

Leaf Rust Resistance in Selected Uruguayan Common Wheat Cultivars with Early Maturity

S. E. Germán and J. A. Kolmer*

ABSTRACT

Leaf rust (caused by *Puccinia triticina* Eriks.) is an important disease of wheat (*Triticum aestivum* L.) in Uruguay; therefore breeding for resistance to this disease has been a long-term objective for the INIA wheat-breeding program. Information on the identity of resistance genes present in the program is required to design strategies to breed for effective and more durable resistance. The leaf rust resistance genes present in seven adapted cultivars that have been extensively used in crosses were studied. Races of *Puccinia triticina* with different virulence combinations were used to determine which seedling resistance genes may be present in six cultivars. Genetic analysis of seedling and adult plant resistance (APR) was conducted on BC₁F₂ and F₃ generations from four cultivars crossed with the susceptible cultivar Thatcher. The cultivar Estanzuela Tarariras was postulated to have *Lr3bg* and genetically determined to have *Lr13* and *Lr34*; INIA Boyero was postulated to have *Lr26* and genetically determined to have *Lr13* and *Lr34*; Estanzuela Benteveo was postulated to have *Lr3a* and *Lr26* and genetically determined to have *Lr13*. Estanzuela Pelón 90 was postulated to have *Lr1*, *Lr17a*, and *Lr26* and genetically determined to have *Lr34*; INIA Caburé was postulated to have *Lr24*; INIA Churrinche was postulated to have *Lr10* and *Lr24*; and INIA Tero was postulated to have *Lr17a* and *Lr24*. INIA Boyero and Estanzuela Benteveo carry additional APR gene(s) that might be useful sources of resistance in addition to *Lr34*.

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Abbreviations: APR, adult plant resistance; INIA, Instituto Nacional de Investigación Agropecuaria; IT, infection type; PCR, polymerase chain reaction; Tc, Thatcher.

Leaf rust (caused by *Puccinia triticina* Eriks.) is one of the most important wheat (*Triticum aestivum* L.) diseases on a worldwide basis (Kolmer, 1996) and in Uruguay (Germán et al., 2007). Facultative (late maturing) and spring (early maturing) hard red wheat cultivars are grown in Uruguay. Many of the commercial cultivars are moderately susceptible or susceptible to leaf rust, allowing *P. triticina* to oversummer on volunteer plants over a large area, which leads to severe epidemics during the crop season. Two or more fungicide applications are required to control leaf rust to prevent grain yield losses that can be higher than 50% in susceptible cultivars (Germán et al., 2007).

The *P. triticina* population in Uruguay is extremely dynamic. A large number of races are generally present every year and their prevalence can change rapidly. New virulent races of leaf rust often overcome the resistance of commercial cultivars within a few years (Germán et al., 2007). Owing to its economic importance, resistance to leaf rust has been a long-term objective for the Instituto Nacional de Investigación Agropecuaria (INIA) wheat-breeding program in Uruguay. During the initial period of wheat breeding in Uruguay (1914–1950), leaf rust resistance was derived from locally adapted regional germplasm (Germán et

Published in Crop Sci. 52:601–608 (2012).

doi: 10.2135/cropsci2011.06.0335

Published online 8 Dec. 2011.

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al., 2007). Other sources of leaf rust resistant germplasm came from the United States Department of Agriculture (USDA) after the 1950s, from the International Maize and Wheat Improvement Center (CIMMYT) after the 1960s, and breeding programs in Argentina and Brazil.

There is limited information about the leaf-rust resistance genes present in the Uruguayan wheat germplasm. Information about the resistance genes used in breeding programs is required to design strategies that will add new genes to the development of cultivars with effective and more durable resistance. The objective of this research was to determine which known leaf-rust resistance genes are present in selected cultivars with early maturity released by the INIA-Uruguay wheat-breeding program and to determine if any cultivars have previously uncharacterized seedling or adult plant resistance.

MATERIALS AND METHODS

Seven early maturity Uruguayan bread wheat cultivars were studied for leaf rust resistance (Table 1). Seedling tests with *P. triticina* races with different virulence combinations were used to postulate the presence of seedling genes in six cultivars. Approximately 10 seedlings per cultivar were planted in clumps in greenhouse flats filled with a mixture of 50% soil, 25% sand, and 25% substrate (Biofer Almáciga, Riverfilco; Biofer Ltd., Montevideo, Uruguay) and were fertilized weekly with Foliar Fertilizer ISUSA NPK (12–8–5; Industria Sulfúrica S. A., San José, Uruguay) plus micronutrients. Seedlings were inoculated when the first leaf was fully expanded with a suspension of urediniospores of single *P. triticina* races in nonphytotoxic light industrial mineral oil (Soltrol 170, Phillips Petroleum Co., Borger, TX). Plants were kept in a humid chamber overnight and maintained in the greenhouse at 15 to 25°C with supplemental lighting after incubation. Inoculum of the 11 different races used for testing was increased on the susceptible wheat 'Little Club', vacuum dried, and stored in sealed glass vials at approximately 5°C. Races were designated with a four-letter code that described virulence of the races to four sets of four near-isogenic lines of 'Thatcher' wheat with different leaf-rust resistance genes. The Thatcher lines with genes *Lr1*, *Lr2a*, *Lr2c*, and *Lr3* were differential Set 1; lines with genes *Lr9*, *Lr16*, *Lr24*, and *Lr26* were Set 2; lines with

