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Leaf rust resistance in selected late maturity, common wheat cultivars from Uruguay

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Abstract Leaf rust (caused by *Puccinia triticina*) is one of the most important diseases of wheat in Uruguay, and breeding for resistance to this disease is a priority for the INIA wheat program. Knowledge of the effective resistance genes present in the germplasm is relevant when selecting for effective and more durable resistance. The leaf rust resistance present in six adapted wheat cultivars that are parents of many advanced lines was studied. Races of *P. triticina* with different virulence combinations were used to determine which seedling resistance genes might be present in the six cultivars and/or derived lines. Genetic analysis of seedling and adult plant resistance (APR) was conducted on BC_1F_2 and F_3 generations from crosses of four cultivars with the susceptible cultivar Thatcher. The presence of APR genes *Lr13* and *Lr34* was confirmed with crosses of the four cultivars and Thatcher lines with these genes. A genetic marker associated with *Lr34* was used to postulate the presence of this gene in all cultivars. The cultivars and resistance genes postulated to be present were: Estanzuela Calandria *Lr3bg*, *Lr16* and *Lr24*; Estanzuela Federal *Lr10*; Estanzuela Halcón *Lr10*, *Lr14a*, and *Lr16*; INIA Tijereta and INIA Garza *Lr16*, *Lr24* and *Lr34*; and INIA Torcaza *Lr10* and *Lr24*. Only *Lr16* and *Lr34* remain effective to the predominant pathotypes. Additional ineffective seedling resistance that could not be identified was present in E. Federal, I. Tijereta and I. Torcaza. Unknown APR gene(s) could be present in E. Calandria and E. Federal.

Keywords *Triticum aestivum* · *Puccinia triticina* · Genetics of resistance · Gene postulation

Abbreviations

APR	Adult plant resistance
IT	Infection type
INIA	Instituto Nacional de Investigación Agropecuaria
I.	INIA
E.	Estanzuela
Tc	Thatcher

Introduction

Leaf rust (caused by *Puccinia triticina* Eriks.) is one of the most important wheat diseases worldwide, including Uruguay (Kolmer 1996; Germán et al. 2007). Hard red spring wheat cultivars with late and early maturity are grown in Uruguay. A highly favorable

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environment and a high proportion of the wheat area sown to leaf rust susceptible or moderately susceptible cultivars allow *P. triticina* to cause severe epidemics and to over-summer in large areas. Two or more fungicide applications are required to prevent grain yield losses that can be as high as 50 % on susceptible cultivars (Germán et al. 2007).

Many races are generally present in the *P. triticina* population in Uruguay and their relative frequencies may change significantly from one year to the next. Races with new virulence combinations often overcome the resistance of cultivars a few years after release (Germán et al. 2007). Due to its economic importance, resistance to leaf rust is a priority for the National Institute for Agricultural Research (INIA)-Uruguay breeding program. During 1914–1950, leaf rust resistant germplasm was obtained from Argentina and Brazil (Germán et al. 2007). After 1950 sources of leaf rust resistance were also selected from germplasm distributed by the United States Department of Agriculture (USDA) and the International Maize and Wheat Improvement Center (CIMMYT).

Germán and Kolmer (2012) indicated that genes *Lr1*, *Lr3a*, *Lr3bg*, *Lr10*, *Lr17a*, *Lr24*, *Lr26*, and the adult plant resistance (APR) genes *Lr13* and *Lr34* were likely present in seven Uruguayan cultivars with early maturity. Among these genes, only *Lr34* remains effective. However, little information about the leaf rust resistance genes present in other Uruguayan wheat cultivars and lines is available. Knowledge of the effective resistance genes used in resistance breeding is helpful to best design strategies to increase the diversity of effective and hopefully durable leaf rust resistance. The objective of this research was to determine the leaf rust resistance genes present in six late maturing cultivars released by the INIA-Uruguay wheat breeding program.

Materials and methods

Six Uruguayan bread wheat cultivars with late maturity (Table 1) were studied for leaf rust resistance. The presence of seedling resistance genes was postulated from seedling tests with *P. triticina* races. The cultivars were planted in clumps of ~10 seeds in greenhouse flats or beds containing a mixture of 50 % soil, 25 % sand and 25 % substrate (Biofer Almáciga, Riverfilco), and were fertilized weekly with Foliar Fertilizer ISUSA NPK (12-8-5 plus micronutrients).

