated bread wheat has usually been short lived (German and Kollmer 1992). This is attributed to the evolution of new virulences in the pathogen to these genes (Samborski 1985).

Roelfs (1988) suggested that *Lr34* in combination with *Lr12* and *Lr13* could have contributed to the durable leaf rust resistance of several wheat cultivars, such as 'Frontana' and 'Inia'. Combinations of *Lr34* with several other known leaf rust resistance genes usually result in higher resistance than when they are present alone (Ezzahiri and Roelfs 1989; German and Kolmer 1992). Singh (1993) identified several Mexican spring wheat cultivars that carry the *Lr34* gene, which confers slow rusting against the Mexican populations of *P. recondita*. However, final disease ratings are often considered unacceptable (Singh and Gupta 1992). The *Lr34* complex, defined as the product of additive interaction involving *Lr34* and two to three additional slow rusting genes, appears to confer highly effective adult plant resistance worldwide

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(Singh and Rajaram 1992). The *Lr34* gene is known to be either pleiotropic or closely linked with leaf tip necrosis (*Ltn*) of adult plants (Singh 1992). The reliable classification of *Ltn* is often difficult, owing to the presence of other biotic and abiotic stresses that can influence its expression.

Molecular markers have the potential to be used as linked markers for traits that are difficult or time-consuming to select through conventional breeding methods. Restriction fragment length polymorphisms (RFLPs) have been widely used in the development of detailed genetic maps of different crop species (Goodfellow et al. 1993). Genetic maps developed using molecular markers spaced throughout the genome offer potential opportunities for marker-assisted selection in breeding programs. Evenly spaced molecular markers can also be utilized in the analysis of quantitative traits by partitioning complex quantitative traits into individual quantitative trait loci (QTLs). Markers based on RFLPs are often codominant and restricted to regions of low-copy sequences.

Markers based on the polymerase chain reaction (PCR; Saiki et al. 1988) have subsequently been utilized in the development of DNA amplification using random primers for the genetic characterization, mapping, and targeting of genomic regions carrying traits of interest (Williams et al. 1990; Welsh and McClelland 1990; Paran et al. 1991; Tanksley et al. 1992). This technique usually provides dominant markers with polymorphisms detected as the presence or absence of amplified fragments.

In wheat, when introgressions from wild relatives are present, RFLP or RAPD (random amplified polymorphic DNA) markers can help locate, with relative ease, alien chromosomal segments, because of the large size of the introgressed gene and the high frequency of molecular variations between these segments and their homoeologous wheat counterparts. For instance, RFLP markers linked to Hessian fly resistance genes that have been transferred from *Triticum tauschii* have been identified (Ma et al. 1993). Similarly, *Lr9*, transferred from a chromosomal segment originating from *Aegilops umbellulata*, has recently been tagged by RFLPs and by sequence tagged assays based on RAPD analysis (Schachermayr et al. 1994). However, success in tagging genes in the absence of alien introgressions in wheat is low. This is mainly due to the large genome size in bread wheat, the low levels of molecular polymorphisms within the species, and the overwhelming presence of repetitive sequences. In RAPD analysis, low levels of polymorphism are coupled with reproducibility problems in wheat (Devos and Gale 1992).

Bulked segregant analysis (BSA) has been used to identify DNA sequences linked to a target region (Michelmore et al. 1991; Eastwood et al. 1994; Reiter et al. 1992). In BSA, DNAs of individuals that share a common characteristic are bulked together and compared for identification of linked molecular markers. In the case of large genomes showing low levels of molecular polymorphism, such as that of wheat, the use of RAPDs in BSA to tag genes can be much enhanced through the use of DNA enriched for low-copy sequences (E.S. Lagudah, personal communication).

Bread wheat cultivars derived from CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) germplasm are currently grown over large areas worldwide (Byerlee and Moya 1993). 'Parula', a CIMMYT variety used in this study, contains *Lr34* and a minimum of two additional genes that confer slow rusting, which together determine its durable leaf rust resistance (Singh and Rajaram 1992). As a first step in developing markers for the durable leaf rust resistance provided by the *Lr34* complex, we here provide evidence for the existence of at least two additional resistance loci, which have been identified using BSA of DNA enriched for low-copy sequences.

Materials and methods

Plant materials

A population of 77 recombinant inbred lines (RILs), at the F_8 generation, derived from the bread wheat cross 'Parula' (resistant) × 'Siete Cerros' (partially susceptible) was studied. The resistant parent, 'Parula', is known to contain *Lr34* and two to three minor leaf rust resistance genes (Singh and Rajaram 1992); the partially susceptible parent, 'Siete Cerros', is known to contain one to two minor genes that confer resistance to leaf rust (R.P. Singh, unpublished results).