genes *Lr3ka*, *Lr11*, *Lr17a*, and *Lr30* were Set 3; and lines with genes *LrB*, *Lr10*, *Lr14a*, and *Lr18* were Set 4 (Kolmer et al., 2008a). Infection types (IT) on seedlings were assessed 12 d after inoculation, according to the scale described by Stakman et al. (1962): IT 0 = immune response, with no uredinia or necrosis; IT fleck (:) = necrotic flecks; IT 1 = small uredinia surrounded by necrosis; IT 2 = small uredinia surrounded by chlorosis; IT 3 = moderate uredinia; IT 4 = large uredinia. Designations of + and – were added to indicate larger and smaller size of uredinia, respectively. Infection type X = a mesothetic response of flecks, small and large uredinia. Infection type 0 to 2+ and X were considered low-infection types and IT 3 and 4 were considered high-infection types. The pattern of high- and low-infection types to the four wheat lines in each of the four differential sets was assigned a letter code as described in Long and Kolmer (1989). To determine which seedling resistant genes might be present, the IT patterns of the cultivars were compared with IT produced on Thatcher lines with single leaf rust resistance genes (Loegering et al., 1971).

For genetic analysis of leaf rust resistance, the cultivars Estanzuela Tarariras (E. Tarariras), INIA Boyero (I. Boyero), Estanzuela Benteveo (E. Benteveo), and Estanzuela Pelón 90 (E. Pelón 90) were crossed and backcrossed to the susceptible cultivar Thatcher and BC₁F₂ and F₃ families developed. Approximately 20 seedlings of BC₁F₂ and F₃ families were planted in clumps in greenhouse flats or beds and tested with leaf rust race BBBB, which is avirulent to most seedling leaf rust resistance genes. BC₁F₂ families from E. Pelón 90 were also tested with race MCRS, which is virulent to *Lr1* and *Lr26*. The inoculation procedure and scoring of ITs were the same as described previously. To determine which seedling resistance genes may be present in the cultivars, Thatcher lines with single resistance genes and homozygous BC₁F₃ families derived from single plants of BC₁F₂ families that segregated for single genes were selected and tested with seven *P. triticina* races with different virulence combinations.

Field tests were performed at INIA La Estanzuela with natural infection of the locally present leaf rust population. Single 1.5-m row plots along with spreader rows of leaf-rust-susceptible cultivars perpendicular to the rows of entries were planted in late July or early August. Fertilization and weed control were performed as required. BC₁F₂ families were field tested in 1994. In field experiments leaf rust severity was described on flag leaves using the modified Cobb scale from

Table 1. Pedigrees, years of release, and leaf rust severity and response of seven Uruguayan wheat (*Triticum aestivum* L.) cultivars with early maturity.

Cultivar	Year	Pedigree	Leaf rust severity [†] and response [‡]	
			1993–1995	1999–present
Estanzuela Tarariras	1974	Bage/4/Thatcher/3/Frontana/Kenya 58/NewThatch	5 M–60 MSS	50 MSS
INIA Boyero	1998	MN72131/BobWhite 'S'	Trace R	30–40 MRMS–70 MSS
Estanzuela Benteveo	1989	Aurora/Kalyansona/Blue Bird/3/Woodpecker (Bobwhite 'S')	Trace–30 MRMS	–
Estanzuela Pelón 90	1990	Kavkaz/Torim	Trace–10 R	5 M–80 MSS
INIA Caburé	1998	Estanzuela Federal/Buck 6//MR 74507	–	40 MSS–90 S
INIA Churrinche	2000	Estanzuela Federal/LE 2154	–	Trace R–70 MSS
INIA Tero	2005	LI 107/C-CH-91-1642	–	2 M–90 S

[†]Modified Cobb scale (Peterson et al., 1948).

[‡]R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

trace level (less than 1%) to 100% (Peterson et al., 1948). Flag leaves with small uredinia surrounded by distinct necrosis were rated as resistant (R), leaves with moderate- to large-sized uredinia surrounded by necrosis were rated as moderately resistant (MR), leaves with moderate to large uredinia surrounded by chlorosis were rated as moderately susceptible (MS), leaves with large uredinia lacking necrosis or chlorosis were rated as susceptible (S), and leaves with a mixture of large and small uredinia were rated as mixed (M). BC₁F₂ families with severity and response similar to the susceptible check cultivar Thatcher were considered as homozygous susceptible.