When the first leaf was fully expanded seedlings were inoculated with urediniospores of single *P. triticina* races suspended in light mineral oil (Soltrol 170, Phillips Petroleum Co.). Plants were kept in a humid chamber overnight and after incubation were maintained in a greenhouse at 15–25 °C with supplemental lighting. The susceptible wheat Little Club was used to increase the inoculum, which was vacuum dried and stored in sealed glass vials at 5 °C or kept in gel capsules at −80° C to maintain viability. Races were designated with a four letter code that described their virulence/avirulence to four sets of four Thatcher near-isogenic lines with different single leaf rust resistance genes. As described by Kolmer et al. (2008a) lines with genes *Lr1*, *Lr2a*, *Lr2c*, and *Lr3* were in differential set 1, lines with genes *Lr9*, *Lr16*, *Lr24*, *Lr26* were in set 2, lines with genes *Lr3ka*, *Lr11*, *Lr17a*, *Lr30* were in set 3, and lines with genes *LrB*, *Lr10*, *Lr14a*, and *Lr18* were in set 4. Infection types (IT) on seedlings were assessed 12 days 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 uredinia, respectively. IT X = a mesothetic response of flecks, small and large uredinia. IT 0–2^+ and X were considered low ITs and IT 3–4 were considered high ITs. The IT patterns of the cultivars were compared to IT produced on Thatcher lines with single *Lr* genes (*Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17a*, *Lr18*, *Lr20*, *Lr21*, *Lr23*, *Lr24*, *Lr26*, *Lr30*, *Lr33*, *LrB*) to postulate which of these genes were present (Loegering et al. 1971).

Cultivars Estanzuela Calandria, E. Federal, E. Halcón and INIA Tijereta were crossed and backcrossed to the susceptible cultivar Thatcher and BC_1F_1 derived BC_1F_2 families, and F_2 derived F_3 families from simple crosses were used to study the genetics of the leaf rust reaction at the seedling stage. In the case of I. Tijereta BC_1F_1 derived BC_1F_3 families and F_3 families were used for the seedling resistance study. I. Tijereta was also crossed with Tc*Lr16* and F_2 seedlings were used to confirm the presence of *Lr16*.

Table 1 Pedigree, year of release and leaf rust severity and response of six late maturing Uruguayan wheat cultivars

Cultivar	Year	Pedigree	Leaf rust severity and response		
			1993–1995	1999–2001	2002–present
Estanzuela Calandria	1986	Prelude/L10/E. Tarariras	1R	1R–20MS	
Estanzuela Federal	1987	E. Hornero/CNT 8	2–70MSS	1–10MR	
Estanzuela Halcón	1991	Buck 6/MR 74507	10–60MRMS	5–10MS	
INIA Tijereta	1997	LE 2132/E. Calandria		1MRMS	20–60MSS
INIA Torcaza	2002	E. Federal/4/Trigal 800/3/K. Impacto/Agatha//K. Impacto/PAT 24		1–5MS	20–80MSS
INIA Garza	2006	I. Caburé/I. Tijereta			1–30MRMS

Modified Cobb scale (Peterson et al. 1948)

R resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible

Families were planted in clumps of ~20 seedlings in greenhouse flats or beds and tested with leaf rust race BBBD, which is avirulent to most seedling leaf rust resistance genes. BC_1F_2 families from E. Calandria were also tested with race MCRS and BC_1F_1 derived BC_1F_3 and F_3 families from I. Tijereta were tested with race MCDS. Inoculation and scoring of ITs were the same as described previously. F_2 seedlings from the cross Tc*Lr16*/I. Tijereta were tested with the *Lr16* avirulent race SPGK to confirm the presence of this gene in the cultivar.

BC_1F_2 plants from Tc*2/E. Calandria, Tc*2/E. Federal, Tc*2/E. Halcón and Tc*2/I. Tijereta, were selected from families that had single seedling gene segregation and had different ITs to race BBBD. The selected BC_1F_2 plants were grown to maturity to obtain BC_1F_3 seed for additional testing.

BC_1F_3 seedlings were tested with seven *P. triticina* races with different virulence combinations (BBBD, MCRS, CGTD SLGD, TBDK, LCGK, TDTD, TGDB) and their ITs were compared with the IT on near isogenic Thatcher lines for postulation of the seedling resistance genes. Lines derived from Tc*2/I. Tijereta F_2 families were tested with a different set of seven races (MCPS, TDDK, KDGK, MRMF, MCDS, CCTK, MHRK).

