# Performance and Mapping of Leaf Rust Resistance Transferred to Wheat from *Triticum timopheevii* subsp. *armeniacum*

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Accepted for publication 11 February 2003.

## ABSTRACT

Brown-Guedira, G. L., Singh, S., and Fritz, A. K. 2003. Performance and mapping of leaf rust resistance transferred to wheat from *Triticum timopheevii* subsp. *armeniacum*. Phytopathology 93:784-789.

Host plant resistance is an economical and environmentally sound method of control of leaf rust caused by the fungus *Puccinia triticina*, which is one of the most serious diseases of wheat (*Triticum aestivum*) worldwide. Wild relatives of wheat, including the tetraploid *T. timopheevii* subsp. *armeniacum*, represent an important source of genes for resistance to leaf rust. The objectives of this study were to (i) evaluate the performance of leaf rust resistance genes previously transferred to wheat from three accessions of *T. timopheevii* subsp. *armeniacum*, (ii) determine inheritance and allelic relationship of the new leaf rust resistance genes, and (iii) determine the genetic map location of one of the *T. timopheevii* subsp. *armeniacum*-derived genes using microsatellite markers. The leaf rust resistance gene transferred to hexaploid wheat from accession TA 28 of *T. timopheevii* subsp. *armeniacum* exhibited slightly different infection types (ITs) to diverse races of leaf rust in inoculated tests of seedlings compared with the gene transferred from TA 870 and TA 874. High ITs were exhibited when seedlings of all the germ plasm lines were inoculated with *P. triticina* races MBRL and PNMQ. However, low ITs were observed on adult plants of all lines having the *T. timopheevii* subsp. *armeniacum*-derived genes for resistance in the field at locations in Kansas and Texas. Analysis of crosses between resistant germ plasm lines showed that accessions TA 870 and TA 874 donated the same gene for resistance to leaf rust and TA 28 donated an independent resistance gene. The gene donated to germ plasm line KS96WGRC36 from TA 870 of *T. timopheevii* subsp. *armeniacum* was linked to microsatellite markers *Xgwm382* (6.7 cM) and *Xgdm87* (9.4 cM) on wheat chromosome arm 2B long. This new leaf rust resistance gene is designated *Lr50*. It is the first named gene for leaf rust resistance transferred from wild timopheevi wheat and is the only *Lr* gene located on the long arm of wheat homoeologous group 2 chromosomes.

Breeding for resistance to leaf rust, caused by *Puccinia triticina* Eriks. (syn. *P. recondita* Roberge ex Desmaz. f. sp. *tritici*), is a major emphasis for many wheat (*Triticum aestivum* L.) breeding programs. The wild and domesticated relatives of wheat are an important source of genes for leaf rust resistance and more than half of the 49 named resistance genes are derived from related species (16).

There are two groups of tetraploid wheats, the emmer wheats (*T. turgidum* L., AABB) and the timopheevi wheats (*T. timopheevii* (Zhuk.) Zhuk., $A^tA^tGG$), which consists of the domesticated form and the wild form known as *T. timopheevii* subsp. *armeniacum* (Jakubz.) van Slageran. The leaf rust resistance gene *Lr18* was transferred to the long arm of chromosome 5B of common wheat from cultivated *T. timopheevii* (8). Resistance to leaf rust is also present in accessions of the wild form of timopheevi wheat (4) and different genes for resistance to leaf rust have been transferred from diverse accessions of *T. timopheevii* subsp. *armeniacum* to common wheat (6). Two hexaploid wheat germ plasms, KS96WGRC35 and KS96WGRC36, were released that have independent leaf rust resistance genes derived from accessions TA 28 and TA 870, respectively, of *T. timopheevii* subsp. *armeniacum* (5). The chromosomal location of leaf rust resistance genes in these germ plasm lines is not known and gene designations have not been assigned. An additional wheat germ plasm having leaf rust resistance derived from another accession of *T. timopheevii* subsp. *armeniacum*, TA 874, has been developed (6). The relationship of the gene transferred to wheat from TA 874 and those in KS96WGRC35 and KS96WGRC36 has not been determined.

In this study, the leaf rust resistance genes derived from TA 28, TA 870, and TA 874 of *T. timopheevii* subsp. *armeniacum* in hexaploid wheat were evaluated at the seedling and adult plant stages. The inheritance and allelic relationships of the genes transferred from these accessions were determined and microsatellite markers were used to determine the genetic map location of one of the resistance genes transferred to wheat.