To estimate the number of effective resistance genes present in the cultivars, the number of segregating to susceptible BC₁F₂ families and the number of segregating plus resistant and susceptible F₃ families were determined and χ^2 values for goodness of fit of observed to expected ratios were calculated (Steel and Torrie, 1980). The Yates correction factor was used when the expected number of families was lower than five (Yates, 1934).

Greenhouse adult plant tests were performed to postulate the presence of adult plant resistance (APR) genes *Lr13* and *Lr34*. Plants were grown in 10-cm-diameter plastic pots, filled with the same mixture of soil, sand, and substrate used for seedlings. Fertilization was as described for seedlings. Single plants that expressed field resistance were selected from BC₁F₂ families that were homozygous susceptible as seedlings. These plants were progeny tested as BC₁F₃ adult plants with races BBBD and LBBS, which are avirulent and virulent to adult plants with *Lr13*, respectively. Flag leaves were inoculated in the same manner as seedlings when the plants were at heading to watery stage (10.5 to 10.5.4 growth stages, Feeks, 1941). Infection types as well as severity of infection were assessed 14 d after inoculation. The BC₁F₃ lines were also tested as seedlings with races BBBD and LBBS to confirm that these lines were seedling susceptible. Thatcher (Tc) lines with *Lr13* (RL4031) and Tc*Lr34* (RL6058) were used as checks.

Two hundred to six hundred F₂ plants derived from crosses of Tc*Lr13* (RL4031) and Tc*Lr34* (RL6058) with E. Tarariras, I. Boyero, E. Bentevio, and E. Pelón 90 were planted in the field during 1993. Selected F₂ plants and F₃ plants within presumably segregating F₃ families with high leaf-rust severity

were progeny tested the following year to confirm if these were susceptible (similar rust severity and response as Thatcher). Thatcher, Tc*Lr13* (RL4031), and Tc*Lr34* (RL6058) were included as checks. Lack of segregation in F₂ and derived F₃ families indicated the presence of *Lr13* or *Lr34* in the cultivar(s). Segregation of leaf rust resistance in the F₂ and derived F₃ families indicated the absence of these genes in the cultivar(s).

The polymerase chain reaction (PCR)-based marker csLV34 (Lagudah et al., 2006) was used to determine the likely presence of the APR gene *Lr34*. The PCR amplification of the wheat cultivars' DNA and determination of marker alleles associated with *Lr34* was done as described by Lagudah et al. (2006).

RESULTS

The cultivars had different levels of effective resistance during the time period of the study, and suffered from erosion of leaf resistance generally within a few years after release (Table 1). In seedling plants, E. Tarariras had very low IT of 0; to ; (fleck) to races BBBD and TLGF, a high IT of 3⁺ to races MFPS and MHDS, and intermediate IT between 2 and 3⁺ to all other races. *Lr3bg* was postulated to be in E. Tarariras on the basis of the similarity of ITs of the cultivar with Tc*Lr3bg* (Table 2). INIA Boyero had intermediate to high ITs to races MFPS, MHDS, KFBJ, and MFPN, which are all virulent to Tc*Lr26*, and was highly resistant to all races that had low IT to *Lr26*. On the basis of these ITs, *Lr26* was postulated to be present in I. Boyero. Additional seedling resistance gene(s) may be present since race THBJ, which is virulent to *Lr26*, had a low to intermediate IT to I. Boyero. Estanzuela Bentevio had intermediate IT to MFPS, KFBJ, and THBJ and very low IT to BBBD, MJB, MBBJ, MBRJ, and TLGF, indicating the possible presence of *Lr26*. Additional seedling resistance is likely present in E. Bentevio since it had low ITs to *Lr26*-virulent isolates TGBG and MHDS.

Seed of E. Pelón 90 germinated poorly, and IT data for only races MFPS and THBJ was obtained, which

Table 2. Seedling infection types (as described in Long and Kolmer [1989]) of six Uruguayan wheat (*Triticum aestivum L.*) cultivars and Thatcher near-isogenic lines (Tc) of wheat to 11 races of *Puccinia triticina*.