The six cultivars and BC_1F_2 families derived from four cultivars were field tested at INIA La Estanzuela under natural infection of leaf rust. Single 1.5 m row plots were planted in late July or early August and spreader rows of susceptible cultivars were planted perpendicular to the entries to support rapid increases in infection. Fertilization and weed control were performed as required. Families from E. Calandria, E. Federal and E. Halcón were tested in 1994, while families from I. Tijereta were tested in 2001. Prevalent races during 1994 were MCRK and MFRF, while during 2001 the most frequent races were MCDS and MFRF. In field experiments leaf rust severity on flag leaves was described using the modified Cobb scale (Peterson et al. 1948). Small uredinia surrounded by distinct necrosis were considered resistant (R); moderate-large sized uredinia surrounded by necrosis were considered moderately resistant (MR); moderate to large uredinia surround by chlorosis were considered moderately susceptible (MS); large uredinia lacking necrosis or chlorosis were considered susceptible (S); and a mixture of large and small uredinia were considered as a mixed (M) response. BC_1F_2 families with severity and response similar to Thatcher were considered to be homozygous susceptible.

The numbers of segregating and homozygous susceptible BC_1F_2 families, and the pooled number of homozygous resistant plus segregating families as a group and homozygous susceptible F_3 families were determined. χ^2 values for goodness of fit of observed to expected ratios were calculated (Steel and Torrie 1980) to estimate the number of effective resistance genes present in the cultivars. The Yates correction factor was used when the expected number of families was lower than 5 (Yates 1934).

F_2 plants derived from crosses of Tc*Lr13* (RL4031) with E. Calandria and E. Halcón and from the crosses of Tc*Lr34* (RL6058) with E. Calandria, E. Federal and E. Halcón were field tested as spaced plants during 1993, and F_2 plants derived from the cross of Tc*Lr34* (RL6058) with I. Tijereta were field tested during 2002. Selected F_2 plants with high or relatively high

leaf rust severities were progeny tested the following year as F_3 families to confirm their field reactions. When there were no homozygous susceptible F_3 families and the presence of susceptible plants within F_3 was not clear, selected plants within F_3 families were progeny tested as F_4 families. Thatcher, Tc*Lr13* (RL4031) and Tc*Lr34* (RL6058) were included as checks. *Lr13* or *Lr34* were considered to be present in the cultivars when the F_2 and derived F_3 or F_4 families did not segregate for susceptibility. The possible presence of APR gene *Lr34* in the cultivars was also determined using the PCR based marker csLV34 (Lagudah et al. 2006).

Results

The six cultivars had low levels of leaf rust severity after release, but as leaf rust races with virulence increased, leaf rust severity also increased on some of the cultivars. For example, E. Calandria was highly resistant in 1993, but by 2001 severity on this cultivar had increased to 20MS (Table 1). From 1993 to 1995 E. Federal had a variable field severity and response due to a new race that was virulent to this cultivar at the seedling stage. After 1999 leaf rust severity decreased on this cultivar. E. Halcón also had a variable level of response of 10–60MRMS from 1993 to 1995 and had a lower level of severity from 1999 to 2001. E. Federal and E. Halcón had lower leaf rust severities from 1999 to 2001 likely due to their replacement by farmers. I. Tijereta was highly resistant during 1999–2001 and had higher leaf rust severity of 20–60MSS after the detection of MFPF and other races with intermediate IT on the cultivar at the seedling stage. I. Torcaza had a low field leaf rust response of 1–5MSS during 1999–2001 and severity increased to 20–80MSS due mainly to race MDRK that was detected in 2003. I. Garza, released in 2006 had a low to intermediate field response (1–30MRMS).

In the seedling stage E. Calandria, I. Tijereta and I. Garza had low IT (0; to ;) to the six *Lr24*-avirulent isolates (Table 2) and intermediate to high IT to races virulent to Tc*Lr24* and intermediate IT to *TcLr16.* The IT data indicated that *Lr16* and *Lr24* are likely present in these three cultivars. It was not possible to postulate the seedling resistance of E. Federal with the leaf rust races used in this study. E. Halcón had high IT to races MJBJ and THBJ that were virulent to *Lr10* and IT 2+ or 2+3 to Tc*Lr16* indicating these genes might be present in the cultivar. *Lr16* is temperature sensitive (Dyck and Johnson 1983) and the ITs of some races characterized as virulent to this gene may vary from intermediate to high on the Tc*Lr16* line, perhaps explaining why E. Halcón had IT 33+ to THBJ while the tester line had IT 2+. The low IT of E. Halcón to race MHDS could be explained by the presence of *Lr23* and the low IT to race TGBG by the presence of *Lr14a*. I. Torcaza was postulated to have *Lr24* since it had low IT with all *Lr24*-avirulent races and high IT to all *Lr24*-virulent races except MFPN and MFPS. I. Torcaza probably has also *Lr10* since it had a low IT to race MFPN, which is virulent to *Lr24* but avirulent to *Lr10*, and additional seedling resistance since it had a low IT to race MFPS, which is virulent to *Lr10* and *Lr24*.

