Association between Gene *Lr34* for Leaf Rust Resistance and Leaf Tip Necrosis in Wheat

Ravi P. Singh*

ABSTRACT

Durable resistance to leaf rust (*Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici*) in bread wheat (*Triticum aestivum* L.) cultivars is known to result from the interaction of *Lr34* with other minor additive genes that are effective in the adult growth state. The *Lr34* gene seems to be present in several CIMMYT germplasm–derived Mexican cultivars that display temperature- and light-sensitive seedling responses similar to those displayed by near-isogenic 'Thatcher' lines that contain *Lr34*. Adult plants of these two Thatcher lines and all Mexican cultivars postulated to carry *Lr34* display resistance to leaf rust and exhibit leaf tip necrosis symptoms. This study was conducted to determine whether *Lr34* and a gene or genes for leaf tip necrosis are linked. The simultaneous presence of *Lr34* and leaf tip necrosis in the two Thatcher near-isogenic lines and Mexican cultivars was confirmed by evaluating F_2 plants or F_3 lines obtained from various intercrosses. *Lr34* and leaf tip necrosis were inherited together in a population of 117 F_3 lines obtained from the cross of leaf rust resistant 'Jupateco 73R' (carrying *Lr34* and leaf tip necrosis) with its susceptible counterpart 'Jupateco 73S'. Linkage or pleiotropism was also evident in the crosses of six *Lr34*-carrying Mexican cultivars with 'Siete Cerros 66' (which does not have *Lr34*). The gene for leaf tip necrosis, designated *Ltn*, could be used as a marker for *Lr34*.

LEAF RUST is one of the most common wheat diseases worldwide. Several genes for resistance have been used to reduce its effects. Roelfs (1988) suggested that *Lr12* or *Lr13* in combination with *Lr34* could have given durable resistances to wheat cultivars, such as Frontana and Era. The adult plant resistances of 'Chinese Spring' and 'Sturdy' were found to be due to the interaction of *Lr12* and *Lr34* (Dyck, 1991). Although *Lr34* can be detected in the seedling stage at low temperatures and low light intensity, its effects can be more easily detected at the adult stage (Dyck and Samborski, 1982; Dyck, 1987). Variable influences of environment and location on the expression of adult plant resistance conferred by *Lr34* have also been observed in Mexico (Singh and Gupta, 1992), and often complicate its detection. Dyck (1987) located *Lr34* in chromosome 7D of wheat. Dyck and Samborski (1982) developed near-isogenic lines of Thatcher, namely, RL6058 (Thatcher *6/PI 58548) and Line 920 (Thatcher/Lageadinho) carrying *Lr34*. Adult plants of these lines display leaf tip necrosis in Mexico. The symptoms include 2 to 3 cm of necrosis at the ends of the leaves, which extend an additional 2 to 4 cm on the edges of the leaf (Fig. 1). Other derivatives of Thatcher with a range of documented genes for leaf rust resistance do not show these symptoms, indicating that *Lr34* may be genetically linked with a gene causing leaf tip necrosis. Dyck (1991) also mentioned the possible association of *Lr34* with leaf tip necrosis. Frontana, several Mexican bread wheat cultivars, and numerous breeding lines from the CIMMYT bread wheat improvement program display leaf tip necrosis.

International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Apdo Postal 6-641, 06600, Mexico, D.F., Mexico.
Recieved 30 Sept. 1991. *Corresponding author.