Wheat Lines	Puccinia triticina race											Postulated seedling resistance gene
	BBBD [†]	TGBG	MFPS	MJB	MHDS	MBBJ	KFBJ	MBRJ	TLGF	THBJ	MFPN	
Estanzuela Tarariras	0;	23	3 ⁺	2 ⁺	3 ⁺	2 ⁺	22 ⁺	2 ⁺ 3	;	2 ⁺ 3	3 ⁺	<i>L3bg</i>
INIA Boyero	;	;	2 ⁺ 3	;	2 ⁺ 3	;	2 ⁺	;	;	12 ⁺	23	<i>Lr26</i> , ⁺
Estanzuela Bentevio	0;	;	2	–	1-	;	2	;	;	1 ⁺ 2	–	<i>Lr26</i> , ⁺
INIA Caburé	0;	0;	1-	33 ⁺	;	;	3	;	0;	;	3	<i>Lr24</i> , ⁺
INIA Churrinche	0;	;	2	12 ⁺	;	;	3	;	0;	;	;	<i>Lr10</i> , <i>Lr24</i> , ⁺
INIA Tero	0;	0;	23 [–]	;	;	;	0;	;	;	;	3 ⁺	<i>Lr17a</i> , <i>Lr24</i> , ⁺
Tc <i>Lr3bg</i>	;	22 ⁺	3 ⁺	22 ⁺	3 ⁺	3 ⁺	2	3 ⁺	2	22 ⁺	3 ⁺	
Tc <i>Lr10</i>	;	3 ⁺	3 ⁺	3 ⁺	3 ⁺	3 ⁺	3 ⁺	3 ⁺	2 [–]	3 ⁺	0;1	
Tc <i>Lr17a</i>	;2	;23	33 ⁺	1 ⁺	3 ⁺	12	1 ⁺ 2	12-	1 ⁺	2	3 ⁺	
Tc <i>Lr24</i>	0;	;	3	3 ⁺	1	;	4	;	;	;	3 ⁺	
Tc <i>Lr26</i>	;12	32 ⁺	3 ⁺	;	3 ⁺	1-	3 ⁺	1	;	3 ⁺	3 ⁺	

[†]Virulence formulae. BBBD: 14a; TGBG: 1,2,2c,3a,16,14a; MFPS: 1,3a,24,26,3ka,17a,30,B,10,14a; MJB: 1,3a,16,24,10,14a; MHDS: 1,3a,16,26,17a,, B,10,14a; MBBJ: 1,3a,10,14a; KFBJ: 2a,2c,3a,24,26,10,14a; MBRJ: 1,3a,3ka,11,30,10,14a; TLGF: 1,2a,2c,3a,9,11,14a,18; THBJ: 1,2a,2c,3a,16,26,10,14a; MFPN: 1,3a,24,26,3ka,17a,30,B,14a.

Table 3. Segregation of leaf rust resistance in seedling plants of BC₁F₂ families and F₃ families of Estanzuela Tarariras (E. Tarariras), INIA Boyero (I. Boyero), Estanzuela Benteveo (E. Benteveo), and Estanzuela Pelón 90 (E. Pelón 90) crossed with 'Thatcher'.

Cultivar	Race	Generation	Number of families				
			Resistant/Segregating	Susceptible	Expected ratio	χ^2	p
E. Tarariras	BBBD [†]	BC ₁ F ₂	31	25	1:1	0.64	0.5–0.3
	BBBD	F ₃	93	26	3:1	0.63	0.5–0.3
I. Boyero	BBBD	BC ₁ F ₂	50	70	1:1	3.33	0.1–0.5
	BBBD	F ₃	139	7	15:1	0.53	0.5–0.3
E. Pelón 90	BBBD	BC ₁ F ₂	41	3	7:1	1.30	0.3–0.2
	MCRS	BC ₁ F ₂	24	20	1:1	0.36	0.7–0.5
	BBBD	F ₃	143	1	63:1	0.25	0.7–0.5

[†]Virulence formulae: BBBD, 14a; MCRS, 1,3a,26,3ka,11,30,B,10,14a.

precluded gene postulation for this cultivar. INIA Caburé (I. Caburé) had high ITs to races KFBJ, MJB, and MFPN, which are virulent to *Lr24*. INIA Caburé was postulated to have *Lr24* plus additional seedling resistance since it had a low IT to race MFPS, which is also virulent to *Lr24*. INIA Churrinche (I. Churrinche) was also postulated to have *Lr24* and additional seedling resistance since it had a high IT to KFBJ and intermediate IT to races MFPS and MJB. INIA Churrinche also probably has *Lr10* since it had low IT to race MFPN, which is virulent to *Lr24* and avirulent to *Lr10*. INIA Tero (I. Tero) was postulated to have *Lr17a* and *Lr24* since it had high IT to race MFPN, which is virulent to both genes. INIA Tero may also have additional seedling resistance since it had intermediate to high IT to race MFPS, which is virulent to both *Lr10* and *Lr24*.