The Tc*2/E. Calandria F_2 families segregated to fit both a 7 segregating : 1 homozygous susceptible and a 15:1 ratio when tested with race BBBD at the seedling stage, that is, a three or four gene model, respectively (Table 3), and a 3:1 ratio when tested with race MCRS, a two gene model. F_3 families of Tc/E. Calandria segregated for at least three genes in a 63 homozygous resistant plus segregating (pooled as one group): 1 homozygous susceptible ratio. Segregation of the Tc*2/E. Federal F_2 families fit a 1:1 ratio, indicating the presence of a single resistance gene. Tc/E. Federal F_3 families segregated in a 3:1 ratio, confirming segregation of one gene for seedling resistance. Segregation of the Tc*2/E. Halcón F_2 and Tc/E. Halcón F_3 families fit 3:1 and 15:1 ratios, respectively, indicating the presence of two genes for seedling resistance. Tc*2/I. Tijereta F_3 families segregated in 3:1 and a 7:1 ratios and the Tc/I. Tijereta F_3 families segregated 63:1 and 255:1 ratio indicating that I. Tijereta probably carries at least three genes for resistance to race BBBD. When tested with race MCDS, BC_1F_2 and F_3 families fit a 3:1 and 15:1 ratio, indicating segregation of two genes.

Homozygous BC_1F_3 lines derived from single BC_1F_2 plants were tested as seedlings with different races to identify the seedling genes likely present in the parents. BC_1F_3 lines derived from Tc*2/E. Calandria were postulated to have *Lr3bg* based on the high ITs to races TBDK and TDTD, which are virulent to Tc*Lr3bg* (Table 4); *Lr16* based on high IT to race CGTD with virulence to Tc*Lr16*, and *Lr24* based on

Table 2 Seedling infection types[a] of six Uruguayan wheat cultivars and Thatcher near-isogenic lines to 10 *Puccinia triticina* isolates

Cultivar	BBBD 59-1	TGBG 04-485	MFPS 06-268	MJBJ 97-406	MHDS 03-237	MBBJ 59-2	KFBJ 97-64	MBRJ 99-60	THBJ 00-588	MFPN 1385-1	Postulated gene
E. Calandria	0;	;	2+3	3+	;	;	;1+	;	;	2	*Lr16,24*
E. Federal	0;	;	;1−	;22+	;1−	;12	2	;1−	22+;	;1=	
E. Halcón	;1=	;	;	2+3	;	1+	;1−	1+2	33+	;	*Lr10,16,23,14a*
I. Tijereta	0;	0;	;2+3	;22+	;	;	;	;	0;	2	*Lr16,24*
I. Torcaza	0;	;	;	3	;	;	3	;	;	;1=	*Lr10,24,+*
I. Garza	0;	;	;2	2+3	;	;	;1−	;	;	22−	*Lr16,24*
Tester lines											
Tc*Lr10*	;	3+	3+	3+	3+	3+	3+	3+	3+	0;1=	
Tc*Lr14a*	3+	;12−	3+	3+	3+	3+	3+	3+	3+	3+	
Tc*Lr16*	;1−	32+	;1+2	2+3	2+	12	;1−	1+	2+	2−	
Tc*Lr23*	;22+	33+	2+	2+	;1	32+	3+4	2+	23	2	
Tc*Lr24*	0;	;	3	3+	;1	;	4	;	;	3+	

[a] Infection types as described in Stakman et al. (1962), Long and Kolmer (1989)

Table 3 Segregation of leaf rust resistance in seedling plants of BC_1F_2, and F_3 families of E. Calandria, E. Federal, E. Halcón and I. Tijereta crossed with Thatcher

Cultivar	Isolate	Generation	Number of families		Expected ratio	Number of genes	χ^2	*p*
			Resistant/ segregating	Susceptible				
E. Calandria	BBBD 59-1	BC_1F_2	59	5	7:1	3	1.29	0.30–0.20
					15:1	4	0.07	0.90–0.70
	BBBD 59-1	F_3	112	3	63:1	3	0.28	0.70–0.50
	MCRS 19-3	BC_1F_2	40	17	3:1	2	0.71	0.50–0.30
E. Federal	BBBD 59-1	BC_1F_2	30	31	1:1	1	0.02	0.90–0.70
	BBBD 59-1	F_3	130	31	3:1	1	2.83	0.10–0.05
E. Halcón	BBBD 59-1	BC_1F_2	43	14	3:1	2	0.01	0.95–0.90
	BBBD 59-1	F_3	160	11	15:1	2	0.01	0.95–0.90
I. Tijereta	BBBD 59-1	BC_1F_3	42	8	3:1	2	2.16	0.20–0.10
					7:1	3	0.56	0.50–0.30
		F_3	137	1	63:1	3	0.22	0.70–050
					255:1	4	1.71	0.20–0.10
	MCDS 689-1	BC_1F_3	39	11	3:1	2	0.24	0.70–0.50
		F_3	131	7	15:1	2	0.33	0.70–0.50