Leaf rust analysis

Field evaluations of leaf rust were conducted in trials at Ciudad Obregón (Sonora, Mexico) in 1992–1993 and 1993–1994. RILs were planted in a randomized complete block design with three replications. Plots consisted of two 1-m rows seeded 15 cm apart with 70 cm between plots. Susceptible spreader rows were inoculated with a mixture of leaf rust pathotypes TCB/TD, TBD/TM, and MCD/SM in equal amounts. Leaf rust severity was recorded thrice at 8- to 12-day intervals, beginning with the appearance of the first symptoms (except for the second trial in which no first reading was taken). Scoring followed the modified Cobb Scale (Peterson et al. 1948). Leaf tip necrosis was scored immediately after the completion of the flowering period. The area under the disease progress curve (AUDPC), which is sometimes considered to be a better measure of foliar disease than the final disease rating, was calculated using a program written at CIMMYT (Van Ginkel and Vivar 1986).

DNA isolation and enrichment

DNA was isolated from ground lyophilized leaf tissue according to the protocols of Hoisington et al (1994). DNA enrichment for low-copy sequences was carried out with hydroxyapatite chromatography according to the recommendations of E.S. Lagudah (personal communication) and Eastwood et al. (1994). DNAs of 10 individuals that were most resistant to leaf rust and had shown leaf tip necrosis were bulked (Bulk 1) and compared with the DNAs of 10 other individuals that were most susceptible and did not express leaf tip necrosis (Bulk 2). Entries that were used to make up the two bulks were also independently enriched for low-copy sequences for PCR analysis. Parental and bulked DNAs (100 µg) were sonicated to a size range of 0.3–6.0 kb, precipitated with ethanol, and resuspended in 500 µL of 0.12 M phosphate buffer (PB; Sambrook et al. 1989). This DNA was heat denatured by boiling for 10 min, followed by re-annealing at 60°C for over 24 h. The kinetics of re-annealing under these conditions would have a C_0t value (log value of moles of nucleotides/litre × re-annealing time in seconds) of over 100 (Smith and Flavell 1975). The hydroxyapatite powder (Bio-Rad, DNA grade) was suspended in 0.12 M PB (1 g in 5 mL 0.12 M PB); the suspension was placed in boiling water for 30 min and the buffer removed and replaced with fresh buffer. The suspension and the column were maintained at 60°C. After packing, the column (0.5 cm × 2.0 cm) was rinsed with three column volumes of 0.12 M PB before the DNA sample was applied. After loading the samples, four column volumes of 0.15 M PB were used to elute the single-stranded DNA. The double-stranded DNA was eluted with three column volumes of 0.4 M PB. All fractions were tested with ethidium bromide spot fluorescence for the presence of DNA (Sambrook et al. 1989). The fractions eluted with

Table 1. Final leaf rust ratings and area under the disease progress curve (AUDPC) of the two parents, and the means and ranges of these values for the RILs, for the 1992–1993 and 1993–1994 seasons at Ciudad Obregón, Mexico.

	1992–1993		1993–1994	
	Final rating	AUDPC	Final rating	AUDPC
Parent				
'Parula'	0.33 ± 0.33	1.7 ± 1.68	0.33 ± 0.33	2.2 ± 2.2
'Siete Cerros'	63.0 ± 3.3	675.8 + 31.9	53.3 ± 6.65	746.7 ± 123.5
RILs				
Mean	20.1 ± 2.6	195.0 ± 29.7	17.2 ± 2.02	217.6 ± 30.9
Range	0–90.0	0–1056.0	1–77	8.3–1098.3

0.15 M PB that had low- or single-copy DNA were pooled and concentrated with 2-butanol to a final volume of 200 µL, according to Sambrook et al. (1989). Salt was removed by Sephadex G-50 spin columns equilibrated with TE (10 mM Tris plus 1 mM EDTA, pH 8.0). The purified concentrated eluate was quantified with a fluorometer and diluted with sterile water to a concentration of 5 ng/µL for PCR assay.