The Tc²/E. Tarariras F₂ families segregated in a 1:1 ratio when tested with race BBBD, which fit a single gene model (Table 3). F₃ families of Tc/E. Tarariras also segregated for a single gene in a 3:1 ratio. Segregation of the Tc²/I. Boyero F₂ families fit a 1:1 ratio, indicating the presence of a single resistance gene effective to race BBBD in this cultivar. Segregation of the Tc²/E. Benteveo F₂ families fit a 3:1 ratio, which indicated two genes for seedling leaf rust resistance. Tc/E. Benteveo F₃ families segregated to fit a 15:1 ratio, confirming segregation of two genes for seedling resistance. Tc²/E. Pelón 90 F₂ families segregated to fit a 7:1 ratio when tested with race BBBD (avirulent to *Lr1*, *Lr17a*, and *Lr26*), which indicated three genes segregating, and a 1:1 ratio, indicating a single gene segregating when tested with race MCRS (avirulent to *Lr17a* and virulent to *Lr1* and *Lr26*). Segregation of Tc/E. Pelón 90 F₃ families fit a 63:1 ratio when tested with race BBBD, which indicated the segregation of at least three seedling genes.

BC₁F₂ lines from Tc²/E. Tarariras, Tc²/I. Boyero, Tc²/E. Benteveo, and Tc²/E. Pelón 90 were selected on the basis of low IT to BBBB and grown to maturity to obtain BC₁F₃ seed for additional testing. Infection types from one of several lines with single seedling genes are shown in Table 4. BC₁F₃ lines derived from Tc²/E.

Tarariras were postulated to have *Lr3bg* on the basis of the high ITs to races TDK and TTD, which are also virulent to Tc*Lr3bg*. BC₁F₃ lines derived from Tc²/I. Boyero were postulated to have *Lr26* on the basis of the high ITs to races MCRS and LCGK, which are also virulent to Tc*Lr26*. Separate BC₁F₃ lines from Tc²/E. Benteveo were postulated to have *Lr3a*, on the basis of low IT to races BBBB, SLGD, and LCGK, and *Lr26* on the basis of high IT to MCRS and LCGK. Separate BC₁F₃ lines from Tc²/E. Pelón 90 were postulated to have *Lr1* on the basis of low IT to BBBB and CGTD; *Lr17a* on the basis of high IT to CGTD, TDK, and TTD; and *Lr26* on the basis of high IT to MCRS and LCGK.

In field plots with the commonly occurring leaf rust races present in Uruguay, the Tc²/E. Tarariras F₂ families segregated for adult plant leaf rust resistance in a 3:1 ratio, which fit the expected ratio for segregation of two genes (Table 5). Segregation of the Tc²/I. Boyero F₂ families fit a 7:1 ratio, which indicated that at least three genes were segregating for leaf rust resistance. BC₁F₂ families derived from E. Benteveo segregated to fit a 1:1 ratio, indicating a single gene for field resistance. The BC₁F₃ families derived from E. Pelón 90 segregated to fit both 3:1 and 7:1 ratios, which indicated the segregation of two or three genes.

Individual BC₁F₂ plants from Tc²/E. Tarariras, Tc²/I. Boyero, Tc²/E. Benteveo, and Tc²/E. Pelón 90 were selected on the basis of adult plant leaf rust resistance, and seed was harvested for further progeny testing. Lines were selected only from BC₁F₂ families that were homozygous for seedling susceptibility to race BBBB, to eliminate any seedling resistance genes. BC₁F₃ lines from Tc²/E. Tarariras and Tc²/I. Boyero had similar ITs to races BBBB and LBBS in the seedling and adult plant stages to either the Tc*Lr13* line or the Tc*Lr34* line (data not shown), therefore *Lr13* and *Lr34* were postulated to be present in E. Tarariras and in I. Boyero. Other BC₁F₃ lines derived from Tc²/I. Boyero had intermediate to high ITs to both BBBB and LBBS in seedling plants and low ITs of 1⁺ to both races as adult plants. These lines likely have additional APR genes other than *Lr13* or *Lr34*.

Table 4. Seedling infection types (as described by Long and Kolmer [1989]) of selected BC₁F₃ lines derived from Tc*2/E. Tarariras, Tc*2/I. Boyero, Tc*2/E. Benteveo, and Tc*2/E. Pelón 90 and 'Thatcher' near-isogenic lines to seven *Puccinia triticina* races. Tc, Thatcher; E., Estanzuela; I., INIA.

