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**Sukhwinder Singh · C. D. Franks · L. Huang · G. L. Brown-Guedira · D. S. Marshall · B. S. Gill · A. Fritz**

# *Lr41, Lr39,* and a leaf rust resistance gene from *Aegilops cylindrica* may be allelic and are located on wheat chromosome 2DS

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**Abstract** The leaf rust resistance gene *Lr41* in wheat germplasm KS90WGRC10 and a resistance gene in wheat breeding line WX93D246-R-1 were transferred to *Triticum aestivum* from *Aegilops tauschii* and *Ae. cylindrica*, respectively. The leaf rust resistance gene in WX93D246-R-1 was located on wheat chromosome 2D by monosomic analysis. Molecular marker analysis of $F_2$ plants from non-critical crosses determined that this gene is 11.2 cM distal to marker *Xgwm210* on the short arm of 2D. No susceptible plants were detected in a population of 300 $F_2$ plants from a cross between WX93D246-R-1 and TA 4186 (*Lr39*), suggesting that the gene in WX93D246-R-1 is the same as, or closely linked to, *Lr39*. In addition, no susceptible plants were detected in a population of 180 $F_2$ plants from the cross between KS90WGRC10 and WX93D246-R-1. The resistance gene in KS90WGRC10, *Lr41*, was previously reported to be located on wheat chromosome 1D. In this study, no genetic association was found between *Lr41* and 51 markers located on chromosome 1D. A population of 110 $F_3$ lines from a cross between KS90WGRC10 and TAM 107 was evaluated with polymorphic SSR markers from chromosome 2D and marker *Xgdm35* was found to be 1.9 cM proximal to *Lr41*. When evaluated with diverse isolates of *Puccinia triticina*, similar reactions were observed on WX93D246-R-1, KS90WGRC10, and TA 4186. The results of mapping, allelism, and race specificity test indicate that these germplasms likely have the same gene for resistance to leaf rust.

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S. Singh (✉) · L. Huang · B. S. Gill
Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA
e-mail: sukh@ksu.edu

C. D. Franks
Department of Soil and Crop Sciences, Texas A&M University, 434 Heep Center, TAMU 274, College Station, TX 77843-2474, USA

G. L. Brown-Guedira
USDA-PSERU Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA

D. S. Marshall
USDA-ARS, Raleigh, NC 27106, USA

A. Fritz
Department of Agronomy, Kansas State University, Manhattan, KS 66506-5501, USA

*Present address:*
S. Singh, Department Plant Pathology, Throckmorton Plant Science Center, Kansas State University, Manhattan, KS 66506, USA

## Introduction

Breeding for resistance to leaf rust (*Puccinia triticina* Eriks.) is of prime importance in most wheat (*Triticum aestivum* L., 2*n*=6*x*=42, AABBDD) improvement programs. Given the rapid evolution of new races of *P. triticina*, the discovery of new resistance genes and their strategic deployment is essential to achieve durability of resistance. *Aegilops tauschii* Coss. (syn. *Ae. squarrosa* L., 2*n*=2*x*=14, DD), and *Ae. cylindrica* (2*n*=4*x*=28, CCDD), are important sources of genes for resistance to leaf rust. Eight leaf rust resistance genes, *Lr21* (on chromosome 1DS), *Lr22a* (2DS), *Lr32* (3D), *Lr39* (2DS), *Lr40* (1DS), *Lr41* (1D), *Lr42* (1D), and *Lr43* (7DS) have been stably transferred from *Ae. tauschii* to hexaploid wheat germplasm (Rowland and Kerber 1974; Raupp et al. 1983; Kerber 1987; Cox et al. 1994; Hussien et al. 1997). No named leaf rust resistance genes have been transferred to common wheat from *Ae. cylindrica*, although the D genome of this species is also derived from *Ae. tauschii* and freely recombines with the D genome of *T. aestivum*.