RAPD analysis

Operon decamer primers (Operon Technologies, Alameda, Calif.) were utilized for PCR analysis using DNA enriched for low-copy sequences. PCR reactions were conducted in 25-µL mixtures containing 20 ng template DNA, 100 µM dNTPs, 15 ng primer, 2.5 mM $MgCl_2$, and 0.001% gelatin in 1× buffer (20 mM Tris–HCl (pH 8.4) plus 50 mM KCl) with 1 U of Taq DNA polymerase (BRL), in an Ericomp Twinblock™ Easy Cycle series thermocycler. The PCR conditions used were 1 cycle at 94°C for 2 min; followed by 45 cycles at 94°C for 1 min, 36°C for 1 min, and 72°C for 1 min; with a final extension period at 72°C for 5 min. The amplified products were separated on 2% agarose with TBE (0.09 M Tris-borate and 0.002 M EDTA) and viewed under UV illumination after ethidium bromide staining.

Recovery of DNA and cloning

PCR fragments of interest were recovered from 3% SeaKem LE (FMC BioProducts) agarose gels and the DNA was eluted using Prep-A Gene DNA purification kit (Bio-Rad). The fragments were re-amplified using the same primers to evaluate the purity of the isolated fragments. Fragments that were re-amplified without smear or additional bands were eluted, ligated to pT7 Blue T-Vector (Novagen), and cloned into the modified *Eco*RV site of the pT7 Blue Vector (Novagen) with X-gal–IPTG (isopropyl β-D-thiogalactopyranoside) based blue–white colony screening. Cloning utilized the terminal transferase activity of the Taq DNA polymerase and its affinity to dATP to produce an adenosine residue at the 3′ end of the amplified products. The vector had been constructed with T overhangs at the *Eco*RV site. White colonies were used for minipreps and were digested with *Eco*RI and *Hin*dIII to release the insert. The molecular weights of the respective inserts were confirmed by agarose gel electrophoresis.

RFLP analysis

Total genomic DNA from the two parental lines was digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I, separated on 0.8% agarose, Southern transferred, and hybridized with the digoxigenin labeled (5%) cloned PCR fragments (Hoisington et al. 1994). DNA of the 77 RILs was digested with the appropriate enzyme and probed with the three cloned fragments. Chromosomal locations of the clones were detected by Southern analysis of nullisomic, tetrasomic, and ditelosomic cytogenetic stocks of 'Chinese Spring' obtained from the late Dr. E.R. Sears and maintained at CIMMYT.

Statistical analysis

Genetic linkage between the three molecular markers and the locus corresponding to leaf tip necrosis (*Ltn*; Singh 1992) was calculated using MAPMAKER 3.0 (Lander et al. 1987). Linkage of molecular markers and of *Ltn* to genetic factors responsible for leaf rust resistance was investigated by single-factor ANOVA at each of the loci, by comparing the mean leaf rust ratings – AUDPC of the two genotype classes at each locus. Simple linear regression at each locus was used to calculate the coefficient of determination (R^2), as a measure of the proportion of the total phenotypic variance explained by each marker. Multiple linear regression that included in the model all the loci linked to a putative QTL was used to calculate partial R^2s, to confirm which loci may be tagging the same genomic region, and the total R^2, as a measure of the total proportion of phenotypic variation explained by those loci. Both the last rating of each of the two trials and the AUDPC (mean of three repetitions) were used as measures of disease.

Results and discussion

Field results

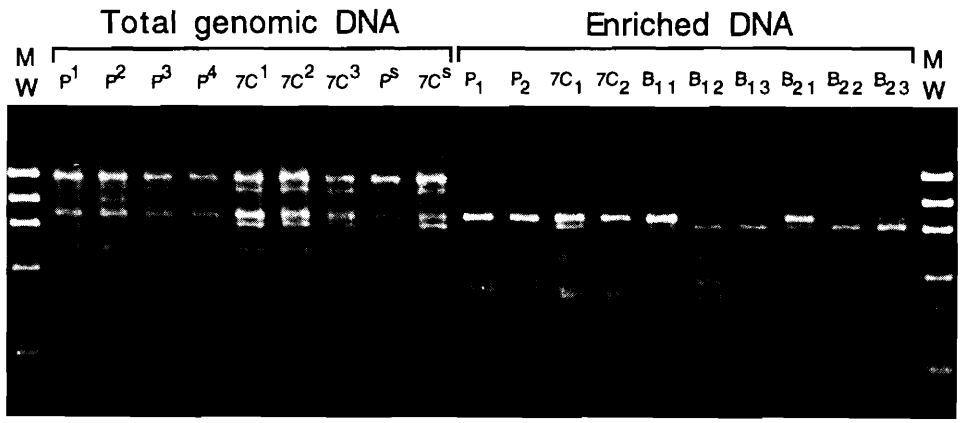
The disease ratings of the two parents and the RILs (mean value and range) are given in Table 1. The resistant parent 'Parula' was almost completely resistant (trace severity), whereas the partially susceptible parent 'Siete Cerros' had moderate disease infection. Since it is suspected that 'Siete Cerros' carries minor genes for leaf rust resistance, it is not surprising to find that some of the RILs showed transgressive segregation, being more susceptible than this parent. The two traits had high heritabilities: h^2 = 0.90 for AUDPC and h^2 = 0.89 for final leaf rust rating.