Wheat line	Gene detected	Puccinia triticina races						
		BBBD [†]	MCRS	CGTD	SLGD	TBDK	LCGK	TDTD
Tc*2/E. Tarariras; BC ₁ F ₃ 15651-3	Lr3bg	0;1	23;	;2-	0;1-	3+	;	4
Tc*2/I. Boyero; BC ₁ F ₃ 12629-6	Lr26	0;1-	3+4	;	;1-	0;	3+	0;1-
Tc*2/E. Benteveo; BC ₁ F ₃ 15706-1	Lr3a	;1-	3+4	33+	;1-	3+	;1	4
BC ₁ F ₃ 15712-3	Lr26	;1-	3+4	;	;1-	;	3+	;1-
Tc*2/E. Pelón 90; BC ₁ F ₃ 15719-8	Lr1	0;	3+4	0;	3	3+	3+	4
BC ₁ F ₃ 15727-3	Lr17a	;	;1-	33+	2	3	;1	4
BC ₁ F ₃ 15723-2	Lr26	0;	3+	;	;1-	;	3+	1-
TcLr1	-	0;	3+	0;	3+	3+	3+	4
TcLr3a	-	;1-	3+	3+	;1-	3+	;1-	4
TcLr3bg	-	;	32;	32;	;1 =	3+	0;	4
TcLr17a	-	;1-	;1-	3+	;1-	3+	12-	4
TcLr26	-	;1-	33+	;	;1 =	0;	3+4	;1 =

[†]Virulence formulae: BBBD: 14a; MCRS: 1,3a,26,3ka,11,30,B,10,14a; CGTD: 3a,16,3ka,11,17a,30,14a; SLGD: 1,2a,2c,9,11,14a; TBDK: 1,2a,2c,3a,17a,10,14a,18; LCGK: 1,26,11,10,14a,18; TDTD: 1,2a,2c,3a,24,3ka,11,17a,30,14a.

Table 5. Segregation for leaf rust resistance in field plots in Uruguay of Tc*2/E. Tarariras, Tc*2/I. Boyero, Tc*2/E. Benteveo, and Tc*2/E. Pelón 90 BC₁F₂ families. Tc, Thatcher; E., Estanzuela; I., INIA.

Cross	Segregating families	Homozygous susceptible families	Expected ratio	χ^2	p
Tc*2/E. Tarariras F ₂	44	13	3:1	0.15	0.9–0.7
Tc*2/I. Boyero F ₂	104	14	7:1	0.44	0.9–0.7
Tc*2/E. Benteveo F ₂	34	46	1:1	1.80	0.2–0.1
Tc*2/E. Pelón 90 F ₂	37	7	3:1	1.94	0.2–0.1
			7:1	0.47	0.5–0.3

Some BC₁F₃ lines derived from Tc*2/E. Benteveo had IT similar to TcLr13, which indicated the possible presence of Lr13, while others had similar resistance phenotype to TcLr34. BC₁F₃ lines derived from Tc*2/E. Pelón 90 had the same IT as TcLr34.

Estanzuela Tarariras, I. Boyero, E. Benteveo, and E. Pelón 90 were crossed with TcLr13 and TcLr34, and F₂ adult plants were tested for segregation of leaf rust resistance (Table 6). All F₂ plants from crosses of E. Tarariras and I. Boyero with TcLr13 and TcLr34 were resistant, which indicated the presence of both genes in the cultivars. F₂ plants of TcLr13/I. Boyero had low field reaction and were clearly resistant. F₂ plants with higher rust severity from TcLr13/E. Tarariras, TcLr34/E. Tarariras, and TcLr34/I. Boyero were progeny tested and confirmed to be resistant. Estanzuela Tarariras and I. Boyero had the allele associated with the presence of Lr34 when tested with marker csLV34. All F₂ plants from TcLr13 crossed with E. Benteveo were resistant, as were all the derived F₃ lines, indicating the presence of Lr13. F₂ plants from TcLr34 crossed with E. Benteveo and the derived F₃ lines segregated for resistance and susceptibility, indicating the absence of Lr34. Estanzuela Benteveo did not have the marker allele for csLV34 associated with Lr34. F₂ plants from TcLr13 crossed with E. Pelón 90, and the derived

F₃ lines segregated for resistance and susceptibility, indicating the absence of Lr13. There was no segregation for resistance in the F₂ plants and F₃ lines of TcLr34 crossed with E. Pelón 90. The presence of Lr34 in E. Pelón 90 was confirmed by the csLV34 marker allele associated with Lr34. INIA Tero had the marker allele associated with the presence of Lr34 while I. Caburé and I. Churrinche did not have the marker allele associated with Lr34.

DISCUSSION

Estanzuela Tarariras was postulated to have Lr3bg and genetically determined to have Lr13 and Lr34; I. Boyero was postulated to have Lr26, plus additional resistance that was effective in seedling and adult plants, and genetically determined to have Lr13 and Lr34; E. Benteveo was postulated to have Lr3a and Lr26, plus additional seedling and APR, and genetically determined to have Lr13; and E. Pelón 90 was postulated to have Lr1, Lr17a, and Lr26 and genetically determined to have Lr34. Comparison of infection types with the Thatcher near-isogenic lines using different races of *P. triticina* and the csLV34 marker indicated that I. Caburé likely had Lr24 and additional seedling resistance, that I. Churrinche likely had Lr10 and Lr24, plus additional seedling resistance, and that I. Tero was postulated to have Lr17a, Lr24, and Lr34. Lr13, Lr17a, and Lr24 conditioned

Table 6. Segregation for leaf rust resistance in field plots of Estanzuela Tarariras (E. Tarariras), INIA Boyero (I. Boyero), Estanzuela Benteveo (E. Benteveo), and Estanzuela Pelón 90 (E. Pelón 90) crossed with 'Thatcher' (Tc) lines with *Lr13* and *Lr34*.