high IT to TDTD that it is virulent to Tc*Lr24*. BC_1F_3 lines derived from Tc*2/E. Federal were postulated to have *Lr10* based on the high ITs to *Lr10* virulent races MCRS, TBDK and LCGK and other lines had seedling resistance that could not be characterized based on the low IT 2-c to race TDTD. Different BC_1F_3 lines derived from Tc*2/E. Halcón probably have *Lr10* or *Lr16* since these lines had the same pattern of high and low IT to the races as did the Tc*Lr10* and Tc*Lr16* lines, respectively. Lines from E. Halcón that were susceptible to *Lr14a*-virulent race BBBD were tested with the *Lr14a*-avirulent race TGDB and expressed the characteristic IT X produced by this gene, indicating the likely presence of *Lr14a* in E. Halcón.

BC_1F_3 lines derived from Tc*2/E. I. Tijereta were tested with a different set of races at the seedling stage (Table 5). The possible presence of *Lr16* in lines derived from I. Tijereta is in agreement with the high

Table 4 Seedling infection types of selected BC_1F_3 lines derived from E. Calandria, E. Federal and E. Halcón crossed with Thatcher and Thatcher near-isogenic lines tested with *P. triticina* isolates

Cross	BC_1F_3 line	Detected gene	BBBD 59-1	MCRS 19-3	CGTD B39	SLGD B37	TBDK B38	LCGK B25	TDTD B29	TGDB B33
Tc*2/E. Calandria	15659-4	*Lr3bg*	0;1=	2–3+;	23	;1=	3+	;	4	–
	15658-8	*Lr16*	1n	1n	33+	1–n	1n	1n	1–;	–
	15659-1	*Lr24*	0;1=	;1=	0;1=	0;	;	;	4	–
Tc*2/E. Federal	12617-1	*Lr10*	0;	3+4	;	;1=	3+	3+	0;	–
	12615-6	*Lr?*	–	–	–	–	–	–	2−c;	2–2;c[a]
Tc*2/E. Halcón	12625-3	*Lr10*	0;	3+4	0;	;1=	3+	3+	0;	–
	12621-10	*Lr16*	1−1+n[b]	1n	33+	11+	1n	1−n	1=1−;	–
	12624-1	*Lr14a*	–	–	–	–	–	–	3	X
Tester lines										
RL 6042		*Lr3bg*	;	32;	32;	;1=	3+	0;	4	–
RL 6004		*Lr10*	;1=	3+	;1=1−	1−;	3+	3+	0;	–
RL 6013		*Lr14a*	33+	3+	33+	3+	3+	4	3	X−
RL 6005		*Lr16*	1−n	1n	3−3+	1−n	1n	1n	1−n	–
RL 6064		*Lr24*	0;	0;	0;	0;	0;	0;	4	–

[a] More chlorosis than normal for IT

[b] More necrosis than normal for IT

IT to MHRK, that is virulent to Tc*Lr16*, and low IT to the *Lr16* avirulent races, except for race CCTK, which produced 3+ IT on the line likely to have *Lr16* and an intermediate IT 22+ on Tc*Lr16*. This may be explained by variability in expression of *Lr16* with temperature (Dyck and Johnson 1983), as already mentioned for E. Halcón. The other BC_1F_3 lines derived from I. Tijereta likely have *Lr24* since their IT pattern resembled Tc*Lr24*. Additional seedling resistance of IT; to race MCPS that could not be assigned to any characterized gene was present in other BC_1F_3 lines derived from I. Tijereta. All 578 F_2 plants from I. Tijereta/RL6005 (Tc*Lr16*) were resistant to isolate SPGK that is virulent to *Lr24* and avirulent to *Lr16*, confirming the presence of *Lr16* in I. Tijereta.

In field plots under natural leaf rust infection, the Tc*2/E. Calandria and Tc*2/I. Tijereta F_2 families segregated for field leaf rust response in a 7 segregating: 1 susceptible ratio, fitting an expected segregation ratio for three genes (Table 6). Segregation of the Tc*2/E. Halcón and Tc*2/I. Federal F_2 families fit 1:1 ratios, indicating segregation of single resistance genes. The Tc*2/E. Halcón F_2 families that were susceptible to race BBBD in the seedling stage were susceptible in the field, whereas the Tc*2/I. Federal F_2 families that were seedling susceptible segregated for field resistance.