RAPD analysis

We first screened 500 decamer primers to reveal RAPDs in the total genomic DNA of the two parental lines 'Parula' and 'Siete Cerros'; only 8–10% of these primers revealed polymorphisms, and none of these were present when the corresponding primers were used on the bulked DNAs (data not shown).

In contrast, 30–35% of 400 decamer primers screened on DNA enriched for low- and (or) single-copy sequences of the parental lines revealed molecular polymorphisms between them. For most primers, amplification of enriched DNAs

Fig. 1. An example of the comparison of RAPD amplification patterns of total genomic DNA (sonicated and not sonicated) with DNA enriched for low-copy sequences from the parental lines, 'Parula' (P) and 'Siete Cerros' (7C), and the bulks (B_1 (resistant) and B_2 (susceptible)). Three to four independent amplifications were compared with the total genomic DNA of P and 7C and two to three independently enriched low-copy DNAs were compared for P, 7C, B_1, and B_2. The primer used here was OPR-03. MW = φX174/*Hae*III. Total genomic DNA: P^1–P^4, independent PCR amplifications of P; $7C^1$–$7C^3$, independent PCR amplifications of 7C; P^s, amplified sonicated DNA of P; $7C^s$, amplified sonicated DNA of 7C. Enriched DNA: P_1 and P_2, amplification of two independently enriched DNAs of P; $7C_1$ and $7C_2$, amplification of two independently enriched DNAs of 7C; B_{11}–B_{13}, amplification of three independently enriched DNAs of Bulk 1; B_{21}–B_{23}, amplification of three independently enriched DNAs of Bulk 2.



tended to yield fragments of lower molecular weight than those observed for total genomic DNA (Fig. 1). Overall, enrichment tended to give more discrete amplification products compared with using total genomic DNA. In some cases, weakly amplified products of the total genomic DNA turned out to be the major amplification products when enriched DNA was used as a template for amplification. Repeatable results were obtained when independent PCR amplifications were compared for total genomic DNA as well as for the enriched DNA of the two bulks (Fig. 1). In RAPD analysis, primer binding to the template is a competitive event and primers have more chances of binding to repetitive sequences than to low- and (or) single-copy ones. This is especially acute in a crop such as wheat in which repetitive DNA is abundant (Smith and Flavell 1975). At a C_0t value above 100, most of the repetitive sequences will have re-annealed (Smith and Flavell 1975). Therefore, by utilizing enriched DNA (the low-copy sequences that remain single stranded after re-annealing) for RAPD analysis, only these sequences seem to be selectively amplified. Primers that showed polymorphisms between the enriched DNAs of the two parents were tested using the enriched DNA of the two bulks. Polymorphisms revealed by three primers were also found between the bulked DNAs and thus represented putative markers associated with leaf rust resistance, the criterion used to construct the bulks.

Primer OPG-05 amplified a fragment (of 500 bp) that was present only in 'Siete Cerros', the susceptible parent; this fragment was present in the DNA bulk of susceptible lines (Bulk 2) and absent from the DNA bulk of the resistant lines (Bulk 1). Analysis of the enriched individual line DNAs confirmed this observation; the fragment was amplified in eight out of nine susceptible lines, but in only one line from the resistant bulk (Fig. 2). Similar data were obtained for the second primer (OPI-16). This primer amplified a fragment (of 1.5 kb) only in the susceptible parent and Bulk 2. This fragment was amplified in 9 out of 10 lines of Bulk 2, but in only one out of nine lines of Bulk 1 (Fig. 2). Finally, in the case of the third primer, OPR-03, a fragment (of 500 bp) was present only in the resistant parent and Bulk 1 (this is more clearly shown in Fig. 1 than in Fig. 2). This fragment was amplified in eight out of nine lines of Bulk 1, but in only 1 out of 10 lines of Bulk 2 (Fig. 2).