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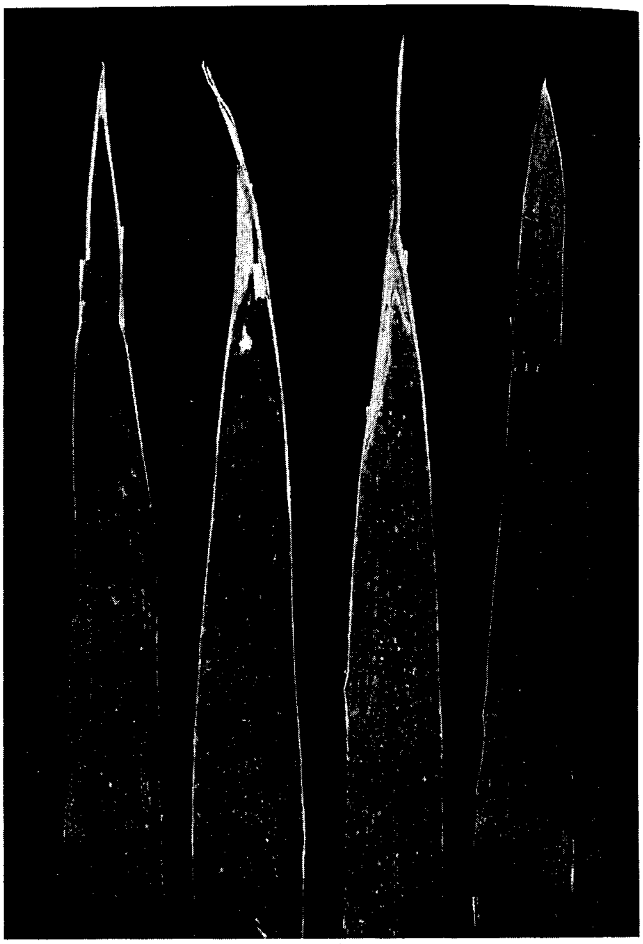

Fig. 1. Flag leaves of wheat plants showing a range of leaf tip necrosis symptoms (A, B, and C) as compared with a leaf without this symptom (D).

From a study of seedling and adult plant responses to leaf rust, Singh and Rajaram (1991) predicted that *Lr34* could frequently occur in CIMMYT-derived bread wheats. This paper describes results from experiments designed to support this postulation and to demonstrate the strong genetic association or pleiotropism that exists between *Lr34* and leaf tip necrosis.

MATERIALS AND METHODS

The wheat cultivars and tester genotypes included in this study are listed in Table 1. Cultivar pedigrees are described in Villareal and Rajaram (1988). The tester lines were near-isogenic lines of Thatcher (developed by P.L. Dyck, Agriculture Canada, Winnipeg) with known *Lr* genes and

Abbreviations: IT, infection type; 40MSS, 40% severity with moderately susceptible to susceptible infection types; T, trace; 10MSS, 10% severity with moderately susceptible to susceptible infection types.

Table 1. Seedling infection types (ITs) displayed by 28 wheat cultivars and eight tester genotypes when inoculated with two pathotypes TBD/TM and TCB/TD of *Puccinia recondita* f. sp. *tritici* at two temperature regimes; field responses with a mixture of the same two pathotypes for two seasons at two locations in Mexico; presence (+) and absence (−) of leaf tip necrosis, and probable *Lr* genes.