Cross/Cultivar	No. of F ₂ plants	F ₃ lines		F ₄ lines	
		No. lines	Response	No. lines	Response
TcLr13/E. Tarariras	322	32	20–60 [†] MS [‡]	4	20 M–70 MSS
TcLr34/E. Tarariras	412	14	50–70 MS	5	30–60 MSS
TcLr13/I. Boyero	565	0	–	–	–
TcLr34/I. Boyero	568	11	20–90 MS	–	–
TcLr13/E. Benteveo	304	13	20–50 MSS	4	20 M–70 MSS
TcLr34/E. Benteveo	211	25	80 S	7	80 S
TcLr13/E. Pelón 90	266	10	60–70 S	3	90 S
TcLr34/E. Pelón 90	222	14	60–70 MS	4	50 M–80 S

[†]Modified Cobb scale (Peterson et al., 1948).

[‡]R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

effective during 1994–1995 but became ineffective in later years as races with these virulences increased. These genes are also commonly found in wheat cultivars from Argentina and Brazil (Germán et al., 2007; 2009).

INIA Boyero and E. Benteveo were postulated to have other seedling resistance in addition to *Lr26* on the basis of the low IT of the cultivars to race THBJ, which is virulent to *Lr26*. However, Tc^{*}2/I. Boyero F₂ families segregated for only a single gene, likely *Lr26* when tested with race BBBD, avirulent to this gene. The additional seedling resistance in I. Boyero was not detected by race BBBD. Similarly, the additional resistance in E. Benteveo was not detected by race BBBD, as the Tc^{*}2/E. Benteveo F₂ families and Tc/E. Benteveo F₃ families segregated for two seedling resistance genes, likely *Lr3* and *Lr26*, which are both detected by race BBBD.

Lr1 is present in Centenario, an old regional cultivar (Dyck and Samborski, 1968), and is common in CIMMYT spring wheats (Singh, 1993; Singh and Rajaram, 1991); however, it is highly ineffective for leaf rust resistance as nearly every race of *P. triticina* in South America is virulent to this gene (Germán et al., 2009). *Lr3a* was found in Americano 25e (Kolmer et al., 2007), a landrace-derived wheat from Uruguay released in 1918 that was an important parent in the initial years of wheat breeding in the Southern Cone region. *Lr3a* is also completely ineffective for leaf rust resistance. *Lr3bg* was derived from the Brazilian cultivar Bagé (Haggag and Dyck, 1973), which was a parent of E. Tarariras. From 1993 to 1995 TcLr3bg varied between 30 MSS and 90 S for leaf rust resistance compared with 70 to 80 S for Thatcher, and has not conferred effective resistance. *Lr17a* was initially isolated from the Argentine wheat 'Klein Lucero' (Dyck and Samborski, 1982) and is also found in CIMMYT cultivars such as Torim 73 (Singh, 1993), one of E. Pelón 90 parents. TcLr17a had very low leaf rust severity in 1993 and an intermediate level of 40 MRMS in 1994. Since 1996 races with virulence to *Lr17a* have increased in frequency, rendering

this gene ineffective for leaf rust resistance (Germán et al., 2007). *Lr26* is common in CIMMYT germplasm (Singh and Rajaram, 1991) and is present in Bobwhite 'S' (McIntosh et al., 1995), a parent of I. Boyero, and in E. Benteveo, a Bobwhite sib line which was released in Uruguay. Virulence to *Lr26* has been common throughout the Southern Cone region (Germán et al., 2009), and this gene has not conditioned effective resistance since the 1980s. The cultivar Agent was the original wheat source of *Lr24*, and derivatives of Agent with *Lr24* such as Cargill Trigal 800 (Antonelli, 2003) have been used in breeding programs in Argentina and Uruguay. Virulence to *Lr24* was low to intermediate in Uruguay from 1991 to 1995, and this gene conditioned effective resistance to leaf rust resistance during this time. However, since 2003 virulence to *Lr24* has increased, likely because of the selective effects of wheat cultivars with this gene.