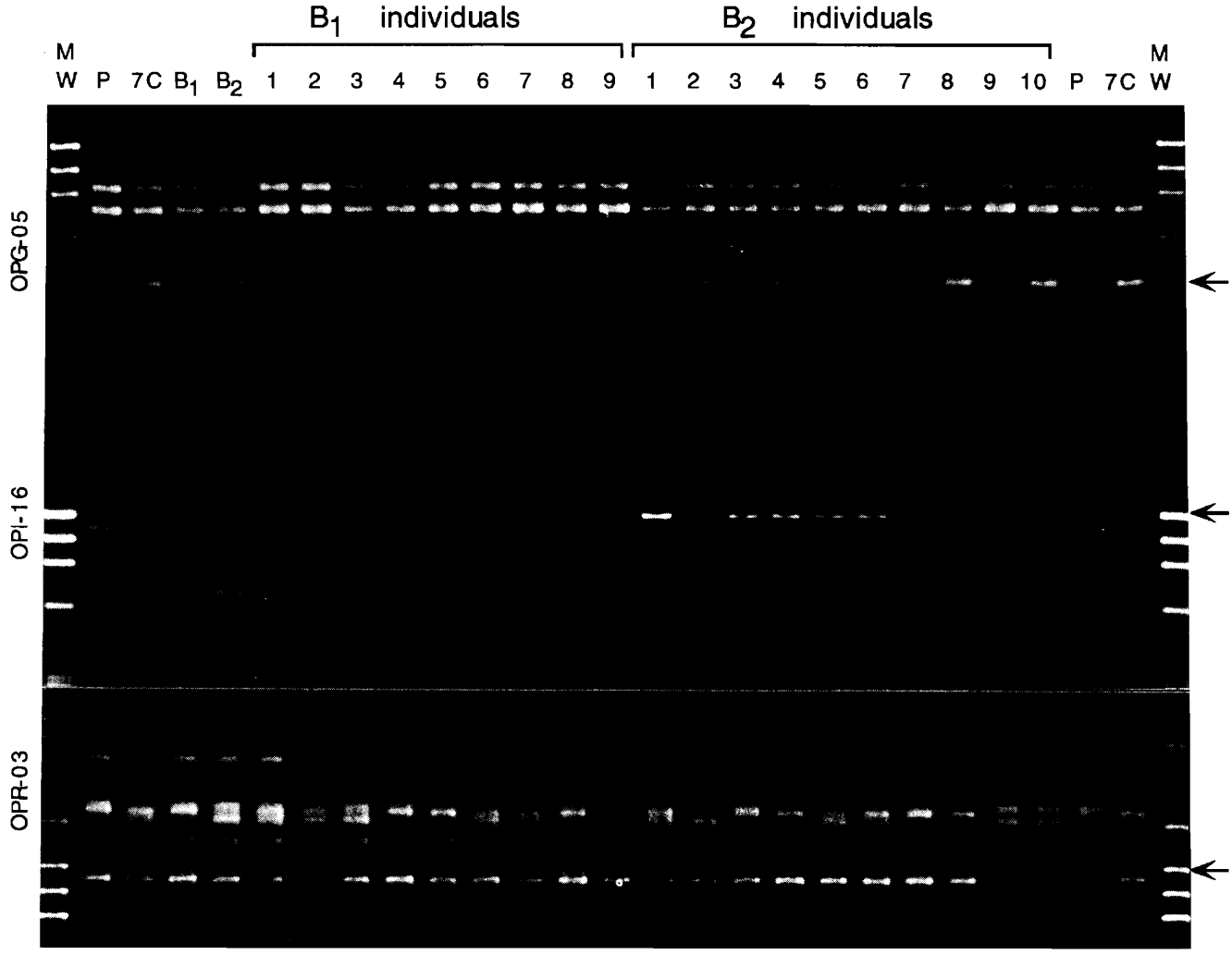
Southern analysis

The three fragments described above were cloned and converted into probes for Southern analysis. The clones derived from primers OPG-05, OPI-16, and OPR-03 were designated CMTG05-500, CMTI16-1500, and CMTR03-500, respectively. Using several restriction enzymes, the DNA of the parental lines was first screened with the three probes to identify polymorphisms. The RIL population was then analyzed with the three probes with the appropriate enzymes. The scoring for the presence or absence of the fragment in the RAPD pattern in the individual entries that comprised the bulks matched the scoring in the Southern blots with the respective clones as probes. The RFLP patterns obtained also indicated that the fragments cloned were low- and (or) single-copy sequences as expected, since low- and (or) single-copy sequences were used for PCR amplification (Fig. 3). This further confirmed the utility of the enrichment procedure using hydroxyapatite chromatography to obtain single- and (or) low-copy sequences in wheat.