		Pathotype and seedling infection types†				Location and field responses‡					
		TBD/TM		TCB/TD		Ciudad Obregón		El Batán			
No.	Wheat	14 to 17 °C	23 to 28 °C	14 to 17 °C	23 to 28 °C	1988-1989	1989-1990	1988	1989	Leaf tip necrosis	*Lr* genes§
1	Thatcher	3+	3+	3+	3+	100S	100S	100S	100S	−	
2	RL6058¶	;3=	3	;3=	3	5MS	5MS	60MSS	30MSS	+	*34*
3	Line 920¶	;3=	3	;3=	3	5MS	5MS	60MSS	30MSS	+	*34*
4	Frontana	;3=	3	;3=	3	TMS	TMS	10MSS	T-5MSS	+	*13,34*
5	Yaqui 50	;3=	3	;3=	3	10MSS	10MSS	60MSS	60MSS	+	*14a,34*
6	Penjamo 62	;3=	3	;3=	3	10MSS	15MSS	60S	60S	+	*14a,34*
7	Sonora 64	3+	3+	3+	3+	100S	100S	100S	100S	−	*1*
8	Inia 66	3+	3+	;	;	100S	100S	100S	100S	−	*13,17*
9	Siete Cerros 66	3C	3+	3C3	3+	60S	60S	80MSS	60S	−	
10	Tobari 66	;3=	3	;3=	3	5MSS	10MSS	20MSS	20MSS	+	*1,13,34*
11	Yecora 70	3+	3+	3+	3+	100S	100S	100S	100S	−	*1,13*
12	Torim 73	;3−	3	;	;	10MSS	10MSS	30MSS	20MSS	+	*1,13,17,34*
13	Jupateco 73R	;3−	3	;	;	15MSS	10MSS	30MSS	20MSS	+	*17,27+31,34*
14	Jupateco 73S	3+	3+	;	;	100S	100S	100S	100S	−	*17,27+31*
15	Anahuac 75	3+	3+	;	;	100S	100S	100S	100S	−	*13,17,27+31*
16	Cocoraque 75	;3=	3	;	;	15MSS	10MSS	30MSS	15MSS	+	*13,17,27+31,34*
17	Nacozari 76	;3=	3	;	;	15MSS	10MSS	30MSS	20MSS	+	*10,34*
18	Sonoita 81	;3=	3	;3=	3	10MS	10MSS	30MSS	20MSS	+	*1,13,34*
19	Tonichi 81	;3=	3	;	X−	5MS	5MSS	5MS	5MS	+	*1,13,27+31,34*
20	Cucurpe 86	;3=	3	;	;	TMS	TS	5MS	TMR	+	*10,34*
21	Esmeralda 86	;3=	3	;	;	TMR	TMR	5MR	TMR	+	*10,14a,34*
22	Ocoroni 86	;3=	3	;	X−	TMS	TS	5MS	TMS	+	*27+31,34*
23	Bacanora 88	;	;	;3=	3	5MS	5MS-S	30MSS	5MS	+	*26,34*
24	Cumpas 88	;	;	;3=	3	TMS	TMS	15MSS	5MS	+	*13,26,34*
25	Rayon 89	;3=	3	;3=	3	5MS	5MSS	30MSS	15MSS	+	*13,34*
26	Mango	;	;	;3=	3	T-5MSS	TMS	10MSS	5MSS	+	*1,13,26,34*
27	Parula	;3=	3	;3=	3	TMSS	TMS	5MSS	TMSS	+	*13,34*
28	Trap	;3=	3	;	;	T-5MSS	TMS	10MSS	T-5MSS	+	*1,3,10,13,34*
						Tester genotypes					
1	RL6003	3+	3+	3+	3+	100S	100S	100S	100S	−	*1*
2	RL6002	3+	3+	3+	3+	100S	100S	100S	100S	−	*3*
3	RL6004	3+	3+	;	;	100S	100S	100S	100S	−	*10*
4	Manitou	3+	3+	3+	3+	100S	100S	100S	100S	−	*13*
5	RL6013	3+	3+	3+	3+	100S	100S	100S	100S	−	*14a*
6	RL6008	3+	3+	;	;	100S	100S	100S	100S	−	*17*
7	RL6078	;	;	3+	3+	100S	100S	100S	100S	−	*26*
8	Gatcher	3+	3+	;	;	100S	100S	100S	100S	−	*10,27+31*

† The seedling ITs reported are ; = no uredia, but small chlorotic flecks present; 3 = medium-sized uredia that may be associated with slight chlorosis; X = random distribution of variable-sized uredia on single-leaf with a pure culture; C = more chlorosis than normal for the IT; − = uredia somewhat smaller than normal for the IT; = = uredia at the lower size limit for the infection type; and + = uredia somewhat larger than normal for the IT.

‡ Field responses are based on modified Cobb Scale (Peterson et al., 1948) and include two components: disease severity and infection type; e.g., T = trace severity; 5 = 5% severity; etc.; MR = moderately resistant IT; MS = moderately susceptible IT; MSS = moderately susceptible to susceptible IT; and S = susceptible IT.

§ *Lr* genes (other than *Lr34*) reported here are based on Singh and Rajaram (1991) and Singh (1991, unpublished data).

¶ Also are tester genotypes for *Lr34*.

'Gatcher', which carries complementary genes *Lr27* + *Lr31* (Singh and McIntosh, 1984) and *Lr10* (Singh and Rajaram, 1991).

