

Expression of Wheat Leaf Rust Resistance Gene *Lr34* in Seedlings and Adult Plants

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ABSTRACT

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The expression of wheat leaf rust resistance genes *Lr34*, *LrT3*, and *Lr12* was studied in seedlings of cv. Thatcher and the near-isogenic Thatcher genotypes RL6058-*Lr34*, line 920-*Lr34*, line 922-*LrT3*, and RL6011-*Lr12* inoculated at 8 and 13 days old. Several Mexican pathotypes of *Puccinia recondita* f. sp. *tritici* were used for inoculation. Incubation was carried out at four temperature regimes (three greenhouse and one growth chamber). Gene *Lr34* conferred resistance to all pathotypes, although the seedling infection types (ITs) varied among tests and ranged from slightly chlorotic flecks to 3+. Low ITs were best recognized in the older first leaves, which indicated that leaf growth stage affected the expression of resistance. The IT was influenced by the genetic background, temperature, and other environmental factors (perhaps light). The pathotype-specific response of *LrT3* could be seen only at lower temperatures, whereas *Lr12* was ineffective to all pathotypes at all temperature regimes. Gene *Lr34* conferred highly effective adult plant resistance at the Ciudad Obregon location, but it was only partially effective at the El Batan location with the same pathotypes. Genes *LrT3* and *Lr12* did not confer any adult plant resistance in the field.

isolates of *P. r. tritici* varied from 0;1- to 3 depending on temperature and light (5). However, very stable, resistant adult plant responses were observed both in the greenhouse and in the field during various crop cycles (5). This gene has been reported to increase the latent period and decrease infection frequency and uredial size (1). Effects of temperature on the above characters also were observed (1). A possible interaction of *Lr34* with *LrT3* has been considered to enhance the resistance expression (2,5). Roelfs (8) suggested the durable resistance to leaf rust in cv. Frontana may be attributable to the interaction of *Lr13* and *Lr34*.

Several studies were conducted recently at the International Maize and Wheat Improvement Center (CIMMYT) to investigate the expression of *Lr34* and its interaction with other known and unknown *Lr* genes and to determine its presence in cultivars derived from CIMMYT germ plasm. The expression of *Lr34* in seedlings at various temperatures with several Mexican patho-

The gene *Lr34*, conferring resistance to *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.

M. Henderson, has been reported to be present in several bread wheat (*Triticum aestivum* L.) cultivars of diverse origin (3-6,10). It was temporarily designated as *LrT2* by Dyck and Samborski (5). In 1987, Dyck (3) renamed it *Lr34* after locating it in chromosome 7D of wheat. The seedling disease response associated with *Lr34* when tested with Canadian

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types of *P. r. tritici* and the observed adult plant responses at two Mexican field locations are reported here, because such information is lacking in the literature.

MATERIALS AND METHODS

The five bread wheat tester lines used included RL6058-*Lr34* (Thatcher*6/PI 58548), line 920-*Lr34* (Thatcher*6/Lageadinho), line 922-*LrT3* (Thatcher*6/Lageadinho), RL6011-*Lr12* (Exchange/6*Thatcher), and Thatcher (susceptible control). These tester lines were developed by P. L. Dyck, Agriculture Canada, Winnipeg, Canada, and were supplied by A. P. Roelfs, Cereal Rust Laboratory, St. Paul, MN.

Eight- and 13-day-old seedlings of the tester lines were inoculated by spraying urediospores of *P. r. tritici* suspended in a lightweight mineral oil, placing them in a dew chamber overnight at 18–20 C, and transferring them to air-conditioned greenhouses. The three ambient temperature regimes used were 14–17, 18–23, and 23–28 C. Inoculated seedlings also were placed in a growth chamber at 12–13 C. Infection type (IT) data were based on a scale of 0–4 (11) and recorded when Thatcher expressed high ITs (10–18 days). The first leaf of 8-day-old plants and first and second leaves of 13-day-old plants were rated. Although tests were carried out with 23 pathotypes described by Singh (9), results with only five selected pathotypes (BBB/BN, MCD/SM, CBJ/QL, TBD/TM, and TCB/TD) are reported. The greenhouse tests were repeated four times during different months to observe the possible effects of light.