Chromosomal locations of the three molecular markers

The three clones were hybridized to *Eco*RV-digested DNA of the nullitetrasomic and ditelosomic stocks of 'Chinese Spring' to establish chromosomal locations of the loci they revealed. The locus revealed by CMTG05-500, designated *Xcmtg05-500*, could be located to the long arm of chromosome 7B in 'Chinese Spring'. The locus detected by the second clone, designated *Xcmti16-1500*, could not be assigned a chromosomal location using the cytogenetic stocks. However, given the

Fig. 2. Amplification patterns of enriched low-copy DNA of the parental lines, 'Parula' (P) and 'Siete Cerros' (7C), the two bulks (B_1 (resistant) and B_2 (susceptible)), and the individual entries used to make up the two bulks. The primers used are indicated on the left and the fragments used for cloning are indicated by arrows.



established linkage between *Xcmtg05-500* and *Xcmti16-1500* (see below), it is reasonable to conclude that they have the same chromosome arm location (7BL).

Finally, in the case of the clone CMTR03-500, two loci were detected, one seemingly located on chromosome 1BS, designated *Xcmtr03-500,* and the other on chromosome 1DS (data not shown).

Inheritance of the three molecular markers and *Ltn*

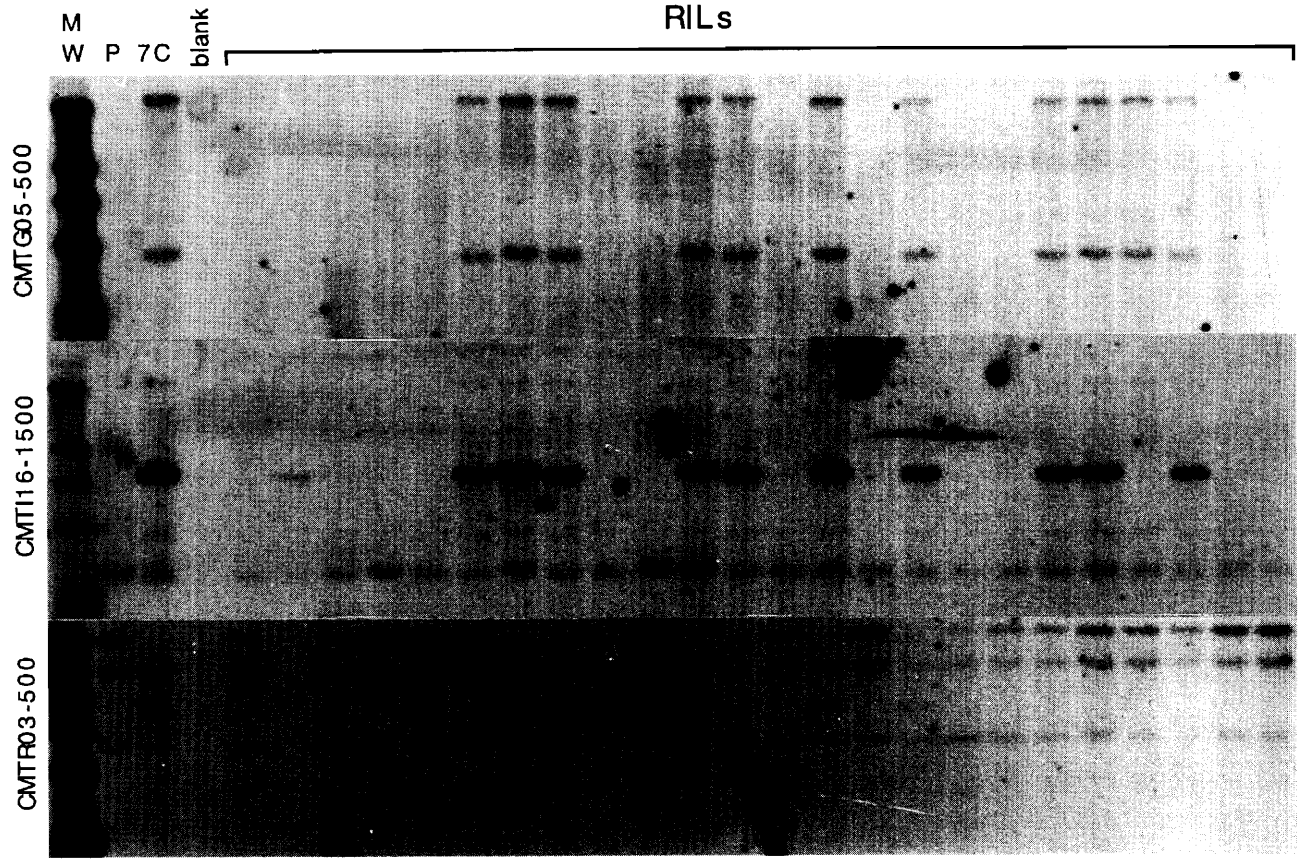
No deviations from Mendelian expectations (1:1) were observed in the segregation of each of the molecular markers and *Ltn*. Thus, *Ltn* behaved as a single Mendelian locus, as reported by Singh (1992).