The naming of new resistance genes requires each gene to be unique in chromosomal location. Prospective new sources of resistance are often crossed with special aneuploid stocks to determine chromosomal location of resistance genes. However, multiple genes for resistance

to leaf rust or other pathogens may be located on the same wheat chromosome arms. In many cases, the uniqueness of a leaf rust resistance gene can be determined with phenotypic screening methods by its virulence/avirulence pattern. However, many genes confer resistance to the same races of leaf rust. New resistance genes may be subjected to tests of allelism to determine relationships to known genes. The availability of DNA markers provides an additional means to determine gene uniqueness. Apart from their indirect use in pyramiding resistance genes, markers also help to verify findings of conventional analyses, which become complicated when large numbers of genes are already known. Such a situation is encountered in the case of leaf rust of wheat where more than 51 resistance genes are named (McIntosh et al. 1995).

In a number of cases, linkage analysis has been used to clarify the relationship of resistance genes in wheat. For example, *Lr39* was transferred to wheat germplasm KS86WGRC02 from *Ae. tauschii* accession TA 1675 and was reported to be a unique gene on chromosome arm 2DS (Raupp et al. 1989). However, Cox et al. (1994) found no segregation in an $F_2$ population from a cross between KS86WGRC02 and the Thatcher isoline having the *Ae. tauschii*-derived gene *Lr21* that is located on chromosome arm 1DS (Browder 1980). Molecular analysis by Huang and Gill (2001) revealed that KS86WGRC02, in fact, had the gene *Lr40,* which was transferred to wheat germplasm KS89WGRC07 from *Ae. tauschii* TA 1649. *Lr40* is located on chromosome 1D and is allelic to *Lr21*, and thus, the molecular analysis confirmed the results of Cox et al. (1994). KS86WGRC02 was likely a selection from a seed mixture or outcross with KS89WGRC07. Raupp et al. (2001) confirmed the location of a gene transferred to wheat germplasm TA 4186 from accession TA 1675 of *Ae. tauschii* on 2DS through telosomic analysis and linkage with SSR marker *Xgwm210*. The germplasm TA 4186, therefore, has *Lr39*.

Additional leaf rust resistance genes from *Ae. tauschii* and *Ae. cylindrica* have been transferred to the D genome of wheat, and it is necessary to determine the uniqueness of these genes. In this study, we report the characterization and mapping of a gene transferred to wheat germplasm WX93D246-R-1 from an accession of *Ae. cylindrica*, and the relationship of that gene with *Ae. tauschii*-derived genes *Lr39* and *Lr41*. The results of analysis of the gene in WX93D246-R-1 led to re-evaluation of the chromosome location of the resistance gene *Lr41* in the wheat germplasm KS90WGRC10, which was reported to be located on chromosome 1D through monosomic analysis (Cox et al.1994). Molecular markers were used to determine the chromosome and genetic map location of *Lr41*.

## Materials and methods

### Plant material

The leaf rust resistant germplasm WX93D246-R-1 is an $F_5$-derived line selected from the cross TAM 300'*2 × TTCC295. TAM 300 is a hard red winter wheat cultivar adapted to the southern Great Plains, but susceptible to *P. triticina* races PMNQ and MFBL (Long and Kolmer 1989), whereas TTCC295 is an accession *Ae. cylindrica* (2*n*=4*x*, CCDD) collected in Turkey and determined to be resistant to MGB-Dal98. The *Ae. cylindrica* accession was crossed with TAM 300 following the protocols of Gill and Raupp (1987). The $F_1$ plants were backcrossed to TAM 300, and $BC_1F_1$ plants were obtained. $BC_1F_1$ plants were screened for resistance to race MGB-Dal98 following the protocols described below. The population was advanced to the $F_5$ with resistant plants being selected in each generation.

Monosomic analysis was used to determine the chromosome location of the resistance gene in WX93D246-R-1. Seeds of the seven D-genome monosomics of Chinese Spring were germinated, and 41-chromosome plants were identified using the root-tip count technique described by Endo and Gill (1984). Crosses were made with each monosomic line using WX93D246-R-1 as the male, and 41-chromosome $F_1$ plants were identified and allowed to self-pollinate. The $F_2$ seed was divided into two lots. The first lot was inoculated with race MGB-Dal98 at the Texas Agricultural Experiment Station, Dallas, Tex. The second lot was inoculated with race MFBL in Manhattan, Kan. The number of $F_2$ plants evaluated ranged from 27 for chromosome 4D to 207 for chromosome 3D. The segregation data were used to determine the chromosomal location of the gene(s) responsible for resistance. Data from non-critical crosses were used to determine the inheritance of resistance to leaf rust in this population. Chi-square tests were used to determine goodness-of-fit to expected segregation ratios.