The two Mexican field locations used to observe adult plant responses during various seasons were Ciudad Obregon

(State of Sonora) (1988–1989 and 1989–1990) and El Batan (State of Mexico) (1988, 1989, and 1990). Field plots (two 3-m rows planted 15 cm apart with 70 cm between plots) were surrounded with spreader rows of the highly susceptible cv. Morocco. Epidemics were initiated by injecting a water suspension of urediospores into the leaf sheath of Morocco plants while the plants were in the late stem elongation growth stage. An equal mixture of pathotypes TCB/TD and TBD/TM was used. Data were recorded on flag leaves following the modified Cobb Scale (7).

RESULTS AND DISCUSSION

The ITs on the first leaves of tester lines inoculated at 8 days old and those on the first and second leaves of the seedlings inoculated at 13 days old at three greenhouse temperature regimes are given in Table 1. These results are from five pathotypes from the test where the lowest ITs were recorded. The ITs on the first leaves of *Lr34*-possessing lines RL6058 and 920 varied from slightly chlorotic flecks to 2+3 and 3– with all 23 pathotypes. This clearly indicated that *Lr34* conferred resistance to all Mexican pathotypes used in the tests. As shown in Table 1 for 8-day-old seedlings inoculated with five pathotypes, a rise in temperature reduced the occurrence of chlorotic flecks and increased the occurrence of uredia classified as 2 without clear chlorosis around them or uredia reaching IT 3. However, ITs on the first leaves of the seedlings inoculated at 13 days old were less influenced by temperature. Second leaves of the same seedlings displayed ITs ranging between 3– and 3+. The ITs displayed by the seedlings of RL6058 tended to be slightly lower than those displayed by the

seedlings of line 920.

The ITs displayed by the tester lines with two pathotypes at 12–13, 14–17, and 18–23 C are given in Table 2. The responses at 14–17 and 18–23 C are those from tests where the *Lr34*-carrying tester lines displayed the highest ITs. This illustrates the variation in ITs that occurs at the same temperature. For the first leaves, only the uredial size was slightly reduced compared with the susceptible check Thatcher. Second leaves of seedlings inoculated at 13 days old displayed ITs similar to the susceptible check Thatcher. In all situations, ITs similar to Thatcher were obtained at 23–28 C (*data not presented*). Only 8-day-old inoculated seedlings were tested at 12–13 C in a growth chamber. The ITs displayed by the seedlings were similar (i.e., slightly chlorotic flecks and occasional small uredia of 3= or 3– type) to those displayed in tests at 14–17 C reported in Table 1.

Results of the two tests reported in Tables 1 and 2 show the range of variation of ITs (from ; to 3) associated with *Lr34*, even when tests are carried out at similar temperatures. This may be associated with a variation in light intensity and duration during different months. Dyck and Samborski (5) also pointed out this feature of *Lr34*. The higher response of the second leaves of the seedlings inoculated at 13 days old was not expected because it is generally believed that second leaves would express higher resistance than first leaves. Current results indicate that the leaf growth stage was extremely important, because first leaves of the seedlings inoculated at 13 days old were more stably resistant than the first leaves of seedlings inoculated at 8 days old. Furthermore, the newly emerging second leaves were more susceptible. It

Table 1. Infection types on five tester lines when 8-day-old (data on first [I] leaf) and 13-day-old (data on first and second [II] leaves) seedlings were inoculated with five pathotypes of *Puccinia recondita* f. sp. *tritici* and incubated for 10–12 days at three greenhouse temperature regimes