## MATERIALS AND METHODS

**Plant materials.** Germ plasm lines homozygous for *T. timopheevii* subsp. *armeniacum*-derived resistance to leaf rust were developed as described by Brown-Guedira et al. (6). The homozygous resistant $BC_3F_3$- and $BC_4F_2$-derived lines used in this study were developed from crosses of *T. timopheevii* subsp. *armeniacum* accessions TA 28, TA 870, and TA 874 with three hard winter wheat cultivars, TAM 107 (PI 495594), Karl 92 (PI 564245), and Wrangler (PI 477288). Pedigrees of the resistant lines and collection sites of the *T. timopheevii* subsp. *armeniacum* parents are listed in Table 1. All accessions of *T. timopheevii* subsp. *armeniacum* were obtained from the Wheat Genetics Resource Center at Kansas State University. The leaf rust susceptible cv. Wichita (CItr 11952) was included as a parent for crossing for genetic analysis.

**Greenhouse and field studies.** Seedlings of lines having resistance derived from *T. timopheevii* subsp. *armeniacum*, along with the recurrent parents, were inoculated with seven races of *P. triticina* (CDBL, KDBL, MBRL, MCDL, MCRL, MFBL, and

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Publication no. P-2003-0512-01R

PNMQ), which elicit high infection types (ITs) on the recurrent wheat parents. Leaf rust isolates and avirulence/virulence phenotypes were provided by M. G. Eversmeyer (USDA-ARS, Plant Science and Entomology Research Unit, Manhattan, KS) and D. L. Long (USDA-ARS, Cereal Disease Laboratory, St. Paul, MN). The avirulence/virulence phenotypes of races are provided in Table 2. Up to 20 seeds of each line 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 (2). Inoculated seedlings were placed in a 16°C moist chamber (100% relative humidity) overnight. Seedlings were then placed in a growth chamber maintained at 22°C day/night temperature. ITs of seedlings were scored 10 to 14 days after inoculation according to the Stakman scale as modified by Roelfs et al. (23).

The resistant germ plasm lines KS96WGRC35, KS96WGRC36, and U2657, and the recurrent parents, were evaluated in field nurseries at Manhattan, KS, in the 1997–98 and 1998–99 growing seasons. Lines were also evaluated at McGregor, TX, in the 1998–99 season. At Manhattan, lines were planted in plots 2.5 m long consisting of three rows with 20 cm between rows. At McGregor, lines were planted in a single row 1 m long. Each year the plots at Manhattan were inoculated when they were between the boot and early heading stages with a mixture of leaf rust isolates collected the previous year. The nursery at McGregor was exposed to natural inoculum. Rust severity was rated according to the modified Cobb scale (18) when the plants were at soft-dough stage of development.

**Genetic studies.** The lines U3172, KS96WGRC36, and U3193 having leaf rust resistance in a TAM 107 background derived from *T. timopheevii* subsp. *armeniacum* accessions TA 28, TA 870, and TA 874, respectively, were crossed with susceptible cv. TAM 107 to determine inheritance of resistance. The resistant lines were also intercrossed to determine allelic relationships. Seedlings of the $F_2$ populations were evaluated for reaction to leaf rust in the greenhouse during October 1998 according to the procedures described previously using race MCDL of *P. triticina*. Fifty-one $F_3$ lines were produced from individual $F_2$ plants from each of the crosses KS96WGRC36 × TAM 107 and U3193 × TAM 107. Up to 25 seedlings of each $F_3$ line were evaluated for IT to leaf rust race MCDL, and lines were classified as homozygous resistant, segregating, or homozygous susceptible. Chi-square analysis was used to determine goodness-of-fit to expected segregation ratios.

A population of 79 $F_3$ lines was also developed from a cross between KS96WGRC36 and leaf rust susceptible cv. Wichita. Up to 25 seedlings of each $F_3$ line were evaluated for resistance to leaf rust race MCDL in a growth chamber at 22°C. The chi-square test was used to determine goodness-of-fit of observed segregation to the expected ratio of 1 homozygous resistant/2 segregating/1 homozygous susceptible line.