Two crosses, TA 4186 × WX93D246-R-1 and KS90WGRC10 × WX93D246-R-1, were made to assess the relationship between the gene in WX93D246-R-1 and the *Ae. tauschii* -derived genes in TA 4186 (*Lr39*) and KS90WGRC10 (*Lr41*) (Cox et al. 1994; Raupp et al. 2001). Three hundred $F_2$ plants from the cross TA 4186 × WX93D246-R-1 and 180 $F_2$ plants from the cross KS90WGRC10 × WX93D246-R-1 were inoculated with leaf rust race MFBL to assess allelism.

The genetic location of the leaf rust resistance gene in wheat germplasm KS90WGRC10 was also determined. KS90WGRC10 is a $BC_2F_3$-derived line from the cross TAM 107'*3 × TA 2460 (Cox et al. 1994). TAM 107 is a hard red winter wheat cultivar adapted to the southern Great Plains, but is susceptible to most United States races of *P. triticina*. TA 2460 is a leaf rust-resistant accession of *Ae. tauschii* from Iran and is the source of resistance in KS90WGRC10. The gene in KS90WGRC10 has been designated *Lr41* and is reported to be on chromosome 1D (Cox et al. 1994). For this study, a population of 110 $F_2$ individuals from the cross KS90WGRC10 × TAM 107 was used to determine inheritance of resistance and for molecular mapping. Spikes of the $F_2$ plants were bagged and allowed to self-pollinate to produce $F_3$ lines. The $F_2$ plants and 20 plants from each $F_3$ line were inoculated with *P. triticina* culture PRTUS6 (PBJL) (Long and Kolmer 1989). KS90WGRC10 and TAM 107 were included as resistant and susceptible checks. The $F_3$ lines were classified as homozygous resistant, segregating, or homozygous susceptible. Chi-square tests were used to determine goodness-of- fit to expected segregation ratios.

### Leaf rust evaluation

Seeds of each entry were planted in 10-$cm^2$ plastic pots filled with vermiculite. Inoculations were done at the two-leaf stage using a suspension of urediospores in lightweight mineral oil according to the method of Browder (1971). Inoculated seedlings were placed in a 16°C moist chamber (100% RH) overnight. Seedlings were then placed in a growth chamber maintained at 22°C. Infection types of

**Table 1** Avirulence/virulence phenotypes of leaf rust races

| Race[a] | Effective/ineffective host *Lr* genes |
|---|---|
| CBBQ | 1, 2a, 2b, 2c, 2d, 3 ka, 3c, 9, 11, 16, 17, 19, 24/3a, 10, 18 |
| CDBL | 1, 2a, 2c, 3 ka, 9, 11, 16, 17, 18, 26, 30/3a, 10, 24 |
| KDBL | 1, 3ka, 9, 11, 16, 17, 18, 26, 30/2a, 2c, 3a, 10, 24 |
| MBRL | 2a, 2c, 9, 16, 17, 18, 24, 26/1, 3a, 3 ka, 10, 11, 30 |
| MCDL | 2a, 2c, 3 ka, 9, 11, 16, 18, 24, 30/1, 3a, 10, 17, 26 |
| MCRL | 2a, 2c, 9, 16, 17, 18, 24/1, 3a, 3 ka, 10, 11, 26, 30 |
| MFBL | 2a, 2c, 3 ka, 9, 11, 16, 17, 18, 30/1, 3a, 10, 24, 26 |
| PBJL | 2a, 9, 10, 11, 16, 19, 24/1, 2b, 2c, 2d, 3a, 15, 17 18 |
| PNML | 2a, 11, 16, 17, 18, 26/1, 2c, 3a, 3 ka, 9, 10, 24, 30 |
| PNMQ | 2a, 11, 16, 17, 26/1, 2c, 3a, 3 ka, 9, 10, 18, 24, 30 |
| TBGL | 3 ka, 9, 16, 17, 18, 24, 26, 30/1, 2a, 2c, 3a, 10, 11 |
| TFGL | 3 ka, 9 16, 17, 18, 30/1, 2a, 2c, 3a, 10, 11, 24, 26 |