Tester line	Temperature regime (C)	Pathotype															
		BBB/BN			MCD/SM				CBJ/QL			TBD/TM			TCB/TD		
		13 days			13 days				13 days			13 days			13 days		
		8 days	I	II	8 days	I	II	8 days	I	II	8 days	I	II	8 days	I	II	
RL6058- <i>Lr34</i>	14–17	;	;	3–	;	;	3–	;23=	;3=	3	;	;	3–	;	;	3–	
	18–23	;23–	;	3	;3=	;	3–	;23–	;3=	3	;	;	3–	;3=	;	3	
	23–28	3–	;	3	;3–	;	3–	3–	;3=	3	;	;	3–	;3=	;3=	3–	
Line 920- <i>Lr34</i>	14–17	23–	;	3	;23=	;	3	23=	;3=	3+	;3–	;	3	;3–	;	3+	
	18–23	3=	;	3	;23–	;3=	3	23–	;3=	3+	;3=	;	3	2+3	;	3+	
	23–28	3–	;	3	23–	;	3	3–	;23=	3	;23–	;	3	3–	;3=	3	
Line 922- <i>LrT3</i>	14–17	2+3	22+	3C	23C	2+3C	3C	3+	3+	3+	3	3	3+	3	3+	4	
	18–23	3+	3	3+	3+	3+	3+	3+	3+	3+	3+	3	3	4	3+	3+	
	23–28	3+	33+	33+	3+	3	3	3+	3+	3+	3+	3	3	3+	3+	3+	
RL6011- <i>Lr12</i>	14–17	3+	3	3	3	3+	3+	3+	3+	3	3+	3+	3+	3	3	3	
	18–23	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	
	23–28	3+	3	3	3	3	3	3+	3+	3+	3+	3	3	3+	3+	3+	
Thatcher	14–17	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	4	4	3+	3+	3+	
	18–23	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	
	23–28	3+	3+	3+	3	3	3	3+	3+	3+	3+	3+	3+	3+	3+	3+	

Table 2. Infection types on the first (I) and second (II) leaves of tester lines when 8-day-old and 13-day-old seedlings were inoculated with two pathotypes (TBD/TM and TCB/TD) of *Puccinia recondita* f. sp. *tritici* in the growth chamber at 12–13 C or in the greenhouse at 14–17 and 18–23 C

Tester line	12–13 C		14–17 and 18–23 C					
	8-day (I)		8-day (I)		13-day (I)		13-day (II)	
	TBD/TM	TCB/TD	TBD/TM	TCB/TD	TBD/TM	TCB/TD	TBD/TM	TCB/TD
RL6058- <i>Lr34</i>	;3–	;3=	3–3	3–3	3–3	3–	3+	3+
Line 920- <i>Lr34</i>	;3–	;3=	3–3	3–3	3–3	3–3	3+	3+
Line 922- <i>LrT3</i>	4	3+	3+	3+	3+	3+	3+	3+
RL6011- <i>Lr12</i>	4	3+	3+	3+	3+	3+	3+	3+
Thatcher	4	3+	3+	3+	3+	3+	3+	3+

may be that the product of the resistance allele *Lr34* involved in the interaction with the product of the avirulence allele in the pathogen accumulates in the leaves at a relatively slower rate. Therefore, the newly emerging leaves would be more susceptible than the older leaves. Genetic background effects also were apparent because RL6058 displayed slightly lower ITs than line 920. Thus, these results demonstrate the complex interaction that influences the expression of *Lr34*.

Leaves of line 922 with *LrT3* displayed ITs ranging from 22+ to 3C only at 14–17 C with pathotypes BBB/BN and MCD/SM (Table 1), indicating that the effectiveness of this gene was pathotype- and temperature-specific. Similar results were reported by Dyck and Samborski (5) and Drijepondt et al (2). Seedlings of RL6011 with *Lr12* (known to confer only adult plant resistance) and Thatcher displayed consistently high ITs with all pathotypes at all temperatures.