Linkage analysis using MAPMAKER determined that the two loci *Xcmtg05-500* and *Xcmti16-1500* were tightly linked to each other with 2% recombination (LOD score of 17.1). The linkage between *Xcmtr03-500* and *Xcmtg05-500* was 25%, with a LOD of 1.8, and that between *Xcmtr03-500* and *Xcmti16-150* was 27%, with a LOD of 1.6. Such distant linkages in RILs cannot be considered significant (Haldane and Waddington 1931). This fact and the observed cytogenetic location of locus *Xcmtr03-500* (see previous section) indicate that this locus segregates quite independently of the other two. There was no significant linkage found between *Ltn* and any of the markers. Given the tight linkage between *Ltn* and *Lr34*, we can conclude that the molecular markers are linked to other putative leaf rust resistance genes.

Association of markers with genetic factors determining leaf rust resistance

Simple one-way analysis of variance showed associations between all three molecular markers and *Ltn* with factors controlling leaf rust resistance irrespective of whether this was measured as the last leaf rust rating or as the AUDPC for each trial (Table 2). As expected from the presence of *Lr34* in 'Parula' and its tight linkage to *Ltn* (Singh 1992), the latter characteristic explained a significant proportion of the total phenotypic variation (20–25%). In the case of the two tightly linked loci detected by OPG-05 and OPI-16 located on chromosome 7BL, each explained similar proportion ranges of

Fig. 3. Southern hybridization showing the segregating patterns of the parental lines, 'Parula' (P) and 'Siete Cerros' (7C), and the RILs with the three clones containing polymorphic RAPD fragments. The clones are indicated on the left. MW = λ/*Hin*dIII.



phenotypic variation (18 and 34 % for the two trials). Note that the alleles detected for both *Xcmtg05-500* and *Xcmti16-150* are present in the susceptible parent and that they are both associated with higher susceptibility. This means that homozygous resistant genotypes (absence of band) could potentially be detected using these two markers. Since the two markers *Xcmti16-150* and *Ltn* explained most of the effects for disease in the population, we listed their joint genotypic means in Table 3. Results indicate that in the presence of either resistant marker genotype, the disease can be effectively controlled. However, a further reduction can be obtained by pyramiding two genes. Although sample size is small, the results are quite consistent between the 2 years. Finally, for *Xcmtr03-500*, the allele that comes from 'Parula' only explained a minor amount of the phenotypic variation (7–10%). The partial R^2 values shown in Table 2 give the proportion of phenotypic variation explained in a multiple regression model that excludes the corresponding locus. Thus, in the case of *Xcmti16-150* and *Xcmtg05-500*, these values are very small and confirm that each of these closely linked loci explains very little variation once the other is included in the model. By contrast, the high partial R^2 values for *Ltn* reflect that it is independent of other markers in the model. When all four loci are included in the multiple regression model, a total of between 45 and 55% of the phenotypic variation is accounted for.

We have demonstrated that in crops such as wheat, where low levels of repeatability with RAPDs have been observed (Devos and Gale 1992), the best approach is to convert observed RAPD polymorphisms into more dependable RFLP assays that can be used in any segregating population to detect the same locus. Eventually, rapid PCR based assays such as sequence tagged sites (STSs) might be the methods of choice.

We have thus found two molecular markers for a leaf rust resistance QTL on chromosome 7BL that seems to be at least as effective as *Lr34* (*Ltn*) in conferring resistance to 'Parula', as well as a third marker for another component of resistance on chromosome 1BS. These findings agree with earlier estimations of the number of slow rusting genes determining the durable leaf rust resistance of 'Parula' (Singh and Rajaram 1992). It is conceivable, however, that other minor genes might be in operation, since only about half the total phenotypic variation for the character could be accounted for by the three regions that were detected. All in all, slow rusting, while clearly quantitative in its expression, might be controlled by relatively few genes and may thus be improved using marker-assisted selection.

If leaf tip necrosis can serve as a tool for selecting resistant lines, the two molecular markers can also serve as two equally effective additional markers at least in this particular population. Since in both cases the evidence for these linkages is based on quantitative analyses of the gene effects at the markers, and since we do not have other

Table 2. Simple and multiple linear regression results for the association between three molecular markers and one morphological marker (*Ltn*) and leaf rust resistance.