Total genomic DNA was isolated from seedlings of the resistant germ plasm lines U3172, KS96WGRC36, and U3193, wheat parents TAM 107 and Wichita, and the $F_3$ lines developed from crosses U3193 × TAM 107, KS96WGRC36 × TAM 107, and KS96WGRC36 × Wichita. DNA isolations were from 10 to 20 seedlings of each line according to the method of Riede and Anderson (20). Polymorphism of resistant lines with the recurrent parent TAM 107 were evaluated with 149 microsatellite markers mapped on A and B genomes of wheat (21,22). The marker GWM382 was evaluated on the $F_3$ lines from crosses U3193 × TAM 107 and KS96WGRC36 × TAM 107. The polymerase chain reaction assays were carried out in 25-µl reactions (22) in a thermocycler (PTC200; MJ Research, Watertown, MA). Products were separated on 2.3% MetaPhor agarose gels (FMC Bioproducts, Rockland, ME) in 1× Tris-borate buffer. Gels were stained with ethidium bromide and visualized with UV light.

The parents of the KS96WGRC36 × Wichita mapping population were evaluated with 51 microsatellite primer pairs specific for homoeologous group 2 chromosomes of wheat (10,17,21,22,25). Polymorphic markers were evaluated on the population of 79 $F_3$ lines. Linkage analysis was performed on marker and phenotypic data with the Mapmaker 2.0 computer program (13) using the Kosambi mapping function (12).

## RESULTS

ITs of seedlings of five wheat germ plasms with *T. timopheevii* subsp. *armeniacum*-derived genes for resistance to leaf rust were evaluated with seven races of *P. triticina*. The leaf rust resistant hexaploid lines were developed by backcrossing accessions TA 28 and TA 870 of the wild species to wheat cvs. Wrangler (KS96WGRC35 and U3064) and TAM 107 (U3172 and KS96WGRC36). Resistance derived from TA 874 was transferred to the TAM 107 background in line U3193 but was not available in the Wrangler background. All the tested germ plasm lines had low ITs when inoculated with leaf rust races CDBL, MCDL, and MFBL (Table 3). An immune IT was observed on seedlings of KS96WGRC35 when inoculated with races CDBL and MCDL. KS96WGRC35 has resistance derived from TA 28 of *T. timopheevii* subsp. *armeniacum* in a Wrangler background. However, when inoculated with these races, a small amount of sporulation was observed on line U3172, which also has resistance derived from accession TA 28 but in a TAM 107 background. Differences in IT when inoculated with CDBL and MCDL were not observed on seedlings of KS96WGRC36 and U3064 that have resistance derived from TA 870 in TAM 107 and Wrangler backgrounds, respectively. Seedlings of KS96WGRC35 and U3172 were more resistant to race MCRL than lines having resistance derived from TA 870 and TA 874. Inoculation with race KDBL resulted in slightly lower ITs on seedlings of KS96WGRC36, U3064, and U3193 than the lines having resistance derived from TA 28. Seedlings of all of the germ plasm lines had high ITs when inoculated with leaf rust races MBRL and PNMQ.

The KS96WGRC35, KS96WGRC36, and U2657 resistant germ plasm lines were selected for field evaluation based on similarity to recurrent parent plant type and agronomic performance. The lines have resistance from TA 28, TA 870, and TA 874 transferred to the recurrent parents Wrangler, TAM 107, and Karl 92, respec-

TABLE 1. Pedigrees of leaf rust resistant wheat lines, with collection sites of parental accessions of *Triticum timopheevii* subsp. *armeniacum*

| Resistant hexaploid line | Pedigree[a] | Collection site of donor parent |
|---|---|---|
| KS96WGRC35 | Wrangler*3/TA 28 | 2 km NW of Salahadin, Iraq |
| U3172 | TAM 107*3/TA 28 | |
| KS96WGRC36 | TAM 107*4/TA 870 | 1 km NE of Salahadin, Iraq |
| U3064 | Wrangler*4/TA 870 | |
| U3193 | TAM 107*4/TA 874 | 2 km NW of Salahadin, Iraq |
| U2657 | Karl 92*3/TA 874 | |

[a] All parents with TA designations are accessions of *T. timopheevii* subsp. *armeniacum*.