Field responses of the tester lines during five location-years (two at Ciudad Obregon and three at El Batan) are given in Table 3. The lines carrying *Lr34*, RL6058 and 920, displayed a response of 5MS during both seasons at Ciudad Obregon. Line 922 with *LrT3*, RL6011 with *Lr12*, and Thatcher displayed 100S responses, indicating that neither *LrT3* nor *Lr12* were effective to the pathotypes used in the adult plant tests. These genes also were ineffective at El Batan. Tester lines RL6058 and 920 displayed seasonal responses ranging between 20MS-S and 60MS-S at El Batan, indicating that the gene *Lr34* was only partially effective. Leaf rust on RL6058 and line 920 reached 100MS-S about 2 wk after Thatcher had obtained 100S response during the 1988 and 1990 seasons (*data not presented*).

Differences in the response of *Lr34* at the two Mexican locations underline the role of the environment. Wheat is grown at Ciudad Obregon (lat. 27°20'N, 38 m above sea level) from November to May. Seedling emergence is initially followed by reduced temperatures (not uncommonly close to 0 C) and a decreasing day length. Subsequently, the temperature rises and wheat matures at relatively warm temperatures and with long day lengths. On the other hand, the summer crop of wheat at El Batan (lat. 19°31'N, 2,249 m above sea level) is

Table 3. Adult plant field responses (based on modified Cobb Scale [7]) of five tester lines when inoculated with a mixture of *Puccinia recondita* f. sp. *tritici* pathotypes TCB/TD and TBD/TM at two locations during several seasons

Tester line	Ciudad Obregon		El Batan		
	1988–1989	1989–1990	1988	1989	1990
RL6058- <i>Lr34</i>	5MS	5MS	60MS-S	20MS-S	40MS-S
Line 920- <i>Lr34</i>	5MS	5MS	60MS-S	20MS-S	40MS-S
Line 922- <i>LrT3</i>	100S	100S	100S	80S	100S
RL6011- <i>Lr12</i>	100S	100S	100S	80S	100S
Thatcher	100S	100S	100S	80S	100S

planted in May when temperatures are moderate and day length is relatively long. Wheat matures at cooler temperatures and shorter day lengths in September and October. The adult plant leaf rust response of *Lr34* in the Thatcher background at Ciudad Obregon may not be entirely realistic. Thatcher's photosensitivity delays flowering by almost 2 mo compared with photo-insensitive wheats. The difference at El Batan is only 2 wk because of the longer day length. In photo-insensitive segregates homozygous for *Lr34*, from a cross involving RL6058 and a susceptible wheat, disease levels up to 40MS-S have been observed (R. P. Singh, *unpublished*).

The durable resistances of cvs. Frontana and Era have been associated with the interaction of *Lr34* with *Lr13* (6,8). Among other known genes, *LrT3* and *Lr12* also were thought to be involved in the interaction with *Lr34* to confer durable resistance (4,5,8). In this study, the genes *LrT3* and *Lr12* did not confer any level of detectable reduction in disease at the adult plant stage in the field with the two Mexican pathotypes of *P. r. tritici*. Recent studies by Singh (*unpublished*) indicate the adult plant resistance of Frontana and three other Mexican wheats involves the additive interaction of *Lr34* with two or three additional adult plant, partially effective genes. Gene *Lr13* was not involved in the interaction, probably because *Lr13*-virulent pathotypes were used. Similarly, we expect that *Lr12* and *LrT3* also are not involved in the interaction because they do not confer any resistance of their own relative to Thatcher. Drijepondt et al (2) also noted race-specific interaction involving the combination of *LrT3* and *Lr34*. Breeding at CIMMYT for leaf rust resistance based on the additive interac-

tion of *Lr34* with other partially effective genes has resulted in stably performing wheat varieties (R. P. Singh, *unpublished*).

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