E. Calandria, and E. Halcón were crossed with Tc*Lr13* and Tc*Lr34* and E. Federal and I. Tijereta were crossed with Tc*Lr34* to test for segregation of these APR genes under field conditions. Plants or derived families or lines with similar field reactions to Thatcher were considered susceptible. The field reaction of Thatcher was 80–90S during 1993–1995 and 2002. Tc*Lr13* was 2MR to 20MS during 1993–1995, and was susceptible in 2002 and the following years. Tc*Lr34* was 5M to 60MSS during 1983–1995 and 40MSS to 60MSS during 2002. F_2 plants from crosses of Tc*Lr13* and Tc*Lr34* with E. Halcón segregated for leaf rust response (Table 7), indicating that this cultivar has neither *Lr13* nor *Lr34*. E. Federal/Tc*Lr34* also segregated and therefore E. Federal does not carry *Lr34*. There were susceptible plants within a few segregating F_4 lines from Tc*Lr13*/E. Calandria (maximum 90S field reaction) and Tc*Lr34*/E. Calandria (maximum 80S field reaction), thus E. Calandria does not carry *Lr13* or *Lr34*. The absence of *Lr34* in E. Halcón, E. Federal and E. Calandria was further confirmed by absence of the marker allele csLV34 associated with the presence of

Table 5 Seedling infection types of selected BC_1F_3 lines derived from crosses of E. Tijereta and Thatcher and Thatcher near-isogenic lines tested with *P. triticina* isolates

	Lr gene	MCPS 550-1	TDDK 481-1	KDGK 611-1	MFRF 712-1	MCDS 689-1	CCTK 345-2	MHRK 674-1
BC_1F_3 lines								
3217	*Lr16*	1	1−;	1−2=	11−;	2	3+	33+
3151	*Lr24*	0;	23;	3+	3+	0;	0;	0;
3200	*Lr?*	;	3+	3+	3+	3+	3+	3+
Tester lines								
RL 6005	*Lr16*	12	1	1−	1−	12	22+	3+
RL 6064	*Lr24*	0;	4	3+	4	0;	;	0;

Table 6 Year of testing and segregation for leaf rust response in field plots of Tc*2/E. Calandria, Tc*2/E. Federal, Tc*2/E. Halcón and Tc*2/I. Tijereta F_2 families

Resistant parent	Years	Number of families		Expected ratio	Number of genes	χ^2	*p*
		Segregating	Susceptible				
E. Calandria	1994	55	9	7:1	3	0.14	0.9–0.7
E. Federal	1994	27	34	1:1	1	0.80	0.5–0.03
E. Halcón	1994	30	25	1:1	1	0.45	0.7–0.5
I. Tijereta	2001	76	12	7:1	3	0.10	0.9–0.7

Table 7 Segregation for leaf rust response in field plots of E. Calandria, E. Federal, E. Halcón and I. Tijereta crossed with Thatcher lines with *Lr13*and *Lr34*

Cross	No. of F_2 plants	F_3 families		F_4 lines	
		No.	Response	No.	Response
Tc*Lr13*/E. Calandria	269	5	5–60MS	3	10R–90S
Tc*Lr34*/E. Calandria	346	11	50–70MS	4	TR–80S
Tc*Lr34*/E. Federal	143	24	90S		
Tc*Lr13*/E. Halcón	544	10	90S		
Tc*Lr34*/E. Halcón	548	68	90S		
Tc*Lr34*/I. Tijereta	690	24	0–60	4	20–40MSS
Tester lines			1993–1995		2002–2004
Thatcher			80–90S		80–90S
Tc*Lr13*			2MR–20MS		80–90S
Tc*Lr34*			5M–60MSS		40–60MSS

Lr34. All F_2 plants and derived F_3 families from Tc*Lr34*/E. Tijereta were resistant, indicating the presence of *Lr34* in this cultivar. The presence of *Lr34* in I. Tijereta was confirmed by the csLV34 marker allele associated with *Lr34*.

The cultivars that were not genetically analyzed were also tested with the molecular marker for *Lr34*. I. Garza had the csLV34 marker allele associated with the presence *Lr34* whereas I. Torcaza did not.

Discussion

Race specific seedling resistance genes *Lr3bg*, *Lr10*, *Lr14a*, *Lr16*, *Lr23* and *Lr24* were identified in late maturing Uruguayan wheat cultivars. During 1994 and 1995 the Tc*Lr16* line had a resistant field response that varied from 20 to 60MR in field plots, but this line had intermediate to high field responses of 60MSS to 80MSS from 2001 to 2007. Isolates virulent to this

gene were not detected until 1997 and only a few races at low frequencies with virulence to *Lr16* have since been identified. Some current isolates produce an intermediate IT on the Tc*Lr16* line at the seedling stage, although the expression of intermediate or high IT on this line with some isolates varies with temperature. The Tc*Lr24* line expressed effective resistance during 1994 and 1995 when the segregating BC_1F_2 families from most crosses were evaluated, however this line has been susceptible since 2001 due to an increase in frequency of races with virulence to *Lr24*. The other genes (*Lr3bg*, *Lr10*, *Lr14a*, *Lr24*) found in the Uruguayan cultivars have not been effective against the *P. triticina* population for the last 20 years. In Uruguay the expression of the race non-specific APR gene *Lr34* is variable with terminal disease responses ranging from 40M to 60MSS.