TABLE 2. Avirulence and virulence phenotypes of *Puccinia triticina* races

| Race[a] | Effective/ineffective host *Lr* genes |
|---|---|
| CDBL | 1, 2a, 2c, 3ka, 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, 3ka, 10, 11, 30 |
| MCDL | 2a, 2c, 3ka, 9, 11, 16, 18, 24, 30 / 1, 3a, 10, 17, 26 |
| MCRL | 2a, 2c, 9, 16, 17, 18, 24 / 1, 3a, 3ka, 10, 11, 26, 30 |
| MFBL | 2a, 2c, 3ka, 9, 11, 16, 17, 18, 30 / 1, 3a, 10, 24, 26 |
| PNMQ | 2a, 11, 16, 17, 26 / 1, 2c, 3a, 3ka, 9, 10, 18, 24, 30 |

[a] Race nomenclature and the first three differential sets based on Long and Kolmer (14). The fourth differential set consisted of *Lr10* and *Lr18*.

tively. Leaf rust resistance was expressed in adult plants of each of the germ plasm lines when exposed to leaf rust in the field at Manhattan and McGregor (Table 4). Lower ITs were observed on both the resistant lines and susceptible recurrent parents at the Manhattan location in 1998 and 1999 than were observed at McGregor in 1999, where a severe natural leaf rust epidemic occurred. KS96WGRC35 exhibited a lower IT at McGregor than did KS96WGRC36 and U2657. However, U2657 had the lowest IT at the Manhattan location in both years. Slightly less disease was observed on Karl 92, the recurrent parent of U2657, than on wheat cvs. TAM 107 and Wrangler at Manhattan in both years. The U2657 line continued to exhibit the lowest IT when the resistant germ plasms were evaluated at locations in Kansas in 2000 and 2001 (G. L. Brown-Guedira, *unpublished data*).

Observed segregation in $F_2$ populations from crosses of resistant lines KS96WGRC36, U3172, and U3193 with TAM 107 fit the expected ratio of 3 resistant/1 susceptible, indicating that these lines each possess a single dominant gene for resistance (Table 5). The observed segregation of the populations of $F_3$ lines from crosses KS96WGRC36 × TAM 107 and U3193 × TAM 107 did not differ significantly from the expected ratio of 1 homozygous resistant/2 segregating/1 homozygous susceptible (Table 5).

Accessions TA 870 and TA 874 donated the same gene for resistance because no segregation was observed in an $F_2$ population from cross KS96WGRC36 × U3193 (Table 5). The leaf rust resistance gene transferred to U3172 from TA 28 segregated independently of the gene transferred from TA 870 and TA 874. The segregation ratios observed in the $F_2$ populations from crosses of U3172 with KS96WGRC36 and U3193 fit the 15 resistant/1 susceptible ratio expected if two independent leaf rust resistance genes were segregating.

To genetically map the resistance genes transferred from *T. timopheevii* subsp. *armeniacum* to wheat, genomic DNA of U3172, KS96WGRC36, U3193 (having resistance from TA 28, TA 870, and TA 874, respectively), the three resistance donor accessions, and the recurrent parent TAM 107 were amplified with 149 wheat microsatellite markers previously mapped to the A and/or B genomes of wheat. Markers from the A and B genomes were selected because resistance was transferred from either the $A^t$ or G genome of tetraploid *T. timopheevii* subsp. *armeniacum*. The $A^t$ and G genomes are partially homologous to the A and B genomes of common wheat but do not recombine with chromosomes of the D-genome. A total of 125 primer pairs amplified fragments in both *T. timopheevii* subsp. *armeniacum* and wheat; 58% of these pairs amplified fragments polymorphic between the wild species accessions and TAM 107. A greater degree of polymorphism was observed between wheat and *T. timopheevii* subsp. *armeniacum* for markers specific to the A genome than markers specific to the B genome. Twenty-four primer pairs amplified a fragment in TAM 107 but did not amplify a fragment in any accession of *T. timopheevii* subsp. *armeniacum.*

Ten primer pairs amplified fragments polymorphic between at least one of the three leaf rust resistant lines and the recurrent parent TAM 107. Markers polymorphic between U3172 and TAM 107 were not associated with resistance when tested on the $F_2$ population. However, a marker was found to be associated with resistance from TA 870 and TA 874. A fragment of approximately 139 bp that was absent in TAM 107 was amplified by primer pair GWM382 in *T. timopheevii* subsp. *armeniacum* accessions TA 870 and TA 874 and the resistant lines KS96WGRC36 and U3193 (Fig. 1). The *T. timopheevii* subsp. *armeniacum*-derived fragment behaved as a dominant marker that was present in all leaf rust resistant $F_3$ lines from crosses KS96WGRC36 × TAM 107 and U3193 × TAM 107 and was absent in all leaf rust susceptible lines.