[a] Race nomenclature and the first three differentials sets based on Long and Kolmer (1989). The fourth differential set consisted of *Lr10* and *Lr18*

the seedlings were scored 10–14 days after inoculation according to the Stakman scale as modified by Roelfs et al. (1992). Race MGB-Dal98 of *P. triticina* was provided by D.S. Marshall (USDA-ARS, Raleigh, N.C.). All other leaf rust isolates and their avirulence/virulence phenotypes were provided by M. G. Eversmeyer (USDA-ARS Plant Science and Entomology Research Unit, Manhattan, Kan.) and D. L. Long (USDA-ARS Cereal Disease Laboratory, St. Paul, Minn.) (Table 1).

Leaf rust-resistant germplasm and parental lines were evaluated for resistance to diverse races of *P. triticina* to determine if there were differences in response among lines with *Lr39, Lr41*, and the gene in WX93D246-R-1. Seedlings of WX93D246-R-1, TAM 300, KS90WGRC10, TA 2460, TAM 107, and TA 4186 were inoculated with seven races of *P. triticina* (CDBL, KDBL, MBRL, MCDL, MCRL, MFBL, and PNML, PNMQ). Seedlings of TA 4186, WX93D246-R-1, TAM 300, and TAM 107 were also inoculated with races CBBQ, PBJL, PNMQ, TBGL, and TFGL.

### Molecular analyses

The chromosomal location of the tentatively new leaf rust resistance gene in WX93D246-R-1 was ascertained, and six microsatellite markers for the critical chromosome were selected from the map constructed by Röder et al. (1998). Total genomic DNA was isolated from $F_2$ seedlings of the cross between WX93D246-R-1 and Chinese Spring according to the procedure of Riede and Anderson (1996). PCR conditions were as described by Röder et al. (1998). The fragments were separated on 5% denaturing polyacrylamide gels and visualized by silver staining as described by Fritz et al. (1999). Three markers were polymorphic between the parents and were tested on 81 $F_2$ individuals from non-critical crosses.

Bulk-segregant analysis was performed to identify markers potentially linked to the leaf rust resistance gene in KS90WGRC10. Genomic DNA of 9-10 homozygous resistant (infection type 1) and 9-10 homozygous susceptible (infection type 4) $F_3$ lines from the cross KS90WGRC10 × TAM 107 were pooled in equal proportions to make resistant and susceptible bulks. Forty-one RFLP markers were selected for wheat chromosome 1D, 18 markers for 1DS, and 23 for 1DL. The markers were first tested on the parents and the resistant and susceptible bulks. Five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII) were used to digest the genomic DNA. Procedures used for genomic DNA isolation, restriction endonuclease digestion, gel electrophoresis, and DNA gel-blot hybridization were as described by Faris et al. (2000).

Polymorphism between KS90WGRC10 and TAM 107 was also evaluated with 149 microsatellite markers mapped to the D genome of wheat (Röder et al. 1998). Polymorphic markers were evaluated on the individual $F_3$ lines. The PCR assays were carried out in 25 $\mu$l reactions as described by Röder et al. (1998) in an MJ Research thermocycler (Watertown, Mass., USA). Products were separated on 2.3% MetaPhor agarose gels in 1× Tris-borate buffer. Gels were stained with ethidium bromide and visualized with UV light.

Linkage analysis was performed using the MAPMAKER 3.0 program (Lander et al. 1987) using an ordering criterion of LOD >3.0 and the Kosambi mapping function (Kosambi 1944).