E. Calandria was postulated to have *Lr16* and *Lr24* based on tests with the cultivar and the derived BC_1F_3 lines, and *Lr3bg* was postulated to be in some Tc*2/E. Calandria F_3 lines, which is in agreement with a three seedling gene ratio of BC_1F_2 families to race BBBD (avirulent to *Lr3bg*, *Lr16* and *Lr24*) and a two gene ratio to race MCRS (virulent to *Lr3bg*, avirulent to *Lr16* and *Lr24*). E. Calandria probably inherited *Lr3bg* from E. Tarariras (Germán and Kolmer 2012). The field-effective resistance of E. Calandria was conditioned by *Lr16*, *Lr24* and a third gene that conferred APR. Segregation for field response in two BC_1F_2 families from E. Calandria with only *Lr3bg* further supported the presence of one APR gene in E. Calandria. This APR gene is different from *Lr13* since families derived from Tc*Lr13*/E. Calandria segregated for susceptibility. E. Calandria did not appear to carry *Lr34*, since F_3 and F_4 families derived from Tc*Lr34*/E. Calandria segregated for susceptibility. Furthermore E. Calandria did not have the marker allele associated to *Lr34*. csLV34 is a robust marker considered highly diagnostic for *Lr34*, (Singh et al. 2007; Kolmer et al. 2008b). Although there were a few disagreements between lines thought to have or lack *Lr34* and the marker data (Kolmer et al. 2008b), csLV34 has been a very reliable marker for *Lr34* in other South American wheat germplasm. In greenhouse progeny tests of selected field resistant and seedling susceptible BC_1F_2 families derived from E. Calandria, adult BC_1F_2 plants had a similar phenotype to Tc*Lr34* with relatively low severity, large uredinia at the leaf base and smaller uredinia at the leaf tip, indicating that E. Calandria carries an APR gene with similar phenotypic expression to *Lr34*.

Lr10, postulated to be present in a group of Tc*2/E. Federal F_3 lines, is probably the single seedling resistance gene segregating to race BBBD in Tc*2/E. Federal F_2 families. E. Federal may have inherited *Lr10* from Lee (Anderson 1961), through ND 84. Additional unidentified seedling resistance was detected by other races in seedling tests of the BC_1F_3 lines. The E. Federal-derived BC_1F_2 segregated for one gene for field resistance that must be an APR gene since *Lr10* and the uncharacterized seedling resistance did not condition effective field resistance. According to the results of allelism tests and the molecular marker for *Lr34* this APR gene is different from *Lr13* and *Lr34*.

E. Halcón was postulated to have *Lr10*, *Lr14a* and *Lr16* based on tests of the cultivar and derived BC_1F_3 lines, which is in agreement with a two seedling gene ratio of BC_1F_2 families to race BBBD (avirulent to *Lr10 and Lr16* and virulent to *Lr14a*). *Lr23* was postulated in E. Halcón based on tests of the cultivar, however the presence of this gene could not be confirmed in backcross lines even when several lines were tested, indicating that it does not have *Lr23*. BC_1F_2 families derived from E. Halcón segregated for one gene in the field plots; this was likely *Lr16* since this gene expressed effective field resistance. E. Halcón does not have any APR genes since all Tc*2/E. Halcón F_2 families that were seedling susceptible to race BBBD were also susceptible in the field. Segregation for susceptibility in Tc*Lr13*/E. Haclón and Tc*Lr34*/E. Halcón F_2 families further confirmed the absence of *Lr13* and *Lr34* in the cultivar.

The presence of *Lr16* in I. Tijereta was confirmed in the allelism test, and *Lr24* was postulated to be in the cultivar and in BC_1F_3 lines. I. Tijereta likely inherited *Lr16* from E. Calandria. An additional seedling resistance gene that expressed low IT to race BBBD, and that was ineffective to race MCDS, was present in some of the BC_1F_3 families but could not be identified. The presence of *Lr34* in I. Tijereta was determined in the allelism test and confirmed by the marker allele associated with *Lr34*. The third gene in addition to *Lr16* and *Lr34* that conferred field resistance could not be identified.