The two pathotypes of *P. recondita* f. sp. *tritici* were TBD/TM and TCB/TD, which are the most prevalent pathotypes in Mexico (Singh, 1991). The avirulence–virulence formula is: TBD/TM *Lr3Ka, 9, 11, 16, 19, 21, 23, 24, 25, 26, 29, 30, 32, 33/1, 2a, 2b, 2c, 3, 3bg, 10, 13, 14a, 14b, 15, 17, 18, 20, 27 + 31, 28;* and TCB/TD *Lr3ka, 9, 10, 11, 16, 17, 19, 21, 24, 25, 27 + 31, 29, 30, 32, 33/1, 2a, 2b, 2c, 3, 3bg, 13, 14a, 14b, 15, 18, 20, 23, 26, 28* (Singh, 1991).

Crosses between cultivars were based on individual plant selections. Each F_3 line was derived from an individual F_2 plant. The F_2– derived F_5 and F_6 lines used in this study were obtained following a pedigree method; a random plant in each generation from each line was harvested for advancing to the next generation. The F_5 or F_6 lines displaying low adult plant leaf rust responses were classified for leaf tip necrosis and seedling infection types for postulating the presence of *Lr34*.

Singh and Rajaram (1991) described the seedling inoculation and incubation methods. Two greenhouse temperature ranges were used for incubation, 13 to 17 °C and 23 to 28 °C. Infection types were recorded after 9 to 12 d using a scale of 0 to 4 similar to that described by Stakman et al. (1962).

Field evaluations for leaf rust were carried out in northwestern Mexico at Ciudad Obregón, Sonora, and at El Batán, near Mexico City. Leaf rust epidemics were initiated by inoculating spreader rows, which consisted of a mixture of susceptible cultivars, with a mixture of the two patho-

Table 2. Classification of F_2 or F_3 lines of wheat for leaf tip necrosis and leaf rust response from 46 intercrosses of the cultivars postulated to carry *Lr34*.

Cross	F_2 plants		F_3 lines	
	No. with tip necrosis†	Leaf rust severity range‡	No. with tip necrosis†	Leaf rust severity range‡
RL6058/Frontana			184	T–30
RL6058/Mango			124	T–30
RL6058/Parula			145	T–30
RL6058/Trap			198	T–40
RL6058/Esmeralda 86	146	T–30		
Line 20/Jupateco 73R§	305	T–40		
Line 20/Tobari 66§	248	T–40		
Line 20/Tonichi 81§	278	T–40		
Line 20/Bacanora 88§	304	T–40		
Frontana/Mango			83	T–5
Frontana/Parula			82	T–10
Frontana/Trap			85	T–10
Frontana/Yaqui 50	139	T–15		
Frontana/Penjamo 62			100	T–10
Frontana/Tobari 66			87	T–15
Frontana/Torim 73	170	T–15		
Frontana/Nacozari 76			100	T–15
Frontana/Sonoita 81			101	T–15
Frontana/Tonichi 81			55	T–15
Frontana/Cucurpe 86			71	T–10
Frontana/Esmeralda 86	178	T–15		
Frontana/Ocoroni 86	141	T–10		
Frontana/Bacanora 88			83	T–30
Frontana/Cumpas 88			76	T–10
Frontana/Rayon 89	170	T–10		
Mango/Parula			82	T–10
Mango/Trap			83	T–10
Mango/Penjamo 62			101	T–15
Mango/Tobari 66			100	T–10
Mango/Tonichi 81			65	T–30
Mango/Cucurpe 86			40	T–30
Mango/Bacanora 88			80	T–30
Parula/Trap			83	T–10
Parula/Rayon 89	164	T–10		
Trap/Yaqui 50	142	T–15		
Trap/Tobari 66			100	T–15
Trap/Torim 73	163	T–10		
Trap/Sonoita 81			100	T–15
Trap/Tonichi 81			46	T–15
Trap/Cucurpe 86			71	T–15
Trap/Esmeralda 86	186	T–5		
Trap/Ocoroni 86	204	T–5		
Trap/Bacanora 88			146	T–30
Jupateco 73R/Cocoraque 75	224	T–15		
Bacanora 88/Cucurpe 86			81	T–30
Bacanora 88/Cumpas 86			74	T–40

† F_2 plants or F_3 lines lacking leaf tip necrosis were not present.
‡ Severities are based on a modified Cobb Scale (Peterson et al., 1948), where T = trace severity, 5, 10, 15, 30, and 40 = % severity.
§ Data for these crosses were from El Batán. All other crosses were evaluated at Ciudad Obregón.

types. Rust severity and response data were based on the Modified Cobb Scale (Peterson et al., 1948).