## Results

### Phenotypic evaluation

A hypersensitive infection type (IT 0 to ;) was observed on seedlings of breeding line WX93D246-R-1 when inoculated with leaf rust races MFBL and TBGQ, whereas seedlings of the recurrent parent TAM 300 were susceptible (ITs of 3–4). These results indicated the resistance in WX93D246-R-1 was derived from the *Ae. cylindrica* parent. High infection types were observed on TAM 107 with all races. When inoculated with races PNML and PNMQ, high ITs were observed on seedlings of WX93D246-R-1 and seedlings of TAM 300. Virulence to *Ae. tauschii*-derived genes *Lr39* and *Lr41* in races PNML and PNMQ was reported by Hussien (1997) and Raupp et al. (2001). High ITs were also observed on seedlings of TA 4186 (*Lr39*) and KS90WGRC10 (*Lr41)* when inoculated with races PNMQ in this study (Fig. 1). TA 4186 and KS90WGRC10 exhibited a hypersensitive reaction (IT 0 to ;) after inoculation with race MCDL, but high ITs on TAM 107. Similarity of race specificity of *Lr39*, *Lr41,* and the *Ae. cylindrica*-derived gene in WX93D246-R-1 indicated that these lines have the same gene for resistance.

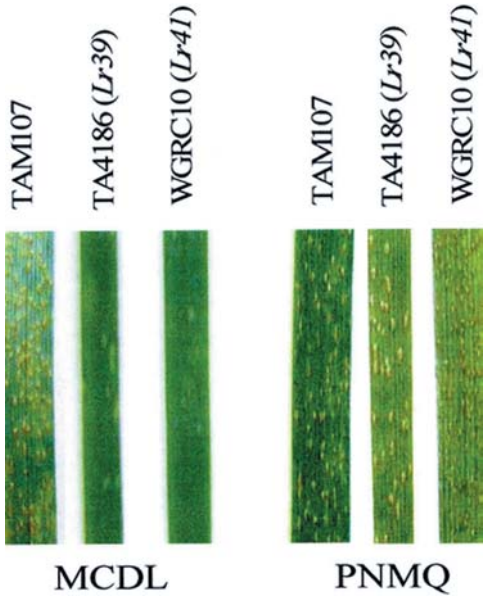


**Fig. 1** Reaction of seedlings from *left* to *right*: TAM 107 (susceptible check), TA 4186 (*Lr39)* (Wichita*3/TA 1675), and KS89WGRC10 (*Lr41*) after inoculation with races MCDL (*left*) and PNMQ (*right*) of *Puccinia triticina*

**Table 2** Segregation for leaf rust resistance in $F_2$ population derived from hybrids of WX93D246-R-1 and the D genome Chinese Spring monosomic. Seedling were inoculated with *Puccinia triticina* races MGB-Dal98 and MFBL

| Monosomic line | Observed segregation | | $\chi^2$ (3:1) | *P* |
|---|---|---|---|---|
| | Resistant | Susceptible | | |
| 1D | 113 | 41 | 0.22 | 0.64 |
| 2D | 173 | 4 | 48.82 | <0.0001 |
| 3D | 158 | 49 | 0.19 | 0.66 |
| 4D | 20 | 7 | 0.01 | 0.91 |
| 5D | 21 | 8 | 0.10 | 0.75 |
| 6D | 73 | 31 | 1.28 | 0.26 |
| 7D | 148 | 40 | 1.39 | 0.24 |

## Chromosome location and mapping of leaf rust resistance in WX93D246-R-1

The $F_2$ populations derived from 41-chromosome plants from crosses of WX93D246-R-1 and the Chinese Spring D-genome monosomics were inoculated with *P. triticina* race MFBL. Observed segregation ratios in all populations fit the 3 resistant:1 susceptible ratio expected if resistance is due to a single dominant gene, except for the cross involving monosomic 2D (Table 2). When tested for goodness-of-fit to a 3:1 ratio, the $\chi^2$ score for the chromosome 2D monosomic cross was significant at the 0.001 level, due to an excess of resistant plants, indicating that the resistance gene in WX93D246-R-1 is located on chromosome 2D.