Comparison of IT of I. Torcaza with the Thatcher near-isogenic lines indicated that this cultivar likely has *Lr10*, *Lr24* and additional seedling resistance, as

determined by Vanzetti et al. (2011). The test with the csLV34 marker suggested that it does not carry *Lr34*. Similarly, based on gene postulation and marker data, I. Garza likely has *Lr16*, *Lr24* and *Lr34*. I. Torcaza may have inherited *Lr24* from Cargil Trigal 800 (Antonelli 2003) and *Lr10* from E. Federal whereas the origin of the leaf rust resistance in I. Garza is most likely I. Tijereta.

Cultivars E. Federal, I. Tijereta and I. Torcaza have seedling resistance that appeared to be conferred by uncharacterized genes, which appear to be common in Uruguayan and Argentinean cultivars. Undesignated seedling resistance genes were present in the Argentinean cultivar Barletta 7D and the Uruguayan cultivars Americano 44d (Antonelli 1994) and Americano 25e (Kolmer et al. 2007). Vanzetti et al. (2011) also found uncharacterized seedling resistances in several recent Argentinean cultivars. However the uncharacterized genes detected in this study did not confer field resistance and thus have limited value for resistance breeding.

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). Frontana, with *Lr34* (Dyck and Samborski 1982) that was probably derived from Mentana (Kolmer et al. 2008b), was also an early source of *Lr34*. Frontana is present in the pedigrees of many wheat cultivars from the Southern Cone region (Germán et al. 2007, 2009) and in a significant proportion of CIMMYT germplasm (Singh 1993; Singh and Huerta-Espino 1995; Kolmer et al. 2008b). *Lr34* was postulated in recent Argentinean cultivars (Vanzetti et al. 2011).

The APR present in E. Federal and probably present in E. Calandria could not be identified and additional genetic studies are required to further characterize these genes. This resistance may be conferred by the presence of a designated gene(s) conferring slow rusting or by an uncharacterized APR gene(s) which could be a potential new source of leaf rust resistance. The slow rusting gene *Lr46* (Singh et al. 1998) is present in many CIMMYT lines (William et al. 2003) while Americano 25e has APR on chromosome 1BL, in the same region as *Lr46* (J. A. Kolmer, unpublished data). *Lr67* (Hiebert et al. 2010) is present in the line RL6077, but the frequency of this gene in modern wheat germplasm is not known. *Lr68* (Herrera-Foessel et al. 2012) was detected in the CIMMYT line Parula, and was likely inherited from Frontana, thus it is probably widespread in CIMMYT wheats. Since Americano 25e and Frontana as well as CIMMYT lines were used as parents in wheat breeding programs in the Southern Cone (Luizzi et al. 1983), it is possible that APR on 1BL, *Lr46* and *Lr68* are widespread in this germplasm, in addition to *Lr34*. Uncharacterized APR genes have been found in the Brazilian cultivars Frontana (Singh and Rajaram 1992), Toropí (Barcellos et al. 2000), BR35 (Brammer et al. 2004), and in Argentinean cultivar Buck Manantial (Altieri et al. 2008). APR genes conferring durable resistance different from *Lr34* and *Lr46* are widely distributed in CIMMYT germplasm (Singh and Rajaram 1992; Singh and Huerta-Espino 1995; Singh et al. 2011).

The six cultivars had high to intermediate levels of resistance after initial release but the leaf rust severities and responses of E. Federal, I. Tijereta and I. Torcaza increased after a few years of commercial cultivation (Table 1) due to the appearance and increased frequencies of virulent races. With the exception of E. Calandria and E. Halcón that were grown commercially for a short period, all other cultivars suffered erosion of resistance over time, but retained levels of partial resistance. After races with virulence to the seedling resistance genes in E. Federal and I. Torcaza became prevalent, these cultivars had maximum leaf rust ratings of 70MSS and 80MSS, respectively. Rust ratings of I. Tijereta also increased over the years to reach 60MSS, whereas I. Garza reached a maximum of 30MRMS. The different levels of residual partial resistance present in the Uruguayan cultivars, with the exception of E. Halcón, are partly explained by APR genes which appear to be common in Uruguayan cultivars.

In summary, late maturing Uruguayan cultivars were found to have combinations of race specific seedling resistance and APR genes. *Lr16* is currently the only effective seedling resistance gene and seems to have better durability than other genes expressed at the seedling stage. *Lr16* was shown to interact with *Lr34* to produce higher levels of resistance to isolates avirulent to *Lr16* (Germán and Kolmer 1992), and with other uncharacterized APR genes (Singh and Huerta-Espino 1995). Cultivars with only *Lr34* or other single APR genes can still have high leaf rust severities and likely suffer significant yield losses, therefore, *Lr34*, the possibly different APR genes found in Uruguayan cultivars and additional different sources of APR should be combined in order to

develop wheat cultivars with long lasting and effective leaf rust resistance.

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