The wheat microsatellite primer pair GWM382 amplifies orthologous loci located distally on the long arm of wheat homoeologous group 2 chromosomes (21,22). Because no other genes for resistance to leaf rust are located on the long arm of wheat group 2 chromosomes, the gene transferred from *T. timopheevii* subsp. *armeniacum* accessions TA 870 and TA 874 and linked to *Xgwm382* is unique and is designated *Lr50*. Due to the lack of polymorphism between TAM 107 and resistant parents KS96WGRC36 and U3193 at other marker loci on the long arm of group 2 chromosomes, we were unable to determine if *Lr50* was transferred to wheat chromosome 2A or 2B using these populations.

To overcome this problem and allow determination of the genomic location of *Lr50*, a mapping population of $F_3$ lines developed from a cross of KS96WGRC36 with leaf rust susceptible cv. Wichita was evaluated. Because KS96WGRC36 and TAM 107 are not closely related to Wichita, more marker polymorphism was expected in this population. Observed segregation in the $F_3$ lines from the cross of 13 homozygous leaf rust resistant lines/36 segregating/25 homozygous susceptible lines did not deviate significantly from the 1:2:1 ratio expected for a single dominant gene ($\chi^2 = 3.95$; $P > 0.20$), although the number of homozygous susceptible individuals was large in comparison with homozygous resistant lines.

Evaluation of KS96WGRC36 and Wichita with microsatellite markers located on the long arm of wheat group 2 chromosomes identified additional polymorphic markers in the region of interest. Three polymorphic markers specific to chromosome 2A (*Xgwm122*, *Xgwm294*, and *Xgwm312*) (21,22) evaluated on the mapping population of $F_3$ lines were linked to each other but were not linked to leaf rust resistance or *Xgwm382*. Röder et al. (22) determined that *Xgwm294* was linked to locus *Xgwm382-2A* on chromosome 2A (25.1 centimorgan [cM]) when mapped in the International Triticeae Mapping Initiative (ITMI) population. Because none of the 2A specific markers were linked to either the *T. timopheevii* subsp. *armeniacum*-derived fragment amplified by GWM382 segregating in the KS96WGRC36 × Wichita population or the *Lr50* gene, transfer of *Lr50* to chromosome arm 2AL can be ruled out.

TABLE 3. Seedling infection types (ITs) of wheat germ plasm lines and cultivars inoculated with seven races of *Puccinia triticina*[a]

| Cultivar or line (source of resistance) | Race of *P. triticina* | | | | | | |
|---|---|---|---|---|---|---|---|
| | CDBL | KDBL | MBRL | MCDL | MCRL | MFBL | PNMQ |
| KS96WGRC35 (TA 28) | 0 | 3 | 4 | 0 | 2+C | 12 | 4 |
| U3172 (TA 28) | 1C | 3C | 3+ | ;1C | 2+C | 2 | 4 |
| U3193 (TA 874) | ;1C | 2C3C | 3 | ;1C | 3 | ;1 | 4 |
| KS96WGRC36 (TA 870) | ;1C | 2C | 3C | 1C | 4 | ;C | 4 |
| U3064 (TA 870) | 1C | 2C | 3C | ;1C | 3+ | nt | 4 |
| TAM 107 | 3+ | 4 | 4 | 3+ | 3+ | 3 | 4 |
| Wrangler | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

[a] The seedling ITs are 0 = no uredinia or other macroscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis (green island may be surrounded by necrotic or chlorotic border), 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, C = more chlorosis than normal for the IT, + = uredinia somewhat larger than normal for the IT. A range of variation between ITs is recorded, with the most prevalent IT listed first (e.g., ;1C, 12, or 2C3C). nt = not tested.

Two markers, *Xgwm382* and *Xgdm87*, were linked to leaf rust resistance in this population. A fragment of approximately 110 bp was amplified by primer pair GDM87 in KS96WGRC36 and TAM 107 that was absent in Wichita (Fig. 1) and was linked to *Lr50* (9.4 cM). Primer pair GDM87 detected homoeologous loci on the distal part of the long arm of chromosomes 2B and 2D of wheat in the ITMI mapping population but was not associated with chromosome 2A (17). This data further supports the transfer of *Lr50* to wheat chromosome arm 2BL instead of 2AL.