Genomic DNA of WX93D246-R-1 and Chinese Spring was amplified using primers for six microsatellite markers on wheat chromosome arm 2DS. Three primers, GWM210, GWM261, and GWM296, amplified fragments polymorphic between the two lines. The polymorphic markers were tested on a population of 81 $F_2$ plants derived from non-critical monosomic crosses. The resistance gene in WX93D246-R-1 was located 11.2 cM from microsatellite *Xgwm210*, which is the most distal microsatellite on the map published by Röder et al (1998). *Xgwm296* and *Xgwm261* were also polymorphic in this population and allowed us to determine that the gene in WX93D246-R-1 is distal to *Xgwm210* (Fig. 2). All markers were mapped at a LOD of 3.0. Raupp et al. (2001) determined that *Lr39* was located in the distal region of chromosome 2DS and 10.7 cM distal to *Xgwm210*.

## Allelism of *Lr39*, *Lr41*, and the gene in WX93D246R-1

Since the race specificity of the leaf rust resistance gene in WX93D246R-1 was similar to that observed for *Lr39*, and both genes were located on chromosome arm 2DS, allelism of the two genes was tested. No leaf rust-susceptible plants were observed in an $F_2$ population of 300 plants from the cross TA 4186 × WX93D246R-1 when inoculated with race MFBL. These data indicated

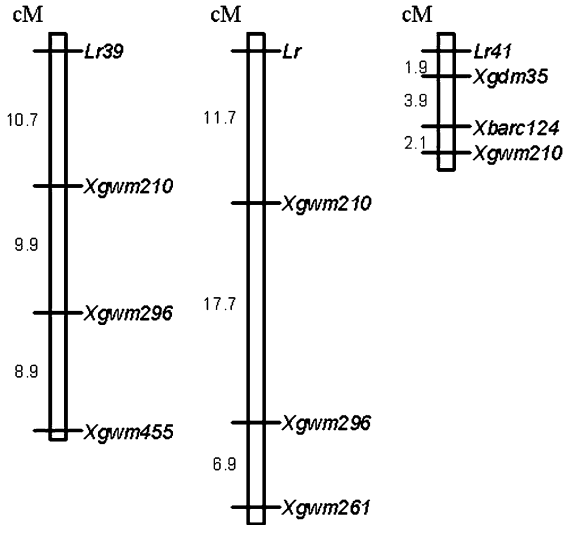


**Fig. 2** Placement of *Lr41*/*Lr39* on genetic map of chromosome 2DS of wheat in three different mapping populations, TA 4186/Wichita (*left*), TAM 300 /*Aegilops cylindrica* (*middle*), and WGRC10/TAM 107 (*right*). The centromere is toward *bottom* of the figure. Marker positions are in cM and scale is drawn at approximately 1/8″ = 1 cM

**Table 3** Numbers of $F_2$ plants or $F_3$ lines resistant or susceptible to leaf rust, $\chi^2$ test for fit to expected segregation ratios for the cross KS90WGRC10 × TAM 107. Plants were inoculated with race PBJL of *P. triticina* (*NS* not significant at 1% level of probability, *R* resistant, *S* susceptible)

| Generation | Number of $F_2$ plants or $F_3$ lines | | | | |
|---|---|---|---|---|---|
| $F_2$ | R | - | S | $\chi^2$ (3:1) | - |
| | 81 | - | 29 | 0.047 | NS |
| $F_3$ | R | Segregating | S | $\chi^2$ (1:2:1) | *P* |
| | 18 | 32 | 17 | 0.253 | N |

that the gene in WX93D246R-1 and *Lr39* are the same or closely linked.

Allelism of the gene in WX93D246R-1 and the gene in KS90WGRC10 was also tested. A sample of 180 $F_2$ plants from the cross KS90WGRC10 × WX93D246R-1 was inoculated with race MFBL, and no susceptible plants were detected. These data suggest that the gene in WX93D246R-1 and *Lr41* is also the same or closely linked. The data also indicate that the previously reported chromosome location of *Lr41* on 1DS was not correct and that *Lr39* and *Lr41* are the same or closely linked.