RESULTS AND DISCUSSION

Table 1 includes seedling ITs displayed by the cultivars and eight tester genotypes, inoculated with pathotypes TBD/TM and TCB/TD and incubated at 14 to 17 °C and 23 to 28 °C. RL6058 and Line 920, which were control genotypes for *Lr34*, displayed a moderately low IT(;3=)at 14 to 17 °C with both pathotypes, whereas a moderately high IT(3) was observed at 23 to 28 °C. Thatcher displayed a high IT(3+) with both pathotypes at both temperatures. The presence of *Lr34* was postulated in Frontana and six other CIMMYT-derived cultivars (namely, Yaqui 50, Penjamo 62, Tobari 66, Sonoita 81, Rayon 89, and Parula), since they displayed similar ITs to the *Lr34* testers. Cultivars Torim 73, Jupateco 73R, Cocoraque 75, Nacozari 76, Tonichi 81, Cucurpe 86, Esmeralda 86, Ocoroni 86, and Trap displayed very low (IT ;) or menothetic (IT X-) responses with pathotype TCB/TD due to the presence of *Lr10*, *Lr17*, or *Lr27*+*Lr31*. However, with pathotype TBD/TM, which is virulent for these and other known *Lr* genes in these cultivars (Singh and Rajaram, 1991), ITs similar to those displayed by the *Lr34*-carrying controls indicated the probable presence of this gene. Cultivars Bacanora 88, Cumpas 88, and Mango displayed IT; with pathotype TBD/TM

Table 3. Classification of a group of F_5/F_6 lines (derived from the crosses of six *Lr34* carrying wheats with Siete Cerros 66) displaying T-10MS adult plant leaf rust reaction for leaf tip necrosis (+ indicates presence), seedling infection types (ITs) displayed at 14 to 17 °C and 23 to 28 °C, and postulation for *Lr34* (+ indicates presence).

Cross	No. of lines	Leaf tip necrosis	Seedling ITs† 14 to 17 °C	Seedling ITs† 23 to 28 °C	*Lr34*
Siete Cerros 66/Parula	19	+	;3=	3	+
Siete Cerros 66/Mango	9	+	;3=	3	+
Siete Cerros 66/Bacanora 88	14	+	;3=	3	+
Siete Cerros 66/Trap	12	+	;3=	3	+
Siete Cerros 66/Tonichi 81	7	+	;3=	3	+
Siete Cerros 66/Cucurpe 86	8	+	;3=	3	+

† Crosses involving Parula, Mango, and Bacanora 88 were tested with *Puccinia recondita* f. sp. *tritici* pathotype TCB/TD, whereas crosses with Trap, Tonichi 81, and Cucurpe 86 with TBD/TM. IT definitions are in Table 1.

due to the presence of *Lr26*. However, the responses with pathotype TCB/TD supported the postulation of *Lr34* in these three cultivars. The *Lr34* gene was considered to be lacking in cultivars Sonora 64, Inia 66, Siete Cerros 66, Yecora 70, Jupateco 73S, and Anahuac 75. Seedling tests were repeated during different months because light intensity was known to influence the expression of resistance associated with *Lr34* (Dyck and Samborski, 1982). The ITs reported are from the test where the clearest and most accurate differences were observed.

Jupateco 73R and Jupateco 73S are partially resistant and susceptible selections, respectively, from the cultivar Jupateco 73. Both genotypes carry *Lr17* and *Lr27*+*Lr31*. Hence, they could be considered as a near-isogenic pair for *Lr34*. Similarly, Anahauac 75 and Cocoraque 75, which are also sister selections of Jupateco 73, appear to differ with respect to *Lr34*. These sister selections carry *Lr13, Lr17,* and *Lr27*+*31* and Cocoraque 75 appears to carry *Lr34*.

All cultivars postulated to carry *Lr34* in seedling tests displayed leaf tip necrosis as adult plants (Table 1). These include RL6058, Line 920, Jupateco 73R, and Cocoraque 75, whereas the Thatcher, Jupateco 73S, and Anahuac 75 near-isogenic counterparts did not show leaf tip necrosis. Moreover, leaf tip necrosis was not present in established control lines carrying well known genes for leaf rust resistance (data for some genotypes included in Table 1). These results clearly indicate an association between *Lr34* and leaf tip necrosis.