Although *Xgwm382* cosegregated with resistance in the populations from crosses of the resistant germ plasms with TAM 107, recombination between *Lr50* and *Xgwm382* was observed in the KS96WGRC36 × Wichita population. Because of the dominant nature of the *Xgdm87* and *Xgwm382* markers, small size of the mapping population, and lack of additional polymorphic markers, we were unable to order the markers with respect to *Lr50* with a LOD > 3.0 or to orient the map with respect to the centromere. The most probable order (LOD = 1.42) places *Lr50* between *Xgdm87* and *Xgwm382* with *Xgwm382* being the closest marker (6.7 cM).

## DISCUSSION

Two independent leaf rust resistance genes from *T. timopheevii* subsp. *armeniacum* were transferred to hard winter wheat germ plasm lines. The gene *Lr50* was transferred to wheat from *T. timopheevii* subsp. *armeniacum* accessions TA 870 and TA 874 and an unnamed gene for resistance was transferred from TA 28. Genetic analyses indicated that these three accessions donated two different resistance genes. This is supported by observed differences in ITs to diverse leaf rust races of lines having resistance derived from TA 870 and TA 874 and those with resistance from TA 28. However, the same two races of leaf rust were virulent on seedlings having either gene.

Previous analyses of intercrosses between accessions of the wild species determined that TA 870 and TA 145 have the same gene for resistance (6). This has been confirmed by lack of segregation for leaf rust resistance in an $F_2$ population from a cross between KS96WGRC36 and a hexaploid line having resistance derived from TA 145 (G. L. Brown-Guedira, *unpublished data*). Additional analysis with the *Xgwm382* marker in our laboratory indicates that we have also transferred *Lr50* to hexaploid wheat from accession TA 895 of *T. timopheevii* subsp. *armeniacum*. All of the accessions of *T. timopheevii* subsp. *armeniacum* thus far determined to have *Lr50* were collected near the village of Salahadin in northeastern Iraq, indicating that *Lr50* is present at a high frequency at this location. Although collected from the same area, accessions TA 870, TA 874, TA 145, and TA 895 are not identical because they differ in their reaction to other pests (4). Interestingly, *T. timopheevii* subsp. *armeniacum* accession TA 28, which donated a different gene for resistance to leaf rust, was also collected near the same village in northeastern Iraq.

TABLE 4. Flag leaf reaction to *Puccinia triticina* of field-grown adult plants of wheat germ plasm lines and cultivars[a]

| Cultivar or line (source of resistance) | 1998 | 1999 | |
|---|---|---|---|
| | Manhattan, KS | Manhattan, KS | McGregor, TX |
| U2657 (TA 874) | R | R | 20MR |
| KS96WGRC35 (TA 28) | tR | 5MR | 5MR |
| KS96WGRC36 (TA 870) | tR | 5MR | 15MR/MS |
| Wrangler | 60S | 80S | 90S |
| TAM 107 | 60S | 80S | 90S |
| Karl 92 | 40S | 50S | 90S |

[a] Ratings are based on the modified Cobb scale and include disease severity (percent leaf area affected) and infection type; R = resistant; tR = trace of pustules; MR = moderately resistant; MS = moderately susceptible; and S = susceptible infection type.

A high percentage (80%) of the evaluated wheat microsatellite markers amplified products in both *T. timopheevii* subsp. *armeniacum* and *T. aestivum*, more than half of which were polymorphic between accessions of the wild species and wheat cv. TAM 107. The lack of amplification of some wheat microsatellite markers in the *T. timopheevii* subsp. *armeniacum* accessions and the high level of marker polymorphism observed between the two species is likely due to differentiation between the $A^t$ and G genomes of *T. timopheevii* subsp. *armeniacum* and the A and B genomes of wheat. Because amplification products of microsatellite markers with dinucleotide repeat units were resolved on high-resolution agarose gels rather than denaturing polyacrylamide gels or capillary electrophoresis, the level of polymorphism we observed probably underestimates the actual degree of microsatellite marker polymorphism between the two species.