## Mapping leaf rust resistance gene *Lr41*

Efforts were underway to map *Lr41* with molecular markers in a population from the cross KS90WGRC10 × TAM 107. The observed segregation for leaf rust resistance in the $F_2$ population fit a 3 resistant:1 susceptible ratio expected for a single dominant gene (Table 3). In addition, the observed segregation of $F_3$ families did not differ significantly from the 1 homozy-

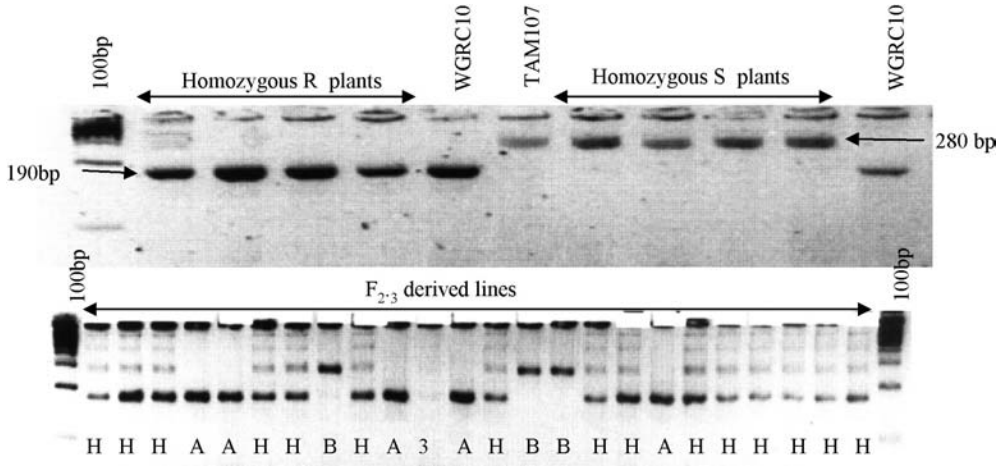


**Fig. 3** Amplification of DNA from homozygous resistant and susceptible $F_2$ plants and in a population of $F_3$ lines of the cross KS89WGRC10 × TAM 107 using microsatellite marker GDM 35 (*A* homozygous resistant lines, *B* homozygous susceptible lines, *H* segregating, *3* no amplification)

gous resistant:2 segregating:1 homozygous susceptible ratio expected for a single gene (Table 3).

Forty-one RFLP markers from chromosome 1D were evaluated for polymorphism between KS90WGRC10 and TAM 107. Seven markers were polymorphic between the parents but were not polymorphic between the resistant and susceptible bulks. The polymorphic markers were mapped in the population, and none was linked with *Lr41*, indicating the gene is not likely located on chromosome 1D.

An additional 75 primer pairs of microsatellite markers mapping to the D genome amplified fragments in both *Ae. tauschii* and wheat. Fourteen markers were polymorphic between KS90WGRC10 and TAM 107. Twenty-four primer pairs of microsatellites did not amplify a fragment in TA 2460. Three markers, GDM 35, BARC124, and GWM210, showed polymorphism between the resistant and susceptible parents and the bulks. These markers are mapped physically on distal bin (2DS-5) of chromosome 2DS using Chinese Spring wheat aneuploid stocks. The SSR primer pairs for GDM 35 amplified a fragment of approximately 190 bp in the resistant bulk and the resistant parent. GDM 35 amplified a 280-bp DNA fragment from TAM 107. The assay of $F_3$ families revealed polymorphism for this fragment. Resistant families yielded the 190-bp fragment and heterozygous families carried both fragments (Fig. 3). The linkage analysis was performed based on recombination between these markers and the gene and placed *Xgdm35* 1.9 cM proximal to *Lr41* (Fig. 2).

## Discussion

The establishment of linkage between molecular markers and resistance genes not only confirms their precise chromosomal placement but also helps in judicious deployment of resistance genes through marker-aided selection. Our results suggest that *Lr39*, *Lr41*, and the unnamed gene in WX93D246-R-1 are identical or closely clustered. Raupp et al. (2001) established that *Lr39* was located 10.7 cM distal to *Xgwm210* in the distal region of chromosome 2DS. Mapping studies in a separate cross of KS90WGRC10 and WX93D246-R-1 also placed the gene(s) responsible for resistance in the distal region of chromosome 2DS. Markers GWM210 and GDM 35 are linked to the leaf rust resistance gene(s) and place the gene(s) in the distal region of chromosome 2DS. In addition, 300 $F_2$ seedlings from the cross TA 4186 × WX93D246 did not segregate for resistance, and we concluded that WX93D246-R-1 carries *Lr39* or a tightly linked gene. A total of 180 $F_2$ plants from the cross of KS90WGRC10 and WX93D246-R-1 also did not segregate. While the population size for this cross is not large enough to definitively determine an allelic relationship between the gene in KS90WGRC10 and the gene in WX93D246-R-1, it does establish that they are linked.