Trait	*F*	Probability	Phenotypic variance (R^2)	Partial R^2
Xcmti16-1500				
AUDPC-92/3	21.71	0.0001	0.22	0.02
AUDPC-93/4	38.14	0.0001	0.34	0.04
LR rating-92/3	26.42	0.0001	0.27	0.02
LR rating-93/4	30.30	0.0001	0.30	0.05
Xcmtg05-500				
AUDPC-92/3	17.18	0.0001	0.18	0.00
AUDPC-93/4	30.06	0.0001	0.29	0.00
LR rating-92/3	20.13	0.0001	0.22	0.00
LR rating-93/4	22.13	0.0001	0.24	0.00
Xcmtr03-500				
AUDPC-92/3	8.26	0.0052	0.10	0.01
AUDPC-93/4	8.67	0.0043	0.10	0.01
LR rating-92/3	5.43	0.0225	0.07	0.01
LR rating-93/4	6.85	0.0108	0.09	0.01
Ltn				
AUDPC-92/3	22.83	0.0001	0.25	0.19
AUDPC-93/4	16.16	0.0002	0.20	0.14
LR rating-92/3	28.96	0.0001	0.31	0.26
LR rating-93/4	16.93	0.0001	0.21	0.18
All four loci				
AUDPC-92/3	12.66	0.0001	0.45	
AUDPC-93/4	17.06	0.0001	0.53	
LR rating-92/3	15.98	0.0001	0.51	
LR rating-93/4	18.67	0.0001	0.55	

Note: R^2, coefficient of determination from the regression model; partial R^2, proportion of phenotypic variation explained in a model excluding the corresponding locus; AUDPC, area under the disease progress curve; LR rating, leaf rust rating.

Table 3. Two-way analysis of the joint effects of the two loci *Xcmti16-150* and *Ltn*.

	Genotypic mean	*n*	SE
	AUDPC (1992)		
Xcmti16-150$^-$			
Ltn$^-$	582.4	13	88.2
Ltn$^+$	128.8	15	27.7
Xcmti16-150$^+$			
Ltn$^-$	99.8	16	17.0
Ltn$^+$	42.1	22	9.2
	AUDPC (1994)		
Xcmti16-150$^-$			
Ltn$^-$	639.8	13	89.0
Ltn$^+$	180.0	14	34.0
Xcmti16-150$^+$			
Ltn$^-$	100.5	16	15.6
Ltn$^+$	51.9	21	12.0

Note: *Xcmti16-150*$^+$ and *Ltn*$^+$ represent the marker genotype correlated with the resistance, and *Xcmti16-150*$^-$ and *Ltn*$^-$ represent the marker genotype correlated with the susceptibility. AUDPC, area under the disease progress curve; SE, standard error.

linked markers, which would allow interval mapping analysis of the traits, we have neither the means for a more precise localization of the detected putative QTLs, nor a definitive measure of their total contribution to the phenotypic variance observed for leaf rust resistance in the trials. A detailed construction of molecular maps of the two chromosome arms involved will have to be completed to resolve these questions. This should allow the establishment of the best molecular markers for these QTLs for use in marker-aided selection in breeding programs for durable leaf rust resistance. The markers identified could then be sequenced with the objective of developing primers that would be useful in a codominant STS based assay on this population, and their utility could be evaluated on other populations that segregate for durable leaf rust resistance.

Finally, we would like to speculate on the possible relationship between the QTLs detected on chromosome 7BL and *Lr34*. Recent cytogenetic data using C-banding to identify wheat chromosome arms, combined with the physical mapping of specific RFLP loci, indicate that the physically shorter chromosome 7DS corresponds to what, genetically, has been called chromosome 7DL (Hohmann et al. 1996). This would mean that 7DS, as defined in the cytogenetic stocks (e.g., the ditelosomic stocks of 'Chinese Spring'), would actually be

homoeologous to chromosomes 7AL and 7BL. The original evidence placed *Lr34* on 7DS using cytogenetic stocks in genetic crosses to lines bearing other genes (*Lr19*, *Lr29*, and *Rc3*). Their location was also based on the use of cytogenetic stocks and thus follows the standard nomenclature for 7DS (Dyck et al. 1994; Dvořák and Knott 1977; Sears 1973; Rowland and Kerber 1974). Therefore, according to the data of Hohmann et al. (1996), the QTLs detected in the present study and *Lr34* would be on homoeologous arms, 7DS (physical) and 7BL (genetical and physical), which could mean that we may have found a leaf rust resistance locus homoeoallelic to *Lr34*.

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