The germ plasm lines used for identification of the *Xgwm382* marker linked to *Lr50* were derived by backcrossing to wheat cv. TAM 107. Lines developed after three backcrosses of the wild tetraploid relative to hexaploid wheat are expected to differ from the recurrent parent at 5% of loci. Only 5 (2.5%) of the 200 total microsatellite markers tested were polymorphic between either KS96WGRC36 or U3193 and the recurrent parent TAM 107. Several generations of selection in the field were done during the development of the resistant hexaploid germ plasms that may have resulted in elimination of much of the genome of *T. timopheevii* subsp. *armeniacum*. In addition, selection against some chromosomes of *T. timopheevii* was observed in the development of chromosome substitution lines in Chinese Spring wheat (3).

Our marker analysis located gene *Lr50* distally on wheat chromosome arm 2BL. Presently, *Lr50* is the only leaf rust resistance gene located on the long arm of wheat group 2 chromosomes. The resistance genes *Sr36*, *Sr40*, and *Pm6*, conferring resistance to stem rust and powdery mildew, respectively, were transferred to the short arm of chromosome 2B from *T. timopheevii* and *T. timopheevii* subsp. *armeniacum* (1,7). The linked genes *Sr36* and *Pm6* were preferentially transmitted to progeny (1). Other studies have also shown that chromosome 2G of *T. timopheevii* is preferentially transmitted (3,9). We did not observe preferential transmission of *Lr50*. In fact, an excess of $F_3$ lines homozygous susceptible to leaf rust in the KS96WGRC36 × Wichita population

TABLE 5. Observed segregation for resistance to leaf rust of populations of $F_2$ plants or $F_3$ lines from crosses of wheat germ plasm having leaf rust resistance derived from accessions of *Triticum timopheevii* subsp. *armeniacum* and $\chi^2$ for fit to expected segregation ratios[a]

| Cross or parent (source of resistance) | Number of $F_2$ plants or $F_3$ lines | | | $\chi^2$ |
|---|---|---|---|---|
| | Res. | Seg. | Sus. | |
| $F_2$ | | | | (3:1)[b] |
| KS96WGRC36 (TA 870) × TAM 107 | 109 | … | 49 | 2.73 |
| U3193 (TA 874) × TAM 107 | 165 | … | 56 | 0.00 |
| U3172 (TA 28) × TAM 107 | 48 | … | 9 | 2.06 |
| $F_3$ | | | | (1:2:1)[c] |
| KS96WGRC36 (TA 870) × TAM 107 | 10 | 25 | 16 | 1.43 |
| U3193 (TA 874) × TAM 107 | 12 | 32 | 7 | 4.29 |
| $F_2$ | | | | (15:1)[b] |
| KS96WGRC36 (TA 870) × U3193 (TA 874) | 138 | … | 0 | – |
| U3172 (TA 28) × KS96WGRC36 (TA 870) | 60 | … | 4 | 0.00 |
| U3172 (TA 28) × U3193 (TA 874) | 167 | … | 14 | 0.45 |
| KS96WGRC36 (TA 870) | 19 | … | … | … |
| U3193 (TA 874) | 20 | … | … | … |
| U3172 (TA 870) | 20 | … | … | … |
| TAM 107 | … | … | 26 | … |
| Wichita | … | … | 20 | … |

[a] Plants were inoculated with race MCDL of *Puccinia triticina*. Res. = resistant, Seg. = segregating, and Sus. = susceptible.
[b] $\chi^2$ for significance = 3.84.
[c] $\chi^2$ for significance = 5.99.