Support for the hypothesis that the genes in the three germplasms are likely identical is also provided by the data on screening with multiple races of leaf rust. Only races PNMQ and PNML gave high infection types on the germplasm lines. All other races produced low infection types on TA 4186, KS90WGRC10, and WX93D246-R-1.

*Lr39* was introgressed into common wheat from at least five accessions of *Ae. tauschii* of diverse geographic origin, as well from an *Ae. cylindrica* accession. This suggests that *Lr39* is common in the gene pool of *Ae. tauschii*. The presence of the gene in *Ae. cylindrica* may indicate that the gene arose prior to the hybridization events that led to the evolution of *Ae. cylindrica.* However, it is also possible the gene evolved separately at later time or represents hybridization between *Ae. tauschii* and *Ae. cylindrica.* One practical implication is that these sources cannot be used for pyramiding leaf rust resistance genes. *Lr39* has already been tagged with PCR-based marker *Xgwm210* (Raupp et al. 2001), but marker *Xgdm35* used in the present study shows tighter linkage with *Lr41* (*Lr39*) in the KS90WGRC10/TAM 107 population and may be useful for marker-assisted selection for *Lr41* (*Lr39*).

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## References

Browder LE (1971) Pathogenic specialization cereal rust fungi especially *Puccinia recondita* f. sp. *tritici* concept, methods of study, and application. USDA Tech Bull 1432:45

Browder LE (1980) A compendium of information about named genes for low reaction to *Puccinia recondita* in wheat. Crop Sci 20:775-779

Cox TS, Raupp WJ, Gill BS (1994) Leaf rust resistance genes *Lr41*, *Lr42*, and *Lr43* transferred from *Triticum tauschii* to common wheat. Crop Sci 34:339–343

Endo TR, Gill BS (1984) Somatic karyotype, heterochromatin distribution, and nature of chromosome differentiation in common wheat, *Triticum aestivum* L. em Thell. Chromosoma 89:361–369

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154:823–835

Fritz AK, Caldwell S, Worrall WD (1999) Molecular mapping of Russian wheat aphid resistance from triticale accession PI 386156. Crop Sci 39:1707–1710

Gill BS, Raupp WJ (1987) Direct genetic transfer from *Aegilops tauschii* L. to hexapolid wheat. Crop Sci 27:445–450

Huang L, Gill BS (2001) An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. Theor Appl Genet 103:1007–1013

Hussien T, Bowden RL, Gill BS, Cox TS (1997) Chromosomal location of leaf rust resistance gene *Lr43* from *Aegilops tauschii* in common wheat. Crop Sci 37:1764–1766

Kerber ER (1987) Resistance to leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *Triticum tauschii*. Crop Sci 27:204–206

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici.* Phytopathology 79:525–529

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, East Melbourne, Australia and Kluwer, Dordrecht, The Netherlands

Raupp WJ, Gill BS, Browder LE (1983) Leaf rust resistance in *Aegilops squarrosa* L., its transfer and expression in common wheat (*Triticum aestivum* L.). Phytopathology 73:818

Raupp WJ, Gill BS, Browder LE, Wilson D (1989) Chromosomal location of two leaf rust resistance genes transferred from *Aegilops squarrosa* to hexaploid wheat. Agron Abstr, p 96

Raupp WJ, Sukhwinder-Singh, Brown-Guedira GL, Gill BS (2001) Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat. Theor Appl Genet 102:347–352

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. International Maize and Wheat Improvement Center (CIMMYT), Mexico, pp 1–18

Rowland GG, Kerber ER (1974) Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can J Genet Cytol 16:137–144