Table 1 includes the adult plant leaf rust responses of the cultivars and tester genotypes for two seasons at both Ciudad Obregón and El Batán. Genotypes with *Lr34* displayed less disease compared with those without *Lr34*. The *Lr34* gene was apparently more effective at Ciudad Obregón than at El Batán, and at the same location, cultivars with *Lr34* showed variable responses. Further studies (Singh and Rajaram, 1992; Singh, 1991, unpublished data) have indicated that the adult plant responses of these CIMMYT-derived cultivars are based on the additive interaction of *Lr34* with from one to three additional genes with minor effects. The responses of the tester genotypes RL6058 and Line 920 with *Lr34* at Ciudad Obregón are perhaps misleading, because heading in these daylength-sensitive genotypes was delayed by up to 8 wk compared with the daylength-insensitive CIMMYT wheat. By that time, CIMMYT wheat are close to physiological maturity. At El Batán, wheat is planted during the summer when the days are long. The difference in heading between Thatcher and its derivatives and the CIMMYT wheat is only 2 wk. A response of up to 40MSS has been observed at Ciudad Obregón in early segregates from crosses of RL6058 and daylength-insensitive wheat with *Lr34* (Table 2).

The F_2 plants or F_3 lines derived from the various intercrosses of wheat containing *Lr34* were evaluated for leaf tip necrosis and adult plant leaf rust response (Table 2). Crosses involving RL6058 or Line 920 with Frontana and eight CIMMYT-derived wheat resulted in homozygous F_2 populations or F_3 lines for leaf tip necrosis, indicating that the same gene caused leaf tip necrosis in all wheats. The highest leaf rust response of some F_2 plants and individual plants within F_3 lines was 40MSS, which is considered within the range expected for *Lr34* when present alone. Similarly, F_2 or F_3 progenies from the crosses of Frontana with 16 CIMMYT-derived wheat were homozygous for leaf tip necrosis, and the leaf rust responses were again within the range expected for genotypes with *Lr34* (Table 2). The results from 21 intercrosses involving CIMMYT wheat also indicate the presence of *Lr34* and the gene causing leaf tip necrosis.

A total of 117 F_3 lines from the cross of Jupateco 73R (with *Lr34* and leaf tip necrosis) and Jupateco 73S (without *Lr34* and leaf tip necrosis) were classified for the segregation of adult plant leaf rust response and leaf tip necrosis. All 29 F_3 lines classified to be homozygous resistant were also homozygous for leaf tip necrosis. Sixty-five lines segregating for leaf rust also segregated for leaf tip necrosis. Moreover, the 23 homozygous susceptible lines lacked leaf tip necrosis. The distribution of F_3 lines for the two traits conformed to that expected for segregation at one locus ($\chi^2 1{:}2{:}1 = 2.06, P < 0.25$). The maximum possible recombination frequency (r) that could have remained undetected in a population of 117 F_3 lines at the 0.05 level of probability can be calculated (Hanson, 1959) from the expression $(1 - r)^2 = (0.05)^{1/117}$ where $r = 0.013$, or 1.3%.

Sixty-nine F_5 or F_6 lines, displaying an adult plant leaf rust response of T to 10MSS and derived from the crosses of Siete Cerros 66 with six wheats carrying *Lr34*, were classified for leaf tip necrosis and seedling ITs at 14 to 17 °C and 23 to 28 °C (Table 3). All lines were homozygous for the gene governing leaf tip necrosis and the seedling response presumed to be conferred by *Lr34*, again indicating that the two genes were either very closely linked or pleiotropic control was involved.

Even though the leaf tip necrosis may not be an attractive trait, wheat developed in Mexico with very high yield potential carry the leaf tip necrosis gene. It is not known, however, if yield reduction is associated with leaf tip necrosis. Since the detection of *Lr34* in seedling tests is quite difficult (Dyck and Samborski, 1982), leaf tip necrosis in adult plants could be used as a marker for its detection. Maintaining this trait in