The APR gene *Lr13* was initially characterized in the Brazilian cultivar Frontana (Dyck et al., 1966), which is present in the pedigree of one of the parents of E. Tarariras. Americano 26n, a Uruguayan landrace that was used in the initial years of wheat breeding in Uruguay (Kolmer et al., 2007), may have been an original donor of *Lr13* in the Uruguayan wheats. *Lr13* is also common in CIMMYT wheats (Singh, 1993; Singh and Rajaram, 1991; 1992) and is likely present in Bobwhite S, a parent of I. Boyero, and E. Benteveo (Bobwhite sib line). From 1993 to 1995 TcLr13 conditioned effective leaf rust resistance from 5 to 30 MRMS in field plot tests in Uruguay. Since 1997 *P. triticina* races with virulence to *Lr13* have increased, rendering this gene ineffective for leaf rust resistance. Frontana was also an early source of *Lr34* (Dyck and Samborski, 1982), which is present in wheat cultivars from the Southern Cone region (Germán et al., 2007; 2009). The Italian cultivar Ardito with *Lr34* (Kolmer et al., 2008b) was used as a parent in the early years of wheat breeding in Uruguay (Luizzi et al., 1983). In Uruguay the expression of *Lr34* resistance is variable, generally ranging from 40 M to 60 MSS.

INIA Tero was postulated to have *Lr34* on the basis of the data from the marker csLV34. However, this cultivar has been considered leaf rust susceptible on the basis of results from field plots. A nonfunctional allele of *Lr34* may be present in I. Tero, as was found in the U.S. wheat 'Jagger' (Lagudah et al., 2009). Some recombinants between csLV34 and *Lr34* have been noted (Lagudah et al., 2009), so another possibility is that I. Tero lacks *Lr34* but has the marker allele associated with the gene.

INIA Caburé and I. Churrinche were both postulated to have *Lr24*, yet both cultivars were resistant to race MFPS as seedling plants, and I. Churrinche was also resistant to race MJB. Since both races are virulent to *Lr24* it is possible that other resistance genes are present in these cultivars. The effectiveness of this additional resistance is likely limited since I. Caburé and I. Churrinche have had high and intermediate levels of leaf rust under field conditions, respectively. INIA Churrinche was highly resistant when released in 2000, and leaf rust severity on this cultivar increased slowly over several years, reaching maximum severity of 70 MSS when Thatcher was at 90 S. The presence of APR may have slowed the erosion of resistance. Since I. Churrinche was negative for *Lr34* on the basis of csLV34, it is possible that different effective APR is present in this cultivar. INIA Boyero was determined to have APR in addition to *Lr13* and *Lr34*. The BC₁F₃ lines with this resistance had lower ITs as adult plants to races BBBB and LBBS compared with Tc*Lr34*. Lines with APR with phenotypic expression similar to *Lr34* were derived from E. Benteveo; however, this cultivar does not have *Lr34* according to the molecular marker data, which indicates that this cultivar may carry different APR gene(s) with similar resistance phenotype as *Lr34*. Additional APR genes are present in South American wheat germplasm. The Brazilian cultivar Toropi was determined to have two APR genes (Barcellos et al., 2000). Americano 25e has adult plant resistance on chromosome 1BL, in the same region as *Lr46* (Kolmer, unpublished data, 2010). Since Americano 25e was used as an early parent in the wheat programs in the Southern Cone (Luizzi et al., 1983), it is possible that adult plant resistance on 1BL is widespread in this germplasm.

In summary, the Uruguayan cultivars with early maturity were found to have combinations of race-specific seedling resistance genes and APR genes. Leaf rust oversummers on volunteer wheat in Uruguay after harvest in December and before planting in April. The year-round presence of leaf rust infections in Uruguay allows the *P. tritici* population to quickly adapt to the race specific seedling resistance genes, as virulent races are routinely selected by wheat cultivars with these genes. INIA Tero and I. Caburé were considered susceptible to leaf rust before or shortly after being released because of the increase of races with *Lr24* virulence. Cultivars such as E.

Tarariras, I. Boyero, and E. Pelón 90 would be expected to maintain some resistance over a period of time because of the nonspecific resistance conditioned by *Lr34*. However, cultivars with only *Lr34* can still have high leaf-rust severities and suffer significant yield loss. Additional sources of APR either from South American (Barcellos et al., 2000; Brammer et al., 2004; Altieri et al., 2008; Kolmer et al., 2007) or CIMMYT (Singh et al., 2011) wheat germplasm will need to be combined with *Lr34* to develop wheat cultivars with long-lasting and highly effective leaf rust resistance. INIA Boyero and E. Benteveo have additional APR gene(s) that may be useful sources of resistance in addition to *Lr34*.

Acknowledgments

Research support was provided by the Cereal Research Centre, Agriculture and Agri-Food Canada Winnipeg MB, and by the USDA-ARS Cereal Disease Laboratory, St. Paul, MN. The research was supported with funding from the Canadian International Development Agency (Wheat Pathology and Tillage CIDA/INIA Project 964/1463). We thank Richard García and Elena Caballero for their technical assistance.

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