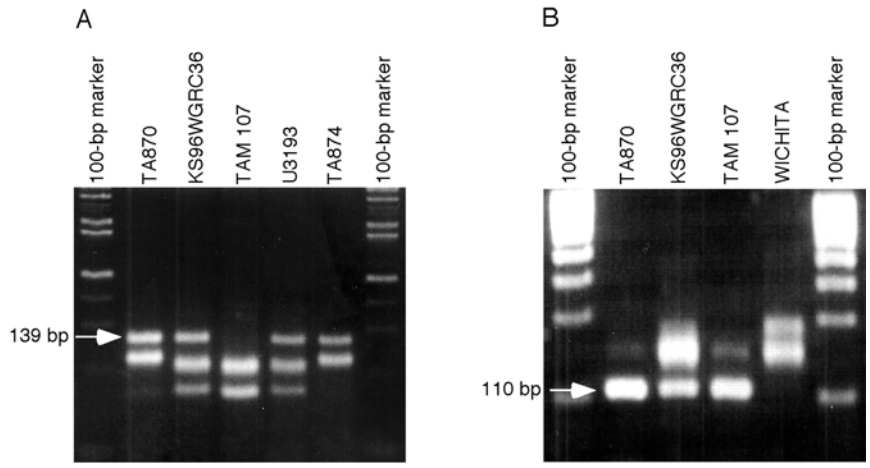


**Fig. 1.** Molecular markers linked to the *Lr50* locus in KS96WGRC36. DNA fragments amplified by primer pairs **A,** GWM382 and **B,** GDM87 from genomic DNA of the leaf rust resistant *Triticum timopheevii* subsp. *armeniacum* accession TA 870, resistant wheat germ plasm line KS96WGRC36, and susceptible wheat cvs. TAM 107 and Wichita. Arrows indicate sizes of polymorphic fragments.

was noted, although the deviation was not significant. Segregation distortion was not noted for the *T. timopheevii* subsp. *armeniacum*-derived *Xgwm382* marker linked to *Lr50*. Because *Lr50* was transferred to the distal part of the long arm of chromosome 2B, recombination may have taken place between it and the genes on 2G responsible for preferential transmission.

Interestingly, only two fragments were amplified by primer GWM382 in wheat cvs. TAM 107 and Wichita, whereas three fragments were present in the resistant germ plasm lines KS96WGRC36 and U3193. The 139-bp GWM382 fragment present in the resistant hexaploid lines was derived from *T. timopheevii* subsp. *armeniacum* chromosome 2G and behaved as a dominant marker. A homoeologous fragment from the B-genome of wheat was not detected in TAM 107 or Wichita. Other studies have noted that a number of microsatellite markers in wheat are dominant (15,24). This may be due to variation in or absence of the primer sequences across genomes or comigration of fragments amplified from different genomes in one parent. A single *Xgwm382* locus on chromosome arm 2DL was mapped physically in Chinese Spring wheat (21), although loci on 2A, 2B, and 2D were mapped genetically in the ITMI mapping population (22).

Although the resistance genes transferred to wheat from *T. timopheevii* subsp. *armeniacum* have not been deployed in any cultivar, virulence to these resistance genes exists in races of *P. triticina* in North America. Races PNMQ and MBRL are virulent on seedlings having *Lr50* and the unnamed gene from TA 28. Virulence to genes transferred to wheat from accessions of *Aegilops tauschii* (*Lr39* and *Lr41*) and *T. monococcum* (an unnamed gene in KS92WGRC23) has been detected prior to deployment of these genes in resistant cultivars (11,19). In each of these cases, virulence was found in *P. triticina* race PNMQ, which is also virulent to the genes *Lr9* and *Lr24* that were transferred to wheat from *T. umbellulatum* and *Thinopyrum ponticum*. It is interesting that this race of leaf rust is virulent to so many genes transferred from wheat relatives.

In this study, low to intermediate ITs were observed on adult plants of wheat having the *T. timopheevii* subsp. *armeniacum*-derived genes when evaluated for multiple years in the field in Kansas, indicating that virulence is not yet common in the southern U.S. Great Plains. However, selection pressure on the pathogen imposed by production of cultivars having genes *Lr9*, *Lr24*, *Lr39*, or *Lr41* may result in an increase in virulence to the new genes from *T. timopheevii* subsp. *armeniacum* in the region. The presence of virulence to new genes derived from wheat relatives prior to development of resistant cultivars will limit the usefulness of these genes unless they are deployed in combination with other effective genes for resistance to leaf rust. Identification of microsatellite markers linked to *Lr50* provides a tool to incorporate this gene into pyramids that include other effective resistance genes. Evaluation of the population segregating for resistance derived from TA 28 with additional markers is needed to identify markers linked to the resistance gene so that it may also be deployed in gene pyramids.

## ACKNOWLEDGMENTS

This research is a joint contribution of USDA-ARS and the Kansas Agriculture Experimentation Station contribution 03-166-J. The authors acknowledge financial support from USDA-IFAFS competitive grant 2001-04462 and the Kansas Wheat Commission. We thank K. D. Howell and C. E. Parker for technical assistance and R. Malik for useful comments on the manuscript.

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